Animal Science Papers and Reports
vol. 38 (2020) no. 2, 181-194

Morphological and biochemical traits of pheasant *Phasianus colchicus* eggs in relation to embryo sex and egg laying date*

Marcin T. Górecki[1], Sebastian Nowaczewski[2], Beata Grzegrzółka[3], Tomasz Szablewski[4], Kinga Stuper-Szablewska[5], Magdalena Rudzińska[6], Renata Cegielska-Radziejewska[4], Agata Biadała[4], Łukasz Tomczyk[4], Marcin Hejdysz[2], Przemysław Racewicz[2], Joanna Gruszczyńska[3]**

[1] Institute of Zoology Poznań University of Life Science, Wojska Polskiego 71C, 60-625 Poznań, Poland

[2] Department of Animal Breeding and Product Quality Assessment, Poznań University of Life Sciences, Słoneczna 1, Złotniki, 62-022 Suchy Las, Poland

[3] Department of Animal Genetics and Conservation, Institute of Animal Sciences, Warsaw University of Life Sciences - SGGW, Ciszewskiego 8, 02-786 Warsaw, Poland

[4] Department of Food and Management, Poznań University of Life Sciences, Wojska Polskiego 31, 60-637 Poznań, Poland

[5] Department of Chemistry, Poznań University of Life Sciences, Wojska Polskiego 75, 60-625 Poznań, Poland

[6] Institute of Plant Products Technology, Poznań University of Life Sciences, Wojska Polskiego 31, 60-624 Poznań, Poland

(Accepted January 2020)

Physical and biochemical characteristics of bird eggs depend on many factors. In turn, the biological quality of eggs influences hatchability and chick fitness. The aim of this study was to compare pheasant *Phasianus colchicus* eggs containing male and female embryos and laid in different months

* Research conducted by the statutory funding: No. 506.569.04.00 of the Faculty of Veterinary Medicine and Animal Science Poznan University of Life Sciences, Poland No. 505-50-740300-FN0000-99 of Department of Animal Genetics and Conservation, Institute of Animal Sciences Warsaw University of Life Sciences - SGGW, Poland

**Corresponding authors: sebastian.nowaczewski@up.poznan.pl

in terms of their morphology (egg weight, egg shape, yolk and white content, eggshell thickness) and biochemical traits (chemical composition, lysozyme content and activity, fatty acid profile as well as contents of macro- and microelements). The experimental material comprised eggs obtained from 1-year-old pheasants. Altogether 200 eggs were collected on April 7 and June 23 (100 eggs on each date). As some eggs were destroyed before and during measurements, numbers of eggs taken into account in the analysis of egg morphology traits were lower than numer of collected eggs. Egg homogenates were used in other analyses, sample size for each date ranged from 12-24. In conclusion, we stated that common pheasant eggs containing embryos of either sex did not differ in terms of morphology, basic chemical composition and a majority of other investigated traits, but eggs laid in April and June differed in terms of many studied traits (egg morphology, basic chemical composition, lysozyme content, some elements and fatty acids). Differences mentioned above can be explained by the egg laying date (female age), but environmental factors such as temperature and photoperiod might also affect egg traits.

KEYWORDS: egg quality / Galliformes / maternal investment / parental investment / Phasianidae

According to the sex allocation theory, parents should bias their investment in sons and daughters when male and female offspring give different fitness returns [Charnov 1982]. Indeed, there is evidence that bird females can control their offspring sex ratio [e.g. Heinsohn *et al*. 1997], which can be dependent i.e. on habitat quality [Prior *et al*. 2011] and mate quality [Pike and Petrie 2005]. It was suggested that bird females can influence the segregation of sex chromosomes during meiosis, possibly by manipulating hormone levels and other yolk traits [e.g. Rutkowska and Badyaev 2008]. It was reported that eggs with male and female embryos differed in terms of size [Anderson *et al*. 1997, Cordero *et al*. 2001, Magrath *et al*. 2003], contents of yolk, white, as well as lipids and non-lipids [Chin *et al*. 2012], concentration of hormones [Petrie *et al*. 2001], antibodies [Saino *et al.* 2003] and carotenoids [Verboven *et al*. 2005]. On the other hand, Rutkowska *et al.* [2014] conducted a meta-analysis and stated that in general eggs containing male and female embryos do not differ significantly in terms of size. Other authors published results of studies, where the relation of embryo sex and other egg characteristics was absent. For example, Pilz *et al*. [2005] found no differences in antibody concentrations in eggs with embryos of different sex, while Rubolini *et al.* [2011] reported no evidence of sex specific allocation of yolk components (carotenoids, vitamins and yolk hormones).

The ring-necked pheasant (also common pheasant) *Phasianus colchicus* is a highly sexually dimorphic species. Sexual dimorphism may be observed as early as in the 3rd week of life, with male chicks being heavier, having longer wings and greater wingspan [Górecki *et al.* 2012]. Adult males are not only heavier, but also have brighter plumage and possess armaments and ornaments absent in females [McGowan 1994]. The common pheasant is also a polygynous species [McGowan 1994], thus reproductive success variation is higher in males. Due to characteristics mentioned above, the ring-necked pheasant seems to be an interesting species in maternal sex allocation studies.

It is well known that the age of bird females can influence egg quality. In many species the weight of eggs is usually greater and the egg shape changes (with eggs

becoming more elongated) with the passage of the laying period [Amem and Al-Daraji 2011, Kontecka *et al.* 2012]. In turn, Sahin *et al.* [2009] reported a relationship between these characteristics and hatchability results. It was also observed that the egg white quality (index, Haugh units) and eggshell traits (weight, thickness or strength) deteriorate as the reproductive season progresses [Van den Brand *et al*. 2004, Akyurek and Okur 2009]. Moreover, Reijrink *et al.* [2008] proved that egg albumen quality plays a significant role in the embryogenetic process. However, a limited number of papers in this field concern niche poultry species, such as the guinea fowl, Japanese quail or the ostrich [Nowaczewski *et al.* 2010, Kontecka *et al.* 2011, Yamak *et al*. 2015]. Moreover, they deal with a comparison of egg quality between successive reproduction seasons [Krystianiak *et al*. 2007, Esen *et al.* 2010], rather than different dates during one season.

The available literature presents many results on the quality of ring-necked pheasant eggs [Kuźniacka *et al*. 2005, Kożuszek *et al*. 2009 a and b, Nowaczewski *et al*. 2011], but few studies focus on detailed biochemical characteristics of pheasant eggs (basic composition, fatty acid profile or content of macro- and microelements; e.g. Nowaczewski *et al*. 2013]. Moreover, to our best knowledge no data were published on the effect of age of ring-neck pheasants on the biochemical characteristics of their eggs. It needs to be stressed here that in our study farmed birds were used. It is known that pheasant female in captivity lay as many as 60-70 eggs during one season [Krystianiak *et al*. 2007], whereas in nature clutch size is between 10 and 20 eggs [e.g. Robertson 1991].

The aim of our study was to investigate possible intersexual differences between ring-necked pheasant eggs with male or female embryoes in terms of their physical traits and some biochemical parameters. Additionally, the effect of egg laying date on the above-mentioned traits was also investigated.

Material and methods

Birds and eggs

The experimental material comprised eggs obtained from 1-year-old pheasants *Phasianus colchicus* kept outdoors in 25 aviaries (702 m^2 surface area per each) belonging to the Polish Hunting Association – the Animal Breeding Center in Moszna, Poland. Pheasants kept in the Center are reproduced to release their offspring into the natural environment. The reproductive flock (females and males of similar age) consisted of 2600 females and 325 males. The mating sex ratio (male to females) was 1:8 in each aviary. A total of 104 females and 13 males were housed in each aviary (stock density was 6 m^2/bird). During the reproductive period (April-June) birds were kept under natural photoperiod. All birds were fed *ad libitum* with a complete diet containing 11.30 MJ/kg metabolisable energy, 17% crude protein, 4% crude fiber, 0.8% lysine, 0.7% methionine, 3.5% calcium and 0.4% total phosphorus.

Altogether 200 eggs were collected on April 7 and June 23 (100 eggs on each date), when hens were approximately 45 and 56 weeks old, respectively. The eggs were

collected on both dates in a random manner from 25 aviaries. As pheasant eggshell color was found to be related to other egg traits, e.g. biochemical composition and microbiological contamination [Nowaczewski *et al.* 2013], we used only eggs with an olive eggshell colour. Such colouration is also typical of eggs in wild pheasants.

Aslam *et al*. [2013], who studied domestic chicken eggs in terms of their weight and dimensions as well as yolk concentrations of hormones and glucose, claimed that results of earlier studies could be influenced by fact that eggs were incubated for several days, as it was necessary to obtain sufficient embryonic tissue for accurate identification of embryo sex. They also wrote that their study "is the first to determine a wide array of egg components […] in relation to the sex of egg in unincubated egg". Consequently, unincubated eggs were also used in our study.

All eggs were weighted directly after collection and immediately transported to the laboratory of the Department of Animal Genetics and Conservation, Institute of Animal Sciences Warsaw University of Life Sciences – SGGW, where embryo sex evaluation was conducted.

Physical traits of eggs

As some eggs were destroyed before and during measurements, numbers of eggs taken into account in the analysis of egg morphology traits were lower than the number of collected eggs. All measurements were made by the same person.

The following traits were recorded:

- eggshell weight (g) determined using a WPS 360C type balance,
- egg length and width were measured using slide calipers, accurate to 0.01 mm
- egg shape index (%) according to the formula: egg width (mm) x 100 / egg length (mm)
- content (%) of the yolk, white and eggshell in the egg mass,
- eggshell thickness (µm) together with shell membranes, measured in the equatorial part and both ends of the egg using a micrometre screw.

Evaluation of embryo sex

The blastoderm was removed from the surface of the egg yolks (eggs several hours after the oviposition without incubation) with a sterile wooden stick (toothpick) and deposited in 1.5 ml Eppendorf type tubes in 70% ethanol. The remaining white and yolk were used in the evaluation of biochemical traits. Each blastoderm was thoroughly fragmented and placed in a 2 ml sample tube. Genomic DNA was extracted from the blastoderm using a GeneMatrix Tissue & Bacterial DNA Purification Kit (EURx). A primer set of P2 (5′-TCTGCATCGCTAAATCCTTT-3') and P8 (5′-CTCCCAAGGATGAGRAAYTG-3′) was used in this study [Griffiths et al. 1998]. The primer set was designed to detect the intronic lenght difference between CHD1W and CHD1Z. The final composition of 18.00 µl PCR mixture was as follows: 10.00 µl JumpStart REDTaq ReadyMix (20 mM Tris-HCl at pH 8.3, 100 mM KCl, 4 mM MgCl2, 0.4 mM, 0.002% gelatine, 0.03 U/µl polymerase Taq DNA) (Sigma-Aldrich); 0.1 µl (2 pmol) of each primer; 5.8 µl MiliQ water and 2.00 µl (25

ng) genomic DNA as the template. An initial denaturing step at 95°C for 5 min was followed by 42 cycles at 94°C for 30 sec, 48°C for 45 sec and 72°C for 45 sec, with a final run at 72°C for 5 min completing the program. PCR products were separated by 2% agarose gel NuSieve™ GTG™ Agarose in 1 x TBE for 60 min at 55 V and visualised by staining with ethidium bromide. The amplified fragments were: 332 bp CHD1Z and 358bp CHD1W [Lee *et al*. 2010].

Egg biochemical analysis

Analyses (for the 1st and 2nd egg collection dates) were carried out using egg homogenates, with 3 eggs comprising 1 sample. As analyses were made in different laboratories and because of technical and financial constrains, sample sizes were not the same in every analysis.

Egg chemical composition. Contents of water, crude protein, crude fat and crude ash were determined using chemical analyses of combined white and yolk. Different methods were used to analyse each chemical component; water – the drier method at 105°C [PN-A-86509: 1994], protein – the Kjeldahl method (N × 6.25) [PN-A-04018: 1975/Az3: 2002], fat – the Soxhlet method [PN-A-86509: 1994] and ash by sample combustion in a muffle furnace at 600°C (BS-A-86509: 1994).

Lysosyme: The quantity (%) of lysozyme in egg white was determined by electrophoresis [Leśnierowski and Kijowski 1995], whereas the hydrolytic activity of this enzyme (U/mL liquid protein) was analysed by spectrophotometry.

Fatty acid profile in yolk: Lipids were extracted from homogeneous yolk samples using a mixture of methylene chloride and methanol. Fatty acid composition was determined after methylation by gas chromatography (ISO/FDIS 17059). Methyl esters of fatty acids (FAME) were prepared according to the AOCS Method Ce 1k-07.

Macro- and microelements. Concentrations of selected trace elements in eggs (yolk and white combined) were analysed by atomic absorption spectrometry (FAAS) using an Agilent Technologies AA Duo – AA280FS/AA280Z spectrometer (Agilent Technologies, Mulgrave, Victoria, Australia) equipped with a Varian hollow-cathode lamp (HCL). Calibration curves were plotted prior to analysis with four replicates per each trace element concentration.

Statistical analysis

Means and the standard errors of the means (SEM) were calculated for each trait. Two-way analysis of variance (ANOVA) was used; embryo sex and date of egg laying were included (as fixed effects) to the models. These computations were performed using the SAS® package [SAS 2011].

Results and discussion

Egg physical traits

In our study eggs containing male or female embryos did not differ significantly in egg weight, length, shape index, share of egg components and eggshell thickness

(p>0.05). Some authors reported dimorphism in the size of bird eggs dependent on the embryo's sex. Some researchers stated that females hatched from bigger eggs [Cordero *et al.* 2001: spotless starling *Sturnus unicolor*; Magrath *et al*. 2003: brown songlark *Cincloramphus cruralis*]. In turn, other researchers observed that males hatched from bigger/heavier eggs [Mead *et al*. 1987: white-crowned sparrow *Zonotrichia leucophrys oriantha*, Anderson *et al.* 1997: American kestrel *Falco sparverius*, Cordero *et al*. 2000: house sparrow *Passer domesticus*, Martyka *et al*. 2010: European blackbird *Turdus merula*]. Results of studies on domestic chicken eggs are ambiguous in terms of egg dimensions in relation to embryo sex, with no inter-sexual differences observed in egg weight [Aslam *et al*. 2013, Yilmaz-Dikmen and Dikmen 2013, Bergoug *et al*. 2015]. Rutkowska *et al*. [2014], when conducting a meta-analysis using data on 51 avian species reported that there is limited evidence for significant differences between eggs with male or female embryos. Even some older reports on egg size sexual dimorphism [Mead *et al.* 1987, Cordero *et al*. 2000] were questioned by newer findings [Bonier *et al*. 2007, Wetzel *et al*. 2012]. We found no significant differences in egg weight and dimensions in relation to embryo sex, thus our results are in line with the general rule proposed by Rutkowska *et al*. [2014]. In turn, Chin *et al*. [2012] observed that ring-billed gull *Larus delawarensis* eggs bearing male and female embryos differed in their relative content of yolk and white; however, we found no significant differences between eggs with male and female embryos of pheasant in terms of contents of egg components and eggshell thickness.

A comparison of physical traits of eggs laid in April and June is presented in Table 1. Our analysis of variability in egg physical characteristics revealed no influence of egg laying date on egg weight. On the other hand, such a relationship is well known in other poultry species. For example, Tůmová and Ledvinka [2009] and Kontecka *et al.* [2012] reported that egg weight increased with the passage of the reproductive period. However, the results were sometimes ambiguous. Similarly as in our research, Lee *et al*. [2016] did not confirm the effect of hens' age on egg weight. In turn, Günlü *et*

Table 1. Physical traits of pheasant eggs laid in April and June

Trait	April		June		*p* value
	mean	SEM	mean	SEM	
Egg weight (g)	32.73 (n=90*)	0.234	33.35 (n=99)	0.290	>0.1
Egg length (mm)	45.64 (n=90)	0.154	46.35 (n=99)	0.191	0.003
Egg width (mm)	36.09 (n=90)	0.093	36.16 (n=99)	0.111	>0.1
Egg shape index (%)	79.13 (n=90)	0.253	78.10 (n=99)	0.298	0.004
White content (%)	56.57 (n=89)	0.237	58.73 (n=99)	0.251	0.002
Yolk content (%)	33.46 (n=89)	0.226	32.38 (n=99)	0.251	≤0.0001
Eggshell content (%)	9.96 (n=89)	0.899	8.97 (n=98)	0.813	≤0.0001
Eggshell thickness - blunt end (μm)	298.10 (n=84)	3.424	271.37 (n=99)	2.631	≤0.0001
Eggshell thickness - equatorial part (μm)	295.69 (n=84)	2.915	277.54 (n=99)	2.511	≤0.0001
Eggshell thickness - sharp end (μm)	324.04 (n=84)	3.730	280.78 (n=99)	3.278	≤0.0001
Average eggshell thickness (μm)	305.94 (n=84)	2.635	276.56 (n=99)	2.340	≤0.0001

*As some eggs were destroyed before and during measurements, numbers of eggs taken into account in analysis of egg morphology traits were lower than the number of collected eggs.

al. [2018], when examining on a weekly basis eggs of pheasant from 41 to 50 weeks of age, observed statistically significant differences only by comparing two above mentioned extreme terms (30.22 vs. 32.19 g, respectively). A significant increase in egg weight with the passage of the reproductive season in pheasants was also confirmed by Uçar and Sarıca [2018].

Our investigations showed that eggs laid in June were significantly longer (by about 0.71 mm) than those produced in April; consequently, their shape index was lower. A similar relationship was observed in broiler breeders [Kontecka *et al.* 2010]. On the other hand, Adamski [2008] proved a reverse tendency in Hubbard Flex hens. Similar results in pheasants were obtained by Lee *et al.* [2016]. The authors observed an increase of this parameter (the eggs became more oval) with the passage of reproductive season. Nowaczewski *et al*. [2010], as well as Wilkanowska and Kokoszyński [2012] reported that there were no significant differences in the egg shape index between eggs laid in different weeks of Japanese quail age. As different authors reported contradictory results in galliforms it is very difficult to explain potential influence of birds' age/egg laying date on egg shape.

We stated that values of yolk and eggshell content in egg mass decreased as reproductive season advanced. In turn, eggs laid in June contained significantly more white, by about 2.2 percentage points. We observed that all measures of eggshell thickness were significantly lower in June. Günlü *et al*. [2018] also noted a significant decrease of the eggshell thickness with the age of birds.

Basic chemical composition and lysozyme

In our study eggs containing male or female embryos did not differ significantly in terms of basic chemical composition and lysozyme level ($p>0.05$). We do not know any papers investigating potential differences in traits mentioned above between male- and female-bearing eggs. Some authors reported difference in immunoglobulins content in relation to embryo sex [Saino *et al*. 2003: barn swallow *Hirundo rustica,* Martyka *et al.* 2011: zebra finch *Taeniopygia guttata*]. Contrary to these statements Pilz *et al.* [2005] did not found differences in antibodies concentration in eggs with embryos of different sex. Our results are in line of those by Pilz *et al*. [2005], we did not observe inter-sexual differences in lysozyme content and activity. However, we investigated lysozyme level, not antibodies mentioned by Saino *et al*. [2003], Pilz *et al.* [2005] and Martyka *et al*. [2011], thus results should be compared with caution, as different substances involved in protection against pathogens were analyzed.

A comparison of basic chemical composition and lysozyme level of eggs laid in April and June is presented in Table 2. Those did not differ significantly in terms of crude protein content. Eggs laid in June contained less crude fat and crude ash and more water than eggs produced earlier (in April). The differences (amounting to 3.24%; 0.30% and 3.50%, respectively) were statistically significant. An increased water content in pheasant eggs at the later part of the reproductive season (June) was probably related to a reduction of crude fat and crude ash. However, the content

Table 2. Basic chemical composition and lysozyme level of pheasant eggs laid in April and June

Trait	April (n=20)*		June (n=20)		*p* value
	mean	SEM	mean	SEM	
Water (%)	71.061	0.189	74.528	0.327	≤0.0001
Crude protein (%)	12.401	0.074	12.534	0.116	>0.1
Crude fat (%)	14.775	0.217	11.526	0.312	≤0.0001
Crude ash (%)	1.262	0.074	0.962	0.059	0.0028
Lysozyme content in liquid white (%)	2.744	0.071	3.123	0.045	≤0.0001
Lysozyme activity (U/mL)	58259.70	1528.00	66294.00	978.579	≤0.0001

*Egg homogenates were used where 3 eggs accounted for 1 sample.

of crude protein remained unchanged and was very similar at both analysed dates. The values of the analysed traits were similar to those reported in pheasants by Nowaczewski *et al*. [2013]. Ahn *et al.* [1997] showed no effect of laying hens' age on lipid and crude protein contents in egg yolk. In turn, Pambuwa and Tanganyika [2017] recorded an increase in the share of crude fat (from 4.57 to 5.05%) and crude protein (from 20.5 to 21.96%) in eggs with the passage of the egg laying period in hens (20 *vs* 28 weeks of life).

We estimated a statistically significantly higher ($p \leq 0.0001$) content and activity of lysozyme in eggs obtained from older pheasant females (June). Similar results were found in laying hens [Lewko and Gornowicz 2013] and pheasants [Kożuszek *et al*. 2009a]. The above-mentioned authors observed that the content and activity of lysozyme in egg white increased with birds' age, reaching its maximum at the end of the laying cycle. This may indicate greater mobilisation of the female organism to produce eggs that are better protected against pathogens at the end of the reproduction period. During that time the thickness of the eggshell usually decreases, which was confirmed in our study. Such a situation facilitates penetration of harmful microorganisms into the eggs. The validity of this thesis may be confirmed partially by other results also obtained in pheasants [Nowaczewski *et al.* 2013]. Those authors found greater lysozyme content and higher activity of this enzyme in eggs with white and blue eggshells. On the other hand, these eggshells are characterised by poorer quality, i.e. lower thickness and a higher number of pores [Kożuszek *et al.* 2009a]. Thus, it seems that such eggs contain a higher antimicrobial (lysozyme) level to compensate for their inferior protection against pathogens, connected with eggshell traits making eggs more vulnerable to microbial infection.

Fatty acids

Contents of only two out of nine analysed fatty acids were related to embryo sex. Eggs with male embryos contained less C16:0 fatty acid than eggs with female ones. The means and standard errors were: 38.528±0.392% for eggs with male embryos and 40.054±0.268% in yolk for eggs with female embryos ($p \leq 0.0001$). The opposite was true in terms of C18:0 fatty acid. Eggs with male embryos contained significantly more of this fatty acid (11.601±1.27%) than eggs with female embryos (8.934±0.633%,

p=0.008). There are papers investigating a possible relation of embryo sex with carotenoids in eggs of birds [Saino *et al.* 2003, Verboven *et al*. 2005, Badyaev *et al.* 2006], but to our best knowledge no articles deal with a potential relation of avian embryo sex and fatty acid composition.

In our study the egg laying date influenced contents of only two fatty acids (Table 3). Eggs laid in June were characterised by a significantly higher content of C16:0 fatty acid and a lower level of C24:0 fatty acid than eggs obtained in April. Neither sex of embryo nor egg laying date influenced contents of SFA, MUFA and PUFA. Our results are in line with those published by Lešić *et al*. [2017]. Those authors assessed the effect of laying hens' age on the fatty acid profile of their eggs. They observed very irregular and ambiguous changes in the levels of n-3 and n-6 PUFA in eggs laid from the 21st to the 55th week of life. It seems, however, that nutrition (components of diet) will play a greater role in the formation of yolk lipids than the age of birds. This thesis was confirmed by e.g. Keum *et al*. [2018].

Table 3. Fatty acid composition (%) of pheasant eggs laid in April and June

Fatty acid	April (n=12)*		June (n=12)		*p* value
	mean	SEM	mean	SEM	
C14:0	1.009	0.807	1.037	0.057	>0.1
C16:0	38.777	0.521	39.806	0.104	0.007
C16:1	6.284	0.251	6.394	0.245	>0.1
C18:0	10.900	1.341	9.635	0.598	>0.1
C18:1	28.228	1.383	28.431	1.026	>0.1
C18:2	13.352	0.686	13.173	0.849	>0.1
C18:3	0.692	0.035	0.723	0.033	>0.088
C20:4	0.186	0.032	0.140	0.022	>0.1
C24:0	0.073	0.022	0.020	0.010	0.036
SFA	50.759	1.341	50.498	0.544	> 0.1
MUFA	34.511	1.528	34.825	1.238	> 0.1
PUFA	14.730	0.620	14.678	0.821	> 0.1

*Egg homogenates were used where 3 eggs accounted for 1 sample.

Macro- and microelements

Eggs with male embryos had significantly lower mean levels of copper (0.916±0.257 mg/L internal egg content) than those with female embryos (1.019±0.452 mg/L; p=0.0495). Eggs containing embryos of both sexes differed in terms of sodium content at p=0.053. In turn, eggs with male embryos contained less sodium (1636.86±8.915 mg/L) than those with female embryos (1665.43±11.497 mg/L). No significant differences were found in this study in contents of other elements in relation to embryo sex. To the best of our knowledge no studies have been published dealing with a potential relation of avian embryo sex and contents of individual chemical elements.

Hen age had no influence on contents of selenium, potassium, calcium, magnesium and sodium in pheasant eggs (Tab. 4). On the other hand, eggs laid in June were characterised by significantly higher levels of copper and manganese, but lower contents of zinc and iron than eggs obtained in April. Nowaczewski *et al.*

Table 4. Macro- and microelements (mg/L internal egg content) of pheasant eggs laid in April and June

Element	April (n=23)*		June (n=24)		*p* value
	mean	SEM	mean	SEM	
Se	0.299	0.029	0.366	0.024	>0.0835
K	1011.90	9.242	1009.21	7.290	>0.1
Cu	0.912	0.027	1.016	0.043	0.0476
Ca	349.761	11.068	362.587	5.334	>0.1
Mg	147.907	3.269	148.022	3.933	>0.1
Na	1657.22	12.263	1644.73	8.765	>0.1
Zn	31.060	0.670	28.420	0.830	0.019
Fe	52.033	0.970	44.015	1.198	≤0.0001
Mn	0.738	0.250	0.910	0.022	≤0.0001

*Egg homogenates were used where 3 eggs accounted for 1 sample.

[2013] reported slightly lower values of all the above-mentioned elements in eggs of pheasants kept both in cages and aviaries.

In summary, our analyses did not confirm any relationship between sex of embryos and the physical traits and quality (biochemical composition) of ring-necked pheasant eggs. Therefore, the phenomenon of sex related investment in offspring, which occurs in many species of wild birds, was not observed in our study. This could be due to the fact that eggs studied by us were laid by females living in captivity and the common pheasant is a bird kept for many generations under specified, optimal environmental conditions with constant access to a complete diet (mixture) covering its living and production requirements. Such a situation could minimise differences which might be observed in natural conditions. It is possible that females in captivity had less need to invest (e.g. deposit nutrients) in a differentiated way depending on the sex of the embryo. It may be one of the reasons why only very few differences were found in the composition of pheasant eggs containing male and female embryos.

On the other hand, the effect of the egg laying date on the physical and biochemical characteristics of eggs was demonstrated. Eggs used in our study were laid by birds kept in outdoor aviaries, thus differences between eggs laid in April and June could be connected not only with differences of birds' age, but also with environmental (weather) factors such as temperature, photoperiod, etc.

Acknowledgements. *We would like to thank Bartosz Kolanoś and Krzysztof Kukulski from the Polish Hunting Association – the Animal Breeding Center in Moszna, Poland for sharing the research material.*

REFERENCES

1. AHN D.U., KIM S.K., SHU H., 1997 – Effect of egg size and strain and age of hen on the solids content of chicken eggs. ***Poultry Science*** 76, 914-919.
2. AKYUREK H., OKUR A.A., 2009 – Effect of storage time, temperature and hen age on egg quality in free-range layer hens. ***Journal of Animal and Veterinary Advances*** 8, 1953-1958.
3. AMEM H.M.M., AL-DARAJI J.H., 2011 – Zinc improves egg quality in Cobb 500 broiler breeder females. ***International Journal of Poultry Science*** 10, 471-476.

4. ANDERSON D.J., REEVE J. BIRD D.M., 1997 – Sexually dimorphic eggs, nestling growth and sibling competition in American Kestrels *Falco sparverius*. ***Functional Ecology*** 11, 331-335.
5. ASLAM M.A., HULST M., HOVING-BOLINK R.A.H., SMITS M.A., DE VRIES B., WEITES I., GROTHUIS T.G.G., WOELDERS H., 2013 – Yolk concentrations of hormones and glucose and egg weight and egg dimensions in unincubated chicken eggs, in relation to egg sex and hen body weight. ***General and Comparative Endocrinology*** 187, 15-22.
6. BADYAEV A.V., SEAMAN D.A., NAVARA K.J., HILL G.E., MENDOCA M.T., 2006 – Evolution of sex-biased maternal effects in birds: III Adjustment of ovulation order can enable sex-specific allocation of hormones, carotenoids, and vitamins. ***Journal of Evolutionary Biology*** 19, 1044-1057.
7. BERGOUG H., GUINEBRETIÈRE M., ROULSTON N., TONG Q., ROMANINI C.E.B., EXADAKTYLOS V., MCGONELL I.M., DEMMERS T., GARAIN P., BAHR C., BERCKMANS D., ETERRADOSSI N., MICHEL V., 2015 – Relationships between hatch time and egg weight, embryo sex, chick quality, body weight and pododermatitis severity during broiler rearing. ***The European Poultry Science*** 79, DOI: 10.1399/eps.2015.93.
8. BONIER F., MARTIN P.R., WINGFIELD J.C., 2007 – Maternal corticosteroids influence primary offspring sex ratio in a free-ranging passerine bird. ***Behavioral Ecology*** 18, 1045-1050.
9. CHARNOV E.L., 1982 – The theory of sex allocation. ***Monographs in Population Biology*** 18, 1-355.
10. CHIN E.H., SHARP C.M., BURNESS G., 2012 - Sex-biased resource allocation *in ovo* in a sexually size-dimorphic species. ***Journal of Avian Biology*** 43, 385-389.
11. CORDERO P.J., GRIFFITH S.C., APARICIO J.M., PARKIN D.T., 2000 – Sexual dimorphism in house sparrow eggs. ***Behavioral Ecology and Sociobiology*** 48, 353-357.
12. CORDERO P.J., VIÑUELA J., APARICIO J.M., VEIGA J.P., 2001 – Seasonal variation in sex ratio and sexual egg dimorphism favouring daughters in first clutches of the spotless starling. ***Journal of Evolutionary Biology*** 14, 829-834.
13. ESEN F, OZBEY O., GENC F., 2010 – The effect of age on egg production, hatchability and egg quality characteristics in pheasants (*Phasianus colchicus*). ***Journal of Animal and Veterinary Advances*** 9, 1237-1241.
14. GÓRECKI M.T., NOWACZEWSKI S., KONTECKA H., 2012 – Body weight and some biometrical traits of ring-necked pheasants (*Phasianus colchicus*) at different ages. ***Folia Biologica (Kraków)*** 60, 79-84.
15. GRIFFITHS R., DOUBLE M.C., ORR K., DAWSON R.J.G., 1998 – A DNA test to sex most birds. ***Molecular Ecology*** 7, 1071-1075.
16. GÜNLÜ A., ÇETİN O., GARİP M., KIRIKÇI K., 2018 - Effect of hen age on some egg quality
17. characteristics of pheasants. ***Manas Journal of Agriculture Veterinary and Life Sciences*** 8, 24-30.
18. HEINSOHN R., LEGGE S., BARRY S., 1997 - Extreme bias in sex allocation in *Eclectus* parrots. ***Proceedings of Royal Society of London Series B*** 264, 1325-1329.
19. KEUM, M.C., AN B.K., SHIN, K.H., LEE, K.W., 2018 – Influence of dietary fat sources and conjugated fatty acid on egg quality, yolk cholesterol, and yolk fatty acid composition of laying hens. ***Revista Brasileira de Zootecnia*** 47, doi.org/10.1590/rbz4720170303.
20. KONTECKA H., NOWACZEWSKI S., SIERSZUŁA M.M., WITKIEWICZ K., 2012 – Analysis of changes in egg quality of broiler breeders during the first reproduction period. ***Annals of Animal Science*** 12, 611-622.
21. KONTECKA H., WOŹNICKA J., WITKIEWICZ K., NOWACZEWSKI S., 2011 – Laying, egg and hatchability characteristics in ostrich (*Struthio camelus*) at different age. ***Folia Biologica (Kraków)*** 59, 163-167.
22. KOŻUSZEK R., KONTECKA H., NOWACZEWSKI S., LEŚNIEROWSKI G., KIJOWSKI J., ROSIŃSKI A., 2009a – Quality of pheasant (*Phasianus colchicus* L.) eggs with different shell colour. ***Archiv für Geflügelkunde*** 73, 201-207.

23. KRYSTIANIAK S., KONTECKA H., NOWACZEWSKI S., ROSIŃSKI A., 2007 – Laying characteristics of one- and two-year old pheasants (*Phasianus colchicus*, L.). ***Folia Biologica (Kraków)*** 55, 65-72.
24. KUŹNIACKA J., ADAMSKI M., BERNACKI Z., 2005 – Wpływ barwy skorupy na jakość jaj i wyniki wylęgu piskląt bażanta łownego (*Phasianus colchicus* L.) (Effect of eggshell colour on egg quality and hatchability results of pheasant (Phasianus colchicus)). ***Prace Komisji Nauk Rolniczych i Biologicznych BTN*** 42, 113-139. In Polish, with English summary.
25. LEE J., TSAI L., HWA P., CHAN C., HUANG A., CHIN S., WANG L., LIN J., LINACRE A., HSIEH H., 2010 – A novel strategy for avian species and gender identification using the CHD gene. ***Molecular and Cellular Probes*** 24, 27–31.
26. LEE M.H., CHO E.J., CHOI E.S., BANG M.H., SOHN S.H., 2016 – The effect of hen age on egg quality in commercial layer. ***Korean Journal of Poultry Science*** 43, 253-261.
27. LEŠIĆ T., KREŠIĆ G., CVETNIĆ L., PETROVIĆ M., PLEADIN J., 2017 – The influence of hen age on fatty acid composition of commercial eggs. ***Croatian Journal of Food Science and Technology*** 9, 158-167.
28. LEŚNIEROWSKI G., KIJOWSKI J., 1995 – Metody badania aktywności enzymatycznej oraz oznaczanie ilościowe lizozymu z białka jaja kurzego (Methods of determination of enzymatic activity and quantification of lysozyme in chicken eggs). ***Przemysł Spożywczy*** 12, 476-479. In Polish.
29. LEWKO L., GORNOWICZ E., 2013 – The enzymatic activity of lysozyme depending on the age of hens. Proc. XXIV International Poultry Science Symposium PB WPSA, "Science for poultry practice – poultry practice for science" 02-04.09.2013 Kołobrzeg, 165.
30. MAGRATH M.J.L., BROUWER L., KOMDEUR J., 2003 – Egg size and laying order in relation to offspring sex in the extreme size dimorphic brown songlark, *Cinclorhamphus cruralis*. ***Behavioral Ecology and Sociobiology*** 54, 240-248.
31. MARTYKA R., RUTKOWSKA J., CICHOŃ M., 2011 – Sex-specific effects of maternal immunisation on yolk antibody transfer and offspring performance in zebra finches. ***Biology Letters*** 7, 50-53.
32. MARTYKA R., RUTKOWSKA J., DYBEK-KARPIUK A., CICHOŃ M., WALASZ K., 2010 – Sexual dimorphism of egg size in the European blackbird *Turdus merula*. ***Journal of Ornithology*** 151, 827-831.
33. MCGOWAN P.J.K., 1994 – Phasianidae (pheasants and partridges) In: Handbook of the Birds of the World. Vol. 2, DEL HOYO J., ELLIOTT A., SARGATAL J., eds. New World Vultures to Guinea Fowl. Lynx Editions, Barcelona, 434-552.
34. MEAD P.S., MORTON M.L., FISH B.E., 1987 – Sexual dimorphism in egg size and implications regarding facultative manipulation of sex in mountain white-crowned sparrow. ***Condor*** 87, 798-803.
35. NOWACZEWSKI S., KONTECKA H., ROSIŃSKI A., KOBERLING S., KORONOWSKI P., 2010 – Egg quality of Japanese quail depends on layer age and storage time. ***Folia Biologica (Kraków)*** 58, 201-207.
36. NOWACZEWSKI S., STUPER K., SZABLEWSKI T., KONTECKA H., 2011 – Microscopic fungi in eggs of ring-necked pheasants kept in aviaries. ***Poultry Science*** 90, 2467-2470.
37. NOWACZEWSKI S., SZABLEWSKI T., CEGIELSKA-RADZIEJEWSKA R., STUPER-SZABLEWSKA K., RUDZIŃSKA M., LEŚNIEROWSKI G., KONTECKA H., SZULC K., 2013 – Effect of housing system and eggshell color on biochemical and microbiological characteristics of pheasant eggs. ***Archiv für Geflügelkunde*** 77, 226-233.
38. PAMBUWA W., TANGANYIKA J., 2017 – Determination of chemical composition of normal indigenous chickens in Malawi. ***International Journal of Avian & Wildlife Biology*** 2, 86-89.
39. PETRIE M., SCHWABL H., BRANDE-LAVRIDSEN N., BURKE T., 2001 – Sex differences in avian yolk hormones. ***Nature*** 412, 498.

40. PIKE T.W., PETRIE M., 2005 – Offspring sex ratio is related to paternal trait elaboration and yolk corticosterone in peafowl. ***Biological Letters UK*** 1, 204-207.
41. PILZ K.M., ADKINS-REGAN E., SCHWABL H., 2005 – No sex difference in yolk steroids concentrations of avian eggs at laying. ***Biological Letters UK*** 1, 318-321.
42. PN-A-04018:1975/Az3, 2002 – Produkty rolniczo-żywnościowe. Oznaczanie azotu metodą Kjeldahla i przeliczanie na białko (Zmiana Az3) (Agricultural and food products. Determination of nitrogen by the Kjeldahl method and conversion into protein (Change Az3)). In Polish.
43. PN-A-86509, 1994 – Przetwory jajowe – Badania fizykochemiczne (Egg products - Physicochemical tests). In Polish.
44. PRIOR G.L., EVANS D.M., REDPATH S., THIRGOOD S.J., MONAGHAN P., 2011 – Birds bias offspring sex ratio in response to livestock grazing. ***Biology Letters*** 7, 958-960.
45. ROBERTSON P.A., 1991 – Estimating the nesting success and productivity of British pheasants *Phasianus colchicus* from nest-record schemes. ***Bird Study*** 38, 73-79.
46. RUBOLINI D., ROMANO M., NAVARA K.J., KARADAS F., AMBROSINI R., CAPRIOLI M., SAINO N., 2011 – Maternal effects mediated by egg quality in the Yellow-legged Gull *Larus michahellis* in relation to laying order and embryo sex. ***Frontiers in Zoology*** 8, 24.
47. RUTKOWSKA J., BADYAEV A.V., 2008 – Meiotic drive and sex ratio determination: molecular and cytological mechanisms of sex ratio adjustment in birds. ***Philosophical Transactions of the Royal Society B: Biological Sciences*** 363, 1675-1686.
48. RUTKOWSKA J., DUBIEC A., NAKAGAWA S., 2014 – All eggs are made equal: meta-analysis of egg sexual size dimorphism in birds. ***Journal of Evolutionary Biology*** 27, 153-160.
49. SAHIN E.A., SENGOR E., YARDIMCI M., CETINGU I.S., 2009 – Relationship between pre-incubation egg parameters from old breeder hens, egg hatchability and chick weight. ***Journal of Animal and Veterinary Advances*** 8, 115-119.
50. SAINO N., ROMANO M., FERRARI R.P., MARTELLINI R., MØLLER A.P., 2003 – Maternal antibodies but not carotenoids in barn swallow eggs covary with embryo sex. ***Journal of Evolutionary Biology*** 16, 516-522.
51. SAS, 2011 – User's Guide. Statistical Analysis System Institute, Inc. Cary North California.
52. TŮMOVÁ E., LEDVINKA Z., 2009 – The effect of time of oviposition and age on egg weight, egg components weight and eggshell quality. ***Archiv für Geflügelkunde*** 73, 110-115.
53. UÇAR A., SARICA M., 2018 – The relationships between egg production, age and the hatching traits of pheasants. ***Turkish Journal of Agriculture - Food Science and Technology*** 6, 1311-1316.
54. VAN DEN BRAND H., PARMENTIER H.K., KEMP B., 2004 – Effects of housing system (outdoor vs cages) and age of laying hens on egg characteristics. ***British Poultry Science*** 45, 745-752.
55. VERBOVEN N., EVANS N.P., D'ALBA L., NAGER R.G., BLOUNT J.D., SURAI P.F., MONAGHAN P., 2005 – Intra-specific interactions influence egg composition in the lesser black-backed gull (*Larus fuscus*). ***Behavioral Ecology and Sociobiology*** 57, 357-365.
56. WETZEL D.P., STEWART I.R.K., WESTNEAT D.F., 2012 – Heterozygosity predicts clutch and egg size but not plasticity in a house sparrow population with no evidence of inbreeding. ***Molecular Ecology*** 21, 406-420.
57. WILKANOWSKA A., KOKOSZYŃSKI D., 2012 – Layer age and quality of pharaoh quail eggs. ***Journal of Central European Agriculture*** 13, 10-21.
58. YAMAK U.S., BOZ M.A., SARICA M., 2015 – Changes in guinea fowl fertility and hatching traits over a 4-month laying season with long-term egg storage conditions. ***Indian Journal of Animal Research*** 49, 532-536.
59. YILMAZ-DIKMEN B., DIKMEN S., 2013 – A morphometric method of sexing white layer eggs. ***Brazilian Journal of Poultry Science*** 15, 203-210.