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Changes in Quail Blastodermal Cell Status as a Result of Selection*

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Genetic selection over many years has significantly improved the growth rate of broilers and increased the number of eggs laid by egg laying chicken breeds. Selection has improved desired parameters, but has caused some negative effects as well. Adverse effects of selection may negatively affect embryonic development. The number of live and apoptotic blastodermal cells (BCs) at the X stage of embryogenesis may be a good indicator of changes in selected individuals. In this paper, a comparison of the number of live and apoptotic BCs was made for three lines of quail: Pharaoh (F33), meat-type line, selected for body weight; egg laying line (S33), selected for egg number; and laying line (S22), additionally selected (for 17 generations) for high yolk cholesterol content. Apoptotic BCs were separated by the magnetic activated cell sorting (MACS) method. The percentage of live and apoptotic BCs was different ($P \leq 0.01$) for F33 (35.8% and 64.2%, respectively) and S33 (60.0% and 36.4%). The number of apoptotic BCs for F33 embryos (45 098) was higher ($P \leq 0.01$) compared to the number of apoptotic BCs for S33 embryos (26 667). The selection for high yolk cholesterol content caused an increase ($P \leq 0.01$) in the total number of BCs from 78 403 (S33) to 140 139 (S22). The percentage of apoptotic BCs was lower ($P \leq 0.01$) in the S22 line (17.1%) compared to the S33 line (36.4%). The results showed that it is possible to evaluate the effects of selection in the early stage of embryonic development.

Key words: Apoptosis, MACS, blastodermal cells, quail.

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As a result of intensive selection carried out for several generations of birds, it was possible to significantly increase the growth rate of meat type birds, as well as to increase the number of eggs obtained from laying hens.

In a study on long-term selection of laying hens, some correlated adverse effects were noted. These effects were connected with a reduction of egg mass, unsolicited shell color, Haugh unit values and others (ALY *et al.* 2010; NESTOR *et al.* 1996). In addition, there is no doubt that a decrease of bird weight was a negatively correlated effect of selection for the number of eggs (EMMERSON *et al.* 2002).

On the other hand, during the last 50 years, the chicken growth rate has increased dramatically as a result of consistent selection. In 1966, broilers needed 60 days to obtain 1.82 kg of body weight, while in 2000 they reached the same weight in only 34 days (HAFEZ & HAUCK 2005). From the biological, as well as practical point of view, the transformations that affected the object of selection are very interesting. The best known and well-documented in various poultry species negative effect of selection for high growth rate is a decrease in reproductive traits (NESTOR *et al.* 1996; ANTHONY *et al.* 1996; DUNNINGTON & SIEGEL 1996). Other adverse effects include inter alia, immunodeficiency diseases, deformed limbs and car-

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cass fatness. Among the undesirable changes, the most important are those associated with reproduction. Quails selected for high growth rate are characterized by reduced fertility (ANTHONY *et al*. 1996) and by a decrease in egg production and extended time of sexual maturation (MARKS 1996). SADEGHI *et al*. (2013) reported a negative phenotypic correlation between body weight, the number of laid eggs, and the fertility and hatchability in three lines of quail.

Moreover, the selection of cocks has resulted in a decrease in semen production and sperm motility. In addition, selection has reduced the ability of sperm to fertilize an egg cell (BARBATO 1999) and increased the number of defective and apoptotic spermatozoon of cocks (TERIC & HOLCMAN 2008).

It is assumed that adverse effects of intensive selection may be observed in the early development of the embryo (the X stage according to EYAL--GILADI & KOCHAV 1976). According to HAMIDU *et al*. (2011) the number of live BCs may be a good indicator of the early development of birds. Therefore, the objective of the present study was to examine the effect of different selective pressures on the number of live and apoptotic BCs in the X stage of development of quail embryos (*Coturnix japonica*).

Material and Methods

Experimental birds

Japanese quail (*Coturnix japonica*) were maintained at the University of Life Sciences in Lublin in three lines: Pharaoh (F33), meat-type line, selected for body weight; egg laying line (S33), selected for egg number; and laying line (S22), additionally selected for high yolk cholesterol content (TAVANIELLO *et al*. 2014). The number of hens was different in each group – 17, 24 and 24 birds in F33, S33, and S22, respectively.

The isolation of blastodermal cells

Eggs laid on 3 consecutive days from each quail were collected and stored for 1 to 3 days before analysis in the same storage room, in the same environmental conditions (the temperature was maintained at 16 to 18°C). Before cell isolation, the eggshell was washed with 70% ethanol. Next, the eggs were opened at the blunt end and the yolk was separated from white. The yolks were transferred onto sterile Petri dishes (Bionovo, Legnica, Poland) and cleaned from the remains of white. Then paper rings were placed onto the blastodiscs and vitelline membranes were cut around the paper ring using sterile microsurgical scissors. Paper rings with blastodiscs were removed from the yolk surface and the blastodiscs were rinsed by a stream of PBS (-Ca^{2+}, -Mg^{2+}) (Invitrogen, Carlsbad, CA, USA) to separate the blastoderm from the vitelline membrane. Obtained blastoderm was suspended in 1 ml of PBS (-Ca^{2+}, -Mg^{2+}) with a solution of antibiotics 1:100 (Penicillin-Streptomycin, Invitrogen, Carlsbad, CA, USA). Next, blastoderm was pipetted briefly in order to obtain homogeneous cell suspensions and subsequently passed through a 30 μm nylon mesh (Miltenyi Biotec, Gladbach, Germany). Each sample was collected from 3 blastodiscs per hen.

Apoptotic BCs were separated by using Magnetic Activated Cell Sorting (MACS) and the Annexin V MicroBead Kit (Miltenyi Biotec, Bergisch Gladbach, Germany). Before separation, the concentration of BCs was evaluated using a Neubauer hemocytometer (Sigmed, Cisek, Poland). Afterwards, suspensions of BCs were centrifuged at 2800 rpm for 5min. and pellets were suspended in 30 μl of binding buffer. The next step involved adding 5 μl of Annexin V to samples and incubation for 15 min. at 6-12°C. After incubation, cells were rinsed by adding 1 ml of binding buffer. Suspensions of BCs were centrifuged at 2800 rpm for 5 min, and the supernatants were replaced with 0.5 ml of binding buffer. The next step included rinsing of the MS column with degassed binding buffer and immediately passing the cell suspension through the MS column. Next, the MS column was rinsed with binding buffer twice, removed from the separator and placed on a collection tube. Finally, the fraction with magnetically labeled apoptotic cells was acquired by washing the MS column with binding buffer. The obtained apoptotic BCs were counted in a Neubauer chamber. The percentage of apoptotic cells was evaluated by comparing the BC concentration before and after MACS separation.

Statistical analysis

Data was evaluated by using a one-way ANOVA, and means were evaluated by a *t*-student test (SAS 2011).

Results

In this work the method of Magnetic Activated Cell Sorting (MACS) was used in order to determine the share of apoptotic cells in the total population of blastodermal cells (BCs). The results of the analysis were used to determine parameters such as the number of apoptotic cells and the percentage of apoptotic cells in the whole population

of blastodermal cells. Prior to analysis on the magnetic sorter VarioMACS ®, the total number of BCs was evaluated. Analyzed embryonic discs were characterized by high heterogeneity in terms of size; this variability appeared at the individual level.

In the X stage of embryonic development (EYAL-GILADI & KOCHAV 1976), embryos of laying (S33) and meat (F33) type quail were characterized by a similar total number of BCs: 78 403 ± 23 792 and 69 804 ± 18 632, respectively (Fig. 1). Embryos of quail selected for high content of cholesterol in yolk (S22) had a statistically significant higher number of BCs (140 139 ± 21 835) compared to S33 and F33 embryos, whereas, in terms of the number of apoptotic cells, S33 and S22 embryos did not differ significantly (Fig. 2). Embryos of meat type quail were characterized by a much higher (45 098 ± 15 040) and statistically significant ($P \leq 0.01$) number of apoptotic cells.

In the light of these results, data shown in Figure 3 is of particular interest. The percentage of live and

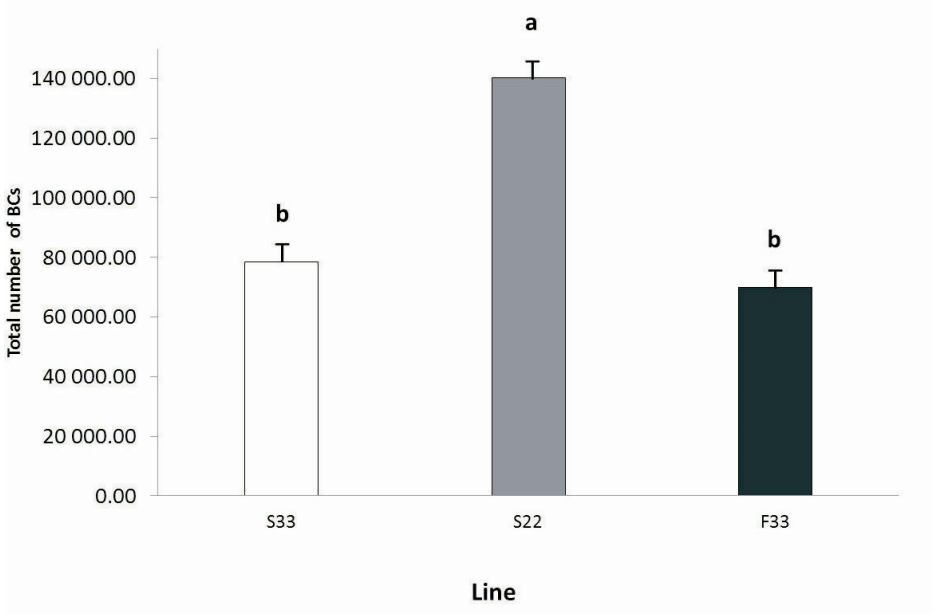

Fig. 1. The total number of BCs of laying quail embryos (S33), quail embryos selected for high content of cholesterol in egg yolk (S22) and embryos of quail of the meat line (F33). BC = blastodermal cells, n = 24 hens S33, n = 24 hens S22, n = 17 hens F33. Values marked with letters a, b differ significantly at $P \leq 0.01$.

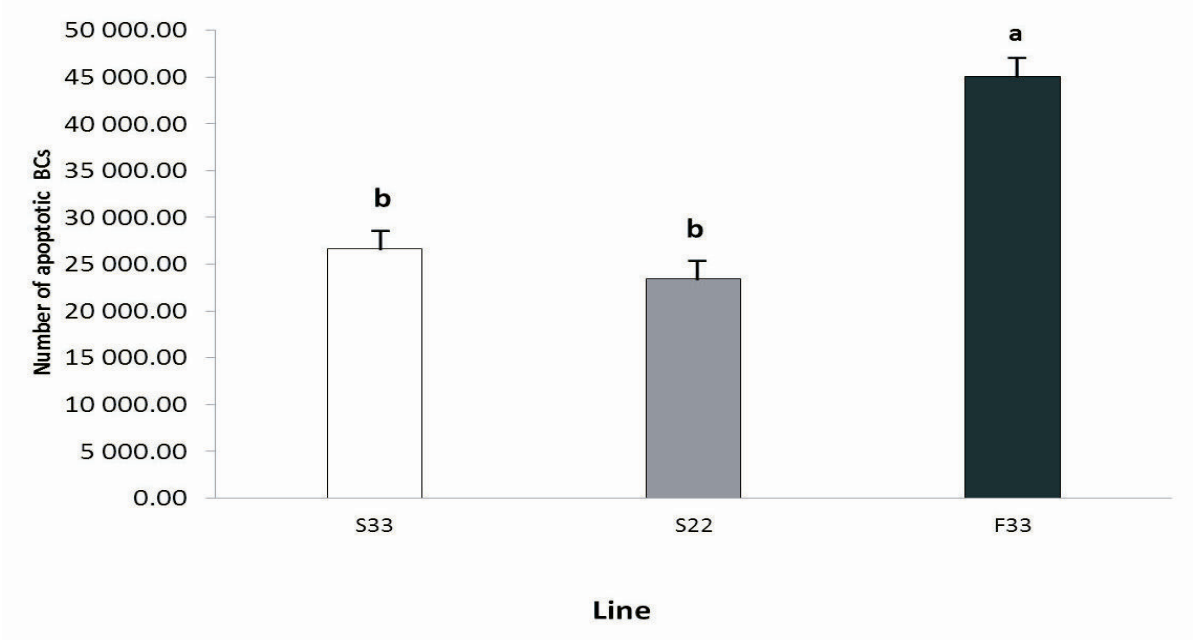

Fig. 2. The number of apoptotic BCs of laying quail embryos (S33), quail embryos selected for high content of cholesterol in egg yolk (S22) and embryos of quail of the meat line (F33). BC = blastodermal cells, n = 24 hens S33, n = 24 hens S22, n = 17 hens F33. Values marked with letters a, b differ significantly at $P \leq 0.01$.

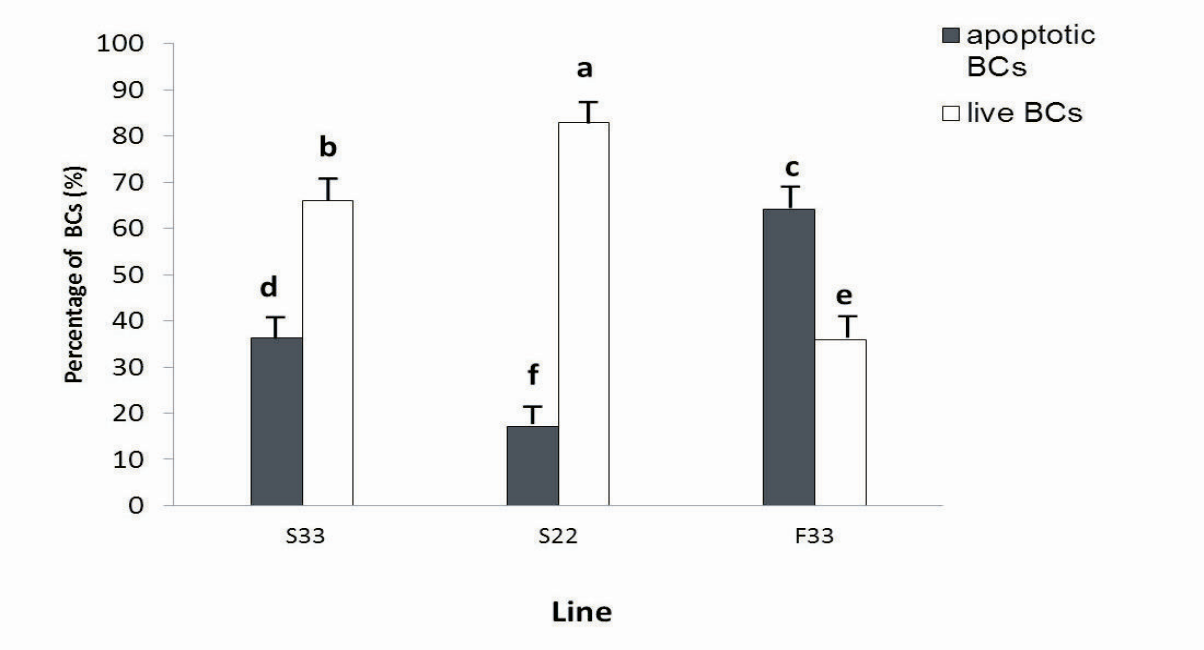

Fig. 3. The percentage of live and apoptotic BCs of laying quail embryos (S33), quail embryos selected for high content of cholesterol in egg yolk (S22) and embryos of quail of the meat line (F33). BC = blastodermal cells, n = 24 hens S33, n = 24 hens S22, n = 17 hens F33. Values marked with letters a-f differ significantly at $P \leq 0.01$.

apoptotic cells was significantly different ($P \leq 0.01$) in each of the compared lines of quail. The highest percentage of live cells (82.9) and the lowest percentage of apoptotic cells (17.1) were noted among embryos from the line selected for high content of cholesterol in yolk (S22). In contrast, in the group of embryos of meat type quail (F33), the percentage of live cells was only 35.8, while the percentage of apoptotic cells was the highest (64.2).

Discussion

Japanese quail are popular as a laboratory animal model, used among others for genetic, physiological, biomedical, behavioural and embryological studies (HUSS *et al.* 2008). Quail selection carried out for many generations greatly affects the characteristics that initially were not its objects (ANTHONY *et al.* 1996; MAIORANO *et al.* 2009; MAIORANO *et al.* 2011; MARKS 1996; NOWACZEWSKI *et al.* 2010; SADEGHI *et al.* 2013). Adverse effects are most visible and best evaluated at the level of adult individuals, especially broilers, and concern a number of problems ranging from bone deformations to reproductive disorders. Since some genetic correlations between selected traits and those not subject to selection were demonstrated, studies were initiated in order to determine whether significant changes occur at the stage of embryonic development.

The growth rate, embryonic weight, time of hatching, embryonic mortality, presence or absence of deformation at the level of morphology, respiratory and metabolic rate, morphology and vitality of blastodermal cells, expression of genes - all of these parameters, indicating the state of embryonic development, are subjected to significant changes under the influence of a variety of factors (ETCHES *et al.* 1997; ROMANOFF 1960).

These include epigenetic, genetic and environmental factors. There are relatively few well-documented investigations showing the impact of selection for production traits on embryonic development. In the light of studies showing, inter alia, the existence of a negative correlation between the length of egg storage and the survival of embryos (BAKST & AKUFFO 1999; FASENKO 2007), it appears that some authors have tried to prove that selection, as an important genetic factor, may affect the development of embryos. However, most of them have limited their interest only to hatching rates (BEDNARCZYK & ROSIŃSKI 1999; NIKOLOVA *et al.* 2011; ROSIŃSKI & BEDNARCZYK 1997).

In our study the percentage of apoptotic cells was higher ($P \leq 0.01$) in meat type embryos (F33) compared to the S22 and S33 embryos. An earlier study (CHRISTENSEN *et al.* 2001) showed that selection for fast growth rate of broilers has an influence on embryonic development and consequently, leads to morphological changes in an embryo. These changes may cause the disruption of homeostasis and may even lead to the death of an embryo.

Disrupted embryogenesis (COLEMAN & SIEGEL 1966 as cited in DUNNINGTON & SIEGEL 1996), deformation of embryos and their increased mortality (HAMIDU *et al*. 2011) were found to be characteristic for embryos of birds selected for higher body weight. JANICKI *et al.* (2003) also noted significant differences in the development of embryos of meat and laying type chickens when measured on the basis of erythropoiesis.

In this study, the increased proportion of apoptotic cells, lower survivability and lower total number of cells in the laying S33 line were observed, compared to the line additionally selected for high cholesterol content in egg yolk (S22). NOWACZEWSKI *et al.* (2010) pointed out the existence of a positive correlation between the cholesterol content and the vitality of the embryos and consequently, hatchability. Thus, high content of cholesterol affected favorably the embryo state, and therefore, may affect the state of the cell population in the X stage. The results obtained in this study seem to confirm this relationship.

These results are in accordance with the very few studies indicating a link between selection for production traits and the development of bird embryos. By analyzing the population of blastodermal cells from embryos of the Japanese quail, subjected to different selective pressures, it was shown for the first time that an assessment of the impact of selection may be carried out even during the early stage of embryonic development.

References

ALY O.M., GHANEM H.H., AFIFI Y.K., NAZLA Y., EL-ELLA A., BALAT M. 2010. Selection for improving egg production in Mandarah chickens 4- Direct and correlated response for some economic traits for four generations of selection. Egyptian Poult. Sci. **30**: 137-156.

ANTHONY N.B., NESTOR K.E., MARKS H.L. 1996. Short-term selection for four-week body weight in Japanese Quail. Poult. Sci. **75**: 1192-1197.

BAKST M.R., AKUFFO V. 1999. Impact of egg storage on embryo development. Proceedings International Congress on Bird Reproduction, Tours, France:125-131.

BARBATO G.F. 1999. Genetic Relationships Between Selection for Growth and Reproductive Effectiveness. Poult. Sci. **78**: 444-452.

BEDNARCZYK M., ROSIŃSKI A. 1999. Comparison of egg hatchability and *in vitro* survival of goose embryos of various origins. Poult. Sci. **78**: 579-585.

COLEMAN J.W., SIEGEL P.B. 1966. Selection for body weight at eight weeks of age 5 Embryonic state at oviposition and its relationship to hatchability. Poult. Sci. **45**: 1008-1011.

CHRISTENSEN V.L., WINELAND M.J., FASENKO G.M., DONALDSON W.E. 2001. Egg storage effects on plasma glucose and supply and demand tissue glycogen concentrations of broiler embryos. Poult. Sci. **80**: 1729-1735.

DUNNINGTON E.A., SIEGEL P.B. 1996. Long-Term Divergent Selection for Eight-Week Body Weight in White Plymouth Rock Chickens. Poult. Sci. **75**: 1168-1179.

EMMERSON D.A., VELLEMAN S.G., NESTOR K.E. 2002. Effect of long-term selection for Increased egg production on the genetics of growth and egg production traits. Poult. Sci. **81**: 316-320.

ETCHES R.J., CLARK M.E., ZAJCHOWSKI L. 1997. Manipulation of Blastodermal Cells. Poult. Sci. **76**:1075-83.

EYAL-GILADI H., KOCHAV S. 1976. From cleavage to primitive streak formation: a complementary normal table and a new look at the first stages of the development of the chick. 1. General morphology. Dev. Biol. **49**: 321-337.

FASENKO G.M. 2007. Egg Storage and the Embryo. Poult. Sci. **86**: 1020-1024.

HAFEZ H.M., HAUCK R. 2005. Genetic selection in turkeys and broilers and their impact on health conditions. World Poultry Science Association, 4th European Poultry Genetics Symposium, Dubrovnik, Croatia, 6-8 October, 2005, **4**: 40-50.

HAMIDU J.A., UDDIN Z., LI M., FASENKO G.M., GUAN L.L., BARREDA D.R. 2011. Broiler egg storage induces cell death and influences embryo quality. Poult. Sci. **90**: 1749-1757.

HUSS D., POYNTER G., LANSFORD R. 2008. Japanese quail (*Coturnix japonica*) as a laboratory animal model. Lab. Anim. **37**: 513-519.

JANICKI B., WINIARSKA M., STASIAK K., NOWAK-TKACZYK A., GRAJEWSKI B., BEDNARCZYK M. 2003. The comparison of erythropoiesis in embryos of laying and meat hens. Vet. Med. **59**: 1145-1147. (In Polish).

MAIORANO G., ELMINOWSKA-WENDA G., MIKA A., RUTKOWSKI A., BEDNARCZYK M. 2009. Effects of selection for yolk cholesterol on growth and meat quality in Japanese quail (*Coturnix coturnix japonica*). Ital. J. Anim. Sci. **8**: 457-466.

MAIORANO G., KNAGA S., WITKOWSKI A., CIANCIULLO D., BEDNARCZYK M. 2011. Cholesterol content and intramuscular collagen properties of pectoralis superficialis muscle of quail from different genetic groups. Poult. Sci. **90**: 1620-1626.

MARKS H.L. 1996. Long-term selection for body weight in Japanese Quail under different environments. Poult. Sci. **75**: 1198-1203.

NESTOR K.E., NOBLE D.O., ZHU J., MORITSU Y. 1996. Direct and correlated responses to long-term selection for increased body weight and egg production in turkeys. Poult. Sci. **75**: 1180-1191.

NIKOLOVA N., KOCEVSKI D., KUZELOV A. 2011. Influence of genotype on eggshell strength and the hatchability of laying parent stock flock. Biotech. Anim. Husbandry **27**: 1659-1666.

NOWACZEWSKI S., KONTECKA H., ELMINOWSKA-WENDA G., ROSIŃSKI A., BEDNARCZYK M., KUCHARSKA A. 2010. Eggs quality traits of Japanese quail divergently selected on yolk cholesterol level. Archiv. Geflügelkd. **74**: 141-144.

ROMANOFF A.L. 1960. The Avian Embryo: Structure and Functional Development. Macmillan, New York.

ROSIŃSKI A., BEDNARCZYK M. 1997. Influence of genotype on goose egg hatchability. Archiv. Geflügelkd. **61**: 33-39.

SADEGHI R., PAKDEL A., MORADI SHAHRBABAK M., MORADI SHAHRBABAK H. 2013. Divergent selection effect on reproductive trait in Japanese quails. Res. J. Poult. Sci. **6**: 18-22.

TAVANIELLO S., MAIORANO G., SIWEK M., KNAGA S., WITKOWSKI A., DI MEMMO D., BEDNARCZYK M. 2014. Growth performance, meat quality traits, and genetic mapping of quantitative trait loci in 3 generations of Japanese quail populations (*Coturnix japonica*). Poult. Sci. **93**: 2129-2140.

TERIC D., HOLCMAN A. 2008. Long-term divergent selection for 8-week body weight in chickens – a review of experiments. Acta Agr. Slov. **92**: 131-138.