A selection study for improving eggshell colour in two parent lines of laying hens and their hybrids

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ABSTRACT
The aim of this research was to evaluate selection studies towards increasing eggshell colour intensity and homogeneity, without reducing egg production of brown-egg-laying hens at the Poultry Research Institute of Ankara, Turkey. This study was based on data obtained from 1342 pedigree Barred Rock-1 (BAR-1), 1158 pedigree Rhode Island Red-1 (RIR-1) and 611 hybrid (RIR-1xBAR-1 or ATAK-S) brown laying hens, where eggshell colour and egg production performances were determined. Hens were selected for dark, uniform shells and egg production performance, as paternal and maternal lines from RIR-1 and BAR-1 base populations. Colour coordinates; L*(brightness), a* (red/green scale), b* (yellow/blue scale) and eggshell colour index (SCI) were determined. The values of L*, b* and a* were significantly changed during the study. The estimated heritabilities for BAR-1 hens of egg weight (EW), L*, a*, b* and SCI were as follows: 0.62, 0.64, 0.62, 0.55 and 0.32; The RIR-1 hen values were as follows: 0.51, 0.74, 0.64, 0.22 and 0.39, respectively. It was concluded that the performance of parents and hybrids was better than that of the base populations, with respect to the characteristics studied.

ARTICLE HISTORY
Received 17 December 2015
Revised 4 March 2016
Accepted 30 May 2016

KEYWORDS
Heritability; colour index; laying hen; egg production

Introduction
Eggshell colour is not a determining factor for internal egg quality but most consumers prefer brown-shelled eggs (Odabasi et al. 2007). A study by Samiullah et al. (2015) reported that the brown colouration of the shell was an important shell quality parameter and had a positive influence on consumer preference. Research by Mizrak et al. (2012), on egg consumers in Turkey, showed that 30.50% of the consumers preferred brown-shelled eggs. In 2004, the Ankara Poultry Research Institute developed a domestic egg-laying hybrid, which was officially registered to the Turkish Patent Institute, and was trademarked as ATAK-S. This egg-laying hybrid was obtained from RIR-1 (Rhode Island Red-1) and BAR-1 (Barred Rock-1) lines and marketed within the country for the first time in 2004. At this point, the field results obtained were good, but variation in egg colour was high (18%); therefore, egg consumption was negatively affected as a result of this situation (Sarica et al. 2010). A study by Liu and Winston (2010) stated that bird eggs showed an enormous diversity of eggshell colours. The major pigments present in bird eggshells are protoporphyrin, biliverdin and biliverdin zinc chelate, which are dependent upon different genes and gene expressions (Kennedy & Vevers 1976). Mengr (2014) determined that avian eggshell colouration was caused by specific pigments deposited into the eggshell. These pigments differed in colour, chemical structure and the site of eggshell deposition. Research by Odabasi et al. (2007) explained changes in eggshell colour with the age of the hen; eggshell mass increased with the hen age but the amount of pigment did not increase in the same ratio as the shell mass, resulting in more shell surface being covered with a given amount of pigment. A study by Solomon (1997) reported that older hens tended to lay larger eggs, with a lighter shell colour. The production of uniformly dark-brown coloured eggshells, through selective breeding, was the goal of poultry breeders of brown-egg laying hens.

Studies at the Poultry Research Institute of Ankara, Turkey, showed that variation in eggshell colour (18%) and the ratio of eggs with pale brown colour (16%) was high. To be able to solve this problem, a breeding selection study was initiated. The purpose of this study was to develop egg-laying parents and hybrids that...
Materials and methods

This study was based on data from 1342 pedigree Barred Rock-1 (BAR-1), 1158 pedigree Rhode Island Red-1 (RIR-1) and 611 hybrid (RIR-1×BAR-1) brown laying hens, which were developed in the Poultry Research Institute of Ankara, Turkey. To obtain the first generation, 1200 eggs from each parent, which laid eggs with dark-coloured eggshells, were hatched. A total of 500 female and 100 male chicks, from each line were selected, and egg production, egg weight (EW), age at first egg (AFE), body weight at first egg (BWFE) and eggshell colour properties were determined. To achieve the desired properties, BAR-1 and RIR-1 lines were formed from 10 families consisting of 1 male and 9 females (9 hens were artificially inseminated with the semen of 1 male), using a similar mating system previously implemented by Goger et al. (2014). To acquire a second generation from these families, hens from each family were artificially mated with their parental cocks, and their eggs were collected for 15 days. After hatching these eggs, 500 female and 100 male chicks were selected. These birds were then examined and their productivity properties were determined, following the same methodology used for the first generation chicks. Following this, a third generation was obtained by hybridising RIR-1 males and BAR-1 females and 500 female hybrid chicks were hatched. To test whether genotypic progression was obtained, selected productivity traits were determined on new parents for a period of 64 weeks and on hybrids up until 72 weeks of age.

Birds were kept individually in cages, in a two-sided battery hen house with three tiers, and fed ad libitum standard commercial diet. The diet formulated was based on National Research Council (NRC) (1994) recommendations. Egg production was recorded individually every day, and AFE was defined as when the hen laid her first egg. BWFE was defined as the weight of the chicken at the time of their first egg laying, which was measured using 20 g precision scales (Dikomsan, Turkey). Egg number (EN) was defined as the number of eggs that each individual hen laid, as a total during the study. After the EW measurement was recorded, using the 0.1 g precision scales (Dikomsan, Turkey), the eggshell colour was measured at the large pole of the egg (Aygun 2014). Eggshell colour measurements were determined by a Minolta CR 10 colorimeter (Konica Minolta Inc., Osaka, Japan). Colour quality can be obtained on a continuous scale for different flocks and compared over a number of years, and was therefore an appropriate measurement for data obtained in our study. The CR-10 Minolta colorimeter measured the colour difference between two colours; for calibration purposes, Roche Yolk Colour Fan (RYCF), was measured first, stored in the memory and was referred to as the target colour. The measurements of eggshell colour and EW were taken 9 times for parent lines (28th–64th week), and 11 times for hybrid lines (28th–72nd week), once in every 4 weeks. The colour of each individual egg was determined by the Minolta CR 10 colorimeter (Konica Minolta Inc., Osaka, Japan) by the three following parameters:

- $L^*$: egg’s lightness (value between $0=black$ and $100=white$)
- $a^*$: egg’s colour tone as a function of a red-green scale ($<0=green$, $>0=red$)
- $b^*$: egg’s colour tone as a function of a blue-yellow scale ($<0=blue$, $>0=yellow$)

Shell colour index (SCI) was calculated on the basis of the three colour parameters ($L^*, a^*$ and $b^*$) and formulated according to Francis (1998) as follows:

$$SCI = 100 - L^* - a^* - b^*$$

the higher the value, the darker the colour.

Descriptive statistics were analysed by the general linear model procedure of Minitab (Minitab 1998). Duncan’s multiple range test (Duncan 1955) was used to compare the means for significant differences. Variance components and genetic parameters were estimated by the animal model, using the MTDFREML packet programme (Boldman et al. 1995).

$$Y_{ivor} = \mu + S_i + d_v (S_i) + b_o + e_{ivor}$$

Table 1. Egg production traits of RIR-1 and BAR-1 lines by years.

| Line | Year | N   | AFE | BWFE   | EN     | EW     |
|------|------|-----|-----|--------|--------|--------|
| BAR-1| 2011 | 293 | 158.10±0.35 | 1730.66±6.70 | 191.90±1.35 | 57.65±0.14 |
|      | 2012 | 539 | 150.45±0.57 | 1663.39±5.62 | 232.83±1.26 | 57.22±0.19 |
|      | 2013 | 510 | 151.21±0.51 | 1748.17±6.62 | 256.63±1.02 | 56.90±0.18 |
| RIR-1| 2011 | 205 | 164.29±0.63 | 1661.37±6.97 | 195.56±1.28 | 56.24±0.24 |
|      | 2012 | 521 | 156.15±0.50 | 1680.49±5.06 | 217.93±1.34 | 61.83±0.21 |
|      | 2013 | 432 | 159.88±0.81 | 1748.17±6.62 | 256.63±1.02 | 56.90±0.18 |

N: number of hen; AFE: age at first egg; BWFE: body weight at first egg; EN: egg number; EW: egg weight; BAR-1: Barred Rock-1; RIR-1: Rhode Island Red-1; a column means with different superscripts differ significantly at ($p < 0.05$).
where $Y_{iwr}$ is the phenotype of the rth offspring, in the oth year, from the family of the ith sire and vth dam ($r$: subscript for offspring), $\mu$: overall mean, $S_i$: the effect of the ith sire ($i$: subscript for sire), $d_v$: the fixed effect of the vth dam, which is mated to the ith sire ($v$: subscript for dam), $b_o$: the fixed effect of the oth year ($o$: subscript for year), $e_{iwr}$ is the random error, and $e$ is assumed as follows: $N (0, \sigma^2_e)$. 

### Results

Descriptive statistics of age, BWFE, EN and EW were determined for 2500 individuals (Table 1). Significant differences were observed (Table 2), with respect to $L^*$, $a^*$ and $b^*$ mean values, dependent upon years ($p < 0.05$), between different breeding lines and within the same breeding lines, except BAR-1 $a^*$ mean value. While the egg shell improved over three generations in brown parental laying hens, egg production traits were also enhanced. Birds of the parent lines reached their sexual maturity at 150.45–164.29 days, and their mean body weight at sexual maturity was 1661.37–1763.97 g (Table 1). Hens laid between 191.90 and 256.63 eggs, with a mean weight of 56.90–62.24 g over the laying period until their 64th week of age.

The first selection studies were conducted using RIR-1 sire lines and BAR-1 dam lines. Considerable progress was achieved in obtaining the desired eggshell colour, by the 3rd generation, by using selection studies of sire and dam lines (Table 2).

For the duration of the study, SCI values were calculated at different weeks and were found to decrease considerably, particularly at an advanced age; the eggshells became lighter as the hen aged (Table 3). Sire line (RIR-1) cocks were crossed with dam line (BAR-1) hens to obtain hybrid chicks. When SCI values were examined, the values for the hybrids were observed to be higher than that of their parents (Table 4).

Genetic and phenotypic correlations, between traits and heritability of traits were estimated for the chickens (Table 5). Although the eggshell colour getting pale with age, hens that laid eggs with a darker shell colour in the early stages of the laying cycle would continue to lay darker coloured eggs until the end of the cycle. This was also true for hens that laid...

### Table 2. $L^*$, $a^*$ and $b^*$ mean values of RIR-1 and BAR-1 lines by years.

| Year | $N$ | $L^*$ | $a^*$ | $b^*$ |
|------|-----|-------|-------|-------|
| BAR-1 | 2011 | 293 | 59.35±0.27 | 12.50±0.25 | 22.97±0.16 |
|      | 2012 | 539 | 61.45±0.22 | 12.81±0.21 | 23.57±0.14 |
|      | 2013 | 510 | 59.21±0.35 | 13.26±0.18 | 22.40±0.19 |
| RIR-1 | 2011 | 205 | 62.09±0.25 | 11.07±0.27 | 22.73±0.12 |
|      | 2012 | 521 | 59.49±0.32 | 13.00±0.25 | 23.02±0.14 |
|      | 2013 | 432 | 58.10±0.47 | 14.21±0.20 | 21.12±0.24 |

### Table 3. Shell colour index values on the basis of weeks at RIR-1 and BAR-1 lines in 2011 and 2012 years.

| Weeks | $N$ | $L^*$ | $a^*$ | $b^*$ |
|-------|-----|-------|-------|-------|
| BAR-1 | 2011 | 76.46±0.42 | 74.94±0.49 | 77.24±0.47 | 12.71±0.55 |
|      | 2012 | 76.43±0.42 | 73.87±0.47 | 75.32±0.45 | 12.70±0.56 |
| RIR-1 | 2011 | 72.21±0.56 | 72.77±0.45 | 70.86±0.66 | 69.56±0.54 |
|      | 2012 | 71.80±0.54 | 71.78±0.46 | 71.40±0.69 | 69.54±0.62 |

### Table 4. Shell colour index values of RIR-1, BAR-1 and their crosses (H) in 2013 by weeks.

| Lines | Week | $N$ | $SCI$ | $L^*$ | $a^*$ | $b^*$ |
|-------|------|-----|-------|-------|-------|-------|
| BAR-1 | 2011 | 510 | 76.07±0.58 | 51.89±0.62 | 69.42±0.42 | 68.23±0.05 |
|      | 2012 | 432 | 76.62±0.61 | 51.89±0.62 | 69.42±0.42 | 68.23±0.05 |
| RIR-1 | 2011 | 555 | 78.15±0.29 | 51.89±0.62 | 69.42±0.42 | 68.23±0.05 |
|      | 2012 | 485 | 75.38±0.43 | 51.89±0.62 | 69.42±0.42 | 68.23±0.05 |
| Lines | Week | $N$ | $SCI$ | $L^*$ | $a^*$ | $b^*$ |
|-------|------|-----|-------|-------|-------|-------|
| H 2011 | 508 | 76.10±0.47 | 51.89±0.62 | 69.42±0.42 | 68.23±0.05 |
|       | 509 | 76.09±0.42 | 51.89±0.62 | 69.42±0.42 | 68.23±0.05 |
| Lines | Week | $N$ | $SCI$ | $L^*$ | $a^*$ | $b^*$ |
|-------|------|-----|-------|-------|-------|-------|
| H 2011 | 510 | 73.05±0.63 | 51.89±0.62 | 69.42±0.42 | 68.23±0.05 |
|       | 511 | 73.06±0.63 | 51.89±0.62 | 69.42±0.42 | 68.23±0.05 |
| Lines | Week | $N$ | $SCI$ | $L^*$ | $a^*$ | $b^*$ |
|-------|------|-----|-------|-------|-------|-------|
| BAR-1 | 2011 | 502 | 72.59±0.48 | 51.89±0.62 | 69.42±0.42 | 68.23±0.05 |
|      | 2012 | 354 | 72.60±0.59 | 51.89±0.62 | 69.42±0.42 | 68.23±0.05 |

### Table 5. Estimated heritabilities, genetic and phenotypic correlations between shell colour parameters and egg weight at BAR-1 (above) and RIR-1 (below) lines weight at BAR-1 (above) and RIR-1 (below) lines.

| EW | $L^*$ | $a^*$ | $b^*$ | SCI |
|----|-------|-------|-------|-----|
| EW | 0.62±0.12 | 0.36±0.01 | 0.26±0.04 | 0.16±0.06 | 0.18±0.05 |
| L* | 0.64±0.10 | 0.43±0.01 | 0.48±0.01 | 0.23±0.08 | 0.33±0.08 |
| a* | 0.98±0.01 | 0.62±0.12 | 0.25±0.03 | 0.11±0.04 | 0.12±0.02 |
| b* | 0.79±0.04 | 0.55±0.14 | 0.29±0.01 | 0.16±0.03 | 0.09±0.01 |
| SCI | 0.24±0.33 | 0.44±0.29 | 0.32±0.18 | 0.19±0.09 | 0.31±0.06 |
| 0.14±0.19 | 0.49±0.16 | 0.51±0.07 | 0.13±0.07 | 0.46±0.09 |
| 0.02±0.12 | 0.07±0.11 | 0.23±0.12 | 0.74±0.06 | 0.17±0.03 |
| 0.13±0.06 | 0.13±0.07 | 0.21±0.06 | 0.64±0.04 | 0.22±0.04 |
| 0.21±0.04 | 0.08±0.30 | 0.25±0.36 | 0.20±0.27 | 0.22±0.05 |
| 0.57±0.18 | 0.02±0.24 | 0.52±0.14 | 0.33±0.10 | 0.39±0.07 |

$*: Heritabilities are given on the diagonal (bold), genetic correlations below, phenotypic correlations above diagonal.
pale brown eggs in the early stages of their laying cycle continued to lay lighter-coloured eggs at the end of their cycle.

In our study, successful results were obtained for the selection of dark shell colour and less variation between the colours of eggshells. Our results show that coefficients of variation (%) of SCI of parents were reduced after three generation selection study, the obtained results from the parents showed similar effects on the egg-laying hybrids (Table 6).

**Discussion**

As indicated by the relatively high heritability in the current study, there was considerable variation in shell colour among families and individual hens within a line. The high values of heritability for eggshell colour reported are 0.46–0.50 (Zhang et al. 2005; Flock et al. 2007; Cavero et al. 2010; Cavero et al. 2012). As indicated by the relatively high heritability, there was considerable variation in shell colour among families and individual hens within a line. According to project objectives, selection was made for low $L^*$ but high $a^*$ and $b^*$ values. In a similar study, estimated genetic correlations between EW and shell colour were found to be 0.00, 0.09 and 0.30 (Francesch et al. 1997). Research by Kim et al. (2014) reported that the hen’s age did not affect eggshell colour and was positively correlated with EW ($r = +0.248, p = 0.082$), which contradicts the results of this study. In 2013, Yildirim et al. observed that the $L^*$ values of the eggshells decreased, while $a^*$ values increased gradually until the period of peak efficiency, indicating that the colour of the eggshell darkened, as the laying hens became older. The findings of this study conflicted with our research results.

The results obtained by Kim et al. (2014) were compared with that of this study, but no similarities were recorded. Their results showed that the shell colour was not change as the hens aged, conflicting with results from this study. In 2007, Odabasi et al. reported that although eggshell mass increased with hen age, the amount of pigment did not increase in the same ratio as that of shell mass; therefore, more shell surface was covered with a given amount of pigment, thereby affecting the eggshell colour. The pigment protoporphyrin-IX is synthesised in the epithelial cells that line the uterus. Inside the epithelial cells, a haem protein, which was once part of a red blood cell, is converted to protoporphyrin-IX (Yang et al. 2009). A report by Bhosale et al. (2013) stated that protoporphyrin IX belonged to a group of biologically active tetrapyrole compounds. Structurally, protoporphyrin IX is a tetrapyrole ring containing a highly conjugated planner and a rigid macrocycle consisting of four pyrrole rings, which are connected by methane groups. Lukanov et al. (2015) conducted a study with 11 purebred groups of laying hens and determined that there was a substantial variation in the colour of eggshells within the groups and the breeds. Eggs with dark brown shells exhibited the highest variation of the trait. The SCI was deemed suitable for reliable colour identification only in brown and non-pigmented eggs. Research by Li et al. (2013) reported that a comparison of protoporphyrin IX in different tissues, of light- and deep-brown egg-laying RIR hens, indicated that eggshell pigment was synthesised in the shell gland. Moreover, there was a significantly higher gene expression of δ-aminolevulinic acid synthase recorded in the deep-brown egg-laying hens as compared with hens laying lighter coloured eggs. Another study by Sekeroglu and Duman (2011) stated that brown eggshell colour was positively correlated with some shell characteristics, such as shell strength and hatchability, while Dearborn et al. (2012) indicated that identity, diet and time were factors affecting eggshell colour of laying hens. Yildirim et al. (2013) reported that adding Korean ginseng (*Panax ginseng* [C.A. Meyer]) root extract to the hens diet increased $L^*$ values of the eggshell and reduced $a^*$ ($p < 0.01$), as compared with the control. Research by Aygun (2013) stated that moulting stress has been shown to severely affect eggshell colour once the hen resumes laying, with the extent of the response differing among individual hens. A study by Samiullah and Roberts (2013) researched 33-, 50- and 67-week-old HyLine Brown hens to determine the amount of protoporphyrin IX in the eggshells. The amount of protoporphyrin IX in 1g of whole eggshell was not significantly different, but in the cuticle itself, was found to be significantly higher in eggs from 50-week hens as compared with the 33- and 67-week hens. Odabasi et al. (2007) reported that shell colour gets lighter with flock age, and the amount of cuticle present on the eggshell surface positively influences the

**Table 6.** Coefficients of variation of SCI by lines and years.

| Year | Lines | $N$ | CV (%) of SCI |
|------|-------|-----|--------------|
| 2011 | BAR-1 | 293 | 0.17         |
|      | RIR-1 | 205 | 0.18         |
| 2012 | BAR-1 | 539 | 0.15         |
|      | RIR-1 | 521 | 0.17         |
| 2013 | Hybrid| 611 | 0.08         |
|      | BAR-1 | 510 | 0.09         |
|      | RIR-1 | 432 | 0.09         |

$N$: number of hen; CV: coefficients of variation (%); SCI: eggshell colour index.
pigment present in the shell. They concluded that eggshell colour generally gets lighter as the hen ages. Seo et al. (2010) warned that feed supplemented with Fe soy proteinate significantly improved eggshell colour in brown-egg-laying hens. Further research by Dearborn et al. (2012), explained that diet had a very limited effect on eggshell spectral reflectance, but individual females differed strongly and consistently from one another in eggshell colour. The heritability estimated for EW and eggshell colour parameters were at a moderate level (Table 5), but generally different in lines, in the following ranges: 0.62–0.64, 0.55–0.62, 0.32–0.51, 0.64–0.74 and 0.22–0.39 for the EW, $L^*$, $a^*$, $b^*$ and SCI values, respectively. Moreover, a reduced variability of eggshell colour increased the visual presentation of eggs at the time of sale. A variety of values of heritability have been reported in the literature ranging at 0.46–0.50 (Zhang et al. 2005). In our study, there was a negative correlation between $L^*$ and $a^*$ values, ranging from −0.44 to −0.49, and it was anticipated that lighter coloured eggshells (higher $L^*$) would have lower $a^*$ values (Odabasi et al. 2007). The $L^*$ and $b^*$ values were also negatively correlated, but at a lower level, particularly for the BAR-1 line, whereas the $a^*$ and $b^*$ values were positively correlated. Although the heritability of the colour index ranged between 0.22 and 0.39, the $a^*$ and $b^*$ values were negatively correlated with EW, in contrast with the positively correlated $L^*$ values. This indicated that hens lay small eggs with a darker eggshell colour but lay large eggs that are lighter coloured. These results were supported by similar studies by Odabasi et al. (2007), Flock et al. (2007), Cavero et al. (2010), Cavero et al. (2012), Lukanov et al. (2015) and Samiullah and Roberts (2013). As a result of these selection studies on these newly developed breeding lines, shell colour will also be considered as a factor in the shell index values. The project team will continue to strive using selective breeding to produce a dark brown eggshell.

**Conclusions**

There was a considerable variation within and between the genetic groups of brown laying hens according to eggshell colour in our study at the Ankara Poultry Research Institute. In addition to this, the lighter coloured eggshell ratio was high. To eliminate these problems, hens laying dark brown coloured eggshells were selected as parents and those laying a paler brown colour were removed from the laying flocks in this selection study. This study has been successful but further research is necessary to achieve the desired result.

**Disclosure statement**

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of this article.

**Funding**

The project was supported by the General Directorate of Agricultural Researches and Policies, Tanım Kampusu İstanbul Yolu Uzeri, No: 38, P.K.51, 06171, Yenimahalle/Ankara, Turkey. Project Number: TAGEM/HAYSÜD 13/10/02/03.

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