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Product Quality and Safety

Quality traits of eggs from autosexing Easter eggers

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Abstract. *The main egg quality traits were investigated in two autosexing F1 Easter egger crosses. Partridge Araucana roosters were used as carriers of the eggshell biliverdin pigmentation gene. Maternal forms used for obtaining the two experimental crosses were high-producing layer hens carrying the sex-linked S and B genes. Experimental groups comprised F1 crosses of partridge Araucana roosters with white mutational Rhode Island hens or hens with barred colour mutation. The aim of the study was to investigated the quality traits of blue-green eggs produced by autosexing hens. The egg quality was evaluated at 38 weeks of age. Studied traits comprised egg weight (g), shape index, albumen index (AI), Haugh units (HU), yolk index (YI), yolk colour (Roche scale), percentages of albumen, yolk and eggshell (%), eggshell surface (cm^2), eggshell density (mg/cm^2), average eggshell thickness (µm) and eggshell colour. The group W was outlined with the highest egg weight – 61.03±0.47 g, and group A – with the lowest (50.91±0.32 g). The albumen and yolk quality was the best in group A, where albumen index was 0.075±0.002, Haugh units – 76.53±0.8 and yolk index – 0.491±0.009. The analysis of eggshell colour showed that eggs of group A were with the highest lightness (L*) value (73.60±0.77), whereas eggs of groups W and AW had egg lightness of 60.45±0.53 and 61.11±0.55 ($p<0.001$) respectively. The shell colour index (SCI) demonstrated a certain overlap of values in eggs with protoporphyrin and biliverdin taints. After introduction of a correction coefficient and recalculation of SCI*, the values of green eggs assumed a negative sign and could be distinguished from brown eggs.*

Keywords: hens, Araucana roosters, Easter eggers, egg quality, eggshell colour, CIE L* a* b* system, shell colour index

Introduction

The early sex identification and separation of male birds is an important factor influencing the costs of commercial egg production. Nowadays, autosexing is the most popular method for sexing day-old chickens. It is based on sex-determined phenotype traits manifested as early as after hatching. Depending on the used sex-linked trait, two types of autosexing are distinguished: feather-sexing and colour-sexing The former is based on the different growth rate of feathers, while the other relies on the different colour of the fluff in both sexes. Today, all contemporary egg-laying hybrids could be sexed at one day of age using the method of autosexing.

In domestic chickens, the eggshell is presented in different shades of white, brown and blue-green (Romanoff and Romanoff, 1959; Lukanov et al., 2015), due to the presence or lack of brown and/or green pigments (Wang et al., 2007). The global stock eggs production involved autosexing hybrid combinations on the basis of high-production White Leghorn and Rhode Island Red strains. That is why, the major share of global egg produce is outlined with uniform shell colour. White-shelled eggs are the commonest on the worldwide markets, followed by brown-shelled. During the last two decades, consumers in Western Europe, North America and Japan exhibited an increased interest to eggs with non-traditional shell colour (Lukanov et al., 2016). This aspect of consumer attitudes could be attributed both to the conservatism in global stock eggs production and to the good self-sustainability of the average consumer in these parts of the world. The application of autosexing in Easter eggers would facilitate their implementation in the practice and the optimisation of blue-green eggs production.

Chicken blue-green shelled eggs are known for centuries. It is presumed that the mutation originates from southeastern Asia and either was introduced on the western coast of South America before its colonisation, or has appeared independently on both continents (Storey et al., 2007; Gongora et al., 2008; Storey et al., 2011; Terry, 2011). Several breeds and breed groups of chickens laying blue-green eggs are acknowledged. Their intrinsic genotype and phenotype variety is a valuable contribution to the genetic diversity of *Gallus gallus domesticus* (Lukanov, 2016).

The quality of eggs intended for human consumption is determined from characteristics making the product more attractive to consumers on one hand, and its safety and nutritional value on the other (Wells, 1968; Stadelman, 1995; Gerber, 2006). Egg quality depends on a number of internal and external factors as genotype, age, physiological condition, feeding, microclimatic parameters, production system etc. (Romanoff and Romanoff, 1959; Gerber, 2006; Ahmadi and Rahimi, 2011; Ledvinka et al., 2012). Although the normal eggshell colour is not a trait directly associated to egg quality, it has a definite role in attracting consumers and finally determining their choice. In the research literature, there is only a scarce information about the quality traits of biliverdin-pigmented eggs produced by different breeds and crosses, hence our interest to this subject.

The purpose of this study was to investigated some of the primary quality traits of eggs of autosexing Easter egger

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combinations and the colour parameters of eggshells in the CIE L*a*b* system.

Material and methods

The experiment was performed with 5 groups of 38-week-old hens (3 control and 2 experimental groups). Control groups comprised purebred partridge Araucana (group A), white mutation of RIR (group W) and barred hens (group B) used for formation of experimental groups. Two of the used groups – W and B were high-productive commercial female strains used for production of autosexing egg-laying hybrids. Both W and B groups lay eggs with cream to light brown shells. The eggs of the Araucana breed have light green shells.

The experimental groups comprised F1 crosses as followed:

- F1partridge Araucana roosters x hens white mutation of RIR (group AW);

- F1partridge Araucana roosters x barred hens (group AB).

All birds were fed compound feed according to the age category of hens. Thirty eggs were collected from each group for morphometric and spectral analysis.

The following quality parameters were determined in each of eggs:

- Egg weight (g): with calibrated electronic balance, precision of 0.01 g;

- Shape index (%): through measurement of big and small egg diameters with Vernier caliper and calculation using the formula:

$$SI\ (\%) = \frac{d}{D} \times 100 \qquad (1),$$

where SI is shape index, d is small diameter of the egg, mm, D is big diameter of the egg, mm (Romanoff and Romanoff, 1959);

- Albumen index: through measurement of big and small thick albumen diameters with Vernier caliper and albumen height with a micrometer. It was determined using the formula:

$$AI = \frac{h}{\frac{d+D}{2}} \qquad (2),$$

where AI is albumen index, h is thick albumen height, mm, d is small diameter of the thick albumen, mm, D is big diameter of the thick albumen, mm (Romanoff and Romanoff, 1959);

- Haugh units were calculated by the formula:

$$HU = 100 \times \log(h + 7.57 - 1.7 \times W^{0.37}) \qquad (3),$$

where: HU – Haugh units, h – thick albumen height, mm, and w –egg weight, g (Haugh, 1937);

- Yolk index: through measurement of yolk diameter with a Vernier caliper and its height with an AMES micrometer (mm) and the equation:

$$YI = \frac{h}{D} \qquad (4),$$

where YI: yolk index, h – yolk height, mm, D – yolk diameter, mm (Romanoff and Romanoff, 1959);

- Yolk colour score: using the La Roche scale (La Roche Yolk Colour Fan).

- The shell surface area (cm^2) – using the formula:

$$SSA = 4.835 \times W^{0.662} \qquad (5),$$

where EW is egg weight (Paganelli et al., 1974).

- Eggshell density (mg/cm^2) – by the formula:

$$SD = \frac{EW \times 1000}{SSA} \qquad (6),$$

where EW is egg weight (g), SSA – shell surface area (Paganelli et al., 1974).

- Average shell thickness (µm): measured with micrometer with precision of 0.0001 mm in the three zones (blunt edge, equatorial region, sharp edge) and retaining the average of three measurements.

For each group, the proportions of the egg parts were determined through their measurement (precision 0.01 g) and calculation of yolk: albumen ratio (Tsarenko, 1988).

Eggshell colour was determined in the CIE L*a*b* space (CIE, 1986). For this purpose, a Konica Minolta CM-700d spectrophotometer was used. The values of L*, a* and b* coordinates were determined in three zones: blunt edge, sharp edge and equatorial region. The chroma (C*) was calculated from a* and b* values (Anonymous, 2006):

$$C^* = (a^{*2} + b^{*2})^{\frac{1}{2}} \qquad (7),$$

The colour index of the eggshell was determined according to the formula (Cavero, 2012):

$$SCI = L^* - a^* - b^* \qquad (8),$$

where lower values corresponded to a darker colour.

For the green pigmented egg shell of the A, AW and AB groups were calculated corrected SCI, according to the formula (Lukanov, 2016):

$$SCI^* = (L^* - a^* - b^*) \times (-1) \qquad (9),$$

The results were submitted to statistical analysis using classic statistical methods via the MS Excel 2010 software.

Results and discussion

The quality characteristics of eggs are divided into 2 group: external and internal (Ahmadi and Rahimi, 2011; Ledvinka et al., 2012), and methods for their evaluation are destructive and non-destructive (Ahmadi and Rahimi, 2011), direct and indirect (Roberts and Brackpool, 1995). The egg weight, shape and eggshell parameters are from the group of external quality traits. The state of shell membranes, albumen and yolk are internal quality traits of eggs.

The analysis of the external traits of eggs from studied groups (Table 1) shows that egg weight was the highest in group W (61.03±0.47 g), and the lowest – in group A (50.91±0.32 g). The significant difference in egg size among the strains with high intensity of selection for production traits (lines W and B) and Araucana breed in which no purposeful selection was performed (group A) should be noted. In both experimental groups – AW and AB, the weight of eggs was similar to that of maternal forms and considerably higher than that of group A ($p<0.001$). A similar relationship was reported by Lukanov et al. (2016) in Araucana × White Leghorn and Schijndelaar × White Leghorn crosses. The calculated relative heterosis (Falconer and Mackay, 1996) for experimental groups revealed comparable low positive values: 2.7

for AW and 3.93 for AB. The tendency towards heavier eggs in group W was not preserved in AW crosses. According to Romanoff and Romanoff (1959) the chicken eggs weighs between 51 and 61 g on the average, and the standard chicken egg is 58 g. Simeonov et al. (1995) established eggs weighing 58-62 g as standard. Today, the eggs sold for consumption are produced entirely from modern autosexing hybrid combinations, and their average weight depending on the hybrid is 60-65 g (Kabakchiev, 2014). The weight of eggs in groups W and B corresponds to that of breeders used for production of coloured stock layers hybrids. The weight of eggs produced by group A is in line with the standard for Araucana breed (EE, 2010). Eggs from both egg groups could be placed into the M category (>53-63<) of class A eggs intended for consumption (EC, 2007).

The shape of eggs is important not only for poultry reproduction, but also when eggs are marketed for consumption. The shape index of eggs from the studied groups ranged between 76.7 and 78.44%, i.e. they were more oval (Delchev et al., 1984). The eggshell surface corresponds to the results about egg weight in the different experimental groups.

Table 1. External characteristics of the eggs

Quality parameters	Control groups			Experimental groups	
	A	W	B	AW	AB
Egg weight, g	50.91 ± 0.32	61.03 ± 0.47	60.91 ± 0.53	57.48 ± 0.42	58.11 ± 0.45
significant difference	1:2,3,4,5***; 2:4,5***; 3:4,5***				
Shape index, %	78.44 ± 0.20	77.42 ± 0.65	78.04 ± 0.72	76.70 ± 0.52	77.37 ± 0.52
significant difference	1:4***				
Shell surface area, cm^2	63.96 ± 0.35	71.61 ± 0.47	71.28 ± 0.47	66.54 ± 0.42	71.18 ± 0.71
significant difference	1:2,3,4,5***; 2:4***; 3:4***; 4:5***				

with 1,2,3,4 and 5 are indicated: A, W, B, AW and AB group respectively, * p< 0.05; ** p< 0.01; c *** p< 0.001;
NS - No significant difference

The analysis of egg parts showed that there were no substantial deviations which could be attributed to shell pigmentation (Table 2). The lowest albumen proportion was established in both experimental groups (56.67±0.24% and 57.47±0.53%). In group B, albumen content was the highest (60.52±0.35%) and that of yolk (29.42**±**0.27%) the lowest, with statistically significant differences vs the other groups. The ratio between yolk and albumen is a relatively accurate criterion for evaluation of the nutritional value of the whole egg (Tsarenko, 1988). On the basis of presented data, the most favourable albumen/yolk ratio was detected in groups AW and AB: 0.56±0.014 and 0.54±0.08 respectively. It could be assumed that 1 g egg content in these groups had a better nutritional value compared to other studied groups. The relative share of the shell in the different groups ranged between 10.06-11.43%, within the normal range for chicken eggs (Stadelman, 1995).

Table 2. Share of the egg components, %

Quality parameters	Control groups			Experimental groups	
	A	W	B	AW	AB
Egg whites share, %	58.17 ± 0.31	59.46 ± 0.31	60.52 ± 0.35	56.67 ± 0.24	57.47 ± 0.53
significant difference	1:2**; 1:4***; 2:3*; 2:4***; 2:5**; 3:4,5***				
Yolk share, %	30.47 ± 0.19	30.35 ± 0.24	29.42 ± 0.27	31.90 ± 0.23	31.12 ± 0.52
significant difference	1:3**; 1:4***; 2:3*; 2:4***; 3:4***; 3:5**				
Eggshell share, %	11.36 ± 0.14	10.19 ± 0.14	10.06 ± 0.13	11.43 ± 0.12	11.42 ± 0.14
significant difference	1:2,3***; 2:4,5***; 3:4,5***				
Yok/albumen ratio	0.52 ± 0.018	0.51 ± 0.011	0.49 ± 0.01	0.56 ± 0.014	0.54 ± 0.08
significant difference	NS				

with 1,2,3,4 and 5 are indicated: A, W, B, AW and AB group respectively, * p< 0.05; ** p< 0.01; c *** p< 0.001;
NS - No significant difference

Table 3 presents the main traits characterising the quality of the different egg components. The primary criteria for albumen quality are the albumen index (AI) and the Haugh units (HU). These traits are based on the content of ovomucin in the thick albumen, which depends on autolytic processes during storage of eggs and its enzymatic system (Mine, 2008). The albumen viscosity is of utmost importance in the calculation of both traits, and it is evaluated through albumen height. In the studied sample, AI varied from 0.054 to 0.075 with highest values in group A (0.075±0.002) – statistically significantly different vs the other groups (p<0.001). This, in the view of Tsarenko (1988) is associated to the size of eggs. The author believes that more oval eggs (with higher shape index values) have higher albumen index, yolk index and Haugh unit values. The AI in crosses (groups AW and AB) are better than those of maternal forms (p<0.01 and p<0.05 respectively). A similar relationship was observed for Haugh units. For Araucana breed, both quality traits had lower values in our study as compared to an earlier published research (Lukanov et al., 2016). Unlike Araucana breed, the Araucana × Rhode Island crosses exhibited similar AI and HU values.

Table 3. Morphological characteristics of the eggs

Quality parameters	Control groups			Experimental groups	
	A	W	B	AW	AB
Albumen index	0.075 ± 0.002	0.054 ± 0.002	0.054 ± 0.002	0.063 ± 0.002	0.061 ± 0.002
significant difference	1:2,3,4,5***; 2:4**; 2:5*; 3:4**; 3:5*				
Haugh units	76.53 ± 0.80	65.51 ± 1.39	64.62 ± 1.59	71.92 ± 1.57	69.81 ± 1.40
significant difference	1:2,3,5***; 1:4**; 2:4**; 2:5*; 3:4**; 3:5*				
Yolk index	0.491 ± 0.009	0.463 ± 0.004	0.463 ± 0.005	0.440 ± 0.006	0.456 ± 0.006
significant difference	1:2,3**; 1:4,5***; 2:4**; 3:4**				
Yolk colour by La Roche	12.16 ± 0.33	11.67 ± 0.12	12.13 ± 0.15	11.71 ± 0.10	11.58 ± 0.11
significant difference	NS				
Shell thickness, µm	395.75 ± 3.48	429.33 ± 4.28	424.63 ± 5 .31	431.5 ± 3.01	380.22 ± 4.87
significant difference	1:2,3,4***; 1:5*; 2:5***; 3:5***; 4:5***				
Eggshell density, mg/cm2	90.40 ± 0.99	86.86 ± 1.28	85.89 ± 1.07	98.73 ± 1.10	93.27 ± 1.28
significant difference	1:2*; 1:3,4**; 2:4,5***; 3:4,5***; 4:5**				

with 1,2,3,4 and 5 are indicated: A, W, B, AW and AB group respectively, * p< 0.05; ** p< 0.01; c *** p< 0.001;
NS - No significant difference

The most important trait characterising the yolk quality is the yolk index (YI). It determines the oval shape of the yolk and the quality of the perivitelline membrane. YI values depend on the level of autolytic processes in eggs (Stadelman, 1995). In this experiment, YI values were relatively high in all groups (from 0.440±0.006 in group AW to 0.491±0.009 in group A). Comparable results are observed by other researchers in close genotypes (Akhtar et al., 2007; Durmuş et al., 2010; Lukanov, 2014).

The colour of yolk in all studied eggs exhibited a significant saturation with pigment (11.58±0.11 – 12.16±0.33). This trait is directly associated to nutrition. Due to the uniform nutrition of birds from all groups, serious inter-group differences were not identified. These parameters, favourable from consumers' point of view, are manifested with pleasant deep yellow to orange-yellow coloration of the egg yolk. The trait is not essential for egg quality and furthermore, is easily manipulatable.

The average thickness of the shell together with the outer shell membrane was within the normal range for chicken eggs with min-max values from 380.22±4.87 µm in group AB to 431.5±3.01 µm in group AW. Our data for shell thickness correspond to those reported by other authors in similar chicken genotypes (Minora et al., 2003; Akhtar et al., 2007; Simeamelak et al., 2013; Lukanov et al., 2016).

The colour characteristics of eggshells are presented in Table 4 and Figure 1. The calculated shell colour index (SCI) as well as the corrected index (SCI*) for eggs with biliverdin pigmentation (Lukanov, 2016) are presented on Figure 2

The highest lightness (L*) value was that of eggs from group A (73.60±0.77). The statistically significantly darkest eggs compared to other genotypes were brown eggs laid by group W and green-gray eggs of group AW (60.45±0.53 and 61.11±0.55). In groups AW and AB, the inheritance of the trait was intermediate. In them, a combined differentiation of both brown and green pigment was observed, resulting in more saturated grayish-greenish colour of the shell. Lukanov et al., (2016) reported higher L* values in Araucana eggs (76.45) than those obtained in the present study. Due to the polygenic pattern of inheritance and the substantial variation in brown-coloured eggs in the different domestic chicken genotypes, our results could be hardly compared to literature data.

The pigmentation in the red-green spectrum did not vary considerably depending on egg colour. The lowest, negative values of *a** were those of green eggs from group A (-5.29±0.24). Li et al. (2006), Lukanov et al. (2015), Lukanov (2016) and Lukanov et al.

Table 4. Eggshell colour characteristics

Colour parameters	Control groups			Experimental groups	
	A	W	B	AW	AB
L*	73.60 ± 0.77	60.45 ± 0.53	68.98 ± 0.44	61.11 ± 0.55	71.30 ± 0.61
significant difference	1:2,3,4***; 2:3,5***; 3:4,5***; 4:5***				
a*	-5.29 ± 0.24	15.92 ± 0.27	10.96 ± 0.23	2.17 ± 0.36	1.53 ± 0.27
significant difference	1:2,3,4,5***; 2:3,4,5***; 3:4,5***				
b*	13.72 ± 0.50	27.60 ± 0.38	22.9 ± 0.43	21.51 ± 1.07	16.43 ± 0.59
significant difference	1:2,3,4,5***; 2:3,4,5***; 3:5***; 4:5***				
C*	14.82 ± 0.39	31.89 ± 0.38	25.40 ± 0.43	21.66 ± 1.08	16.54 ± 0.60
significant difference	1:2,3,4***; 1:5*; 2:3,4,5***; 3:4**; 3:5***; 4:5***				

with 1,2,3,4 and 5 are indicated: A, W, B, AW and AB group respectively, * p< 0.05; ** p< 0.01; c *** p< 0.001;
NS - No significant difference

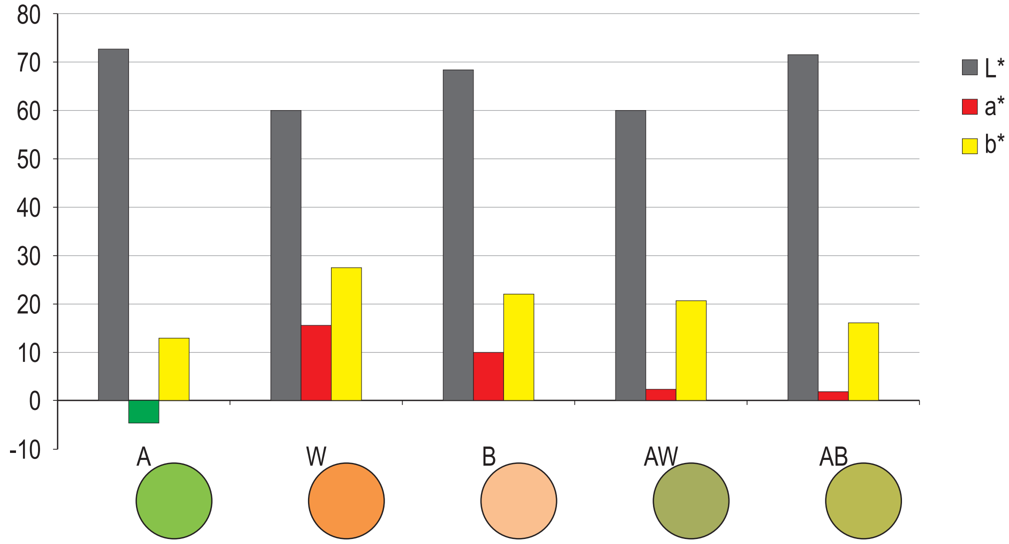

Figure 1. Eggshell colour characteristics in CIE L*a*b*

(2016) also established negative *a** colour coordinates in eggs laid by breeds carrying the biliverdin shell pigmentation gene. In the other groups used in this experiment, *a** values were positive which placed them in the red spectrum of the system. The two experimental groups were with combined biliverdin-porphyrin deposition in eggshells. Although externally they appeared gray-green, *a** values were positive evidencing the greater influence of protoporphyrin in the determination of this trait. Durmuş et al. (2010) reported lower *a** values (8.1) in Rhode Island Red eggs than those obtained by us in group W (15.92). In other studies with modern Rhode Island Red strains, Cavero et al. (2012), Lukanov et al. (2015), Lukanov et al. (2016) found out values comparable with those for group W.

The pigmentation of eggshell in the yellow-blue spectrum had positive values in the yellow spectrum of the *b** colour coordinate. The highest average values of *b** were established in group W (27.60±0.38), whereas the lowest – in group A (13.72±0.50). In both experimental groups AW and AB b* values were intermediate vs breeder groups (controls). The b* values of Araucana eggs were similar although higher than those published previously (Lukanov et al., 2016). Our data for group W were comparable to those reported by Durmuş et al. (2010) in Rhode Island Red. Cavero et al. (2012) and Lukanov et al. (2015) demonstrated higher values of the *b** colour coordinate in eggs produced by the same breed.

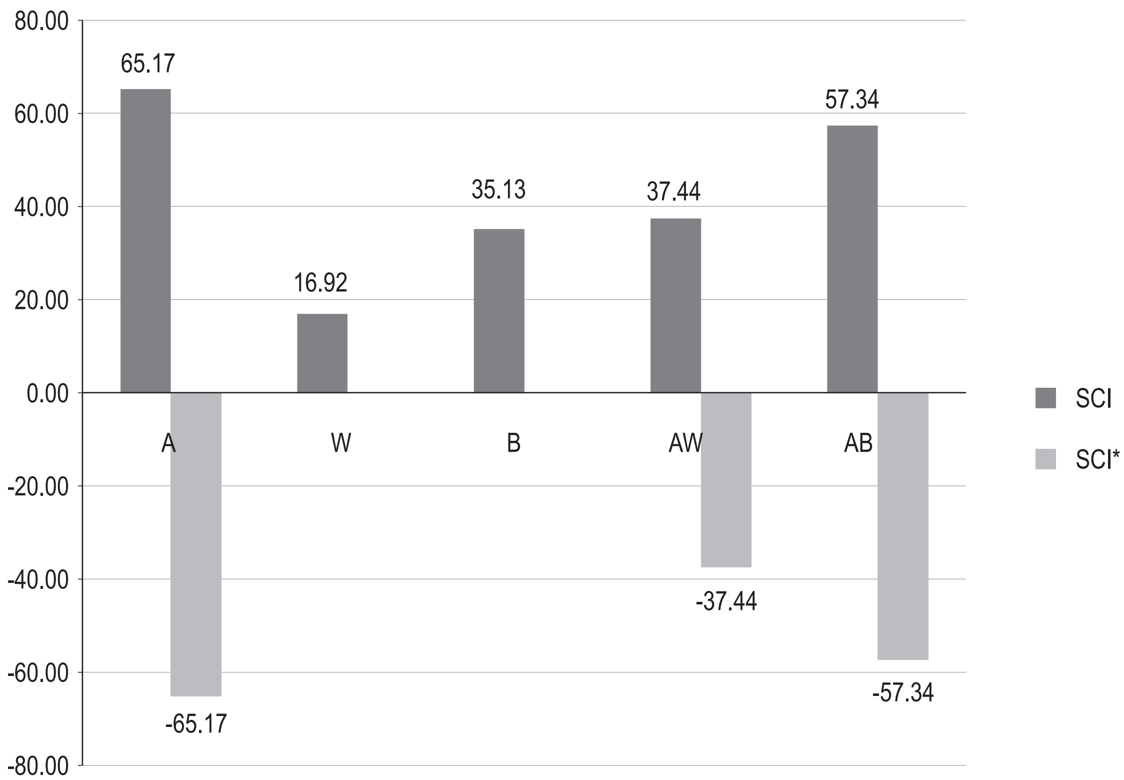

Figure 2. Eggshell color indices (SCI/SCI*)

The chroma (C*) together with lightness (*L**) depicts numerically the colour traits of the eggshell comprising both coordinates *a** and *b**. This trait is not yet implemented for evaluation of the colour characteristics of eggshells as could be concluded from the few studies in this field. This allows it future use for elaboration of a common criterion for determination of eggshell colour (Lukanov, 2016). In this study, the highest values of C* were established in group A (14.82±0.39). The two experimental groups were closer to the sire breed, indicating the increasing important of the green pigment in determination of the trait.

Logically, at the background of presented main colour parameters, the shell colour index (SCI) was the highest in eggs from group A (65.17), and the lowest in both maternal forms – cream eggs of group B (35.13) and brown eggs of group W (16.92). In crosses, SCI values were intermediate, with a greater effect of the sire breed. In eggs from groups B and AW, which had cream and gray-green shells respectively, SCI values had overlapped. This did not allow the differentiation of colour of eggs with visually different pigmentation. Similar data were reported in eggs with biliverdin, protoporphyrin and mixed pigmentation and white eggs by Lukanov (2016) and Lukanov et al. (2016).

The corrected shell colour index (SCI*) in eggs with biliverdin pigmentation (groups AW, A and AB) allowed to distinguish them numerically from eggs with brown pigmentation (see SCI values in groups B and AW). Using a negative coefficient of correction, SCI values of green-shelled eggs assumed negative values (Fig. 2).

Conclusion

The group W (white mutation of RIR) was outlined with the highest egg weight, and group A (Araucana) – with the lowest. Group A exhibited the superior values of albumen index, Haugh units and yolk index. The analysis of eggshell colour showed that eggs of group A were with the lightest whereas eggs of groups W and AW (Araucana roosters x hens white mutation of RIR) – the darkest. The combination of biliverdin and protoporphyrin pigmentation resulted in a dilution effect of the former one, resulting in higher lightness values. The eggs with biliverdin pigmentation only had negative values of the a* colour coordinate. The combination of biliverdin and protoporphyrin pigments in shells lead to low positive a* values and variation in both positive and negative spectrum of this colour coordinate. The pigments of the shell did not influence the values of the *b** coordinate. The brown, cream, gray-green and blue-green eggs had positive b* values, i.e. were positioned in the yellow spectrum of the colour space. The corrected shell colour index (SCI*) allowed distinguishing the numerical values of the parameter in blue-green eggs from those with non-pigmented or brown-pigmented eggshells.

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