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(RESEARCH ARTICLE)

Phenotypic correlations between egg quality traits, albumen pH and ovalbumin levels in four varieties of Japanese quail (*Coturnix coturnix japonica*)

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Abstract

This study aimed to reveal phenotypic correlations of some internal and external egg quality traits between egg white (albumen) pH and ovalbumin levels in four different varieties of Japanese quail. A total of 480 eggs were used belong to gray, white, golden and black plumage color Japanese quail obtained from a commercial quail breeding company. Each group consisted of 120 eggs collected from the birds which were same age. After determined the external traits, all eggs were broken, albumen and yolk separated and internal traits were examined. Albumen pH was measured after the separation process. SDS-PAGE was applied for determining the ovalbumin level. A positive correlation was observed between egg weight and albumen (0.838) and yolk weight (0.599). There was a negative correlation determined between egg weight and albumen pH (-0.431). The percentage of yolk and albumen pH had a moderately significant positive correlation (0.350). We observed significant correlation between yolk color and albumen pH as well as between genotype of birds ($P<0.01$). The ovalbumin level high positively correlated with only the birds' genotype (0.814). Black plumage color (67.69%) had the highest ovalbumin level amongst golden (64.76%), white (49.28%) and gray (46.84%) plumage colors, respectively. The novel data is presented in this study about ovalbumin level comparing in four varieties of Japanese quail. The ovalbumin level is not affected by any quality features except for the genotype. The studies need to be conducted under different storage conditions for revealing more accurate phenotypic correlations between egg quality traits and albumen pH.

Keywords: Egg quality; Phenotypic correlations; Albumen pH; Ovalbumin; Japanese quail

1. Introduction

Quail is a bird species that belongs to the *Phasianidae* family. It is believed that quail was first domesticated in Japan in the 11th century. After domestication, this bird has been raised as a pet (songbird). At the beginning of the 20th century, quails were raised for egg and meat production [1]. It is even used as a lab animal in scientific researches [2]. The most common domesticated type is the coturnix quail also known as the Japanese quail has an important place in poultry farming of the developing countries and rural areas with their remarkable features such as having fast-growing (maturity at 4-5 weeks), need small space, their short incubation period and generation interval [3]. Different plumage color varieties of Japanese quail emerged as a result of crossbreeding. The 18 different plumage color mutants were previously described for the Japanese quail. Wild-type (Pharoah) is the most common one which has gray plumage color amongst them. The other common varieties are Manchurian Golden, Recessive White (English White), and Tuxedo plumage color [4].

Eggs are a valuable food source because of their protein ingredient and, offers a moderate energy level. Although consumers focus on chicken eggs, quail eggs are also known to be consumed in many countries [5]. It is stated that the nutritional value of quail eggs was 3-4 times greater than chicken eggs [6]. In addition, quail eggs are rich in protein,

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folate, vitamin B12, iron, phosphorus, selenium and riboflavin [7]. On the other hand, egg quality is also another issue in point of consumers' attention. External characteristics of an egg such as its weight, shape index and internal characteristics such as egg white (albumen), the yolk are evaluated mainly under egg quality. Moreover, phenotypic correlations of egg quality traits give significant information for genetic estimations in fowls [8].

The egg albumen consists of 88% water and more than 10% protein [9]. Albumen is utilized in many areas from the bakery to the wine industry in the food sector due to its physical properties [10, 11]. One of these properties, pH, gives an important idea about the storage. Albumen is naturally mild alkaline with the pH 7.6-7.9 shortly after oviposition [9]. However, increasing in pH is occurred by storage due to stream of carbon dioxide throughout the egg shell [12]. The most important factor that determines the quality of albumen is protein content except physical quality characteristics. Ovalbumin is the most abundant protein in albumen. This protein is a globular, acidic protein that comprises a single polypeptide chain of 385 amino acid residues with a molecular weight of approximately 45 kDa [11, 13]. Ovalbumin has an effect on the physical characteristics of albumen such as the foaming capacity which is important for the food sector. It is stated that high ovalbumin level is related to the high foaming capacity [14].

In this context, the aim of this study is determine some phenotypic correlations between egg quality traits, albumen pH and, ovalbumin levels of four different plumage color (gray, white, golden and black) of Japanese quails (*Coturnix coturnix japonica*).

2. Material and methods

The conducted research is not related to animals' use. No ethical approval was obtained because this study did not involve laboratory animals and only involved non-invasive procedures (egg collection).

2.1. Egg quality analyses

In this study, a total of 480 eggs were used from gray, white, golden and black plumage color Japanese quail obtained from a commercial quail breeding company. Each group consisted of 120 eggs collected from the birds which were same age. The quails fed *ad libitum* with the maize and soybean meal basis standard laying quail diet (17 % crude protein and 2750 kcal ME/kg energy content). The eggs kept at 4 °C for 1 day prior to egg quality analysis. All eggs were weighed by a 0.001 g sensitive digital scales (A&D, FX-300i, Japan). Width and length (mm) of the eggs were measured by a digital caliper accurate to 0.01 mm (Tresna, 0-300 mm, USA). The eggs were broken and the eggs' albumen was separated from the yolk. The yolks were rolled on filter paper to remove any albumen residues. Both albumen and yolk were weighed separately in sterile cups. The yolk color was determined by a Roche yolk color fan with a specific 15-slices yellow color. Every 10 albumen of eggs were pooled from each group and homogenized. Albumen pH was measured with pH meter (Hanna Instruments, HI99163, USA) after a double calibration (pH 4.01 and 7.01). Shape index, percentages of albumen and yolk were calculated according to following formula:

Shape index: [Width (mm) /Length (mm)] x 100;

Percentage of albumen: (albumen weight/egg weight) x 100;

Percentage of yolk: (yolk weight/egg weight) x 100 [15].

2.2. SDS-PAGE analysis of albumen

The each pooled albumen were homogenized (1/10 w/v) with 25 mM Tris (pH: 7.4). The total protein concentration of albumen was measured with a nano drop spectrophotometer (Thermo Fisher, NanoDrop 2000c, USA). The protein samples were mixed with 4 X sample buffer (1 M Tris-HCl pH 6.8, SDS, 0.1 % Bromophenol blue, glycerol and 14.3 M β-mercaptoethanol) and boiled at 97 °C for 5 min. The 30μg/30μl from each protein samples were loaded into discontinuous polyacrylamide gel (4 % stacking and 10 % separating gel). The prestained protein weight marker was added to first well of the gel. Electrophoresis was carried out at a constant voltage of 135 V for 90 min (Bio-Rad, Mini Protean Tetra, USA). The gels removed carefully when the end of the 90 min and stained with the 1 % coomassie brilliant blue dye solution for 1 hour. Thereafter, the dye solution was removed with the destaining solution (mixture of pure methanol, glacial acetic acid, distilled H_2O). Each band were analyzed with image processing software (NIH Image) after grayscale calibration for obtaining relative density values (RD, %) [15].

2.3. Statistical analysis

In this study, the sample size was determined by GPower 3.1 power analysis software [16]. The power of test was adjusted as 0.95 which stated for correlation (point biserial) analysis according to this software. The Pearson correlation test was applied for determining coefficients of egg quality, albumen pH and ovalbumin level with IBM®SPSS 22 statistical package program. The Spearman correlation test was utilized for comparing plumage color with other parameters. The data were subjected to analysis of variance (One-way ANOVA) for comparing of ovalbumin level in four varieties of quails. Tukey post hoc test was performed for determined which group was different. The statistical significance was considered when $P \leq 0.05$ [17].

3. Results and discussion

Egg quality is influenced by many environmental factors and genetic structure (plumage genotype) of Japanese quails [2, 18]. There are many studies conducted on determining phenotypic correlations between egg internal and external quality either in hen and quail [19, 20]. Phenotypic correlations coefficients between some external and internal egg quality between albumen pH and ovalbumin level in four varieties of Japanese quail are presented in Table 1.

In this study, a positive correlation was observed between egg weight and albumen (0.838) and yolk weight (0.599), which similar to some studies [19, 21]. However, there was a negative correlation determined between egg weight and albumen pH (-0.431). This result may suggest that bigger eggs also have many pores on egg surface and this leads to susceptible for much loss of carbon dioxide via those pores [22]. Contrary, the egg which has high weight may resist to loss of carbon dioxide, thus according to this study the pH decreases as egg weight increases. However, this fact needs to study especially under different storage conditions. There was no correlation between egg weight and shape index ($P>0.05$) which disagree with findings of Kul and Seker [21] and Duman et al. [23] however; agree with the findings of Begli et al. [8] and Alkan et al. [24]. In addition, yolk color and egg weight had a negative moderate correlation (-0.322) in this study. The shape index also was exhibited no significant correlations between in any quality traits as well as with between ovalbumin level ($P>0.05$). Albumen weight had the positive correlations between the percentage of albumen (0.751) and yolk weight (0.373), however; the negative correlations were determined with albumen weight between the percentage of yolk (-0.389) and yolk color (-0.365). On the other hand, percentage of albumen is showed negative correlations with yolk color (-0.345) and albumen pH (-0.487). This result may be attributed to egg weight due to albumen proportion depends on the weights of the egg [25]. Indeed, both egg weight and percentage of albumen had a negative correlation with albumen pH in this study. In the present study, yolk weight was only correlated with the percentage of yolk (0.580). The percentage of yolk and albumen pH had a moderately significant positive correlation (0.350). Yolk color is influenced by genotype, age and diet [26, 27, 28]. In addition, dos Santos et al. [29] reported that the yolk color decreased during storage under both at room temperature and refrigerator. Moreover, we observed a significant correlation between yolk color and albumen pH ($P<0.01$). In our study, the yolk color and the plumage color had a significant correlation which had an agreement in findings of Sokolowicz et al. [28] In contrary to our findings, Sokolowicz et al. [28] reported albumen pH had also correlated to birds' genotype. There was no significant correlation between plumage color and albumen pH in our study ($P>0.05$).

Ovalbumin levels in albumen which belong to four different varieties of Japanese quails are presented in Figure 1. In Figure 2, the SDS-PAGE gel image of examined for ovalbumin is shown. The ovalbumin level had high positively correlated with only the birds' genotype (0.814) except for the other internal and external egg quality traits (Table 1). Black plumage color (67.69 %) had the highest RD value amongst golden (64.76 %), white (49.28 %) and gray (46.84 %) plumage colors, respectively. In Figure 2, the ovalbumin levels are presented in the groups. The ovalbumin level was similar in gray and white colors ($P>0.05$). Together with this, it was also similar in black and golden colors ($P>0.05$). However, gray and white colors were different from black and golden colors ($P<0.05$).

Table 1 Phenotypic correlations between some external and internal egg quality between albumen pH and ovalbumin level in four varieties of Japanese quail

	Egg weight	Shape index	Albumen weight	Percentage of albumen	Yolk weight	Percentage of yolk	Yolk color	pH	Ovalbumin
Plumage color	-0.281	-0.126	-0.277	-0.229	-0.110	0,165	0.468**	0.231	0,814**
Egg weight		0.265	0.838**	0.319*	0.599*	-0.269	-0.322*	-0.431**	-0.185
Shape index			-0.283	-0.117	-0.193	-0.024	0.119	0.055	-0.108
Albumen weight				0.751**	0.373*	-0.389*	-0.365*	-0.544	-0.165
Percentage of albumen					-0.041	-0.365	-0.345*	-0.487**	-0.157
Yolk weight						0.580**	-0.112	-0.073	-0.085
Percentage of yolk							0.175	0.350*	0.124
Yolk color								0.415**	0.307

** ($P < 0.01$), *($P < 0.05$)

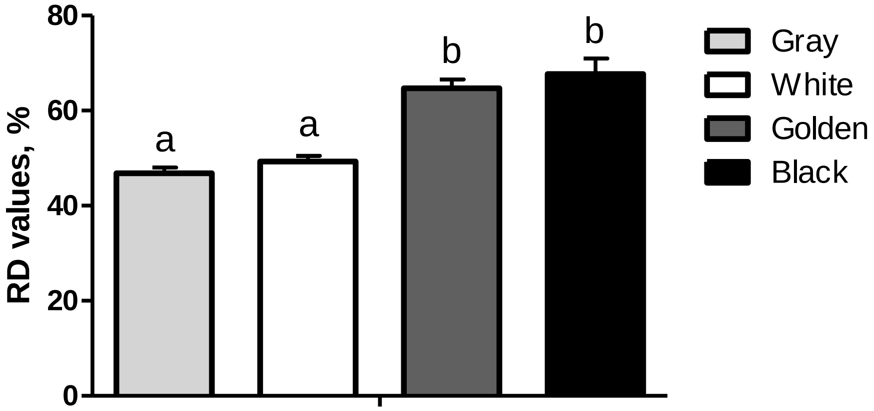
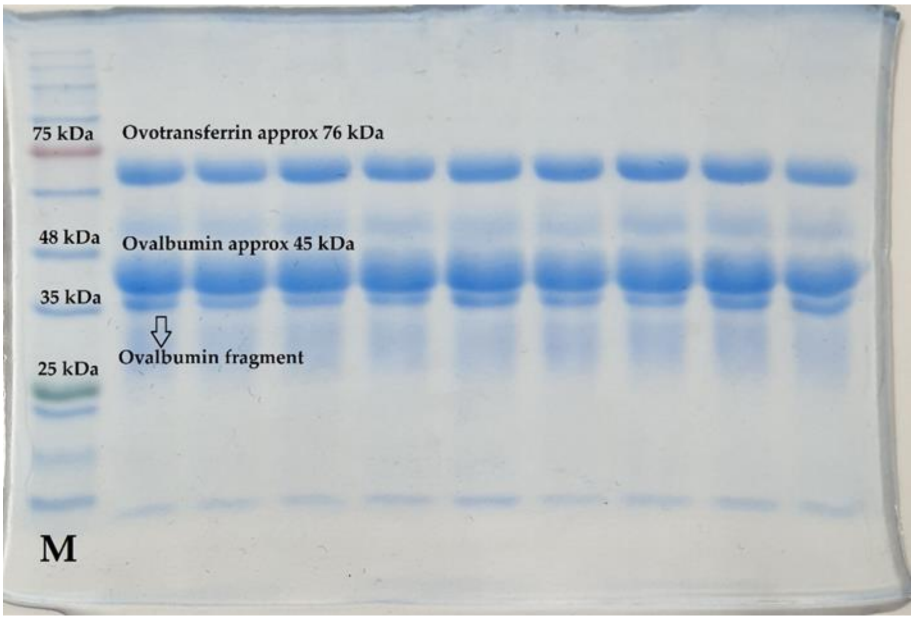


Figure 1 Ovalbumin levels in albumen belong to four varieties of Japanese quail



M: molecular weight marker

Figure 2 SDS-PAGE gel image of studied eggs

4. Conclusion

The novel data is presented in this study because of comparing the ovalbumin levels in four varieties of Japanese quail. The black and golden colored Japanese quails had the highest ovalbumin level. In this study, significant correlations were observed between both internal and external egg quality traits. The ovalbumin level is not affected by any quality features except for the genotype. However, the studies need to be conducted under different storage conditions for revealing more accurate phenotypic correlations between egg quality traits and albumen pH.

Compliance with ethical standards

Acknowledgments

The authors declare that they have no conflict of interest.

Disclosure of conflict of interest

There is no conflict of interest among all the authors.

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