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Effects of Age and Strain on Relationships among Albumen Quality Traits and Egg Weight in Commercial Brown Layers

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Abstract: A total of 240 eggs from two commercial layer strains (ATAK and ATAK-S) at 30 and 40 weeks of ages were used to investigate relationships among measures of thick albumen quality and Egg Weight (EW). Eggs were weighed and broken onto non-reflective glass surface and Albumen Height (AH), Albumen Index (AI), Haugh Unit (HU) and Albumen Area (AA) were determined. The AA was calculated by digital image analysis. The correlation coefficients among AH, AA and EW and the linear regressions of EW on AH and AA were determined. Regression coefficients of the AH on the EW were insignificant and were ranged 0.02-0.059 mm g^{-1} showing that the fixed regression of 0.05 mm AH per gram of egg implied by the HU is wrong. The R^2 values were ranged 0.4-4.5% and were fairly poor. The regression of the AA on the EW was significant but a bit higher than of the AH. The slope was always positive and was ranged 0.611-1.430 cm^2 of AA g^{-1} of egg. The R^2 values were ranged 7.5-21.1%. The EW was insufficient to determine both of AH and AA with a well fitted model. The fixed HU regression of AH or AA on EW is not adequate for diverse strain and age groups of eggs. The AH or AA alone will give a measure that is at least more accurate. However, image analysis to calculate irregular AA will provide an opportunity to easier and time-independent evaluation.

Key words: Albumen quality, albumen height, albumen area, digital image analysis, egg weight, evaluation

INTRODUCTION

Albumen quality is a standard measure of egg quality that is most often measured as the height of the thick albumen or a function of this such as the Haugh Unit (HU). The unit proposed by Haugh (1937) implies a positive regression of 0.05 mm Albumen Height (AH) per g of egg weight (Eisen *et al*., 1962). That is to say, the HU adjusts the thick albumen height according to the weight of the Egg (EW) and it uses a log scale because AH declines with storage in a logarithmic fashion (Silversides and Budgell, 2004). The HU has been used extensively (Williams, 1992) although, many researchers (Eisen *et al*., 1962; Nestor and Jaap, 1963; Kidwell *et al*., 1964; Silversides and Villeneuve, 1994) have criticized it and have shown that the adjustment for EW implied by the HU is incorrect except possibly in the sample of eggs measured by Haugh (1937). Silversides and Villeneuve (1994) proposed simply measuring the height of the thick albumen without a correction for EW. Genotype and age are two of the major influences on AH (Asharf *et al*., 2003). As the age of the hen increases, the AH decreases even as the EW and total amount of albumen increase (Hill and Hall, 1980; Silversides, 1994). The determinants of AH are not completely understood (Williams, 1992) although, the components of the albumen and their chemical and functional characteristics have been described (Robinson, 1987; Li-Chan and Nakai, 1989). The content and nature of ovomucin appear to be primarily responsible for determining AH but the chemical changes in storage that cause the reduction in AH are less clear. Reduced AH has been variously attributed to proteolysis of ovomucin, cleavage of disulfide bonds interactions with lysozyme and changes in the interaction between α and β ovomucins with no clear favorite (Stevens, 1996). But there is no practical method to determine these involved biochemical occurrences. However, the height of the thick albumen when the egg is broken onto a flat surface has largely defined the quality of sound eggs for many years. Because it is easily measured and relates well to the freshness of the egg.

On the other hand, albumen spreading area as another well-known indicator to freshness is also easily observed by consumers when the egg is broken open but calculation of an irregular area is time-consuming and impractical via traditional methods (Use of a planimeter). In such case, digital image analysis may be applicable as an alternative, quick and reliable method to calculate irregular areas by tracing the perimeters of the region of interest (Aktan, 2004a, b, 2005). Besides, once the digital images of broken eggs are shot and stored, images may be time-independently analysed.

In this study, it was aimed that to investigate relations between EW, AH and AA and to clarify whether the fixed relations among these traits and any correction could be used and could be generalized. For this purpose, two brown layer strains and two ages of hen were considered as main factors on albumen quality variables.

MATERIALS AND METHODS

Brown eggs were collected from commercial ATAK (Rhode Island Red x Line 54) and ATAK-S (Rhode Island Red x Barred Rock) hens. While an ATAK female is gold-feathered on the other hand, an ATAK-S female is non-barred and black-feathered. All hens were fed and housed under same conditions. When they were 30 and 40 weeks of age, randomly collected 60 eggs per each strain were stored 1 day at room temperature (Approximately 21°C). During sampling, cracked, soft-shelled and double-yolked eggs were excluded. At the time of sampling, each egg was weighed by an electronic scale with 0.01 g sensitivity and then broken onto non-reflective glass surface to prevent any reflection during digital images were shot. One of the nominative thick albumen traits is AH and it was determined by an electronic micrometer with 0.01 mm sensitivity. The latter is AA and for this purpose, digital photos of broken eggs were taken by a Canon EOS 400 D camera which was equipped with a Tamron 17-50 lens. While images were shot, a length-known reference line was placed near to region of interest because of it was needed to apply spatial calibration (Pixel to metric unit conversion) to calculate thick albumen spreading Area (AA) by digital image analysis. Digital images were analysed by Image-Pro Plus 5 software. On the other hand, two of derivative parameters were calculated by using EW, AH and the average of albumen width and length. First of these parameter is HU and the latter is AI and they were calculated by using equation as follows:

$$HU = 100 \log [H+7.57-1.7W^{0.37}]$$

Where:

H = AH in mm

W = EW in g

$$AI = \left[\frac{AH(mm)}{\text{Average of albumen width and length (mm)}}\right] \times 100$$

Data on EW, AH, HU and AA were analyzed using STATISTICA package. A General Linear Model included the main effects of age and strain of hen and 2 way interactions between these factors. Correlation coefficients (r) were calculated using Pearson correlation for each combination of age and strain. Correlations among nominative and derivative ones were not considered because of HU and AI includes AH and/or EW thus it leads to collinearity and a possible high level of association. Correlation coefficients were also compared by Fisher's -r to -z transformation (Papoulis, 1990), when it was needed. Moreover, the linear regressions of EW and nominative thick albumen traits (AH and AA) on both AH and AA were investigated. Linear regressions and R^2 values were evaluated in both case of the main effects were considered or purposely combined. The p-value of ($p<0.05$) were considered significant for all analyses.

RESULTS AND DISCUSSION

Nominative (AH and AA) and derivative (HU and AI) thick albumen traits in groups were shown in Table 1. As shown in the Table 1 only strain effect was found to be significant for AH. However, age was affected all of the examined thick albumen traits. As the age of the hens increase, AH, HU and AI decrease even as AA increases. That is to say, all of the thick albumen traits were run down by the increasing age as expected. Although, primary aim of this study was to investigate relationships among egg weight and nominative and derivative thick albumen quality variables on the other hand interactions were not to be found significant.

Correlation coefficients among EW and nominative thick albumen traits were shown in Table 2. The derivative thick albumen traits were excepted for calculate correlation coefficients because of both use and overlap AH and/or EW and this leads to the collinearity and a high level of correlation.

In factorial groups, there is no significant association between EW and AH. This is thought-provoking because of the HU formula assumes a fixed association among EW and AH. If the strain or the age is ignored, there are some significant statistical associations between these traits. But this theoretical and purposely ignorance was led to the significant statistical associations in an irregular manner. While EW and AH were generally associated in a negative manner both were only associated in a positive manner at 40 weeks of age ($p<0.01$).

Table 1: Nominative and derivative albumen quality traits in groups

Main effect		AH (mm)	NS		
Strain	N	p<0.05	HU	AI	AA (cm^2)
ATAK	120	6.01±0.15	74.52±1.42	7.42±0.28	60.99±1.56
ATAK-S	120	6.39±0.17	76.47±1.24	7.78±0.25	63.33±1.65
Age (weeks)		p<0.01	p<0.01	p<0.01	p<0.01
30	120	7.52±0.09	86.43±0.53	9.86±0.15	49.15±0.53
40	120	4.89±0.12	64.57±1.12	5.35±0.17	75.16±1.43
S x A		NS	NS	NS	NS

NS: Not Significant

Table 2: Pearson correlation coefficients among the thick albumen traits by strain and age groups

Strain	All		ATAK		ATAK-S	
Age (weeks)	AH (mm)	AA (cm^2)	AH (mm)	AA (cm^2)	AH (mm)	AA (cm^2)
All						
EW (g)	-0.144*	0.471**	-0.173	0.450**	-0.266**	0.533**
AA (cm^2)	-0.756**	-	-0.784**	-	-0.761**	-
30						
EW (g)	0.156	0.439**	0.175	0.337**	0.123	0.459**
AA (cm^2)	-0.433**	-	-0.525**	-	-0.369**	-
40						
EW (g)	0.239**	0.313**	0.213	0.274*	0.064	0.359**
AA (cm^2)	-0.483**	-	-0.477**	-	-0.555**	-

*p<0.05, **p<0.01

As another indicator of albumen quality, the AA was always positively associated with EW. While the highest association was observed in ATAK-S (0.533 and age-independent) this coefficient was significantly differed with eggs from 40 weeks age (0.313 and strain-independent).

On the other hand, AH and AA were always significantly and negatively associated. As expected, when the AH was decreased, AA was increased. This is also evidence to albumen liquefaction. Where the thick albumen liquefies and spreads as a natural result AH will decrease. Although, the lowest and the highest associations among AH and AA were observed as 0.369 and 0.555 in ATAK-S strain at 30 and 40 weeks of ages, respectively there was no significant difference among these correlation coefficients.

When the age and the strain effects were ignored, the linear regressions of nominative thick albumen traits (AH and AA) and EW on AH and AA were shown in Table 3 and 4, respectively. Moreover, linear regressions of AH, AA and EW on AH and AA at examined ages by strains were also shown in Table 5.

When the age effect was ignored, larger eggs were associated with lower albumen for only ATAK-S strain with regression coefficient was 0.094 mm g^{-1} of egg. The R^2 value was 7.1% and indicated that the EW was not noteworthy to determine AH. On the contrary, the larger eggs were associated with wider AA for ATAK and ATAK-S with regression coefficients were 1.997 and 2.080 cm^2 g^{-1} of egg, respectively. The R^2 values were 20.2 and 28.4%, respectively. This was also indicated that the EW was relatively unimportant in determining AA. On the other hand as expected AH and AA were had a relatively strong association with each other. The R^2 values were 61.5 and 57.9% in strains (Table 3).

When the strain effect was ignored on the contrary, larger eggs were associated with higher albumen for 30 and 40 weeks of age with regression coefficient was 0.038 and 0.067 mm g^{-1} of egg, respectively. But the R^2 values were still poor (2.4 and 5.7%, respectively). The larger eggs were associated with wider AA for ATAK

Table 3: Linear regressions of nominative thick albumen traits and EW on AH and AA in strains

Dependent variables	Intercept with slope (b)	Independent variable	R^2	Significance
ATAK				
AH (mm) =	10.975 - 0.084	×EW	0.030	NS
AH (mm) =	11.233 - 0.086	×AA	0.615	**
AA (cm^2) =	-56.961 + 1.997	×EW	0.202	**
AA (cm^2) =	104.188 - 7.184	×AH	0.615	**
ATAK-S				
AH (mm) =	12.363 - 0.094	×EW	0.071	**
AH (mm) =	10.761 - 0.069	×AA	0.579	**
AA (cm^2) =	-68.492 + 2.080	×EW	0.284	**
AA (cm^2) =	117.118 - 8.406	×AH	0.579	**

**p<0.01; NS: Not Significant

Table 4: Linear regressions of nominative thick albumen traits and EW on AH and AA at ages

Dependent variables	Intercept with slope (b)	Independent variable	R^2	Significance
30 weeks				
AH (mm) =	5.275 + 0.038	×EW	0.024	NS
AH (mm) =	11.149 - 0.074	×AA	0.187	**
AA (cm^2) =	12.250 + 0.620	×EW	0.193	**
AA (cm^2) =	68.179 - 2.531	×AH	0.187	**
40 weeks				
AH (mm =	0.701 + 0.067	×EW	0.057	**
AH (mm) =	7.984 - 0.041	×AA	0.233	**
AA (cm^2) =	10.509 + 1.027	×EW	0.098	**
AA (cm^2) =	102.973 - 5.680	×AH	0.233	**

**p<0.01; NS: Not Significant

and ATAK-S with regression coefficients were 0.620 and 1.027 cm^2 g^{-1} of egg, respectively. The R^2 values were 19.3 and 9.8%, respectively. This finding also indicated that the EW was relatively unimportant in determining AA. On the other hand, AH and AA had a bit higher association with each other. The R^2 values were observed as 18.7 and 23.3% in strains (Table 3) and were lower than in case of the strain effect was combined.

When all of the strain and the age factors were considered (Table 5), it was observed that all of the regression coefficients of the AH on the EW were positive but insignificant. Regression coefficients were varied from 0.02-0.059 mm g^{-1} meaning that these regression coefficients were sometimes less than half that which the HU correction implies or the fixed regression of 0.05 mm AH g^{-1} of egg implied by the HU is not valid in all cases. The regression coefficient whereas all data

Table 5: Linear regressions of nominative thick albumen traits and EW on AH and AA at ages by strains

Dependent variable	Intercept with slope (b)	Independent variable	R^2	Significance
All				
AH (mm) =	9.46 - 0.053	×EW	0.021	*
AH (mm) =	10.91 - 0.076	×AA	0.571	**
AA (cm^2) =	-44.198 + 1.737	×EW	0.222	**
AA (cm^2) =	109.021 - 7.551	×AH	0.571	**
ATAK at 30 weeks				
AH (mm) =	4.067 + 0.059	×EW	0.031	NS
AH (mm) =	11.993 - 0.095	×AA	0.275	**
AA (cm^2) =	11.768 + 0.628	×EW	0.114	**
AA (cm^2) =	69.719 - 2.914	×AH	0.275	**
ATAK at 40 weeks				
AH (mm) =	0.427 + 0.069	×EW	0.045	NS
AH (mm) =	7.690 - 0.042	×AA	0.227	**
AA (cm^2) =	13.562 + 0.999	×EW	0.075	*
AA (cm^2) =	98.631 - 5.393	×AH	0.227	**
ATAK-S at 30 weeks				
AH (mm) =	5.899 + 0.027	×EW	0.015	NS
AH (mm) =	10.673 - 0.062	×AA	0.136	**
AA (cm^2) =	12.806 + 0.611	×EW	0.211	**
AA (cm^2) =	67.039 - 2.207	×AH	0.136	**
ATAK-S at 40 weeks				
AH (mm) =	3.912 + 0.020	×EW	0.004	NS
AH (mm) =	8.539 - 0.043	×AA	0.308	**
AA (cm^2) =	-17.222 + 1.430	×EW	0.129	**
AA (cm^2) =	113.399 - 7.098	×AH	0.308	**

*p<0.05; **p<0.01; NS: Not Significant

combined was negative but significant. Besides, the R^2 values were varied from 0.4-4.5% and were fairly low. This finding also indicated that the EW was fairly insufficient to determine AH.

The regression of the AA on the EW was significant but it was only a bit higher than of the AH. The regression coefficient was always positive and ranged 0.611-1.430 cm^2 of AA g^{-1} of egg. The R^2 values were ranged 7.5-21.1%. In the same way, the EW was insufficient to determine AA with a well fitted model and any correction of AA by the strain or the age will not be appropriate.

On the other hand as expected, AH and AA were had a relatively stronger association with each other. The R^2 values were ranged 13.6-30.8% within the factorial groups and they were lower than in case of that the main effects were combined (57.1%).

The statistical association among EW and AH is poor and insignificant. The fixed HU regression of AH on EW is not adequate for diverse groups of eggs. Where the age or strain of the hens are unknown or when diverse groups of eggs are being compared, the AH alone will give a measure that is at least as accurate as the HU and is considerably easier to take. These results are agreed with both of the old and relatively actual reports of various researchers (Eisen *et al*., 1962; Nestor and Jaap, 1963; Kidwell *et al*., 1964; Silversides, 1994). This is also a thought-provoking situation because of the questioned but widely used HU that is a logarithmic expression in which the thick albumen height is corrected for the EW. Another previous report (Silversides and Villeneuve, 1994) concluded that measuring the AH alone was sufficient to describe deterioration in albumen quality.

In the same way, there is a weak but significant association among AA and EW from diverse groups of eggs. The AA is preferable because of measuring AH and applying HU to eggs is originally very tedious. Both are time-consuming and tiresome, moreover this limits the sample size per any duration. However, the AA by using digital image analysis provides an opportunity to measure and to evaluate of results independently from egg broken time.

CONCLUSION

In this study, the results confirm that there is no inherent association between EW and both of AH and AA. As Silversides (1994) was criticised that the HU correction for EW is designed to remove statistical association between EW and AH. The HU formula assumes that a larger egg has more albumen and that more albumen will be higher. The first association is true but the latter is of minor importance. Thus, neither the AH nor the AA must be corrected by EW to compare eggs from diverse groups. It is more correctly that to use AH and/or AA alone. If a correction for EW is to be used, correlations and regressions must be determined for each group of eggs. Any correction will improve the measure only if the eggs being compared come from hens which have similar genetic background of the same age. However in further studies, validity of the correction for EW must be investigated for each factor (Age, strain, storage duration and conditions and bird species etc.) which has any possible effect on albumen quality variables.

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