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Original Article

Eggshell and Bone Quality in Two Different Genetic Groups of Aged Layer Breeders

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ABSTRACT

The present study was conducted on pair aged commercial laying breeder strains (Hy-Line Brown and Hy-Line W-36) to evaluate genetic differences of eggshell and bone quality. In addition, egg production performance, mortality rate, internal organs and reproductive measurements were determined. Correlation coefficients among some eggshell and bone traits were calculated. White layer breeder hens had significantly higher percentages of hen-day and hen-housed egg production compared to the brown one. Brown Hy-Line layer breeder strain had better eggshell quality compared to W-36 Hy-Line layer breeder strain. The white layer breeder strain had a significantly higher relative oviduct weight compared to the brown one. Brown layer breeder strain recorded the higher humerus width in comparison with the white one. Tibia bone weight of brown layer breeder strain was significantly heavier by 22.87% as compared to the white strain. Moreover, the brown breeder strain recorded the higher figure of tibia width, thickness, and strength. In conclusion, at the end of the laying cycle, brown Hy-Line layer breeder strain exhibited better eggshell quality, body weight and tibia bone quality than those of W-36 breeder strain. Positive correlation coefficients were detected between humerus bone strength and both eggshell thickness and strength in brown and white strains.

INTRODUCTION

Recently, there has been increased interest from the scientific community and poultry industry regarding eggshell and bone quality of laying breeder strains. Modern commercial layer breeder hens produce more than 260 hatching eggs during a production period, and the amount of calcium for eggshell formation corresponds to such numbers of eggs during that time, with the advancing of layer breeder hen's age in late stages of egg production. It is hard to imagine how this rather small animal can cope with this effort, which requires a high ability to process feed for eggs and high eggshell quality. Another problem that occurrs on commercial laying breeder hens is the loss of bone strength specifically at the end of the laying season. Modern laying breeder hens face a major physiological challenge when they must produce large quantities of hatching eggs and maintain the strength of their skeleton.

Egg production is a dependent variable and is influence by several factors like the strain of chicken, feeding, mortality, culling, health, and management practices, peak lay and persistency of lay. Tolimir & Masic (2000) observed variable egg production performance for various strains of chicken. The mortality plays the main role in the determining profits from layer breeders as it is a function of dead and culled birds over the growth and production period. The higher mortality rate has been stated to harmfully affect production performance of layer breeders. The mortality and its negative association with net income was also reported by Asghar *et al*. (2000); and Farooq *et al*. (2001). North (1984) stated weak economic performance of breeders at mortality levels of more than 10%.

The eggshell quality is an important factor in poultry industry due to reproductive and economic implications; therefore, it is very essential to assess the egg quality characteristics and factors affecting them. Eggshell quality is assumed through its weight and percentage of the eggshell, thickness, and strength. The main differences in eggshell quality depend on the chicken strain and the age of the birds (Hanusová *et al.,* 2015). Eggshell quality is related to the hen age (Park & Sohn, 2018). Brown eggs had a thicker eggshell than the white ones (Ledvinka *et al.,* 2000). Conversely, Leyendecker *et al.* (2001) found a thinner shell in brown eggs. Lower values of eggshell thickness and percentage caused a significant decrease in the number of hatching chicks.

Silversides & Scott (2001) noticed a relative decrease in the shell as a percentage of egg weight as the hen ages. Also, the white-egg strain had a greater decrease in eggshell quality than the brown-egg strain. Poor shell quality will increase the incidence of cracked eggs. Tona *et al*. (2001) stated that the age of layer and broiler breeders is important to be considered by the hatchery manager. They also demonstrated that eggs produced by young or older breeders do not hatch in addition to the eggs from the breeders of 40 to 42 weeks of age.

Riczu *et al*. (2004) decided that the occurrence of both bone fractures and weakness in older laying hens at the end of lay is a concern in the poultry industry, and they stated a positive correlation might be indicative of the brown-egg hens' ability to replenish bone Ca reserves. Bone strength decreases with age in response to the high demand for calcium from bone for egg formation. Roland & Rao (1992) estimated that 15 to 30% of layer mortality in the USA is due to weak bones.

Bone strength has been presented to have a positive relationship with body weight (Bishop *et al*., 2000). Knowles *et al*. (1993) observed heavier chickens to have stronger bones but also have an increased incidence of bone breakage. They hypothesized that the increased bone strength was not sufficient to offset the stress of supporting the extra weight placed on the hen's skeletal system. Whitehead *et al*. (1998) reported that some hens are accomplished of high egg production and good bone quality at the end of lay. Thus, depending on the strain and breed, bone quality may not be deleteriously affected by high rates of egg production. Farmer *et al*. (1986) reported that egg production with good shell quality depends on the Ca in the feed and bones of birds.

Several studies have been conducted concerning bone strength in layers. It focused on the effects of nutrition (Rath *et al.,* 2000; Cransberg *et al.,* 2001) and housing (Appleby *et al.,* 2002) as factors affecting bone quality. To date, few researches have examined the differences in bone quality between brown and white layer breeder strains at the end of lay. Consequently, the aim of this research was to investigate the genetic variations of eggshell and bone quality in two aged layer breeder strains (Hy-Line Brown and Hy-Line W-36).

MATERIALS AND METHODS

The hens in the present study were reared in open-sided houses under standard managemental conditions. Feed amount was adjusted for each house depending on a number of breeders. Each hen from both strains was fed a constant amount of diet according to the manufacture (management) guide of Hy-Line. Managerial conditions were the same for the two laying breeder strains from 20 until 72 weeks of age. Water was available ad libitum and natural light was supplemented with artificial light to give the required scheduled photoperiod. The photoperiod was increased gradually according to the age of the birds until 17 hours per day was reached. All flocks laid eggs at a normally expected rate. Feed composition values of brown and W-36 Hy-Line layer breeder strains are presented in Table (1).

Table 1 – Feed composition of brown and W-36 Hy-Line layer breeder strains (60 - 70 wks of age).

Ingredients	Strain	
	Brown	W-36
ME, kcal/kg	2740–2840	2822–2910
Crude protein, g/day	14.3	14.5
Calcium, g/day	3.93	4.51
Phosphorus (available), %	0.36	0.41
Sodium, %	0.16	0.17
Chloride, %	0.16	0.17
Linoleic acid, %	0.89	0.97
Lysine, %	0.71	0.74
Methionine, %	0.32	0.34
Methionine+Cystine, %	0.56	0.58
Threonine, %	0.54	0.56
Tryptophan, %	0.16	0.17
Arginine, %	0.75	0.78
Isoleucine, %	0.55	0.58
Valine, %	0.65	0.67

Mortality rate and egg production performance

Mortality rate (number and percentage) for both males and females of brown and W-36 Hy-Line layer breeder strains was recorded from 60 to 70 weeks of age. Total numbers of hens housed were 7573 hens and 1034 males for W-36 Hy-line layer breeder strain; whereas brown Hy-line layer breeder strain were 2081 hens and 243 males. Total egg production was recorded daily for Brown and W-36 Hy-Line layer breeder strains beginning from 60 till 70 wks of layer breeder age. Percentages of hen-day and Hen-housed egg production were calculated using the following equations given by North (1984);

Hen-day egg production (%) = Eggs Number produced on daily basis / Number of birds available in the flock on that day * 100

Hen-housed egg production (%) = Total number of eggs produced by a flock / Total number of hens housed * 100

Eggshell quality traits

At the late stage of egg production (at 71 wks of layer breeders' age), 120 hatching eggs were collected from the two strains (60 Brown and 60 White). All eggs were individually weighed to the nearest 0.01 g using an electronic digital balance. Length and width of egg were individually measured by using a digital caliper. Shape index was calculated by (width / length) x100. Eggshell strength was determined according to Attar & Fathi (2014) using eggshell strength apparatus. The egg breakage force, Pa, was calculated, to within an accuracy of 0.02 kg/cm^2, from the registered gauge reading Pt and the apparatus correction factor f:

$Pa = f * Pt$

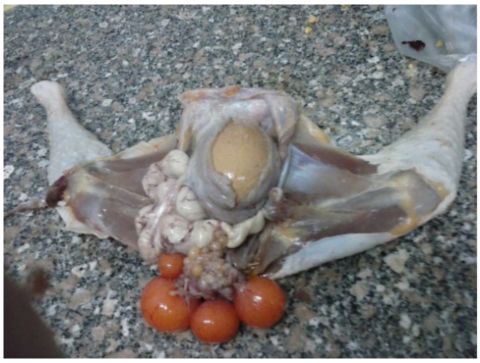
$f = 0.0109 * e^{5.73 * I}$ (where I represents the egg length/width ratio).

Wet and dry shell weight (to the nearest 0.01 gram) was determined using second decimal scales. The shell percentage was calculated according to the following equation: shell % = wet shell weight / egg weight x 100. Shell thickness (mm) with membranes was measured using a digital micrometer, the average of three measurements (two at both pointed and broad polar and one at the equator) was recorded.

Internal organs and reproductive measurements

At 72 wks of breeders' age, twenty-four layer breeder chickens (12 Hy-Line W-36 and 12 Hy-Line Brown) were randomly selected from the birds as part of a larger population. All hens were weighed as live body weight and slaughtered. Shank length (the distance from leg pad to the tarsus bone) was measured individually (in millimeter) by digital caliper. Chickens were dissected and weights of (the abdominal fat, liver, total ovary, and oviduct) to the nearest 0.01 g using digital balance. All parts were expressed as a proportion of the live body weight. Furthermore, number and weight of total large yellow follicles were also estimated (photo 1).

A



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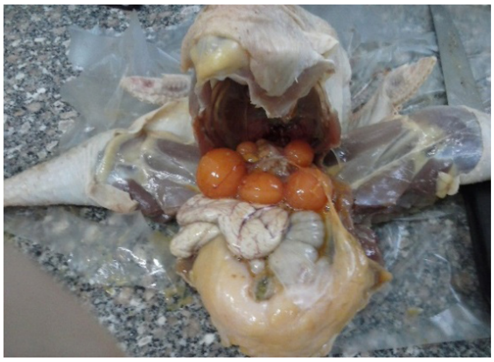

Photo 1 – (a) Total ovary, oviduct, number and weight of total large yellow follicles; (b) All of the above, in addition to abdominal fat.

Bone measurements

A total of 48 bone samples of left tibia and humerus of all breeder hens (24 Hy-Line W-36 and 24 Hy-Line Brown) were removed and then well cleaned of soft tissue. Bones were weighed to the nearest 0.01 gram. Bone length, width, and thickness were individually measured by digital caliper (mm) and breaking strength of bone was recorded. Tibia and humerus strengths were measured using an Instron Universal Testing Machine, which was set at a maximum load of 50 kg, testing was conducted under displacement control at a rate of speed 50 mm/min, the obtained reading from two channels (axis), representing maximal load (the force causing breakage, N). Bone put on stand take U-shape; a rod is lowered until it rests on the bone. A small pressure was then applied to the bone via this bar; breaking strength was recorded as the resultant change of reading on the dial gauge.

Statistical analysis:

Data were analyzed using one-way analysis of variance with the layer breeder strain as the main effect using the General Linear Model (GLM) procedure of SAS (2002) in accordance with the following model;

$Y_{ij} = \mu + S_i + e_{ij}$

Where;

Y_{ij} = Trait measured,

μ = Overall mean,

S_i = Strain effect

e_{ij} = Experimental error.

When significant differences among means were found, means were separated using Duncan's multiple range tests. The procedure CORR was used to calculate the phenotypic correlation among some eggshell quality and bone traits.

RESULTS AND DISCUSSION

Mortality rate and egg production

Data presented in Table 2 clarify mortality rate and egg production performance of Brown and W-36 Hy-Line layer breeder strains during the period from 60 to 70 wks of age. Results demonstrated a highly significant difference between strains for mortality rate (%), whereas the brown layer breeder strain had significantly higher mortality rate in comparison with the white one during the late stage of layer breeders' age. In this concern, Yakubu *et al.* (2007) reported a significant effect of strain on mortality rate and egg production. The lower mortality rate of Hy-Line W-36 layer breeder strain also indicated a better adaptation to the prevailing environmental conditions compared to Brown strain.

Table 2 – Mortality rate and egg production performance (Means ± SE) of brown and W-36 Hy-Line layer breeder strains (60 - 70 wks of age).

Trait	Strain		Probability
	Brown	W-36	
Mortality rate, %	1.37 ±0.09	0.96 ±0.06	<0.001
Hen-day egg production, %	55.27 ±0.95	59.91 ±1.57	<0.02
Hen-housed egg production, %	35.27 ±1.29	45.82 ±1.72	<0.0001

n=1169 hens for W-36, and 2961 for brown Hy-line layer breeder strain.

The eggs produced by layer breeders were calculated by hen-day and hen-housed egg production. It was noticed that the white layer breeder hens had significantly higher percentages of hen-day and hen-housed egg production (59.91 and 45.82%) compared to the brown layers (55.27 and 35.27%), respectively during the late stage of laying cycle. Such result may not reflect egg production accurately throughout the entire production cycle because egg production was only recorded during the late stage from 60 to 70 wks of layer breeders' age. It does, however, give an indication of egg production at the end of lay and can be compared with early egg production by the same strains as reported by Renema *et al.* (2001). They reported a 5.8% higher percentage of hen-day egg production for the brown-egg strain from 21 to 45 wks of age. The present results show 8.4% higher percentage of hen-day egg production for the white-egg layer breeder strain during the late stage of egg production. This finding indicates that the brown-egg layer breeder strain had a greater decrease in egg production close to the end of lay. Because the brown-egg strain has previously been shown to have a higher level of egg production through the early stages (Renema *et al.,* 2001), the total egg production of white and brown strains may be similar.

Eggshell quality

Eggshell quality traits of Brown and W-36 Hy-Line layer breeder strains at 71 wk of age are presented in Table 3. No significant difference between layer breeder strains for egg weight or egg shape index (%) was observed. Shell weights (wet or dry) were significantly affected by strain. The brown breeder hens had significantly heavier wet and dry shell weight compared to the white one. A similar trend was observed by Scott & Silversides (2000); Renema *et al*. (2001); Riczu *et al*. (2004), indicated that eggshell weights for brown eggs were heavier compared to white eggs strain. Some studies have shown that eggshell was heavier in older hens (Suk & Park, 2001; Roberts, 2004; Singh *et al.,* 2009; Rayan *et al.,* 2010). Therefore, the reason for heavier eggshells of older layers may be due to their production of heavier eggs than those of young layers.

Shell percentage can be used to estimate the eggshell quality (Mertens *et al.,* 2006). The present results clarify that brown eggs had significantly higher shell percentage (11.24%) compared to the white ones (10.12%). A similar trend was reported by Scott & Silversides (2000). Likewise, Silversides & Scott (2001) noticed that the shell as a percentage of egg weight decreased more for ISA-White eggs with increasing hen's age than it did for ISA-Brown eggs.

Table 3 – Eggshell quality of brown and W-36 Hy-Line layer breeder strains at 71 wks of age (Means ± SE).

Trait	Strain		Probability
	Brown	W-36	
Egg weight, g	65.36 ±0.60	66.13 ±0.64	NS
Egg shape index, %	75.16 ±0.39	75.71 ±0.35	NS
Wet shell weight, g	7.32 ±0.08	6.69 ±0.11	<0.0001
Dry shell weight, g	6.45 ±0.08	5.75 ±0.10	<0.0001
Shell percentage, %	11.24 ±0.12	10.12 ±0.14	<0.0001
Shell thickness with membranes, mm	0.40 ±0.004	0.36 ±0.005	<0.0001
Eggshell breaking strength (kg/cm2)	4.63 ±0.18	3.69 ±0.14	<0.0001

n=120 hatching eggs (60 Brown and 60 White).
NS= Non-significant.

Shell thickness was significantly affected by layer breeder strain; the brown eggs recorded significantly higher shell thickness with membranes compared to the white counterparts, confirmed by previous result from Ledvinka *et al.* (2000); Badawe (2006); and Rayan *et al.* (2010). Eggshell breaking strength of the brown eggs was significantly higher than that of the white ones. Generally, Brown Hy-Line layer breeder strain at the end of the laying cycle (71 wk of age) had better eggshell quality than W-36 Hy-Line layer breeder strain. Poor shell quality would increase the incidence of cracked eggs and reduce the amount of settable eggs.

Body weight, internal organs, and reproductive measurements

Table 4 clarifies internal organs and reproductive measurements of brown and W-36 Hy-Line layer breeder strains at 72 wks of age. The brown layer breeder strain had significantly heavier body weight compared to the white one. This result was consistent with the findings of Shalev (1995); and Rayan (2013). No significant difference between breeder strains for shank length, relative liver weight, and abdominal fat percentage were noticed. Regarding reproductive measurements, the white layer breeder strain had significantly higher relative oviduct weight (4.54%) compared to the brown layers (3.23%). No significant differences between breeder strains for relative total ovary weight, number and weight of large yellow follicles were observed. Renema & Robinson (2001); and Riczu *et al.* (2004), didn't report any differences in reproductive system component weights between brown and white layer strains.

Table 4 – Internal organs and reproductive measurements of brown and W-36 Hy-Line layer breeder strains at 72 wks of age (Means ± SE).

Trait	Strain		Probability
	Brown	W-36	
Live body weight, g	1593.42 ±57.88	1430.50 ±36.42	<0.03
Shank length, mm	96.79 ±0.94	95.09 ±0.57	NS
Liver weight, %	2.09 ±0.07	2.19 ±0.10	NS
Abdominal fat, %	1.03 ±0.24	0.53 ±0.16	NS
Oviduct weight, %	3.32 ±0.21	4.54 ±0.38	<0.01
Total ovary weight, %	2.36 ±0.14	2.88 ±0.20	NS
Large yellow follicles, n	4.63 ±0.18	4.92 ±0.19	NS
Large yellow follicles, g	39.28 ±2.72	36.37 ±3.00	NS

n= 24 layer breeder chickens (12 Hy-Line W-36 and 12 Hy-Line Brown)
NS= Non-significant.

Bone quality

Humerus and tibia bones characteristics of brown and W-36 Hy-Line layer breeder strains at 72 wks of age are summarized in Table (5). No significant differences between breeder strains for humerus bone weight, length, thickness, and breaking strength were found. On the contrary, a significant difference between breeder strains was detected for humerus bone width; brown layer breeder strain recorded higher humerus width (8.25 mm) compared to the white layers (7.90 mm). In the hen, the humerus has one of the greatest fracture rates of all bones (Fleming *et al.,* 1998). It is naturally a pneumatized bone of the hen's wing but has been found to contain variable quantities of trabecular bone (Fleming *et al.,* 1996).

Regarding tibia bone, it could was observed that the brown breeder strain recorded heavier tibia weight than that of the white breeder. Tibia length was not significantly different between the brown and W-36 breeder strains, although the tibia bone weight of brown layer breeder strain was about 22.87% greater as compared with that breeder strain (Table 5). This result likewise supports the observing that the overall thickness of the bone is greater for the brown breeder strain and suggests there is more bone tissue in an equivalent length of the bone. There was a significant difference between strains for tibia bone width, thickness, and breaking strength; the brown breeder strain recorded higher tibia width, thickness, and strength in comparison with the white breeder.

Tibia bone strength of brown layer breeder strain was greater than in W-36 layer breeder strain by 33.02%.

Table 5 – Humerus and tibia bones characteristics of brown and W-36 Hy-Line layer breeder strains at 72 wks of age (Means ± SE).

Measurement	Strain		Probability
	Brown	W-36	
Humerus bone			
Weight, g	4.63 ±0.35	4.96 ±0.31	NS
Lenght, mm	73.61 ±0.72	72.96 ±0.33	NS
Width, mm	8.25 ±0.11	7.90 ±0.08	<0.01
Thickness, mm	6.57 ±0.11	6.36 ±0.08	NS
Breaking strength, Kg	13.14 ±1.26	12.77 ±1.33	NS
Tibia bone			
Weight, g	11.37 ±0.40	8.77 ±0.19	<0.0001
Lenght, mm	115.64 ±1.21	113.47 ±0.52	NS
Width, mm	8.02 ±0.11	7.08 ±0.11	<0.0001
Thickness, mm	6.90 ±0.10	5.92 ±0.09	<0.0001
breaking strength, kg	21.43 ±2.62	14.22 ±1.61	<0.03

n= 48 bone samples (24 Hy-Line W-36 and 24 Hy-Line Brown)

NS= Non-significant.

Correlation coefficients among traits

Data presented in Table 6 show correlation coefficients among some eggshell and bone traits of brown and W-36 Hy-Line layer breeder strains during the late stage of laying cycle. Tibial bone thickness and strength was not correlated with eggshell thickness or eggshell strength in brown or W-36 layer breeder strains. In the brown layer breeder strain, A significant negative correlation coefficient was detected between the humerus bone thickness and eggshell strength (r = -0.62), while this correlation was not observed for the W-36 strain. Humerus bone strength was positively correlated with both eggshell thickness and strength in the two strains, but these coefficients were not statistically significant.

CONCLUSION

Brown Hy-Line layer breeder strain at the end of laying cycle had better eggshell quality, body weight and tibia bone quality than W-36 Hy-Line layer breeder strain. The better tibia bone quality of brown layer breeder strain may indicate that this strain is less susceptible to bone problems such as osteoporosis and osteomalacia. Also, although the larger body weight of the brown layer breeder hens results in a greater feed consumption, the loss of white strain hens may offset this cost due to poor tibia bone quality. The present results indicate that there may be a potential for improving the welfare of layer breeder hens through the selection of certain strains. A positive correlation coefficient was detected between the humerus bone strength and both eggshell thickness and strength in brown and white strains.

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Table 6 – Correlation coefficients among some eggshell and bone traits of brown and W-36 Hy-Line layer breeder strains.

Eggshell traits Bone characteristics	Eggshell Thickness, mm	Eggshell Strength, kg/cm2	Strain
Tibial thickness, mm	0.06	-0.21	Brown
	0.08	0.17	W-36
Tibial strength, kg	-0.04	-0.04	Brown
	-0.10	0.25	W-36
Humerus thickness, mm	-0.19	-0.62*	Brown
	-0.17	-0.08	W-36
Humerus strength, kg	0.22	0.37	Brown
	0.25	0.30	W-36

(*) $p \leq 0.05$

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