



chickens line. A total of 150 eggs from College of Natural Resources farm were sampled for the present experiment. About 70 eggs each were stored in home refrigerator (temperature, 7.46 ± 0.23 °C; relative humidity, $24.80\pm0.05\%$) and room temperature (temperature, 22.98 ± 1.12 °C; relative humidity, $48.93\pm2.50\%$). The correctness of the conditions was ensured by installing data logger. Egg quality traits monitored included egg weight, egg shape index, specific gravity, egg volume, shell thickness, haugh unit, and yolk index and albumen pH. The results present effects of prolonged storage on the physical qualities significantly (P<0.05). Quality of eggs rapidly deteriorated when stored in a room temperature compared to those kept in a cold environment. Among the studied egg traits, the pH of albumen seems to be reliable in testing the freshness quality of an egg because of its consistent trend. According to albumen pH information, egg preserved for 10 days under cool conditions remained similar to that of freshly laid eggs, but the following days seems to be significant (P<0.05). Moreover, the study verified the importance of the correlation coefficient in determining the downgrading of eggs for extended storage.

KEY WORDS albumen pH, egg physical quality, egg production, environmental conditions, Hyline chickens.

INTRODUCTION

Egg is one of the most essential cheap sources of protein in human diet. Egg is also an essential economic trait generating income instantly through sell of eggs and chickens (Monira *et al.* 2003; Jones and Musgrove, 2005). In fact, quality of an egg ascertains the success of a poultry business because it is associated with the acceptability among the consumers. Based on consumer needs and likings, the eggs are categorized with respect to shell, albumen and yolk information. Similarly, quality of the eggs in ISA-Brown and ISA-White (Silversides and Scott, 2001), historic strains of single White Leghorn (Anderson *et al.* 2004), Sudanese indigenous chicken (Mohammed *et al.* 2005) and Nigerian indigenous chicken (Yakubu *et al.* 2008) have been already assessed. Generally, popularity of eggs from native hens over is improved among the consumers recent times because they are better in taste and flavor (Mohammed *et al.* 2005; Dorji *et al.* 2011). Nevertheless, the native eggs produced are comparatively low favoring the rearing of improved birds by the farmers.

Recently, there is an increasing numbers of exotic chickens in Bhutan (especially, Hyline) owned usually by parttime and / or full-time poultry entrepreneurs in small scale. This has lead to a boom in egg production in the country. For instance, statistic reports from department of Livestock have showed that improved birds contributed about 25% and 53% of the country's total poultry population in 2008 and 2010, respectively. Correspondingly, the egg sale was observed to increase from 36% to 42% in two years (Livestock Statistics, 2008; Livestock Statistics, 2010). It is, therefore, expected that the eggs from exotic hens contribute to the maximum supply of the country's egg demand. However, the egg production is a seasonal and socio regional based and thus, storing the eggs is an inevitable process. The period when the eggs are stored and transported to retail market is likely to produce more damaged eggs. This results in greater losses and risks to the smallscaled entrepreneurs. Therefore, the objective of this study was to evaluate physical egg quality of Hyline chickens under different storage periods and assessment of recommended storage temperature by FAO (2003).

MATERIALS AND METHODS

Layers

The College of Natural Resources Poultry farm maintains about 300 Hyline birds imitating a typical semi-intensive production system existing in the country. The birds were purchased from the Regional Poultry Breeding Centre (RBPC) at the age of eight weeks. They were reared under deep litter system and fed with layer feed and treated against the worms. A poultry shed constructed consists of five compartments with 20.3 ft (length), 15.1 ft (width) and 11.2 ft (height) facing east west direction to avail maximum natural light. As it was suggested by Sonaiya and Swan (2004), the inner wall of the shed was whitewashed. A stock density of three birds per square meter was considered (Sonaiya and Swan, 2004).

Clean light bulbs were used to meet the light requirement by birds (Sonaiya and Swan, 2004) and they were exposed to artificial lighting for about eight hours / day. About two millimeter deep, saw dust litter was used to cover the floor. Market feeders and drinkers were provided and were cleaned before providing feed and water to the birds. The birds were fed twice a day. Animals were also supplemented with vitamins and minerals. Grower feed was fed from eight to seventeen weeks of age. At the age of 17 weeks, layer feed was provided to the birds. The grower feed contained crude protein (CP) of 16% and calcium value of 1.5% respectively. On the other hand, layer feed contained CP, metabolizable energy and calcium value of 15.7%, 2883-2900 kcal and 4%, respectively. The layers were 36 weeks of age when were considered for the present study. This was because the temperature was considered suitable (about 20-24 °C) for the layers' performance (FAO, 2003).

Egg sampling

A total of 150 eggs were randomly collected at same day from the college poultry farm. The eggs were about 18 hours old at the time of the collection from nests and / or soft litter floor. Subsequently, about 70 eggs were randomly assigned to storage chambers set at a temperature and relative humidity of home refrigerator $(7.46\pm0.23 \text{ °C} \text{ and } 24.80\pm0.05\%$, respectively) and room temperature $(22.98\pm1.12 \text{ °C} \text{ and } 48.93\pm2.50\%$, respectively). A data logger was used to ensure correct conditions during the entire study period.

The eggs were manually screened for detecting any cracks. The eggs with persisting dirt after cleaning with edible oil were also rejected from further experiment. About 10 eggs from each storage chamber were randomly selected to assess egg quality for extended storage time (0, 3, 5, 7, 10, 14 and 18 days).

Traits measured

A list of traits was recorded for evaluating the quality of eggs.

During the data collection, eggs were measured for weight, width and length by digital electronic weighing machine and electronic vernier caliper respectively. Egg shape index was estimated by the following formula:

Egg shape index= width of egg (short perimeter) / length of egg (length perimeter)

The specific gravity was determined by Stadelman and Cotterill (1995) method and the equation was as follows:

Egg specific gravity= egg weight (gm) / egg volume (cm³)

Egg volume was estimated using the method of some studies.

Egg volume (cm³)= 0.51 (egg length) × (egg breadth)²

The eggs were subjected to breakout analysis. The height of the thick albumen was measured according to Silversides and Scott (2001) procedure by electronic vernier caliper. The yolk was separated from the white egg and, then, it was weighed by digital electronic weighing machine. The pH of the albumen was estimated immediately using the pH meter (microprocessor pH 211).

The weight of the shell was determined as it was described by Anderson *et al.* (2004). The thickness of egg shell was measured at the equator of the eggshell by electronic vernier caliper thrice. Albumen weight was measured as:

Weight of albumen= weight of egg - (weight of yolk+weght of shell)

Haugh unit (HU) for individual egg was estimated by Monira *et al.* (2003):

 $HU=100 \log (H-1.7W^{0.37}+7.6)$

Where: HU: is Haugh unit. H: is height of albumen (mm). W: is weight of egg (gm).

The percent of the three principle egg components were taken as a proportion of the total egg weight as:

Egg component %= (component weight (gm)) / (total egg weight (gm)) \times 100

Statistical analyses

The data were analyzed using analysis of variance (ANO-VA) of SPSS version 16.0 to record the effect of extended storage duration and conditions (Raji *et al.* 2009). When the significance difference was observed (P<0.05), least square difference (LSD) mean was used to separate (Yakubu *et al.* 2008). Pearson coefficient (r) was also employed to determine the relationships among various egg quality traits.

RESULTS AND DISCUSSION

Egg quality traits

There are several important factors that potentially affect the quality of an egg such as breed and age of the laying hen, prevalence of diseases and handling processes (FAO, 2003). The scientific literature proposed that nutrition has little importance in determining the quality of an egg (Silversides and Scott, 2001). The egg characteristics become downgraded and the rate of deterioration is influenced by storage duration and conditions such as temperature and moisture. For example, egg chemical and physical changes for 7 to 10 days at 27-29 °C were equivalent to those stored at -1 °C and 85% relative humidity (RH) for several months (FAO, 2003). The egg quality of Hyline birds were quantified as described by Mohammed *et al.* (2005), Yakubu *et al.* (2008) and Biladeau and Keener (2009).

Exterior quality

An exterior egg quality trait refers to the weight, size (shape index) and specific gravity of the egg. The variation among egg weight is observed to be moderate and it has a direct impact on its economic value. Conversely, shell thickness has an indirect influence on its economic value although variation is also moderately occurring. The presence of large standard deviation (SD) is assumed that it is due to large variations and small sample sizes.

There was no significant difference (P<0.05) across the storage durations and the egg conditions from day 0 to 7 for all the mentioned exterior traits (Table 1). Samli *et al.* (2005) also found that the mean values of egg weight were

not significant (P<0.05) using Bovans White hens. By contrast, the evidence from Monira and coworkers (2003) showed a significant decreasing trend in egg weight with increasing storage period.

The egg weight and specific gravity decreases as the holding period prolongs in both storing conditions (Table 1). This result could be attributed to the loss of water through the shell (FAO, 2003; Silversides and Budgell, 2004) and probably more for lager eggs. On the other hand, shape index change was insignificant (P<0.05) with the storage time. This study provides an additional support to Monira *et al.* (2003), Silversides and Budgell (2004) and Jones and Musgrove (2005) documentation. However, the performed experiment represents inconsistent trends exemplifying the bias and, therefore, shape index may be considered as an inappropriate parameter to present the quality of an egg.

The inconsistent trend of this quality parameter might be explained by the differences in weight of the hens (Anderson *et al.* 2004; Mohammed *et al.* 2005) and the differences in temperature and relative humidity. For example, the ambient temperature and the relative humidity (in particular) were high from day 0 to 10. Furthermore, egg weight depends on feeding system and temporary and permanent environmental conditions (Mohammed *et al.* 2005; Yakubu *et al.* 2008). The egg size is dependent on strains and age of hens (Anderson *et al.* 2004; Mohammed *et al.* 2005; Yakubu *et al.* 2008). They also reported that the differences in egg weight are highly dependent on hen's genotype and age.

Interior quality

The interior egg quality description includes yolk weight, height and index, albumen weight, height, HU (height is expressed as HU to adjust the effect of egg weight), pH and the weight and thickness of the shell.

Previous studies conducted claims that interior quality measure is the best indicators of an egg quality (Silversides and Budgell, 2004; Yakubu *et al.* 2008; Biladeau and Kenner, 2009). The mean yolk indices decreased with passage of time irrespective to store conditions, this was dramatically under room temperature in ordered direction. Furthermore, the differences in yolk indices due to different storage condition counterparts were significant (Table 2).

The mean of yolk index was the lowest at 18-days of ambient temperature storage (28.77 ± 2.27). It was also noted that in the refrigerated eggs that were kept for 18-days, the yolk index was insignificant during 3-days of room temperature storing (P<0.05).

Nevertheless, the decrease in the yolk index of those eggs stored in room temperature from the current study was lower than the corresponding days of st orage in Raji *et al.* (2009) study.

Day	Egg weig	Egg weight (gm)*		e index*	Specific gravity*		
0	62.23	62.23 ± 3.22^{ab}		76.67 ± 2.22^{a}		1.12±0.04 ^a	
	Room temp.	Refrigerator	Room temp.	Refrigerator	Room temp.	Refrigerator	
3	64.18±3.17 ^{ab}	63.66±4.30 ^{ab}	76.86±2.71ª	76.18±6.50 ^a	$1.12{\pm}0.08^{a}$	1.19±0.19 ^b	
5	62.50±3.56 ^{ab}	61.23±6.00 ^{ab}	77.25±1.59 ^a	76.65 ± 2.82^{a}	$1.08{\pm}0.02^{a}$	1.09±0.01ª	
7	62.11±3.45 ^{ab}	62.37±6.50 ^{ab}	76.50±1.91ª	76.85 ± 1.82^{a}	$1.08{\pm}0.01^{a}$	1.11±0.01 ^a	
10	63.56±3.30 ^{ab}	59.49±5.53ª	$77.43{\pm}1.28^{a}$	76.99±2.63ª	$1.08{\pm}0.04^{a}$	1.09±0.01ª	
14	61.03±4.79 ^{ab}	59.73±1.31 ^a	$78.37{\pm}1.09^{a}$	75.93 ± 3.57^{a}	$1.07{\pm}0.01^{a}$	1.08 ± 0.04^{a}	
18	60.76 ± 4.83^{ab}	58.44 ± 4.40^{d}	77.26±3.04 ^a	78.44 ± 2.22^{a}	$1.07{\pm}0.01^{a}$	$1.08{\pm}0.04^{a}$	

Table 1 Description of external quality in Hyline eggs for different storage time and conditions

The means within the same column with at least one common letter, do not have significant difference (P>0.05).

SD: standard deviation.

* Mean \pm SD.

This evidence may probably be explained by differences in room temperature and RH.

The loss of albumen weight (expressed in percentage) and Haugh unit (HU) followed similar manner with extended storing (Table 2 and Table 3). However, the changes in albumen percentages and HU were much slower in refrigerated eggs than in their counterparts. On the other hand, with the advancing of storage time, the yolk percentage increased.

These result highlightss similar findings to those of Silversides and Budgell (2004), Samli *et al.* (2005) and Raji *et al.* (2009). This is due to the water and proteins travel to yolk through vitelline membrane from the egg white (Silversides and Scott, 2001; Silversides and Budgell, 2004; Raji *et al.* 2009).

The mean HU of 81.60 estimated for 0 week in old eggs was almost near to Biladeau and Keener (2009) experiment using Hyline W36 layers under same temperature (7 °C) but higher than that reported by Raji et al. (2009) from brown strain Bovans. Scientific literature suggest that the yolk index and HU are the best indicators of internal egg quality (Silversides and Budgell, 2004; Yakubu et al. 2008; Biladeau and Keener, 2009). Both HU and yolk indices calculated under ambient temperature for corresponding day were higher than those obtained by Raji et al. (2009). The difference in storage conditions from earlier could explain the varied estimation for stated quality. However, direction of change in HU was not consistent contradicting to previous studies of Jones and Musgrove (2005); Raji et al. (2009) and Biladeau and Keener (2009). It was also concluded from the earlier studies that the HU, an empirical expression of albumen height, is affected by the age and the stains of the hens (Silversides and Budgell, 2004) and the age of the eggs (Biladeau and Keener, 2009). In addition, the height of albumen can be measured by different techniques resulting to different calculation of HU (Silversides and Budgell, 2004).

There were significant differences (P<0.05) in albumen pH for increasing period of storage as well as for different storage conditions. However, the increase in albumen pH was more pronounced in non-refrigerated eggs (Table 2).

This is in line with Silversides and Scott (2001), Silversides and Budgell (2004), Samli *et al.* (2005) and Biladeau and Keener (2009) researches. When keeping the eggs for longer storage time, the increase in albumen pH with passage of time is found to be associated with the loss of carbon dioxide and movement of water from albumen to yolk (Biladeau and Keener, 2009). Therefore, the impact of different storage conditions on egg weight was observed to be significant.

The pH of albumen stored at 7 °C for a week was as per Biladeau and Keener (2009) recorded value. However, the increase in pH of albumen in their study was slower than in the current study. On the other hand, the pH value from Silversides and Budgell (2004) observation was comparatively lower than in the present study. A probable explanation for this similar consistent trend but different degree of changes may be related to the use of different strains, age and storage conditions (Silversides and Budgell, 2004).

More interestingly, the results revealed that the home refrigerator is likely to preserve the freshness of an egg for more days. For instance, the albumen pH of cold stored eggs for 10 days was not significant compared to the albumen pH observed in those eggs kept under an open environment for three days (P < 0.05) as it is shown in Table 2. The fate of albumen pH increased sharply from day 14th of storage in both storing conditions. Our research has similar results to formerly studies on deducing the important role of albumen pH in representing the freshness of an egg quality (Silversides and Scott, 2001; Silversides and Budgell, 2004). Similarly, Silversides and Budgell (2004) also mentioned that the changes in the pH of albumen with respect to the age of the egg were distinct while not the case with the lines and the age of the hens. Therefore, Biladeau and Keener (2009) suggested to use edible food grade film such as oil, wax and whey protein isolate to maintain the freshness of an egg for longer duration. Egg borne poisoning has been reported repeatedly caused by Salmonella spp. in many poultry industries. Thus, the pathogens growth can be effectively reduced by controlling the temperature and when the temperature is above 30 °C, they grow rapidly Mukhopadhyay et al. 2012).

Day	Yolk index*		Haugh	n unit*	Albumen pH*		
0	46.73 ± 3.10^{a}		81.60 ± 1.76^{a}		8.43±0.24 ^a		
	Room temp.	Refrigerator	Room temp.	Refrigerator	Room temp.	Refrigerator	
3	40.17±2.53 ^b	46.61±3.12 ^a	73.93±3.05 ^{bd}	76.23±3.21°	$9.18{\pm}0.24^{b}$	9.00±0.05°	
5	37.25±2.19 ^c	48.96±1.35 ^a	73.61±1.18 ^{bd}	75.82±1.57°	9.31±0.3 ^{bd}	9.12±0.08 ^{bc}	
7	35.02±3.07 ^c	$46.94{\pm}3.47^{a}$	$72.88 {\pm} 1.75^{bd}$	76.23±1.02 °	9.56±0.15 ^e	9.13±0.06 ^{bc}	
10	33.43±2.50°	44.01±2.53 ^b	73.56±1.15 ^{bd}	76.19±1.59°	9.54±0.11e	$9.28{\pm}0.04^{bd}$	
14	$29.34{\pm}0.87^{d}$	41.77 ± 1.82^{b}	71.51 ± 1.18^{d}	76.19±1.31°	9.67±0.09 ^e	$9.34{\pm}0.03^{d}$	
18	28.77 ± 2.17^{d}	40.65±3.25 ^b	71.83 ± 1.39^{d}	75.03±1.27 ^c	$9.95{\pm}0.05^{ m f}$	9.51±3.25 ^e	

 Table 2
 Description of interior quality in Hyline eggs for different storage time and conditions

The means within the same column with at least one common letter, do not have significant difference (P>0.05).

SD: standard deviation.

* Mean \pm SD.

Table 3 Percentage of the three principle egg components for extended storage time and conditions

Day	Albumen percentage*		Yolk per	centage*	Shell percentage*			
0	$66.45{\pm}1.56^{a}$		$23.78{\pm}1.62^{a}$		9.77±	9.77±0.59 ^{ab}		
	Room temp.	Refrigerator	Room temp.	Refrigerator	Room temp.	Refrigerator		
3	64.55±2.14 ^{ab}	64.57±2.32 ^{ab}	25.96±1.85 ^b	25.56±2.34ª	9.49±0.49 ^{ab}	9.88±0.39 ^{ab}		
5	59.59±5.41 ^{ab}	59.72 ± 4.14^{ab}	30.69±5.07 ^{bc}	30.36±3.77 ^a	9.71 ± 0.68^{ab}	$9.92{\pm}0.62^{ab}$		
7	61.36±3.08 ^b	63.12±2.52 ^b	$28.65 \pm 3.15^{\circ}$	27.13±2.35 ^a	9.99 ± 0.40^{ab}	$9.76{\pm}0.49^{ab}$		
10	62.06±2.61 ^{ab}	$63.34{\pm}1.66^{b}$	27.98 ± 2.16^{cd}	27.01 ± 1.72^{ab}	$9.96 {\pm} 0.60^{ab}$	9.65±0.43 ^{ab}		
14	62.29 ± 3.16^{ab}	$62.79 {\pm} 1.67^{b}$	27.79±2.81 ^{bd}	27.19 ± 1.49^{b}	$9.92{\pm}0.59^{ab}$	10.02 ± 0.35^{ab}		
8	$61.82{\pm}1.59^{b}$	$63.08 {\pm} 2.75^{ab}$	$27.66{\pm}2.61^{d}$	26.89±2.61 ^b	10.52±0.49 ^a	10.03 ± 0.43^{ab}		

The means within the same column with at least one common letter, do not have significant difference (P>0.05). SD: standard deviation.

* Mean ± SD.

Thus, it would be very useful to observe the presence of pathogens in the eggs stored for the same period of study.

Correlation traits

To ensure the influence of ageing eggs on various egg traits, a Pearson correlation coefficient was estimated (Table 4). Correlation was used to describe the degree of relationship between the two random traits. The correlation coefficient (r) was hesitantly classified as weak, moderate and strong for 0 < r < 0.30, 0.30 < r < 0.70 and r > 0.70, respectively. The statistical relationships among time of storage to the studied egg traits were negatively correlated for egg weight, specific gravity, yolk index, albumen weight and HU while were positively correlated for shape index, albumen pH, yolk weight and shell weight. The negative correlation values ranged from -0.251 (P<0.01) between day and egg weight to -0.562 (P<0.01) between day and yolk index.

Conversely, positive value varied from 0.129 between day and shape index to 0.779 (P<0.01) between day and albumen pH. The study favored an additional support to the similar results that some egg quality traits decreases while other increases as storage period extends (Silversides and Scott, 2001; Silversides and Budgell, 2004; Samli et al. 2005; Biladeau and Keener, 2009). The correlation values have very important implications while breeders want to improve any traits because almost all the economic traits are somehow connected. The values estimated as egg weight and albumen percentage were positively correlated (P<0.01), as it was shown in Table 4. The improvements made on egg weight will increase the albumen percentage (Yakubu et al. 2008). On the other hand, significant (P<0.01) negative correlations (r=-0.988) between albumen percentage and yolk percentage were observed indicating the improvement made on albumen percentage will have

Table 4 Correlation relationships among the various egg traits studied in Hyline layers

Table 4 Contration relationships among the various egg traits studied in Hynne rayers									
Trait	EW	ESIN	SPG	YLIN	Al pH	Al %	YL %	SH %	HU
Day	-0.251**	0.129	-0.286**	-0.562**	0.779^{**}	-0.261**	0.226^{*}	0.284^{*}	-0.523**
EW	-	0.06	-0.005	0.085	-0.129	0.271^{**}	-0.224*	-0.363**	0.141
ESIN	-	-	-0.317**	-0.05	0.084	-0.042	0.045	-0.011	0.007
SPG	-	-	-	0.196^{*}	-0.214*	0.195^{*}	-0.211*	0.043	0.160
YLIN	-	-	-	-	-0.707**	0.305^{**}	-0.288**	-0.201*	0.687^{**}
Al pH	-	-	-	-	-	-0.397**	0.376^{**}	0.237**	-0.781**
Al %	-	-	-	-	-	-	-0.988**	-0.365**	0.530^{**}
YL %	-	-	-	-	-	-	-	0.215^{*}	-0.521**
SH %	-	-	-	-	-	-	-	-	-0.219^{*}

** P<0.01 and * P<0.05.

EW: egg weight; ESIN: egg shape index; SPG: specific gravity; YLIN: yolk index; Al pH: albumen pH; Al %: albumen percentage; YL %: yolk percentage; SH %: shell percentage and HU: haugh unit.

adverse effect on yolk percentage. Similarly, a progress made on yolk index will also result in a decrease for yolk percentage (r=-0.288). Silversides and Scott (2001) also reported a significant relationship between various egg traits.

CONCLUSION

The results recorded from this experiment suggest that the ageing of eggs had a significant effect on exterior and interior egg quality. However, except for albumen pH, all quality traits expressed inconsistent direction changes. Therefore, Pearson correlation values were used to confirm the relationships existing among various quality traits included in this study. Storage conditions also greatly influenced the quality of eggs. From our results, it is suggested that eggs may not be stored for more than 10 days at home refrigerator.

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