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Effect of Pigment Intensity and Region of Eggshell on the Spectral Transmission of Light That Passes the Eggshell of Chickens

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Abstract: The effects of intensity of brown pigment and region (small pole, equator and large pole) of eggshell on eggshell thickness and per cent transmittance of light (PT) that passes through the eggshell of chicken eggs were investigated. The PT was measured over the wavelength (WL) range of 200 to 800 nm (near-ultra violet (near-UV) WL range from 200 to 380 nm, visible (V) WL range from 380 to 780 nm and near-infra red (near-IR) WL range from 780 to 800 nm). Brown pigment eggshells [light (LBP), medium (MBP) and dark (DBP)] from meat-type breeder (Hybro) eggs of comparable weight were used. The intensity of brown pigment and region of eggshells did not affect eggshell thickness, but significantly ($P<0.01$) influenced the PT that passes through the eggshell over the WL range of 200 to 800 nm. LBP had higher PT and spectral transmission ratio of the near-UV:V and lower spectral transmission ratio of the near-IR:V when compared with those of the MBP and DBP. Whilst, the MBP had a higher PT than that of the DBP. The equator of eggshell transmitted more light when compared with those of the small (over the near-UV range) and large (over the WL range measured) poles regions. The small pole region of eggshell had higher PT and lower spectral transmission ratios of the near-UV:V and near-IR:V than those of the large pole region. There was an interaction between brown pigment and region of eggshell on PT of light. MBP and DBP eggshells have similar PT at the equator but differ at the poles regions of eggshells. In the MBP, the equator region of eggshell had a lower PT than that of the small pole. Whilst the equator region of the DBP had a higher PT than that of the large pole. The PT of the small pole was higher than that of the large pole in the LBP and DBP groups. It is concluded that the intensity of brown pigment and region of eggshell influence light transmission that passes through the eggshell and consequently the spectral characteristics of eggshell.

Key words: Pigment intensity of eggshell, eggshell region, light transmittance

Introduction

To date, investigations into the effects on embryonic growth and hatchability of exposing hatching eggs to light during incubation have been inconsistent. For example the growth rate of chick embryo increases (Lowe and Garwood, 1977; Shafey and Al-Mohsen, 2002), hatching period decreases (Siegel *et al*., 1969; Walter and Voitle, 1972; Shafey and Al-Mohsen, 2002); and hatchability per cent improves (Gimeno *et al*., 1967; Walter and Voitle, 1972; Shafey and Al-Mohsen, 2002) indicating a beneficial role for incubating eggs under light. In contrast, Tamimie (1967) and Tamimie and Fox (1967) reported a delay in hatching time, reduced hatchability and increased incidence of embryonic abnormalities in those chicks exposed to light during incubation. Whilst, Lauber and Shutze (1964); Siegel *et al*. (1969) and Zakaria (1989) found no effect on hatchability when eggs were exposed to light during incubation period. This disagreement among authors suggests that there are some factors influencing the spectral characteristics of light that reaches the embryos and consequently the outcome of incubated eggs under light. Ghatpande *et al*. (1995) reported that the light induced-acceleration of embryonic development *in ovo* depends on the amount of light that reaches the embryos. Shafey *et al*. (2002) compared pigmented and non-pigmented eggshells and found that the pigmentation of eggshell influenced the spectral transmittance of light into the egg.

The eggs of the domestic hen show great variety in the pigment of their shells. Eggshell pigment is identified with breed (Romanoff and Romanoff, 1949). However, birds from the same breed lay eggs with different intensities of eggshell pigment. Hybro birds lay eggs with different intensity of brown eggshell pigments. The objectives of this study are to examine the effects of intensity of brown pigment and region of eggshell on the transmission of light that passes through the eggshell.

Materials and Methods

A total of 45 fresh-laid eggs of approximately comparable weight 59.7 ± 0.32 (g mean ± SEM) obtained from a commercial meat-type (Hybro) breeder flock at 32 weeks of age were used in this study. Eggs were assigned according to the intensity of their brown eggshell pigments into three groups [light (LBP), medium (MBP) and dark (DBP)]. A paint colour chart (Natural Colour System (NCS), Scandinavian Colour Institute), used to select and distribute the eggs into the three brown eggshell pigments. LBP, MBP and DBP eggs were selected according to the NCS numbers of *S*1005/*y*30*r* to *S*1005/*y*40*r*, *S*1010/*y*40 to *S*1010/*y*50 and *S*2020/*y*40 to *S*2020/*y*50, respectively, numbered for identification and broken open. Eggshells were washed with water and dried with paper towel. Pieces (1 to 1.5 cm^2) from the three different regions (small pole, equator and large pole) of each eggshell with the membranes intact were selected. Three measurements of thickness were taken from each region of eggshell with a micrometer (Ames, Walthman, MA, USA). Eggshells of nine eggs from each intensity of brown pigment (LBP, MBP and DBP) were randomly selected for the determination of light transmission. Per cent transmittance of light (PT) that passed through each region of eggshell (0.25 cm^2) was measured using a spectrophotometer (Model UV-1601 PC, Shimadzu, Japan). Spectral transmission of eggshell was recorded every 2 nm over the wavelength (WL) range of 200 to 800 nm (near-ultra violet (near-UV) range from 200 to 380 nm, visible (V) range from 380 to 780 nm and near-infra red (near-IR) range from 780 to 800 nm. Calculations of PT were based on twenty-five nm-groups of WL ranges. The WL range of the first nm-group was from 200 to 225 nm and this was increased by 25 nm for each of the next twenty-four WL groups (226 to 250, 251 to 275, 276 to 300 nm, ……etc..).

Data collected were subjected to analysis of variance as 3 x 3 factorial arrangements with brown pigment and region of eggshells as main effects and their interaction fitted into the model using the Statistical Analysis System (SAS Institute, 1985). Correlations between brown pigment (LBP, MBP and DBP) or region of eggshell (small pole, equator and large pole) and egg weight, eggshell thickness and PT of light were calculated. All reported values represent the mean ± standard error and the level of significance was taken as ($P<0.05$). When significant variance ratios were detected, differences between treatment means were tested using the least significant difference (LSD) procedures.

Results

The effects of intensity of brown pigment and region of eggshells on eggshell thickness and PT of light over the WL range of 200 to 800 nm are shown in Table 1. The intensity of brown pigment and region of eggshells did not influence the thickness of eggshell. The intensity of brown pigment of eggshell was not significantly correlated with egg weight ($r=0.12$, $P<0.4329$, $n=45$) and eggshell thickness ($r=0.06$, $P<0.7096$, $n=45$). The transmission of light across the eggshell was significantly ($P<0.01$) reduced as the intensity of eggshell pigment increased. The LBP had significantly ($P<0.01$) higher PT of light over the WL range of 200 to 800 nm as a whole and near-UV, V, near-IR WL ranges and the PT ratio of the near-UV:V and lower PT ratio of the near-IR:V than those of the MBP and DBP groups. Whilst the MBP had significantly ($P<0.01$) higher PT of light over the WL range of 200 to 800 nm as a whole and near-UV, V and near-IR WL ranges than those of the DBP.

The region of eggshell influenced the transmission of light across the eggshell. The equator region of eggshell had significantly ($P<0.01$) higher PT of light over the WL range of 200 to 800 nm as a whole and near-UV than those of the poles regions. The large pole region of eggshell had significantly ($P<0.01$) lower PT of light over the V and near-IR WL ranges and higher PT ratio of near-IR:V when compared with those of small pole and equator regions of eggshell. The small pole region of eggshell had a significantly ($P<0.01$) lower PT ratio of near-UV:V (Table 1).

There were significant interactions between the region and intensity of brown pigment of eggshell ($P<0.05$) on the PT of light over the WL range of 200 to 800 nm as a whole (Fig. 1). MBP and DBP eggshells have similar PT of light at the equator but differ at the poles regions of eggshells. In the MBP, the equator region of eggshell had a lower PT of light than that of the small pole. Whilst the equator region of the DBP had a higher PT of light than that of the large pole. The PT of light of the small pole was higher than that of the large pole in the LBP and DBP groups. There was no significant difference in the PT of light between poles regions of eggshell in the MBP group. In the LBP eggshells, the greatest difference between shell regions is observed in the near-UV region, where the PT of light for the small region is consistently lower than the equator and large pole, but towards the opposite end of the V range, the larger pole of the eggshell has slightly lower PT than the equator and the small pole, which are comparable (Fig. 2). However, in the MBP eggshells, the biggest differences are observed within the near-UV spectrum such that the equator has the lowest PT of light and the poles are variably higher, whilst the PT of light of all regions of the remaining spectrum are similar (Fig. 3). For DBP eggshells, the pattern is again different with poles having lower PT values within the near-UV range whilst towards the higher V range, the large pole again display a significantly lower PT when compared with the equator and small pole (Fig. 4).

There were significant ($P<0.01$) negative correlations between the intensity of brown eggshell pigment and PT of light over the WL range of 200 to 800 nm as a whole and near-UV, V and near-IR WL ranges and the PT ratio of near-UV:V and between region of eggshell (from mall pole, equator to large pole) and PT of light over the WL range of V and near-IR. There were significant ($P<0.01$)

Table 1: Egg weight, eggshell thickness and per cent transmittance of light over the wavelength (WL) of 200 to 800 nm at the small pole, equator and large pole of three different intensity of brown pigment of eggshells[1]

	Intensity of brown pigment of eggshell			Region of eggshell		
	Light	Medium	Dark	Small pole	Equator	Large pole
Egg weight (g)	59.0 ± 0.58	60.4 ± 0.58	59.6 ± 0.48	60.1 ± 0.53	60.2 ± 0.59	58.7 ± 0.50
Eggshell thickness (mm)	43.1 ± 0.36	43.5 ± 0.27	43.3 ± 0.44	43.6 ± 0.29	43.4 ± 0.33	42.8 ± 0.43
Transmittance of light %						
200 <WL< 800	0.070 ± 0.003^{a}	0.043 ± 0.002^{b}	0.037 ± 0.001^{c}	0.050 ± 0.003^{b}	0.057 ± 0.004^{a}	0.043 ± 0.003^{c}
200 <WL< 380 (near-UV)[2]	0.088 ± 0.004^{a}	0.030 ± 0.003^{b}	0.023 ± 0.002^{b}	0.041 ± 0.005^{b}	0.060 ± 0.008^{a}	0.041 ± 0.005^{b}
380 <WL< 780 (V)[3]	0.034 ± 0.002^{a}	0.025 ± 0.001^{b}	0.021 ± 0.001^{c}	0.029 ± 0.001^{a}	0.030 ± 0.002^{a}	0.020 ± 0.001^{b}
780 <WL< 800 (near-IR)[4]	0.089 ± 0.004^{a}	0.074 ± 0.002^{b}	0.066 ± 0.002^{c}	0.082 ± 0.002^{a}	0.081 ± 0.003^{a}	0.066 ± 0.002^{b}
Near-UV : V ratio	2.79 ± 0.18^{a}	1.25 ± 0.12^{b}	1.16 ± 0.11^{b}	1.33 ± 0.13^{b}	1.95 ± 0.23^{a}	1.91 ± 0.19^{a}
Near-IR : V ratio	2.73 ± 0.07^{b}	3.06 ± 0.05^{a}	3.22 ± 0.11^{a}	2.88 ± 0.06^{b}	2.81 ± 0.06^{b}	3.23 ± 0.11^{a}

[1]Values are mean ± SEM of the number of replicates given in parentheses.
[2]Near-ultra violet WL range. [3]Visible WL range. [4]Near-infra red WL range.
^{a,b,c,d} Means within row followed by different superscripts are significantly different (P<0.05).

Table 2: Correlation coefficients between of intensity of brown pigment (light, medium and dark) or region of eggshell (small pole, equator and large pole) and per cent transmission of light over the wavelength (WL) of 200 to 800 nm[1]

Transmission of light %	Intensity of brown pigment	Region of eggshell
200 <WL< 800	-0.778, P<0.0001	-0.180, P<0.1086
200 <WL< 380 (near-UV)[2]	-0.785, P<0.0001	0.010, P<0.9319
380 <WL< 780 (V)[3]	-0.581, P<0.0001	-0.398, P<0.0002
780 <WL< 800 (near-IR)[4]	-0.582, P<0.0001	-0.391, P<0.0003
Near-UV : V ratio	-0.651, P<0.0001	0.233, P<0.0364
Near-IR : V ratio	0.437, P<0.0001	0.396, P<0.0003

[1]N=81 for each parameter estimated. [2]Near-ultra violet WL range. [3]Visible WL range. [4]Near-infra red WL range.

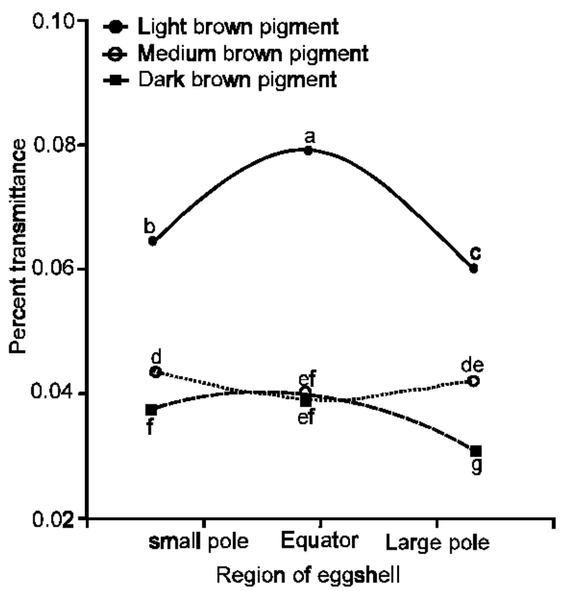

Fig. 1: The effect of brown pigment intensity of eggshell on the per cent transmittance of light over the wavelength range of 200 to 800 nm at the small pole, equator and large pole regions of eggshell [a, b, c Brown pigments and regions of eggshell followed by different superscripts are significantly different (P<0.05)]

positive correlations between the intensity of brown eggshell pigment and the PT ratio of near-IR:V and between region of eggshell and the PT ratios of the near-IR:V and near-UV:V (P<0.05, Table 2).

Discussion

Hybro birds lay brown eggs with a great variety in the intensity of colour of their shells (Shafey *et al*., 2002). The main pigment of brown eggshell is protoporphyrin (Butcher and Miles, 1995). The colour of a pigment depends on its selective absorption of certain WL of light and its reflection of others (Clifford and Philpott, 2002). Results from this study showed that the intensity of brown pigment of eggshell influenced the spectral characteristics of eggshell. Light brown pigment allowed for more light to cross the eggshell when compared with those of darker pigments. The LBP eggshell had a higher PT of light when compared with those of the MBP and DBP by approximately 62.8 and 89.2%, respectively with more light transmitted in the near-UV, V and near-IR ranges. Whilst, the spectral transmission of the near-UV:V ratio was higher and near-IR:V ratio was lower in the LBP eggshell when compared with those of the MBP and DBP by approximately 123.2 and 10.8% and 140.7 and 15.2%, respectively. The MBP eggshell had a higher PT of light when compared with that of the DBP by approximately 16.2% over the same WL range. The

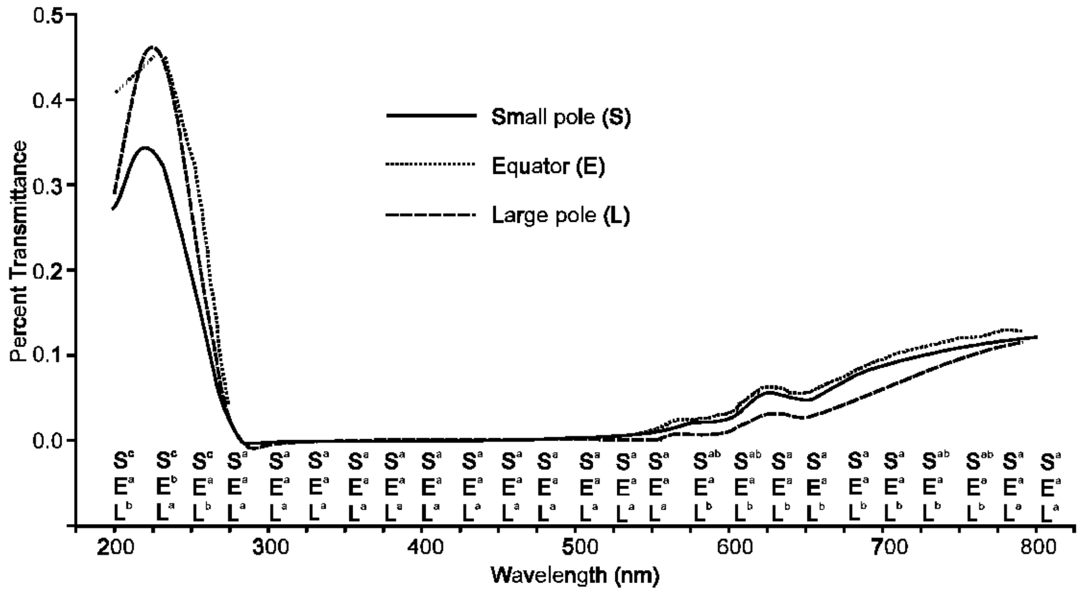

Fig. 2: Per cent transmittance of light over the wavelength range of 200 to 800 nm at the small pole, equator and large pole of light brown pigment eggshells [a, b, c regions of egg shell (S, E and L) within wavelength group followed by different superscripts are significantly different (P<0.05)]

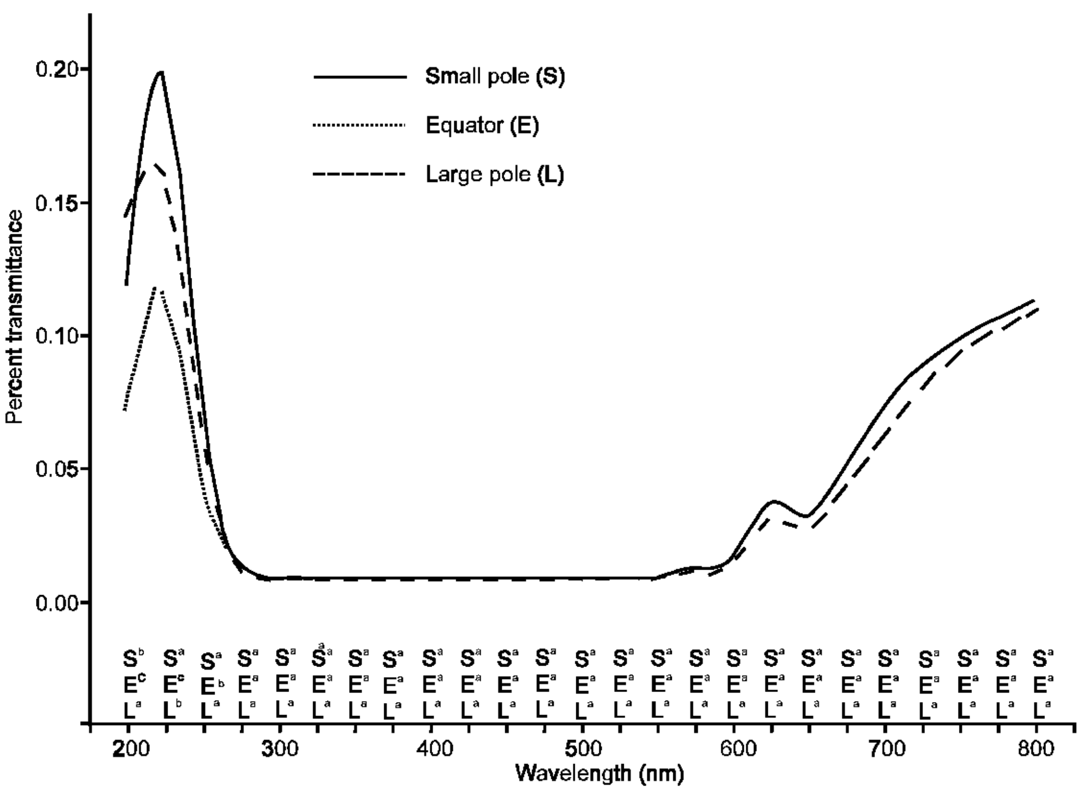

Fig. 3: Per cent transmittance of light over the wavelength range of 200 to 800 nm at the small pole, equator and large pole of medium brown pigment eggshells [a, b, c REGIONS OF EGG SHELL (S, E and L) within wavelength group followed by different superscripts are significantly different (P<0.05)]

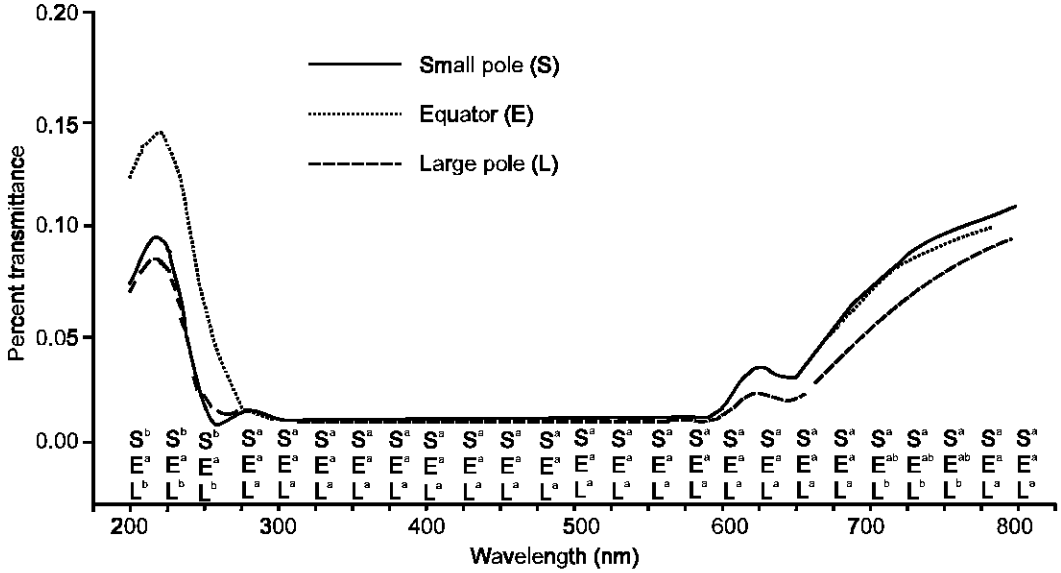

Fig. 4: Per cent transmittance of light over the wavelength range of 200 to 800 nm at the small pole, equator and large pole of dark brown pigment eggshells [a, b, c regions of egg shell (S, E and L) within wavelength group followed by different superscripts are significantly different (P<0.05)]

increase in the PT of MBP eggshell was due to the non-significant increase in the spectral transmission of the V and near-IR WL ranges. It appears that the increase in the intensity of brown pigment of eggshell from medium to dark reduced the transmission of light across the eggshell, without altering the spectral transmission ratios of UV:V and IR:V. These results suggest that the intensity of protoporphyrin pigment of brown eggshells altered the spectral characteristics of the transmitted light. Shafey *et al.* (2002) found that the pigmentation of eggshell influenced the spectral transmission of eggshell when they compared pigmented and unpigmented eggshells.

Results from this study showed that the region of eggshell influenced the transmission of light through the eggshell. The equator of eggshell had a higher PT of light when compared with those of the small and large poles by approximately 14 (with more light transmitted in the near-UV range) and 32.5% (with more light transmitted over the WL range of 200 to 800), respectively. The small pole of eggshell had a higher PT of light than that of the large pole region by approximately 16.3%, with more light transmitted in the V and near-IR ranges. Shafey *et al.* (2002) found that eggshells with high conductance had a higher transmission of light when compared with their counterparts of low conductance values. This finding suggests that differences in the transmission of light among the three regions of eggshell may be associated with differences in the distribution of pores over the surface of eggshell, since there was no significant difference in eggshell thickness among the three regions of eggshell. Eggshell thickness and total function cross-sectional area of pores are known to determine eggshell conductance (Ar *et al.*, 1974). This suggestion was in agreement with Peebles and Brake (1986) who reported that pores are not uniformly distributed over the surface of the egg and pore concentration in each region of eggshell varies among its parts (Romanoff and Romanoff, 1949). Generally, the equator and wide pole regions of eggshell have more pores than the small pole (Romanoff and Romanoff, 1949; Peebles and Brake, 1986). However, the finding that the small pole of eggshell had a higher PT than that of the large pole may be due to differences in the active pore area of the measured samples from these two regions. Birds have the constructional ability to change the distribution of pores over the surface of eggshell probably to maximize the strength of eggshell (Tyler, 1955). The finding that regions of eggshell differed in their ability for the transmission of light may have some implications on the brooding behavior of ground nesting birds. Further research are needed to address the impacts of spectral transmission of region of eggshell on brooding behavior of birds.

Little information available on factors that cause variation in the response of lighted incubation of chicken eggs. Gold and Kalb (1976) using 700-1100 lux fluorescent light and Ghatpande *et al.* (1995) using different intensities of fluorescent light (800-1500 vs. 1500-3000 lux) found that the light induced-acceleration of embryonic development *in ovo* depends on the amount of light that reaches the embryos. Coleman and McNab (1975) found that under the influence of light, the development of embryos from Japanese quail with pigmented shells is slower than those of unpigmented shells and that depigmentation of eggs results in early hatching. Results from this study suggested that the intensity of pigmentation and region of eggshell influenced the spectral transmission of eggshell. This finding may help to explain partly some of the inconsistency found in the literature. It has been suggested that eggshell pigmentation represents a natural adaptation for shielding the egg contents from sunlight (Romanoff and Romanoff, 1949; Kennedy and Vevers, 1976). Differences in the spectral properties of different brown eggshell pigments may influence the outcome of lighted incubation. Further study may be needed to clarify this point.

The non-significant correlation between pigment intensity and thickness of eggshell may indicate that there is no relationship between pigment intensity and quality of eggshell. This finding is supported by Butcher and Miles (1995) who reported that the majority of eggshell pigment is localized in the cuticle and the contribution of eggshell to the intensity of brown colour is negligible compared to that of cuticle.

It is concluded that region and brown pigment of eggshell influenced the spectral characteristics of eggshell.

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