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NON-DESTRUCTIVE PREDICTION FOR YOLK-ALBUMEN RATIO

Non-Destructive Prediction Method for Yolk-Albumen Ratio in Chicken Eggs by Computer Image Analysis

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ABSTRACT The purpose of this study was to develop a non-destructive prediction method for the yolk-albumen ratio by computer image analysis for candling inspection. Twenty-two to forty-nine eggs per line were randomly sampled from 4 chicken lines. After weighing the eggs, the eggs were illuminated by an overhead projector beam through a small hole in dark room. Video images were taken of the eggs at 4 directions, rotated each time by 90 degrees. The eggs were broken for measuring egg traits including the yolk-albumen ratio. The average value obtained from 4 directions was used for statistical analysis. The ratio of the number of pixels of light and dark parts (light-dark ratio), and the coefficients of variation (CV) of R, G, and B components for the whole egg and for light and dark parts of the egg were calculated and defined as image analysis traits. Correlation coefficients between the yolk-albumen ratio and CV of R and G components of the whole egg were significant (0.42-0.79) in all the lines. The determination coefficient of multiple regression of the yolk-albumen ratio on the CV of R and G components of the whole egg and the light-dark ratio was 0.83. Observed and predicted yolk-albumen ratios were classified into 5 levels. The ratio of zero difference between observed and predicted values was 76.1%, and the percentage of 0 to ±1 difference between observed and predicted values was 100.0%. These results indicated that the image analysis method could accurately predict the yolk-albumen ratio without breaking the egg.

(*Key words*: Yolk-albumen ratio, Computer image analysis, Prediction method)

INTRODUCTION

Accurate prediction of the characteristics of egg composition and egg quality for the food processor makes use of the egg effectively. There is a business category in which only yolk or albumen is used as an ingredient in food processing such as mayonnaise factories. Production cost might be decreased by predicting egg composition more efficiently. Miyoshi and Mitsumoto (1994) pointed out the importance of displaying egg quality for various uses of the egg for food and as an ingredient in food processing. If the yolk-albumen ratio, which represents egg quality, could be predicted by a non-destructive method, then eggs could be graded according to egg quality during the candling process. This method would give additional value to eggs used as ingredients in food processing. Voisey and Hamilton (1976) used ultrasonic equipment to measure the eggshell thickness and reported that the correlation coefficient between observed and predicted values was 0.74. Pugh *et al.* (1993) determined the embryological characteristics of the vitellus and embryo by ultrasonic measurement, and they reported that a small hole to pass a sound wave through the eggshell was needed for ultrasonic measurement of the vitellus. This report suggests that it is difficult to predict egg components by ultrasonic measurement due to absorption of the sound waves by the eggshell.

Sauter *et al.* (1953) found a high correlation between the candling value of the egg, based on the U.S. grading standard, and the egg color, yolk index and albumen score. However, the liquidization of the albumen may have been a factor contributing to the high correlation, because most of the eggs used in their experiment were stored for a long period of time (some more than 6 months). In a study which used only fresh eggs, an obvious relationship was not seen among these parameters (Stewart *et al.*, 1932).

Computer image analysis is a suitable method for measuring an object with a complex shape and for calculating the strength of color. Newman (1984) and Kuchida *et al.* (1991) reported that the chemical fat percentage in minced meat could be predicted by computer image analysis.

When a beam of light is projected onto an egg from the sharp or dull end, the egg is separated into light and dark parts. This suggests the possibility of predicting egg quality by using the area ratio or the strength of color. The purpose of this study was to develop a non-destructive prediction method for the yolk-albumen ratio using image analysis.

MATERIALS AND METHODS

The eggs used in this study were from four lines of White Leghorn. The first two lines were selected for their high and low yolk-albumen ratios (coded H-line and L-line, respectively) for the purpose of changing egg composition raised at the Animal Breeding laboratory, Obihiro University of Agriculture and Veterinary Medicine in Japan (Miyoshi and Mitsumoto, 1994; Miyoshi *et al.*,1996). The remaining two lines were two commercial laying hen groups (coded A-line and B-line), raised in Tokachi district, Hokkaido in Japan. The age of the layers were 6-7 mo for H- and L-line and 8-9 mo for A- and B-line.

Eggs were randomly sampled from hens of the 4 lines, and egg composition was measured within 2 days after laying. Forty-nine eggs each were collected from H- and L-line, and 22 eggs each from A- and B-line hens. The total number of egg used in this study was 142. The eggs were kept in a refrigerator at 4 degrees Celsius until measurement.

Non-destructive measurement was performed as follows. A circular pipe made from vinyl chloride was placed on the lens part of the overhead projector (OHP) and a rubber stopper with a small hole (13mm in diameter) in it was set on the circular pipe. The light source of the OHP is a 300-W halogen lamp. The OHP was covered to prevent light from leaking. An egg was placed on the small hole with the sharp end down. The illuminated egg was photographed in a dark room using a digital video camera (SONY:DCR-VX1000). The image was read into the computer using a digital still image capture board (SONY:DVBK-1000). The above equipment allowed an image of 640×480 pixels to be read into the computer without any degradation of picture quality. An example of the image is shown in Fig. 1. The eggs were calmly put 30 min before setting them on the irradiation stand. The light and dark parts, shown in Figure 1 and observed in all eggs, were separated by the discriminant analysis method (Otsu, 1980). Each pixel has brightness information of 256 levels for each of the red (R), green (G) and blue (B) components. The parameters calculated in this study were: (1) the pixel number of the whole egg, (2) the average and standard deviation of each R, G and B component of the whole egg, (3) the pixel number of the light part of the egg, (4) the average and standard deviation of each R, G and B component of the light part of the egg, (5) the pixel number of the dark part of the egg, and (6) the average and standard deviation of each R, G and B component of the dark part of the egg. The image analysis traits (described later) were calculated using these values. The values were calculated in four directions by rotating the egg on the irradiation stand four times by 90 degrees each time in order to eliminate the bias caused by direction of the egg on the irradiation stand.

The light and dark parts of the egg shown in Fig. 1 might indicate the albumen and the yolk, respectively. The light-dark ratio was calculated by dividing the number of pixels in the dark part by the number of pixels in the light part of the egg. This coefficient was used to determine the yolk-albumen ratio of the egg as a weight ratio. The coefficients of variation (CV) of the R, G and B components were calculated based on the average and the standard deviation of each R, G and B component. By using this CV, the bias of brightness information by the distance between the camera and the egg could be eliminated to some degree. The 10 image analysis traits were defined as the CV of R, G and B components for the whole egg, for the light part and for the dark part of the egg, and the light-dark ratio.

The yolk-albumen ratio was estimated by the multiple regression equation on ten independent variables of the image analysis traits. To obtain accurate results, the data set was halved and multiple regression equation was estimated for only one half of the data set. The other half of data set was used to predict the yolk-albumen ratio, based on regression coefficients estimated from the first half of the data set. The acceptance or rejection of independent variables in the multiple regression equation was performed by the Stepwise method of SAS (1985).

After measuring egg weight, egg length and egg width, the eggs were broken and separated into the yolk and albumen. The albumen was divided into thick albumen and thin albumen by a sieve with a 2-mm lattice, and the weight of each type of albumen was measured. The albumen weight was calculated as the sum of the thick and thin albumen weights. The yolk-albumen ratio was calculated by the following equation:

$$\text{Yolk-albumen ratio} = \text{Yolk weight} / \text{Albumen weight} \times 100$$

The eggshell weight (containing eggshell membrane) was also measured. The eggshell thickness was determined by averaging values measured by a dial pipe gage at three points on the equator surface of the egg.

RESULTS AND DISCUSSION

Means and standard deviations of egg weight, yolk weight, albumen weight, eggshell weight, eggshell thickness and yolk-albumen ratio for each line of hens are shown in Table 1. The yolk-albumen ratios of eggs from the commercial hens (A-line:37.0%, B-line:40.3%) used in this study agree with the results by Miyoshi and Mitsumoto (1994). The mean values of image analysis traits are shown in Table 2. The mean values of image analysis traits for the H-line were significantly higher than those for other lines.

The video images of the eggs may have been influenced by the eggshell thickness. The correlation coefficients between the ten image analysis traits and eggshell thickness were -0.19 to 0.03 for the H-line, -0.02 to 0.44 for the L-line, -0.22 to 0.24 for the A-line and -0.33 to 0.03 for the B-line. It was difficult to estimate eggshell thickness by this method because the correlation coefficient was relatively low and insignificant, except for the L-line.

The correlation coefficients between the yolk-albumen ratio and image analysis traits for each line are shown in Table 3. The light-dark ratio, which is considered to be equivalent to the yolk-albumen ratio, showed no significant correlation with the yolk-albumen ratio in all 4 lines. Therefore, it might be impossible to predict egg composition using only the shadow on an illuminated egg. However, significant correlation coefficients were found between the yolk-albumen ratio and CV of the R and G components for the whole egg in all lines, suggesting that these traits may be used to predict the yolk-albumen ratio without breaking eggs.

An egg illuminated by a beam from OHP appeared yellow. The beam penetrated into the eggshell and the albumen from a small hole in the rubber stopper and might have been reflected by the yolk floating in the albumen. A small hole was made in the eggshell, and internal egg content was removed. Only the albumen was injected back into the egg, and then the small hole was closed. This egg was called a "yolk-removal egg" in this study. The irradiation and image analysis procedures were repeated in the same manner as for the normal eggs. The CV of the R, G and B components of the whole egg in the case of "yolk-removal eggs" (n=12) were 7.2, 8.3 and 46.6%, respectively. Whereas, the corresponding R, G and B values for the normal eggs in all lines were 12.9 to19.9%, 17.9 to 32.9% and 22.0 to 43.8%, respectively. The CV of the R and G components in the "yolk-removal eggs" were lower than those in normal eggs, indicating that there was a smaller variation in the strength of color of "yolk-removal eggs". The higher variation for normal eggs may be explained by the reflection of the penetrating light at the yolk.

The data set was randomly divided into two halves, and multiple regression of the yolk-albumen ratio on image analysis traits was estimated using one of the data sets (n=71). The independent variables for the equation selected by the Stepwise selection method were the CV of R and G components for the whole egg and the light-dark ratio with a determinant coefficient (R^2) of 0.83 ($p<0.01$). The yolk-albumen ratio was predicted for the other part of the data set (n=71) using the parameters estimated from the first part of the data set. The relationship between observed and predicted values of the yolk-albumen ratio is shown in Fig. 2. A significant correlation coefficient (r=0.85) was detected between observed and predicted values.

The only one set of "light" was for penetrating in this study. There might be more proper light sources such as different light color, brightness, or the size of the hole to penetrate the light beam.

The observed and predicted values of the yolk-albumen ratio were classified into 5 levels (level 1:less than 35%, level 2:35-45%, level 3:45-55%, level 4:55-65% and level 5:more than 65%). The accuracy of the prediction was examined by the degree of agreement with each score determined by observed and predicted values. The frequencies of the difference between those scores are shown in Table 4. The ratio of zero difference between levels of observed and predicted values was 76.1%, and the percentage of 0 to ±1 difference between observed and predicted values was 100.0%. These results indicate that classification of the yolk-albumen ratio (roughly in 5 levels in this study) by the non-destructive method is feasible.

Hutchison *et al.* (1992) examined the inner structure of the egg using magnetic resonance imaging (MRI) and concluded that MRI could be used successfully in assessing the microanatomy of eggs. However, the inspection of yolk-albumen ratio for numerous eggs using MRI is impractical, because the equipment is very expensive and not in popular use yet.

Hussein *et al.* (1993) pointed out that the difference in the yolk-albumen ratio become increasingly important because a demand for liquid eggs continues to increase every year. This study has shown that the yolk-albumen ratio could be predicted by penetrating a beam of light into the egg and calculating the strength of the color. Although there were good agreement between image analysis and composition determinations, the model might be strengthened by including a term for eggshell texture. This should be considered in future studies.

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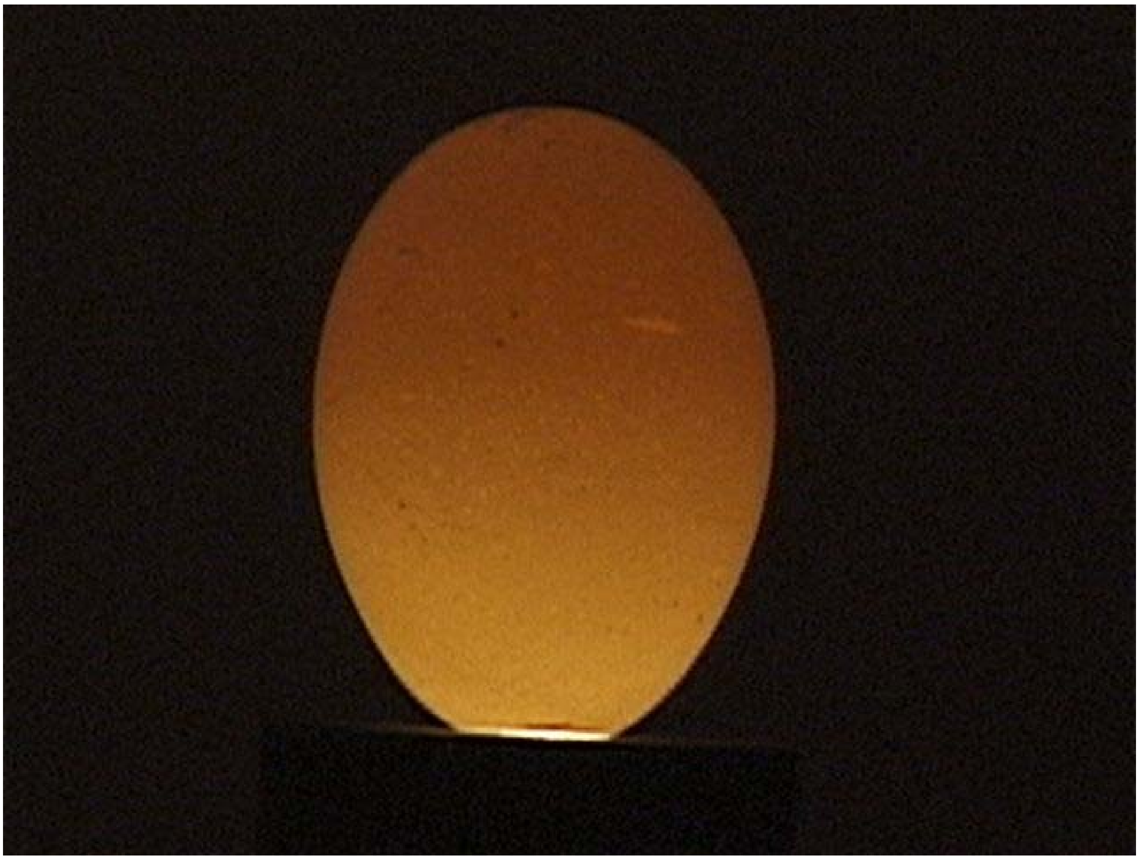

FIGURE 1. Example of illuminated egg.

Table 1. Means and standard deviations of egg component traits for each line of hens

	Selected line		Commercial line	
	H-line	L-line	A-line	B-line
n	49	49	22	22
EW (g)	60.9±3.0^{b}	62.2±3.6^{ab}	55.9±2.9^{c}	62.9±3.8^{a}
YW (g)	19.1±1.4^{a}	16.9±1.4^{b}	13.1±1.0^{d}	16.0±1.2^{c}
AW (g)	35.6±2.5^{b}	39.0±3.1^{a}	35.6±1.9^{b}	39.7±2.7^{a}
SW (g)	6.31±0.61^{b}	6.28±0.80^{b}	7.14±0.55^{a}	7.14±0.44^{a}
EST (mm)	0.322±0.026^{bc}	0.310±0.035^{c}	0.370±0.034^{a}	0.336±0.018^{b}
YAR (%)	53.8±5.6^{a}	43.6±5.1^{b}	37.0±2.6^{d}	40.3±2.3^{c}

EW: egg weight, YW: yolk weight, AW: albumen weight,
SW: shell weight, EST: eggshell thickness, YAR: yolk-albumen ratio
a,b,c,d: different superscript means significantly difference ($p<0.05$) in each trait

Table 2. Means and standard deviations of image analysis traits for each line of hens

Image analysis	Selected line		Commercial line	
Traits	H-line	L-line	A-line	B-line
N	49	49	22	22
D-L ratio	111.8±48.4^{a}	109.8±41.3^{a}	64.6±10.8^{b}	75.0±13.7^{b}
CV R(whole)	19.9±3.2^{a}	15.7±2.6^{b}	13.2±1.3^{c}	12.9±1.2^{c}
CV G(whole)	32.9±5.5^{a}	25.9±4.8^{b}	17.9±1.6^{c}	17.9±1.5^{c}
CV B(whole)	43.8±10.7^{a}	37.8±8.4^{b}	22.0±1.2^{c}	22.6±1.4^{c}
CV R(light)	9.2±1.9^{a}	7.1±1.0^{b}	6.2±0.4^{c}	6.3±0.5^{c}
CV G(light)	17.5±3.6^{a}	13.8±2.0^{b}	7.5±0.7^{c}	7.8±0.6^{c}
CV B(light)	35.8±7.8^{a}	30.8±5.3^{b}	19.4±1.6^{c}	18.9±1.1^{c}
CV R(dark)	9.9±1.8^{a}	8.7±1.2^{b}	9.6±1.0^{a}	8.9±0.7^{b}
CV G(dark)	15.2±2.5^{a}	13.3±2.2^{b}	11.0±1.3^{c}	10.1±1.0^{c}
CV B(dark)	34.0±6.7^{a}	32.3±5.9^{a}	21.6±0.9^{b}	20.8±0.7^{b}

D-L ratio: ratio of numbers of pixel for Dark part and Light part of egg

CV R, G and B: coefficient of variance of R, G and B components

CV R, G and B (whole): CV of R, G and B components for whole egg.

CV R, G and B (light): CV of R, G and B components for light part of egg.

CV R, G and B (dark): CV of R, G and B components for dark part of egg.

a,b,c: different superscript means significantly difference ($p<0.05$) in each trait

Table 3. Correlation coefficient between yolk-albumen ratio and image analysis traits for each line of hens

Image analysis	Selected lines		Commercial lines	
Traits	H-line	L-line	A-line	B-line
N	49	49	22	22
D-L ratio	0.25	0.17	-0.03	0.01
CV R(whole)	0.60**	0.79**	0.64**	0.49*
CV G(whole)	0.65**	0.63**	0.67**	0.42*
CV B(whole)	0.18	0.17	0.04	0.11
CV R(light)	0.21	0.45**	0.51*	0.21
CV G(light)	0.21	0.30*	0.70**	0.37
CV B(light)	-0.06	0.10	0.07	0.09
CV R(dark)	0.39**	0.76**	0.51*	0.41
CV G(dark)	0.48**	0.52**	0.62**	0.26
CV B(dark)	-0.17	-0.11	-0.29	-0.15

D-L ratio: ratio of numbers of pixels for dark part and light part of egg

CV R, G and B: coefficient of variance of R, G and B components

CV R, G and B (whole): CV of R, G and B components for whole egg.

CV R, G and B (light): CV of R, G and B components for light part of egg.

CV R, G and B (dark): CV of R, G and B components for dark part of egg.

*: $p<0.05$, **: $p<0.01$

Table 4. Differences between classified values (5 levels[a]) based on observed and predicted yolk-albumen ratio (number of eggs and percentage)

	Selected lines		Commercial lines		
Difference[b]	H-line	L-line	A-strain	B-strain	Total
-2	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)
-1	10(20.4)	6(12.2)	0(0.0)	1(4.5)	17(12.0)
0	34(69.4)	37(75.5)	16(72.7)	21(95.5)	112(76.1)
+1	5(10.2)	6(12.2)	6(27.3)	0(0.0)	17(12.0)
+2	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)

a: Yolk-albumen ratio was classified into 5 levels (level 1:less than 35%, level 2:35-45%, level3:45-55%, level 4:55-65% and level 5:more than 65%).

b: Difference = (level of yolk-albumen ratio based on predicted value)
- (level of yolk-albumen ratio based on observed value)