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1 NON-DESTRUCTIVE PREDICTION FOR YOLK-ALBUMEN RATIO

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

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1 **ABSTRACT** The purpose of this study was to develop a
2 non-destructive prediction method for the yolk-albumen
3 ratio by computer image analysis for candling inspection.
4 Twenty-two to forty-nine eggs per line were randomly
5 sampled from 4 chicken lines. After weighing the eggs, the
6 eggs were illuminated by an overhead projector beam through
7 a small hole in dark room. Video images were taken of the
8 eggs at 4 directions, rotated each time by 90 degrees. The
9 eggs were broken for measuring egg traits including the
10 yolk-albumen ratio. The average value obtained from 4
11 directions was used for statistical analysis. The ratio of
12 the number of pixels of light and dark parts (light-dark
13 ratio), and the coefficients of variation (CV) of R, G, and
14 B components for the whole egg and for light and dark parts
15 of the egg were calculated and defined as image analysis
16 traits. Correlation coefficients between the yolk-albumen
17 ratio and CV of R and G components of the whole egg were
18 significant (0.42-0.79) in all the lines. The determination
19 coefficient of multiple regression of the yolk-albumen
20 ratio on the CV of R and G components of the whole egg and
21 the light-dark ratio was 0.83. Observed and predicted
22 yolk-albumen ratios were classified into 5 levels. The
23 ratio of zero difference between observed and predicted

1 values was 76.1%, and the percentage of 0 to ± 1 difference
2 between observed and predicted values was 100.0%. These
3 results indicated that the image analysis method could
4 accurately predict the yolk-albumen ratio without breaking
5 the egg.

6

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

INTRODUCTION

1
2 Accurate prediction of the characteristics of egg
3 composition and egg quality for the food processor makes
4 use of the egg effectively. There is a business category
5 in which only yolk or albumen is used as an ingredient in
6 food processing such as mayonnaise factories. Production
7 cost might be decreased by predicting egg composition more
8 efficiently. Miyoshi and Mitsumoto (1994) pointed out the
9 importance of displaying egg quality for various uses of
10 the egg for food and as an ingredient in food processing.
11 If the yolk-albumen ratio, which represents egg quality,
12 could be predicted by a non-destructive method, then eggs
13 could be graded according to egg quality during the candling
14 process. This method would give additional value to eggs
15 used as ingredients in food processing. Voisey and Hamilton
16 (1976) used ultrasonic equipment to measure the eggshell
17 thickness and reported that the correlation coefficient
18 between observed and predicted values was 0.74. Pugh *et al.*
19 (1993) determined the embryological characteristics of the
20 vitellus and embryo by ultrasonic measurement, and they
21 reported that a small hole to pass a sound wave through the
22 eggshell was needed for ultrasonic measurement of the
23 vitellus. This report suggests that it is difficult to

1 predict egg components by ultrasonic measurement due to
2 absorption of the sound waves by the eggshell.

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

12 Computer image analysis is a suitable method for
13 measuring an object with a complex shape and for calculating
14 the strength of color. Newman (1984) and Kuchida *et al.*
15 (1991) reported that the chemical fat percentage in minced
16 meat could be predicted by computer image analysis.

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

MATERIALS AND METHODS

1
2 The eggs used in this study were from four lines of White
3 Leghorn. The first two lines were selected for their high
4 and low yolk-albumen ratios (coded H-line and L-line,
5 respectively) for the purpose of changing egg composition
6 raised at the Animal Breeding laboratory, Obihiro
7 University of Agriculture and Veterinary Medicine in Japan
8 (Miyoshi and Mitsumoto, 1994; Miyoshi *et al.*, 1996). The
9 remaining two lines were two commercial laying hen groups
10 (coded A-line and B-line), raised in Tokachi district,
11 Hokkaido in Japan. The age of the layers were 6-7 mo for
12 H- and L-line and 8-9 mo for A- and B-line.

13 Eggs were randomly sampled from hens of the 4 lines,
14 and egg composition was measured within 2 days after laying.
15 Forty-nine eggs each were collected from H- and L-line, and
16 22 eggs each from A- and B-line hens. The total number of
17 egg used in this study was 142. The eggs were kept in a
18 refrigerator at 4 degrees Celsius until measurement.

19 Non-destructive measurement was performed as follows.
20 A circular pipe made from vinyl chloride was placed on the
21 lens part of the overhead projector (OHP) and a rubber
22 stopper with a small hole (13mm in diameter) in it was set
23 on the circular pipe. The light source of the OHP is a 300-W

1 halogen lamp. The OHP was covered to prevent light from
2 leaking. An egg was placed on the small hole with the sharp
3 end down. The illuminated egg was photographed in a dark
4 room using a digital video camera (SONY:DCR-VX1000). The
5 image was read into the computer using a digital still image
6 capture board (SONY:DVBK-1000). The above equipment
7 allowed an image of 640×480 pixels to be read into the
8 computer without any degradation of picture quality. An
9 example of the image is shown in Fig. 1. The eggs were calmly
10 put 30 min before setting them on the irradiation stand.
11 The light and dark parts, shown in Figure 1 and observed
12 in all eggs, were separated by the discriminant analysis
13 method (Otsu, 1980). Each pixel has brightness information
14 of 256 levels for each of the red (R), green (G) and blue
15 (B) components. The parameters calculated in this study
16 were: (1) the pixel number of the whole egg, (2) the average
17 and standard deviation of each R, G and B component of the
18 whole egg, (3) the pixel number of the light part of the
19 egg, (4) the average and standard deviation of each R, G
20 and B component of the light part of the egg, (5) the pixel
21 number of the dark part of the egg, and (6) the average and
22 standard deviation of each R, G and B component of the dark
23 part of the egg. The image analysis traits (described later)

1 were calculated using these values. The values were
2 calculated in four directions by rotating the egg on the
3 irradiation stand four times by 90 degrees each time in
4 order to eliminate the bias caused by direction of the egg
5 on the irradiation stand.

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

20 The yolk-albumen ratio was estimated by the multiple
21 regression equation on ten independent variables of the
22 image analysis traits. To obtain accurate results, the data
23 set was halved and multiple regression equation was

1 estimated for only one half of the data set. The other half
2 of data set was used to predict the yolk-albumen ratio,
3 based on regression coefficients estimated from the first
4 half of the data set. The acceptance or rejection of
5 independent variables in the multiple regression equation
6 was performed by the Stepwise method of SAS (1985).

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

$$15 \quad \text{Yolk-albumen ratio} = \text{Yolk weight} / \text{Albumen weight} \times$$

16 100

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

21

22 **RESULTS AND DISCUSSION**

23 Means and standard deviations of egg weight, yolk

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

9 The video images of the eggs may have been influenced
10 by the eggshell thickness. The correlation coefficients
11 between the ten image analysis traits and eggshell
12 thickness were -0.19 to 0.03 for the H-line, -0.02 to 0.44
13 for the L-line, -0.22 to 0.24 for the A-line and -0.33 to
14 0.03 for the B-line. It was difficult to estimate eggshell
15 thickness by this method because the correlation
16 coefficient was relatively low and insignificant, except
17 for the L-line.

18 The correlation coefficients between the yolk-albumen
19 ratio and image analysis traits for each line are shown in
20 Table 3. The light-dark ratio, which is considered to be
21 equivalent to the yolk-albumen ratio, showed no significant
22 correlation with the yolk-albumen ratio in all 4 lines.
23 Therefore, it might be impossible to predict egg

1 composition using only the shadow on an illuminated egg.
2 However, significant correlation coefficients were found
3 between the yolk-albumen ratio and CV of the R and G
4 components for the whole egg in all lines, suggesting that
5 these traits may be used to predict the yolk-albumen ratio
6 without breaking eggs.

7 An egg illuminated by a beam from OHP appeared yellow.
8 The beam penetrated into the eggshell and the albumen from
9 a small hole in the rubber stopper and might have been
10 reflected by the yolk floating in the albumen. A small hole
11 was made in the eggshell, and internal egg content was
12 removed. Only the albumen was injected back into the egg,
13 and then the small hole was closed. This egg was called a
14 "yolk-removal egg" in this study. The irradiation and image
15 analysis procedures were repeated in the same manner as for
16 the normal eggs. The CV of the R, G and B components of the
17 whole egg in the case of "yolk-removal eggs" (n=12) were
18 7.2, 8.3 and 46.6%, respectively. Whereas, the
19 corresponding R, G and B values for the normal eggs in all
20 lines were 12.9 to 19.9%, 17.9 to 32.9% and 22.0 to 43.8%,
21 respectively. The CV of the R and G components in the
22 "yolk-removal eggs" were lower than those in normal eggs,
23 indicating that there was a smaller variation in the

1 strength of color of "yolk-removal eggs". The higher
2 variation for normal eggs may be explained by the reflection
3 of the penetrating light at the yolk.

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

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

22 The observed and predicted values of the yolk-albumen
23 ratio were classified into 5 levels (level 1:less than 35%,

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

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

20 Hussein *et al.* (1993) pointed out that the difference
21 in the yolk-albumen ratio become increasingly important
22 because a demand for liquid eggs continues to increase every
23 year. This study has shown that the yolk-albumen ratio could

1 be predicted by penetrating a beam of light into the egg
2 and calculating the strength of the color. Although there
3 were good agreement between image analysis and composition
4 determinations, the model might be strengthened by
5 including a term for eggshell texture. This should be
6 considered in future studies.

7

8

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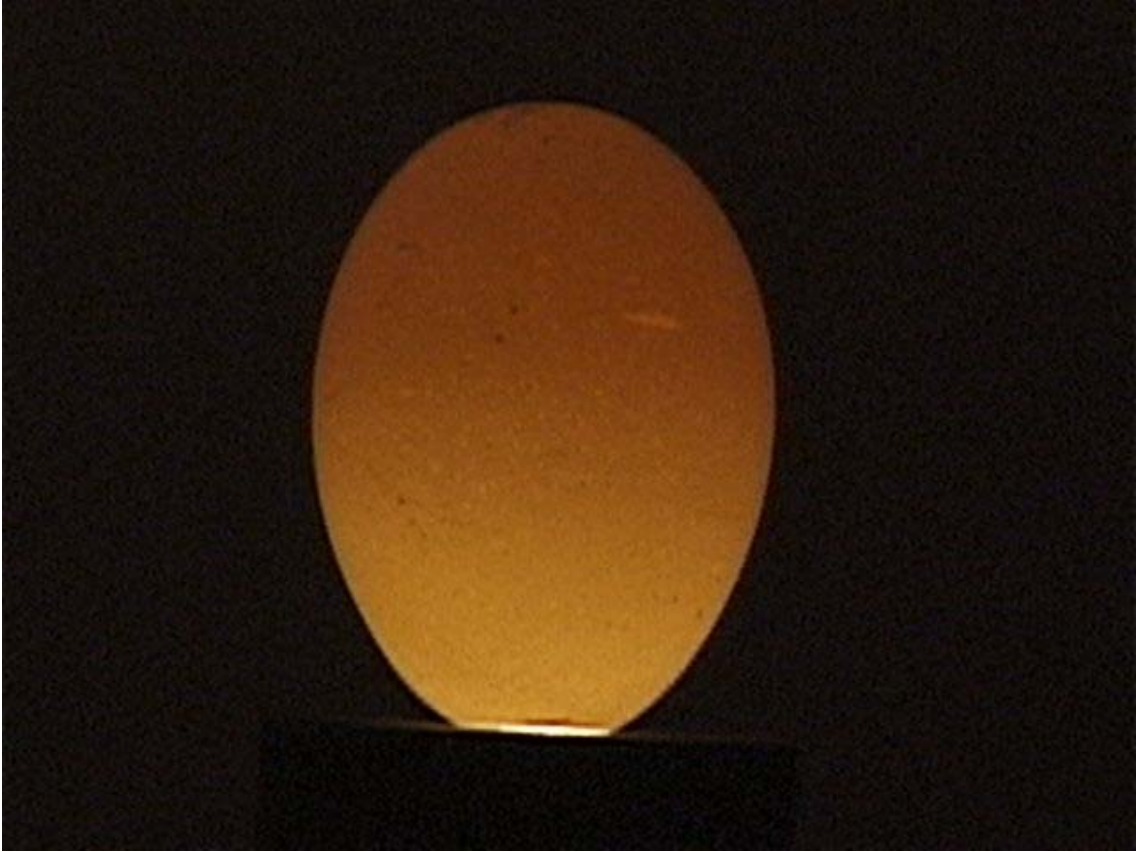


FIGURE 1 . Example of illuminated egg .

1 Table 1. Means and standard deviations of egg component traits for each line of
 2 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

3 EW: egg weight, YW: yolk weight, AW: albumen weight,

4 SW: shell weight, EST: eggshell thickness, YAR: yolk-albumen ratio

5 a,b,c,d: different superscript means significantly difference (p<0.05)

6 in each trait

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

Image analysis Traits	Selected line		Commercial line	
	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

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

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

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

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

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

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

8 in each trait

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

Image analysis Traits	Selected lines		Commercial lines	
	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

- 3 D-L ratio: ratio of numbers of pixels for dark part and light part of egg
 4 CV R, G and B: coefficient of variance of R, G and B components
 5 CV R, G and B (whole): CV of R, G and B components for whole egg.
 6 CV R, G and B (light): CV of R, G and B components for light part of egg.
 7 CV R, G and B (dark): CV of R, G and B components for dark part of egg.
 8 *: p<0.05, **:p<0.01

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

Difference ^b	Selected lines		Commercial lines		Total
	H-line	L-line	A-strain	B-strain	
-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)

- 3 a: Yolk-albumen ratio was classified into 5 levels (level 1:less than 35%,
 4 level 2:35-45%, level 3:45-55%, level 4:55-65% and level 5:more than 65%).
 5 b: Difference = (level of yolk-albumen ratio based on predicted value)
 6 - (level of yolk-albumen ratio based on observed value)