Validating Egg Component Proportions in Quail Receiving Methionine Supplementation

A Ratriyanto, S Nurcahyo, S Prastowo and N Widyas

Department of Animal Science, Faculty of Agriculture, Universitas Sebelas Maret, Surakarta, Indonesia

E-mail: ratriyanto@staff.uns.ac.id

Abstract. Essential amino acid methionine is a methyl donor which involved in protein and fat metabolism; thus, it holds an important role in the synthesis of albumen and egg yolk precursors. This research aimed to observe the effect of methionine on the weight of egg and its components and to prove if egg weight (EW) could be used to predict the albumen weight (AW) and yolk weight (YW). In total, 225 quails were allotted into three treatments and five replicates each with fifteen individuals. The treatments were basal diet (T0), basal diet with methionine supplementation at 0.6 (T1) and 1.2 g/kg (T2). ANOVA was conducted to test the effect of treatments. Simple linear regressions were built to predict AW and YW based on EW. Cross validation procedure was applied to test the predictability of the models. Quails in T2 and T1 produced heavier eggs (9.29±0.52 and 9.04±0.76 g) compared to T0 (8.77±0.55 g). The AW follow similar trends which were 5.37±0.38, 5.53±0.54, 5.75±0.44 g for T2, T1 and T0, respectively. The YW slightly differ where T1 had higher weight (2.64±0.30 g) followed by T2 (2.62±0.27 g) and T0 (2.50±0.32 g). Pearson’s correlation between EW and AW were between 69-87% whereas for EW and YW were between 61-71%. Simple linear regression predicting AW and YW from EW had accuracies between 94.3-96.2% and 92.7-94.3%, respectively. Thus, methionine supplementation had significant effect on egg and egg components weights. Linear regression can be utilized to predict AW and YW based on EW with high accuracy.

Keywords : quail, egg, methionine

1. Introduction

Egg quality is determined by several egg traits such as the weight of egg, eggshell, albumen and yolk as well as shell thickness [1]. In the egg processing industries, the shell weight (SW), albumen weight (AW), and yolk weight (YW) affect the amount of the product [2]. Furthermore, the interior quality of the egg is affected by nutrient content in the diet [3]. The yellow yolk is composed of lipoproteins while albumen is composed of proteins. The protein requirements for laying quail is about 20% [4]. However, feeding with high-protein diet, particularly in hot, tropical climate such as in Indonesia, stimulates metabolic heat production leading to heat stress. Thus, it is required to provide the precise protein content in the diet. Feeding low-protein diet with supplementation of feed additive or amino acids may become an alternative to avoid heat stress while maintaining the quails’ performances [5,6].
Methionine is considered to be the first essential amino acid in poultry nutrition, which limits the biological value of protein [7]. It also acts as a methyl group donor which plays a role in energy and protein metabolism [8]. Supplementation of methionine to low-protein diet showed beneficial effect on the performance of laying hens. On the contrary, methionine supplementation to high-protein diet did not show beneficial effect [9]. Harms et al [10] demonstrated that egg weight (EW) of laying hens increased as the methionine content in the diet increased. Accordingly, previous observation revealed that methionine supplementation increased EW or egg mass in quails [11,12]. Furthermore, increasing methionine consumption enhanced the albumen total solid and protein as well as yolk protein contents [9].

Egg is a multi-purpose animal product, which is utilized in various ways; either as a whole or separated between its albumen and yolk. For the later purpose, the information about egg composition is essential. Except EW, other egg traits including AW and YW are determined only after breaking the egg [13]. This method is complicated, takes a lot of time and causes economic losses due to broken eggs [14]. Prediction of AW and YW with the regression equation using EW as predictor allows to measure these variables without breaking the eggs [15]. Several studies have been conducted to determine the correlation between external and internal quality traits in laying hens, but only few studies have been performed on this subject in quails. For example, Kul and Seker [13] observed a positive correlation between EW and the internal quality traits such as AW and YW in quails. However, there is paucity of information on the predictability with cross validation method, particularly in quails fed diet supplemented with methionine. Therefore, the objective of this study was to build models to predict the AW and YW using EW data of quails (Coturnix coturnix japonica) receiving methionine supplementation in the diet.

2. Methodology

Experimental design and diet formulation
In total, 225 laying quails (Coturnix coturnix japonica) were allotted into three treatments and five replicates each with fifteen individuals. The basal diet was formulated according to the recommendation of Nutrition Research Council [4] for laying quails, except the crude protein which was formulated below the standard (16.5%), as presented in Table 1. The basal diet was fed without methionine supplementation (T0) or with methionine supplementation at the levels of 0.6 g/kg (T1) and 1.2 g/kg (T2) according to the procedure of Ratrifianto et al. [16]. The dietary treatments were given for two periods of 28 days (2×28 days). The egg quality traits data were collected at the last 3 days of each period, namely days 26, 27 and 28. The eggs were individually weighed and then cracked. The yolks were separated from the albumen using yolk separator and weighed thereafter. The AW was obtained by reducing the EW with the YW and SW [17].

| Nutrients                  | Content |
|----------------------------|---------|
| Metabolizable energy (kcal/kg) | 2,800   |
| Crude protein (g/kg)        | 165.0   |
| Lysine (g/kg)               | 11.3    |
| Methionine (g/kg)           | 4.1     |
| Calcium (g/kg)              | 34.2    |
| Phosphorus (g/kg)           | 6.2     |

Management and analysis of the data
The data of EW, AW and YW were obtained from two measurement periods. They were separated between T0, T1 and T2. The data were the subjected to analysis of variance, correlation, regression and cross validation. All data analyses were performed using custom scripts of R program[18]. Analysis of variance was performed to determine the effect of dietary methionine on EW,
AW and YW. If the treatments showed significant effect on these traits, it was continued with Duncans test[19].

Correlation between EW and AW or YW, and the regression model was calculated to determine the relation between them. The correlation of EW~AW and EW~YW were calculated according to Ott et al. [20] as follows:

\[ R_{EW,AY} = \frac{\text{cov}(EW,AY)}{\sigma_{EW} \sigma_{AY}} \]

\[ \text{cov}(EW,AY) = \frac{\sum_{i=1}^{n}(EW_{i} \times AY_{i})}{n} \]

\[ \sigma = \sqrt{\bar{\sigma}^2} \]

\[ \bar{\sigma}^2 = \frac{\sum_{i=1}^{n}(x_{i} - \mu)^2}{n} \]

Where: \( R_{EW,AY} = \) correlation of EW~AW or EW~YW; \( \text{cov}(EW,AY) = \) covariant between EW~AW or EW~YW; \( \sigma_{EW} = \) standard deviation of EW; \( \sigma_{AY} = \) standard deviation of AW and YW; \( n = \) number of data; \( x = \) data; \( \mu = \) average.

A linear regression model was built to predict AW and YW using EW data according to Gujarati [21]:

\[ y_i = \alpha + \beta x_i \]

Where: \( y_i = \) response (AW and YW); \( \alpha = \) intercept; \( \beta = \) slope of independent variable; and \( x_i = EW \).

The model built was compared to the predictor's ability by using the accuracy of the prediction model. Predictability was obtained by employing the crossvalidation method to measure the performance of a prediction model [15] using most of the data to create a prediction model and save the rest for validation process [22]. Cross validation applied a regression formula obtained in another data to measure the accuracy of the formula in predicting another set of data. The crossvalidation was determined by selecting a certain amount of data (test set) and the remaining data are training set (Figure 1).

**Figure 1** shows the data 2–10 were defined as the training set that were used to create a regression equation, then to predict the test set of the data 1. The actual value of the test set data and predicted value based on the regression was then compared to determine the predictability of the regression equation.

**Figure 1.** Illustration of cross-validation method

3. Result and Discussion

### Actual weight of the egg, yolk and albumen

The average EW of all treatments ranged between 8.77 and 9.29 grams (**Table 2**), which were categorized in the normal range of quails’ EW [23]. The AW and YW obtained in this study are also in the normal range, namely 5.37–5.75 grams and 2.50–2.60 grams, respectively. The EW is in direct proportion to albumen, yolk and eggshell. The albumen and yolk quality are the parameters which determine the internal egg quality [24]. Dietary methionine administration yielded heavier eggs...
associated with enhanced in AW and YW. Quails in T2 and T1 produced heavier eggs (9.29±0.52 and 9.04±0.76 g) compared to T0 (8.77±0.55 g). The AW follow similar trends which were 5.37±0.38, 5.53±0.54, 5.75±0.44 g for T2, T1 and T0, respectively. The YW slightly differ where T1 had higher weight (2.64±0.30 g) followed by T2 (2.62±0.27 g) and T0 (2.50±0.32 g). Methionine is an essential amino acid and serves as a methyl group donor which involved in the protein and energy metabolism process leading to improvement in EW[11]. Albumen composed of protein while yolk composed mainly of lipoproteins, thus involvement of methionine in the metabolism increase the synthesis of the albumen and yolk precursors. In this study, each variable (EW, AW and YW) was affected by methionine supplementation, thus further analyses were conducted separately based on the treatment.

**Table 2.** Average actual weight of the eggs and its components

| Variables | Treatments | Total Data | Min  | Max  | Mean±SD  |
|-----------|------------|------------|------|------|----------|
| Egg weight| T0         | 80         | 7.68 | 10.38| 8.77±0.55b |
|           | T1         | 79         | 7.31 | 10.49| 9.04±0.76a |
|           | T2         | 80         | 7.56 | 10.44| 9.29±0.52a |
| p value   |            |            |      |      | < 0.01   |
| Albumen weight | T0      | 80         | 4.38 | 6.41 | 5.37±0.38b |
|            | T1        | 79         | 4.39 | 6.49 | 5.53±0.43b |
|            | T2        | 80         | 4.87 | 6.91 | 5.75±0.34a |
| p value   |            |            |      |      | < 0.01   |
| Yolk weight | T0        | 80         | 1.76 | 3.47 | 2.50±0.32b |
|            | T1        | 79         | 1.91 | 3.29 | 2.64±0.30a |
|            | T2        | 80         | 1.62 | 3.08 | 2.62±0.27ab |
| p value   |            |            |      |      | < 0.01   |

*a,b* Different superscript in the same column and variable indicated significant difference (*p* < 0.01).

**Correlation of egg weight–yolk weight and egg weight–albumen weight**

The statistical analyses showed the positive correlations of EW–AW and EW–YW (Table 3). Correlations of EW–AW are classified as high correlation with the correlation value above 0.7 while correlations of EW–YW are classified as moderate(0.3–0.7) to high correlation (above 0.7) [25]. Supplementation of 0.6 g/kg methionine generated the highest correlation both for EW–AW and EW–YW. In agreement with this result, previous observation indicated positive correlation of EW–AW and EW–YW in quails and chickens [13,26]. However, the correlation values obtained in this study are lower than those observed in previous study, where correlation between EW–AW was 0.94 and correlation between EW–YW was 0.77 [27].

**Table 3.** Correlation of egg weight–albumen weight and egg weight–yolk weight

| Treatments | Egg Weight–Albumen Weight | Egg Weight–Yolk Weight |
|------------|---------------------------|------------------------|
| T0         | 0.69                      | 0.69                   |
| T1         | 0.87                      | 0.77                   |
| T2         | 0.78                      | 0.61                   |

Predicting the interior quality of the egg components using the EW data is considered easier and without breaking the egg. Moreover, EW has proven as a good predictor for some egg [13] since the correlations between external and internal quality traits were high [28]. Kul and Seker[13] showed that almost all internal egg quality traits changed depending on the change occurred in the EW as indicated by positive correlations.

**Linear regression model and cross validation**
The simple linear regression model that was built in this study used the dependent variable (y), that is AW or YW and independent variable (x), that is EW. In total, six regression models were built in this study since there were 3 treatments in each variable of AW or YW (Table 4). The coefficient of determination ($R^2$) indicates the capability of the model in explaining variations in the dependent variable [21]. The $R^2$ values ranged between 0 and 1, with the higher value indicating the better fit to explain the relationship between the EW and the AW or YW. In agreement with this study, Seker [27] showed that EW can be used as a predictor for AW and YW with $R^2$ values for linear regression were 0.88 and 0.59 for AW and YW, respectively. In this regard, linear regression was accurate in predicting the relation between EW and AW or YW [27].

All the six models were then subjected to K-fold cross validation resulting the predicted values of AW and YW. The prediction accuracy were obtained by comparing the actual values with the prediction values [15] as presented in Table 5. Regression models had predictability ranged between 94.3–96.2% for AW and 92.7–94.3% for YW. The predictability values of all treatments were above 90%, thus its classified as very high accuracy. Similar to this study, a study in Japanese quail revealed that AW and YW were predictable from EW with enough accuracy [1]. The finding of this study supports that forecasting the weight of the egg and egg components without recourse to sensitive electronic scale has been a serious problem in egg production enterprise [26,29].

**Table 4. Linear regression model and $R^2$**

| Variables   | Treatments | Regression Models | $R^2$ |
|-------------|------------|-------------------|-------|
| Albumen weight | T0        | AW = 1.23+0.47.EW  | 0.47  |
|             | T1        | AW = –0.09+0.62.EW | 0.75  |
|             | T2        | AW = 0.44+0.67.EW  | 0.61  |
| Yolk weight  | T0        | YW = –0.04+0.41.EW | 0.47  |
|             | T1        | YW = –0.10+0.30.EW | 0.53  |
|             | T2        | YW = –0.10+0.30.EW | 0.59  |

AW = albumen weight; EW = egg weight; YW = yolk weight; $R^2$ = coefficient of determination.

**Table 5. Accuracy of prediction model**

| Variables   | Treatments | Actual Value | Predicted Value | Accuracy (%) |
|-------------|------------|--------------|-----------------|--------------|
| Albumen weight | T0        | 5.37±0.38    | 5.37±0.26       | 96.20        |
|             | T1        | 5.53±0.54    | 5.53±0.47       | 94.30        |
|             | T2        | 5.73±0.44    | 5.73±0.35       | 94.30        |
| Yolk weight  | T0        | 2.50±0.32    | 2.50±0.22       | 92.70        |
|             | T1        | 2.55±0.30    | 2.55±0.23       | 94.30        |
|             | T2        | 2.47±0.27    | 2.74±0.16       | 94.30        |

4. Conclusion
Linear regression model with EW as a predictor was considered as good model for predicting AW and YW in quails receiving methionine supplementation in the diet. All models for predicting AW and YW generated very high predictability. Based on this result, it is possible to use the EW as predictor of the AW and YW of quail eggs with high accuracy.

References

[1] A. Kurshid, M. Farooq, F. R. Durrani, K. Sarbiland and N. Chand. 2013. Predicting egg weight, shell weight, shell thickness and hatching chick weight of Japanese quails using various egg traits as regressors. *Int. J. Poult. Sci.* 2:164–167.

[2] S. O. Olawumi and J. T. Ogunlade. 2008. Phenotypic correlations between some external and internal egg quality traits in the exotic Isa Brown layer breeders. *Asian J. Poult. Sci.* 2:30–35.
[3] M. M. Shanaway. 1994. Quail Production Systems: A Review (Rome: FAO).
[4] Nutrition Research Council. 1994. Nutrient Requirements of Poultry. Washington DC: National Academic Press.
[5] S. V. R. Rao and E. T. Reddy. 2016. Effect of different levels of dietary crude protein, lysine and methionine on performance of White Leghorns laying hens. *Asian J. Poult. Sci*. 10:147–152.
[6] A. Ratriyanto, R. Indreswari and A. M. P. Nuhiawangsa. 2017. Effects of dietary protein level and betaine supplementation on nutrient digestibility and performance of Japanese quails. *Rev. Bras. Cienc. Avic*. 19:445–454.
[7] J. Jankowski, M. Kubińska and Z. Zduńczyk. 2014. Nutritional and immunomodulatory function of methionine in poultry diets - A review. *Ann. Anim. Sci*. 14:17–31.
[8] A. Ratriyanto and R. Mosenthin. 2018. Osmoregulatory function of betaine in alleviating heat stress in poultry. *J. Anim. Physiol. Anim. Nutr. (Berl)*. 102:1634–1650.
[9] C. Bunchasak. 2009. Role of dietary methionine in poultry production. *J. Poult. Sci*. 46:169–179.
[10] R. H. Harms, G. B. Russell, H. Harlow and F. J. Ivey. 1998. The influence of methionine on commercial laying hens. *J. Appl. Poult. Res*. 7:45–52.
[11] R. Ratriyanto, R. Indreswari, A. M. P. Nuhiawangsa and A. A. Arifin. 2017. Dietary metabolizable energy and methionine affect performance of quails. *Proceedings of the 1st International Conference on Tropical Agriculture* (Cham: Springer International Publishing): 329–335
[12] K. Khairani, S. Sumiati and K. G. Wiryawan. 2015. Egg production and quality of quails fed diets with varying levels of methionine and choline chloride. *Media Peternak* 39:34–39.
[13] S. Kul and I. Seker. 2004. Phenotypic correlation between some external and internal egg quality traits in Japanese quail (*Coturnix japonica*). *Int. J. Poult. Sci*. 3:400–405.
[14] A. Kurshid, F. R. Farooq, K. S. Durrani. 2003. Predicting egg weight, shell weight, shell thickness and hatching chick weight of japanese quails using various egg traits as regressor. *Int. J. Poult. Sci*. 2:164–167.
[15] Y. Zhang and Y. Yang. 2015. Cross-validation for selecting a model selection procedure. *J. Econom*. 187:95–112.
[16] A. Ratriyanto, R. Indreswari and Sunarto. 2014. Effects of protein levels and supplementation of methyl group donor on nutrient digestibility and performance of broiler chickens in the tropics. *Int. J. Poult. Sci*. 13:575–581.
[17] A. Ratriyanto and S. Prastowo. 2019. Floor space and betaine supplementation alter the nutrient digestibility and performance of Japanese quail in a tropical environment. *J. Therm. Biol*. 18:80–86.
[18] R Core Team. 2019 *R: A Language and Environment for Statistical Computing*. Vienna: R Foundation for Statistical Computing.
[19] R. G. D. Steel, J. H. Torrie and D. A. Dickey. 1996. *Principles and Procedures of Statistics: A Biometrical Approach* (New York: McGraw-Hill).
[20] R. L. Ott, M. Longnecker, M. Taylor and M. A. Payumo. 2010. *An Introduction to Statistical Methods and Data Analysis* Sixth Edition. USA: Brooks/Cole, Cengage Learning.
[21] D. Gujarati. 2012. *Econometrics by Example*. New York: Palgrave MacMillan.
[22] R. Kohavi. 1995. A study of cross-validation and bootstrap for accuracy estimation and model selection. *Int. Jt. Conf. Artif. Intell*. 14:1137–1143.
[23] A. Ratriyanto. 2018. Egg production pattern in quails fed diet supplemented with betaine and methionine. *Caraka Tani J. Sustain. Agric*. 33:1–7.
[24] S. Alkan, A. Galic, T. Karsli, and K. Karabal. 2015. Effects of egg weight on egg quality traits in partridge (*Alectoris Chukar*). *J. Appl. Anim. Res*. 43:450–456.
[25] B. B. Gerstman. 2008. Basic biostatistics: Statistics for the public health practice. USA: Jones and Bartlett Publishers.
[26] U. H. Udoh, B. Okon and A. P. Udoh. 2012. Egg quality characteristics, phenotypic correlations and prediction of egg weight in three (naked neck, frizzled feather and normal feathered) Nigerian local chickens. *Int. J. Poult. Sci.* 11:696–699.

[27] I. Seker. 2004. Prediction of albumen weight, yolk weight, and shell weight as egg weight in Japanese quail eggs. *Uludag Univ. J. Fac. Vet. Med.* 23:98–92.

[28] H. Orhan, E. Eyduran, A. Tatliyer and H. Saygici. 2016. Prediction of egg weight from egg quality characteristics via ridge regression and regression tree methods. *Rev. Bras. Zootec.* 45:380–385.

[29] V. O. Chimezie, T. R. Fayeye, K. L. Ayorinde and A. Adebunmi. 2017. Phenotypic correlations between egg weight and some egg quality traits in three varieties of Japanese quail (*Coturnix coturnix japonica*). *Agrosearch*:44–53.