Egg quality of quails fed low methionine diet supplemented with betaine

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Abstract. This experiment investigated the effect of betaine supplementation to low methionine diet on egg quality of quails. A total of 340 laying quails (Coturnix coturnix japonica) was divided into 4 dietary treatments with 5 replicates of 17 quails each. The experiment was assigned in a completely randomized design. The four dietary treatments were the low methionine diet (0.3% methionine) without betaine supplementation and the low methionine diet supplemented with 0.07, 0.14, and 0.21% betaine. The experimental diets were applied for 8 weeks and the egg quality traits were measured at the age of 16 and 20 weeks. The data were subjected to analysis of variance, and when the treatment indicated significant effect, it was continued to orthogonal polynomial test to determine the optimum level of betaine. Increasing dietary levels of betaine increased the fat content of the egg with the linear regression of $y = 11.0949 + 4.1914x$ ($R^2 = 0.18$). However, supplementation of betaine did not affect protein content, yolk, albumen, and eggshell percentage. It can be concluded that betaine supplementation up to 0.21% to low methionine diet only had little effect in improving the quality traits of quail eggs.

1. Introduction
Methionine and betaine are the potential feed additives as methyl group (CH$_3$) donor [1]. Methionine is an essential amino acid whose metabolites are used in a variety of fundamental biological processes including protein deposition and synthesis of S-adenosyl methionine [2]. Providing other methyl group donor (e.g. betaine) may spare methionine as methyl group donor or provide the methyl group necessary to convert homocysteine to methionine, thus the requirement for methionine in the diet can be reduced [3].

Betaine (trimethyl glycine) has two main biological functions, i.e. as a methyl group donor and an organic osmolyte. As a methyl group donor, betaine can spare the use of other methyl group donors such as methionine and choline. Thus, betaine is expected to increase the availability of methionine for protein synthesis, resulting in optimal performance [1]. As an organic osmolyte, betaine protects cells from various osmotic pressures, such as cell hydration [4].

Methyl group cannot be synthesized in the body of poultry, thus it must be supplied in the diet [1]. Previous studies showed that betaine supplementation to methionine-deficient diet increased betaine homocysteine-methyltransferase (BHMT) activity in chickens, indicating that poultry have specific requirements for methyl groups [5]. Observation in poultry revealed that betaine maintained performance of laying hens fed diets reduced in methionine and without choline chloride [6]. Supplementation of 0.1% betaine increased eggshell thickness, haugh unit and eggshell percentage but did not affect yolk percentage in laying hens [7]. Studies on the potential of betaine as a methyl group...
donor in laying poultry are still limited and the results are inconsistent [6, 8]. Betaine supplementation to low methionine diet is expected to provide methyl groups which have the potential to improve egg quality. The objective of this study was to investigate the effect and optimum level of betaine supplementation in low methionine diet egg quality of quails.

2. Methods
A total of 340 laying Japanese quails (Coturnix coturnix japonica) aged 4 weeks with an average body weight of 98.31 ± 8.67 grams was used in this study. The study was designed as a completely randomized design with 4 dietary treatments, each treatment with 5 replicates containing 17 quails. The basal diet was formulated to meet nutrient requirement of laying quails according to the recommendation of Indonesian National Standard [9] except for the amino acid methionine which was set at 0.3%, lower than the methionine requirement standard of 0.4 (Table 1).

| Ingredients                  | Proportion (%) |
|------------------------------|----------------|
| Yellow corn                  | 25.00          |
| Rice bran                    | 21.00          |
| Soybean meal                 | 25.00          |
| Fish meal                    | 5.50           |
| Cassava by product meal      | 15.50          |
| Dicalcium phosphate          | 1.75           |
| Limestone                    | 5.50           |
| Premix                       | 0.50           |
| NaCl                         | 0.25           |
| Nutrient content             |                |
| Metabolizable energy (kcal/kg)| 2,887.61       |
| Crude protein (%)            | 18.51          |
| Calcium (%)                  | 3.41           |
| Available phosphorus (%)     | 0.70           |
| Lysine (%)                   | 1.02           |
| Methionine (%)               | 0.30           |

The diet was fed without betaine supplementation or supplemented with 0.07, 0.14, and 0.21% betaine. Betaine was supplemented at the expense of rice bran [10]. During the experiment, the quails had free access to water and feed. The experimental diets were applied for 8 weeks after the egg production has reached 10%. The egg quality traits were measured for 3 consecutive days at 16 and 20 weeks. In addition, standard managemental practice was applied during this study.

The observed variables were yolk, albumen, and eggshell percentage, and protein and fat content of the eggs. Data were subjected to variance analysis and if it showed an effect of treatment, then it was continued with orthogonal polynomial test to determine the optimum level of treatment.

3. Results and Discussion
Betaine supplementation to low methionine diet did not affect yolk, albumen, and eggshell percentage as well as protein content of the eggs (Table 2). Egg size is mostly determined by the yolk and albumen. The results of this study were in accordance with previous study in Lohmann Brown laying hens, in which supplementation of 0.035% betaine did not affect yolk and eggshell percentage [6]. Similarly, Ezzat et al. [7] did not observed any effect of 0.1% betaine supplementation on yolk, albumen, and eggshell percentage in Matrouh laying hens.
Table 2. Effect of betaine supplementation to low methionine diet on egg quality traits of quails

| Variables              | Betaine Levels (%) | P Value |
|------------------------|--------------------|---------|
|                        | 0                  | 0.07    | 0.14 | 0.21 |         |
| Yolk percentage (%)    | 38.94              | 33.21   | 35.05 | 34.46 | 0.10 |
| Albumen percentage (%) | 53.33              | 58.94   | 55.40 | 57.36 | 0.14 |
| Eggshell percentage (%)| 8.73               | 8.27    | 7.37  | 8.47  | 0.29 |
| Protein content (%)    | 11.76              | 11.80   | 11.59 | 11.41 | 0.21 |
| Fat content (%)        | 10.94              | 11.33   | 12.26 | 11.61 | 0.03 |

Previous studies revealed that betaine supplementation increased protein synthesis and decreased protein degradation as indicated by increased in serum protein, globulin and albumin levels [11]. However, increasing dietary betaine levels in this study could not optimize the availability of methionine for protein synthesis. Presumably methionine is used for immune systems, where the requirement of methionine for immunity is greater than for protein synthesis [1]. This results was in support with previous observation, in which betaine supplementation did not affect egg quality traits in laying hens [12]. Different to this result, Ratriyanto et al. [13] observed that supplementation of 0.06 and 0.12% betaine to methionine adequate diet enhanced egg quality traits of quails including yolk, albumen, and eggshell weight.

Furthermore, the homeostasis mechanism regulates the amount of calcium absorbed according to the requirement of mineral [14], thus the eggshell percentage was not affected by the treatment. In agreement with this study, supplementation of 0.035% betaine did not affect eggshell percentage in Lohmann Brown laying hens [6]. Meanwhile, according to Ryu et al. [15] betaine supplementation at levels of 0.05% to 0.2% increased eggshell strength in laying hens.

Increasing dietary betaine supplementation to low methionine diet increased linearly (P<0.05) fat content of the eggs, with the regression $y = 11.0949 + 4.1914x$ ($R^2 = 0.18$) (Figure 1). Betaine donates the methyl group during the transmethylation reaction to synthesize important metabolically substances such as carnitine and creatine that play an important role in fat metabolism [1]. Betaine is also a lipotropic agent and involved in the synthesis of chylomicron that play a role in fat absorption [16]. In addition, betaine donates its methyl groups to synthesis of lecithin which is required in the transportation of fat in the body, thus providing the fat for egg formation [17].

![Figure 1. Effect of betaine supplementation on fat content of the eggs](image)

4. Conclusion
Betaine supplementation to low methionine diet increased fat content but did not affect the percentage of yolk, albumen, eggshell, and protein content of quail eggs. Furthermore, increasing dietary betaine supplementation linearly increased fat content of the eggs with the regression $y = 11.0949 + 4.1914x$ ($R^2 = 0.18$).
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