

## Egg Production and Quality of Quails Fed Diets with Varying Levels of Methionine and Choline Chloride

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### ABSTRACT

The aim of the present study was to determine the effect of choline chloride supplementation at 1500 ppm in diets containing various levels of methionine on egg production and egg quality in quails. A total of 180 birds, at 6 week-old quail were divided into 18 experimental units, and assigned to a 2 x 3 factorial design experiment with 3 replications (10 birds each) in each treatment. The birds were offered diets containing choline chloride at either 0 (A1) or 1500 ppm (A2), with three levels of methionine namely, low (0.19%, B1), standard (0.79%, B2) and, high (1.05%, B3). The feeding trial lasted for 8 weeks. Supplementation of choline chloride in low methionine diet significantly ( $P < 0.05$ ) increased egg production, egg mass, and egg weight as compared to those without choline chloride supplementation. Supplementation of choline chloride significantly ( $P < 0.05$ ) increased egg yolk weight but decreased albumen and egg shell weight as compared to those fed diets without choline chloride supplementation. It can be concluded that supplementation of choline chloride to a diet containing low methionine increased egg production, without affecting egg quality.

*Key words: choline chloride, egg quality, methionine, quail*

### ABSTRAK

Tujuan penelitian ini adalah untuk mengevaluasi efek penambahan *choline chloride* 1500 ppm pada ransum yang berbeda kadar metioninnya pada performa produksi dan kualitas telur puyuh. Penelitian menggunakan 180 ekor puyuh petelur yang berumur 6 minggu, yang dipelihara selama 8 minggu, dan percobaan dibagi menjadi 18 unit, setiap unit percobaan terdiri atas 10 ekor puyuh. Penelitian menggunakan rancangan acak lengkap dengan pola faktorial 2 x 3 dan 3 ulangan. Perlakuan faktor A adalah 2 level penambahan *choline chloride* (0 ppm, 1500 ppm), faktor B adalah 3 level metionin ransum (rendah (0,19%), standar (0,79%), tinggi (1,05%)). Hasil penelitian menunjukkan bahwa penambahan *choline chloride* 1500 ppm pada ransum rendah metionin nyata ( $P < 0,05$ ) meningkatkan produksi telur, massa telur, bobot telur dibandingkan tanpa penambahan *choline chloride*. Suplementasi *choline chloride* 1500 ppm nyata ( $P < 0,05$ ) meningkatkan persentase kuning telur dan menurunkan persentase putih dan kerabang telur. Kesimpulan penelitian adalah pemberian *choline chloride* 1500 ppm pada ransum rendah metionin dapat meningkatkan produksi telur tanpa menurunkan kualitas telur.

*Kata kunci: choline chloride, kualitas telur, metionin, puyuh*

### INTRODUCTION

Quail is one of commodities of meat and egg producing poultry that is increasingly interested to be reared by the community, especially farmer groups. This is evidenced by the increasing population of

quail of *Coturnix coturnix japonica* or Japanese quail in Indonesia. According to Directorate General of Livestock and Animal Health (2013), quail population in Indonesia increased from 7.054 million to 12.594 million birds from 2010 until 2013.

Balance feed containing either macro or micro nutrients is one factor that influences and has important role in laying quail production. Poultry feed which is commonly composed of 80% cereals, usually maize, and soybean meal, usually contains low methionine.

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Therefore, this feed is necessary to be supplemented with synthetic DL-Methionine in order to fulfill the requirement of the quail. However, considering that the price of DL-Methionine is quite high for farmers, it is necessary to use other alternative substances with lower price, that can replace the role of DL-Methionine partially or in a whole, such as choline chloride. Choline chloride is a substance to be regarded as an effective source of choline (EFSA, 2011).

Choline is water soluble vitamin and plays an important role in the synthesis of membrane phospholipids (Hollenbeck, 2010). Choline is an essential nutrient for poultry, and its function is divided into four broad categories, namely (1) as an essential metabolic for building and maintaining cell structure, (2) plays an essential role in hepatic fat metabolism and prevent abnormal accumulation of fat (fatty liver), (3) essential for the formation of acetylcholine that is responsible in the transmission of nerve impulse which cannot be formed without the availability of choline in the body (Zeisel, 2012; Garrow, 2007) and (4) provides methyl groups that is necessary for the formation of methionine from homocysteine during the oxidation to betaine is non-essential function of choline (Workel 2005; Garrow, 2007; Zhang *et al.* 2013).

Several studies on supplementation of choline chloride indicated that dietary choline chloride supplementation at 750 mg/kg in broiler chickens had a positive impact on body weight gain, final body weight and feed conversion (Sumiati *et al.*, 2006). Diet containing 2000 mg choline/kg could be recommended for feeding Japanese quail during the growing period (7 to 42 days of age) without adverse effects on growth performance or feed utilization (Alagawany *et al.*, 2015). Choline concentration at 1500 ppm is necessary to maintain a maximal laying performance (Danicke *et al.* 2006). In hens fed diet that contained 15% protein, 0.30% methionine and 1600 ppm choline showed a significant increase in hen-day egg production and egg weight (Omara, 2012).

Choline and methionine are interrelated to each other, and are possible methyl group donors which play important roles in methylation reactions (Omara, 2012). Both choline and methionine are methyl group donors, so the lack of methyl donation derived from methionine can be replaced by choline, and further can be used for the synthesis of methionine and vice versa. According to Sun *et al.* (2008), methionine is a competent amino acid for the formation of protein and S-adenosylmethionin (SAM). Therefore, the existence of alternative donor of methyl groups derived from choline chloride can reduce the usage of methionine, and methionine can be more efficiently used for protein synthesis. It is clear that there is a similarity in roles between choline and methionine as methyl group donors, thus supplementation of choline can help to alleviate the role of methionine.

The present study was to determine the productive performance of quail (*Coturnix coturnix japonica*) at laying period and the physical quality of eggs due to supplementation of choline chloride in diets containing different levels of methionine. It is expected that the dietary supplementation of choline chloride for laying

quail can reduce the use of DL-Methionine, thus lowering feed cost.

## MATERIALS AND METHODS

Feed ingredients were obtained from PT. Indofeed Bogor. Experimental animals were 180 birds of 6 week-old laying quails, raised in a cage of 60 x 60 x 40 cm in size. The experiment was arranged in a 2 x 3 factorial scheme of completely randomized design (CDR) with 3 replications (10 birds each). Factor A was choline chloride supplementation with 2 levels (none/A1 and 1500 ppm/A2). Factor B was methionine supplementation with 3 levels, namely low (0.19%/B1), standard (0.79%/B2) according to the recommendation of Leeson & Summers (2005), and high (1.05%/B3). Experimental diets were formulated isoprotein and isoenergy according to the recommendation of Leeson & Summers (2005) with protein and energy contents of 18% and 2950 kcal/kg, respectively (Table 1).

Quails were reared for 10 weeks divided into 3 steps, namely one week for environmental adaptation, one week for dietary treatment adaptation, and eight weeks for feeding trial. Diet and drinking water were offered *ad libitum*. Variables observed were feed consumption, feed conversion ratio, egg production (QDP), egg weight, and egg physical quality. Egg weight and egg production were recorded daily while feed consumption and feed conversion ratio were recorded weekly. Physical quality of egg was observed at the fifth, sixth, seventh, eight weeks of the treatment by taking randomly 3 eggs from the respective replication.

Data were subjected to analysis of variance (ANOVA) based on a 2 x 3 factorial design and the means were subjected to Duncan's multiple range test (Steel & Torrie, 1995).

## RESULTS AND DISCUSSION

### Quail Productive Performances

Feed consumption during the study ranged between 17.64-20.52 g/bird/d (Table 2). When no choline chloride was supplemented in low methionine diet (A1B1) feed consumption was significantly decreased ( $P < 0.05$ ) as compared to diet containing standard methionine (A1B2). This decrease is due to the diet containing standard methionine has a relatively better amino acid balance compared to the low methionine diet. Similar result has also been reported by Bunchasak & Silaparsorn (2005) that feed consumption decreased in chickens given diet containing low methionine. Feed consumption was the lowest in laying hens fed diet containing low protein (14%) supplemented with methionine at 0.26% (relatively low) as compared to that of birds given diets with higher supplementation of methionine (0.30%, 0.38%, and 0.44%). Methionine deficiency is known to decrease feed consumption due to the imbalance of dietary amino acids. Under such condition quails lost their potentials to adjust feed intake to satisfy their amino acids requirements. According to

Table 1. Composition of the experimental diets and calculated nutrient contents

Composition	Treatments					
	A1B1	A1B2	A1B3	A2B1	A2B2	A2B3
Feed ingredients						
Yellow corn (%)	58.60	58.40	58.33	58.60	58.40	58.33
Rice bran (%)	1.00	0.30	0.20	1.00	0.30	0.20
Corn gluten meal (CGM) (%)	3.00	3.00	3.00	3.00	3.00	3.00
Soybean meal (%)	22.50	22.50	22.50	22.50	22.50	22.50
Fish meal (%)	3.80	3.80	3.80	3.80	3.80	3.80
Palm oil (%)	2.60	2.85	2.88	2.60	2.85	2.88
Dicalcium diphosphate (DCP) (%)	0.80	0.80	0.71	0.80	0.80	0.71
CaCO <sub>3</sub> (%)	7.10	7.10	7.00	7.10	7.10	7.00
NaCl (%)	0.10	0.10	0.10	0.10	0.10	0.10
Premix (%)	0.50	0.40	0.40	0.50	0.40	0.40
DL-Methionine (%)	0.00	0.75	1.075	0.00	0.75	1.075
Total	100	100	100	100	100	100
Choline chloride (ppm)				1500	1500	1500
Nutrient contents						
ME (kcal/kg)	2951.55	2951.65	2950.01	2951.55	2951.65	2950.01
Crude protein (%)*	18.14	18.03	18.01	18.14	18.03	18.01
Crude fat (%)*	5.14	5.35	5.37	5.14	5.35	5.37
Crude fiber (%)*	2.37	2.28	2.27	2.37	2.28	2.27
Methionine + Cystine (%)**	0.22	0.82	1.08	0.22	0.82	1.08
Methionine (%)**	0.19	0.79	1.05	0.19	0.79	1.05
Lysine (%)	1.07	1.07	1.07	1.07	1.07	1.07
Calcium (%)	3.18	3.18	3.12	3.18	3.18	3.12
Phosphorus available (%)	0.47	0.46	0.45	0.47	0.46	0.45
Choline mg/kg**	1498.30	1498.30	1498.30	2998.30	29998.30	2998.30

Note: A1B1= low methionine (0.19%) plus 0 ppm choline chloride containing diets, A1B2= standard methionine (0.79%) plus 0 ppm choline chloride containing diets, A1B3= high methionine (1.05%) plus 0 ppm choline chloride containing diets, A2B1= supplementation of choline chloride at 1500 ppm in low methionine diet (0.19%), A2B2= supplementation of choline chloride at 1500 ppm in standard methionine diet (0.79%), A2B3= supplementation of choline chloride at 1500 ppm in high methionine diet (1.05%); \*Laboratory of Research Center for Bioresources and Biotechnology, Bogor Agricultural University, 2014; \*\*PT. Saraswanti Indo Genetech Bogor, 2014

Table 2. Feed consumption, egg production, egg mass, and feed conversion ratio of quail during 8 week treatments

Variable	Choline (A)	Diet (B)		
		B1	B2	B3
Feed consumption (g/bird/d)	A1	17.64±1.73 <sup>b</sup>	20.52±0.33 <sup>a</sup>	18.87±1.01 <sup>ab</sup>
	A2	19.42±0.80 <sup>ab</sup>	19.40±0.78 <sup>ab</sup>	20.44±0.46 <sup>a</sup>
Egg production (%)	A1	44.93±8.04 <sup>b</sup>	70.24±3.88 <sup>a</sup>	65.56±4.87 <sup>a</sup>
	A2	65.86±4.96 <sup>a</sup>	64.58±2.15 <sup>a</sup>	68.72±2.91 <sup>a</sup>
Egg mass (g/bird)	A1	204.19±36.48 <sup>b</sup>	387.71±37.28 <sup>a</sup>	356.55±23.50 <sup>a</sup>
	A2	352.90±27.79 <sup>a</sup>	374.54±2.85 <sup>a</sup>	400.02±12.43 <sup>a</sup>
Feed conversion ratio	A1	4.88±0.44 <sup>a</sup>	3.08±0.31 <sup>b</sup>	2.96±0.20 <sup>b</sup>
	A2	3.10±0.30 <sup>b</sup>	2.87±0.08 <sup>b</sup>	2.90±0.11 <sup>b</sup>

Note: A1= no supplementation of choline chloride; A2= supplementation of choline chloride at 1500 ppm; B1= low methionine diet; B2= standard methionine diet; B3= high methionine diet; Means in the same variable with different superscript differ significantly (P<0.05).

Bunchasak & Keawarun (2006), amino acid imbalance on a wider scale would decrease feed consumption and egg production. However, feed consumption as a result of either 1500 ppm supplemental choline chloride in whatever levels of methionine containing diets (A2B1, A2B2, and A2B3) or control diet without supplementation (A1B2) indicated similar feed consumptions. Danicke *et al.* (2006) reported that there were no sig-

nificant differences in feed intake among hens receiving diets containing choline levels up to 4000 mg/kg.

The average of egg production for eight weeks (6-13 wk of age) is shown in Table 2. Low methionine diet without choline chloride supplementation (A1B1) produced significantly lower (P<0.05) egg production (quail day) as compared to both standard (A1B2) and high (A1B3) dietary methionine diets. Supplementation

of choline chloride at 1500 ppm on low methionine diet (A2B1) significantly increased ( $P < 0.05$ ) daily egg production (quail day) by 46.58% from 44.93% (without supplementation/A1B1) to 65.86%. However, no effect was observed in two other levels of dietary methionine with choline chloride supplementation, A2B2 and A2B3, as compared to diets without supplementation, A2B2 and A1B3, respectively. Methionine is the first limited amino acid and provides an important function with choline in donating methyl group for egg production. Methionine supplementation as a positive factor resulted in higher laying productive performances (Pourreza & Smith, 2007; Koreleski & Swiatkiewicz, 2011). The relationship between methionine and choline is the role of choline as a methyl group donor necessary for the formation of methionine from homocysteine via betaine (Workel, 2005; Garrow, 2007; Zhang *et al.* 2013). Increased methionine in the diet can maximize egg production on laying birds (Bunchasak, 2009). In addition to its relations with methionine as methyl group donor, choline is also functions as an essential nutrient required by laying quail in influencing the egg production, because it is a major component in the formation of phospholipid lecithin, a component of the egg yolk. Egg yolk phospholipid is very rich in phosphatidylcholine, it comprises 76% of the total phospholipids (Huopalahti *et al.* 2007). Supplementation of choline chloride at 1500 ppm in the standard methionine (A2B2) and high methionine (A2B3) diets did not affect egg production. This is presumably due to the same nutrients supply, such as protein, energy and fat, derived from the similar amount of feed consumption. The amount of energy, protein, and fat consumptions can affect egg production. Energy and nutrient needs for the formation of eggs are obtained from daily feed intake (Ghazvinian *et al.*, 2011).

Egg mass (g/bird) is the total eggs weight produced by each bird of quail during the study. Supplementation of choline chloride at 1500 ppm in the low methionine diet (A2B1) significantly increased ( $P < 0.05$ ) egg mass from 204.19 g/bird (without supplementation/A1B1) to 352.90 g/bird. The high yield of egg mass due to the effect of supplemental choline chloride in the diet containing low methionine is closely related to high egg production. High egg production is usually positively correlated with increasing egg mass, because egg mass is the result of egg production multiplied by egg weight (Sh *et al.* 2013). According to Koreleski & Swiatkiewicz (2011), methionine supplementation significantly increased egg weight and daily egg mass per hen. Further, Vercese *et al.* (2012) explained that the egg mass is influenced by egg weight and egg production.

Feed conversion ratio (FCR) was calculated as gram feed consumption per day per hen divided by gram egg mass per day per hen. The value of feed conversion ratio in layer indicates the efficiency of nutrient utilization for egg synthesis. Supplementation of choline chloride at 1500 ppm in low methionine diet (A2B1) significantly decreased ( $P < 0.05$ ) feed conversion ratio by 36.81% from 4.89 (without supplementation/A1B1) to 3.09. Supplementation of choline chloride at 1500 ppm increased egg production, egg mass, and egg weight, which affected the value of feed conversion ratio. FCR

were calculated from comparison between the amounts of diet consumed with the egg mass. According to Danicke *et al.* (2006), feed conversion ratio was significantly reduced by choline addition up to 4000 mg/kg.

### Dietary Treatments on Physical Quality of Quail Eggs

Low methionine diet without choline chloride supplementation (A1B1) produced egg with a significantly lower ( $P < 0.05$ ) weight as compared to the same diet with supplementation, and the egg weight was the lowest ( $P < 0.05$ ) among treatments (Table 3). It was clear that supplementation of choline chloride in low methionine diet (A2B1) significantly increased ( $P < 0.05$ ) egg weight by 18.002%, from 8.11 g (without supplementation/A1B1) to 9.57 g. Similarly, the significant increase ( $P < 0.05$ ) in egg weight was also found in the high methionine diet with choline chloride supplementation (A2B3) when compared to A1B3, and indicated the highest value among other treatments. This result is due to the rich nutrient contents of A2B3 diet that play a role in affecting egg weight which contains methionine and choline. Methionine is the most important nutrient in controlling egg weight (Zeweil *et al.*, 2011; Francis *et al.*, 2012). According to Leeson & Summers (2005), amino acids (especially methionine) are nutrient responsible for controlling the size of the eggs, in addition to genetic and avian body size. The increased egg weight in quail fed low methionine diet supplemented with choline chloride indicating an important relationship between choline and methionine which both are methyl group donors for egg synthesis. The lack of methyl donation from methionine can be replaced by choline, and methyl of choline can be used for the synthesis of methionine and vice versa. Supplementation of choline chloride at 1600 mg/kg in laying hen diets increased egg weight (Omara, 2012). According to Zhai *et al.* (2013), dietary choline level up to 1700 mg/kg is sufficient to maintain egg productive performances.

Supplementation of choline chloride at 1500 ppm (A2) significantly reduced ( $P < 0.05$ ) albumen weight percentage compared to without supplementation (A1), from 56.20% (without supplementation/A1) to 54.85%. Supplementation of choline chloride at 1500 ppm (A2) significantly increased ( $P < 0.05$ ) yolk weight percentage, 33.17%, as compared to without supplementation (A1), 31.06%. Increasing yolk weight percentage due to choline is a major component in the formation of phospholipid lecithin, a component of the egg yolk. Egg yolk phospholipid is very rich in phosphatidylcholine, it comprises 76% of the total phospholipid (Houopalahti *et al.*, 2007).

Low methionine diet without choline chloride supplementation (A1B1) resulted in a higher ( $P < 0.05$ ) eggshell weight percentage as compared to the same diet with supplementation, and the eggshell weight percentage was the highest ( $P < 0.05$ ) among treatments. This is due to the reduced eggshell thickness and eggshell weight with the increased egg size. Eggshell weight percentage of the study was ranged from 11.47%-14.13% yield is still within the normal range. According to Mine & D'Silva (2008), standard yolk composition of bird's

Table 3. Egg quality of quail fed diets with varying levels of methionine and supplemented with choline chloride

Variables	Choline (A)	Diet (B)			Average
		B1	B2	B3	
Egg weight (g)	A1	8.11 ±0.02 <sup>d</sup>	9.84 ±0.46 <sup>b</sup> <sup>c</sup>	9.72 ±0.46 <sup>c</sup>	
	A2	9.57 ±0.14 <sup>c</sup>	10.37 ±0.27 <sup>ab</sup>	10.40 ±0.19 <sup>a</sup>	
Albumen weight (%)	A1	55.86 ±0.74	57.25 ±2.23	55.49 ±0.68	56.20 ±2.00 <sup>a</sup>
	A2	54.02 ±0.58	55.60 ±1.71	54.93 ±0.55	54.85 ±1.17 <sup>b</sup>
	Average	54.94 ±1.17	56.43 ±1.99	55.21 ±0.63	
Yolk weight (%)	A1	30.02 ±0.39	30.81 ±2.192	32.37 ±0.77	31.06 ±1.57 <sup>b</sup>
	A2	33.60 ±0.97	32.30 ±2.05	33.61 ±0.42	33.17 ±1.32 <sup>a</sup>
	Average	31.81 ±2.07	31.55 ±2.07	32.99 ±0.88	
Eggshell weight (%)	A1	14.13 ±0.64 <sup>a</sup>	11.95 ±0.39 <sup>b</sup>	12.14 ±0.13 <sup>b</sup>	
	A2	12.38 ±0.57 <sup>b</sup>	12.09 ±0.76 <sup>b</sup>	11.47 ±0.14 <sup>b</sup>	
Eggshell thickness (mm)	A1	0.178±0.020	0.182±0.007	0.182±0.002	0.180±0.010
	A2	0.177±0.005	0.200±0.027	0.200±0.017	0.193±0.020
	Average	0.177±0.011	0.191±0.020	0.191±0.042	
Haugh unit	A1	92.84 ±1.35	92.08 ±1.87	91.77 ±1.37	92.23 ±1.42
	A2	93.53 ±3.69	85.59 ±6.42	90.54 ±1.35	89.89 ±5.12
	Average	93.18 ±2.51	88.83 ±5.52	91.15 ±1.39	
Yolk color score	A1	8.06 ±0.54	8.11 ±0.49	8.39 ±0.05	8.19 ±0.40
	A2	8.00 ±0.17	8.08 ±0.22	7.94 ±0.21	8.01 ±0.18
	Average	8.03 ±0.36	8.10 ±0.34	8.17 ±0.28	

Note: A1= none supplementation of choline chloride; A2= supplementation of choline chloride at 1500 ppm; B1= low methionine diet; B2= standard methionine diet; B3= high methionine diet; Means in the same column/raw with different superscript differ significantly (P<0.05).

egg ranging from 32%-35%, egg whites 52%-58%, and eggshell 9%-14%.

Supplementation of choline chloride at 1500 ppm in diets containing different methionine did not affect the eggshell thickness. The average of eggshell thickness was 0.177-0.200 mm. The eggshell thickness had the same relative value because the treatment diets have relatively similar Ca and P contents. The quality of eggshell thickness can be influenced by many factors including minerals; calcium, magnesium and phosphorus are the major inorganic constituents of eggshell (King'ori, 2011). According to Kebreab *et al.* (2009), the higher calcium intake can improve the quality of the eggshell.

Supplementation of choline chloride at 1500 ppm in the diet containing different methionine concentrations did not affect the Haugh unit with the average value of 85.59-93.53. Choline chloride and methionine levels did not affect the freshness of eggs produced. Haugh unit value was not different because each egg was stored in the same place and time. Factors affecting the value of Haugh units were time and place of egg storage, age, strain of livestock, nutrients, disease, and supplementation (vitamin C or E) (Ahmadi & Rahmini, 2011). According to El-Husseiny *et al.* (2005), shell weight and Haugh unit were not influenced by dietary methionine up to 0.50%, choline up to 900 mg/kg, and their combinations. Further results by Omara (2012) reported that the dietary combination of protein, methionine and choline did not significantly affect Haugh unit.

Supplementation of choline chloride at 1500 ppm in diets containing different methionine did not affect yolk color score. Yolk color was influenced by xanthophylls

in diet. Leeson & Summers (2005) reported that feed ingredients rich in xanthophylls are corn and corn gluten meal (CGM). The average score of the yolk during the study ranged from 7.94-8.39. The yolk score was not different because the amount of CGM and corn in the diet were the same.

## CONCLUSION

Supplementation of choline chloride at 1500 ppm in low methionine diet (A2B1) increased egg production, egg mass, and egg weight without negative effect on egg quality. Choline chloride at 1500 ppm could replace 75.95% dietary DL- methionine of egg laying quail.

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