Revista Brasileira de Ciência Avícola
ISSN: 1516-635X
revista@facta.org.br
Fundação APINCO de Ciência e Tecnologia Avícolas
Brasil

Ratriyanto, A; Indreswari, R; Nuhriawangsa, AMP

Effects of Dietary Protein Level and Betaine Supplementation on Nutrient Digestibility and Performance of Japanese Quails

Revista Brasileira de Ciência Avícola, vol. 19, núm. 3, julio, 2017, pp. 445-453
Fundação APINCO de Ciência e Tecnologia Avícolas
Campinas, Brasil

Brazilian Journal of Poultry Science
Revista Brasileira de Ciência Avícola

ISSN 1516-635X Jul - Sept 2017 / v.19 / n.3 / 445-454

http://dx.doi.org/10.1590/1806-9061-2016-0442

Effects of Dietary Protein Level and Betaine Supplementation on Nutrient Digestibility and Performance of Japanese Quails

Author(s)

Ratriyanto A[I]
Indreswari R[I]
Nuhriawangsa AMP[I]

[I] Department of Animal Science, Faculty of Agriculture, Sebelas Maret University, Jl. Ir. Sutami 36A, Surakarta, Indonesia 57126

Note: Part of the data presented here were presented at the 6th International Seminar on Tropical Animal Production in Yogyakarta, Indonesia (ISTAP 2015).

Mail Address

Corresponding author e-mail address
Adi Ratriyanto
Department of Animal Science, Faculty of Agriculture, Sebelas Maret University, Jl. Ir. Sutami 36A, Surakarta, Indonesia 57126
Tel: +62-271-637457
Email: ratriyanto@staff.uns.ac.id

Keywords

Betaine, digestibility, laying Japanese quails, performance, protein.

Submitted: 04/December/2016
Approved: 11/April/2017

ABSTRACT

This study investigated the effects of dietary protein levels and betaine supplementation on nutrient digestibility and performance of Japanese quails. In total, 765 Japanese quails were randomly assigned to a 3×3 factorial arrangement, with five replicates of 17 quails each. Three basal diets were formulated to contain three crude protein levels (16.5, 18.0, and 19.5%). Each protein level was supplemented with 0, 0.06, and 0.12% betaine. The diet with 16.5% dietary crude protein with no betaine supplementation resulted in the lowest crude fiber digestibility, while the 18.0% CP diet supplemented with 0.12% betaine generated the highest crude fiber digestibility ($p<0.05$). The diets with 18.0 and 19.5% crude protein increased crude fiber digestibility, but reduced ether extract digestibility ($p<0.01$). Moreover, betaine supplementation increased dry matter, crude protein, crude fiber, and crude ash ($p<0.01$) digestibility and tended to increase ether extract digestibility (p=0.09). The increase in egg weight for the 18.0 and 19.5% protein diets was correlated with a decrease in feed conversion ratio ($p<0.05$). However, feed intake and egg production were not affected by protein levels. Betaine supplementation enhanced all performance variables ($p<0.01$). The diets with 18.0 and 19.5% crude protein resulted in heavier yolks and eggshells than the 16.5% crude protein diet ($p<0.05$), whereas betaine supplementation increased yolk, albumen, and eggshell weight ($p<0.01$). The 18.0 and 19.5% protein diets produced similar responses in most evaluated parameters. Laying Japanese quails can be fed diets with 18.0% crude protein. Moreover, betaine supplementation provided several benefits, and particularly improved nutrient digestibility, performance, and egg quality.

INTRODUCTION

Layers are susceptible to heat stress because egg formation results in a high level of metabolic heat production (Blem, 2000). Moreover, the high ambient temperature in the tropics may limit protein synthesis, negatively affecting poultry production (Rashid *et al*., 2012). Protein-rich diets do not have a positive impact on poultry performance in hot climates and may even depress performance due to the high heat increment of protein digestion (Daghir, 2009). Although many studies have been performed to evaluate the effects of dietary crude protein (CP) in layers, the responses have been inconsistent among studies, possibly due to differences in experimental conditions, bird strains and age, laying period, and parameters evaluated (Li *et al*., 2013; Ding *et al*., 2016). Previous studies demonstrated that dietary CP influences laying performance and egg quality (Nahashon *et al*., 2007; Gunawardana *et al*., 2008; Li *et al*., 2013). Under tropical conditions, Musa *et al*. (2008) recommended 18% CP for laying quails, which is lower than the 20% recommended by the NRC (1994). Garcia *et al*. (2005) observed that, in quails, increasing dietary CP from 16% to 18% improved egg production and feed conversion ratio, while increasing dietary CP to 20% did not result in the further performance improvements. According to Junqueira *et al*. (2006), for brown hens, feeding with 16% CP was adequate and resulted in better performance and egg quality than 18% and 20% CP.

Betaine is a trimethyl derivative of the amino acid glycine. It is a methyl group donor, donating its labile methyl group (CH_3), and plays an important role in the metabolism of protein and energy (Metzler-Zebeli *et al*., 2009; Ratriyanto *et al*., 2009a). Because poultry cannot synthesize the methyl group, it must be supplied in their diet. Unlike other methyl group donors, such as choline and methionine, betaine can act directly as a methyl group donor. The methyl groups of choline become available when choline is oxidized to betaine, while methionine must be converted to S-adenosylmethionine to donate its methyl group (Ratriyanto *et al*., 2009a). Betaine has the potential to substitute for some part of methionine in the diet by providing a methyl group during the conversion of homocysteine to methionine (Metzler-Zebeli *et al*., 2009). Consequently, betaine may improve methionine availability for protein synthesis, thus achieving optimal performance (Rao *et al*., 2011). Moreover, due to its osmotic properties, betaine has the potential to improve nutrient digestibility by supporting the growth and survival of intestinal cells, as well as intestinal microbes (Kettunen *et al*., 2001; Ratriyanto *et al*., 2009a). It has been shown that betaine supplementation alters the microbial population of the digestive tract of poultry (Kettunen *et al*., 1999). Dietary betaine may protect intestinal cells and intestinal microbes from osmotic variation along the digestive tract (Ratriyanto *et al*., 2009a).

Furthermore, there are only few studies evaluating dietary CP levels and betaine supplementation in relation to nutrient digestibility in poultry, particularly in laying quails, even though dietary CP levels or betaine supplementation affect nutrient digestibility in quails (Li *et al*., 2011; Ratriyanto *et al*., 2012). Therefore, the objective of this study was to determine the effects of dietary CP levels and betaine supplementation on nutrient digestibility and performance in quails.

MATERIAL AND METHODS

In total, 765 42-day-old female Japanese quails (*Coturnix coturnix japonica*), with an average body weight of 126.34 ± 7.29 g, were used in the study. The birds were randomly distributed according to a completely randomized experimental design in a 3 × 3 factorial arrangement: CP levels (16.5, 18.0, and 19.5%) and betaine supplementation (0, 0.06, and 0.12%). Each treatment included five replicates of 17 quails each.

The birds were reared in 45 colony battery cages (75×50 cm, one cage per replicate). A uniform managemental practice was applied to all the experimental groups.

The three basal diets were formulated to contain the same amount of metabolizable energy: 2,800 kcal/kg (Table 1). Betaine (anhydrous betaine 96%) was supplemented in the basal diets at the expense of corn, according to the procedure of Ratriyanto *et al.* (2009b).

Table 1 – Ingredient composition (%) and calculated nutrient content of the basal diets.

Ingredients	Protein Levels		
	16.5%	18.0%	19.5%
Yellow corn	45.63	45.50	44.12
Rice bran	21.43	18.04	15.00
Soybean meal	18.15	20.20	24.15
Fishmeal	5.00	6.75	7.00
Coconut oil	1.28	1.37	1.80
DL-methionine	0.08	0.05	0.03
L-lysine HCl	0.18	0.09	0.00
Dicalcium phosphate	1.65	1.50	1.40
Limestone	5.90	5.80	5.80
Premix*	0.35	0.35	0.35
NaCl	0.35	0.35	0.35
Nutrient contents			
Metabolizable energy (kcal/kg)	2800.50	2800.20	2800.10
Crude protein (%)	16.50	18.01	19.51
Ether extracts (%)	5.09	4.85	4.60
Crude fiber (%)	4.58	4.24	4.03
Crude ash (%)	5.00	5.36	5.49
Calcium (%)	3.40	3.41	3.40
Available phosphorus (%)	0.61	0.63	0.62
Lysine (%)	1.13	1.14	1.14
Methionine (%)	0.41	0.41	0.41
Cystine (%)	0.29	0.30	0.31
Methionine+cystine (%)	0.70	0.71	0.72

* The premix supplied the following per kilogram diets: 42,000 IU vitamin A; 7,000 IU vitamin D_3; 28 mg vitamin E; 7 mg vitamin K; 7 mg vitamin B1; 18 mg vitamin B2; 2 mg vitamin B6; 42 mg vitamin B12; 88 mg vitamin C; 21 mg calcium *D*-pantothenate; 140 mg niacin; 35 mg choline chloride; 420 mg manganese; 70 mg iron; 0.7 mg iodine; 350 mg zinc; 0.7 mg cobalt; 14 mg copper, 35 mg antioxidant (santoquin).

The birds were housed under natural temperature conditions, with an average ambient temperature during experiment at 25.4 ºC in the morning, 32.9 ºC in the afternoon, and 28.7 ºC in the evening. During the pre-experimental period (42 days until 50% hen day average - HDA), the birds were fed the same diet, which contained 18% CP. The dietary treatments started to be applied after egg production reached 50% HDA. Water and feed were provided *ad libitum*. The treatment diets were fed for two periods of 28 days each (2×28 days). Feed intake, egg production, and egg weight were recorded daily. Feed conversion ratio (FCR) was calculated as the ratio of feed intake per egg mass (Junqueira *et al*., 2006). Protein efficiency ratio (PER) and energy efficiency ratio (EER) were also calculated for each experimental period. PER was calculated as grams of egg mass per gram of protein intake, and EER was calculated as grams of egg mass×100/total ME intake (Suprijatna *et al*., 2009). Physical egg quality was assessed during the last three days of each period (days 26, 27, and 28). In total, 405 eggs from each period (nine eggs per replicate) were randomly collected to evaluate yolk weight, albumen weight, and eggshell weight and thickness. The eggs were weighed and cracked, and the egg components were weighed thereafter. Eggshell thickness was measured with a digital micrometer (0-25 × 0.001 mm).

At the end of experiment, 90 birds (two per replicate) were randomly selected to determine nutrient digestibility. The digestibility trial was performed over a 5-day total excreta collection, following the procedure proposed by Ratriyanto *et al*. (2014a). During the collection period, the excreta were periodically spread with 0.2 N H_2SO_4 to minimize further bacterial fermentation. The excreta collected during the 5 days were dried under the sun. The samples of excreta for the various diets were milled through a 0.5 mm mesh screen prior to analysis. Excreta CP content was determined by the Kjeldahl method, while dry matter (DM), ether extract (EE), crude fiber (CF), and crude ash (CA) analyses were performed as outlined by the AOAC (1990). The nutrient digestibility coefficients were calculated according to Emamzadeh & Yaghobfar (2009).

The data were checked for outliers and tested to ensure the homogeneity of variances (Bartlett test). Then, the data were submitted to analysis of variance using the R statistic (R Core Team, 2015). Statistically different means were compared via Duncan's multiple-range test at 5% probability.

RESULTS AND DISCUSSION

Nutrient Digestibility

There was no influence of the interaction between protein levels and betaine supplementation on nutrient digestibility (Table 2), except for CF. The16.5% CP diet without betaine supplementation resulted in lower CF digestibility than the other dietary treatments ($p<0.05$). Also, for the three CP levels, 0.12% betaine supplementation increased CF digestibility, which ranged between 12.87 and 26.89%, as compared with the non-supplemented diets ($p<0.05$). Regardless of the CP level in the diets, these findings indicate that betaine supplementation improved CF digestibility possibly due to stimulation of microbial fermentation in the digestive tract (Ratriyanto *et al*., 2014a).

Dietary CP levels did not affect the digestibility of DM, CP, CA, or nitrogen free extracts (NFE). This result is in agreement with Li *et al*. (2011), who did not find any effect of dietary CP levels (17.75 or 19.95% CP) on organic matter (OM) digestibility in yellow quails. Feeding 17 and 20% CP did not affect CP digestibility in broilers either (Faria Filho *et al*., 2007). In Japanese quails, during the rearing period, Omidiwura *et al*. (2016) did not find any effect of CP levels on the digestibility of DM and CP.

In the present experiment, dietary 18.0 and 19.5% CP decreased ($p<0.01$) EE digestibility by 7.10 and 7.56%, respectively, as compared with 16.5% CP, indicating better EE utilization at lower dietary CP levels. According to Rosebrough *et al*. (1999), dietary CP levels modulate the metabolic effects of dietary fat, as increasing dietary CP reduces lipogenesis and the levels of precursors of fat synthesis, which may explain the decrease in the digestibility coefficient of EE with increasing dietary CP levels in the present experiment. Moreover, dietary 18.0 and 19.5% CP improved ($p<0.01$) CF digestibility by 7.12 and 6.45%, respectively, as compared with 16.5% CP, indicating more efficient CF degradation and absorption (Faria Filho *et al*., 2007). This result was in agreement with the previous observation that CF digestibility increased as dietary CP increased (Ratriyanto *et al*., 2014a). In addition, according to Li *et al*. (2011), the effect of CP level on nutrient digestibility can be attributed to an imbalance between protein and energy in the diet.

The supplementation of 0.06 and 0.12% betaine improved DM, CP, CF, and CA digestibilities ($p<0.05$) and tended to improve EE digestibility ($p=0.09$), which is in agreement with previous studies with quails (Ratriyanto *et al*., 2012). Similarly, dietary betaine supplementation was also shown to improve the digestibility of CP (Ezzat *et al*., 2011; Attia *et al*., 2016) and EE in laying chickens (Ezzat *et al*., 2011). Moreover, studies with broilers have revealed that betaine improved DM (Ratriyanto *et al*., 2014a; Amerah & Ravindran, 2015), OM (El-Husseiny *et al*., 2007), CP, CF (El-Husseiny *et al*., 2007; Ratriyanto *et al*., 2014a), EE, and NFE digestibilities (El-Husseiny *et al*., 2007). These results indicate that betaine supplementation promotes better nutrient utilization, as confirmed by the improvement in production performance in the present experiment (Table 3).

There is growing evidence that betaine supplementation improves nutrient digestibility due to its osmoprotective properties, supporting intestinal cells and the growth of intestinal microbes (Ratriyanto *et al*., 2009a; Ratriyanto *et al*., 2010). Previous reports have shown that betaine decreased crypt:villus height ratio in broilers, which may partially explain the increased digestibility of nutrients (Kettunen *et al*., 2001; Ratriyanto *et al*., 2014b). Moreover, because poultry do not produce fiber-degrading enzymes, the improvement in CF digestibility indicates that betaine has the potential to stimulate the bacterial fermentation of dietary fiber (Ratriyanto *et al*., 2010; Ratriyanto 2014a). These results confirm previous observation that intestinal bacteria require compatible osmolytes, such as betaine (Ratriyanto *et al*., 2010). The higher CF digestibility due to betaine supplementation corresponded to higher CA digestibility. Previous studies have shown the interaction between fiber fermentation and mineral absorption (Ohta *et al*., 1995; Aulrich & Flachowsky, 1997). Obviously, the fiber fraction holds nutrients that can be released during fiber degradation (Aulrich & Flachowsky, 1997). Higher mineral absorption, as indicated by improved CA digestibility, supports the previous observations that betaine reduces the osmolality of the digesta of chickens (Klasing *et al*., 2002; Hamidi *et al*., 2010).

Production Performance

There was no significant interaction between dietary CP levels and betaine supplementation levels for the evaluated performance parameters. Dietary CP levels did not affect feed intake or egg production, but the19.5% CP diet improved feed conversion ratio ($p<0.05$). These results are in agreement with those of Dos Santos *et al*. (2016), who did not find any significant effect on feed intake or egg production of quails fed 16 or 20% CP. In addition, previous observations in Japanese quails determined that increasing dietary CP from 17 to 20% (Tuleun *et al*., 2013) or from 15 to 20% (Muhammad *et al*., 2016) did not affect egg production, but did improve feed conversion ratio. A recent study showed that increasing dietary CP from 18 to 24% did not affect egg production, egg weight, or feed conversion ratio in Japanese quails (Agboola *et al*., 2016). On the other hand, Azghadi *et al*. (2014) found

Table 2 – Nutrient digestibility in Japanese quails (%) fed diets with varying levels of crude protein and betaine.

Treatments		Dry Matter	Crude Protein	Ether Extracts	Crude Fiber	Crude Ash	Nitrogen Free Extracts
Interaction effects between protein (%) and betaine (%)							
16.5	0	70.14	71.80	89.65	27.46^{d}	46.72	85.25
16.5	0.06	73.31	73.05	90.25	33.18^{bc}	52.91	81.32
16.5	0.12	78.08	80.74	90.68	36.41^{ab}	61.92	81.14
18.0	0	69.42	69.25	81.62	31.60^{c}	49.26	82.82
18.0	0.06	74.54	74.20	83.80	35.32^{abc}	54.97	82.03
18.0	0.12	74.17	76.00	85.95	37.56^{a}	61.73	80.43
19.5	0	70.65	68.18	81.44	31.67^{c}	50.76	82.78
19.5	0.06	74.03	71.05	83.55	35.73^{abc}	55.17	84.06
19.5	0.12	75.72	76.36	85.12	36.35^{ab}	60.70	83.18
SEM		0.41	0.26	0.31	0.51	0.63	0.67
p value		0.52	0.87	0.72	0.03	0.65	0.18
Effects of protein (%)							
16.5		73.84	75.20	90.19^{a}	32.35^{b}	53.85	82.57
18.0		72.71	73.15	83.79^{b}	34.83^{a}	55.32	81.76
19.5		73.46	71.86	83.37^{b}	34.58^{a}	55.55	83.34
SEM		0.15	1.24	1.82	0.91	0.69	0.31
p value		0.76	0.09	<0.01	<0.01	0.47	0.53
Effects of betaine (%)							
0		70.07^{b}	69.74^{b}	84.23	30.24^{c}	48.91^{c}	83.62
0.06		73.96^{a}	72.77^{b}	85.87	34.74^{b}	54.35^{b}	82.47
0.12		75.99^{a}	77.70^{a}	87.25	36.77^{a}	61.45^{a}	81.58
SEM		1.74	1.49	0.87	1.23	1.15	0.59
p value		<0.01	<0.01	0.09	<0.01	<0.01	0.26

^{a,b,c} Means in the same column and treatment with no common superscript differ significantly ($p<0.05$).

that increasing dietary CP from 18 to 22% increased egg production, but did not affect feed intake, egg weight, or feed conversion ratio. In addition, Garcia *et al*. (2005) observed that feeding with 18% CP resulted in the best performance of laying quails.

In the present study, dietary 18.0 and 19.5% CP resulted in higher egg weight compared with 16.5% CP ($p<0.05$), which may be attributed to the fact that birds fed 18.0 and 19.5% CP consumed more protein than those fed the 16.5% CP diet. It is well-established that protein is the main nutrient required for egg formation (Ding *et al*., 2016). Egg weight is highly correlated with daily CP intake because laying hens are not able to store large amounts of protein (Li *et al*., 2011). Moreover, feed intake and CP levels are important in controlling protein intake (Soares *et al*., 2003). The discrepancies in the results obtained by different studies may be due to differences in experimental conditions and diets.

The observed increase ($p<0.01$) in egg mass in birds fed 18.0 and 19.5% CP compared with those fed a 16.5% CP diet may be due to higher egg weight, and not to higher egg production, which was not significantly influenced by dietary CP level. Consequently, the diets containing 18.0 and 19.5% CP were more effective in improving egg mass production. In addition, the egg mass obtained with the 18.0% CP diet was similar to that observed with the 19.5% CP diet. According to Rashid *et al*. (2012), feeding poultry reared in hot climates with high protein levels is not recommended because of the high heat increment resulting from protein digestion. The egg mass results of the present study are in line with those of Azghadi *et al*. (2014), who did not observe any increase in egg mass of Japanese quails when dietary CP levels were increased from 18 to 22%. In laying chickens, previous observations indicated that increasing dietary CP from 14 to 16% increased egg mass (Park & Ryu, 2011).

The reduction ($p<0.05$) of PER with increasing dietary CP levels may be attributed to higher CP intake (Dos Santos *et al*. 2016), as high-protein diets increase protein excretion, resulting in lower PER. This result is in agreement with previous findings (Li *et al*., 2011; Ratriyanto *et al*., 2014b; Dos Santos *et al*., 2016).

The 19.5% CP diet increased EER compared with the 16.5% CP ($p<0.05$). Because the birds were fed isocaloric diets, this result is explained by the higher egg mass produced by the birds fed 19.5% CP.

Table 3 – Production performance of Japanese quails fed diets with varying levels of crude protein and betaine.

Treatments		FI (g)	EP (%)	EW (g)	EM (g)	FCR	PER	EER
Interaction effects between protein (%) and betaine (%)								
16.5	0	19.08	62.77	8.45	5.25	3.60	1.69	9.95
16.5	0.06	20.05	71.70	8.72	6.25	3.21	1.89	11.16
16.5	0.12	20.69	71.68	9.02	6.47	3.20	1.89	11.16
18.0	0	19.96	64.16	8.57	5.54	3.64	1.53	9.85
18.0	0.06	20.80	71.92	8.84	6.38	3.28	1.70	10.91
18.0	0.12	20.68	75.29	9.07	6.83	3.03	1.83	11.79
19.5	0	19.69	66.48	8.50	5.73	3.51	1.47	10.23
19.5	0.06	20.38	70.58	9.06	6.41	3.19	1.61	11.22
19.5	0.12	21.28	75.77	9.09	6.83	3.09	1.66	11.56
SEM		0.04	0.70	0.04	0.14	0.06	0.01	0.06
p value		0.93	0.48	0.21	0.40	0.35	0.35	0.47
Effects of protein (%)								
16.5		19.94	68.72	8.73[b]	5.99[b]	3.35[a]	1.82[a]	10.72[b]
18.0		20.48	70.46	8.83[a]	6.25[a]	3.30[ab]	1.69[b]	10.89[ab]
19.5		20.45	70.94	8.88[a]	6.34[a]	3.24[b]	1.59[c]	11.07[a]
SEM		0.21	0.95	0.06	0.51	0.05	0.09	0.14
p value		0.13	0.25	0.04	<0.01	0.02	<0.01	0.03
Effects of betaine (%)								
0		19.58[b]	64.47[b]	8.51[a]	5.51[c]	3.56[a]	1.57[c]	10.01[c]
0.06		20.41[a]	71.40[a]	8.87[b]	6.35[b]	3.22[b]	1.74[b]	11.10[b]
0.12		20.89[a]	74.25[a]	9.06[c]	6.73[a]	3.11[c]	1.80[a]	11.50[a]
SEM		0.38	0.89	0.16	1.27	0.14	0.07	0.43
p value		<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01

FI: feed intake; EP: egg production; EW: egg weight; EM: egg mass; FCR: feed conversion ratio; PER: protein efficiency ratio; EER: energy efficiency ratio.

[a,b,c] Means in the same column and treatment with no common superscript differ significantly ($p<0.05$).

The dietary supplementation with 0.06 and 0.12% betaine improved the production performance of quails ($p<0.05$), which is in accordance with previous observations in laying chickens (Lu & Zou, 2006; Park *et al*., 2006; Ezzat *et al*., 2011; Gudev *et al*., 2011). Moreover, except for feed intake and egg production, the dietary supplementation with 0.12% betaine promoted better responses compared with 0.06% betaine ($p<0.05$). Compared with the diet with no betaine, the higher feed intakes observed when birds were fed 0.06 and 0.12% betaine ($p<0.01$) were correlated with 10.45 and 15.17% higher egg production, and 4.23 and 6.46% higher egg weights, respectively.

The performance improvement obtained with the dietary supplementation of betaine has been previously described and may be attributed to its osmotic properties and its role as a methyl group donor (Metzler-Zebeli *et al*., 2009; Ratriyanto *et al*., 2009a; Ezzat *et al*., 2011). Kettunen *et al*. (2001) observed that betaine increased the length of the small intestine and reduced the crypt:villi ratio of broilers, indicating a larger surface area for nutrient absorption, resulting in better live performance. Moreover, betaine is involved in the synthesis of metabolically-active substances and in protein and energy metabolism (Metzler-Zebeli *et al*., 2009). This was confirmed by the enhanced PER and EER in this study, indicating better protein and energy utilization. In addition, betaine stimulates the anterior pituitary to secrete follicle-stimulating hormone and luteinizing hormone. These hormones promote follicle growth and ovulation, increasing egg production (Zou & Feng, 2002; Xing & Jiang, 2012). Previous observations indicated that betaine supplementation in the diets of laying hens increased egg production (Lu & Zou, 2006; Ezzat *et al*., 2011; Gudev *et al*., 2011) and egg weight (Park *et al*., 2006), and improved feed conversion ratio (Ezzat *et al*., 2011). In contrast, Harms & Russel (2002) did not find any effect of betaine on the performance of laying hens that were fed a diet containing adequate choline and methionine as additional sources of methyl group donors. In addition, Park & Ryu (2011) showed that 0.06% betaine supplementation did not affect the performance of laying hens.

Egg Quality

The interaction between protein levels and betaine supplementation influenced yolk weight (Table 4), but not the other evaluated egg-quality parameters. For the three CP levels, betaine supplementation increased yolk weight ($p<0.01$). Regardless of CP level, this finding indicates that betaine improved the synthesis of yolk precursors, such as very-low-density lipoproteins and vitellogenin, as described previously by Lu & Zou (2006).

The diets with 18.0 and 19.5% CP increased yolk and eggshell weight ($p<0.01$), compared with the 16.5% CP diet, and these results may be attributed to higher egg weight in those groups. Akbar *et al*. (1983) also observed that higher dietary CP levels increased yolk percentage in laying hens. Previous studies showed that increasing dietary CP from 16 to 20% did not affect albumen, yolk, or eggshell weight (Garcia *et al*., 2005; Abdel-Azeem, 2011), neither eggshell thickness in quails (Abdel-Azeem, 2011). In addition, Agboola *et al*. (2016) reported that increasing dietary CP from 18 to 24% did not affect yolk or albumen weight or eggshell thickness in quails. A similar result was obtained by Muhammad *et al*. (2016), who observed that dietary CP did not affect yolk or albumen weight in quails.

The observed increase ($p<0.01$) in the weight of egg components, such as the yolk, albumen, and eggshell with betaine supplementation was correlated with higher egg weight. These results may be attributed to the methyl donor function of betaine, which is involved in protein and energy metabolism (Ratriyanto *et al*., 2009a). The higher albumen weight when betaine was supplemented suggests an increase in protein synthesis, was in agreement with previous studies showing that betaine supplementation improved protein synthesis (Apicella *et al*., 2013), which increased albumen weight (Joseph *et al*., 2000). Moreover, the 4.26 and 5.18% increases in eggshell weight determined with the supplementation of 0.06 and 0.12% betaine, respectively, may be due the higher availability of minerals for eggshell formation, which is related to improved CA digestibility. However, eggshell thickness was not affected by betaine supplementation in this study, which was in line with previous observations in laying hens (Park & Ryu, 2011) and ducks (Awad *et al*., 2014).

CONCLUSIONS

Dietary protein levels had minor effects on nutrient digestibility coefficients. The diets containing 18.0 and 19.5% crude protein promoted similar production performance, which was better than that obtained with the 16.5% crude protein diet, independently of dietary betaine level. The dietary supplementation of 0.06 and 0.12% betaine improved nutrient

Table 4 – Egg quality of Japanese quails fed diets with varying levels of crude protein and betaine.

Treatments		Yolk Weight (g)	Albumen Weight (g)	Shell Weight (g)	Shell Thickness (mm)
Interaction effects between protein (%) and betaine (%)					
16.5	0	2.52[c]	5.40	0.88	0.189
16.5	0.06	2.58[bc]	5.59	0.84	0.198
16.5	0.12	2.75[ab]	5.79	0.82	0.194
18.0	0	2.51[c]	5.40	0.86	0.199
18.0	0.06	2.76[ab]	5.77	0.89	0.195
18.0	0.12	2.84[a]	5.82	0.85	0.198
19.5	0	2.49[c]	5.26	0.92	0.192
19.5	0.06	2.83[a]	5.78	0.93	0.194
19.5	0.12	2.81[a]	5.73	0.86	0.196
SEM		0.03	0.05	0.00	0.01
p value		<0.01	0.19	0.13	0.15
Effects of protein (%)					
16.5		2.61[b]	5.59	0.85[b]	0.194
18.0		2.70[a]	5.66	0.87[ab]	0.197
19.5		2.71[a]	5.59	0.91[a]	0.194
SEM		0.04	0.02	0.02	0.01
p value		<0.01	0.56	<0.01	0.78
Effects of betaine (%)					
0		2.51[c]	5.35[b]	0.87[b]	0.193
0.06		2.72[b]	5.71[c]	0.91[a]	0.196
0.12		2.81[a]	5.78[a]	0.91[a]	0.196
SEM		0.09	0.10	0.01	0.01
p value		<0.01	<0.01	<0.01	0.14

[a,b,c] Means in the same column and treatment with no common superscript differ significantly ($p<0.01$).

digestibility, production performance and egg quality, independently from dietary protein level. Therefore, the findings of this experiment show that Japanese quails can be fed diets contain 18.0% crude protein and supplemented with betaine.

ACKNOWLEDGMENTS

The authors wish to thank the Directorate General for Higher Education of the Republic Indonesia for financial support of this work. We are grateful to J. Haryadi, M.S. Giri, R.A. Setyobudi, S. Kusharini, A.E. Haryanti, S.N. Hikmah, and R.D. Cahyanti for their excellent work during the feeding trial of this study.

REFERENCES

Abdel-Azeem F. Influence of qualitative feed restriction on reproductive performance of Japanese quail hens. Egyptian Poultry Science Journal 2011;31:883-897.

Agboola AF, Omidiwura BRO, Ologbosere DY, Iyayi EA. Determination of crude protein and metabolisable energy of Japanese quail (*Coturnix coturnix japonica*) during laying period. Journal of World Poultry Research 2016;6(3):131-138.

Akbar MK, Gavora JS, Friars GW, Gowe RS. Composition of eggs by commercial size categories: Effects of genetic group, age and diet. Poultry Science 1983;62:925-933.

Amerah AM, Ravindran V. Effect of coccidia challenge and natural betaine supplementation on performance, nutrient utilization, and intestinal lesion scores of broiler chickens fed suboptimal level of dietary methionine. Poultry Science 2015;94:673-680.

AOAC. Official methods of analysis. 15th ed. Arlington: Association of Official Analytical Chemists;1990.

Apicella JM, Lee EC, Bailey BL, Saenz C, Anderson JM, Craig SA, Kraemer WJ, Volek JS, Maresh CM. Betaine supplementation enhances anabolic endocrine and Akt signaling in response to acute bouts of exercise. European Journal of Applied Physiology 2013;113:793-802.

Attia YA, Abd-El-Hamid E, Abedalla AA, Berika MA, Al-Harthi MA, Kucuk O, Sahin K, Abou-Shehema BM. Laying performance, digestibility and plasma hormones in laying hens exposed to chronic heat stress as affected by betaine, vitamin C, and/or vitamin E supplementation. SpringerPlus 2016;5:1619-1631.

Aulrich K, Flachowsky G. Wirksamkeit NSP-spaltender enzyme - in vitro -studien an modellfuttermitteln. Proceedings of the 6th Symposium Vitamine und Zusatzstoffe in der Ernaehrung von Mensch und Tier; 1997 Sep 24-25; Jena (GER): Weimar Kessler GmbH; 1997. p.322-328.

Awad AL, Fahim HN, Ibrahim AF, Beshara MM. Effect of dietary betaine supplementation on productive and reproductive performance of domyati ducks under summer conditions. Egyptian Poultry Science Journal 2014;34:453-474.

Azghadi MA, Kermanshahi H, Golian A. The effect of dietary energy and protein levels on growth performance and antibody responses of offspring of laying Japanese quails. Iranian Journal of Applied Animal Science 2014;4:185-190.

Blem CR. Energy balance. In: Whittow GC, editor. Sturkie's avian physiology. 5th ed. San Diego: Academic Press; 2000. p. 327–341.

Daghir NJ. Nutritional strategies to reduce heat stress in broilers and broiler breeders. Lohmann Information 2009;44:6-15.

Ding Y, Bu X, Zhang N, Li L, Zou X. Effects of metabolizable energy and crude protein levels on laying performance, egg quality and serum biochemical indices of Fengda-1 layers. Animal Nutrition 2016;2:93-98.

Dos Santos GC, Garcia EA, Filho JAV, Molino ADB, Pelicia K, Berto DA. Performance of Japanese quails fed diets with low-protein and isoleucine. Acta Scientiarum, Animal Sciences 2016;38:219-225.

El-Husseiny OM, Abo-El-Ella MA, Abd-Elsamee MO, Ab-Elfattah MM. Response of broiler chick performance to dietary betaine and folic acid at different methionine levels. International Journal of Poultry Science 2007;6:515-525.

Emamzadeh AN, Yaghobfar A. Evaluation of protein digestibility in canola meals between caecectomised and intact adult cockerels. World Academy of Science, Engineering and Technology 2009;57:113-115.

Ezzat W, Shoeib MS, Mousa SMM, Bealish AMA, Ibrahiem ZA. Impact of betaine, vitamin C and folic acid supplementations to the diet on productive and reproductive performance of Matrouh poultry strain under Egyptian summer condition. Egyptian Poultry Science Journal 2011;31:512-537.

Faria Filho DE, Campos DMB, Alfonso-Torres KA, Vieira BS, Rosa PS, Vaz AM, Macari M, Furlan RL. Protein levels for heat-exposed broilers:performance, nutrients digestibility, and energy and protein metabolism. International Journal of Poultry Science 2007;6:187-194.

Garcia EA, Mendes AA, Pizzolante CC, Saldanha ESPB, Moreira J, Mori C, Pavan AC. Protein, methionine+cystine and lysine levels for Japanese quails during the production phase. Brazilian Journal of Poultry Science 2005;7:11-18.

Gudev D, Popova-Ralcheva S, Yanchev I, Moneva P, Petkov E, Ignatova M. Effect of betaine on egg performance and some blood constituents in laying hens reared indoor under natural summer temperatures and varying levels of air ammonia. Bulgarian Journal of Agricultural Science 2011;17:859-866.

Gunawardana P, Roland DA, Bryant MM. Effect of energy and protein performance, egg components, egg solids, egg quality, and profits in molted Hy-line W-36 hens. Journal of Applied Poultry Research 2008;17:432-439.

Hamidi H, Jahanian R, Pourreza J. Effect of dietary betaine on performance, immunocompetence and gut contents osmolarity of broilers challenged with a mixed coccidial infection. Asian Journal of Animal and Veterinary Advances 2010;5:193-201.

Harms RH, Russell GB. Betaine does not improve performance of laying hens when the diet contains adequate choline. Poultry Science 2002;81:99-101.

Joseph NS, Robinson FE, Korver DR, Renema RA. Effect of dietary protein intake during the pullet-to-breeder transition period on early egg weight and production in broiler breeders. Poultry Science 2000;79:1790-1796.

Junqueira MO, de Laurentiz AC, da Silva Filardi R, Rodrigues EA, Casartelli EM. Effects of energy and protein levels on egg quality and performance of laying hens at early second production cycle. Journal of Applied Poultry Research 2006;15:110-115.

Kettunen H, Tiihonen K, Peuranen S, Saarinen MT, Remus JC. Dietary betaine accumulates in the liver and intestinal tissue and stabilizes the intestinal epithelial structure in healthy and coccidia-infected broiler chicks. Comparative Biochemistry and Physiology Part A 2001;130:759-769.

Kettunen H, Peuranen S, Jatila H, Nurminen P, Saarinen M, Apajalahti J. Effect of betaine on the microbiology of the chicken gastrointestinal tract. Proceedings of the 12th European Symposium on Poultry Nutrition; 1999 Aug 15-19; Veldhoven (NL): World's Poultry Science Association; 1999. p 186.

Klasing KC, Adler KL, Remus JC, Calvert CC. Dietary betaine increases intraepithelial lymphocytes in the duodenum of coccidian-infected chicks and increases functional properties of phagocytes. The Journal of Nutrition 2002;132(8):2274-2282.

Li F, Zhang LM, Wu XH. Effects of metabolizable energy and balanced protein on egg production, quality, and components of Lohmann Brown laying hens. Journal of Applied Poultry Research 2013;22:36-46.

Li YX, Wang YQ, Pang YZ, Li JX, Xie XH, Guo TJ, Li WQ. The effect of crude protein level in diets on laying performance, nutrient digestibility of yellow quails. International Journal of Poultry Science 2011;10:110–112.

Lu JJ, Zou XT. Effects of adding betaine on laying performance and contents of serum yolk precursors VLDL and VTG in laying hen. Journal of Zhejiang University, Agriculture and Life Science 2006;32:287-291.

Metzler-Zebeli BU, Eklund M, Mosenthin R. Impact of osmoregulatory and methyl donor functions of betaine on intestinal health and performance in poultry. World's Poultry Science Journal 2009;65:419-441.

Muhammad N, Altine S, Abubakar A, Chafe UM, Saulawa LA, Garba MG, Yusuf A. Effect of varying protein levels and preservation methods on egg production performance and internal egg qualities of Japanese quails in a semi-arid environment. European Journal of Basic and Applied Science 2016;3:8-19.

Musa U, Haruna ES, Lombin LH. Quails production in the tropics. Vom (NGR): National Veterinary Research Institute Press; 2008.

Nahashon SN, Adefope A, Amenyenu A, Wright D. Effect of varying concentrations of dietary crude protein and metabolizable energy on laying performance of pearl grey guinea fowl hens. Poultry Science 2007;86:1793-1799.

NRC - National Research Council. Nutrient requirements of poultry. 9th ed. Washington: National Academic Press; 1994.

Ohta A, Ohtsuki M, Baba S, Takizawa T, Adachi T, Kimura S. Effects of fructooligosaccharides on the absorption of iron, calcium and magnesium in iron-deficient anaemic rats. Journal of Nutritional Science and Vitaminology 1995;41:281-291.

Omidiwura BRO, Odu O, Agboola AF, Akinbola DD, Iyayi EA. Crude protein and energy requirements of Japanese quail (*Coturnix coturnix japonica*) during rearing period. World Poultry Research 2016;6(2):99-104.

Park JH, Kang CW, Ryu KS. Effects of feeding betaine on performance and blood hormone in laying hens. Korean Journal of Poultry Science 2006;33:323-328.

Park JH, Ryu KS. Relationship between dietary protein levels and betaine supplementation in laying hens. Journal of Poultry Science 2011;48:217-222.

R Core Team. R: a language and environment for statistical computing. Vienna: R Foundation for statistical computing; 2015.

Rao SVR, Raju MVLN, Panda AK, Saharia P, Sunder GS. Effect of supplementing betaine on performance, carcass traits and immune responses in broiler chicken fed diets containing different concentration of methionine. Asian-Australasian Journal of Animal Science 2011;24:662-669.

Rashid HOS, Huwaida EEM, Ibrahim AY. Effect of dietary protein level and strain on growth performance of heat stressed broiler chicks. International Journal of Poultry Science 2012;11:649-653.

Ratriyanto A, Indreswari R, Dewanti R, Sofyan A. Nutrient digestibility and protein efficiency ratio in quails (*Coturnic coturnix japonica*) fed high methionine diet with betaine supplementation. Proceedings of the 4th National Conference on Sustainable Animal Agriculture Development; 2012; Bandung (ID): Faculty of Animal Science, Padjadjaran University; 2012. p.146-150.

Ratriyanto A, Indreswari R, Sunarto. Effects of protein levels and supplementation of methyl group donor on nutrient digestibility and performance of broiler chickens in the tropics. International Journal of Poultry Science 2014a;13:575-581.

Ratriyanto A, Indreswari R, Sunarto. The effect of protein levels and betaine supplementation on digestible nutrient and small intestine characteristic of broilers. Proceedings of the 6th National Conference on Sustainable Animal Agriculture Development. Bandung (ID): Faculty of Animal Science, Padjadjaran University; 2014b. p.1-8.

Ratriyanto A, Mosenthin R, Bauer E, Eklund M. Metabolic, osmoregulatory and nutritional functions of betaine in monogastric animals. Asian-Australasian Journal of Animal Science 2009a;22:1461-1476.

Ratriyanto A, Mosenthin R, Jezierny D, Eklund M. Effect of graded levels of dietary betaine on ileal and total tract nutrient digestibilities and intestinal bacterial metabolites in piglets. Journal of Animal Physiology and Animal Nutrition 2010;94:788-796.

Ratriyanto A, Mosenthin R, Jezierny D, Sauer N, Eklund M. Betaine, organic acids and inulin do not affect ileal and total tract nutrient digestibility or microbial fermentation in piglets. Journal of Animal and Feed Sciences 2009b;18:453-464.

Rosebrough RW, McMurtry JP, Vasilatos-Younken R. Dietary fat and protein interactions in the broiler. Poultry Science 1999;78:992-998.

Soares R. Fonseca JB, Santos AS, Mercandante MB. Protein requirement of Japanese quail (*Coturnix coturnix japonica*) during rearing and laying periods. Brazilian Journal of Poultry Science 2003;5:153-156.

Suprijatna E, Sunarti D, Mahfudz LJ, Ni'mah U. The efficiency of the use of protein for the egg production in quails fed low protein diet supplemented with lysine synthesis. Proceedings of the National Seminar on Animal Agriculture Development; 2009; Semarang (ID); 2009. p. 648-654.

Tuleun CD, Adenkola AY, Yenle FG. Performance and erythrocyte osmotic membrane stability of laying Japanese quails (*Coturnix coturnix japonica*) fed varying dietary protein levels in a hot-humid tropics. Agriculture and Biology Journal of North America 2013;4:6-3.

Xing J, Jiang Y. Effect of dietary betaine supplementation on mRNA level of lipogenesis genes and on promoter CpG methylation of fatty acid synthase (FAS) gene in laying hens. African Journal of Biotechnology 2012;11(24):6633-6640.

Zou XT, Feng J. Effect of betaine on performance of laying hens. Chinese Journal of Animal Science 2002;38:7-9.