ISSN 1682-8356
ansinet.org/ijps

INTERNATIONAL JOURNAL OF POULTRY SCIENCE

ANSInet
308 Lasani Town, Sargodha Road, Faisalabad - Pakistan
Mob: +92 300 3008585, Fax: +92 41 8815544
E-mail: editorijps@gmail.com

International Journal of Poultry Science 13 (10): 575-581, 2014
ISSN 1682-8356

Effects of Protein Levels and Supplementation of Methyl Group Donor on Nutrient Digestibility and Performance of Broiler Chickens in the Tropics

Adi Ratriyanto, Rysca Indreswari and Sunarto
Department of Animal Science, Faculty of Agriculture, Sebelas Maret University,
Jl. Ir. Sutami 36A, Surakarta-57126, Indonesia

Abstract: This study was conducted to investigate the effects of protein levels and supplementation of methyl group donors on nutrient digestibility and performance of broiler chickens. Three experimental diets were formulated to have three levels of crude protein (23, 21.5 and 20%). Each protein level was supplemented with methyl group donor (methionine or betaine) and was categorized by: without supplementation (control), with supplementation of 0.14% methionine, 0.14% betaine and combination of 0.14% methionine plus 0.14% betaine. The diets were applied to 540 broiler chickens, which were randomly assigned to 3 x 4 factorial arrangement. Dietary with 21.5 and 20% protein and the supplementation of methyl group donor increased the digestibility of dry matter, crude protein and crude fiber ($p<0.05$). Dietary with 23 and 21.5% protein resulted in a higher daily gain and a lower abdominal fat compared to that with 20% protein ($p<0.05$). All methyl group donor supplementations increased carcass and breast yield, while only supplementation with betaine and methionine plus betaine decreased abdominal fat deposition ($p<0.05$). Therefore, it is concluded that dietary with 21.5% protein can be applied to broiler chickens. Supplementation of methyl group donor provided benefits, particularly to improve carcass characteristics of broilers.

Key words: broiler, protein, methionine, betaine, nutrient digestibility, performance

INTRODUCTION

High ambient temperature in the tropics is a fundamental factor that obstructs poultry production due to the limited protein synthesis (Rashid *et al.*, 2012). Previous studies discovered that the decreased protein synthesis under high temperature cannot be restored by increasing the dietary protein level (Lin *et al.*, 2006; Moosavi *et al.*, 2011; Rashid *et al.*, 2012). Due to the high heat increment it has, part of the protein's decreased performance may be associated with the increased heat production (Daghir, 2009). Furthermore, the high nutrient content leads to inefficiency since many nutrients are not absorbed, but rather excreted from the body (Faria Filho *et al.*, 2007; Moosavi *et al.*, 2011).

Meanwhile, methyl group donor donates its labile methyl group (CH_3) and is involved in the metabolism of protein and energy (Metzler-Zebeli *et al.*, 2009; Ratriyanto *et al.*, 2009). Methyl group cannot be synthesized by chickens and have to be provided from the diet. Regarding the diets for livestock, methionine, betaine and choline are the most potential sources for preformed, transferable methyl groups (Ratriyanto *et al.*, 2009). However, livestock diets' methyl groups are not equally available since methionine is used for protein synthesis, while choline predominantly serves in the formation of cell membranes and neurotransmitters (Metzler-Zebeli *et al.*, 2009; Ratriyanto *et al.*, 2009). Moreover, betaine may be directly used as methyl group donor, while choline needs to be converted to betaine (Kidd *et al.*, 1997).

Protein and energy requirements are associated with the methionine in the diet due to its role as lipotrophic agents in poultry. Protein and methionine could increase protein deposition and decrease fat deposition as a result of methionine metabolites used in a variety of fundamental biological processes, including protein synthesis (Metzler-Zebeli *et al.*, 2009). However, the methionine's role in protein synthesis competes with its role as a methyl group donor for the formation of S-adenosylmethionine in the transmethylation reaction (Ratriyanto *et al.*, 2009). Therefore, other alternative methyl group donors (e.g., betaine) can substitute methionine or provide the required methyl group in converting homocysteine into methionine (Metzler-Zebeli *et al.*, 2009). The application of betaine as a methyl group donor may improve the availability of methionine for protein synthesis and optimal performance (Rao *et al.*, 2011). Moreover, there is a growing evidence that performance improvements may be associated with nutrient digestibility improvements (El-Husseiny *et al.*, 2007; Ratriyanto *et al.*, 2009; Ratriyanto *et al.*, 2012). Currently, there are only a small number of studies focusing on protein levels and methyl group donor supplementation in relation to nutrient digestibility in poultry, even though there is an evidence that protein levels, methionine or betaine affect nutrient digestibility in broilers (El-Husseiny *et al.*, 2007; Faria Filho *et al.*, 2007). Therefore, the objective of this study was to

Corresponding Author: Adi Ratriyanto, Department of Animal Science, Faculty of Agriculture, Sebelas Maret University, Jl. Ir. Sutami 36A, Surakarta-57126, Indonesia

investigate the effects of protein levels and supplementation of methyl group donors on nutrient digestibility and performance of broiler chickens.

MATERIALS AND METHODS

Animals and diets: In total, 540 one-day-old unsexed Lohmann broiler chickens were randomly allocated to the 12 dietary treatments with five replicates of nine chickens each. The arrangement of treatments corresponds to a 3 x 4 factorial design with three levels of protein and four supplementations of methyl group donor. The chickens were reared in 60 floor pens (1 x 1 m) covered with rice hulls.

The basal diet was formulated according to the standard of National Research Council or NRC (1994) except for the protein that contained 23, 21.5 and 20% and each protein level contained the same metabolizable energy of 3,200 KCal/kg (Table 1). Each protein level was fed without supplementation (control) or supplemented with methyl group donor consisting of 0.14% methionine, 0.14% betaine and combination of 0.14% methionine plus 0.14% betaine. The supplementation of methionine, betaine or combination of the two to the basal diets was performed at the expense of corn, following the procedure as done by Ratriyanto *et al.* (2010).

Performance trial: The chickens were housed under natural temperature condition until 35 days of age. The average ambient temperature during the experiment was 25.7°C in the morning, 32.9°C in the afternoon and 28.8°C in the evening. Water and feed were supplied *ad libitum*. Feed intake was recorded daily and body weight gain was recorded weekly. Feed conversion ratio (FCR) was calculated by measuring the ratio between feed intake and weight gain. Protein efficiency ratio (PER) was calculated by dividing the weight gain with protein consumption (Nasr *et al.*, 2011).

At the end of performance trial, two chickens per pen were randomly selected (ten per treatment) after 12 h fast, weighed and slaughtered for carcass characteristics measurement according to the procedure outlined by Sun *et al.* (2008). Blood samples were collected (two chickens per pen) from the wing vein and put into eppendorf tubes containing coagulant and centrifuged at 2,500 rpm for ten minutes. The samples were stored at -20°C before being analyzed for plasma total protein (TP), serum cholesterol, high density lipoprotein (HDL) and low density lipoprotein (LDL) using diagnostic kits (Boehringer-Ingelheim, Germany).

Digestibility trial: At the end of the experiment, 120 chickens (two per replicate) were randomly selected for a digestion trial to measure the nutrient digestibility of each experimental diet. The chickens were reared in individual cages and fed on the tested diet for five days collection period according the procedure as done by Attia *et al.* (2005). The chickens were fed experimental diets containing 0.4% Fe_2O_3 as an indicator to determine the start and the end of excreta collection (Marais, 2000; Indreswari *et al.*, 2009). At the end of collection period, the chickens were fed diets without indicator. Excreta collection was started when red color of the excreta appeared and terminated when the red color of the excreta disappeared (Indreswari *et al.*, 2009). During excreta collection, 2 mL 0.2 N H_2SO_4 was spread periodically on excreta to minimize further bacterial fermentation. The excreta were pooled and dried under the sun thereafter. Samples of diets and excreta were milled through a 1.0 mm mesh screen prior to analyses. Determination of dry matter (DM), crude protein (CP), ether extracts (EE), crude fiber (CF) and crude ash (CA) was performed as outlined by AOAC (1990).

Statistical analyses: Data were analyzed statistically by conducting analysis of Variance (ANOVA) with replicate as the statistical unit. Differences among means were tested using Duncan's multiple-range tests. The significance level was set at $\alpha = 0.05$. Significant differences between treatments were represented by different superscript letters using algorithm for letter based representation of all pair-wise comparisons (Piepho, 2004).

RESULTS AND DISCUSSION

Nutrient digestibility: Dietary with 21.5 and 20% protein improved ($p<0.05$) DM digestibility by 2.3 and 2.2% compared to dietary with 23% protein (Table 2). In accordance with this result, CP and CF digestibilities for dietary 21.5 and 20% protein were higher ($p<0.05$) compared to that with 23% protein, indicating more efficient nutrient degradation and absorption (Faria Filho *et al.*, 2007). However, digestibility of EE, CA and NFE was not affected by protein levels. These results confirm previous observation where the digestibility coefficient of DM and CP decreased as dietary protein levels increased (Faria Filho *et al.*, 2007). The oxidation of amino acid excesses in feeding high protein diet for broilers might be associated with the low protein utilization (Blair *et al.*, 1999). According to Li *et al.* (2011), the high protein in the diet did not only interfere with the metabolic processes that can lead to feed inefficiency, but also caused an increase in protein excretion. In addition, reduced nitrogen excretion has been observed as dietary protein decreased (Ferguson *et al.*, 1998; Faria Filho *et al.*, 2007).

The results of the study showed that the supplementation of methionine, betaine and methionine plus betaine enhanced ($p<0.05$) DM digestibility, ranging between 2.7 and 3.5% higher compared to the control treatment. The improvement in DM digestibility corresponds to the higher digestibility of CP and CF

Table 1: Composition (%) and nutrient content of experimental diets

	Protein diets		
Ingredients	23%	21.5%	20%
Yellow corn	55.00	55.11	56.75
Rice bran	4.93	8.00	10.35
Soybean meal	25.00	23.55	20.60
Fishmeal	10.00	8.00	7.00
Coconut oil	3.95	3.90	3.60
L-lysine HCl	0.00	0.00	0.08
DL-methionine	0.07	0.09	0.11
Dicalcium phosphate	0.10	0.30	0.50
Limestone	0.50	0.60	0.56
Premix*	0.20	0.20	0.20
NaCl	0.25	0.25	0.25
Nutrient content			
Crude protein (%)	23.00	21.50	20.00
Metabolizable energy (KCal/kg)	3.200	3.200	3.200
Methionine (%)	0.50	0.50	0.50
Lysine (%)	1.20	1.19	1.18
Calcium (%)	1.01	1.01	1.01
Available phosphorus (%)	0.48	0.48	0.48

*Premix supplied the following per kilogram diets: 12,000 IU vitamin A; 2,400 IU vitamin D; 5 mg vitamin E; 6 mg vitamin K; 4 mg vitamin B1; 6 mg vitamin B2; 2 mg vitamin B6; 4 mg vitamin B12; 28 mg vitamin C; 30 mg nicotinic acid; 10 mg calcium D-pantothenate; 150 mg electrolyte containing Na, K, Ca and Mg

(Table 2). These results were in line with the previous observation according to which the digestibility of DM and organic matter (OM) was higher with 0.32% methionine level compared to that with 0.25 or 0.27% level (Naulia and Singh, 2002). Furthermore, El-Husseiny *et al.* (2007) noted that betaine supplementation improved the digestibility of OM, CP, EE, CF and NFE. In this observation, the supplementation of methyl group donor led to the better use of nutrients, which was reflected in the digestibility improvement of several nutrients and confirmed with the improvement in carcass characteristics (Table 3). Furthermore, since poultry lacks of fiber degrading enzymes, improvement in CF digestibility indicated that bacterial fermentation of dietary fiber has been facilitated as suggested by Ratriyanto *et al.* (2010). On the contrary, methyl group donor supplementation did not affect the digestibility of EE, CA and NFE. In addition, Attia *et al.* (2005) did not find any effect of methionine or betaine supplementation on nutrient digestibility in slow-growing chickens.

Performance and carcass characteristics: Dietary with 23 and 21.5% protein resulted in a higher ($p<0.01$) average daily gain (ADG) than that with 20% protein. However, chickens fed with 20% protein had a higher ($p<0.05$) PER than chickens fed with 23% protein (Table 3). The indication of lower ADG but higher PER of the chickens fed with 20% protein might be attributed to the lower protein consumption even though the nutrient digestibility of those fed with 20% protein appeared to be higher compared to those fed with 23% protein as described previously. Moreover, low protein diet led to the decrease of protein excretion, resulting higher PER, which was in agreement with previous reports (Li *et al.*, 2011; Moosavi *et al.*, 2011). This also supports Cheng *et al.* (1997) who found that dietary with 20% protein improved PER compared to that with 24% protein.

Moreover, dietary protein levels did not affect feed intake and feed conversion of broilers, which was in agreement with previous report (Azizi *et al.*, 2011). This result indicated that in the tropical condition, dietary with 21% protein resulted in a similar output with 23% protein. Increasing dietary protein levels was not recommended to apply to chickens reared in hot environment since protein has a high heat increment (Cheng *et al.*, 1999; Musharaf and Latshaw, 1999; Daghir, 2009). In accordance with this finding, Rahman *et al.* (2002) reported no significant differences of performance resulted from feeding with 23 and 21% protein to broilers raised in hot and humid environment.

Dietary protein levels did not affect carcass and breast yield. However, feeding with 23 and 21.5% protein resulted in a lower abdominal fat compared to feeding with 20% protein albeit small magnitude (Table 3). In agreement with this result, Nguyen and Bunchasak (2005) showed that dietary protein did not affect carcass and breast yield of the chickens, while abdominal fat tended to decrease as dietary protein increased. Similarly, Marcu *et al.* (2012) reported that feeding with high protein increased protein deposition and decreased lipids deposition. Meanwhile, according to Smith and Pesti (1998), abdominal fat pad was negatively related to dietary protein levels.

The supplementation of methyl group donor did not affect broiler performance (Table 3), but rather improved carcass yield and breast yield compared to control treatment ($p<0.05$). The abdominal fat percentages of the betaine and methionine plus betaine supplemented chickens appeared to be lower than those of the control chickens, but neither group was different from the methionine supplemented group. This result confirms previous findings in which betaine or methionine supplementation has been shown to exert positive effects on carcass characteristics of broilers (Attia *et al.*, 2005; Zhan *et al.*, 2006; Rao *et al.*, 2011; Alirezaei *et al.*, 2012), even though without influencing performance (Zulkifli *et al.*, 2004; Pillai *et al.*, 2006). In this experiment, betaine seemed to be more effective in serving as lipotrophic agent than methionine, which was in accordance with the observation of Wang *et al.* (2004). The methyl group donor properties of betaine or methionine may be associated with its influence on carcass characteristics (Ratriyanto *et al.*, 2009).

Blood parameters: Significant interaction was found between protein and supplementation of methyl group donor on blood TP (Table 4). For chickens' fed controlled diets, decreasing protein level from 23 to 21.5 and 20% lessened the TP level. Meanwhile, for chickens

Table 2: Effects of protein levels and methyl groups donor supplementation on nutrient digestibility of broiler chickens (%)

Treatments		DM	CP	EE	CF	CA	NFE
Interaction effects between protein and methyl group donor							
23	C	74.70	81.37	78.02	27.11	47.14	77.63
21.5	C	77.44	84.35	77.39	30.01	53.29	78.15
20	C	76.01	84.58	77.53	29.55	52.80	78.65
23	M	77.51	86.83	78.75	31.72	52.86	77.34
21.5	M	79.27	85.69	77.04	33.21	55.11	79.09
20	M	79.61	87.30	77.34	31.51	56.40	78.02
23	B	77.07	83.31	79.07	31.35	51.37	79.46
21.5	B	79.39	87.66	76.99	33.38	58.16	75.77
20	B	79.82	87.10	77.05	31.15	52.69	76.75
23	M+B	77.75	84.18	79.72	29.81	52.70	78.43
21.5	M+B	80.24	87.62	78.21	32.12	54.91	77.78
20	M+B	80.56	89.04	82.02	31.68	55.17	78.81
SEM		0.68	0.38	0.33	0.21	0.51	0.36
Significance		NS	NS	NS	NS	NS	NS
Main effects of protein							
23		76.76^{b}	83.92^{b}	78.89	29.99^{b}	51.02	78.22
21.5		79.09^{a}	86.33^{a}	77.41	32.18^{a}	55.57	77.70
20		79.00^{a}	87.00^{a}	78.48	30.97^{a}	54.27	78.06
SEM		0.63	0.94	0.44	0.63	1.31	0.15
Significance		*	*	NS	*	NS	NS
Main effects of methyl group donor							
C		76.05^{b}	83.43^{b}	77.65	28.89^{b}	51.08	78.15
M		78.80^{a}	86.61^{a}	77.71	32.15^{a}	54.79	78.15
B		78.76^{a}	86.02^{a}	77.70	31.96^{a}	54.07	77.33
M+B		79.52^{a}	86.94^{a}	79.98	31.20^{a}	54.26	78.34
SEM		0.76	0.80	0.58	0.75	0.84	0.23
Significance		*	*	NS	**	NS	NS

[a,b]Means within a treatment and column with different superscripts differ significantly (p<0.05)

DM: Dry matter; CP: Crude protein; EE: Ether extracts; CF: Crude fibre; CA: Crude ash; NFE: Nitrogen free extract; C: Control; M: Methionine; B: Betaine; *p<0.05; **p<0.01; NS: Not significant

Table 3: Effects of protein levels and methyl groups donor supplementation on performance of broiler chickens

Treatments		FI (g/d)	ADG (g)	FCR	PER	CY (%)	BY (%)	AF (%)
Interaction effects between protein and methyl group donor								
23	C	87.39	43.08	2.03	2.14	65.52	29.79	1.86
21.5	C	87.84	42.88	2.05	2.29	64.71	28.59	1.88
20	C	91.34	41.63	2.20	2.28	64.90	28.96	1.90
23	M	88.64	47.96	1.85	2.36	67.29	31.08	1.60
21.5	M	86.17	44.34	1.94	2.41	66.93	30.32	1.71
20	M	83.73	40.91	2.05	2.47	66.00	29.72	1.92
23	B	81.25	42.51	1.92	2.32	68.43	31.10	1.39
21.5	B	80.23	41.91	1.92	2.43	69.25	31.08	1.55
20	B	82.87	42.54	1.95	2.60	66.33	31.19	1.76
23	M+B	85.62	43.77	1.95	2.25	66.68	30.34	1.39
21.5	M+B	83.21	44.23	1.88	2.48	66.20	30.29	1.55
20	M+B	79.72	40.24	1.98	2.53	66.16	29.72	1.76
SEM		0.72	0.49	0.01	0.01	0.21	0.13	0.03
Significance		NS	NS	NS	NS	NS	NS	NS
Main effects of protein								
23		85.72	44.33^{a}	1.94	2.27^{b}	66.98	30.58	1.61^{b}
21.5		84.36	43.34^{a}	1.95	2.40^{ab}	66.77	30.07	1.66^{b}
20		84.41	41.33^{b}	2.05	2.47^{a}	65.85	29.90	1.85^{a}
SEM		0.45	0.88	0.04	0.06	0.35	0.16	0.07
Significance		NS	**	NS	*	NS	NS	<0.01
Main effects of methyl group donor								
C		88.86	42.53	2.10	2.24	65.04^{c}	29.12^{b}	1.88^{a}
M		86.18	44.41	1.95	2.41	66.74^{ab}	30.38^{a}	1.74^{ab}
B		81.45	42.32	1.93	2.45	68.00^{a}	31.13^{a}	1.57^{b}
M+B		82.85	42.75	1.94	2.42	66.35^{b}	30.12^{a}	1.64^{b}
SEM		1.67	0.48	0.04	0.05	0.61	0.42	0.07
Significance		NS	NS	NS	NS	**	**	**

[a,b,c]Means within a treatment and column with different superscripts differ significantly (p<0.05)

CY: Carcass yield; BY:Breast yield; AF: Abdominal fat; FI: Feed intake; ADG: Average daily gain; FCR: Feed conversion ratio; PER: Protein efficiency ratio; C: Control; M: Methionine; B: Betaine; NS: Not significant; *p<0.05; *p<0.01

Table 4: Effects of protein levels and methyl groups donor supplementation on several blood parameters of broiler chickens

Treatments		TP (g/dL)	TG (mg/dL)	Cholesterol (mg/dL)	HDL (mg/dL)	LDL(mg/dL)
Interaction effects between protein and methyl group donor						
23	C	3.65^{a}	129.17	121.06	19.25	106.21
21.5	C	2.95^{bc}	129.17	134.21	29.15	101.21
20	C	2.95^{bc}	116.67	118.42	30.25	88.17
23	M	3.50^{ac}	95.83	113.16	19.25	92.50
21.5	M	3.10^{abc}	116.67	128.95	25.85	103.10
20	M	2.75^{bd}	116.67	121.06	33.55	87.51
23	B	2.75^{bc}	108.33	110.53	31.35	85.78
21.5	B	3.05^{abc}	116.67	107.90	28.60	81.50
20	B	3.00^{bc}	112.50	107.90	38.50	69.40
23	M+B	2.55^{b}	129.17	126.31	29.70	96.61
21.5	M+B	3.15^{abc}	100.00	118.42	25.85	99.05
20	M+B	3.20^{acd}	120.83	113.16	33.00	87.86
SEM		0.12	3.49	1.87	1.08	1.15
Significance		*	NS	NS	NS	NS
Main effects of protein						
23		3.11	115.62	117.76	24.89	95.27
21.5		3.06	115.63	122.37	27.36	96.22
20		2.98	116.67	115.13	23.83	93.23
SEM		0.02	0.35	2.12	2.66	4.18
Significance		NS	NS	NS	NS	NS
Main effects of methyl group donor						
C		3.18	125.00	124.56^{a}	26.22	98.53^{a}
M		3.12	109.72	121.05^{a}	26.22	94.37^{a}
B		2.97	112.50	108.77^{b}	32.82	78.89^{b}
M+B		2.93	116.67	119.30^{ab}	29.52	94.51^{a}
SEM		0.05	3.33	3.40	1.58	4.34
Significance		NS	NS	*	NS	**

^{a,b,c,d}Means within a treatment and column with different superscripts differ significantly (p<0.05)

TP: Total protein; TG: Trigliseride; HDL: High density lipoprotein
LDL: Low density lipoprotein; C: Control; M: Methionine
B: Betaine; *p<0.05, **p<0.01; NS: not significant

supplemented with methionine plus betaine, feeding with 20% protein resulted in higher TP than feeding with 23%. For chickens fed with 23% protein, supplementation of betaine and methionine plus betaine decreased the TP content. Dietary protein levels did not affect blood parameters, which corresponds to previous observation (Sharifi *et al.*, 2011). Moreover, only supplementation of betaine decreased blood cholesterol and LDL (p<0.05). This result was in accordance with previous observation in which the supplementation of 0.08% betaine decreased cholesterol content in broilers (Rao *et al.*, 2011). Based on this observation, decrease in blood cholesterol following betaine supplementation was due to the changes in LDL rather than HDL contents. In strengthening this experiment, previous studies can be referred to, which showed that methionine or betaine supplementation facilitated the synthesis of carnitine (Zhan *et al.*, 2006) and lecithin (Saunderson and MacKinlay, 1990). It is well known that carnitine and lecithin play an important role in lipid metabolism, which may be associated with the decreased in cholesterol and LDL contents. In addition, an enhance in lipase activity and decrease in the trigliseride and cholesterol contents in the serum of laying hens have been observed (Zou *et al.*, 1998).

Conclusion: The findings of this experiment showed that dietary with 21.5% protein can be applied for broiler chickens as indicated by a higher or a similar response in nutrient digestibility and performance compared to that of dietary 23 or 20% protein. Furthermore, dietary with 21.5% protein resulted an improvement in daily gain and a reduction in abdominal fat compared to dietary 20% protein. Dietary supplementation of methyl group donor could improve the dry matter, crude protein and crude fiber digestibilities as well as carcass and breast yield. Furthermore, betaine is more effective in reducing abdominal fat deposition than methionine.

REFERENCES

Alirezaei, M., H.R. Gheisari, V.R. Ranjbar and A. Hajibemani, 2012. Betaine: a promising antioxidant agent for enhancement of broiler meat quality. Br. Poult. Sci., 53: 699-707.

AOAC, 1990. Official Methods of Analysis, 15th Ed. Association of Official Analytical Chemists. Arlington, VA.

Attia, Y.A., R.A. Hassan, M.H. Shehatta and S.B. Abd-El-Hady, 2005. Growth, carcass quality and serum constituents of slow growing chicks as affected by betaine addition to diets containing 2. Different levels of methionine. Int. J. Poult. Sci., 4: 856-865.

Azizi, B., G. Sadeghi, A. Karimi and F. Abed, 2011. Effects of dietary energy and protein dilution and time of feed replacement from starter to grower on broiler chickens performance. J. Centr. Eur. Agric., 12: 44-52.

Blair, R., J.P. Jacob, S. Ibrahim and P. Wang, 1999. A quantitative assessment of reduced protein diets and supplements to improve nitrogen utilization. J. Appl. Poult. Res., 8: 25-47.

Cheng, T.K., M.L. Hamre and C.N. Coon, 1997. Effect of environmental temperature, dietary protein and energy levels on broiler performance. J. Appl. Poult. Res., 6: 1-17.

Cheng, T.K., M.L. Hamre and C.N. Coon, 1999. Effect of constant and cyclic environmental temperatures, dietary protein and amino acid levels on broiler performance. J. Appl. Poult. Res., 8: 426-439.

Daghir, N.J., 2009. Nutritional strategies to reduce heat stress in broilers and broiler breeders. Lohmann Inf., 44: 6-15.

El-Husseiny, O.M., M.A. Abo-El-Ella, M.O. Abd-Elsamee and M.M. Ab-Elfattah, 2007. Response of broiler chick performance to dietary betaine and folic acid at different methionine levels. Int. J. Poult. Sci., 6: 515-525.

Faria Filho, D.E., D.M.B. Campos, K.A. Alfonso-Torres, B.S. Vieira, P.S. Rosa, A.M. Vaz, M. Macari and R.L. Furlan, 2007. Protein levels for heat-exposed broilers: performance, nutrients digestibility and energy and protein metabolism. Int. J. Poult. Sci., 6: 187-194.

Ferguson, N.S., R.S. Gates, J.L. Taraba, A.H. Cantor, A.J. Pescatore, M.L. Straw, M.J. Ford and D.J. Burnham, 1998. The effect of dietary protein and phosphorus on ammonia concentration and litter composition in broilers. Poult. Sci., 77: 1085-1093.

Indreswari, R., H.I. Wahyuni, N. Suthama and P.W. Ristiana, 2009. Calcium utilization for egg shell formation in laying hens due to the different feeding regimes of morning and afternoon portion. J. Indon. Trop. Anim. Agic., 34: 134-138.

Kidd, M.T., P.R. Ferket and J.D. Garlich, 1997. Nutritional and osmoregulatory functions of betaine. World's Poult. Sci. J., 53: 125-139.

Li, Y.X., Y.Q. Wang, Y.Z. Pang, J.X. Li, X.H. Xie, T.J. Guo and W.Q. Li, 2011. The effect of crude protein level in diets on laying performance, nutrient digestibility of yellow quails. Int. J. Poult. Sci., 10: 110-112.

Lin, H., H.C. Jiao, J. Buyse and E. Decuypere, 2006. Strategies for preventing heat stress in poultry. World's Poult. Sci. J., 62: 71-85.

Marais, J.P., 2000. Use of Markers. In: D'Mello, J.P.F. (Ed), Farm Animal Metabolism and Nutrition. CABI Publishing, Wallingford, pp: 255-277.

Marcu, A., I. Vacaru-Opris, A. Marcu, D. Dronca and B. Kelciov, 2012. The influence of feed protein and energy level on meat chemical composition at "Hybro PN+" broiler chickens. Lucrari Stiintifice-Seria Zoot., 57: 260-265.

Metzler-Zebeli, B.U., M. Eklund and R. Mosenthin, 2009. Impact of osmoregulatory and methyl donor functions of betaine on intestinal health and performance in poultry. World's Poult. Sci. J., 65: 419-441.

Moosavi, M., M. Eslami, M. Chaji and M. Boujarpour, 2011. Economic value of diets with different levels of energy and protein with constant ratio on broiler chickens. J. Anim. Vet. Adv., 10: 709-711.

Musharaf, N.A. and J.D. Latshaw, 1999. Heat increment as affected by protein and amino acid nutrition. World's Poult. Sci. J., 55: 233-240.

Nasr, J., F. Kheiri, A. Solati, A. Hajibabaei and M. Senemari, 2011. The efficiency of energy and protein of broiler chickens fed on diets with different lysine concentration. J. Anim. Vet. Adv., 10: 2394-2397.

Naulia, U. and K.S. Singh, 2002. Replacement of dietary groundnut-cake by soybean meal supplemented with DL-methionine on the performance of layer chickens. Ind. J. Anim. Sci., 72: 1173-1176.

NRC, National Research Council, 1994. Nutrient Requirements of Poultry. 9th ed. National Academic Press, Washington DC.

Nguyen, T.V. and C. Bunchasak, 2005. Effects of dietary protein and energy on growth performance and carcass characteristics of Betong chicken at early growth stage Songklanakarin J. Sci. Technol., 27: 1171-1178.

Piepho, H.P., 2004. An algorithm for a letter-based representation of all-pairwise comparisons. J. Comput. Graph. Stat., 13: 456-466.

Pillai, P.B., A.C. Fanatico, K.W. Beers, M.E. Blair and J.L. Emmert, 2006. Homocysteine remethylation in young broilers fed varying levels of methionine, choline and betaine. Poult. Sci., 85: 90-95.

Rahman, M.S., M.A.H. Pramanik, B. Basak, S.U. Tarafdar and S.K. Biswas, 2002. Effect of feeding low protein diets on the performance of broilers during hot humid season. Int. J. Poult. Sci., 1: 35-39.

Rao, S.V.R., M.V.L.N. Raju, A.K. Panda, P. Saharia and G.S. Sunder, 2011. Effect of supplementing betaine on performance, carcass traits and immune responses in broiler chicken fed diets containing different concentration of methionine. Asian-Aust. J. Anim. Sci., 24: 662-669.

Rashid, H.O.S., E.E.M. Huwaida and A.Y. Ibrahim, 2012. Effect of dietary protein level and strain on growth performance of heat stressed broiler chicks. Int. J. Poult. Sci., 11: 649-653.

Ratriyanto, A., R. Mosenthin, E. Bauer and M. Eklund, 2009. Metabolic, osmoregulatory and nutritional functions of betaine in monogastric animals. Asian-Aust. J. Anim. Sci., 22: 1461-1476.

Ratriyanto, A., R. Mosenthin, D. Jezierny and M. Eklund, 2010. Effect of graded levels of dietary betaine on ileal and total tract nutrient digestibilities and intestinal bacterial metabolites in piglets. J. Anim. Physiol. Anim. Nutr., 94: 788-796.

Ratriyanto, A., R. Indreswari, Sudiyono and A. Sofyan, 2012. Potential use of betaine to substitute methionine in broiler diets. Proceedings National Seminar on Zootechniques for Indogenous Resources Development. Faculty of Animal Agriculture, Diponegoro University, Indonesia and Indonesian Society of Animal Agriculture, pp: 135-138.

Saunderson, C.L. and J. MacKinlay, 1990. Changes in bodyweight, composition and hepatic enzyme activities in response to dietary methionine, betaine and choline levels in growing chicks. Br. J. Nutr., 63: 339-349.

Sharifi, M.R., M.S. Shargh, B. Dastar and S. Hassani, 2011. The effect of dietary protein levels and synbiotic on performance parameters, blood characteristics and carcass yields of Japanese quail (*Coturnix coturnix japonica*). It. J. Anim. Sci., 10: e4: 17-21.

Smith, E.R. and G.M. Pesti, 1998. Influence of broiler strain cross and dietary protein on the performance of broilers. Poult. Sci., 77: 276-281.

Sun, H., W.R. Yang, Z.B. Yang, Y. Wang, S.Z. Jiang and G.G. Zhang, 2008. Effects of betaine supplementation to methionine deficient diet on growth performance and carcass characteristics of broilers. Am. J. Anim. Vet. Sci., 3: 78-84.

Wang, Y.Z., Z.R. Xu and J. Feng, 2004. The effect of betaine and *DL*-methionine on growth performance and carcass characteristics in meat ducks. Anim. Feed Sci. Technol., 116: 151-159.

Zhan, X.A., J.X. Li, Z.R. Xu and R.Q. Zhao, 2006. Effects of methionine and betaine supplementation on growth performance, carcase composition and metabolism of lipids in male broilers. Br. Poult. Sci., 47: 576-580.

Zou, X.T., Y.L. Ma and Z.R. Xu, 1998. Effects of betaine and thyroprotein on laying performance and approach to mechanism of the effects in hens. Acta Agric. Zhejiang, 10: 144-149.

Zulkifli, I., S.A. Mysahra and L.Z. Jin, 2004. Dietary supplementation of betaine (Betafin®) and response to high temperature stress in male broiler chickens. Asian-Aust. J. Anim. Sci., 17: 244-249.