Journal homepage www.jzs.univsul.edu.iq
Journal of Zankoy Sulaimani
Part-A- (Pure and Applied Sciences)

Effect of Adding Methionine or Choline Chloride to the Low Protein Diets During Production Period of Japanese Quail

Fatma Mustafa Jalal & Mohammad Ibrahim AL-Neemi

Animal Production Department-Agriculture Collage-Kirkuk University
E. Mail: fatimamustafa991@gmail.com

Article info

Original: 20/12/2017
Revised: 20/01/2018
Accepted: 06/02/2018
Published online:

Keywords:**:**
Methionine, Choline Chloride , low protein diets , Japanese Quail.

Abstract

The experiment was conducted in the farm of Poultry - Animal Production department of the faculty of Agriculture - University of Kirkuk from the period of 28.12.2016 until 25.2.2017. The aim of this experiment was to determine the effect of adding Methionine and Choline Chloride more than the NRC recommended to the low protein diets on production phase of Japanese Quail. Distributed randomly 140 female Japanese Quail (65 days old) to7 treatments 20 birds each, in five replicates containing 4 birds each cage with dimensions 40×30×20 cm as replicate. Birds fed dietary treatment: control (20% crude protein), T1(18% crude protein), T2(16% crude protein), T3(18% crude protein + adding 20% more than the nutritional requirement of methionine), T4(16% crude protein + adding 20% more than the nutritional requirement of methionine), T5(18% crude protein + adding 20% more than the nutritional requirement of choline) and T6(16% crude protein + adding 20% more than the nutritional requirement of choline) . The statistical analysis obtained no significant differences in egg production (H.D %), egg mass, egg weight, feed intake, feed conversion ratio, energy intake and energy conversion ratio. While significant differences have obtained in the mean conversion ratio of protein, methionine and choline, the T1 (control) was significantly ($p<0.05$) higher than all other treatments in mean of protein intake. The T4 and T5 were significantly higher than all other treatments in the methionine intake. The T6 and T7 were significantly higher than all other treatments in choline intake. The T5 was the beast treatment in protein conversion ratio among treatments, there was significant improvement in mean of methionine conversion ratio in all treatments except T4 and T5. There was significant improvement in mean of choline conversion ratio in all treatments except T6 and T7. The T2 was significantly higher than T6 in mean of shape index and egg specific gravity. The results showed higher shell thickness significantly in (T1) and (T2) treatment compared to other treatment. The T2 and T7 significantly higher than T6 in the percentage of egg shell. The T1 and T4 significantly higher than T7 in The mean of yolk index. The T4 was significantly higher than T7 in mean of yolk percentage and there was no significant differences found in the mean albumen weight percentage, albumen index and haugh unit.

Introduction

The feed constitutes approximately 60-70% of the total production cost *[1; 2]* The aim of modern poultry enterprise is to reduce feed cost for optimal economic returns *[3]* one of the ways leading to reduced cost of feeds is to reduce their protein content *[4]* Because one of the most expensive and deficient compounds of feeds for farm animals is the protein source *[5].* Methionine is an essential amino acid for livestock animals

particularly in poultry *[6]*. Therefore supplemental methionine is routinely used in layer feeds to solve lower dietary protein content problem *[7; 8]*. The importance of methionine is indicated by three major functions in poultry : a methyl donor , in protein synthesis *[9]* and as precursor to cysteine *[7]*. Choline is an essential nutrient for the poultry [10, 11, and 12] one of his functions is to furnish methyl groups similar to methionine *[12]*. Choline is water soluble vitamin *[13; 14]* and has long been used as a vitamin supplement for poultry diets. Numerous studies reported the interrelationship between Choline and methionine as donors of methyl group with variable results *[15; 16]*. According to N.R.C. (1994) *[17]* diets of layer Japanese Quail should contain 20% crude protein, 0.45% methionine and 1500 mg/kg of choline. This study was aimed to determine the effect of reducing percentage of crude protein in diet contents of layer Japanese Quail from 20% to 18% and 16% with increasing 20% methionine or choline to diet content more than the *[17]* recommended.

Material and method

The experiment was conducted in the farm of Poultry - Animal Production department of the faculty of Agriculture - University of Kirkuk during the period of 28.12.2016 until 25.2.2017. One hundred and forty females of Japanese Quail have randomly distributed of Japanese Quail (65 days old) to seven treatments 20 birds each, in five replicates with four birds in each cage as replicate . The following treatments (Table 1) were prepared:

T1(control): (20% crude protein) , T2(18% crude protein) , T3(16% crude protein) , T4(18% crude protein + adding 20% more than the nutritional requirement of methionine), T5(16% crude protein + adding 20% more than the nutritional requirement of methionine) ,T6(18% crude protein + adding 20% more than the nutritional requirement of choline) and T7(16% crude protein + adding 20% more than the nutritional requirement of choline).

Hen-day egg production was recorded whereas egg weight, feed intake, feed conversion ratio, egg mass were determined 3 weeks interval (3period). Egg mass was calculated by multiplying egg weight by hen-day egg production percentage, feed conversion ratio (FCR) was calculated as gram feed consumption/day /bird divided by gram egg mass per day per bird. In the end of each period, 10 eggs from each group were randomly taken in order to determine egg weight, egg components (percentage of egg yolk, egg albumen, egg shell and egg shell thickness). All data were statistically analyzed by the Completely Randomized Design (CRD)by the SAS system and the differences between the means of groups were separated by Duncans Multiple Range Test statements of statistical significance are based on $p<0.05$.

Table -1: Composition of experimental diets of Laying Japanese Quail.

	Experimental Treatments						
Ingredients (%)	*T1*	*T2*	*T3*	*T4*	*T5*	*T6*	*T7*
Wheat	*60.31*	*60.91*	*64.87*	*60.90*	*64.86*	*60.86*	*64.82*
Barley	*-----*	*5.00*	*8.00*	*5.00*	*8.00*	*5.00*	*8.00*
Soybean meal(44%cp)	*27.58*	*22.00*	*15.37*	*22.00*	*15.37*	*22.00*	*15.37*
Sun flower oil	*3.96*	*3.90*	*3.53*	*3.90*	*3.53*	*3.90*	*3.53*
Di-calcium phosphate	*1.94*	*1.94*	*1.94*	*1.94*	*1.94*	*1.94*	*1.94*
Limestone	*5.34*	*5.34*	*5.34*	*5.34*	*5.34*	*5.34*	*5.34*
Na Cl	*0.20*	*0.20*	*0.20*	*0.20*	*0.20*	*0.20*	*0.20*
*Enzyme premix**	*0.10*	*0.10*	*0.10*	*0.10*	*0.10*	*0.10*	*0.10*
Vit.&Min. Premix	*0.10*	*0.10*	*0.10*	*0.10*	*0.10*	*0.10*	*0.10*
L-Lysine	*0.05*	*0.05*	*0.05*	*0.05*	*0.05*	*0.05*	*0.05*
DL-Methionine	*0.17*	*0.21*	*0.25*	*0.22*	*0.26*	*0.21*	*0.25*
Choline Chloride 60%	*0.25*	*0.25*	*0.25*	*0.25*	*0.25*	*0.30*	*0.30*
Total	*100*	*100*	*100*	*100*	*100*	*100*	*100*
*Calculated Analysis***							
Energy(Kcal ME/Kg)	*2900*	*2900*	*2900*	*2900*	*2900*	*2900*	*2900*
Crude Protein %	*20*	*18*	*16*	*18*	*16*	*18*	*16*
Methionine %	*0.45*	*0.45*	*0.45*	*0.54*	*0.54*	*0.45*	*0.45*
Lysine %	*1.00*	*1.00*	*1.00*	*1.00*	*1.00*	*1.00*	*1.00*
Choline (mg/kg)	*1500*	*1500*	*1500*	*1500*	*1500*	*1800*	*1800*
Ca %	*2.50*	*2.50*	*2.50*	*2.50*	*2.50*	*2.50*	*2.50*
Av. P %	*0.35*	*0.35*	*0.35*	*0.35*	*0.35*	*0.35*	*0.35*

*Enzyme premix: Xylanase 1400 IU , B-Glucanase 1200 IU and Cellulase 5000 IU .

** According to *[17]*.

Results and discussion

Results of Egg production, egg weight, egg mass, feed intake and feed conversion ratio are shown in table (2). The results of overall mean of egg production ,egg weight, egg mass, feed intake and feed conversion ratio was not affected significantly by two levels of proteins T2 (18% crude protein) and T3 (16% crude protein). These results are agreement with Garcia et al., (2005) *[18]*, Zeweil et al., (2011) *[19]* who reported that protein levels had no effect on Egg production, egg weight, egg mass, feed intake and feed conversion ratio, on the other hand Aami Azghadi et al., (2014) *[20]* reported significant effects on egg production by feeding on low diet proteins, Zofia et al., (2006) *[4]* who reported also significant differences in feed intake, feed conversion ratio with fed proteins, Sangilimadan et al., (2013) *[21]* reported increased egg weight with increase protein level diet of laying Japanese Quail and Bunchasak and Silapasorn (2005) *[22]* also found decreased egg mass with decrease level of protein diets of Layer. Therefore, the low crude protein level with methionine (T4 and T5) and choline (T6 and T7) supplementations did not had any effect on Egg production, egg weight, egg mass, feed intake and feed conversion ratio. These results agreed with that of Garcia et al., (2005) *[18]*who reported that methionine supplement had no effect on these measurements and Zhai et al., (2013)*[23]* who seen no significant effect on egg mass and egg weight with choline supplement in diet of laying hen, while these results disagreement with William et al., (2005) *[24]* who seen significant differences in mean of egg production, egg weight, egg mass, feed intake and feed conversion ratio when methionine supplemented to laying hen diets and Khairani et al., (2016) *[14]* who also noticed significant

effect on egg weight, egg mass and feed conversion ratio with addition of 1500 ppm of choline chloride to the diet of laying Japanese Quail.

Table -2: Effect of adding Methionine or Choline to the low protein diets during production period of Japanese Quail on Egg production, egg weight, egg mass, feed intake and feed conversion ratio. (SE ±mean)

*Treatments	traits				
	Egg production (H.D%0	Egg weight (gm)	Egg mass (gm/bird/day)	Feed intake (gm/bird/day)	FCR (gm feed/gm egg mass)
T1	0.83±88.05 a	0.37±12.72 a	0.42±11.20 a	1.01±30.86 a	0.14±2.77 a
T2	0.85±87.69 a	0.26±12.55 a	0.26±11.00 a	0.21±31.11 a	0.07±2.83 a
T3	2.41±84.68 a	0.24±12.89 a	0.35±10.88 a	0.61±32.54 a	0.10±3.00 a
T4	2.28±86.01 a	0.23±13.10 a	0.19±11.25 a	0.48±31.01 a	0.02±2.75 a
T5	1.15±90.65 a	0.18±12.37 a	0.27±11.22 a	0.60±31.19 a	0.11±2.79 a
T6	1.57±89.83 a	0.21±13.16 a	0.34±11.81 a	0.55±31.55 a	0.08±2.67 a

The results in table (3) show it effect of adding methionine or choline chloride more than the nutritional requirements to low protein diet for laying Japanese Quail on consumption and Conversion ratio of Energy, Protein and Methionine showed that there was no significant differences between all seven treatments in energy consumption and energy conversion, protein consumption was significantly ($p<0.05$) decreased with decrease the level of protein in the diet (T2 and T3) compare to the control treatment (T1) which significantly higher than all treatments, and the best protein conversion was recorded by the birds in T5. Methionine consumption was significantly ($p<0.05$) increased by the birds in T4 and T5 compared to all treatments due to metionine supplement 20% more than the requirement. There were significant ($p<0.05$) improvement in methionine conversion for all treatments except the T4 and T5 which recorded bad protein conversion.

Table -3: Effect of adding Methionine or Choline to the low protein diets during production period of Japanese Quail on Consumption and Conversion ratio of Energy, Protein and Methionine. (SE ±mean)

*Treatments	Traits					
	Energy consumption (kcal/bird/day)	Energy conversion (kcal / gm of egg mass)	Protein consumption (gm/bird/day)	Protein conversion ratio (gm protein/gm egg mass)	Methionine consumption (mgm/bird/day)	Methionine conversion (mgm methionine/ gm egg mass)
T1	2.94±89.51 a	0.40±8.03 a	0.20±6.17 a	0.02±0.55 a	4.57±138.90 b	0.63±12.47 c
T2	0.61±90.23 a	0.23±8.22 a	0.03±5.60 b	0.01±0.51 ab	0.95±140.01 b	0.35±12.75 c
T3	1.78±94.36 a	0.29±8.70 a	0.09±5.20 c	0.01±0.48 bc	2.76±146.43 b	0.45±13.50 bc
T4	1.40±89.93 a	0.08±7.99 a	0.08±5.58 b	0.00±0.49 bc	2.62±167.46 a	0.15±14.88 ab
T5	1.76±90.46 a	0.34±8.09 a	0.09±4.99 c	0.01±0.44 c	3.29±168.45 a	0.63±15.07 a
T6	1.60±91.50 a	0.25±7.76 a	0.09±5.67 b	0.01±0.48 bc	2.49±141.99 b	0.38±12.05 c
T7	1.27±92.04 a	0.35±8.36 a	0.07±5.07 c	0.01±0.46 bc	1.97±142.83 b	0.55±12.97 c

The results in the table (4) showed that there were no significant differences between control treatment (T1) which contained 20% crud protein and T2 (18%crude protein) and T3 (16%crude protein) in the mean of shape index and egg specific gravity. These results are agreement with the results reported by Sangilimadan et al., (2013) *[21]***,** while Zofia et al., (2006) *[4]* reported that shape index significantly decreased with low-cp diets.

Therefore, no significant differences were observed in shape index, egg specific gravity and eggshell percentage with supplementation 20%methionine (T4 and T5) or choline (T6 and T7) more than the nutritional requirement compare to the control treatment (T1). These results were agreed with Zeweil et al., (2011) *[19]* who also observed no significant effect on shape index by methionine supplement to the diet, while disagreed with Saki et al., (2011)*[7]* who seen significant differences on the shape index with methionine supplement to the diet.

There were no significant differences between control treatment (T1, T2 and T3) in eggshell percentage egg surface area similar results were reported by Garcia et al., (2005) *[18]*while these results disagreement with the results obtained by Sangilimadan et al., (2013) *[21]* who they found significant differences in eggshell percentage and Bunchasak and Silapasorn (2005) *[22]* who they found significant effect of level proteins (14 and 16%) on eggshell thickness.

There were no significant differences in eggshell percentage between control treatment (T1:20% crude protein) and T4 (18% crude protein + adding 20% more than the nutritional requirement of methionine) and T5 (16% crude protein + adding 20% more than the nutritional requirement of methionine) similar results reported by Garcia et al., (2005) *[18];* Zeweil et al., (2011) *[19].* These results were disagreement with Gomez and Angeles (2009) *[25]* who observed increase eggshell percentage with methionine supplement to the low protein diet. On the other hand there were significant differences ($p<0.05$) in eggshell percentage with choline supplement to diet protein (T6 and T7) which was eggshell percentage significant ($p<0.05$) decreased in T6 compare to the control treatment (T1). These results were in agreement with the results obtained by Khairani et al., (2016) *[14];* Kiran *et al., (2013) [26].*

Table -4: Egg quality of Quail fed diets on various level of protein with adding 20% methionine or choline more than the nutritional requirement. (SE±mean)

*Treatments	traits				
	Shape index	Egg specific gravity	Egg surface area	Eggshell thickness	Eggshell percentage
T1	0.37±75.93 ab	0.001±1.104 ab	0.86±38.90 ab	0.005±0.202 a	0.33±13.15 ab
T2	0.67±77.75 a	0.001±1.107 a	0.79±37.58 ab	0.005±0.201 a	0.28±13.58 a
T3	0.47±76.45 ab	0.002±1.100 ab	0.71±38.58 ab	0.004±0.185 ab	0.37±12.38 ab
T4	0.78±77.19 ab	0.001±1.101 ab	0.71±39.24 ab	0.005±0.198 ab	0.19±12.65 ab
T5	0.69±76.44 ab	0.001±1.102 ab	0.55±37.04 b	0.007±0.179 b	0.30±12.73 ab
T6	0.65±75.50 b	0.002±1.098 b	0.63±39.39 a	0.006±0.198 ab	0.51±12.08 b
T7	0.85±77.05 ab	0.003±1.105 a	0.44±38.52 ab	0.007±0.189 ab	0.53±13.32 a

The results in the table (5) showed that the mean of albumen percentage, yolk index, yolk percentage, albumen index and haugh unit were insignificantly affected by protein levels similar, results were reported by Sangilimadan et al., (2013) *[21]* who also found that these traits was not affected by dietary protein levels .However Zofia et al., (2006) *[4]* indicated significantly increasing albumen percentage with feeding

on the low protein diets. Contrary to that Zeweil et al., (2011) *[19]* who observed that haugh unit was significantly increased with decreasing protein level diet.

Regardless of adding 20% more than the nutritional requirement of methionine to the low protein diet (T4 and T5), there were no significant differences between the control treatment and T4 and T5 in albumen percentage, yolk index, yolk percentage, albumen index and haugh unit these results were agreement with Zeweil et al., (2011) *[19]* who reported that yolk percentage, yolk index and albumen percentage was not affected by dietary methionine level contrary to that William et al., (2005) *[24]* who also observed that albumen index was not affected by methionine supplement. However, Husseiny et al., (2007)*[27]* indicated that yolk index were unchanged when methionine supplement to the diet and Saki et al., (2011)*[7]* who observed increasing albumen index and decreasing haugh unit with increasing methionine percentage in diet.

Yolk percentage and yolk index decreased significantly ($p<0.05$) as dietary choline (T6 and T7), these results are disagreement with the findings of Zhai et al., (2013)*[23];* Kiran *et* al., (2013) *[26]*who they showed that the yolk percentage was not affected by choline supplement.

There were no significant effects on the albumen percentage, albumen index and haugh unit by supplement 20% more than the nutritional requirement of choline to the low protein diet (T6 and T7) compare to the control treatment (T1) similarly, Zhai et al., (2013)*[23]*reported that the albumen percentage was not affected by choline supplement and Khairani et al., (2016) *[14]*who also observed no significant effect on haugh unit by choline supplement. Based on the results of this study, it can be reduced crude protein in the diet of laying Japanese Quail from 20% to 16% with supplement 20% more than the nutritional requirement of methionine or choline for optimum production performance of laying Japanese Quail.

Table -5: Effect of adding Methionine or Choline Chloride to low protein diets during production period of Japanese Quail on the egg components. (SE±mean)

*Treatments	traits				
	Yolk percentage	Yolk index	Albumen percentage	Albumen index	Haugh unit
T1	0.44±31.70 ab	0.003±0.168 a	0.56±55.14 a	0.005±0.120 a	0.67±88.99 a
T2	0.43±30.58 ab	0.002±0.161 ab	0.64±55.82 a	0.003±0.117 a	0.84±88.28 a
T3	0.77±31.44 ab	0.004±0.167 ab	0.86±56.17 a	0.010±0.129 a	1.50±89.58 a
T4	0.49±31.93 a	0.003±0.169 a	0.59±55.41 a	0.004±0.114 a	0.77±88.42 a
T5	0.53±31.63 ab	0.001±0.160 ab	0.67±55.62 a	0.002±0.118 a	0.78±89.32 a
T6	0.38±31.49 ab	0.002±0.165 ab	0.73±56.41 a	0.002±0.117 a	0.48±89.81 a
T7	0.98±29.94 b	0.005±0.157 b	1.25±56.73 a	0.003±0.121 a	0.53±89.13 a

References

[1] Qureshi, M. S. *"Quality control in poultry feed production in Pakistan",* Feed International, Vol.(8), pp. 94-95. (1987).

[2] Younis, D. Th. *"Effect of sorghum and methionine supplementation in productive performance and the quality of hatching eggs of two quail strains",* The Iraqi Journal of Veterinary Medicine, Vol.(38), No.2, pp.22-27. (2014).

[3] Moosavi , M. ; Chaji1, M.; boujarpour, M.; Rahimnahal, S.; and Kazemi, A.R . *"Effect of different levels of energy and protein with constant ratio on performance and carcass characteristics in broiler chickens",* Intl. Res. J. Appl. Basic. Sci. Vol.(3), No.12, 2485-2488. (2012).

[4] Zofia,T.; Marek, L. ; Danuta, S.; Danuta, M.; and Alicja, D. *"Different level of crude protein and energy – protein ratio in adult quail diets",* Arch. Tierz., dummerstorf, Vol.(49), special issue, pp. 325-331. (2006).

[5] Djouvinov, D. and Mihailov. R. *"Effect of low protein level on performance of growing and laying japanese quails",* Bulgarian Journal of Veterinary Medicine, Vol.(8), No.2, p.9198. (2005).

[6] Bunchasak, C. *"Role of Dietary Methionine in poultry Production",* Japan Poult. Sci. Association, Vol.(46), pp.169-179. (2009).

[7] Saki, A. A.; Harsini, R. N.; Tabatabaei, M. M.; Zamani, P.; Haghighat, M. and Matin, H. R. *"Thyroid function and egg characteristics of laying hens in response to dietary methionine levels",* African Journal of Agricultural Research, Vol.(6), No.20, pp. 4693-4698. (2011).

[8] Mehrdad, B. *"Effects of excess dietary lysine and methionine on performance and economic efficiency of broiler chick",* Scholars Research Library, Annals of Biological Research, Vol.(4), No.5, pp.241-246. (2013).

[9] Rafeeq,M.; Pasha, T. N.; Rashid, N.; Hilal, B. and Shahzad, I. *"Effect of supplementation of methionine, betaine and choline on the performance of broiler chicken in early life fed methionine deficient ration",* The Journal of Animal & Plant Sciences, Vol.(21), No.4, pp. 778-780. (2011).

[10] Algawany,M.; El-Hindawy, M.; Attia, A.; Farag, M. and Abd El-Hack, M. *"Influence of Dietary Choline Levels on Growth Performance and Carcass Characteristics of Growing Japanese Quail",* Journal.Aavs, Vol(109), pp.2307-8316.(2015).

[11] Calderano,A.A. ; Nunes, R. V.; Rodrigueiro, R. J. B.; César, R. A. *"Replacement of choline chloride by a vegetal source of choline in diets for broilers",* Cienc. Anim. Bras., Vol.(16), No.1, pp. 37-44. (2015).

[12] Omara, I.I. *"Protein-sparing effect of dl-methionine and choline supplementation of low protein diets on productivity in laying hens",* Egyptian J. Anim. Prod., Vol.(49), No.2, pp.219-231. (2012).

[13] Hollenbeck, C. B. *"The importance of being choline",* J. Am. Diet. Assoc., Vol.(110), pp.1162-1165. (2010).

[14] Khairani ; Sumiatib; and Wiryawanb, K. G. *"Egg Production and Quality of Quails Fed Diets with Varying Levels of Methionine and Choline Chloride",* 39 (1) : 34-39 (2016).

[15] Garcia,N.M.; Pestiو G. M. and Bakalli, R. I. *"Influence of Dietary Protein Level on the Broiler Chicken's Response to Methionine and Betaine Supplements",* Poultry Science, Vol.(79), pp.1478–1484. (2000).

[16] Pillai, B.P.; Fanatico, C.A.; Beers, W.K.; Blair, E.M. and Emmert, L.J. *"Homocysteine Remethylation in Young Broilers Fed Varying Levels of Methionine, Choline, and Betaine",* Poult. Sci., Vol.(85), pp.90–95. (2006).

[17] N.R.C. "*National Research council. Nutrient Requirement of Poultry",* (9th rev. ed.). National Research Council. National Academy Press, Washington, D.S; USA. (1994) .

[18]Garcia, E.A.; Mendes, A.A.; Pizzolante C.C.; Saldanha, E.S.; Moreira, J.; Mori, C.; Pavan, A.C. *"Protein, Methionine+Cystine and Lysine Levels for Japanese Quails During the Production Phase",* Brazilian Journal of Poult.Sci., Vol.(7), No.1, pp.11–18. (2005).

[19] Zeweil , H. S. ; Abdalah, A. A.; Ahmed, M. H. and Marwa , R. S. A. *"Effect of different levels of protein and methionine on performance of baheij laying hens and environmental pollution",* Egypt. Poult. Sci., Vol (31) No.II, pp.621-639. (2011).

[20] Aami Azghadi,M.; Kermanshahi, H. and 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, Vol.(4), No.1, pp.185-190. (2014).

[21] Sangilimadan,K.; AshaRajini, R.; Prabakaran, R.; Ahmed, M. and Murugan, M. *"Effect of different dietary protein on egg qualitytraits in layer Japanese Quails",* Tamilnadu j. Veterinary & Animal Sciences, Vol.(9), No.1, pp.32 - 40. (2013).

[22] Bunchasak, C. and Silapasorn, T. *"Effects of Adding Methionine in Low-Protein Diet on Production Performance, Reproductive Organs and Chemical Liver Composition of Laying Hens under Tropical Conditions",* Nternational J . Poult. Sci., Vol.(4), No.5, pp.301-308. (2005).

[23] Zhai,Q.H.; Dong, X. F.; Tong, J. M.; Guo, Y. M. and Bao, Y. E. *"Long-term effects of choline on productive performance and egg quality of brown-egg laying hens",* Poultry Science, Vol.(92), pp.1824–1829. (2013).

[24] William, N.S.; Rostagno, H.S.; Soares, P. R.; Silva, M.A. and Velasquez, L. F. *"Nutritional Requirements in Methionine + Cystine for White-Egg Laying Hens During the First Cycle of Production",* International Journal of Poult.Sci., Vol.(4), No.12, pp.965-968. (2005).

[25] Gomez ,S. and Angeles, M. *"Effect of threonine and methionine levels in the diet of laying hens in the second cycle of production",* Poult. Sci. Association, Inc., Vol.(18), pp.452–457. (2009).

[26] Kiran,K.; Tiwari, S.P.; Ravikanth K. and Thakur. A. *"Study on Comparative Efficacy of Polyherbal Preparation and Synthetic Methionine, Choline, Lysine and Biotin on Performance of Rhode Island Red Layers",* Indian Journal of Research, Vol. (2) No. 8. 33-35. (2013).

[27] Husseiny ,O.M.; Soliman, A.Z.; Omara, I.I.; and El – Sherif, H.M.R. *"Evaluation of Dietary Methionine, Folic Acid and Cyanocobalamin (B) and Their Interactions in Laying Hen Performance",* International Journal of Poult. Sci., Vol. (7), No.5, pp.461-469. (2008).