J. Animal and Poultry Prod., Mansoura Univ., Vol.9 (12): 471 – 480, 2018

Effect of Dietary Folic Acid Supplementation to Diets of Low Levels of Energy and Methionine of Developed Laying Hens in Summer Season on Performance, Physiological Status and Immune Response

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ABSTRACT

The objective of this study was to evaluate the effect of different levels of folic acid (FA) supplementation on productive and physiological performance and immune response of laying hens fed diets varying in methionine (M) and metabolizable energy (ME) levels. A total number of two hundred and sixteen of Silver Montazah laying hens (28 weeks old), were randomly divided into 12 treatment groups (18 hens / each). All birds were housed in individual cages. The experimental design was conducted in 2 x 2 x 3 factorial arrangement of treatments, 2 levels of ME (2800 and 2600 Kcal/Kg diet), two levels of DL-methionine (0.40% and 0.30%) and three levels of folic acid (0.0, 10, and 20 mg /kg feed). The results can be summarized as follows:- Increasing ME significantly increased final body weight ($P \leq 0.01$), shell thickness ($P \leq 0.01$), plasma total protein, globulin, glucose, cholesterol, triglycerides, egg folate content and hatchability of total fertile eggs while, daily feed intake and feed conversion ratio (FCR) were decreased. - Methionine at a level of 0.40% in the diet increased significantly egg number, albumin%, plasma total protein, globulin and HDL. On the other hand daily feed intake, FCR, shell%, egg shape index% and egg specific gravity were significantly decreased.- Significant increases were observed in egg number, shell thickness, plasma total protein, globulin and egg folate content due to increasing FA supplementation in the diet while, Haugh units, plasma albumin and plasma folate recorded significantly their lowest values.- Feeding hens diet with ME of 2800 kcal/kg and a level of 0.40% M without FA supplementation significantly increased albumen % and plasma triglycerides. In this respect, FA supplementation at a level of 10 mg/kg diet significantly increased plasma HDL. On the other side, FA supplementation at a level of 20 mg/kg diet significantly increased body weight change, daily egg mass, shell thickness, plasma total protein, albumin, globulin and significantly improved hatchability of fertile eggs. In addition, antibody titre against NDV recorded the highest value with insignificant difference as compared to other treatments.- Hens fed diet with 2600 kcal/kg ME and a level of 0.30% M without FA supplementation laid eggs with the highest mean of specific gravity while increasing FA to 10 mg/kg decreased plasma HDL. Increasing the supplementation level of FA in the diet significantly increased daily feed intake while, plasma triglycerides was significantly decreased.- A significant increase was observed in Haugh units attributed to feeding hens diet of ME at level of 2600 kcal/kg and 0.40% M without FA supplementation.

Keywords: Folic acid, Methionine, Metabolizable energy, Laying hens.

INTRODUCTION

The formulation of hens'diet has been based on the nutritional requirements and feed intake but should be based also on the ratio of energy to amino acids (Harms 1999). Hens will perform very well over a wide range of energy to methionine ratio. The response of hens received two levels of energy with three levels of methionine was evaluated by Harms *et al*. (1998). They found a linear response for egg contents as the daily methionine intake increased. In practical poultry diets, where soybeans represent the primary protein component, methionine is the first limiting essential amino acid. Supplementation of methionine to poultry diets will improve the efficiency of protein utilization. Increasing the energy content of the diet has been reported to increase egg production, egg mass, energy efficiency [kcal of nitrogen-corrected apparent metabolizable energy/g of egg (AMEn)] and BW gain but decreased feed conversion, Haugh units and egg shell% and feed intake (Pérez-Bonilla *et al.,* 2012). A large egg naturally contain folate at level of 22 mg (USDA, 2000). Increasing the level of crystalline folic acid in the laying hens 'diet to 4 mg/kg can be increase the folate content of egg by approximately three folds. But excessive folic acid supplementation caused a depression in feed intake and hen-day egg production rate, while egg weight, fertility and hatchability were not affected. House *et al.* (1999) reported that folic acid status is linked to increased serum level of the sulfur amino acid homocysteine, due to the role of folic acid as a co-factor in the remethylation of homocysteine to form methionine. Herbert *et al.* (2005) reported that increasing dietary folic acid level had a significant impact on egg folate content. Increasing folic acid content of the egg makes it an important source of dietary folic acid and leads to consumer acceptance of this commodity as a healthful product (House *et al.* 2002). Ozek and Bahtuyarca (2004) indicated that dietary (CP) and metabolizable energy (ME) concentrations affected blood parameters in chucker partridges. They added that CP by ME interactions in their diets had a significant effect on serum triglycerides of this class of birds.

Blood plasma levels of Low-and high-density lipoproteins (LDL and HDL) increased significantly with increasing dietary ME level in broiler chicks (Ghazalah *et. al*., 2007). In addition, Alderey *et al*., (2017) stated that, cockerels fed low energy diet (2600 kcal/ kg) displayed significantly higher New Castle diseases Virus (NDV) titre than those fed high energy diet (2700 kcal/ kg).

The objective of the present paper was to evaluate the effect of different dietary levels of methionine (M) and folic acid (FA) supplementation on the performance, egg quality and immune response of laying hens fed diets with to metabolizable energy (ME) levels. Additionally, this study aimed to improve the utilization low-energy and methionine marginally-deficient diets by laying hens via fortification of their diets with folic acid.

MATERIALS AND METHODS

The present study was carried out at Al-Gimmizah Research Station, Animal Production Research Institute, Agricultural Research Center, Ministry of Agriculture during summer season. Two hundred and sixteen Silver Montazah hens (28 weeks old) with similar body weights were housed in individual wire cages. During the experimental period (12 weeks), feed and water were provided *ad libitum* and hens were exposed to 16-hr light daily. Hens were randomly distributed into 12 treatments (18 hens per treatment). The basal diet was formulated to meet the nutrient requirements of laying hens (2800 Kcal ME/kg, 0.40 % M) according to (NRC, 1994). Twelve experimental diets were formulated to contain two levels of metabolizable energy (ME): 2800 (normal energy control) and 2600 Kcal of ME/kg diet (low energy). With each dietary energy level two levels of M (0.40

or 0.30%) within each level of dietary methionine three levels of folic acid (0, 10 and 20 mg/kg diet) were added. Methionine (M) and folic acid (FA) supplements were added as DL-methionine (98%) and folic acid (96%), respectively.

All birds were individually weighed at the beginning of the experiment and at the end of the experimental period (40 weeks old). Egg number and egg weight were recorded daily, feed intake was recorded twice weekly. One hundred and twenty eggs (10 eggs from each experimental group) were taken at the end of experiment for measuring the external and internal egg quality. Eggs from each treatment were individually weighed (g). Egg length (EL, mm) and width (EWd, mm)) were measured by a venire caliper to the nearest 0.01 mm then, egg shape index was calculated as a percentage of EWd to EL. Egg specific gravity was calculated according to Harms *et al.* (1990). Eggs were broken individually and the inner contents were placed on a leveled glass surface to determine inner quality. The average of two measurements of thick albumen height (H) with egg weight were used to compute the Haugh unit score for each individual egg according to Haugh (1937) as follows: Haugh units = 100 × log (H - 1.7 × W0.37 + 7.57). Yolk index was calculated as follows: Yolk index = (yolk height / yolk diameter) x 100. Egg shell thickness (mm) excluding shell membranes was determined using a dial pipe gauge digital micrometer at three locations on the egg. Shell including membranes, albumin and yolk for each egg were weighed then, expressed as percentages to egg weight. Three samples of pooled yolk and albumen for each experimental group were freeze-dried and stored at –20°C. The extraction and analysis of the egg yolk folate content was performed as described by House *et al.,* (2002). In brief, eggs were weighed, placed in boiling water for 10 min, cooled, and the yolks were separated, weighed and kept for analysis by storing at −80°C. Egg folate in the form of 5-methyltetrahydrofolate was extracted. The extracts were analyzed for 5-methyltetrahydro folate via reverse-phase HPLC with fluorescence detection by using the method of Vahteristo *et al*. (1997). An external standard curve with purified 5- methyltetrahydro folate was used to quantify egg folate concentrations. The content of egg folate was expressed as micrograms of folic acid per egg.

Physiological parameters:

At the end of the experimental period, blood samples were collected from the wing veins of 5 hens from each treatment into heparinized tubes. Blood plasma samples were separated by centrifugation at 4000 rpm for 10 minutes. Plasma concentrations of total protein, albumin, glucose, triglycerides, cholesterol, Low-density lipoprotein (LDL) and high-density lipoprotein (HDL) and activity of alanine amino transferase (ALT), and aspartate amino transferase (AST) in blood plasma were colorimetrically determined using commercial kits (Bio-Merieux Morcyl Etiols Charbon Rains/France). Blood plasma globulin was calculated by subtracting the level of plasma albumin from the total protein content.

Plasma folate concentrations were determined through the use of a competitive binding assay, according to the manufacturer's recommended protocol.

Determination of Newcastle disease virus (NDV) antibody titre:

Serum samples from 40 weeks old hens were used for determination NDV antibody titre by using the method described by Liu *et al. (*1999).

Fertility and hatchability:

Hens in all experimental groups were artificially inseminated with a fixed volume of freshly collected semen. Three hatches were done at three successive weeks (32, 33 and 34 weeks of age) by using eggs of each group of hens to determine egg fertility and hatchability.

Statistical Analyses

A factorial trial (2 x 2 x 3) in a completely randomized design was performed; 2 levels of dietary ME, 2 levels methionine and 3 levels of folic acid supplementation. The data were analyzed according to General Liner Model (GLM) procedure by means of three- way analysis of variance using SPSS computer program (SPSS, 2011). The following mathematical model was used:

$$Y_{ijkl} = \mu + ME_i + M_j + FA_K + (ME\ M)_{ij} + (ME\ FA)_{ik} + (M\ FA)_{jk} + (ME\ M\ FA)_{ijk} + e_{ijkl}.$$

Where Y_{ijkl} **= An observation. μ = Overall mean. MEi = The metabolizable energy effect (i= 1,...2),** M_j **= The methionine level effect (j=1….2),** FA_K **= The folic acid level effect (k= 1….3); (ME x M)**$_{ij}$ **= The interaction effect between energy and methionine (ij =(1….4); (ME x FA)**$_{ik}$ **= The interaction effect between energy and folic acid (ik =(1….6); (M x FA)**$_{jk}$ **= The interaction effect between methionine and folic acid (jk =(1….6); (ME x M x FA)**$_{ijk}$ **= The interaction effect among energy, methionine and folic acid (ijk =(1….12), and** e_{ijkl} **= The random residual error. Duncan's multiple range test was used to determine the significant differences among means of different variables (Duncan, 1955).**

RESULTS AND DISCUSSION

Productive performance:

Body weight and body weight change:

It can be seen from Table 2 that hens fed on the diet with 2800 kcal/kg were significantly (P≤0.01) heavier in their final BW and gained more weight than those fed on the lower (2600 kcal/kg) ME diet. These results are in harmony with those obtained by Omara *et al.* (2009), who indicated that the estimated body weight gain for Lohmann Brown hens were significantly higher in response to feeding the energy-adequate diets, than that of hens received the lower energy diets. Lower dietary energy levels tended to reduce body weight gain when hens fed diets containing a wide range of ME (2500, 2600, 2700 and 2800 kcal ME/kg). This may be attributed to less available energy for fat deposition when lower dietary ME levels are utilized resulting in reducing the amount of weight gain that occurs during the laying period (Stilborn and Waldroup, 1990). Balnave and Robinson (2000) observed that body weight gain of laying hens increased with increasing dietary ME level (2500, 2700 and 2900 kcal ME/kg) in the diet, and concluded that this result may be due to the fact that abdominal fat pad weight (as a proportion of body weight) at termination of the trial was lower for the low ME diet than for the other diets.

In the present study, there were insignificant differences in finial body weight of laying hens and its change due to M or FA supplementation. Our results agree with those obtained by Amaefule *et al*. (2004), who found that the differences in body weight gain among treatments due to dietary M levels (from 0.289 to 0.422%) were not significant. Folic acid supplementation up to 4.0 mg/kg had no significant effect on body weight of laying hens (Herbert *et al.,* 2009). In this respect, El-Husseiny *et al*. (2005) found that body weight gain of layers increased by increasing the dietary energy level from 2600 to 3000 kcal ME/kg without supplemental M and FA. However, average body weight gain decreased gradually with increasing M and FA levels to the 3000 kcal ME/kg diet. On the contrary, Pesti (1991) and Summers and Leeson (1993) found no differences in body weight gain of laying hens fed dietary energy ranged from 2600 to 3200 kcal ME/kg. It is obviously that energy consumption is the main factor influencing weight gain of the pullet to onset of production. Along the same line, Harms and Russell (2003) reported that the loss of body weight was reduced as the level of M was increased in the diet from 0.20 to 0.38%. Keshavarz (2003) observed that reducing dietary folic acid led to a reduction in body weight. It's evident from Table 2 that finial BW and change in BW of laying hens significantly affected by the interaction among ME, M and FA during the experimental period. It was observed that feeding hens diet with ME of 2800 kcal/kg, 0.40% M and supplemented with 20 mg FA/kg diet led to the highest final body weight and body weight gain. Our results are in agreement with those obtained by El–Husseiny *et al*. (2008), who observed significant differences in BW change among hens fed on the dietary three levels of methionine.

Table 1. Composition and calculated analysis of the experimental diets.

Ingredients (%)	1	2	3	4	5	6	7	8	9	10	11	12
Yellow corn	57.56	57.56	57.56	57.95	57.95	57.95	56. 2	56. 2	56. 2	56. 2	56. 2	56. 2
Soybean meal (44%)	30.8	30.8	30.8	30.6	30.6	30.6	29.6	29.6	29.6	29.4	29.4	29.4
Wheat bran	0.0	0.0	0.0	0.0	0.0	0.0	4.56	4.56	4.56	4.85	4.85	4.85
Cotton seed oil	2.02	2.02	2.02	2.02	2.02	2.02	0.0	0.0	0.0	0.0	0.0	0.0
Dicalcium phosphate	1.53	1.53	1.53	1.53	1.53	1.53	1.55	1.55	1.55	1.55	1.55	1.55
Limestone	7.50	7.50	7.50	7.30	7.30	7.30	7.40	7.40	7.40	7.40	7.40	7.40
Sodium chloride	0.30	0.30	0.30	0.30	0.30	0.30	0.30	0.30	0.30	0.30	0.30	0.30
Vit. & Min - mixture*	0.30	0.30	0.30	0.30	0.30	0.30	0.30	0.30	0.30	0.30	0.30	0.30
DL- Methionine	0.09	0.09	0.09	0.00	0.00	0.00	0.09	0.09	0.09	0.00	0.00	0.00
Folic acid (mg)	0.00	0.10	0.20	0.00	0.10	0.20	0.00	0.10	0.20	0.00	0.10	0.20
Total	100	100	100	100	100	100	100	100	100	100	100	100
Calculated analysis												
C P %	17.99	17.99	17.99	17.93	17.93	17.93	18.09	18.09	18.09	17.98	17.98	17.98
ME, kcal/kg	2792	2792	2792	2799	2799	2799	2603	2603	2603	2599	2599	2599
Ca, %	3.21	3.21	3.21	3.14	3.14	3.14	3.18	3.18	3.18	3.18	3.18	3.18
NPP, %	0.43	0.43	0.43	0.43	0.43	0.43	0.44	0.44	0.44	0.44	0.44	0.44
Methionine %	0.40	0.40	0.40	0.30	0.30	0.30	0.40	0.40	0.40	0.30	0.30	0.30
Lysine, %	1.04	1.04	1.04	1.04	1.04	1.04	1.03	1.03	1.03	1.03	1.03	1.03

*** Each 3 Kg of Vit and Min. premix contains: 10000000 IU Vit. A; 2000000 IU.vit D3; 10000 mg Vit. E; 1000 mg Vit.K3; 1000 mg Vit. B1;5000 mgVit. B2; 10 mg Vit. B12; 1500 mg Vit . B6;30000 mg Niacin; 10000 mg Pantothenic acid; 1000 mg Folic acid ;50 mg Biotin; 300000 mg Choline; 4000 mg Copper; 300 mg Iodine; 30000 mg Iron; 50000 mg Zinc; 60000 mg Manganese; 100 mg Selenium; 100 mg Cobalt and CaCO3 as carrier to 3000.**

Feed intake and feed conversion:

Table 2 shows that differences in dietary feed intake attributed to ME or M levels were significant (P≤0.01). Hens fed the lower ME diet (2600 kcal /kg diet) consumed more feed than those fed on the normal ME (2800 kcal/kg) diet. Hens consumed more feed in an attempt to meet their energy requirement. Feeding hens diet had the lower level of M (0.30%) significantly increased their feed intake in comparing with that fed the normal level (0.40%). FA supplementation up to 20 mg/kg had no significant effect on the amount of feed intake (Table 2). The synergistic effect due to the interaction among ME x M x FA on feed intake was significant (P≤0.01).These results are close in agreement with those concluded by Omara *et al.* (2009), who found that hens fed the 2600 kcal ME/kg diets consumed the highest amount of feed, while those fed the high-energy diet (2800 kcal ME/kg) consumed the least amount of feed. The present results are supported by Hebert *et al.* (2009), who reported that there was no significant difference in feed consumption due to folic acid supplementation up to 4 mg/kg of the diet. The amount of feed intake decreased with increasing ME, M and FA levels up to 3000 kcal/kg, 0.50% and 6.0 mg/kg, respectively (El-Husseiny *et al.* 2005). On the other hand, feed intake was not affected by a range of dietary ME from 2400 to 2900 kcal ME/kg (Adeyemo and Longe, 1996). Depression in feed intake is a highly sensitive response to excessive amino acid supplementation because diets supplemented with 0.10% DL-methionine significantly reduced feed intake (Wideman *et al.* 1994).

Significant effect on FC (g feed/ g eggs /day) was observed due to ME level (P≤0.01) and M level (P≤0.05), while no significant effect was attributed to FA supplementation in the diet. These results are in harmony with the findings of El- Husseiny *et al.* (2008), who found significant differences due to M level on FCR, where, it was improved with increasing M level, being least at 0.5% M level. They also observed no significant differences in FCR due to the supplementation level of FA. These inconsistent results could be attributed to the different amounts of feed intake and egg production. While folic acid levels up to 20 mg/kg had no significant effect on FCR of laying hens. The effect of the interaction among ME, M and FA on FCR was significant (P≤0.01) in which, hens fed on the diet with 2800 kcal/kg ME, 0.40% M and 20 mg /kg FA recorded the best value of FCR in comparison with other dietary treatments. These results agree with those obtained by Omara *et al.* (2009), who found a significant effect of the interaction among ME x M x FA on FCR of laying hens. Adeyemo and Longe (2000) and Rezvani *et. al.* (2000) indicated that birds fed on a diet containing 2600 kcal ME/kg had better FCR when the ME contents of the diets ranged from 2500 to 2900 kcal/kg. But feed conversion ratio was not affected by adding M up to 0.383% to laying hen diets (Balnave and Robinson 2000; and Amaefule *et al.*, 2009). Hebert *et al.* (2005) suggested that there was no significant difference in FCR of laying hens due to dietary FA supplementation up to 4 mg/kg. Our results agree with those of Colvara *et al.* (2002), Balnave and Robinson (2000) and Totsuka *et al.* (1993) who found that increasing the dietary energy levels up to 3000 kcal ME/kg improved FCR, due to the decrease in feed intake. The best value of FCR was recorded for diets containing 3000 kcal ME/kg with different levels of M (0.40 and 0.50%) and folic acid (2 and 6 mg/kg) as compared to the control group (El-Husseiny *et al*., (2005).

Table 2. Means of initial body weight, finial body weight, change in BW, daily feed intake (g) / day and feed conversion ratio as affected by dietary levels of metabolizable energy, methionine and added folic acid in Silver Montazah laying hens

Main effects	Initial BW (g)	Final BW (g)	Chang in BW (g)	Daily feed intake (g)	FC / day
		Metabolizable Energy (A)			
2800 Kcal / kg (A1)	1718.82	1800.93^{a}	82.11^{a}	112.56^{b}	3.27^{b}
2600 K cal / kg (A2)	1715.35	1762.28^{b}	46.93^{b}	116.51^{a}	3.55^{a}
Significance level	NS	**	**	**	**
SEM	4.10	3.74	4.74	0.52	0.07
		Methionine (B)			
0.40% (B1)	1715.72	1786.28	70.56	113.11^{b}	3.30^{a}
0.30% (B2)	1718.44	1776.93	58.49	115.96^{a}	3.52^{b}
Significance level	NS	NS	NS	**	*
SEM	4.10	4.56	5.25	0.56	0.70
		Folic acid (C)			
0.0 mg/kg (C1)	1717.58	1782.58	65.00	113.71	3.50
10 mg/kg (C2)	1721.31	1777.72	56.41	114.80	3.38
20 mg/kg (C3)	1712.36	1784.50	72.14	115.09	3.35
Significance level	NS	NS	NS	NS	NS
SEM	4.97		6.45	0.72	0.08
		Interactions			
A1XB1XC1	1720.78	1805.44	84.67	109.66	3.27
A1XB1XC2	1721.11	1806.11	85.00	111.33	2.95
A1XB1XC3	1707.44	1816.22	108.78	112.73	2.85
A1XB2XC1	1721.89	1811.11	89.22	114.02	3.69
A1XB2XC2	1722.00	1780.00	58.00	114.42	3.42
A1XB2XC3	1719.67	1786.67	67.00	113.19	3.44
A2XB1XC1	1713.56	1763.33	49.78	114.85	3.58
A2XB1XC2	1721.56	1765.67	44.11	114.47	3.75
A2XB1XC3	1709.89	1760.89	51.00	115.61	3.40
A2XB2XC1	1714.11	1750.44	36.33	116.30	3.46
A2XB2XC2	1720.56	1759.11	38.55	118.96	3.40
A2XB2XC3	1712.44	1774.22	61.78	118.84	3.69
Significance level	NS	**	**	**	**
SEM	10.26	8.42	11.08	1.23	0.14

a-b: For each of the ma .0in effects, in the same column bearing different superscripts differ significantly (P≤0.05). NS: not significant.
***: Significant at (P≤0.05). **: (P≤0.01). SEM is standard errors of the means.**

Egg Production traits:

Data presented in Table 3 showed that, egg number, hen-day egg production rate, egg weight (g) and daily egg mass (g /day) were not significantly affected by decreasing ME level to 2600 kcal/kg in the diet. These findings were supported by many authors (Pesti, 1991; Harms *et al*., 2000; De-Acosta *et al*., 2002). They found that no significant differences in egg production when laying hens fed diets containing ME level ranged from 2396 to 3200 kcal/kg ME/kg. However, Valkonen *et al.* (2008) found that hens received the lower energy diet produced fewer eggs per day (p ≤ 0.05) than the birds fed the high-energy diet. As presented in Table 3, hen-day, egg production rate, egg weight and daily egg mass were not affected significantly by increasing M or FA in the diet, but egg number was significantly increased (P≤0.05) by increasing either M level or FA supplementation in the diet. These results are agreed with those obtained by Shafer *et al*. (1996) and Balnave and Robinson (2000), who found that egg production of laying hens was not significantly affected by dietary methionine level (0.283 to 0.40%). Also, hens received diet containing 0.40% methionine and FA from 0 to 20 mg/kg exhibited no significant differences in egg weight (Omara *et al*. 2009). Our results were also confirmed by De-Acosta *et al*. (2002) and Oke *et al*. (2003), who reported that egg weight was not affected by dietary energy levels from 2500 to 3000 kcal ME/kg. In addition, (Novak *et al*. (2004) and Amaefule *et al.* (2004) noticed that, M supplementation at 0 and 0.10% of laying hens`diet containing 2790 kcal ME/kg did not affect egg weight. Moreover, egg weight was not affected by FA supplementation up to 4 mg crystalline folic acid/kg of laying hen diet (Hebert *et al*., 2009; Roth-Maier Bohmer, 2007). Increasing dietary levels of ME, M and FA up to 3000 kcal ME/kg, 0.50% and 6 mg/kg did not affect egg weight of laying hens (EL-Husseiny *et al.*, 2005). On the other hand, egg weight was higher (P≤0.05) in hens receiving diets with 2800 kcal of ME/kg of feed than those fed diets containing 2900 kcal of ME/kg of diet (Nahashon *et al*., 2007). Also, it has been reported that egg weight increased with increasing dietary M level in the diet (Okazaki *et al.* 1995).

The interactions among studied factors had no significant on egg number, hen-day egg production rate and egg weight, while, the highest daily egg mass was recorded by hens fed a diet with 2800 kcal/kg ME that supplemented with 0.40% M and 20 mg FA/kg. Our results are in harmony with those of Omara *et al.* (2009), who observed significant differences in egg production due to ME X M X FA interaction.

Egg quality:

It was detected from Table 4 that the level of ME in the diet had no significant effect on all egg quality traits except shell thickness (P≤0.01). It was clear that, feeding hens a diet with low ME (2600 kcal/kg diet) led to a decrease in shell thickness. Hens fed a diet with the level of 0.40% M displayed significantly higher egg shape index and albumen percentage, while eggs of the former were significantly lower in shell percentage and specific gravity than those of the latter. Feeding hens on a diet supplemented with 10 mg FA/kg led to an increase in yolk percentage, while Haugh units was significantly decreased compared with those fed on the control diet. Increasing FA supplementation to 20 mg /kg diet improved shell thickness compared with the control diet or the diet supplemented with 10 mg FA/kg diet. These results are in agreement with those of Harms and Russell (2003), who found that egg contents increased as the level of dietary M increased in the diet. There were no significant differences in egg yolk weight and egg yolk percentage due to FA supplementation (Hebert *et al*. 2009). However, Grobas *et al*. (1999) found that dietary levels of ME (2676 or 2820 kcal/kg) had no significant effect on yolk and albumen weights. On the other side, albumen and yolk weights showed irregular trends with different dietary energy levels (Salah Uddin *et al.,* 1991). At the beginning of the laying cycle, methionine plus cystine had no effect on yolk and albumen weight (Vogt, 1993). These results also agree with those of El_Husseiny *et al*. (2007), who noticed no significant effect of FA supplementation on shell %. Also they added that M level in the diet had no significant effect on shell thickness. Junqueira *et al*. (2006) did not detect any differences in Haugh units or eggshell quality in brown egg-laying hens fed diets varying in AMEn content from 2,850 to 3,050 kcal/kg. On the other hand, Gunawardana *et al*. (2008) reported similar proportions of yolk and albumen in eggs from laying hens fed diets varying in AMEn content from 2,750 to 3,055 kcal/kg. In contrast, Wu *et al.* (2005) reported that yolk weight increased and Haugh units decreased as the AMEn of the diet increased from 2,720 to 2,955 kcal/kg.

Table 3. Means of egg number, egg weight, daily egg mass, and hen-day egg production rate as affected by dietary levels of metabolizable energy, methionine and added folic acid in Silver Montazah laying hens

Main effects	Egg number	Egg weight (g)	Daily Egg mass (g)	hen-day egg production rate %
Metabolizable Energy (A)				
2800 Kcal / kg (A1)	57.00	55.37	35.13	61.53
2600 Kcal / kg (A2)	55.33	54.37	33.46	60.42
Significance level	NS	NS	NS	NS
SEM	0.87	0.48	0.66	0.54
Methionine (B)				
0.40% (B1)	57.35[a]	54.92	35.08	61.02
0.30% (B2)	54.98[b]	54.83	33.52	60.92
Significance level	*	NS	NS	NS
SEM	0.86	0.49	0.67	0.54
Folic acid (C)				
0.0 mg/kg (C1)	54.25[b]	55.09	33.19	61.21
10 mg/kg (C2)	57.19[a]	54.60	34.79	60.67
20 mg/kg (C3)	57.06[a]	54.93	34.91	61.04
Significance level	*	NS	NS	NS
SEM	1.03	0.60	0.55	0.66
Interactions				
A1XB1XC1	56.00	54.54	34.08	60.72
A1XB1XC2	62.44	55.31	38.43	61.46
A1XB1XC3	61.67	57.94	39.78	64.38
A1XB2XC1	50.67	55.93	31.49	62.15
A1XB2XC2	56.22	53.93	33.67	59.93
A1XB2XC3	55.00	54.47	33.36	60.52
A2XB1XC1	54.00	54.60	32.74	60.67
A2XB1XC2	53.33	53.06	31.49	58.95
A2XB1XC3	56.67	53.97	33.95	59.96
A2XB2XC1	53.33	55.17	34.46	61.30
A2XB2XC2	56.78	56.10	35.59	62.33
A2XB2XC3	54.89	53.36	32.56	59.28
Significance level	NS	NS	**	NS
SEM	1.83	1.14	1.43	1.26

a-b: For each of the main effects, in the same column bearing different superscripts differ significantly (P≤0.05). NS: not significant.
***:Significant at (P≤0.05). **:(P≤0.01). SEM is standard errors of the means**

Petersen *et al*. (1983) reported that reducing dietary M improved shell quality without affecting egg production. It has been found that M supplementation to layer diets did not significantly influence egg shell thickness of laying hens (Amaefule *et al*. 2004). El-Husseiny *et. al*. (2005) found that increasing FA level from 2.0 to 6.0 mg/kg had no effect on shell weight but egg shell thickness was insignificantly increased with high FA level. This may be attributed to the availability of nutrients in the blood at their convenience.

Harms and Russell (2003) and Novak *et al*. (2004) concluded that albumen percentage was affected by dietary M levels. On the other side, El_Husseiny *et al*. (2007) detected no significant effect due to M and FA levels on yolk and albumen percentage.

In this study, the ME x M x FA interaction significantly affected all studied egg quality traits except egg shape index and yolk percent. The highest value of Haugh units (90.26) was recorded by hens received the dietary ME level of 2600 Kcal/ kg with 0.40% M without FA supplementation in the diet. While, the least value of Haugh units (68.86) was obtained by hens received diet contained ME 2800 kcal/ kg with 0.40% M and 10 mg FA /kg diet. Feeding hens the control diet (2800 kcal/kg ME, 0.40% M without FA supplementation) significantly decreased shell% (11.86%), yolk index (0.43), and egg specific gravity (1.099), while albumen percent was significantly increased (56.58%). In addition, shell thickness significantly increased in eggs laid by hens fed diet with 2800 kcal/kg ME, 0.40% M and supplemented with 20 mg FA/kg as compared to other treatments (Table 4). These findings agree with those reported by Omara *et al.* (2009) and Stilborn and Waldroup (1990), who reported that dietary energy level did not significantly influenced Haugh units values. Haugh units were not influenced significantly by energy and folic acid levels up to 3000 kcal ME/kg and 6 mg/kg, respectively, but influenced by M level up to 0.50% and the effect of interaction among ME, M and FA on Haugh unit was significant (El-Husseiny *et al*. 2005). On the other hand, Salah Uddin *et al*. (1991) found irregular Haugh units trend with different levels of dietary energy. However, Haugh units were not affected by total sulfur amino acid level up to 0.877% (Novak *et al*., 2004 and Amaefule *et al*., 2004). El-Husseiny *et al*. (2008) found no significant differences due to M and FA levels on Haugh units of laying hens. Pérez-Bonilla *et al*. (2012) noticed that increasing energy concentration of the diet reduced the Haugh units (HU) and the proportion of shell in the egg.

Fertility and hatchability:

Data presented in Table 5 showed that egg fertility percentage was not affected by all studied factors. On the other hand, differences due to ME level in the diet in hatchability of fertile eggs, were significant (P≤0.01). Hens fed the normal ME diet (2800 kcal/kg) achieved significantly higher hatchability percentage in comparison with that fed the low ME diet. These results are in agreement with those of Van Emous *et al*. (2015), who noted that, high energy diet had no effect on fertility, but improved hatchability of fertile eggs resulting from a decrease in egg embryonic mortality during incubation. Interaction among the studied factors affected significantly both the percentages of egg fertility and hatchability of total fertile eggs. Feeding hens diet with 2600 kcal/kg ME, 0.30% M and supplemented with 20 mg FA/kg decreased egg fertility in comparison with other treatments, however, differences in egg fertility among other treatments were not significant. Hens fed a ration had 2800 kcal/kg ME, 0.40% M and supplemented with 10 or 20 mg FA/ kg diet were the highest in hatchability of fertile eggs (87.54 and 89.04%, respectively)

Table 4. Means of egg quality measurements as affected by dietary levels of metabolizable energy, methionine and added folic acid in Silver Montazah laying hens

Main effects	Egg shape Index %	Egg components: Albumen %	Yolk %	Shell %	Shell thickness (mm)	Hough Unit	Yolk index %	Specific gravity
Metabolizable Energy (A)								
2800 Kcal / kg (A1)	74.09	52.13	33.56	14.31	40.50^{a}	76.48	47.73	1.114
2600 K cal / kg (A2)	74.18	52.13	33.03	14.85	38.19^{b}	79.44	49.32	1.118
Significance level	NS	NS	NS	NS	**	NS	NS	NS
SEM	0.69	0.71	0.52	0.54	0.70	1.90	0.63	0.003
Methionine (B)								
0.40% (B1)	72.97^{b}	53.73^{a}	32.79	13.47^{b}	39.46	77.48	48.41	1.109^{b}
0.30% (B2)	75.30^{a}	50.52^{b}	33.79	15.69^{a}	39.22	78.44	48.64	1.123^{a}
Significance level	*	**	NS	**	NS	NS	NS	**
SEM	0.63	0.62	0.48	0.45	0.75	1.94	0.66	0.003
Folic acid (C)								
0.0 mg/kg (C1)	74.46	53.34	31.87^{b}	14.78	37.50^{b}	80.56^{a}	47.56	1.117
10 mg/kg (C2)	73.52	51.23	34.44^{a}	14.32	39.04^{b}	74.20^{b}	49.17	1.114
20 mg/kg (C3)	74.42	51.60	33.56^{ab}	14.63	41.49^{a}	79.11^{ab}	48.86	1.116
Significance level	NS	NS	*	NS	**	*	NS	NS
SEM	0.82	0.82	0.60	0.65	0.79	2.22	0.78	0.01
Interactions								
A1XB1XC1	71.22	56.58	31.56	11.86	37.00	74.27	43.29	1.099
A1XB1XC2	72.52	53.78	33.72	12.50	40.33	68.86	49.11	1.103
A1XB1XC3	72.03	52.61	34.11	13.29	43.50	80.12	48.63	1.108
A1XB2XC1	77.55	51.84	32.46	15.70	39.67	83.51	49.81	1.123
A1XB2XC2	73.83	49.44	35.30	15.26	41.00	69.11	45.73	1.119
A1XB2XC3	77.36	48.51	34.20	17.28	41.50	83.00	49.83	1.133
A2XB1XC1	75.60	54.13	31.34	14.53	34.67	90.26	49.46	1.115
A2XB1XC2	72.55	51.79	34.33	13.87	37.83	76.68	50.97	1.111
A2XB1XC3	73.88	53.49	31.72	14.79	43.44	74.67	49.00	1.117
A2XB2XC1	73.47	50.82	32.13	17.04	38.67	74.18	47.68	1.131
A2XB2XC2	75.16	49.91	34.41	15.67	37.00	82.17	50.87	1.122
A2XB2XC3	74.40	52.30	34.23	13.17	37.50	78.65	47.96	1.107
Significance level	*	*	NS	*	**	*	*	**
SEM	1.23	1.24	0.91	0.78	0.92	3.23	0.90	0.005

$^{a-b}$: For each of the main effects, in the same column bearing different superscripts differ significantly (P≤0.05). NS: not significant.
***: Significant at (P≤0.05). **: (P≤0.01). SEM is standard errors of the means.**

Table 5. Means of fertility% and hatchability% as affected by dietary levels of metabolizable energy, methionine and added folic acid levels in Silver Montazah laying hens

Main effects	Egg fertility %	Hatchability% of fertile eggs
Metabolizable energy (A)		
2800 Kcal / kg (A1)	80.17	84.82^{a}
2600 K cal / kg (A2)	77.61±1.86	80.16^{b}
Significance level	NS	**
SEM	1.28	1.11
Methionine (B)		
0.40% (B1)	80.00	83.83
0.30% (B2)	77.78	81.15
Significance level	NS	NS
SEM	1.36	1.20
Folic acid (C)		
0.0 mg/kg (C1)	78.15±1.33	80.64±1.13
10 mg/kg (C2)	80.75±1.35	82.59±1.62
20 mg/kg (C3)	77.50±2.35	84.24±1.64
Significance level	NS	NS
SEM	1.68	1.46
Interactions		
A1XB1XC1	76.67	81.03
A1XB1XC2	81.67	87.48
A1XB1XC3	80.66	88.89
A1XB2XC1	77.67	83.36
A1XB2XC2	81.67	82.82
A1XB2XC3	82.66	85.33
A2XB1XC1	76.33	81.21
A2XB1XC2	83.00	83.28
A2XB1XC3	81.67	81.11
A2XB2XC1	83.00	76.96
A2XB2XC2	76.60	76.79
A2XB2XC3	65.00	81.61
Significance level	**	*
SEM	2.05	2.05

$^{a-b}$: For each of the main effects, in the same column bearing different superscripts differ significantly (P≤0.05).
NS: not significant. *: Significant at (P≤0.05). **: (P≤0.01). SEM is standard errors of the means.

Physiological traits:

Plasma protein fractions and glucose:

Data presented in (Tables 5 and 6) summarize the effect of ME, M and FA in hen's diet on some plasma constituents, plasma folate and egg folate content. ME level in hens diet had a significant effect on plasma total protein and globulin. Hens fed a diet had 2800 kcal ME/kg had significantly higher levels of plasma total protein (P≤0.01) and globulin (P≤0.01) but plasma albumin was not affected (Table 5). Similar results in plasma total protein and globulin were observed due to M level. Hadinia *et al.* (2014) demonstrated that increasing dietary M levels led to an increase in globulin concentrations and a decrease in albumin concentrations in broiler chickens. Jankowski *et al.* (2017) noticed significant differences in the concentrations of plasma albumin and total protein in turkeys in response to added dietary methionine. In previous studies performed on turkeys fed diets with a low and high M content for 4 and 8 wk (0.45% vs. 0.71% and 0.40% vs. 0.57%, respectively). On the other hand no significant differences were observed in blood parameters (Kubińska *et al.,* 2014; Kubińska *et al.,* 2015a,b). Data presented in Table 5 showed significant differences in plasma total protein, albumin and globulin due to FA supplementation level. Increasing FA significantly increased plasma total protein and globulin while, plasma albumin was significantly decreased. Significant differences in plasma total protein, albumin and globulin due to the synergistic effect attributed to the interaction among ME, M and FA. Hens fed diet with 2800 kcal/kg ME 0.40% M and supplemented with 20 mg FA achieved the highest levels of plasma total protein and globulin in comparing with other treatments.

These results agree with the findings by Zeweil *et al.* (2011), who showed that plasma total protein and globulin were significantly increased by increasing the M levels in Baheij hen diets, while plasma albumin was not affected.

Plasma folic acid and egg folate content:

As present in Table 5, it is clear that ME level in the diet affected significantly egg folate content. Egg folate content increased in eggs of hens fed a diet with ME of 2800 kcal/kg with a significant ($P \leq 0.05$) difference compared with those fed on the 2600 kcal/kg ME diet. But M level in diet had no significant effect on plasma folate concentration or egg folate content. Plasma folate concentration was significantly increased ($P \leq 0.01$) in hens fed the diet supplemented with 10 or 20 mg FA/kg compared with the control group. Egg folate was affected significantly by the synergistic effect among ME, M and FA in which, hens fed on diet had ME of 2800 kcal/kg and supplemented with 0.30% M and 20 mg FA / kg produced eggs with the highest folate content in comparison with other treatments. The lowest folate content was found in eggs laid by hens fed diet with ME of 2600 kcal/kg, 0.30% M without FA supplementation. The highest plasma folate concentration was recorded for hens fed on a diet had ME of 2800 kcal/kg, 0.40% M and 10 mg FA/kg diet or 2600 kcal/kg, 0.40% M and 10 mg FA/kg diet.

These results are in agreement with the findings of Tactacan *et al.* (2012), who found that the egg and plasma folate concentrations of birds fed 10 or 100 mg of FA-supplemented diets significantly increased compared with those of birds fed the control diet. A proportionate increase in both plasma folate and egg folate concentrations is an indication that circulating blood folate are transferred efficiently into the egg (Sherwood *et al.,* 1993; House *et al.,* 2002; Hebert *et al.,* 2005). On the other hand, blood folate has been reported to serves as the precursor pool for folate deposition into the egg (Sherwood *et al.,* 1993).

Table 6. Means of some blood plasma constituents, and plasma and egg contents of folic acid as affected by dietary levels of metabolizable energy, methionine and added folic acid in Silver Montazah laying hens

Main effects	Total protein g/dl	Albumin g/dl	Globulin g/dl	Glucose g/dl	Plasma folate ng/ml	Egg folate µ/egg
			Metabolizable Energy (A)			
2800 Kcal / kg (A1)	4.69^{a}	2.30	2.39^{a}	141.51	31.42	32.56^{a}
2600 K cal / kg (A2)	4.38^{b}	2.25	2.13^{b}	139.03	31.14	31.33^{b}
Significance level	**	NS	*	NS	NS	*
SEM	0.70	0.50	0.90	1.13	2.94	2.95
			Methionine (B)			
0.40% (B1)	4.63^{a}	2.25	2.38^{a}	141.07	31.43	32.03
0.30% (B2)	4.45^{b}	2.30	2.15^{b}	139.47	31.13	31.87
Significance level	*	NS	**	NS	NS	NS
SEM	0.80	0.50	0.90	1.16	3.03	2.95
			Folic acid (C)			
0.0 mg/kg (C1)	4.33^{b}	2.45^{a}	1.88^{c}	138.33	14.23^{c}	14.87^{b}
10 mg/kg (C2)	4.52^{b}	2.23^{b}	2.29^{b}	140.32	40.30^{a}	39.90^{a}
20 mg/kg (C3)	4.78^{a}	2.15^{b}	2.63^{a}	142.17	39.32^{b}	41.08^{a}
Significance level	**	**	**	NS	**	**
SEM	0.08	0.05	0.07	1.38	0.30	0.42
			Interactions			
A1XB1XC1	4.50	2.40	2.10	139.97	15.00	16.17
A1XB1XC2	4.87	2.20	2.67	142.00	41.00	40.00
A1XB1XC3	5.10	2.10	3.00	142.07	38.00	40.70
A1XB2XC1	4.30	2.60	1.70	139.00	14.80	15.50
A1XB2XC2	4.50	2.30	2.20	141.03	39.00	41.00
A1XB2XC3	4.90	2.20	2.70	145.00	40.70	42.00
A2XB1XC1	4.30	2.50	1.80	138.00	13.90	14.80
A2XB1XC2	4.40	2.20	2.20	141.27	41.00	39.50
A2XB1XC3	4.60	2.10	2.50	143.10	39.67	41.00
A2XB2XC1	4.20	2.30	1.90	136.33	13.20	13.00
A2XB2XC2	4.30	2.20	2.10	136.97	40.20	39.10
A2XB2XC3	4.50	2.20	2.30	138.5	38.90	40.60
Significance level	**	*	**	NS	**	**
SEM	0.11	0.08	0.03	0.18	0.29	0.53

a-b: For each of the main effects, in the same column bearing different superscripts differ significantly ($P \leq 0.05$). NS: not significant. *: Significant at ($P \leq 0.05$). **: ($P \leq 0.01$). SEM is standard errors of the means.

Plasma lipid profile:

It is obvious from Table 6 that plasma cholesterol and triglycerides were increased significantly ($P \leq 0.01$) due to increasing ME level in the diet. Hens fed a diet with 2800 kcal/kg displayed higher levels of plasma cholesterol and triglycerides compared with those fed 2600 kcal/kg diet. Plasma HDL was significantly increased by increasing M level. FA supplementation in the diet had no significant effect on plasma lipid profile. There was a significant effect attributed to the interaction among ME, M and FA on plasma triglycerides and HDL. Feeding hens diet with ME 2600 kcal/kg and supplemented with 0.40 or 0.30% M and 20 mg FA/kg caused the lowest level of plasma triglycerides when compared with other treatments applied. The highest level of plasma HDL was recorded by hens fed on the diet contained ME of 2800 kcal/kg, 0.40% M and supplemented 10 mg FA/kg while the lowest value was recorded by hens fed on the lower ME (2600 kcal/kg), lower M level (0.30%) and the low supplemental level of FA (10 mg/kg). This may attributed to the fact that, FA supplementation helps to reduce circulating homocysteine levels which, in turn, may cause on improvement in serum lipid profile.

Plasma liver enzymes activity:

Data listed in Table 6 shows the effect of ME, methionine and folic acid supplementation level on liver enzymes activity (AST and ALT). Studied factors on their interactions had no significant effect on liver enzymes activity. It was clear that liver enzymes (AST and ALT) were insignificantly decreased by increasing ME or FA supplementation in the diet.

Newcastle disease virus (NDV) antibody titre:

Data presented in Table 7 indicated that, ME, M or FA supplementation level had no significant effect on NDV titre. It was clear that the titre against NDV value improved with insignificant differences by increasing FA supplementation level in the diet. Feeding hens a diet contained 2800 kcal/kg of ME with 0.40 or 0.30% M and supplemented with 20 mg/kg diet of FA improved the titre against NDV (6.00) when compared with other treatments applied.

Table 7. Means of some blood plasma constituents, Plasma folic acid and egg folic acid as affected by dietary levels of metabolizable energy, methionine and added folic acid levels in Silver Montazah laying hen.

Main effect	Cholesterol (mg/dl)	Triglycerides (mg/dl)	HDL (mg / dl)	LDL (mg / dl)	AST (U/L)	ALT (U/L)	NDV-Titre
			Metabolizable Energy (A)				
2800 Kcal / kg (A1)	120.78^{a}	127.43^{a}	34.00	61.29	28.72	26.50	4.83
2600 K cal / kg (A2)	118.96^{b}	118.81^{b}	33.96	61.14	27.28	26.00	4.50
Significance level	*	**	NS	NS	NS	NS	NS
SEM	0.58	0.20	0.25	0.58	0.59	0.63	0.29
			Methionine (B)				
0.40% (B1)	120.41	123.01	34.58^{a}	61.14	28.22	26.22	4.67
0.30% (B2)	119.33	123.22	33.37^{b}	61.30	27.78±	26.35	4.67
Significance level	NS	NS	**	NS	NS	NS	NS
SEM	0.61	1.25	0.20	0.58	0.61	0.63	0.30
			Folic acid (C)				
0.0 mg/kg (C1)	120.25	123.70	33.70	61.93	28.42	26.50	4.25
10 mg/kg (C2)	120.08	123.11	34.15	61.04	27.92	26.36	4.50
20 mg/kg (C3)	119.28	122.54	34.08	60.68	27.67	26.00	5.25
Significance level	NS	NS	NS	NS	NS	NS	NS
SEM	0.76	1.55	0.30	0.70	0.75	0.78	0.34
			Interactions				
A1XB1XC1	121.67	128.33	33.40	62.67	29.33	27.00	4.00
A1XB1XC2	122.00	127.17	35.50	60.90	29.00	26.33	5.00
A1XB1XC3	121.00	126.00	35.00	60.80	28.67	26.00	6.00
A1XB2XC1	120.67	128.07	33.20	62.05	29.00	27.00	4.00
A1XB2XC2	120.23	127.00	33.80	60.83	28.33	27.10	4.00
A1XB2XC3	119.10	128.00	33.10	60.50	28.00	26.00	6.00
A2XB1XC1	119.67	119.34	35.00	60.80	28.00	26.00	5.00
A2XB1XC2	119.10	119.17	34.70	60.27	27.33	26.00	4.00
A2XB1XC3	119.00	118.07	33.90	61.39	27.00	26.00	4.00
A2XB2XC1	119.00	119.07	33.20	62.19	27.33	26.00	4.00
A2XB2XC2	119.00	119.10	32.60	62.18	27.00	26.00	5.00
A2XB2XC3	118.00	118.10	34.33	60.05	27.00	26.00	5.00
Significance level	NS	**	**	NS	NS	NS	NS
SEM	1.60	1.91	0.25	1.56	1.66	1.79	0.60

a-b: For each of the main effects, in the same column bearing different superscripts differ significantly (P≤0.05). NS: not significant.
***: Significant at (P≤0.05). **: (P≤0.01). SEM is standard errors of the means.**

CONCLUSION

Feeding hens on a diet ME level of 2800 kcal/kg with a level of 0.40% DL-methionine and supplemented with 20 mg folic acid /kg can improve daily egg mass, shell thickness, hatchability of fertile eggs and antibody titre against NDV of Silver Montazah laying hens.

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تأثير إضافة حمض الفوليك إلى العلائق التى تحتوى على مستويات منخفضة من الطاقة الممثلة والمثيونين فى الدجاج المستنبط خلال موسم الصيف على الأداء والحالة الفسيولوجية والاستجابة المناعية

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تهدف هذه الدراسة الى تقييم تأثير إضافة مستويات مختلفة من حمض الفوليك على الاداء الانتاجي والاستجابة المناعية للدجاج المغذى على مستوبات مختلفة من المثيونين والطاقة اللمثلة. وقد استخدم لهذا الغرض 216 دجاجة من سلالة المنتزة الفضى (عمر 28 أسبوع) متماثلة فى الوزن وقد قسمت الى 12 مجموعة (18 دجاجة فى كل مجموعة) وقد تم ايوائها فى أقفاص فردية. صممت هذه التجربة عامليا 3x2x2 (مستويين من الطاقة الممثلة 2800, 2600 كيلو كالورى لكل كيلو جرام ومستويين من د.ل مثيونين 0,40, 0,30%) وثلاث مستويات من حمض الفوليك 0, 10, 20 مليجرام لكل كيلو جرام عليقة) وكانت النتائج كما يلى: - أدت زيادة الطاقة الممثلة الى 2800 كيلو كالورى الى زيادة معنوية فى وزن الجسم النهائى وسمك القشرة والبروتين الكلى والجلوبيولين والكوليسترول والدهون الثلاثية فى بلازما الدم ومحتوى البيضة من حمض الفولات ونسبة الفقس من البيض المخصب بينما تناقص معنويا الاستهلاك اليومى للغذاء ومعامل التحويل الغذائى اليومى. - كان للمستوى0,40% من المثيونين فى العليقة تأثيرا معنويا فى زيادة عدد البيض ونسبة الالبيومين فى البيضة والبروتين الكلى والجلوبيولين و HDL فى بلازما الدم. على الجانب الآخر تناقص كلا من معدل الغذاء اليومى ومعامل التحويل ونسبة القشرة ودليل شكل البيضة والكثافة النوعية للبيضة. - كان لزيادة معدل إضافة حمض الفوليك فى الغذاء تأثيرا معنويا فى زيادة عدد البيض وسمك القشرة والبروتين الكلى والجلوبيولين فى بلازما الدم ومحتوى البيضة من الفولات بينما تناقصت وحدات هاو والالبيومين و الفولات فى بلازما الدم. - كان لتغذية الدجاج على عليقة تحتوى 2800 كيلوكلورى طاقة ممثلة بمستوى 0,40% مثيونين بدون اضافة خمض فوليك تأثيرا معنويا فى زيادة نسبة البياض والدهون الثلاثية فى بلازما الدم. وفى هذا السياق كان لاضافة حمض الفوليك بمعدل 10 مليجرام لكل كيلوجرام تأثرا معنويا فى زيادة محتوى البلازما من HDL. على الجانب الآخر أدت إضافة 20 مليجرام من حمض الفوليك لكل كيلوجرام غذاء تأثرا معنويا فى زيادة معدل التغير فى وزن الجسم وكتلة البيض وسمك القشرة ومحتوى بلازما الدم من البروتين الكلى والالبيومين والجلوبيولين كما حسن معنويا نسبة الفقس من البيض المخصب علاوة على ذلك زاد مستوى المناعة لفيروس النيوكاسل مقارنة بالمعاملات الأخرى بينما كانت الفروق غير معنوية . - أثر تغذية الدجاج على عليقة تحتوى 2600 كيلوكالورى و 0,30% مثيونين بدون إضافة حمض الفوليك تأثيرا معنويا فى زيادة الكثافة النوعية للبيضة وكان لزيادة معدل اضافة حمض الفوليك إلى معدل 10 مليجرام تأثيرا معنويا فى نقص محتوى البلازما من HDL. ومن ناحية أخرى كان لزيادة معدل إضافة حمض الفوليك تأثيرا معنويا فى زيادة معدل استهلاك الغذاء ونقص محتوى البلازما من الدهون الثلاثية. - كانت هناك زيادة معنوية لوحدات هاو نتيجة تغذية الدجاج على عليقة 2600 كيلوكالورى و0,40% مثيونين دون اضافة حمض الفوليك. الخلاصة: أدت تغذية دجاج المنتزه الفضى على عليقة تحتوي على 2800 كيلوكالورى لكل كيلوجرام عليقة و0,40% مثيونين ومعدل اضافة 20 مليجرام حمض الفوليك لكل كيلوجرام الى زيادة معنوية فى كتلة البيض وسمك قشرة البيضة كما حسنت نسبة الفقس من البيض المخصب ومستوى المناعة لفيروس النيوكاسل.