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Energy and Methionine Utilization in Laying Hen Diets Supplementation with Folic Acid

[1]Islam I. Omara and [2]Ramy M. Romeilah

[1]Department of Animal Production, Faculty of Agriculture, Cairo University.
[2]Department of Biochemistry, Faculty of Agriculture, Cairo University.

Abstract: In this experiment, the factors were control and low energy concentration (2800 vs. 2600 kcal ME/kg), control and deficiency methionine concentration (0.40 vs. 0.35%), and control and excess folic acid concentration (0, 10 and 20 mg/kg) in a 2 × 2 × 3 factorial arrangement design. This experiment lasted 20 week. Lohmann Brown (L.B.) laying hens, 25 weeks of age (n=1800) were randomly assigned into 12 treatments (10 replicates of 15/treatment). Hen-day egg production percentage increased when methionine and folic acid added to any energy levels. Increasing energy, methionine and folic acid up to 2800 kcal ME/kg, 0.40% and 20 mg/kg, respectively did not affected egg weight and egg mass. The hens fed 2600 kcal ME/kg diets consumed the highest ($P \leq 0.05$) amount of feed, while those fed the diets 2800 kcal ME/kg consumed the least amount of feed. Increasing dietary methionine and folic acid levels in the diets slightly decreased the amount of feed intake, without any significant differences. Feed conversion ratio improved when added 0.35 and 0.40% methionine and folic acid up to 20 mg/kg to low energy diets. Live body weight gain significantly affect by energy levels, but not affected by methionine and folic acid levels. In general, the diet containing 2600 kcal ME/kg diet within methionine and folic acid proved to be adequate for good external (egg shell percentage and shell thickness) and internal (yolk and albumen weight, Haugh units and egg yolk color) egg quality, while the decline in egg yolk color could be overcome by xanthophyll pigmentation. However, the diets containing 2600 kcal ME/kg tends to increase yolk total lipids and cholesterol compared to diets containing 2800. Methionine and folic acid levels did not affect on average yolk total lipids and cholesterol values. Moreover, serum total immunoglobulin titres was significantly affected by energy levels, but little affected by methionine and folic acid supplementation in the diet. The nutrients digestibility coefficient values improved with diets containing 2600 kcal ME/kg and supplementation methionine and folic acid did not appear any effect. The results of the study indicate that improving the laying hens performance, egg quality and immune response when fed these on diets containing the low dietary levels of energy and methionine by increasing the right amount from folic acid supplementation.

Key words: Laying hens, dietary energy, Methionine deficiency, Folate, Performance, Egg quality, Immunity, nutrients digestibility.

INTRODUCTION

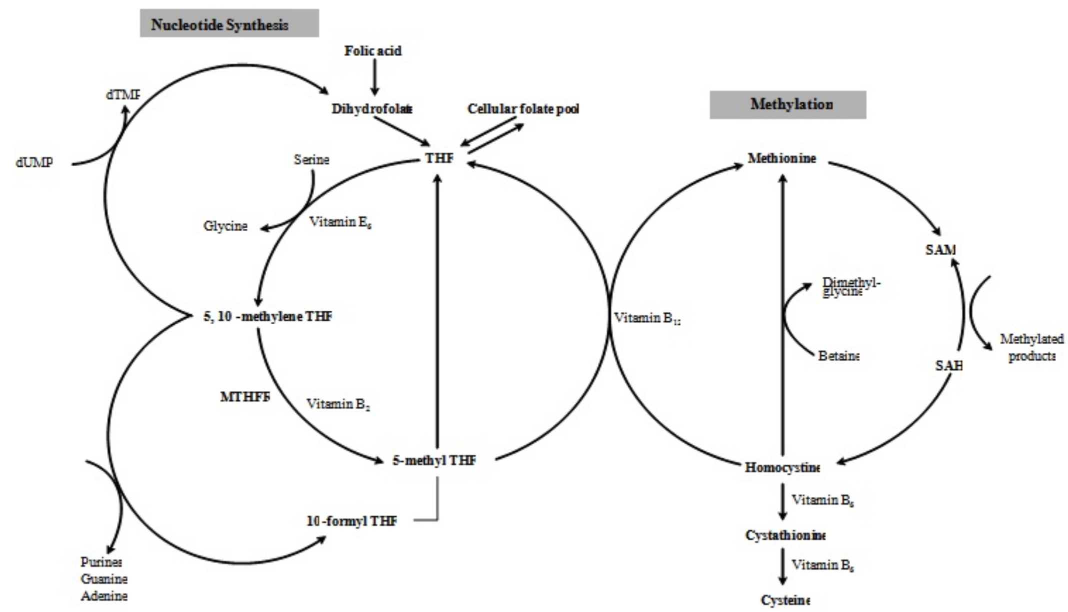
A number of researches have been conducted to investigate the effects of dietary energy on feed intake of commercial Leghorns. It is generally accepted that laying hens are capable of adjusting their feed consumption to maintain constant energy intake and that the intake of all nutrients, except water, can be regulated by including them in the diet in a specific ration to the amount of the energy present[1]. There is a wide range of dietary energy levels (2684 to 2992 kcal of ME/kg) currently being used by the egg industry. Wu *et al.*[2] reported that when dietary energy increased from 2719 to 2956 kcal of ME/kg, hens adjusted feed intake from 107.6 to 101.1 g/hen per day to achieve a constant energy intake.

The formulation of feed for commercial laying hens has traditionally been based on nutritional requirements and feed intake but upon further examination should be based on the ratio of energy and amino acids[3]. Hens will perform very well over a wide range of energy: methionine ratio, however the feed should be formulated to the energy : methionine ratio that meet the hen's need for the most efficient production of eggs. Harms[3] found a new program for formulating feed for laying hens. This program based on the ideal amino acid: energy ratio for the amount of egg content produced and was used to determine the percentage of methionine needed for laying hens. The response of hens received two levels of energy with three levels of methionine was evaluated by Harms *et al.*[4]. They found a linear response for egg content as the daily methionine intake increased.

Corresponding Author: Ramy M. Romeilah Department of Biochemistry, Faculty of agriculture, Cairo University.

In practical poultry diets, where soybeans are the primary protein-aceous component of poultry rations, methionine is the first limiting essential amino acid. Supplementation of methionine to poultry diets provides a way of improving the efficiency of protein utilization[5]. Its need in poultry rations for growth has been studied extensively[1,6], although there is considerable disagreement as to factors that influence its dietary requirement[1]. Many aspects of a methionine deficiency on avian metabolism have been studied such as its interaction with choline, betaine, folic acid, and vitamin B_{12} as well as effects on the immune system[7].

Folate is a collective term for a group of compounds with a pteroylglutamic acid backbone but differing oxidation states (i.e., folic acid, 5 methyltetrahydrofolate) whose primary function includes one-carbon transfer reactions. Examples of one-carbon transfer reactions include the remethylation of homocysteine, glycine-serine interconversion and purine synthesis[8]. House *et al.*[9] reported that folic acid status is linked to increased serum levels of the sulfur amino acid homocysteine, due to the role of folic acid that plays as co-factor in the remethylation of homocysteine to form methionine. We have shown increasing the folic acid content of eggs make the egg as an important source of dietary folic acid and lead to consumer acceptance of this commodity as a healthful product[10,11].



Folate Metabolism: Folate provides methyl groups for nucleotide synthesis via 5,10- methylenetetrahydrofolate (5,10-methyleneTHF), or to methylation reactions via 5-methyltetrahydrofolate (5-methyl THF) and S-adenosylmethionine (SAM). These two pathways of folate metabolism are separated by an irreversible reaction mediated by the methylenetetrahydrofolate reductase (MTHFR) enzyme. A polymorphism in MTHFR, reduces enzyme activity and is detrimental to folate status. dUMP, deoxyuridylate monophosphate; dTMP, deoxythymidylate monophosphate; SAH, S-adenosylhomocysteine[12].

The object of the present paper was to evaluate the effect of different levels of dietary methionine and folic acid supplementation on the performance, egg quality and immune response of laying hens fed diets varying in metabolizable energy (ME) levels. Therefore, this study aimed to improve the low dietary energy and methionine deficiency levels in laying hen diets by increasing folic acid supplementation.

MATERIAL AND METHODS

Experimental Design: In this experiment, birds received experimental diets in a 2 × 2 × 3 factorial arrangement. Laying hens were fed various levels of adequate or low energy (2800 vs. 2600 kcal ME/kg), adequate or deficient methionine (0.40 vs. 0.35%) and adequate or excess folic acid (0 vs. 10 and 20 mg/kg).

Birds, Management and Diets: One-thousand and eight-hundred Lohmann Brown (L.B.) laying hens were kept in cleaned and fumigated cages of wire floored batteries in closed system house. Hens were randomly distributed into 12 treatments of 150 hens in 10 replicates of 15 hens each. Feed and water were offered *ad-libitum* all over the experimental period (20 weeks) from 25 to 45 week of age, under a total of 16 hours light per day regimen. The basal experimental diets were formulated to meet the nutrient requirements according to the recommended allowances of the Lohmann Brown breed, where 2800 kcal ME/kg, 0.40% methionine and 0 mg folic acid/kg considered as a control (Table 1). Twelve diets were formulated to contain the following levels of metabolizable energy (ME) 2600 (low energy) and 2800 ME/kg diet (control). Each energy level containing either 0.35 or 0.40% methionine level, within each methionine level there was 3 levels of folic acid i.e. 0, 10 and 20 mg/kg diet. Methionine and folic acid sources were added as DL-methionine (98% methionine) and folic acid (96% folic acid), respectively.

Data Collection: The daily feed consumed per hen and hen-day egg production percentage were calculated every 4 weeks interval during the experimental period. Eggs were collected and weighed every 4 weeks during the experimental periods (20 weeks). Records of egg production, egg weight and feed consumption were used to calculate the amount of feed (kg.) which was required to produce one kilogram of eggs per hen or to calculate feed conversion ratio (FCR). All birds of each treatment were weighed at the beginning (initial live body weight) and at the end of experimental period (final live body weight) to calculate body weight gain. Egg shell thickness was determined using a dial pipe gauge digital. Haugh units were calculated based up on the height of albumen determined by a micrometer and egg weight according to Eisen *et al.*[13]. Yolk and albumen were separated, then 5 samples of pooled yolk and albumen for each replicate were freeze-dried and stored at –20°C. The egg yolk total lipid was extracted according to Folch *et al.*[14], while total cholesterol of egg yolk was determined by the method of Henly and Zak[15]. Serum total immunoglobulin titres were also determined according to Van der Zipp *et al.*[16]. Gross calorific values of feed and excreta were determined using the programmable isothermal jacket bomb calorimeter. Proximate analysis of the feed and dried excreta were done following the methods of A.O.A.C.,[17]. Faecal nitrogen was determined according to Jakobsen *et al.*[18].

Statistical Analysis: The data pooled through this study were procceded by General Linear Model procedures (GLM) described in SAS User's Guide[19] as follows:

$$Y_{ijkl} = m + E_i + M_j + F_k + (EMF)_{ijk} + e_{ijkl}$$

where, Y_{ijkl} is the observation of the parameter measured, m is the overall means, E_i is the energy levels effect, M_j is the methionine levels effect, F_k is the folic acid levels effect, $(EMF)_{ijk}$ is the interaction among energy, methionine and folic acid and e_{ijkl} is the random error term. Differences among treatments were tested using Duncan's multiple range test[20] and differences were significant at ($P \leq 0.05$).

RESULTS AND DISCUSSION

Laying Hen Performance: No significant differences due to energy and methionine levels in hen-day egg production percentages (Table 2). Folic acid at levels of 0, 10 and 20 mg/kg to hen diets, had a significant effect on the average values of egg production percentage. Egg production values were increased with increasing folic acid level from 0 to 20 mg/kg. The differences in egg production were significant due to energy x methionine x folic acid interaction. The highest egg production (91.67%) was recorded for group received T_9, while the least percentage (86.57%) was obtained for T_{10}. Significant differences among the control treatment (T_1) and the most of different experimental treatments. Generally, the improved hen-day egg production percentage due to the addition of methionine and vitamin (folic acid) was more apparent at the low energy level which could be due to the impact of amino acid or vitamin on nutrient utilization and especially energy. The same findings were supported by many authors[21,22,23,24]. They found that no significant differences in egg production when fed laying hen diets containing ME level ranged from 2396 to 3200 kcal ME/kg. The hens received the low-energy diet produced fewer eggs per day ($P \leq 0.05$) than the birds fed the high-energy diet[25]. Adeyemo and Longe [26] observed that birds fed the 2600 kcal ME/kg diet performed best egg production compared to those fed diets containing 2500, 2700, 2800 and 2900 kcal ME/kg and that egg production was significantly ($P \leq 0.05$) reduced by excessive dietary energy intake. Mean egg production during the laying period (22-44 weeks of age) with low energy (2600 kcal ME/kg) diet was greater than those for high energy (2900 kcal ME/kg) diet. They explained that low energy intake to protein intake ratio in low energy diet versus high energy diet may be a reason for greater egg production in lower energy than in higher energy diets[27]. Egg production decreased as energy levels increased and laying hens responded positively to diets containing as low as 2700 kcal ME/kg[28]. Shafer *et al.*[29] and Balnave[30] found that egg production was not significantly different due to methionine treatments when laying hens were fed diets containing methionine from 0.283 to 0.40%. Also, egg production was not

Table 1: Composition and chemical analysis for the basal diets:

Ingredients	%			
Yellow corn	50.87	50.82	50.47	50.28
Soybean meal	20.38	20.36	19.28	18.80
Corn gluten	5.06	5.12	5.00	5.34
Wheat bran	9.42	9.48	13.40	13.78
Corn oil	3.42	3.42	1.00	1.00
Limestone	8.00	8.00	8.00	8.00
Bone meal	2.20	2.20	2.20	2.20
Salts	0.30	0.30	0.30	0.30
Vitamin and mineral Premix *	0.30	0.30	0.30	0.30
DL-Methionine	0.05	-----	0.05	-----
Total	100.00	100.00	100.00	100.00
Calculated analysis**				
ME (kcal / kg)	2800	2800	2600	2600
Protein (%)	18.00	18.00	18.00	18.00
Lysine (%)	0.83	0.83	0.82	0.81
Methionine (%)	0.40	0.35	0.40	0.35
Methionine + cystine %	0.70	0.65	0.70	0.65
Calcium, %	3.80	3.80	3.80	3.80
Available Phosphorus, %	0.42	0.42	0.43	0.43
folic acid, mg/kg.	2.09	2.09	2.12	2.12
Linoliec acid, (%)	2.88	2.88	1.78	1.78
Linolenic acid, (%)	0.11	0.11	0.10	0.10
Energy : methionine ratio.	1 : 1.43	1 : 1.25	1 : 1.25	1 : 1.35
Energy : folic acid ratio.	1 : 0.001	1 : 0.001	1 : 0.001	1 : 0.001

*Each one kg of vitamin and mineral premix containing: Vit.A 8000000 I.U, vit.D3 2400000 I.U, vit.E 14000 mg, vit. K3 2000 mg, vit.B1 2000 mg, vit.B2 5000 mg, vit.B6 2000 mg, vit.B12 10 mg, pantothenic acid 8000 mg, niacin 27000mg, folic acid 500 mg, biotin 34 mg, choline chloride 400 gm, manganese 54 gm, zinc 50 gm, iron 27 gm, copper 7 gm, iodine 2 gm, selenium 0.2 gm, cobalt 0.20 gm and carrier (CaCo3) to 1 kg.
** According to NRC,[1].

significantly affected by different total sulfur amino acid (TSAA) levels up to 0.80%[31]. Increasing folic acid from 0.54 to 1.5 mg/kg in laying hen diets increased egg production[32]. El-Husseiny *et al.*[33] found that the addition of 0.5% methionine and folic acid up to 6.0 mg/kg to low energy diet (2600 kcal ME/kg) improved egg production. Nevertheless, Hens fed the 2800 kcal ME/kg diets showed a significant increase in egg production than those fed the diets containing 2700 kcal ME/kg[34]. Egg production of groups fed 0.40% dietary methionine significantly inferior, but there were no differences between other groups containing 0.46 and 0.52% methionine[35]. Also, egg production was significantly improved when methionine was supplemented to laying hen diets up to 0.44%[36,37].

Energy, methionine and folic acid levels and their interaction had no effect on egg weight (Table 2). Data showed that increasing folic acid level from 0 to 20 mg/kg decreased egg weight, but without significant effect. Moreover, the differences between the control treatment (2800 kcal ME/kg + 0.40% methionine + 0 mg folic acid/kg) and the other experimental treatments were not significant. Generally, increasing energy levels from 2600 to 2800 kcal ME/kg, methionine from 0.35

Table 2: Effect of experimental treatments on laying hen performance:

Treatments	Egg production (%)	Egg weight (g)	Egg mass (g of egg/ hen/day)	Feed intake (g/hen/day)	Feed conversion ratio (g of feed/g of egg)	Body weight gain (g)
T1 (2800 kcal/kg, 0.40%, 0 mg/kg).	89.58 ^{cd}	68.34	61.22	112.03 ^{cd}	1.83 ^{d}	180.45 ^{ab}
T2 (2800 kcal/kg, 0.40%, 10 mg/kg).	90.28 ^{bc}	67.74	61.14	111.78 ^{cd}	1.83 ^{d}	210.44 ^{a}
T3 (2800 kcal/kg, 0.40%, 20 mg/kg).	91.02 ^{ab}	66.42	60.46	111.22 ^{d}	1.84 ^{cd}	211.25 ^{a}
T4 (2800 kcal/kg, 0.35%, 0 mg/kg).	89.68 ^{cd}	68.18	61.12	112.44 ^{c}	1.84 ^{cd}	172.22 ^{ab}
T5 (2800 kcal/kg, 0.35%, 10 mg/kg).	90.22 ^{bcd}	67.74	61.12	112.16 ^{cd}	1.84 ^{cd}	173.00 ^{ab}
T6 (2800 kcal/kg, 0.35%, 20 mg/kg).	90.81 ^{ab}	67.05	60.89	111.97 ^{cd}	1.84 ^{cd}	173.50 ^{ab}
T7 (2600 kcal/kg, 0.40%, 0 mg/kg).	89.68 ^{cd}	68.77	61.66	116.14 ^{a}	1.89 ^{bc}	152.83 ^{b}
T8 (2600 kcal/kg, 0.40%, 10 mg/kg).	90.53 ^{bc}	68.13	61.67	115.66 ^{ab}	1.88 ^{bcd}	153.17 ^{b}
T9 (2600 kcal/kg, 0.40%, 20 mg/kg).	91.67 ^{a}	67.12	61.53	115.04 ^{b}	1.87 ^{bcd}	168.00 ^{b}
T10 (2600 kcal/kg, 0.35%, 0 mg/kg).	86.57 ^{e}	69.48	60.15	116.72 ^{a}	1.94 ^{a}	144.33 ^{b}
T11 (2600 kcal/kg, 0.35%, 10 mg/kg).	89.18 ^{d}	68.80	61.36	116.36 ^{a}	1.90 ^{ab}	153.95 ^{b}
T12 (2600 kcal/kg, 0.35%, 20 mg/kg).	91.10 ^{ab}	67.99	61.93	116.17 ^{a}	1.88 ^{bcd}	155.50 ^{b}
± Pooled SEM.	± 0.33	± 0.53	± 0.46	± 0.34	± 0.02	± 12.66
Probability	< 0.0001	0.2671	0.3351	< 0.0001	0.0012	0.0133
Main effect of energy level						
Control energy level (2800 kcal/kg).	90.27	67.58	60.99	111.93 ^{b}	1.84 ^{b}	186.81 ^{a}
Low energy level (2600 kcal/kg).	89.79	68.38	61.38	116.01 ^{a}	1.89 ^{a}	154.63 ^{b}
± Pooled SEM.	± 0.32	± 0.25	± 0.19	± 0.16	± 0.01	± 5.38
Probability	0.2991	0.2930	0.1607	< 0.0001	< 0.0001	0.0002
Main effect of methionine level						
Control methionine level (0.40%).	90.46	67.75	61.28	113.64	1.86	179.36
Deficiency methionine level (0.35 %).	89.59	68.20	61.10	114.30	1.87	162.08
± Pooled SEM.	± 0.31	± 0.26	± 0.20	± 0.52	± 0.01	± 6.31
Probability	0.0548	0.2265	0.5087	0.3728	0.2456	0.0613
Main effect of folic acid level						
Control folic acid level (0 mg / kg).	88.88 ^{c}	68.69	61.04	114.33	1.88	162.46
10 mg folic acid level (10 mg / kg).	90.05 ^{b}	68.10	61.32	113.99	1.86	172.64
20 mg folic acid level (20 mg / kg).	91.15 ^{a}	67.15	61.20	113.60	1.86	177.06
± Pooled SEM.	± 0.29	± 0.27	± 0.24	± 0.64	± 0.01	± 8.06
Probability	< 0.0001	0.1330	0.7129	0.7224	0.4997	0.4310

a, b, c,.... etc. means in same column, within each factor with different superscripts are significantly ($p \leq 0.05$) different.

to 0.40% and folic acid from 0 to 20 mg/kg did not affected egg weight. This indicates that Lohmann Brown laying hens perform well over a wide range of energy, methionine and folic acid. These results were confirmed by[24,38] who indicated that egg weight was not affected by dietary energy levels from 2500 to 3000 kcal ME/kg. Methionine supplementation at 0 and 0.10% of laying hen diets containing 2790 kcal ME/kg did not affect egg weight[31,39]. Egg weight was not affected by folic acid supplementation up to 4 mg crystalline folic acid/kg of laying hen diet[40,41]. Increasing energy, methionine and folic acid up to 3000 kcal ME/kg, 0.50% and 6 mg/kg did not affect egg weight[33]. On the other hand, mean 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[42]. Average egg weight increased with increasing ME level (2500, 2700 and 2900 kcal/kg) in the diet. The reasons for these differences are not clear, but may be due to the colder weather combined with behavioral factors[43]. Increasing dietary concentration of several nutrients including methionine increases egg weight[44]. Egg weight increased significantly ($P\leq 0.05$) when DL-methionine was added to the basal diet from 0.012 to 0.060%. The inconsistent response could be due to low sensitivity, large variation and different statistical methods[45]. Egg weight increased with increasing dietary methionine level (0.40, 0.46 and 0.52%)[35]. These contradictions could be related to body weight and amino acid levels among different experiments. Also, reducing dietary folic acid resulted in reduced egg weight[44].

The differences in egg mass were not significant due to either metabolizable energy, methionine or folic acid levels (Table 2). Moreover, the effect of interaction among energy x methionine x folic acid on egg mass was not significant. Generally, decreasing energy and methionine levels up to 2600 kcal ME/kg and 0.35%, respectively with increasing folic acid up to 20 mg/kg did not effect on egg mass. These results were confirmed by[2] who showed that there was no significant dietary energy (2719, 2798, 2877 and 2956 kcal ME/kg) effect on egg mass. However, Nahashon *et al*. (2007)[42] reported that mean egg mass was higher ($P\leq 0.05$) in hens receiving diets with 2800 kcal of ME/kg of feed than those fed diets containing 2900 kcal of ME/kg of diet. Egg mass responded significantly ($P\leq 0.05$) to methionine supplementation[46].

A significant linear effect on feed intake (g/hen/day) was observed due to ME levels (Table 2). Hens received the low ME diet significantly ($P\leq 0.05$) consumed more feed than those received the control ME diets. Hens received the low ME diet consumed 3.65% more feed than did hens receiving the control diet. Hens increased feed intake to meet their increased energy requirement. Methionine and folic acid levels up to 0.40% and 20 mg/kg, respectively had no effect on the amount of feed intake. The effect of interaction among energy x methionine x folic acid on the amount of feed intake was significant ($P\leq 0.05$). T_{10} recorded the highest value of feed intake (116.72 g/hen/day), while T_3 consumed the least amount (111.22 g/hen/day). Additionally, there were highly significant ($P\leq 0.05$) differences in feed intake values between the control treatment (T_1) and the other experimental treatments which containing the low energy level but, the differences between the control diet and groups containing 2800 kcal ME/kg did not significant. Generally, the hens fed the 2600 kcal ME/kg diets consumed the highest ($P\leq 0.05$) amount of feed, while those fed the other diets at 2800 kcal ME/kg consumed the least amount of feed. Increasing dietary methionine and folic acid levels in the diets slightly decreased the amount of feed intake, without any significant differences. These results supported by[25,47,48] observed that birds fed the lower energy diets (2600 kcal ME/kg) consumed significantly more feed than those fed the higher energy diets (2900 kcal ME/kg). They concluded that dietary energy is the main factor controlling the feed intake of pullets and laying hens. Also, Totsuka *et al*.[49] noticed that feed consumption decreased with increasing ME levels (2700, 2850 and 3000 kcal/kg) and mentioned that many factors may influenced feed intake in laying hens, among those are environmental temperature, stage of production and energy content of the diet. Similarly, Robinson[43] observed that hen is inefficient at adjusting feed intake to meet their energy requirement, since feed intake of low energy diet (bulky diet), was substantially lower than predicted on the basis of energy requirement of the birds and energy content of the diet, suggesting that gut capacity is a limiting factor. Harms *et al*.[50] who concluded that there was no significant difference in feed consumption among 6 methionine levels up to 0.422%. Hebert *et al*.[40] 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 consumed decreased with increasing energy, methionine and folic acid levels up to 3000 kcal/kg, 0.50% and 6.0 mg/kg, respectively[33]. On the other hand, Feed intake was not affected by a range of dietary energy between 2400 to 2900 kcal ME/kg[51,52]. Depression of feed intake is a highly sensitive response to excessive amino acid supplementation because diets supplemented with 0.10% DL-methionine significantly reduced feed intake[53]. The precise mechanisms responsible for the depressive effects of excess amino acids on feed intake remain to be fully clarified, although the available results indicate neither the sulfur content nor the presence of an amino nitrogen group are critical[54]. Average daily feed consumption was significantly higher for birds consuming the diets with 32 mg folic acid/kg compared to birds eating folic acid at 0, 1, 2, 4, 8 and 16 mg/kg[10].

There were significant differences ($P \leq 0.05$) in FCR between different ME levels (Table 2). These results could be attributed to the different amounts of feed consumed and egg production. Neither methionine nor folic acid levels up to 0.40% and 20 mg/kg, respectively had significant effect on FCR for egg production. The effect of interaction among energy x methionine x folic acid on FCR values was significant. FCR was improved (1.83) when hens fed T_1 and T_2. Meanwhile, hens received T_{10} recorded the worst value of FCR (1.94). It is clear that, the addition of high folic acid level 20 mg/kg diet with 0.35 and 0.40% methionine levels to low energy diet (2600 kcal/kg) improved FCR compared to all diets containing 2800 kcal/kg with different methionine and folic acid levels. Generally, the addition of 0.35 and 0.40% methionine and folic acid up to 20 mg/kg to low energy diets improved FCR. This means that 2600 kcal ME/kg diet with 0.40% methionine and 20 mg folic acid/kg diet was adequate for Lohmann Brown egg type strain during 25 – 45 wk of age. These results are in agreement with those obtained by[26,27] which, indicated that birds fed on diet containing 2600 kcal ME/kg had better FCR when the ME content of the diets ranged from 2500 to 2900 kcal/kg. FCR was not affected by adding DL-methionine up to 0.383% to laying hen diets[30,39]. Hebert *et al.*[40] who suggested that there was no significant difference in FCR due to of laying hens dietary folic acid supplementation up to 4 mg/kg. These results disagree with those of[28,43,49] who indicated that increasing the dietary energy levels up to 3000 kcal ME/kg improved FCR, due to the decrease in feed intake. Also, Feed conversion was improved as TSAA level increased from 0.484 to 0.684% in laying hen diets. The explanation for improved feed efficiency with increasing TSAA level might be attributed to more balanced amino acids[55]. The best value of FCR was all diets containing 3000 kcal ME/kg with different levels methionine (0.40 and 0.50%) and folic acid (2 and 6 mg/kg) compared to the control group (2800 kcal ME/kg, 0.40% methionine and 2.0 mg folic acid/kg)[33].

The average live body weight gain for Lohmann Brown hens was significant effect by dietary energy level (Table 2). Laying hens received control ME diets gained the most weight while hens received the low energy diets gained the least weight. No significant differences between the mean values of body weight gain due to methionine and folic acid levels. The average values of body weight gain ranged from 144.33 to 211.25g for T_{10} and T_3, respectively. While, no significant differences in live body weight gain between the control diet (2800 kcal ME/kg + 0.40% methionine + 0 mg folic acid/kg) and all the experimental treatments. Generally, the average live body weight gain increased gradually with increasing the energy level from 2600 to 2800 kcal ME/kg without supplemental methionine and folic acid. However, average live body weight gain did not significantly effect by methionine and folic acid levels. It was worthy to note that energy level 2800 kcal ME/kg, with 0.40% methionine and 20 mg folic acid/kg resulted in higher body weight gain values than 2600 kcal ME/kg diets. It is perhaps surprising that reduced final live body weight and live body weight gain on the low ME diets did not result in adverse effect on performance. This may be due to circulating T_3 (3,3,5- triiodothyronine) and T_4 (thyroxine) where the low ME diet resulted in higher plasma T_3 and lower plasma T_4 concentrations, suggesting a change in the rate of conversion of T_3 and T_4 between growing and laying hens which is dependent either on dietary ME intake or on the amount of feed consumed. This leads to the conclusion that the diet (or feed consumption or energy intake) affects plasma thyroid hormone concentrations and that during the laying stage; this effect of diet is as important or greater than the effect of climate. This conclusion may be modulated by the somewhat inconsistent nature of field conditions, which may have resulted in some uncontrolled factor influencing the observed effects[56]. The significant differences in body weight gain among experimental treatments were supported by the findings of Stilborn and Waldroup[57] who concluded that lower dietary energy levels tended to reduce body weight gain when fed hens diets containing 2500, 2600, 2700 and 2800 kcal ME/kg. Less energy is available for fat deposition when lower dietary ME levels are utilized and high levels of fibrous feed with low energy diets will reduce the amount of weight gain that occurs during the laying period[58]. Robinson[43] observed that body weight gain increased with increasing dietary ME level (2500, 2700 and 2900 kcal ME/kg) in the diet. This result may be due to abdominal fat pad weight (as a proportion of body weight) at termination of the trial was lower ($P \leq 0.01$) for the low ME diet than for the other diets. Harms *et al.*[50] and Amaefule *et al.*[39] who found that body weight gain did not differ among treatments which containing methionine from 0.289 to 0.422%. Hebert *et al.*[40] who noticed that there was no significant difference in body weight due to folic acid supplementation up to 4.0 mg/kg. El-Husseiny *et al.*[33] found that live body weight gain increased with increasing the energy level from 2600 to 3000 kcal ME/kg without supplemental methionine and folic acid. However, average live body weight gain decreased gradually with increasing methionine and folic acid levels to 3000 kcal ME/kg diet. On the contrary to the present results, Pesti[21] and Summers and Leeson[47] found no differences in body weight gain of laying hens fed dietary energy ranged from 2600 to 3200 kcal ME/kg. Obviously, energy consumption is the main factor influencing weight gain of the pullet to onset of production. Along the same line, Harms and Russell[59] reported that the loss of body weight was reduced as the level of methionine was increased in the diet from 0.20 to 0.38%. Keshavarz[44] observed that reducing dietary folic acid resulted in reduced body weight.

Egg Quality: There were detectable changes in yolk and albumen percentage due to energy and methionine levels (Table 3). While, the differences among folic acid levels were not significant on yolk and albumen percentage. The interaction among energy x methionine x folic acid significantly effect edible percentage. The values of yolk and albumen percentage ranged from 89.83 to 91.92 g for T_{12} and T_1, respectively. These results were in agreement with those by[60,61] found that egg content increased as the level of dietary methionine increased in the diet.

There were no significant difference in yolk weight and percent yolk of egg weight due to folic acid supplementation[40]. However, Grobas *et al.*[62] who found that different levels of ME (2676 or 2820 kcal/kg) had no significantly effect on yolk and albumen weights. Also, Albumen and yolk weights showed irregular trends with dietary energy levels (2600, 2800, 3000 and 3100 kcal ME/kg)[63]. At the beginning of the laying cycle, methionine plus cystine (TSAA) had no effect on yolk and albumen weight[64].

A significant ($P \leq 0.05$) decrease in shell percentage (%) and shell thickness (μm) due to feeding laying hen diets containing 2800 kcal ME/kg when compared to diets containing 2600 kcal ME/kg (Table 3). It is clear that egg shell percentage and shell thickness decreased gradually with increasing ME level from 2600 to 2800 kcal/kg of the diet. Methionine deficiency level (0.35%) improved egg shell percentage and shell thickness. Increasing folic acid level from 0 to 20 mg/kg had no effect on shell percentage. But, egg shell thickness increased by increasing folic acid level from 0 to 20 mg/kg. The differences in shell percentage and shell thickness were significant due to energy x methionine x folic acid interaction. However, the highest value of shell percentage and shell thickness (10.17 % and 0.477 μm, respectively) was recorded by T_{12}, while the least value (8.08 % and 0.412 μm, respectively) was obtained by T_1 (control diet). Moreover, there were highly significant differences in shell percentage and shell thickness between the birds fed the control diet (T_1) and those fed the other different experimental diets were observed. Generally, all diets which formulated to contain 2600 kcal ME/kg with methionine and folic acid supplementation improved egg shell percentage and shell thickness compared to 2800 kcal ME/kg diets. In this regard, mean shell thickness was higher ($P \leq 0.05$) in hens receiving low energy diets than those fed high energy diets[42]. Atteh and Leeson[65] indicated that the reasons of improved both egg shell weight and egg shell thickness with diets containing 2600 kcal ME/kg was due to the fecal calcium losses on the low ME diet were lower than on the high ME diet due to formation of indigestible ca-soaps between Ca and fats/oils in high energy (high fat diet). The problem of bone density loss with resultant deformities and fractures associated with old age is a problem. Bone density loss, or osteoporosis, is a definite health problem that can be managed and prevented if one understands what is happening. One of the mechanisms, and the one that usually gets the most attention, is the reduction of hormone levels associated with aging. There are two kinds of cells found in bone. The osteoclasts are constantly destroying old bone while osteoblasts are constantly building new bone. It is necessary for these two processes, bone destruction and bone building to be balanced in order to have solid healthy bones. There are two control mechanisms by which osteoblasts are stimulated to create new bone mass. One is hormonal regulation and the other is the piezoelectric stimulation of bone growth that comes from exercise. One of the principal factors in the hormonal regulation of bone health is estrogen. Estrogen is actually a group of hormones. Another contributing factor in the development of osteoporosis is homocysteine. Homocysteine is a metabolite of the amino acid methionine. It has been implicated in several degenerative diseases including heart disease, arteriosclerosis, and osteoporosis. High homocysteine levels cause osteoporosis by the formation of defective bone (protein) matrix. Homocysteine is detoxified into methionine by specific nutrients which are able to donate methyl groups to the homocysteine molecule. These nutrients include folic acid, B_{12}, B_6, and betaine (trimethylglycine). Over eighteen nutrients are required to build bone. Calcium is the most abundant element in bone, but without the others new bone cannot be built regardless of how much calcium is available. These nutrients include calcium, phosphorus, magnesium, manganese, zinc, copper, boron, silica, fluorine, vitamins A, C, D, B_6, B_{12}, K, folic acid, essential fatty acids and protein. The body only uses minerals well when they are in a proper balance. An excess of phosphorus, for example, can cause loss of bone calcium and reduced bone mass[66]. The reason for improved egg shell quality is due to high calcium intake resultant from high feed intake[67].

Table 3: Effect of experimental treatments on egg quality:

Treatments	Albumen and yolk percent	Egg shell percent	Shell thickness(µm)	Haugh unit	Egg yolk color
T1 (2800 kcal/kg, 0.40%, 0 mg/kg).	91.92 a	8.08 h	0.412 e	66.66 ab	7.22 a
T2 (2800 kcal/kg, 0.40%, 10 mg/kg).	91.65 b	8.35 g	0.425 de	65.28 abc	7.19 a
T3 (2800 kcal/kg, 0.40%, 20 mg/kg).	91.44 bc	8.56 fg	0.434 bcde	65.44 abc	7.22 a
T4 (2800 kcal/kg, 0.35%, 0 mg/kg).	91.64 b	8.36 g	0.416 e	62.03 d	6.99 ab
T5 (2800 kcal/kg, 0.35%, 10 mg/kg).	91.51 b	8.49 g	0.430 cde	63.97 cd	7.25 a
T6 (2800 kcal/kg, 0.35%, 20 mg/kg).	91.27 cd	8.73 ef	0.436 bcde	65.18 abc	6.84 abcd
T7 (2600 kcal/kg, 0.40%, 0 mg/kg).	91.11 de	8.89 de	0.456 abcd	66.93 a	6.71 bcd
T8 (2600 kcal/kg, 0.40%, 10 mg/kg).	90.91 ef	9.09 cd	0.465 ab	66.16 abc	6.54 cd
T9 (2600 kcal/kg, 0.40%, 20 mg/kg).	90.76 f	9.24 c	0.472 a	64.77 abcd	6.46 d
T10 (2600 kcal/kg, 0.35%, 0 mg/kg).	90.86 f	9.14 c	0.460 abc	64.52 abcd	6.60 bcd
T11 (2600 kcal/kg, 0.35%, 10 mg/kg).	90.26 g	9.74 b	0.468 ab	63.51 cd	6.75 bcd
T12 (2600 kcal/kg, 0.35%, 20 mg/kg).	89.83 h	10.17 a	0.477 a	65.32 abc	6.94 abc
± Pooled SEM.	±0.072	±0.072	±0.010	±0.859	±0.123
Probability	< 0.0001	< 0.0001	0.0006	0.0276	0.0003
Main effect of energy level					
Control energy level (2800 kcal/kg).	91.57 a	8.43 b	0.425 b	64.76	7.12 a
Low energy level (2600 kcal/kg).	90.62 b	9.38 a	0.466 a	65.20	6.67 b
± Pooled SEM.	0.086	0.087	± 0.004	± 0.430	± 0.05
Probability	< 0.0001	< 0.0001	< 0.0001	0.4743	< 0.0001
Main effect of methionine level					
Control methionine level (0.40%).	91.30 a	8.70 b	0.444	65.87 a	6.89
Deficiency methionine level (0.35 %).	90.90 b	9.10 a	0.448	64.09 b	6.89
± Pooled SEM.	± 0.135	± 0.135	0.006	0.375	0.078
Probability	0.0419	0.0419	0.6622	0.0019	0.9644
Main effect of folic acid level					
Control folic acid level (0 mg / kg).	91.38	8.62	0.436 b	65.03	6.88
10 mg folic acid level (10 mg / kg).	91.08	8.92	0.447 ab	64.73	6.93
20 mg folic acid level (20 mg / kg).	90.83	9.17	0.455 a	65.17	6.86
± Pooled SEM.	± 0.165	± 0.165	±0.219	± 0.535	± 0.097
Probability	0.0718	0.0718	0.035	0.8361	0.8753

a, b, c,…. etc. means in same column, within each factor with different superscripts are significantly ($p \leq 0.05$)different.

Petersen *et al.*[68] reported that reducing dietary methionine improved shell quality without affecting egg production. Methionine supplementation to layer diets did not significantly influence egg shell thickness values[39,69]. El-Husseiny *et al.*[33] found that increasing folic acid level from 2.0 to 6.0 mg/kg had no effect on shell weight and shell thickness. But, egg shell thickness was insignificantly increased with high folic acid level. This may be attributed to the necessary of nutrients available in the blood at their convenience. However, Shell thickness was similar at all dietary ME levels ranged from 2600 to 3200 kcal ME/kg, but egg shell weight was not affected by dietary energy levels[70,71]. Shell weight increased when increasing the level of methionine from 0.33 to 0.45%[72]. Reducing dietary folic acid improved egg shell quality[44].

Haugh units decreased with increasing ME level in the diet but, no significant effects were observed (Table 3). A significant effect in Haugh unit values was shown due to methionine level. Increased folic acid levels from 0 to 20 mg/kg insignificantly improved Haugh units. Energy x methionine x folic acid interaction significantly affected ($P \leq 0.05$) on Haugh units. The highest value of Haugh unit (66.93) was recorded by T_7. While, the least value (62.03) was obtained by T_4. No significant difference in Haugh units between the control treatment (T_1) and most of other treatments. These findings agreed with those reported by Stilborn and Waldroup[57] who reported that dietary energy level did not significantly influenced Haugh units values. Rao *et al*.[73] who line with those that Haugh unit score was better at 0.60% TSAA in the diet. There was no significant difference in Haugh units due to folic acid supplementation up to 4 mg/kg[40]. 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 methionine level up to 0.50% and the effect of interaction among energy, methionine and folic acid on Haugh unit was significant[33]. On the other hand, Uddin *et al*.[63] found irregular Haugh units trend with dietary energy 2600, 2800, 3000 and 3100 kcal ME/kg. However, Haugh units were not affected by TSAA level up to 0.877%[31,39].

Significant differences ($P \leq 0.05$) in egg yolk color were found due to ME levels (Table 3). The diets containing 2800 kcal ME/kg recorded the darkest color values when compared with diets containing 2600 kcal ME/kg. The reason of the differences on egg yolk color may be due to the use of different levels of yellow corn in the experimental diets being formulated to contain different levels of energy. Egg yolk color did not statistically differ between 0.35 and 0.40% methionine level. Addition of folic acid up to 20 mg/kg did not affect egg yolk color. Differences in egg yolk color were significant due to energy x methionine x folic acid interaction. The darkest value of egg yolk color (7.25) was recorded by T_5. Moreover, a significant differences in average values of egg yolk color between control group (T_1) and the other treatments. The results of the yolk color agreed with those reported by[57] who noticed that there were significant differences for energy levels 2500, 2600, 2700 and 2800 kcal ME/kg for yolk color. These differences may be due to the xanthophyll content of the major feed ingredients rather than actual energy levels as xanthophyll levels were not controlled within the diets. Also, egg yolk color was higher in high energy corn diets. This could be due to high carotenoid content of corn[71]. Diet containing 2600 kcal ME/kg diet within 0.40% methionine and 2.0 mg folic acid proved to be adequate for good external and internal egg quality, while the decline in egg yolk color could be overcome by xanthophyll pigmentation[33]. However, Adeyemo and Longe[51] observed that yolk color did not vary significantly with dietary energy when they formulated diets containing 2400, 2500, 2600 and 2700 kcal ME/kg. In general the addition of 20 mg folic acid/kg to low energy (2600 kcal ME/kg) and low methionine (0.35%) levels due to improved external and internal egg quality (Table 3).

Chemical Traits: The low ME diets which containing 2600 kcal ME/kg increased egg yolk total lipids significantly ($P \leq 0.05$) when compared to diets containing 2800 kcal ME/kg (Table 4). No significant effect in egg yolk total lipids was observed due to methionine or folic acid supplementation. No significant effects in egg yolk total lipids due to energy x methionine x folic acid interaction. These results are in agreed with the findings of Guenter *et al.*[74] who reported that increasing dietary linoleic acid contents for laying hens, caused a decrease in lipid contents of egg yolk when compared with those fed lower dietary levels of linoleic acid. Diets containing 3000 kcal ME/kg (high linoleic acid contents, 4%) decreased significantly egg yolk total lipids. However, Methionine (0.40 and 0.50%) and folic acid (2 and 6 mg/kg) levels did not affect egg yolk total lipids[33]. On the contrary to this result, yolk fat was similar and not significantly influenced by dietary energy levels up to 3100 kcal ME/kg[63,70].

A significant difference in egg yolk total cholesterol was observed among hens fed two different ME levels (Table 4). Results indicated that hens received low ME diets recorded the highest egg yolk cholesterol compared to the other level of ME. Differences due to methionine or folic acid levels on egg yolk total cholesterol were not significant. The differences in egg yolk total cholesterol values were significant due to energy, methionine and folic acid interaction. The highest value of egg yolk total cholesterol (15.65 mg/g of yolk) was recorded by T_{10}, while the least value (14.56 mg/g of yolk) was obtained by T_2. However, no significant differences in egg yolk total cholesterol between control treatment (T_1) and most of the other experimental treatments. The results are in agreement with those reported by

Table 4: Effect of experimental treatments on chemical traits and immuneresponses:

Treatments	physiological traits		Immune response
	Egg yolk total lipids (gm/100gm)	Egg yolk total cholesterol(mg/kg)	Immunoglobulin titres
T1 (2800 kcal/kg, 0.40%, 0 mg/kg)	25.37	15.55 [ab]	8.76 [c]
T2 (2800 kcal/kg, 0.40%, 10 mg/kg)	25.65	14.56 [d]	8.93 [bc]
T3 (2800 kcal/kg, 0.40%, 20 mg/kg)	25.78	14.96 [abcd]	9.19 [bc]
T4 (2800 kcal/kg, 0.35%, 0 mg/kg)	25.36	14.69 [cd]	9.12 [c]
T5 (2800 kcal/kg, 0.35%, 10 mg/kg)	25.46	14.82 [bcd]	9.29 [bc]
T6 (2800 kcal/kg, 0.35%, 20 mg/kg)	25.34	14.60 [d]	9.18 [bc]
T7 (2600 kcal/kg, 0.40%, 0 mg/kg)	26.16	15.40 [abc]	9.28 [bc]
T8 (2600 kcal/kg, 0.40%, 10 mg/kg)	26.53	15.26 [abcd]	9.18 [bc]
T9 (2600 kcal/kg, 0.40%, 20 mg/kg)	26.39	15.53 [ab]	9.51 [b]
T10 (2600 kcal/kg, 0.35%, 0 mg/kg)	26.54	15.65 [a]	9.09 [bc]
T11 (2600 kcal/kg, 0.35%, 10 mg/kg)	26.12	15.38 [abc]	9.51 [b]
T12 (2600 kcal/kg, 0.35%, 20 mg/kg)	26.25	15.54 [ab]	10.14 [a]
± Pooled SEM.	± 0.377	± 0.228	± 0.205
Probability	0.1776	0.0087	0.0144
Main effect of energy level			
Control energy level (2800 kcal/kg).	25.49 [b]	14.86 [b]	9.08 [b]
Low energy level (2600 kcal/kg).	26.33 [a]	15.46 [a]	9.45 [a]
± Pooled SEM.	± 0.135	± 0.099	± 0.097
Probability	0.0001	0.0002	0.0101
Main effect of methionine level			
Control methionine level (0.40%).	25.98	15.21	9.14
Deficiency methionine level (0.35 %).	25.84	15.11	9.39
± Pooled SEM.	± 0.168	± 0.122	± 0.103
Probability	0.5739	0.9644	0.0984
Main effect of folic acid level			
Control folic acid level (0 mg / kg).	25.86	15.32	9.06 [b]
10 mg folic acid level (10 mg / kg).	25.94	15.00	9.23 [ab]
20 mg folic acid level (20 mg / kg).	25.94	15.16	9.51 [a]
± Pooled SEM.	± 0.210	± 0.147	± 0.121
Probability	0.9521	0.3290	0.0432

a, b, c,…. etc. means in same column, within each factor with different superscripts are significantly ($p \leq 0.05$) different.

Oke *et al*.[38] who found that cholesterol level maximized at 2650 kcal ME/kg compared to other diets containing 2750 and 2850 kcal ME/kg. This suggested that a basal level is required for egg formation. The persistence of abnormally high level of cholesterol can cause despite of cholesterol plaques to occur in the aorta which can ultimately contribute to health hazard. Hens had lower cholesterol and packed cell volume levels as a result of higher egg production due to an increase in demand for egg production. In general, egg yolk total lipids paralleled egg yolk total cholesterol where, the diets containing 2600 kcal ME/kg tends to increase yolk total lipids and yolk total cholesterol compared to diets containing 2800. The different levels of methionine and folic acid did not affect on average yolk total lipids and yolk total cholesterol values.

Immune Response: The effect of ME level on serum total immunoglobulin titres was significant (Table 4). However, low ME (2600 kcal ME/kg) diets resulted in the highest average of serum immunoglobulin titres and 2800 kcal ME/kg diets which recorded the least value. Moreover, low methionine level (0.35%) improved serum total immunoglobulin titres compared to that obtained at level of 0.40%, but, without significant differences. However, significant differences in serum total immunoglobulin titres were observed due to feeding laying hen diets containing different levels of folic acid up to 20 mg/kg. The effect of interaction among energy, methionine and folic acid on serum total immunoglobulin titres was significant. T12 gave the highest value of serum total immunoglobulin titres (10.14). While, T1 resulted in the least value (8.76). Moreover, significant differences in serum total immunoglobulin titres between most of the experimental treatments and the control diet (T1) were observed. Generally, Immune functions (serum total immunoglobulin titres) were significantly affected by energy levels, but little affected by methionine and folic acid supplementation in the diet. A slightly decrease was observed in serum total immunoglobulin titres with increased methionine and folic acid. However, deficiency level of methionine with high level of folic acid or vice-versa to low energy diet significantly improved serum total immunoglobulin titres compared to their respective control. In this regard, the reason for the appear discrepancy is most probably relates to the amounts of oil (fatty acids) in the experimental diets.

Calder[75] concluded that high fat diets are associated with suppressed immune functions (T-cell proliferation). Kelly and Daudu[76] and Calder[75,77] reported that lower natural killer cell activity, lymphocyte proliferation and antibody production are associated with the feeding of high fat diet including oils rich in linoleic acid (maize, sunflower and safflower oils) or in linolenic acid (linseed oil) when compared with feeding high saturated fat diets. These data suggested that linoleic acid has the potential to suppress innate and acquired immune functions. Also, Balnave[30] reported that serum total immunoglobulin titres decreased with increasing dietary methionine level in laying hen diets. Energy level up to 3000 kcal ME/kg had a significant effect on immunoglobulin titres but, methionine and folic acid levels up to 0.50% and 6 mg/kg, respectively did not significant effect on immunoglobulin titres. Moreover, the interaction among energy, methionine and folic acid for immunoglobulin titre values were significant[33].

Nutrients Digestibility: Generally, the diet containing 2600 kcal ME/kg gave the best digestion coefficient values of almost the nutrients compared to the other diets which containing 2600 kcal ME/kg (Table 5). Metabolizability increased with increasing ME level in the diet from 2600 to 2800 kcal ME/kg (92.55 vs. 93.24%). However, methionine and folic acid levels up to 0.40% and 20 mg/kg, respectively did not any significant effect on both nutrients digestion coefficient and metabolizability. However, a significant difference in the digestion coefficient of almost the nutrients and metabolizability values were observed depends on energy level but, methionine and folic acid supplementation did not effect on digestibility coefficient of nutrients. These results are supported by the findings of Hunton[78] reported that feed intake as well as nutrient utilization is increased by low level of energy in diet. Also, energy metabolizability was significantly ($P \leq 0.01$) lower when pullets were fed the low ME diet[79]. Along the same line, Saki[80] reported that high level of crude fiber content reduced the bioavailability of energy. On the contrary to the result, Digestibility of DM and OM were significantly higher ($P \leq 0.05$) on 0.323% methionine level compared to 0.248 and 0.267% level[69]. However, The losses of crude protein were significantly ($P \leq 0.01$) greater from pullets fed the low ME diet[79].

In conclusions and applications, based on results of the present study, the addition of methionine and folic acid up to 0.35% and 20 mg/kg to low energy Lohmann Brown laying hen diets (2600 kcal ME/kg) improved performance, egg quality and increased immune response compared to adequate energy level (2800 kcal ME/kg). Therefore, we can decrease the usage of yellow corn in diets. Although, the calorie: protein ratio was found to be important and is considered in the formulation of broiler feed, it has not been given much consideration in the formulation of feed for laying hens. Obviously, very good performance, egg quality and immune response were obtained even when the ratio of calories of metabolizable energy to sulfur amino acid (methionine) and vitamin (folic acid) in the diet, were 1:1.35 and 1:0.008, respectively. Moreover, added folic acid in layer diets increased egg shell thickness through estrogen hormone relations mechanism in which the

Table 5: Effect of experimental treatments on the digestibility coefficient of nutrients and metabolizability of the experimental diets:

Treatments	DM	CP	EE	CF	NFE	OM	Metabolizability
T1 (2800 kcal/kg, 0.40%, 0 mg/kg).	72.70 [bcd]	96.01 [ab]	86.12 [c]	24.65 [cd]	83.72 [a]	80.59 [b]	93.71 [a]
T2 (2800 kcal/kg, 0.40%, 10 mg/kg).	70.47 [de]	95.63 [abc]	85.76 [cd]	24.93 [cd]	81.53 [b]	78.91 [c]	93.39 [ab]
T3 (2800 kcal/kg, 0.40%, 20 mg/kg).	68.27 [e]	95.21 [bc]	85.39 [de]	25.20 [c]	81.57 [b]	78.65 [c]	93.07 [abc]
T4 (2800 kcal/kg, 0.35%, 0 mg/kg).	73.43 [abc]	96.45 [a]	85.06 [ef]	25.01 [cd]	84.70 [a]	80.71 [b]	93.25 [abc]
T5 (2800 kcal/kg, 0.35%, 10 mg/kg).	71.86 [cd]	96.09 [ab]	84.62 [fg]	24.52 [cd]	84.73 [a]	80.79 [b]	93.10 [abc]
T6 (2800 kcal/kg, 0.35%, 20 mg/kg).	70.75 [d]	95.68 [abc]	84.18 [g]	24.05 [d]	84.57 [a]	80.64 [b]	92.96 [abc]
T7 (2600 kcal/kg, 0.40%, 0 mg/kg).	75.35 [a]	95.70 [abc]	89.29 [a]	28.28 [a]	85.12 [a]	83.26 [a]	93.37 [ab]
T8 (2600 kcal/kg, 0.40%, 10 mg/kg).	74.61 [ab]	95.35 [bc]	89.17 [a]	27.62 [ab]	84.08 [a]	82.33 [a]	92.42 [bcd]
T9 (2600 kcal/kg, 0.40%, 20 mg/kg).	73.87 [abc]	95.00 [c]	89.05 [a]	26.96 [b]	85.75 [a]	83.26 [a]	91.48 [d]
T10 (2600 kcal/kg, 0.35%, 0 mg/kg).	74.17 [abc]	95.85 [abc]	88.33 [b]	27.41 [ab]	85.53 [a]	82.94 [a]	93.18 [abc]
T11 (2600 kcal/kg, 0.35%, 10 mg/kg).	74.16 [abc]	95.28 [bc]	88.30 [b]	27.14 [b]	85.37 [a]	82.92 [a]	92.68 [abc]
T12 (2600 kcal/kg, 0.35%, 20 mg/kg).	74.16 [abc]	95.72 [abc]	88.26 [b]	26.90 [b]	84.91 [a]	82.92 [a]	92.18 [cd]
± Pooled SEM.	± 0.756	± 0.266	± 0.159	± 0.321	± 0.658	±0.495	± 0.324
Probability	0.0007	0.0466	< 0.0001	< 0.0001	0.0073	0.0001	0.0182
Main effect of energy level							
Control energy level (2800 kcal/kg).	71.24[b]	95.84	85.19[b]	24.72[b]	83.47[b]	80.05[b]	93.24[a]
Low energy level (2600 kcal/kg).	74.38[a]	95.48	88.73[a]	27.38[a]	85.12[a]	82.94[a]	92.55[b]
± Pooled SEM.	± 0.437	± 0.132	± 0.175	± 0.161	± 0.376	±0.252	± 0.174
Probability	< 0.0001	0.0670	< 0.0001	< 0.0001	0.0051	< 0.0001	0.0101
Main effect of methionine level							
Control methionine level (0.40%).	72.54	95.48	87.46	26.27	83.63	81.16	92.90
Deficiency methionine level (0.35 %).	73.09	95.84	86.46	25.83	84.96	81.82	92.89
± Pooled SEM.	± 0.639	± 0.132	± 0.541	± 0.427	± 0.403	± 0.493	± 0.203
Probability	0.5539	0.0657	0.2032	0.4765	0.0686	0.3610	0.9589
Main effect of folic acid level							
Control folic acid level (0 mg / kg).	73.91	96.00	87.20	26.33	84.77	81.87	93.37
10 mg folic acid level (10 mg / kg).	72.77	95.59	86.96	26.05	83.92	81.23	92.90
20 mg folic acid level (20 mg / kg).	71.76	95.40	86.72	25.77	84.20	81.36	92.42
± Pooled SEM.	± 0.736	± 0.152	± 0.701	± 0.534	± 0.550	± 0.622	± 0.208
Probability	0.1426	0.0619	0.8890	0.7629	0.5522	0.7468	0.0714

a, b, c,.... etc. means in same column, within each factor with different superscripts are significantly ($p \leq 0.05$) different.

estrogen regulate the activity of osteoclasts and slow the process of bone dissolution. This lead to increasing egg shell thickness and decreased the eggs broken percentage and therefore, increased economic profits. Moreover, an equation model was proposed for predicting the contributions of folic acid in hen performance as sparing effect from methionine:

`Folic acid = 771.93 + 0.80 EP – 9.23 EW + 0.17 BW – 0.066 E – 154.48 M. ($r2$ = 0.93)

where, EP (egg production) ranged between 86.57 and 91.67%, EW (egg weight) ranged between 66.42 and 69.48 gm, BW (body weight) ranged between 1820.0 and 2098.3 gm, E energy level existed in the diet and M methionine level existed in the diet.

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