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EFFECT OF DIETARY ENERGY, METHIONINE, CHOLINE AND FOLIC ACID LEVELS ON LAYERS PERFORMANCE.

By

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Abstract: *Two experiments were conducted in a 3 x 2 x 2 factorial arrangement of treatments. Three levels of metabolizable energy 2600, 2800 and 3000 Kcal ME/kg diet, two levels of methionine (0.40 and 0.50%) and two levels of choline (300 and 900 mg/kg diet for experiment I) or two levels of folic acid (2.0 and 6.0 mg/kg diet for experiment II) were used. The experimental diets were formulated to cover the nutrient requirements according to the recommended allowances of the breed, where 2800 kcal ME/kg, 0.40% methionine, 300 mg choline/kg and 2.0 mg folic acid/kg considered as a control. The diets were fed to 2400 (1200/experiment) Lohmann Brown (L.B.) laying hens, 28 weeks of age for 16 weeks. Hens in each experiment were randomly distributed into 12 treatments, of 100 hens each, in 4 replicates. In both experiments, feed was given in all mash form and offered with water ad-libitum under a total of 16 hours light per day regimen.*

The results in experiment I and II indicated that hens fed the high energy diets produced more eggs than those fed the medium or low energy diets with no significant differences. Increasing energy, methionine, choline and folic acid levels did not affected egg weight. In both experiments, the amount of feed consumed decreased with increasing dietary energy level in the diet. The best feed conversion ratios were noticed with all diets containing 3000 kcal ME/kg, the ratio improved by 13.20 and 19.72% for experiment I vs. 11.68 and 19.82% for experiment II than the control diet and low ME diet, respectively. In the first and second experiments, the average live body weight gain increased gradually with increasing the energy level from 2600 to 3000 kcal ME/kg with supplemental methionine, choline and folic acid.

The results indicated that experimental treatments which contained 2600 kcal ME/kg with methionine and choline (experiment I) and folic acid (experiment II) supplementation improved egg shell weight, while the diets containing 3000 kcal ME/kg improved Haugh units compared to the diets containing 2800 kcal ME/kg (control) and these results were inversely to *those of egg yolk lipid. The diets containing 2600 kcal ME/kg in both experiments tend to increase yolk total lipid and yolk total cholesterol compared to diets containing 2800 (control) and 3000 kcal ME/kg. Immune functions (serum total immunoglobulin titres) were significantly affected by energy levels, but not affected by methionine, choline (experiment I) or folic acid (experiment II) supplementation in the diet. A slightly decrease was observed in serum total immunoglobulin titres with increased choline or folic acid. In both experiments, the high energy diets which containing 3000 kcal ME/kg improved the digestion coefficient values of almost all the nutrients compared to the control diet (2800 kcal ME/kg) and diets containing 2600 kcal ME/kg. The addition of methionine, choline or folic acid had no significant effect on nutrients digestibility coefficient. The economic study was affected by different energy, methionine, choline or folic acid levels, where increasing the dietary energy level increased economic efficiency in both experiments.*

INTRODUCTION

It is a widely accepted principle in poultry nutrition that dietary energy and the essential nutrients must be considered as an entity. A change in the energy content of the diet will normally result in an inverse change in the total amount of feed consumed and will therefore influence the intake of essential nutrients **(Slagter and Waldroup, 1990)**. **Hunton (1995)** found that nutrients intake can be influenced by different levels of energy in diet. Therefore, deficiency of nutrients may be occur in poultry by more increasing of energy content in the diet. In contrast, feed intake as well as nutrients utilization are increased by low level of energy in the diet. **Oke *et al.* (2003)** reported that high dietary energy levels inhibit feed intake and observed that egg production and feed conversion ratio were improved when laying hens were fed diet containing 2750 kcal ME/kg compared with the other diets which containing 2650 or 2850 kcal ME/kg.

In most poultry diets, methionine is the first limiting amino acid. This means that an adequate quantity of methionine is necessary for most practical diets to obtain optimum performance. Therefore, it is common practice to supplement diets with synthetic methionine source such as DL-methionine **(Liu *et al.* 2004 b)**. In this respect, **Cortes *et al.* (2001), Silpasorn *et al.* (2003)** and **Abd-Elsamee (2005)** indicated that hen diets supplemented with methionine at levels up to 0.55% improved egg production, egg weight, feed conversion ratio and egg quality.

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 (1999)** 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.* (1998)**. They found a linear response for egg content as the daily methionine intake increased.

Choline is an essential nutrient for the poultry. One of choline functions is to furnish methyl groups that can also be furnished by methionine **(Harms and Russell, 2002)**. The level of choline in the diet can affect the methionine requirement **(Harms *et al.* 1990)**. **House *et al.* (1999)** reported that folic acid status is linked to increased serum levels of the sulfur amino acid homocystine, due to the role of folic acid that plays as co-factor in the remethylation of homocystine to form methionine. **House *et al.* (2002)** reported that 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.

Therefore, this study aimed to improve the laying hen performance, egg quality and immune response at lower levels of metabolizable energy as a result of adding the right amounts of both amino acid (methionine) and vitamins (choline and folic acid).

MATERIALS AND METHODS

The experimental work of the present study was carried out at the poultry farm of the Animal Production Islamic Company (APICO) from September 2003 to January 2004.

Experimental design:

Two experiments were conducted in a 3 x 2 x 2 factorial arrangement of treatments. Three levels of metabolizable energy (2600, 2800 and 3000 Kcal ME/kg), two levels of methionine (0.40 and 0.50%) and two levels of choline (300 and 900 mg/kg) for the first experiment or two levels of folic acid (2.0 and 6.0 mg/kg) for the second experiment.

Experimental diets:

The experimental diets and their calculated analysis for the first and second experiments are presented in Table 1. The experimental diets were designed to contain three levels of metabolizable energy 2600 (low level), 2800 (medium level) and 3000 Kcal ME/kg diet (high level). Each level of energy was provided with either 0.40 or 0.50% methionine. Each level of the methionine was also provided with either choline at 300 (basal) or 900 mg/kg of the diet (experiment I) or folic acid at 2.0 or 6.0 mg/kg of the diet (experiment II). Methionine, choline or folic acid were added as DL-methionine (98% methionine), choline chloride (60% choline) and folic acid (100%), respectively. The experimental diets were formulated to cover the nutrient requirements according to the recommended allowances of the breed, where 2800 kcal ME/kg, 0.40% methionine, 300 mg choline/kg or 2.0 mg folic acid/kg considered as a control. Twenty four experimental diets (12 per experiment) were formulated using Linear Programming to be isonitrogenous (18.00 % CP).

Experimental birds and management:

A total number of 1200 Lohmann Brown (L.B.) laying hens, 28 weeks of age were used for each experiment. Hens were randomly divided into 12 groups of 100 hens in four replicates of 25 hens each. Hens were kept in cleaned and fumigated cages of wire floored batteries in closed system house. Feed and water were offered *ad-libitum* all over the experimental period (16 weeks) from 28 to 43 weeks of age, under a total of 16 hours light per day regimen.

Measurements:

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. The daily feed consumed per hen and hen-day egg production percentage were calculated every four weeks interval during the experimental period. Eggs were collected and weighed every 4 weeks during the experimental periods (16 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. Egg shell thickness was determined using a dial pipe gauge digital. Haugh units were calculated based upon the height of albumen determined by a micrometer and egg weight according to **Eisen *et al.* (1962)**. Yolk and albumen were separated, then 5 samples of pooled yolk and albumen for each replicate were freeze-dried and stored at –20 ^{o}C. The egg yolk total lipid was extracted according to **Folch *et al.* (1957)**, while total cholesterol of an egg yolk was determined by the method of **Henly and Zak (1957)**. Serum total immunoglobulin titres were also determined according to **Van der Zipp (1983)**. Gross calorific values of feed and excreta were determined using the programmable isothermal jacket bomb calorimeter. To determine the economic efficiency of egg production, the amount of feed consumed during the entire experimental period and the total eggs produced per treatment were considered. The price of experimental diets was calculated according to the local market price of DL-methionine, choline chloride and folic acid as well as the prices of the ingredients at the time of the experiment.

Digestion trials:

At the end of the experimental period, 43 weeks of age, a total number of 288 hens, 12 from each treatment were randomly taken for carrying digestion trials to estimate the nutrients digestibility, nitrogen balance and energy utilization. Feeds and fresh water were offered *ad-libitum*, excreta was collected quantitatively every 24 hours, during a three days collection period. Proximate analysis of the feed and dried excreta were done following the methods of **(A.O.A.C., 1990)**. Faecal nitrogen was determined according to **Jakobsen *et al.* (1960)**.

Statistical analysis:

Data were statistically analyzed by using the General Linear Model procedures (GLM) described by **SAS Institute (2004)**. Differences among treatment means were tested using Duncan's multiple range test **(Duncan, 1955)** and differences were significant at ($P \leq 0.05$).

RESULTS AND DISCUSSION

Laying Hen Performance:

Egg production:

The effects of experimental treatments on egg production determined as hen-day (H.D., %) are summarized in Table (2) for experiment I and II. Results showed that egg production (H.D., %) increased gradually with increasing metabolizable energy (ME) levels while, there were no significant differences ($P>0.05$) among ME levels in egg production percentages in both experiments. Moreover, the differences in egg production due to methionine, choline and folic acid levels were also of not significant. In the first and second experiment, there was a significant effect ($P \leq 0.05$) on the egg production due to energy x methionine x choline or folic acid interaction, where T_{11} and T_{12} recorded the highest value (91.1%), however T_1 and T_3 gave the lowest percentage (86.6%) for experiment I. In experiment II, the highest egg production (90.4%) was recorded by T_{12}, while the least percentage (85.3%) was obtained by T_1. These results may be attributed to the supplementation of sulfur amino acid (methionine) and vitamins (choline or folic acid) to high energy diet (3000 kcal ME/kg) that improved egg production. These results agreed with the findings of **Rosa *et al.* (1996)** who reported that hens fed the 2940 kcal ME/kg diet showed a significant increase in egg production than those fed the diet containing 2710 kcal ME/kg. **De-Acosta *et al.* (2002)** reported that the level of dietary energy (2600 and 2800 kcal ME/kg) did not affect the laying performance. **Keshavarz (2003)** observed that the methionine and choline supplementation did not statistically influence the egg production. **Hebert *et al.* (2004)** found that there was no significant difference ($P \leq 0.05$) in egg production rate due to folic acid supplementation from 0 to 32 mg/kg and the results disagree with those of **Rezvani *et al.* (2000)** who found that 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. **Abd-Elsamee (2005)** showed that feeding laying hens diets supplemented with methionine up to 0.55% recorded significantly higher egg production compared to diets containing 0.42 (control) and 0.49% methionine. **Harms *et al.* (1999)** obtained a significant ($P<0.05$) increase in egg production when 878 mg choline/kg was added to the diet containing 0.033% supplemental methionine. **Liu and Feng (1992)** reported that folic acid increased egg production when added from 0.54 to 1.5 mg/kg to the diet.

Egg weight:

The effects of treatments on egg weight are shown in Table (2) for experiment I and II. Data showed that the differences in egg weights were not significant due to either metabolizable energy, methionine, choline or folic acid levels. Moreover, the effect of interaction among energy x methionine x choline or folic acid on egg weight were not significant ($P>0.05$). Generally, increasing energy levels from 2600 to 3000 kcal ME/kg, methionine from 0.40 to 0.50%, choline from 300 to 900 mg/kg or folic acid from 2.0 to 6.0 mg/kg did not affect egg weight. These results were confirmed by **Novak *et al.* (2004)** and **Amaefule *et al.* (2004)** who reported that methionine supplementation at 0 and 0.10% of laying hen diets containing 2790 kcal ME/kg did not affect egg weight. However, **Abd-Elsamee (2005)** reported that egg weight increased with increasing dietary methionine level up to 0.55%. In this connection, **Bhardwaj *et al.* (2000)** reported that egg weights did not differ among dietary treatments containing 2600 kcal ME/kg and 500 mg choline/kg. **Hebert *et al.* (2004)** observed that egg weight was not affected by folic acid supplementation up to 4 mg folic acid/kg. While, results obtained herein disagree with those obtained by **Colvara *et al.* (2002)** and **Keshavarz (2003)** who reported that increasing the concentration of several nutrients in the diet increases egg weight. These nutrients include methionine and choline or combinations of related nutrients. Moreover, **Liu *et al.* (2004 a, b)** found that there was a difference ($P<0.05$) in egg weight among dietary methionine supplementation (0.02, 0.04, 0.06, 0.08 and 0.10%). This indicated that egg weight was a more sensitive criterion than egg production, egg mass and body weight. On the other hand, **House *et al.* (2002)** noticed that birds consuming diets containing 8 and 16 mg folic acid/kg produced less egg weight than those observed for birds consuming the diets containing 0 to 4 and 32 mg folic acid/kg.

Feed consumption and conversion:

Data presented in Table (2) showed the effect of treatments on the amount of feed intake for different experimental birds in the first and second experiment. There was a significant effect on the amount of feed consumed (g/hen/day) due to metabolizable energy levels in both experiments. Hens receiving the low metabolizable energy diet consumed significantly ($P\leq0.05$) more feed than those receiving the control and high metabolizable energy diets. Hens increased feed intake to meet their increased energy requirement. No significant differences in feed intake due to increasing dietary supplementation of methionine, choline and folic acid in the diets. T_1 recorded the highest value of feed intake (126 and 128 g/hen/day) for experiment I and II, respectively, while, T_{12} gave the least value (105 g/hen/day) for experiment I and II. Generally, the amount of feed consumed decreased with increasing energy, choline and folic acid levels. These results supported by **Harms *et al.* (2000)** and **Robinson (2000)** who observed that feed intake declined with increasing ME levels (2500, 2700 and 2900 kcal/kg) in the diet and the results indicated that the hen is inefficient at adjusting feed intake to meet their energy requirement. However, **Ghaisari and Golian (1996)** suggested that feed intake was not affected by dietary energy levels (2500, 2700 and 2900 kcal/kg). Additionally, feed intake of low energy diet, which was a very bulky (low density) diet, was substantially lower than predicted on the basis of energy requirement of the birds and energy content of the diet, suggesting that dietary bulk was a limiting factor. **Abd-Elsamee (2005)** indicated that no significant differences ($P>0.05$) in feed intake values were observed due to the different levels of methionine (0.42, 0.49 and 0.55%) in laying hen diets. **Wideman *et al.* (1994)** suggested that the precise mechanisms responsible for the depressive effects of excess amino acids (1% DL-methionine) on feed intake remain to be fully clarified. On the other hand, **Hebert *et al.* (2004)** reported that there was no significant difference ($P>0.05$) in feed consumption due to folic acid supplementation up to 4 mg/kg to the diets. However, **House *et al.* (2002)** observed that average daily feed consumption was significantly higher for birds consuming the diets with 32 mg folic acid/kg compared to birds have folic acid at 0, 1, 2, 4, 8 and 16 mg/kg in their diet.

Average values of feed conversion ratio (FCR), calculated as amount of feed (kg) required to produce 1 kg of eggs are shown in Table (2) for experiment I and II. There were significant differences ($P \leq 0.05$) among metabolizable energy levels, where diets containing 2600 kcal ME/kg recorded the worst FCR. The diets containing 3000 kcal ME/kg improved FCR by 13.20 and 19.72% for experiment I vs. 11.68 and 19.82% for experiment II compared to the control diet and low ME diet, respectively. These results may be attributed to two reasons, firstly, the different amounts of feed consumed and egg production. Secondly, the supplemental sulfur amino acid (methionine) and vitamins (choline or folic acid) to high energy diet (3000 kcal ME/kg) which improved FCR. Results in Table (2) revealed that methionine, choline or folic acid levels did not affect FCR for egg production. The effect of interactions among energy x methionine x choline or folic acid on FCR was significant. The best FCR (1.70) was recorded by T_{10} and T_{11} for the first experiment, and T_{12} for the second experiment, while the worst FCR was obtained by T_1. Generally, the best feed conversion ratio was noticed with all diets containing 3000 kcal ME/kg with methionine, choline or folic acid supplementation compared to the control diets (2800 kcal ME/kg) and low metabolizable energy diets. These results are in agreement with those obtained by **Totsuka *et al.* (1993)** who indicated that increasing the dietary energy levels (2700, 2850 and 3000 kcal ME/kg) decreased feed intake and consequently improved feed conversion ratio. **Amaefule *et al.* (2004)** showed that supplementation of 0.10% methionine did not affect feed conversion ration (FCR). **Rao *et al.* (2001)** found that supplementation of 0, 750 and 1520 mg choline/kg did not influence the efficiency of feed utilization. **Hebert *et al.* (2004)** suggested that there was no significant difference ($P<0.05$) in feed conversion ratio due to folic acid supplementation up to 4 mg/kg. These results disagree with those of **Adeyemo and Longe (2000)** who indicated that hens fed diet containing 2600 kcal ME/kg performed best feed conversion ratio when the ME content of the diets ranged from 2500 to 2900 kcal/kg. **Novak *et al.* (2004)** and **Liu *et al.* (2004 b)** reported that the addition of DL-methionine at level up to 0.10% improved feed conversion ratio. The reason that the differences between methionine levels were not statistically detected was that laying hens were not sensitive enough to supplemental methionine. In this connection, **Khan *et al.* (1991)** reported that feed conversion efficiency showed a positive response to choline supplementation as a substitute for methionine when they fed hens on diets containing 2900 kcal ME/kg with 0.128% DL-methionine and 600 or 900 mg choline/kg.

Live body weight gain:

The effect of treatments on body weight gain is presented in Table (2) for experiment I and II. In both experiments, there was a significant difference in average live body weight gain among hens fed the three different metabolizable energy levels while, hens receiving the high energy diets gained the best, however those receiving the low energy diets gained the least weight. There was no significant difference between the average values of body weight gain due to methionine, choline or folic acid levels. A significant difference was observed in the average live body weight gain due to energy x methionine x choline or folic acid interaction. Generally, the average live body weight gain increased gradually with increasing the energy level from 2600 to 3000 kcal ME/kg with supplemental methionine, choline (experiment I) or folic acid (experiment II). These results supported the findings of **Stilborn and Waldroup (1990)** who concluded that lower dietary energy levels tended to reduce body weight gain of hens fed diets containing 2500, 2600, 2700 and 2800 kcal ME/kg. In this regard, **Piliang *et al.* (1982)** stated that 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. **Robinson (2000)** observed that body weight gain increased with increasing dietary ME level (2500, 2700 and 2900 kcal ME/kg) in the diet. Accordingly, 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. **Amaefule *et al.* (2004)** found that body weight gain did not differ among treatments which containing methionine from 0.289 to 0.422%. **Harms and Russell (2002)** observed no significant differences in body weight gain among the hens receiving diets containing choline at level of 1220 mg/kg. **Hebert *et al.* (2004)** noticed that no significant difference ($P<0.05$) in body weight due to folic acid supplementation up to 4.0 mg/kg. On the contrary, **Oke *et al.* (2003)** suggested that diet containing 16% CP and 2750 kcal ME/kg is ideal for optimum body weight gain for laying hens probably because the diet contains a good balance between energy and protein. **Okazaki *et al.* (1995)** concluded that body weight gain of hens fed 0.52% methionine diet was heavier than other treatments containing 0.40 and 0.46% methionine. **Keshavarz (2003)** observed that reducing dietary folic acid resulted in reduced body weight.

Egg Quality:

Shell thickness:

The effects of treatments on average values of shell thickness (µm) including shell membrane are presented in Table (3) for experiment I and II. Shell thickness was significantly ($P \leq 0.05$) affected by ME levels, while no significant differences were detected between the average values of shell thickness due to different levels of methionine and choline (experiment I) or folic acid (experiment II). Table (3) showed that T_1 had the best shell thickness (0.468 µm), where T_9 recorded the least shell thickness (0.421 µm) for the first experiment. However, in experiment II, no significant differences were observed in shell thickness due to energy x methionine x folic acid interaction and the values of shell thickness ranged from 0.432 to 0.458 µm for T_{10} and T_4, respectively. Generally, all diets which formulated to contain 2600 kcal ME/kg with methionine, choline or folic acid supplementation improved egg shell thickness compared to 2800 kcal ME/kg (control) and 3000 kcal ME/kg diet. In this regard, **Atteh and Leeson (1985)** reported that the reason of improved egg shell thickness with diets containing 2600 kcal ME/kg is due to the calcium losses on the low ME diet which were lower than on the high ME diet. The greater loss of calcium from pullets fed on the high ME diet was attributed to that calcium can combine with excess dietary fat to form indigestible soaps. **Hebert *et al.* (2004)** noticed was no significant difference ($P>0.05$) in egg shell thickness due to folic acid supplementation up to 4 mg/kg of the diet. On the contrary, **Ciftci *et al.* (2003)** found that shell thickness was not significantly affected by dietary energy level (2650 and 2750 kcal ME/kg). **Abd-Elsamee (2005)** reported that hens fed diets containing 0.55% methionine significantly showed higher values of shell thickness compared with those fed either 0.42 (control) or 0.49% methionine. **Keshavarz (2003)** reported that dietary choline level up to 1256 mg/kg and reducing dietary folic acid from 1.12 to 1.08 mg/kg improved egg shell thickness.

Haugh units:

Data in Table (3), showed that the average values of Haugh units were not influenced significantly ($P>0.05$) by energy, choline or folic acid levels, but influenced by methionine level in both experiments. The effect of interaction among energy x methionine x choline or folic acid on Haugh unit were significant. In experiment I, the values ranged between 61.1 and 67.3 for T_1 and T_{10}, respectively. While, in experiment II, the values varied from 59.8 and 68.2 for T_1 and T_7, respectively. These findings agree with those reported by **Uddin *et al.* (1991)** who found that Haugh units were in irregular trend with dietary energy 2600, 2800, 3000 and 3100 kcal ME/kg. **Abd-Elsamee (2005)** observed that the use of high levels of methionine (0.49 and 0.55%) significantly increased Haugh unit values compared with the control group (0.42% methionine). **Rao *et al.* (2001)** observed that amount of supplemental choline at levels up to 1520 mg/kg did not influence Haugh unit score. **Hebert *et al.* (2004)** showed no significant difference (P>0.05) in Haugh units due to folic acid supplementation up to 4 mg/kg but, that disagree with the findings of **Novak *et al.* (2004)** and **Amaefule *et al.* (2004)** who reported that supplementing layer diets with 0.10% methionine did not significantly influenced Haugh units.

Egg yolk total lipids:

Egg yolk total lipids were calculated as a percentage of the total yolk weight and listed in Table (3) for experiment I and II. The low ME diets (2600 kcal ME/kg) significantly increased ($P \leq 0.05$) egg yolk total lipids when compared to the medium (2800 kcal ME/kg) and high (3000 kcal ME/kg) energy diets in both experiments. Methionine and folic acid levels did not affect egg yolk total lipids. A significant effect was due to choline levels. In experiment I and II, the effect of interaction among energy x methionine x choline or folic on egg yolk total lipids were significant. In experiment I, T_1 recorded the highest value of egg yolk total lipids (27.9 mg/100 g yolk), while T_2 and T_6 gave the least value (25.8 mg/100 g yolk). However, the values of the different experimental treatments of experiment II ranged between 25.6 to 28.0 g/100 g yolk for T_9 and T_3, respectively. On the contrary **Uddin *et al.* (1991)** and **Uddin *et al.* (1997)** reported that yolk fat was similar and did not significantly influenced by dietary energy levels up to 3100 kcal ME/kg.

Egg yolk cholesterol:

Egg yolk cholesterol values (mg/g of yolk) are presented in Table (3) for experiment I and II. In both experiments, ME, methionine and folic acid levels did not affect average yolk total cholesterol values, while in the first experiment the difference between two choline levels was significant ($P \leq 0.05$). The interaction effect of energy x methionine x choline or folic acid for egg yolk total cholesterol was significant ($P \leq 0.05$). Concerning the various experimental diets of experiment I, the highest value (16.9 mg/gm) was for T_1, while the lowest value (12.5 mg/g) was for T_4. While, the values of egg yolk total cholesterol in experiment II ranged from 13.5 to 17.1 mg/gm for T_{12} and T_9, respectively. The results are in agreement with those reported by **Oke *et al.* (2003)** 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 of 2750 kcal ME/kg 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.

Immune Response:

Values of serum total immunoglobulin titres are listed in Table (3) for experiment I and II. Low ME diets resulted in the highest average of serum titres (9.7 and 9.5), followed by medium energy diets (9.2 and 9.4) and high energy diets which recorded the least values (8.9 and 8.9) for the first and second experiment, respectively. But, in both experiments, no significant differences in serum total immunoglobulin titres were observed with increases dietary methionine, choline or folic acid concentration. The interaction of energy x methionine x choline or folic acid for the average serum total immunoglobulin titre values were significant. Concerning the various experimental diets, the values ranged between 8.2 and 10.2 for T_8 and T_2, respectively (experiment I). In experiment II, the highest value of immunoglobulin titre (10.3) was obtained by T_1, while T_9 gave the least value (8.3). The reason for this discrepancy probably relates to the amounts of oil (fatty acids) in the experimental diets. **Calder (1998 a)** concluded that high fat diets are associated with suppressed immune functions (T-cell proliferation). **Kelley and Daudu (1993)** and **Calder (1998 a, b)** reported that lower natural killer cell activity, lymphocyte proliferation and antibody production following 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. On the other hand, **Balnave (2000)** reported that decreased serum total immunoglobulin titres go parallel with increasing dietary methionine concentrations.

Digestibility Coefficient and metabolizability:

Digestion coefficient of the nutrients and metabolizability of the experimental diets are shown in Table (4) for experiment I and II. Results revealed that different ME levels, especially high ME diets which containing 3000 kcal ME/kg improved the digestion coefficient values of almost all the nutrients compared to the control diet (2800 kcal ME/kg) and diets containing 2600 kcal ME/kg except for CP and NFE digestibility in both experiments. The addition of methionine, choline (experiment I) or folic acid (experiment II) had no significant effect on nutrients digestibility coefficient. This may be due to different amounts of feed consumed which produced the lower rapid passage of the feed for a high ME diets (3000 kcal ME/kg) and in contrast of this, a low ME diets (2600 kcal ME/kg) appeared the more rapid passage of feed which resulted in a decrease in the utilization of feed nutrients.

The high ME diets (3000 kcal ME/kg) improved significantly ($P \leq 0.05$) the coefficient of ME utilization (metabolizability) when compared to diets containing 2800 (control) and 2600 kcal ME/kg meaning that metabolizability increased gradually with increasing energy levels in both experiment Table (4). No significant differences in metabolizability was observed due to methionine and folic acid supplementation, however choline addition significantly ($P \leq 0.05$) influenced the metabolizability. The effect of interaction among energy x methionine x choline or folic acid on metabolizability were highly significant in experiment I and II. Concerning the metabolizability of experiment I, the best value (93.4%) was for T_{10}, followed by T_{11} (93.3%) and the least value (89.2%) was recorded for T_2. While, in experiment II, the values of metabolizability varied from 90.3 and 94.3 % for T_2 and T_9, respectively. These results supported the findings of **Scott and Balnave (1991)** who reported that energy metabolizability was significantly ($P<0.01$) lower when pullets were fed on the low ME diet. Moreover, the losses of crude protein were significantly ($P<0.01$) greater from pullets fed the low ME diet. **Saki (2005)** reported that high level of crude fiber content reduced the bioavailability of energy. **Hunton (1995)** reported that deficiency of nutrients may occur in poultry by more increasing of energy content in diet. In contrast, feed intake as well as nutrient utilization is increased by low level of energy in diet. **Naulia and Singh (2002)** reported that the digestibility of dry matter (DM) and organic matter (OM) were significantly higher ($P<0.05$) on 0.323% methionine level compared to 0.248 and 0.267% level.

Economic efficiency:

The economic efficiency and money return per hen fed the different formulated diets are summarized in Table (5). The net revenue and economic efficiency values ranged between 10.9–13.3 and 0.65–0.87 (first experiment) or varied from 11.1–13.0 and 0.71–0.85 (second experiment), respectively. The lowest values were recorded for T_4 and T_1 for experiment 1 and 2, respectively, while, the highest values were listed for T_{12} for both experiments. The results are in agreement with those reported by **Totsuka *et al.* (1993)** who found that cost per unit was minimal with the diet of 2700 kcal ME/kg compared to diets containing 2850 and 3000 kcal ME/kg, but increasing the dietary energy levels increased economic efficiency.

It may be concluded that: diets containing 3000 kcal ME/kg would be ideal for the achievement of optimum performance and revenue cost ratio for laying hens. The addition of amino acid (methionine) and vitamins (choline or folic acid) to low energy diet (2600 kcal ME/kg) improved egg quality and increased immune response compared to medium (2800 kcal ME/kg) and high energy (3000 kcal ME/kg) diets. Methionine, choline or folic acid supplementation up to 0.50%, 900 mg choline /kg and 6.0 mg folic acid/kg, respectively did not cause any adverse effect on laying hens performance, egg quality and immune response.

Table (1): Composition and calculated analysis of the experimental diets for experiment I and II.

Ingredients	Experimental treatments											
	Metabolizable energy levels											
	Low energy, 2600 Kcal ME / kg				Medium energy, 2800 Kcal ME / kg				High energy, 3000Kcal ME / kg			
	0.40% methionine		0.50% methionine		0.40% methionine		0.50% methionine		0.40% methionine		0.50% methionine	
	300 C or 2.0 F mg/kg	900 C or 6.0 F mg/kg	300 C or 2.0 F mg/kg	900 C or 6.0 F mg/kg	300 C or 2.0 F mg/kg	900 C or 6.0 F mg/kg	300 C or 2.0 F mg/kg	900 C or 6.0 F mg/kg	300 C or 2.0 F mg/kg	900 C or 6.0 F mg/kg	300 C or 2.0 F mg/kg	900 C or 6.0 F mg/kg
	T1	**T2**	**T3**	**T4**	**T5**	**T6**	**T7**	**T8**	**T9**	**T10**	**T11**	**T12**
Ground yellow corn	42.50	42.50	42.35	42.35	47.60	47.60	47.50	47.50	52.70	52.70	52.60	52.60
Soybean meal (44%)	26.60	26.60	26.45	26.45	28.10	28.10	27.95	27.95	29.60	29.60	29.50	29.50
Rice bran	18.20	18.20	18.40	18.40	9.60	9.60	9.75	9.75	1.00	1.00	1.10	1.10
Vegetable oil	2.00	2.00	2.00	2.00	4.00	4.00	4.00	4.00	6.00	6.00	6.00	6.00
Ground limestone	8.00	8.00	8.00	8.00	8.00	8.00	8.00	8.00	8.00	8.00	8.00	8.00
Bone meal	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00
NaCl	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. and min. premix *	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	0.10	0.10	0.20	0.20	0.10	0.10	0.20	0.20	0.10	0.10	0.20	0.20
Total	**100.00**	**100.00**	**100.00**	**100.00**	**100.00**	**100.00**	**100.00**	**100.00**	**100.00**	**100.00**	**100.00**	**100.00**
Supplemental choline chloride (60%) mg/kg. (experiment I)	------	**1000**	------	**1000**	------	**1000**	------	**1000**	------	**1000**	------	**1000**
Supplemental folic acid (100%) mg/kg. (experiment II)	-------	**4.00**	-------	**4.00**	-------	**4.00**	-------	**4.00**	-------	**4.00**	-------	**4.00**
Calculated analysis **												
ME, k cal / kg	2600	2600	2600	2600	2800	2800	2800	2800	3000	3000	3000	3000
CP, %	18.00	18.00	18.00	18.00	18.00	18.00	18.00	18.00	18.00	18.00	18.00	18.00
Total choline **(C)** mg/ kg.	300	900	300	900	300	900	300	900	300	900	300	900
Total folic acid **(F)** mg/kg.	2.00	6.00	2.00	6.00	2.00	6.00	2.00	6.00	2.00	6.00	2.00	6.00
Methionine, %	0.40	0.40	0.50	0.50	0.40	0.40	0.50	0.50	0.40	0.40	0.50	0.50
Methionine + Cystine %	0.71	0.71	0.81	0.81	0.70	0.70	0.80	0.80	0.70	0.70	0.80	0.80
Lysine, %	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
Calcium, %	3.72	3.72	3.72	3.72	3.72	3.72	3.72	3.72	3.72	3.72	3.72	3.72
Available P. %	0.40	0.40	0.40	0.40	0.40	0.40	0.40	0.40	0.40	0.40	0.40	0.40

*** Vitamin and mineral choline-free premix at 0.3% of the diet supplies the following per kg of the diet:**
vit.A 10000 I.U., vit.D$_3$ 3000 I.U., vit. E 20 mg, vit. K$_3$ 3 mg, vit.B$_1$ 2 mg, vit.B$_2$ 6 mg, pantothenic acid 10 mg, Folic acid 1 mg, biotin 5 mcg, niacin 66 mg, vit. B$_6$ 5 mg, vit.B$_{12}$ 20 mcg, Mn 100 mg, Fe 100 mg, Zn 75 mg, Cu 8 mg, I 45 mcg and Se 10 mcg.

**** According to N.R.C. (1994).**

Table (2): Effect of experimental treatments on laying hen performance.

Experimental treatments	Laying hen performance									
	Experiment I					Experiment II				
	EP	EW	FI	FCR	BWG	EP	EW	FI	FCR	BWG
E. effect:										
E1	87.2	67.6	125^{a}	2.13^{a}	95.9^{c}	87.7	66.8	127^{a}	2.17^{a}	141.2^{b}
E2	88.1	67.0	116^{b}	1.97^{b}	241.7^{b}	87.9	67.6	117^{b}	1.97^{b}	163.8^{b}
E3	90.3	68.0	105^{c}	1.71^{c}	341.1^{a}	89. 5	68.0	106^{c}	1.74^{c}	197.9^{a}
M. effect:										
M1	87.9	67.3	115	1.96	227.5	87.5	67.2	116	1.99	174.9
M2	89.2	67.8	115	1.91	225.0	89.2	67.7	116	1.93	160.4
C. or F. effect:										
C1 or F1	88.1	67.4	116	1.96	232.1	87.7	67.0	117	2.00	174.0
C2 or F2	88.9	67.7	115	1.92	220.4	89.0	67.9	116	1.93	161.2
E. x M. x C. or F. effect										
T1 (E1+ M1 + C1 or F1)	86.6^{b}	67.1	126^{a}	2.17^{a}	92.5^{e}	85.3^{b}	66.7	128^{a}	2.26^{a}	141.3^{b}
T2 (E1+ M1 + C2 or F2)	87.2^{ab}	67.0	125^{a}	2.16^{a}	102.5^{e}	87.8^{ab}	65.9	126^{b}	2.18^{a}	141.8^{b}
T3 (E1+ M2 + C1 or F1)	86.6^{b}	68.0	125^{a}	2.13^{ab}	93.8^{e}	89.0^{ab}	66.6	126^{b}	2.14^{ab}	141.7^{b}
T4 (E1+ M2 + C2 or F2)	88.6^{ab}	68.3	125^{a}	2.06^{ab}	95.0^{e}	88.8^{ab}	67.9	126^{b}	2.10^{ab}	140.0^{b}
T5 (E2+ M1 + C1 or F1)	87.2^{ab}	66.2	116^{b}	2.02^{b}	242.5^{cd}	85.7^{b}	66.7	117^{c}	2.05^{bc}	168.5^{b}
T6 (E2+ M1 + C2 or F2)	87.4^{ab}	66.9	116^{b}	1.98^{b}	225.0^{d}	88.3^{ab}	68.2	116^{c}	1.93^{de}	166.8^{b}
T7 (E2+ M2 + C1 or F1)	88.0^{ab}	65.9	116^{b}	1.94^{b}	220.0^{d}	89.0^{a}	66.1	117^{c}	1.98^{bcd}	163.3^{b}
T8 (E2+ M2 + C2 or F2)	89.6^{ab}	68.0	116^{b}	1.90^{b}	279.3^{bc}	89.8^{ab}	68.3	117^{c}	1.91^{cd}	156.5^{b}
T9 (E3+ M1 + C1 or F1)	89.1^{ab}	67.7	106^{c}	1.76^{c}	389.0^{a}	89.0^{a}	67.4	107^{d}	1.78^{ef}	245.0^{a}
T10 (E3+ M1 + C2 or F2)	89.6^{ab}	68.7	104^{c}	1.70^{c}	313.7^{b}	90.0^{a}	68.4	106^{d}	1.74^{f}	185.7^{b}
T11 (E3+ M2 + C1 or F1)	91.1^{a}	68.3	105^{c}	1.70^{c}	355.0^{a}	89.6^{a}	67.7	106^{d}	1.75^{f}	184.3^{b}
T12 (E3+ M2 + C2 or F2)	91.1^{a}	67.3	105^{c}	1.71^{c}	306.8^{b}	90.4^{a}	68.6	105^{d}	1.70^{f}	176.5^{b}

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

EP: Egg Production, H.D. (%), **EW:** Egg Weight (g), **FI:** Feed Intake (g/hen/day), **FCR:** Feed Conversion Ratio (kg. feed/kg. egg) and **BWG:** Body Weight Gain (g).

E. (Metabolizable energy level); **E1:** 2600 kcal ME/kg; **E2:** 2800 kcal ME/kg; **E3:** 3000 kcal ME/k

M (Methionine level); **M1:** 0.40% methionine; **M2:** 0.50% methionine.

C (Choline level); **C1:** 300 mg choline/kg; **C2:** 900 mg choline/kg.

F (Folic acid level); **F1:** 2.0 mg folic acid/kg; **F2:** 6.0 mg folic acid/kg.

T5 (control diet).

Table (3): Effect of experimental treatments on egg quality and immune response.

Experimental treatments	Experiment I					Experiment II				
	ST	**HU**	**YTL**	**YTC**	**Ig**	**ST**	**HU**	**YTL**	**YTC**	**Ig**
E. effect:										
E1	0.450 ^{a}	63.3	27.1 ^{a}	14.4	9.7 ^{a}	0.454 ^{a}	64.2	27.5 ^{a}	16.0	9.5 ^{a}
E2	0.438 ^{b}	65.1	26.4 ^{ab}	14.2	9.2 ^{ab}	0.449 ^{b}	64.5	26.8 ^{ab}	15.9	9.4 ^{a}
E3	0.426 ^{c}	65.5	26.2 ^{b}	14.1	8.9 ^{b}	0.436 ^{c}	65.2	26.1 ^{b}	15.1	8.9 ^{b}
M. effect:										
M1	0.437	64.0 ^{b}	26.3	14.6	9.4	0.446	63.0 ^{b}	26.7	15.7	9.4
M2	0.440	65.3 ^{a}	26.8	13.9	9.1	0.446	66.3 ^{a}	26.8	15.6	9.2
C. or F. effect:										
C1 or F1	0.440	64.2	26.9 ^{a}	14.8 ^{a}	9.5	0.445	63.9	26.7	15.9	9.3
C2 or F2	0.436	65.0	26.2 ^{b}	13.7 ^{b}	9.1	0.447	65.4	26.9	15.4	9.2
E. x M. x C. or F. effect										
T1 (E1+ M1 + C1 or F1)	0.468 ^{a}	61.1 ^{d}	27.9 ^{a}	16.9 ^{a}	9.9 ^{ab}	0.451	59.8 ^{d}	27.2 ^{abc}	16.0 ^{abc}	10.3 ^{a}
T2 (E1+ M1 + C2 or F2)	0.442 ^{b}	62.6 ^{cd}	25.8 ^{b}	15.0 ^{bc}	10.2 ^{a}	0.456	63.6 ^{c}	27.7 ^{ab}	16.3 ^{ab}	9.6 ^{b}
T3 (E1+ M2 + C1 or F1)	0.451 ^{ab}	64.7 ^{abc}	27.7 ^{a}	13.4 ^{def}	9.3 ^{bc}	0.451	66.4 ^{abc}	28.0 ^{a}	15.9 ^{abc}	9.3 ^{bc}
T4 (E1+ M2 + C2 or F2)	0.440 ^{b}	64.7 ^{abc}	27.0 ^{ab}	12.5 ^{f}	9.3 ^{bc}	0.458	67.0 ^{ab}	27.0 ^{abc}	15.7 ^{abc}	9.0 ^{bc}
T5 (E2+ M1 + C1 or F1)	0.432 ^{bc}	64.7 ^{abc}	25.9 ^{b}	15.5 ^{b}	9.8 ^{ab}	0.449	60.3 ^{d}	26.4 ^{abc}	14.2 ^{de}	9.6 ^{b}
T6 (E2+ M1 + C2 or F2)	0.434 ^{bc}	65.1 ^{abc}	25.8 ^{b}	13.2 ^{ef}	8.9 ^{cd}	0.457	65.4 ^{bc}	26.5 ^{abc}	15.9 ^{abc}	9.3 ^{bc}
T7 (E2+ M2 + C1 or F1)	0.440 ^{b}	64.9 ^{abc}	27.7 ^{a}	14.6 ^{bcd}	10.0 ^{ab}	0.440	68.2 ^{a}	27.0 ^{abc}	17.0 ^{a}	9.3 ^{bc}
T8 (E2+ M2 + C2 or F2)	0.446 ^{ab}	65.7 ^{ab}	26.0 ^{b}	13.7 ^{def}	8.2 ^{d}	0.449	64.2 ^{bc}	27.3 ^{abc}	16.5 ^{ab}	9.5 ^{b}
T9 (E3+ M1 + C1 or F1)	0.421 ^{c}	62.9 ^{bcd}	25.9 ^{b}	13.3 ^{def}	8.9 ^{cd}	0.433	63.9 ^{c}	25.6 ^{c}	17.1 ^{a}	8.3 ^{d}
T10 (E3+ M1 + C2 or F2)	0.425 ^{bc}	67.3 ^{a}	26.4 ^{ab}	13.9 ^{cde}	8.8 ^{cd}	0.432	65.1 ^{bc}	26.9 ^{abc}	14.6 ^{cde}	9.2 ^{bc}
T11 (E3+ M2 + C1 or F1)	0.431 ^{bc}	66.8 ^{a}	26.3 ^{b}	15.2 ^{b}	8.6 ^{cd}	0.446	64.8 ^{bc}	26.0 ^{bc}	15.1 ^{bcd}	9.2 ^{bc}
T12 (E3+ M2 + C2 or F2)	0.429 ^{bc}	64.9 ^{abc}	26.2 ^{b}	14.0 ^{cde}	9.0 ^{cd}	0.433	67.1 ^{ab}	25.9 ^{bc}	13.5 ^{e}	8.8 ^{cd}

a, b, c,…. etc. means in same column, within each factor with different superscripts are significantly (P≤0.05) different.

ST: Shell Thickness (μm), **HU:** Haugh Unit, **YTL:** Yolk Total Lipid (gm/100 g yolk), **YTC:** Yolk Total Cholesterol (mg/g) and **Ig:** Immunoglobulin titre

E. (Metabolizable energy level); **E1:** 2600 kcal ME/kg; **E2:** 2800 kcal ME/kg; **E3:** 3000 kcal ME/kg.

M (Methionine level); **M1:** 0.40% methionine; **M2:** 0.50% methionine.

C (Choline level); **C1:** 300 mg choline/kg; **C2:** 900 mg choline/kg.

F (Folic acid level); **F1:** 2.0 mg folic acid/kg; **F2:** 6.0 mg folic acid/kg.

T5 (control diet).

Table (4): Effect of experimental treatments on the digestibility coefficient of nutrients a metabolizability.

Experimental Treatments	Experiment I							Experiment II						
	Digestion coefficient %						EU	Digestion coefficient %						EU
	DM	CP	EE	CF	NFE	OM		DM	CP	EE	CF	NFE	OM	
E. effect:														
E1	65.7^{c}	96.3^{b}	83.6^{c}	24.2^{c}	80.4	75.9^{c}	90.8^{c}	69.3^{c}	95.8^{a}	82.7^{c}	23.4^{c}	84.9	78.1^{c}	92.2^{b}
E2	70.7^{b}	97.9^{a}	86.7^{b}	27.1^{b}	76.0	80.4^{b}	92.3^{b}	73.2^{b}	95.9^{a}	87.7^{b}	26.1^{b}	82.2	82.0^{b}	92.9^{a}
E3	73.1^{a}	96.7^{b}	88.8^{a}	28.8^{a}	78.8	82.6^{a}	93.0^{a}	75.6^{a}	95.1^{b}	89.7^{a}	28.7^{a}	81.2	83.9^{a}	93.6^{a}
M. effect:														
M1	69.3	96.8	86.5	27.2	79.1	79.5	91.9	72.5	95.4	87.4	26.5	82.7	81.0	92.9
M2	70.4	97.1	86.3	26.2	77.7	79.8	92.2	72.9	95.8	86.1	25.6	82.9	81.7	92.8
C. or F. effect:														
C1 or F1	71.1	97.1	86.5	26.7	80.5	80.2	92.4^{a}	74.1	95.9	87.0	26.5	83.1	81.9	93.5
C2 or F2	68.7	96.8	86.2	26.7	76.4	79.2	91.6^{b}	71.3	95.3	86.4	25.7	82.4	80.8	92.3
E. x M. x C. or F. effect														
T1 (E1+ M1 + C1 or F1)	68.9^{h}	97.0^{f}	85.4^{h}	25.8^{e}	81.8^{a}	78.0^{f}	91.6^{f}	72.5^{e}	95.9^{abc}	84.2^{i}	23.8^{g}	84.6^{ab}	79.8^{e}	93.6^{b}
T2 (E1+ M1 + C2 or F2)	60.0^{k}	96.3^{i}	83.7^{i}	25.8^{e}	70.4^{b}	72.3^{h}	89.2^{j}	64.0^{g}	95.0^{de}	83.3^{j}	24.9^{f}	83.9^{ab}	74.1^{f}	90.3^{l}
T3 (E1+ M2 + C1 or F1)	66.7^{j}	96.7^{g}	82.8^{j}	22.9^{f}	82.7^{a}	77.7^{f}	91.2^{h}	73.2^{d}	96.5^{a}	82.7^{k}	23.4^{h}	89.7^{a}	79.4^{e}	93.4^{e}
T4 (E1+ M2 + C2 or F2)	67.3^{i}	95.0^{k}	82.7^{j}	22.1^{g}	86.6^{a}	75.8^{g}	91.0^{i}	67.6^{f}	95.8^{abc}	80.4^{l}	21.5^{i}	81.5^{ab}	79.0^{e}	91.3^{k}
T5 (E2+ M1 + C1 or F1)	70.4^{f}	97.1^{e}	86.5^{f}	26.9^{d}	85.3^{a}	80.5^{cde}	92.2^{e}	72.9^{de}	96.2^{ab}	88.0^{e}	25.6^{e}	79.3^{b}	81.2^{d}	93.1^{f}
T6 (E2+ M1 + C2 or F2)	69.6^{g}	98.1^{b}	86.0^{g}	27.0^{d}	69.1^{b}	80.3^{de}	92.1^{e}	72.5^{e}	95.5^{bcd}	87.5^{g}	25.4^{e}	80.5^{ab}	82.4^{bcd}	92.6^{j}
T7 (E2+ M2 + C1 or F1)	74.1^{b}	98.3^{a}	86.8^{e}	26.8^{d}	77.5^{ab}	81.5^{cd}	93.3^{b}	73.4^{d}	96.4^{a}	87.5^{h}	26.7^{d}	83.9^{ab}	82.2^{cd}	93.0^{h}
T8 (E2+ M2 + C2 or F2)	68.7^{h}	98.2^{a}	87.4^{d}	27.7^{c}	72.2^{b}	79.5^{e}	91.5^{g}	73.2^{c}	95.6^{bcd}	88.0^{f}	26.6^{d}	85.0^{ab}	82.2^{cd}	93.0^{g}
T9 (E3+ M1 + C1 or F1)	72.5^{d}	96.4^{h}	88.8^{b}	29.1^{a}	85.9^{a}	81.8^{bc}	92.7^{c}	77.8^{a}	95.2^{cde}	90.6^{b}	31.1^{a}	83.8^{ab}	84.8^{a}	94.3^{a}
T10 (E3+ M1 + C2 or F2)	74.3^{a}	95.8^{j}	88.4^{c}	28.7^{ab}	82.0^{a}	84.1^{a}	93.4^{a}	75.2^{b}	94.5^{e}	90.7^{a}	28.4^{b}	84.0^{ab}	83.4^{bc}	93.5^{c}
T11 (E3+ M2 + C1 or F1)	73.7^{c}	97.4^{c}	88.8^{b}	28.9^{ab}	69.5^{b}	81.6^{cd}	93.3^{a}	75.0^{b}	95.3^{cd}	89.2^{c}	28.3^{b}	77.5^{b}	83.7^{ab}	93.5^{d}
T12 (E3+ M2 + C2 or F2)	71.9^{e}	97.2^{d}	89.1^{a}	28.5^{b}	77.9^{ab}	83.0^{ab}	92.5^{d}	74.4^{c}	95.2^{cde}	88.6^{d}	27.1^{c}	79.7^{b}	83.5^{abc}	92.9^{i}

a, b, c,…. etc. means in same column, within each factor with different superscripts are significan (P≤0.05) different.

EU: Energy utilization (Metabolizability %).

E. (Metabolizable energy level); **E1:** 2600 kcal ME/kg; **E2:** 2800 kcal ME/kg; **E3:** 3000 kcal M

M (Methionine level); **M1:** 0.40% methionine; **M2:** 0.50% methionine.

C (Choline level); **C1:** 300 mg choline/kg ; **C2:** 900 mg choline/kg.

F (Folic acid level); **F1:** 2.0 mg folic acid/kg ; **F2:** 6.0 mg folic acid/kg.

T5 (control diet).

Table (5): Effect of experimental treatments on economic efficiency of the experimental diets.

Experimental treatments	Experiment I							Experiment II						
	Input			Output		N.R.	E.Ef.	Input			Output		N.R.	E.Ef.
	FI / hen (kg)	Price / Kg feed (L.E.)	Cost of FI (L.E.)	EN / hen	Price of egg (L.E.)			FI / hen (kg)	Price / Kg feed (L.E.)	Cost of FI (L.E.)	EN / hen	Price of egg (L.E.)		
E. effect:														
E1	14.0	1.1	15.8	98	27.4	11.6	0.74	14.2	1.1	15.6	98	27.5	12.0	0.77
E2	13.0	1.2	15.6	99	27.6	12.0	0.77	13.1	1.2	15.7	99	27.7	12.0	0.77
E3	11.8	1.3	15.3	101	28.3	13.0	0.85	11.9	1.3	15.5	101	28.1	12.7	0.82
M. effect:														
M1	12.9	1.2	15.5	99	27.6	12.1	0.79	13.1	1.2	15.6	98	27.5	11.9	0.76
M2	12.9	1.2	15.7	100	28.0	12.3	0.79	13.0	1.2	15.5	100	28.0	12.5	0.81
C. or F. effect:														
C1 or F1	13.0	1.2	15.5	99	27.7	12.2	0.79	13.1	1.2	15.6	99	27.6	12.0	0.77
C2 or F2	12.9	1.2	15.6	100	27.9	12.2	0.78	13.0	1.2	15.5	100	28.0	12.4	0.80
E. x M. x C. or F. effect														
T1 (E1+ M1 + C1 or F1)	14.1	1.1	15.5	97	27.2	11.7	0.75	14.3	1.1	15.7	96	26.8	11.1	0.71
T2 (E1+ M1 + C2 or F2)	14.0	1.1	15.4	98	27.4	12.0	0.78	14.1	1.1	15.5	98	27.5	12.0	0.77
T3 (E1+ M2 + C1 or F1)	14.0	1.1	15.4	97	27.2	11.8	0.77	14.1	1.1	15.5	100	27.9	12.4	0.80
T4 (E1+ M2 + C2 or F2)	14.0	1.2	16.8	99	27.7	10.9	0.65	14.1	1.1	15.5	99	27.8	12.3	0.79
T5 (E2+ M1 + C1 or F1)	13.0	1.2	15.6	98	27.4	11.8	0.76	13.1	1.2	15.7	96	26.9	11.2	0.71
T6 (E2+ M1 + C2 or F2)	13.0	1.2	15.6	98	27.4	11.8	0.76	13.0	1.2	15.6	99	27.7	12.1	0.78
T7 (E2+ M2 + C1 or F1)	13.0	1.2	15.6	99	27.7	12.1	0.78	13.1	1.2	15.7	100	27.9	12.2	0.78
T8 (E2+ M2 + C2 or F2)	13.0	1.2	15.6	100	28.0	12.4	0.79	13.1	1.2	15.7	101	28.2	12.5	0.80
T9 (E3+ M1 + C1 or F1)	11.9	1.3	15.5	100	28.0	12.5	0.81	12.0	1.3	15.6	100	27.9	12.3	0.79
T10 (E3+ M1 + C2 or F2)	11.6	1.3	15.1	100	28.0	12.9	0.85	11.9	1.3	15.5	101	28.2	12.7	0.82
T11 (E3+ M2 + C1 or F1)	11.8	1.3	15.3	102	28.6	13.3	0.87	11.9	1.3	15.5	100	28.1	12.6	0.81
T12 (E3+ M2 + C2 or F2)	11.8	1.3	15.3	102	28.6	13.3	0.87	11.8	1.3	15.3	101	28.3	13.0	0.85

L.E = 1 pound Egyptian currency = 100 piasters.
Price of total egg prod./ hen (L.E.) = Total number of eggs / hen x price of one egg (0.28 L.E.).
Net revenue (N.R.) / hen (L.E.) = price of total egg production / hen (L.E.) - total feed cost / hen (L.E.).
Economic Efficiency (E.Ef.) = Net revenue / price of total feed intake.
E. (Metabolizable energy level); **E1:** 2600 kcal ME/kg; **E2:** 2800 kcal ME/kg; **E3:** 3000 kcal ME/kg.
M (Methionine level); **M1:** 0.40% methionine; **M2:** 0.50% methionine.
C (Choline level); **C1:** 300 mg choline/kg ; **C2:** 900 mg choline/kg.
F (Folic acid level); **F1:** 2.0 mg folic acid/kg ; **F2:** 6.0 mg folic acid/kg. **T5** (control diet).

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الملخص العربى

تأثير المستويات المختلفة من الطاقة والمثيونين والكولين وحامض الفوليك على الأداء الإنتاجى للدجاج البياض.

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اشتملت هذه الدراسة على تجربتين ، بحيث تضمنت التجربة الأولى ثلاثة مستويات من الطاقة ٢٦٠٠ ، ٢٨٠٠ و ٣٠٠٠ كيلو كالورى طاقة ممثلة/كجم عليقة ومستويين من الحامض الأمينى المثيونين (٠,٤٠ و ٠,٥٠%) ومستويين من الكولين (٣٠٠ و ٩٠٠ ملليجرام/كجم عليقة) بينما تحتوى التجربة الثانية نفس مستويات الطاقة والمثيونين وعلى مستويين من حامض الفوليك (٢,٠ و ٦,٠ ملليجرام/كجم عليقة) بدلا من الكولين، كل من التجربتين فى تصميم عاملى ٣ x ٢ x ٢ . تم تكوين العلائق بحيث تفى بالإحتياجات الغذائية تبعا لتوصيات دليل تربية السلالة مع الأخذ فى الإعتبار أن مستويات الكنترول للطاقة ، المثيونين ، الكولين و حامض الفوليك هى ٢٨٠٠ كيلو كالورى طاقة ممثلة/كجم عليقة ، ٠,٤٠% ، ٣٠٠ ملليجرام/كجم عليقة و ٢,٠ ملليجرام/كجم عليقة على الترتيب. اجريت الدراسة على عدد ٢٤٠٠ دجاجة بياضة (بعدد ١٢٠٠ دجاجة / تجربة) من النوع اللوهمان البنى عمر ٢٨ إسبوعاً. تم تقسيم الطيور فى كل تجربة إلى ١٢ مجموعة بكل منها ٤ مكررات. تم تربية الطيور لكلا التجربتين فى بطاريات داخل عنبر من النوع المقفول تحت نفس الظروف من الرعاية لمدة ١٦ إسبوعاً مدة إجراء التجربة. تم تقديم الغذاء على صورة سائبة مع ماء الشرب حتى الشبع مع إعطاء الطيور ١٦ ساعة إضاءة فى اليوم.

Layers, Performance, Energy, Mathionine, Choline, Folic acide.

أوضحت النتائج عدم وجود فروق معنوية نتيجة إستخدام المستويات المختلفة من الطاقة ، المثيونين ، الكولين أوحامض الفوليك فى نسبة الإنتاج اليومى للبيض حيث سجلت الطيور التى تم تغذيتها على علائق مرتفعة فى الطاقة أعلى نسبة من الإنتاج اليومى للبيض بالمقارنة بالطيور التى تم تغذيتها على علائق متوسطة ومنخفضة الطاقة ولكن بدون وجود فروق معنوية بين مستويات الطاقة الثلاثة فى كلا من التجربتين. أشارت النتائج أيضا إلى أن زيادة مستوى الطاقة ، المثيونين ، الكولين أوحامض الفوليك بالعلائق لم يكن له أى تأثير معنوى على وزن البيضة. فى التجربة الأولى والثانية حدثت زيادة ملحوظة فى كمية الغذاء المستهلك اليومى مع انخفاض طاقة العليقة وسجلت العلائق المحتوية على ٣٠٠٠ كيلو كالورى طاقة ممثلة/كجم عليقة أفضل معامل لتحويل الغذاء حيث أدت هذه العلائق الى تحسين معامل التحويل الغذائى بمعدل 13.20 و19.72% (التجربة الأولى) وبمعدل 11.68 و 19.82% (التجربة الثانية) بالمقارنة بالعلائق المحتوية على ٢٨٠٠ و ٢٦٠٠ كيلو كالورى طاقة ممثلة/كجم عليقة على الترتيب. كما وجد فروق معنوية فى قيم مقدار الزيادة فى الوزن نتيجة لإختلاف مستوى الطاقة بالعلائق حيث إزداد مقدار الزيادة فى الوزن تدريجياً مع زيادة مستوى الطاقة فى العلائق المضاف اليها المثيونين، الكولين وحامض الفوليك فى التجربة الأولى والثانية. أدت المعاملات المحتوية على ٢٦٠٠ كيلو كالورى طاقة ممثلة/كجم عليقة المضاف إليها المثيونين ، الكولين وحامض الفوليك إلى تحسين الصفات الخارجية لجودة البيضة والمتمثلة فى سمك القشرة بينما أدت العلائق المحتوية على ٣٠٠٠ كيلو كالورى طاقة ممثلة/كجم عليقة إلى تحسين الصفات الداخلية لجودة البيضة والمتمثلة فى وحدات هاوف بالمقارنة بعليقة الكنترول المحتوية على ٢٨٠٠ كيلو كالورى طاقة ممثلة/كجم عليقة. أدت أيضا التغذية على العلائق المحتوية على ٢٦٠٠ كيلو كالورى طاقة ممثلة/كجم عليقة فى التجربة الأولى والثانية إلى زيادة الدهون الكلية والكوليسترول الكلى فى صفار البيضة بالمقارنة بالعلائق المحتوية على ٢٨٠٠ و ٣٠٠٠ كيلو كالورى طاقة ممثلة/كجم عليقة. تأثرت الإستجابة المناعية والمتمثلة فى الجلوبيولين المناعى (الأجسام المضادة) معنويا بمستويات الطاقة فى العلائق فى حين لم يتأثر الجلوبيولين المناعى باضافة المثيونين ، الكولين وحامض الفوليك إلى العلائق مع ملاحظة حدوث انخفاض طفيف فى مستوى الجلوبيولين المناعى مع زيادة مستوى الكولين وحامض الفوليك المضاف إلى العلائق ولكن بدون أى تاثير معنوى واضح. أدى إستخدام مستويات مختلفة من الطاقة وخاصة مستوى الطاقة المرتفع (٣٠٠٠ كيلو كالورى طاقة ممثلة/كجم عليقة) إلى الحصول على نتائج أفضل لمعاملات الهضم فى حين لم يؤثر إضافة المثيونين ، الكولين وحامض الفوليك على معاملات الهضم المختلفة. تأثرت الكفاءة الإقتصادية بالمستويات المختلفة من الطاقة، المثيونين، الكولين وحامض الفوليك حيث ازدادت بزيادة مستوى الطاقة فى العلائق.

بصفة عامة نستخلص من هذه الدراسة أن التغذية على العلائق المحتوية على ٣٠٠٠ ك.كالورى/كجم عليقة أعطى أفضل أداء انتاجى للطيور وأعلى عائد اقتصادى. أدت اضافة المثيونين ، الكولين و حامض الفوليك الى العلائق المنخفضة الطاقة (٢٦٠٠ ك.كالورى/كجم عليقة) الى تحسن فى صفات جودة البيضة وزيادة فى الاستجابة المناعية للطيوربالمقارنة بالعلائق المحتوية على ٢٨٠٠ و٣٠٠٠ ك.كالورى/كجم عليقة. لم يكن لاضافة المثيونين، الكولين وحامض الفوليك حتى مستوى ٠,٥٠% ، 900 مللجم كولين/كجم و ٦,٠ مللجم حامض الفوليك/كجم على الترتيب أى تأثير عكسى على الأداء الانتاجى للطيور، جودة البيضة والاستجابة المناعية.