Effect of dietary energy and protein on the performance, egg quality, bone mineral density, blood properties and yolk fatty acid composition of organic laying hens

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Abstract

An experiment was conducted to evaluate the effect of dietary metabolizable energy (ME) and crude protein (CP) on the performance, egg quality, bone characteristics and yolk fatty acid composition of organic laying hens. At 23 weeks, a total of 600 Brown nick laying hens were randomly distributed into 24 outdoor pens (4 replicate pens/treatment; 25 birds/pen) and were given (2750, 2775 and 2800 kcal of ME/kg) and CP (16 and 17%) resulting in a 3x2 factorial arrangement of organic dietary treatments. The experiment lasted 23 weeks. The performance of laying hens were not affected by the dietary treatment while the egg weight was increased with energy and CP levels in the diet (P<0.05). Serum total protein was not affected by dietary energy and protein level. Total cholesterol and triglyceride tend to reduce with the increasing amount of CP in the diet. Thereafter, bone and egg quality characteristics were numerically increased in dietary 2775 kcal of ME/kg and 16% CP treatment. On the other hand, docosahexanoic acid content in egg yolk was higher (P<0.01) in 2750 kcal of ME/kg and 17% CP treatment. As a result, the performance, blood and fatty acid composition were maximized in 2750 kcal of ME/kg and 16% CP treatment. Thus, dietary 2750-2775 kcal of ME/kg and 16% CP may enhance performance, blood and fatty acid composition of organic laying hens.

Introduction

In recent years global consumers are concerned about the livestock and poultry product due to the addition of additives and veterinary drugs into the feedstuff. Therefore, attention is now centered upon on organic poultry production due to its significant implications for environmental preservation, food safety and health promotion. After 2012, European Union would not allow genetically modified (GM) grain, antibiotics, hormones, animal slaughter by-products or chemically extracted feeds and amino acids and also coccidiostats cannot be included in the feed (European Commission, 2007). In such global circumstances, feeding the poultry organic diet is becoming popular worldwide. For example, in the Republic of Korea, the commercial poultry industry has greatly increased and the production volume more than doubled in the last 10 years. As of October 2012, there were 139.44 million birds and 37.6 million of eggs produced per day and out of which 26% of eggs were produced under the organic system in Korea (Statistics Korea, 2012). Currently, the Korean society pays enormous attention to food due to recent economic growth and rising national incomes equal to the advanced nations. Therefore, many consumers are willing to pay the higher prices for organically produced food (Ki and Lee, 2011). In poultry production the organic farming, ruled by the National Agricultural Product Quality Management Services (2006) in Korea (Announcement number No. 2006-20) allows the hens to have access to outdoor pens (0.22 m²/hen). Moreover, the diet must be composed of at least 90% organic ingredients.

The effect of dietary energy and protein concentrations on the performance of domestic fowl has been the subject of several studies. Previous researchers reported that egg production can be enhanced by increasing protein and decreasing energy level in the diet (Rose et al., 2004, Yu et al., 2008), protein level enhanced egg production (Zanaty, 2006 and Gunawardana et al., 2009), egg mass and feed conversion (Rose et al., 2004, Liu et al., 2005 and Nahashon et al., 2007). On the other hand, Harms et al., (2000), Elwinger et al., (2008) and Guangbing et al., (2007) did not find any effects of dietary energy on egg production and quality (Guangbing et al., 2007). Recently, Niekerk and Reuvekamp, (2009) and Elwinger et al., (2008) mentioned that egg production was not influenced by the organic feed. But, organic dietary concentrations of metabolizable energy and protein on bone health, blood properties and fatty acid composition of laying hens have not previously been investigated, as far as we are aware.

Organic feed is generally more expensive than conventional feed. Therefore it is more important for the egg producers to optimize dietary energy and protein level in the diet. Meanwhile, the challenge for nutritionists will be to obtain well-balanced cost effective organic feed. These factors may be critical in the Republic of Korea, where most of the feed ingredients have been imported from USA, Brazil, and China etc. to meet their domestic demands. Thus, there is an urgent need to optimize the organic dietary concentrations of energy and protein to enhance the performance of laying hens. Therefore, this study was undertaken to evaluate the levels of organic dietary metabolizable energy (ME) and crude protein (CP) on the performance, egg quality, blood properties, bone mineral density and yolk fatty acid composition of organic laying hens.

Materials and methods

Feeding trial and management

For this experiment, organic housing system was prepared to the criteria of organic livestock product (No. 2006-20, NAQS). The official announcement is published at 29th June by the National Agricultural Product Quality Management Service (2006) in South Korea. The guideline described briefly that stock density was 0.22 cm²/bird and lighting period was limited to 14 h with artificial lighting source in a day. The pen should be provided...
with perches and laying nest and the diet must be composed of at least 90% organic ingredients. Eggs were considered organic only when produced by laying hens reared in an organic housing system for at least 3 months. Therefore, a total of 600 pullets of 11 weeks old were purchased from a local farm. Upon arrival pullets were reared under organic conditions such as diet contained above 90% of organic ingredients, 0.26 m²/hen of stock density, natural lighting source, ad libitum of diet and water and outdoor floor with litter and perch for 12 weeks. As final trial, in a 3×2 factorial arrangement, pullets were randomly divided into 6 groups having 25 birds in each group and were assigned to the diet containing (2750, 2775 and 2800 kcal of ME/kg) and CP (16 and 17%) respectively. During the trial, hens were reared in an outdoor floor pens of 6.57 m² (0.26 m²/hen) which was covered with rice husk. The floor was equipped with long feeder, nipple drinker, perches and laying nest. The outdoor floor pens were located near the battery cage housing system at the farm of the Department of Animal Science, Chonbuk National University, South Korea. Feed and drinking water was available on an ad libitum basis. Day light was provided less than 14 h in addition with artificial light. The beaks of all birds were remained intact. Ingredients and nutrient composition of experimental diets were shown in Table 1.

### Laying performance and egg quality

During the laying period, egg production, egg weight and feed intake were recorded daily. Egg mass (g of egg/hen per day) and feed conversion ratio (FCR) was calculated as the feed to egg weight ratio (g·g⁻¹ egg weight). At the end of experiment, 30 eggs were randomly collected from each experimental group to assess egg quality parameters. The eggshell breaking strength was measured by using an egg multi-tester instrument (QC-SPA, TSS, York, UK) and expressed as unit of compression force was exposed to unit egg shell surface area (kg/cm²). Then, eggs were singularly weighed and carefully broken on a glass plate and egg shell color, albumin height, Haugh unit and yolk color were measured by egg quality equipment (QCM+-System, TSS).

### Blood test and bone mineralisation

At the end of the experiment (46 weeks), 10 blood samples per treatment were taken by puncturing the wing vein and serum was isolated and stored at -20°C until analysis. Individual serum samples were analyzed for total cholesterol, triglyceride and HDL by enzymatic calorimetric method using commercial Blood analysis kits (AM 202-K; Asan Pharmaceutical Co., Ltd., Hwaseong-si, Korea). Similarly, Automatic blood analyzers (ADVIA 1650, JEOL, Tokyo, Japan) were used to measure the blood total protein and albumin levels. At 46 weeks of age, five hens per treatment with similar body weight (150±50 g) were selected and killed by cervical dislocation and tibia was removed from the muscle. Bone mineral density (BMD) and bone breaking strength (BBS) of the tibia (n=5/group) were measured by using Bone Densitometer (pDEXA, Norland Medical Systems Inc., White Plains, NY, USA) and Texture Analyser (TA. HD. plus, Stable Micro Systems, Godalming, UK), respectively.

### Measurement of fatty acid

Determination of fatty acid composition, one g of fresh egg yolk was precisely weighed in a glass tube and dissolved in 4 mL of methanol-benzene (1:4, v/v). Thereafter, 200 µL of acetyl chloride was slowly added over a period of 1 min and tubes were tightly closed with Teflon-lined caps and subjected to methanolsysis at 100°C for 1 h. After providing a cooling period of 15 min at room temperature, 2 mL of 6% K₂CO₃ was added in the tubes followed by the addition of 2 mL hexane for vortex. The tubes were then shaken and centrifuged at 1700 g for 20 min. An aliquot of the upper phase hexane contained fatty acid methyl esters (FAME) was injected into the chromatograph. Fatty acids were chromatographed as methyl esters on a 30-m fused silica column having an internal diameter of 0.25 µm. The column was wall-coated with 0.20 mm Supelco™ 10. Analysis was performed on an Agilent Technologies 6890N, a gas chromatograph, equipped with a flame ionization detector. Helium was used as a carrier gas and nitrogen as a make-up gas. The split ratio was 100:1. The injection port temperature in oven condition and the detector was 240°C. The column temperature rose in a stepwise manner from 180°C up to 230°C at the rate of 3°C/min and then holds for 15 min. The fatty acids identified using a FAME standard and were expressed as percentage of total known FAME.

### Statistical analysis

To investigate the effects of organic dietary energy and protein level, data were analyzed as 3 (energy: 2750, 2775 and 2800 kcal of ME/kg)×2 (protein: 16 and 17%) factorial design by 2-way ANOVA plus interaction (SAS, 1998). Significant differences among treatment means were separated using the Duncan’s new multiple-range test (Steel and Torrie, 1980).

### Table 1. Experimental diet composition.

| Ingredients, % | 2750 kcal/kg ME | 2775 kcal/kg ME | 2800 kcal/kg ME |
|---------------|-----------------|-----------------|-----------------|
| ME, kcal/kg   | 2750            | 2750            | 2775            | 2800            |
| CP, %         | 16              | 16              | 16              | 16              |
| Lysine, %     | 0.74            | 0.74            | 0.74            | 0.74            |
| Methionine, % | 0.32            | 0.34            | 0.32            | 0.34            |
| Calcium, %    | 3.75            | 3.75            | 3.75            | 3.75            |
| Sodium, %     | 0.17            | 0.17            | 0.17            | 0.17            |

#### Calculated composition

| ME, kcal/kg   | 2750            | 2750            | 2775            | 2800            |
|---------------|-----------------|-----------------|-----------------|-----------------|
| CP, %         | 16              | 16              | 16              | 16              |
| Lysine, %     | 0.74            | 0.74            | 0.74            | 0.74            |
| Methionine, % | 0.32            | 0.34            | 0.32            | 0.34            |
| Calcium, %    | 3.75            | 3.75            | 3.75            | 3.75            |
| Sodium, %     | 0.17            | 0.17            | 0.17            | 0.17            |

# Table 1. Experimental diet composition.

| Ingredients, % | 16% CP | 17% CP | 16% CP | 17% CP | 16% CP | 17% CP |
|----------------|--------|--------|--------|--------|--------|--------|
| Corn           | 66.08  | 63.98  | 66.64  | 64.56  | 67.23  | 65.14  |
| Soybean meal   | 21.13  | 21.64  | 19.24  | 19.79  | 17.38  | 17.93  |
| Corn gluten meal | 1.74  | 3.32  | 3.01  | 4.55  | 4.24  | 5.78  |
| Limestone      | 0.44   | 9.42   | 9.42   | 9.42   | 9.42   | 9.42   |
| Calcium phosphate | 0.87  | 0.89  | 0.92  | 0.91  | 0.94  | 0.92  |
| Salt           | 0.37   | 0.38   | 0.38   | 0.38   | 0.38   | 0.38   |
| L-Lysine       | -      | -      | 0.02   | 0.03   | 0.05   | 0.07   |
| DL-Methionine  | 0.04   | 0.04   | 0.04   | 0.03   | 0.03   | 0.03   |
| Vitamin premix | 0.18   | 0.18   | 0.18   | 0.18   | 0.18   | 0.18   |
| Mineral premix | 0.15   | 0.15   | 0.15   | 0.15   | 0.15   | 0.15   |

*contain per kg: retinol, 3.6 mg; cholecalciferol, 0.125 mg; tocopherol, 50 mg; menadione, 3 mg; thiamine, 2 mg; riboflavin, 6 mg; pyridoxine, 4 mg; cyanocobalamin, 0.025 mg; biotin, 0.15 mg; pantothénac acid, 20 mg; folic acid, 2 mg; nicotinac acid, 7 mg.¢contain per kg: Cr, 66,729 mg; Cu, 41,700 mg; Mn, 63,400 mg; Zn, 64,72 mg; I, 1,854 mg; Se, 250 mg; ME, metabolizable energy; CP, crude protein.
Results

Egg production, feed intake and feed conversion ratio

There was no organic dietary interaction between ME and CP on egg production, egg mass, feed intake and FCR of laying hens (Table 2) except egg weight. With increasing dietary energy and CP levels (2800 kcal of ME/kg and 17% CP), egg weight increased (P<0.05) from 61.48 to 62.48 g. On the other hand, higher egg production and egg mass were obtained when 2750 kcal of ME/kg and 16% CP were fed to the laying hens than that of other dietary treatments. The feed intake was higher in 2800 kcal of ME/kg and 17% CP and FCR was better for 2775 kcal of ME/kg and 16% CP in the diet as compared to other dietary treatments.

Blood composition in laying hens

Total protein content in blood was not influenced by the interaction of dietary ME and CP level (Table 3). On the other hand, variation of energy and protein did not affect the blood albumin, total cholesterol, triglyceride and HDL contents of laying hens. Blood albumin contents was non-significantly increased with CP content but declined with the ME concentration in the diet. Consequently, total cholesterol and triglyceride contents were non-significantly higher in dietary 2750 kcal of ME/kg and 16% CP content than that of 2800 and 2775 kcal of ME/kg and 17% CP treatments. Maximum blood triglyceride level was reduced in 2775 kcal of ME/kg and 17% CP treatment (P>0.05). Moreover, HDL content in blood was non-significant among ME and CP levels in the diet, but HDL content was negatively influenced due to levels of ME and CP in the diets.

Bone mineral density and bone breaking strength in laying hen

Bone mineral density (BMD) and bone breaking strength (BBS) were not influenced due to the interactions of ME and CP in the diet (Table 4). From 23 to 46 weeks of age, feeding a diet with 2775 kcal/kg ME and 16% CP level numerically increased the BMD and BBS of laying hen’s tibia than those of 2750 and 2800 kcal/kg ME and 17% CP treatment.

Table 2. Effect of various levels of organic dietary metabolizable energy and crude protein on the performance in laying hens from 23 to 46 weeks.

| Treatments          | 2750 kcal/kg ME | 2775 kcal/kg ME | 2800 kcal/kg ME | P value ME×CP |
|---------------------|-----------------|-----------------|-----------------|---------------|
|                     | 16% CP          | 17% CP          | 16% CP          | 17% CP        | 16% CP        | 17% CP        | 16% CP        | 17% CP        | 16% CP        |
| Egg production, %   | 93.74±1.8       | 90.99±1.5       | 92.01±1.3       | 90.66±2.8     | 91.18±3.3     | 92.09±2.5     | 0.3085        |
| Egg weight, g       | 61.48±0.36 b    | 62.15±0.9 a     | 62.41±0.5 a     | 61.85±0.5 a   | 61.28±0.5 a   | 62.48±0.6 a   | 0.0448        |
| Egg mass            | 57.64±1.6       | 56.49±1.5       | 57.42±0.6       | 56.15±1.6     | 55.91±1.9     | 56.84±2.5     | 0.3760        |
| Feed intake, g/d    | 145.06±2.9      | 145.73±5.1      | 143.85±0.5      | 143.71±0.6    | 145.05±3.4    | 147.63±5.5    | 0.7415        |
| FCR                 | 2.51±0.1        | 2.58±0.1        | 2.50±0.2        | 2.56±0.1      | 2.59±0.1      | 2.59±0.1      | 0.7697        |

Values are mean±SE. ME, metabolizable energy; CP, crude protein; FCR, feed conversion ratio. a,bValues with the same letters are not significantly different at 5% level.

Table 3. Effect of various levels of organic dietary metabolizable energy and crude protein on blood composition in laying hens.

| Treatments          | 2750 kcal/kg ME | 2775 kcal/kg ME | 2800 kcal/kg ME | P value ME×CP |
|---------------------|-----------------|-----------------|-----------------|---------------|
|                     | 16% CP          | 17% CP          | 16% CP          | 17% CP        | 16% CP        | 17% CP        | 16% CP        |
| TPN, g/dL           | 5.19±0.5        | 6.00±0.8        | 5.05±0.4        | 5.26±0.5      | 5.38±0.5      | 5.38±0.5      | 0.0557        |
| ALB, g/dL           | 1.28±0.1        | 1.38±0.1        | 1.26±0.1        | 1.39±0.3      | 1.34±0.2      | 1.28±0.1      | 0.2012        |
| TCL, mg/dL          | 115.9±47.9      | 113.6±31.8      | 101.0±35.1      | 93.9±14.7     | 121.3±43.3    | 93.9±19.8     | 0.4733        |
| TG, mg/dL           | 1204.7±247.3    | 1114.7±216.4    | 1010.4±228.8    | 850.4±235.3   | 1245.4±211.6  | 853.8±188.9   | 0.6444        |
| HDL, mg/dL          | 37.8±7.9        | 37.9±4.6        | 37.4±4.2        | 37.2±6.1      | 36.8±4.2      | 35.3±5.7      | 0.8932        |

Values are mean±SE. ME, metabolizable energy; CP, crude protein; TPN, total protein; ALB, albumin; TCL, total cholesterol; TG, triacylglyceride; HDL, high density lipoprotein.

Table 4. Effects of various levels of organic dietary metabolizable energy and crude protein on the bone mineral density (BMD) and bone breaking strength in laying hens.

| Treatments          | 2750 kcal/kg ME | 2775 kcal/kg ME | 2800 kcal/kg ME | P value ME×CP |
|---------------------|-----------------|-----------------|-----------------|---------------|
|                     | 16% CP          | 17% CP          | 16% CP          | 17% CP        | 16% CP        | 17% CP        | 16% CP        |
| BMD, g/cm²          | 0.249±0.01      | 0.222±0.01      | 0.271±0.04      | 0.265±0.02    | 0.231±0.02    | 0.217±0.01    | 0.6345        |
| BBS, kg             | 21.05±0.39      | 21.45±0.14      | 22.50±0.68      | 25.28±0.22    | 22.82±0.41    | 20.55±0.42    | 0.5989        |

Values are mean±SE. BMD, bone mineral density; BBS, bone breaking strength.
Egg quality in laying hens

Data describing the effects of energy and protein level on egg quality traits are shown in Table 5. In the present results, egg shell breaking strength was not significantly affected by the ME and CP level in the diet. Egg shell color and albumin height showed similar pattern among the ME and CP level in the diet. Though diet has no significant impact on haugh unit but higher in 2775 kcal of ME/kg and CP 16% level followed by other organic dietary ME and CP was determined. Yolk color was not affected by the interaction of ME×CP but numerically influenced with the increasing levels of ME and decreasing level of CP content.

Fatty acid composition in egg yolk

In the present results, the fatty acid compositions of egg yolk were influenced by the experimental diets (Table 6). A significant ME×CP interaction was found in the docosahexaenoic acid (DHA, C22:6n3) content in egg yolk which was higher in 2750 kcal of ME/kg and CP 17% diet as compared with other dietary treatments. On the other hand, myristic acid (C14:0), palmitic acid (C16:0), palmitoleic acid (C16:1n7), stearic acid (C18:0), oleic acid (C18:1n9), linoleic acid (C18:2n6), linolenic acid (C18:3n3) and arachidonic acid (C20:4n6) were not significantly affected by the diets (Table 6).

Table 5. Effects of various feeding organic dietary metabolizable energy and crude protein on egg quality in laying hens.

| Treatments | 2750 kcal/kg ME | 2775 kcal/kg ME | 2800 kcal/kg ME | P value |
|------------|----------------|----------------|----------------|---------|
| ESC        | 28.70±4.3      | 27.03±3.4      | 27.60±4.7      | 0.4234  |
| ESBS, kg/cm²| 4.83±1.1      | 4.58±0.9       | 5.03±1.2       | 0.0793  |
| AH, mm     | 7.08±1.1       | 6.81±1.1       | 7.39±1.2       | 0.0793  |
| HU         | 82.12±7.6      | 79.17±7.8      | 83.40±7.2      | 0.7264  |
| YC         | 8.40±0.6       | 8.50±0.6       | 9.06±0.4       | 0.5409  |

Values are mean±SE. ESC, egg shell color; ESBS=egg shell breaking strength; AH, albumin height; HU, Haugh unit; YC, yolk color.

Table 6. Effects of various feeding organic dietary metabolizable energy and crude protein on fatty acid composition of egg yolk.

| Treatments | 2750 kcal/kg ME | 2775 kcal/kg ME | 2800 kcal/kg ME | P value |
|------------|----------------|----------------|----------------|---------|
| C14:0      | 0.29±0.0       | 0.27±0.0       | 0.26±0.0       | 0.07    |
| C16:0      | 26.15±0.2      | 26.13±1.4      | 25.16±0.7      | 0.83    |
| C16:1n7    | 2.55±0.4       | 2.49±0.5       | 2.48±0.5       | 0.33    |
| C18:0      | 11.50±0.6      | 11.28±0.3      | 11.74±0.9      | 0.16    |
| C18:1n9    | 38.78±1.4      | 38.62±1.2      | 40.02±2.4      | 0.74    |
| C18:2n6    | 15.49±1.4      | 16.00±2.7      | 15.31±1.6      | 0.82    |
| C18:3n3    | 0.59±0.2       | 0.55±0.1       | 0.38±0.0       | 0.08    |
| C20:4n6    | 3.15±0.0       | 3.15±0.1       | 3.17±0.2       | 0.71    |
| C20:5n3    | 1.21±0.2       | 1.47±0.0b      | 1.29±0.1c      | 0.02    |

Values are mean±SE. **Value with the same letters are not significantly different at 5% level.
differential interaction between ME and CP had a significant effect on egg weight and which was increased with increasing dietary energy and protein. In previous, Yu et al. (2008) and Gunawardana et al. (2008), did not get any energy and protein interaction effects on egg weight, but egg weight was increased with CP (Gunawardana et al., 2008) or ME (Harms et al., 2010) in a concentration dependent manner. Yu et al. (2008) increased dietary energy from 3360 kcal of ME/kg had decreased the egg weight of organic hens and it is partially consistent with the present results. Furthermore, feed consumption was higher in 2800 kcal of ME/kg and 17% CP in the diet but did not reach to the significant level. It might be due to small chemical differences among the dietary treatments.

In the current experiment, as expected, blood protein contents were increased with protein and decreasing energy level in the diet and it might be due to dietary protein. A lower level of ME and higher level of CP in diets did not increase blood albumin and it is consistent with the results of Yu et al. (2008). On the other hand, blood total cholesterol has shown opposite direction with the level of albumin and triglyceride content. The possible reasons might be due to the ingredients (soyabean meal and corn gluten meal contain a higher percentage of protein) used in the diets. This result is consistent with previous studies which reported that different protein and energy levels did not influence the blood components (Hussein et al., 2010).

Protein and carbohydrate supplement as energy sources are important factors for bone health. There was no interaction between ME×CP on BMD and BBS of organic laying hens. When dietary energy level increased from 2750 to 2775 kcal of ME/kg with 16% CP level, BMD and BBS also increased linearly but the effect was non-significant. However a further increase in dietary energy level from 2775 to 2800 kcal of ME/kg had no additional effect on bone characteristics. Due to the lack of previous studies regarding the implementation of organic dietary ME on bone health of laying hens, a direct comparison was not possible with the present results. In consistent with dietary protein and energy levels did not affect bone strength of toms (Kirk and Firman, 1993) and pouls (Cook et al., 1984; Patterson et al., 1986). Thus, the laying hen, feeding a diet of low crude protein might improve bone quality in hens (Rennie et al., 1997).

Dietary interaction between ME×CP had no significant effect on egg quality. This result is consistent with previous studies which reported that either energy or protein did not affect egg quality of hens (Hussein et al., 2010; Yu et al., 2008; Guangbing et al., 2007) and guinea fowl (Nashahon et al., 2007) which is comparable with the present findings. Yolk color increased with the ME and CP in a dependent manner. In the present trial, dietary energy level decreased by replacing of corn and corn gluten meal with the soybean meal and this could be affected on yolk color and which corresponds with the findings of Gunawardana et al., (2008) and Karunajeewa, (1972).

Dietary interaction of ME and CP significantly influenced the docosahexaenoic acid (DHA) content in egg yolk which was higher in 2750 kcal of ME/kg and 17% CP diet. It was also observed that 2750 kcal of ME/kg and 17% CP in layer rations could induce a reduction in the ratio of n-6/n-3 fatty acids in egg yolk which influences the desaturase activity of hens. Thereby, the ratio of n-6/n-3 fatty acids may be affected the DHA synthesis. The present results also found that, increased the dietary energy level decreased the SFA and increased the UFA in the yolk due to lower level of MUFA. The other fatty acid (UFA, MUFA, PUFA, UFA/SFA and n-6/n-3) showed a tendency to increase by increasing the energy in the diet. The possible mechanism behind this effect is not clear but high level of dietary energy increased the UFA in the egg yolk.

This result shows that a higher UFA/SFA ratio was obtained from the 2800 kcal/kg ME and 16% CP treatment and such a proportion improves the nutritional value of eggs. In the present results, hens fed with 2750 kcal/kg ME and 17% CP had the lowest n-6/n-3 ratios than that of other groups and this n-6 to n-3 fatty acid ratio may be more important than the absolute amount of dietary n-3 fatty acids to reduce the arachidonic acid metabolism (Simopoulos, 2000).

**Conclusions**

It may be concluded that the performance, blood and fatty acid composition were maximized in 2750 kcal of ME/kg and CP 16% and bone and egg quality characteristics were numerically increased in 2775 kcal/kg of ME and 16% CP diet. Thus, dietary 2750-2775 kcal of ME/kg and 16% CP may enhance performance, blood and fatty acid composition of organic laying hens. Meanwhile, further follow-up studies should be conducted between 2750 and 2775 kcal of ME/kg in organic laying hens diet.
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