Influence of Different Dietary Levels of Energy and Protein on Reproductive and Post Hatch Growth Performance in Japanese Quails

E. Lotfi, N. Karimi, B. Parizadian Kavan and M.R. Sharifi

1 Young Researchers and Elite Club, Gorgan Branch, Islamic Azad University, Gorgan, Iran
2 Department of Animal Science, Gorgan University of Agricultural Science and Natural Resources, Gorgan, Iran
3 Department of Animal Science, Faculty of Agriculture, Lorestan University, Khoramabad, Iran
4 Department of Animal Science, Faculty of Agriculture, Shahrekord University, Shahrekord, Iran

ABSTRACT

The response of laying Japanese quails to dietary levels of energy and protein on performance, egg quality, hatchability, fertility and their effect on subsequent offspring live body weight was investigated. A total of 432 Japanese quails (13 weeks old) were divided into nine treatments. Each treatment comprised of four replicates of twelve birds (9 females and 3 males). Nine diets including three levels of metabolizable energy (ME) (11.51, 12.41 and 12.77 MJ/kg diet) and three levels of crude protein (CP) (180, 200 and 220 g/kg diet) in a 3 × 3 factorial design were formulated. Weight gain and egg production were higher in quails fed diets with high level of ME (12.77 MJ/kg diet) and CP (220 g/kg diet). Feed conversion ratio (FCR) improved linearly with the increase in dietary energy level, and the best FCR was obtained by 12.77 MJ ME/kg diet (P<0.05). Increasing energy and protein levels resulted in an increase in egg shell thickness, egg shell strength, albumen and yolk indexes (P<0.01). Percentage of hatchability and subsequent offspring live body weight at hatch were significantly affected by dietary ME and CP levels (P<0.01). Offspring live body weight were higher in chicks from hens fed diets high in energy and protein level. Based on the results of the present study, ME and CP levels of laying Japanese quails diets had significant effects on performance, egg quality and offspring live body weight at hatch.

KEY WORDS egg quality, energy, Japanese quail, offspring hatch weight, protein.

INTRODUCTION

Egg production traits and quality of produced eggs is an important factor for human consumption or hatchability. The egg quality traits can be discussed under two broad categories namely “external” and “internal” quality (Zaheer, 2015). The external quality of the egg is determined by features such as the weight, size and shape of the egg as well as the structure, thickness and strength of the shell (Bain, 2005). The internal quality is measured on the basis of the weight, size and percentage of albumen and yolk. Fertility and hatchability are important traits which has a great economical impact on hatcheries. Also it has a strong effect on chick output. Several environmental factors are known to affect hatchability, such as storage length and conditions, age of dam and quality of the egg (Kingori et al. 2010; Miranda et al. 2015). It is clear that nutrition is one of the most important factors affecting egg production and egg quality, since it may influence fertility, hatchability, quality of chick output and offspring growth rate (Kidd, 2003; Zaheer, 2015). The nutrients required for chicken embryo development are derived from the nutrients stored in the egg, whose nutrient profile changes with the maternal diet (An et al. 2010).
Energy and protein are primary nutritional requirements of all classes of animals. These requirements must be met before requirements for other nutrients are addressed. Generally, the energy and protein requirement in Japanese quails were considered to be similar to laying chickens. Therefore, there is need of updating optimal nutritional requirement of Japanese quails. It is well known that dietary energy and protein level influences the productive and reproductive traits of quails (Gunawardana et al. 2008). If dietary protein is too low or the amino acid requirements are not met, poor egg production and hatchability will occur (Tarasewicz et al. 2006b). When hens first begin laying eggs, during the early laying period, they are still growing and maturing. During this period they need an increased amount of protein. Energy is considered to be the most important nutrient required from the standpoint of total cost and quantity of quails feed. The importance of dietary energy has long been recognized for achieving maximum growth rate and efficiency of feed conversion with poultry (Gamal, 2005; Miranda et al. 2015).

As a general rule, the quail eats to satisfy its energy requirements. So, within limits, the energy content of the diet determines the quantity of feed consumed, including the quantity of protein, minerals and vitamins contained in that feed (Gamal, 2005). The worldwide competitiveness of the poultry industry has made it necessary to develop new methods that relate nutrition to production cost and optimize the feeding strategies and the efficiency of nutrient utilization, according to input ingredients, production cost, and market price of poultry production (Rivera-Torres et al. 2011). Due to this perspective, the objective of this study was to investigate the potential of dietary with various levels of ME and CP on overall egg production performance, egg quality, hatchability and their effect on offspring growth rate of laying Japanese quails throughout the peak production period.

MATERIALS AND METHODS

Birds and housing
A total of 432 Japanese quails (Coturnix coturnix japonica) aged 13 weeks and with uniform body weight were housed in cages and randomly allocated to nine treatments. Duration of the study was 6 weeks and quails were reared from 13 to 19 weeks of age. Each treatment comprised of 4 replicates of 12 birds (9 females and 3 males). Quails house was provided with programmable lighting and ventilation. Feed and water were provided ad libitum.

Experimental diets
The ingredients and nutrient composition of the experimental diets are shown in Tables 1 and 2.

All of the diets were formulated based on National Research Council (NRC, 1994) recommended levels for nutrients. The experimental birds were fed with three levels of metabolizable energy (ME) (11.51, 12.41 and 12.77 MJ/kg diet) and crude protein (CP) (180, 200 and 220 g/kg diet) in a 3 x 3 factorial design. A diet containing 12.41 MJ ME and 240 g CP/kg was used for growth period of the resulted offspring (Table 2).

Performance and egg production
Body weight of the quails were measured at the beginning and end of the experiment. Feed intake (FI) was measured weekly. Eggs were collected daily and egg production was calculated on a bird-day basis. Feed conversion ratio (FCR) was calculated as g of feed intake for g of egg production. Egg mass was calculated by multiplying egg production to average egg weights for each replicate.

Egg quality
Random samples of 12 eggs from each treatment were collected biweekly to measure egg quality parameters. Individual eggs were weighed and broken. Egg shell strength (kg/cm²) was measured by egg shell strength tester (Ogawa Seiki Co., Tokyo, Japan). Shell thickness was a mean value of measurements at three places on the egg (air cell, equator and sharp end) measured by using an egg shell thickness meter (Ogawa Seiki Co., Tokyo, Japan). The shape index (100 times the ratio of width to length of an egg) was determined by measuring the length and width of the egg using Vernier calipers (Cd-15cpx., Kawasaki. Mitutoyo, Japan). Egg shell was broken and the albumen and yolk height was measured with spherometer (Ogawa Seiki Co., Tokyo, Japan), whereas, the length and width of albumen and yolk was measured using Vernier calipers. The albumen index (the ratio of average albumen height to the average of the width and length) and yolk index (the ratio of yolk height to its average width) were determined as described by Heiman and Carver (1936). Haugh units were calculated from the records of albumen height and egg weight using the formula:

\[ HU= 100 \log_{10}(H-1.7 W^{0.37}+7.56) \]

Where:
- HU: Haugh unit.
- H: height of the albumen (mm).
- W: egg weight (g).

Fertility and hatchability
Random samples of 720 eggs (80 per treatment) were collected biweekly and stored in a storage room at 16 °C and 60% relative humidity.
Eggs had been stored for up to 6 d when set. Before setting the eggs into incubator cracked eggs, thin shells, dirty and abnormal eggs in size or shape, were eliminated and incubated at 37.9 °C and 65% RH in a commercial incubator with automatic egg turning. These eggs were labeled individually and placed in plastic trays, which were set randomly in the incubator. On day 14, egg turning was stopped, and eggs were transferred to the hatchery. At the end of the incubation period the hatched chicks moved to rearing house while non-hatched.
eggs were broken and examined for fertility. Fertility (FERT) and hatchability of total incubated eggs (HTE) were calculated as follows:

\[
\text{FERT} = \left( \frac{\text{number of fertilized eggs}}{\text{total number of eggs placed into incubator}} \right) \times 100
\]

\[
\text{HTE} = \left( \frac{\text{number of hatched chicks}}{\text{total number of eggs placed in incubator}} \right) \times 100
\]

**Rearing the resulted offspring**

The newly hatched chicks were transferred to rearing house. The temperature of the house was 37 °C and it was reduced weekly by 3 °C until 24 °C. Weighing of birds was performed weekly from hatch to 28 days with a 0.01 g sensitive electronic scale. Feed, but not water, was removed 6 h prior to weighing. A diet containing 12.41 MJ ME and 240 g CP/kg was used *ad libitum*. Illumination was 24 h/d during rearing period. The ingredients and nutrient composition of the used experimental diets for growth period are shown in Table 2.

**Statistical analysis**

The experiment was conducted as a completely randomized design with 3 × 3 factorial arrangement with 3 levels of ME (11.51, 12.41 and 12.77 MJ/kg) and three levels of CP (180, 200 and 220 g/kg CP). The obtained data were subjected to statistical analysis using the general linear model (GLM) procedure of the SAS software (SAS, 2001). Before statistical analysis, univariate procedure of SAS was also used to test the normality of data. Output data are given as means with standard error. Significant differences among the means of treatments were determined by using Tukey test.

**RESULTS AND DISCUSSION**

**Performance and egg production**

The performance characteristics of quails fed with different energy and protein levels are depicted in Table 3. The results showed that the body weight, feed intake, feed conversion ratio, egg mass and egg production were affected by the interaction between ME and CP levels (P<0.05). Body weight, egg production, feed intake and egg mass were higher in quails fed with high ME and CP diet. The FCR improved with the increase of dietary energy level, and the best FCR was achieved from the diet with 12.77 MJ ME/kg (P<0.05).

When hens first begin laying eggs, during the early laying period, they are still growing. A part of the energy and protein of dietary are used for growth and this may affect the performance.

Growth of the organism as an extreme form of growth, demands an intensive supply of amino acids, especially during fattening. Because of increased needs of proteins, the liver increases the synthesis of serum proteins, which results in the growth of their concentration in blood. Fluctuations in concentrations of individual protein fractions, considering their function, can also be related to the rapid increase in body mass (Filipovic *et al.*, 2007). Protein deficiency might have impaired the development of the reproductive system (Soares *et al.*, 2003). Studies by Junqueira *et al.* (2006) showed that performance and egg production is not affected by dietary energy and protein levels. On the other hand, some reports showed that there were no significant differences in body weight of laying birds fed diets differing in energy and protein contents (Abdel-Azeem, 2011). The higher feed intake with the increase in CP level is well corroborated with the earlier observations of Kaur *et al.* (2006a) and Kaur *et al.* (2008). The reason for higher feed intake with the increase in dietary CP level is to compensate energy requirement for growth (Mosaad and Iben, 2009; Perez-Bonilla *et al.*, 2012). Underscoring the various nutrient levels in commercial laying hens, the ratio of feed to egg is an indicator of feed utilizable efficiency, reflecting the digestion and absorption of feed and nutritional balance. Feed consumption per 1 kg of eggs was the lowest if the diet with 17% crude protein was applied (Tarasewicz *et al.*, 2006a). According to the reports of Tarasewicz *et al.* (2006b) the energy: protein ratio and amino-acids level had a stronger influence on performance than the protein level alone. Pinto *et al.* (2002) found that diet containing 2850 kcal ME/kg was the best for Japanese quails to produce higher egg mass which in agreement with the results of the current study. Wu *et al.* (2005) reported that increase of dietary energy and other nutrients (amino acids, Ca, and available P) resulted to more improvement in egg mass and feed conversion than increasing just in dietary energy. Egg production increased with the increase in dietary protein which is in consistent with the previous report of Soares *et al.* (2003).

**Egg quality**

The egg quality parameters showed significant variations as seen in Table 4. For the interaction effects between dietary ME and CP, the effects on egg weight were significant (P<0.01). There was a linear increase in egg weight with the increase in ME and CP levels. The shape index of eggs across dietary treatments was relatively similar (P=0.05). But, increasing energy and protein levels resulted in an increase in egg shell thickness, egg shell strength, albumen index and yolk index (P<0.01). Haugh unit score did not differ between dietary treatments.
### Table 3: Effects of dietary metabolizable energy (ME, MJ/kg) and crude protein (CP, g/kg) levels on performance of laying Japanese quails during 13-19 weeks of age

<table>
<thead>
<tr>
<th>Dietary treatments</th>
<th>ME (MJ/kg)</th>
<th>CP (g/kg)</th>
<th>BW change (g/bird)</th>
<th>FI (g/d)</th>
<th>FCR (g feed/g egg)</th>
<th>Egg mass (g egg/bird/d)</th>
<th>Egg production (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>11.51</td>
<td>180</td>
<td>-13.01z</td>
<td>29.07*</td>
<td>3.26*</td>
<td>8.94*</td>
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<tr>
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<td>11.51</td>
<td>200</td>
<td>2.78*</td>
<td>29.97*</td>
<td>3.35*</td>
<td>8.97*</td>
<td>72.09*</td>
</tr>
<tr>
<td></td>
<td>11.51</td>
<td>220</td>
<td>4.09*</td>
<td>32.07*</td>
<td>3.27*</td>
<td>10.01**</td>
<td>78.74*</td>
</tr>
<tr>
<td></td>
<td>12.41</td>
<td>180</td>
<td>-4.08z</td>
<td>32.25*</td>
<td>3.29*</td>
<td>10.23*</td>
<td>79.99**</td>
</tr>
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<td>200</td>
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<td>34.01*</td>
<td>2.66*</td>
<td>11.08*</td>
<td>83.01*</td>
</tr>
<tr>
<td></td>
<td>12.41</td>
<td>220</td>
<td>3.69*</td>
<td>33.91*</td>
<td>2.76*</td>
<td>11.18*</td>
<td>84.12*</td>
</tr>
<tr>
<td></td>
<td>12.77</td>
<td>180</td>
<td>5.48*</td>
<td>31.57*</td>
<td>3.18*</td>
<td>11.02*</td>
<td>82.19*</td>
</tr>
<tr>
<td></td>
<td>12.77</td>
<td>200</td>
<td>6.50*</td>
<td>34.25*</td>
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<td>11.12*</td>
<td>84.03*</td>
</tr>
<tr>
<td></td>
<td>12.77</td>
<td>220</td>
<td>9.61*</td>
<td>33.00*</td>
<td>2.71*</td>
<td>11.49*</td>
<td>86.32*</td>
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</table>

**Main effects**

<table>
<thead>
<tr>
<th>ME (MJ/kg)</th>
<th>CP (g/kg)</th>
<th>BW: body weight; FI: feed intake and FCR: feed conversion ratio. The means within the same column with at least one common letter, do not have significant difference (P&gt;0.05). SEM: standard error of the means.</th>
</tr>
</thead>
</table>

### Table 4: Effects of dietary metabolizable energy (ME, MJ/kg) and crude protein (CP, g/kg) levels on eggs quality in Japanese quails

<table>
<thead>
<tr>
<th>Dietary treatments</th>
<th>ME (MJ/kg)</th>
<th>CP (g/kg)</th>
<th>Egg weight (g)</th>
<th>Shape index</th>
<th>Egg shell thickness (mm)</th>
<th>Egg shell strength (kg/cm²)</th>
<th>Haugh unit</th>
<th>Albumen index</th>
<th>Yolk index</th>
</tr>
</thead>
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<tr>
<td></td>
<td>11.51</td>
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<td>76.90</td>
<td>0.240x</td>
<td>1.45*</td>
<td>90.12</td>
<td>0.110x</td>
<td>0.469*</td>
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<td>11.51</td>
<td>200</td>
<td>12.87w</td>
<td>77.23</td>
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<td>1.57*</td>
<td>90.74</td>
<td>0.116x</td>
<td>0.472*</td>
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<td>13.88x</td>
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<td>0.470*</td>
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<tr>
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<td>12.41</td>
<td>180</td>
<td>11.29x</td>
<td>76.97</td>
<td>0.240x</td>
<td>1.49*</td>
<td>90.82</td>
<td>0.124*</td>
<td>0.481*</td>
</tr>
<tr>
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<td>12.41</td>
<td>200</td>
<td>13.67x</td>
<td>78.19</td>
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<td>1.61*</td>
<td>91.27</td>
<td>0.127*</td>
<td>0.473*</td>
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<tr>
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<td>12.41</td>
<td>220</td>
<td>13.97x</td>
<td>78.21</td>
<td>0.229x</td>
<td>1.36**</td>
<td>91.63</td>
<td>0.119*</td>
<td>0.479*</td>
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<tr>
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<td>12.77</td>
<td>180</td>
<td>12.20x</td>
<td>77.39</td>
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<td>1.45*</td>
<td>90.82</td>
<td>0.117*</td>
<td>0.473*</td>
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<td>78.67</td>
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<td>1.33**</td>
<td>91.31</td>
<td>0.115*</td>
<td>0.480*</td>
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**Main effects**

<table>
<thead>
<tr>
<th>ME (MJ/kg)</th>
<th>CP (g/kg)</th>
<th>Egg weight (g)</th>
<th>Shape index</th>
<th>Egg shell thickness (mm)</th>
<th>Egg shell strength (kg/cm²)</th>
<th>Haugh unit</th>
<th>Albumen index</th>
<th>Yolk index</th>
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<td>11.51</td>
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**SEM**

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<th>ME (MJ/kg)</th>
<th>CP (g/kg)</th>
<th>Egg weight (g)</th>
<th>Shape index</th>
<th>Egg shell thickness (mm)</th>
<th>Egg shell strength (kg/cm²)</th>
<th>Haugh unit</th>
<th>Albumen index</th>
<th>Yolk index</th>
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<td>0.01</td>
<td>0.04</td>
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</table>

**Probability**

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<th>Energy</th>
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<th>0.015</th>
<th>0.041</th>
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<th>0.019</th>
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<tbody>
<tr>
<td>Protein</td>
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<td>0.076</td>
<td>0.127</td>
<td>0.270</td>
<td>0.037</td>
</tr>
<tr>
<td>Energy x protein</td>
<td>0.004</td>
<td>0.001</td>
<td>0.039</td>
<td>0.042</td>
<td>0.019</td>
</tr>
</tbody>
</table>

The means within the same column with at least one common letter, do not have significant difference (P>0.05). SEM: standard error of the means.
However, there was a linear but not significant increase in Haugh unit in response to protein levels (P<0.05).

Usually, the average egg weights are increasing with the increase in dietary protein levels (Chen et al. 1999) which is in agreement with the results of this study. Soares et al. (2003) reported that egg size depends greatly on daily crude protein intake, since layers do not store large amounts of protein. Dietary energy regulates the egg weight through influencing egg yolk weight, which is mediated by a mechanism of estrogen synthesis and metabolism. Estrogen has some important effects on metabolism. Estrogen increases adiposity in poultry. For instance, synthetic estrogens increase adipose tissue in chickens. Further, estrogens are responsible for the dramatic increase in the hepatic synthesis of the yolk lipoproteins (Wu et al. 2005). In the groups of quails that were fed more energetic feeds, significantly higher egg weights were achieved. Tarasewicz et al. (2006a), found a trend of the egg weight to increase under growing dietary protein level and a trend to diminish the weight of egg when the energy content increased. Gunawardana et al. (2008) reported that dietary energy had no influence on egg weight. The effect of dietary protein on egg weight in this study is consistent with Wu et al. (2005), and Sohail et al. (2003), who reported that egg weights of hens with high protein diets were greater than of those hens with low protein diets, which is a consequence of the increase in albumen and yolk weights. Shafer et al. (1998) also indicated that increasing amino acid (lysine and total sulfur amino acids) intake had a significant effect on albumen weight. However, Sehu et al. (2005) did not find any significant effects for dietary energy and protein on eggs quality. The increase of albumen weight might be partially attributed to the improved nutrient (protein, total sulfur amino acids, Ca, and available P) utilization with increased dietary energy. Increasing dietary nutrients (energy, amino acids, Ca, and available P) significantly increased yolk and albumen weights at the same time, resulting in a significant increase in egg weights during early egg production. However, Wu et al. (2007) reported that the nutrients density had no significant effect on shell weight, percentage of yolk, percentage of albumen and percentage of shell. Increasing dietary protein significantly decreased percentage of egg shell. This was due to a significant increase of egg weight as protein level increased (Gunawardana et al. 2008). In contrast, Hassan et al. (2000) reported no significant effect for dietary protein on egg shell thickness. Tollba and El-Nagar (2008) found a significant increase in Haugh unit and shell thickness by increasing dietary protein level.

According to Murakami et al. (1993) egg shell percentage and egg shell thickness were not affected by feeding Japanese quails on diets containing 2500, 2700, 2900 or 3100 ME kcal/kg and 16, 18, 20 or 22% CP.

On the other hand, Novak et al. (2006) reported that shell quality was affected by protein and its ratio in diet, which could potentially have serious consequences for commercial egg-laying operations. The shell protein matrix is comprised of 70% protein. Also, increasing the sulfate groups existing in the shell matrix significantly increases the Ca binding ability, which in turn may increase both shell percentage and specific gravity and overall shell quality.

**Fertility, hatchability and offspring growth rate**

Statistical analysis indicated significant differences between dietary treatments, due to energy but not protein and the ME and CP interaction, in percentage of fertility and subsequent offspring live body weight at the age of 21 days (Table 5). There was a linear increase of hatchability, and subsequent offspring live body weight at hatching, 7, 14 and 21 days of age with the increase in ME and CP.

Fertility and hatchability are parameters of reproductive performance that are most sensitive to genetic and environmental influences. Factors affecting fertility and hatchability of produced eggs are nutrition, storage conditions and its duration, bird strain, egg quality and mating ratio. Diet mainly affects the number and size of eggs rather than their composition (Hassan et al. 2005; Kingori et al. 2010). Donald et al. (2002) reported that hatchability of fertile broiler eggs may be related to the weight of the eggs. Effects of CP and ME levels on hatchability and embryo development is confounded. Insufficient dietary protein and poorly balanced protein will reduce egg production and have negative effect on the hatchability of eggs (Barker, 2003). Marie et al. (2009) found that the high-energy diet fed hens had significantly higher egg fertility than those fed with the lower energy levels which is consistent with our results. This reduction in hatchability appeared to be the result of increased embryonic mortality in mid-incubation. Vanemous et al. (2015) showed that high crude protein diet improved hatchability of the eggs of broiler breeders. However, relatively severe reductions of protein and energy intake during the growing period of broiler did not affect subsequent fertility or hatchability (Fattori et al. 1991).

Furthermore, different dietary energy and protein levels in broiler breeder feeding programs during the grower period did not affect on egg fertility, hatchability (percentage of fertile eggs), embryonic mortality or offspring chick body weights (Hussein, et al. 2010). The chicken hatch weight is closely correlated with egg weight. Chicks hatched from large eggs were heavier than those hatched from comparatively smaller eggs. It is possible that the decreased hatch weight of eggs produced by low-protein hens can be a result of lower egg weights. The nutritional state of the bird dramatically affects endocrine status of chickens (Rosebrough et al. 2005).
Chicken embryo develops independent of the mother and all the nutritive substances, especially protein content in diet (Kingori et al. 2010). Embryonic growth is largely affected by regular signals including hormones contained in the egg. The yolk sac envelops the yolk and produces enzymes and transporters that help to mobilize and transport the nutrients from the yolk to the developing embryo (Powell et al. 2004). The hypothalamus is one of the most susceptible targets for fetal programming induced by maternal nutrition (Symonds et al. 2007). The chicken embryo is structurally completed by 14 d of incubation (Moran, 2007), and most of the neuroendocrine regulatory networks in the hypothalamus establish and start to function about this time (Degroef et al. 2008).

In a comprehensive research, Rao et al. (2009) speculated that the profiles of hypothalamic expression of genes involved in metabolic and energy homeostasis regulation may be modified by maternal protein restriction at this stage of chicken embryonic development. Generally, in quails and broiler chickens, protein content of feed affects the birds’ growth, mostly in the first stage of life (Ocak and Erener, 2005) which is confirmed with the results of current study. In this study, body weights of chicks at different age (except at hatching) are significantly affected by dietary energy levels which is in agreement with those obtained by Hasanien (1995). In the same study, this effect was interpreted as evidence that the offspring of calorie-restricted mothers were more likely to be ill compared with the offspring of control ones (Grindstaff et al. 2005). In contrast, birds on protein-restricted diets fed ad libitum may be able to overcome protein deficiencies by increasing total food intake and protein production. Previous research suggests that both egg size and egg contents have important consequences for offspring fitness (Styrsky et al. 1999; Milos, 2009).

**CONCLUSION**

In the current study, the productive and reproductive performance of laying Japanese quails are affected by energy and protein levels, especially at high dietary levels. Since the nutritional status of maternal quails showed significant effects on growth capacity the offspring and their subsequent development, fortification of quail breeder diets with nutrients, especially protein and energy, is suggested.

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