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Original research article

Effects of metabolizable energy and crude protein levels on laying performance, egg quality and serum biochemical indices of Fengda-1 layers

Yang Ding, Xingchen Bu, Nannan Zhang, Lanlan Li, Xiaoting Zou*

Key Laboratory of Animal Nutrition and Feed Science, Ministry of Agriculture, Zhejiang Provincial Laboratory of Feed and Animal Nutrition, Feed Science Institute, Zhejiang University, Hangzhou 310058, China

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ABSTRACT

This study was conducted to investigate the effects of dietary ME and CP levels on laying performance, egg quality and serum biochemical indices of Fengda-1 layers. In a 2 × 3 factorial arrangement, 2,400 Fengda-1 layers (32 wk of age) were randomly assigned to 6 experimental diets with 2,650 and 2,750 kcal of ME/kg of diet, each containing 14.50%, 15.00% and 15.50% CP, respectively. Each dietary treatment was replicated 5 times, and feed and water were provided *ad libitum*. The trial lasted for 10 wk, including a 2-week acclimation period and an 8-week experimental period. Our results showed that ADFI decreased as the ME level of diet increased from 2,650 to 2,750 kcal/kg ($P < 0.05$). Layers fed diets with 2,750 kcal/kg ME exhibited higher mortality than those fed with 2,650 kcal/kg ME ($P < 0.05$). Birds fed with 14.50% and 15.00% CP had higher egg production (EP) and egg mass (EM) than those fed with 15.50% CP ($P < 0.05$). Yolk color increased as the ME level of the diet increased from 2,650 to 2,750 kcal/kg, however, the eggshell thickness decreased ($P < 0.05$). Serum concentrations of uric acid and triglyceride in layers fed diets with 2,750 kcal/kg ME were higher than those fed diets with 2,650 kcal/kg ME ($P < 0.05$). There was no significant interaction between ME and CP on laying performance, egg quality, or serum biochemical indices ($P > 0.05$). Based on the data under the experimental conditions, the optimal dietary ME and CP levels of Fengda-1 layers are 2,650 kcal/kg and 15.00% (33 to 41 wk of age).

1. Introduction

"Fengda-1" layer, cultivated independently by Anhui Rongda Poultry Development Co., Ltd (Xuancheng, China), is an excellent layer breed. It has already been promoted to large-scale market and shown superior economic profits with broad market prospects for its outstanding performance including strong disease-resistance ability, high egg production rate, good egg quality, etc. However, the nutrient requirements for this kind of birds were formulated according to the nutrient recommendation of other layers. Therefore, studies on nutritional requirements and for evaluating practical levels of nutrients in diets of Fengda-1 layers have become indispensable. The ME and CP levels, which should be firstly considered when the diets are formulated, were 2 major nutritional parameters for evaluating feed nutrition value. The ME and CP levels for layers recommended by the NRC were usually for ideal management and environmental conditions (NRC, 1994). Previous studies had found that dietary ME and CP levels had significant influences on laying performance and product quality (Gunawardana et al., 2008; Li et al., 2013; Nahashon et al., 2007).

Li et al. (2013) conducted an experiment with 4 dietary ME levels (2,400, 2,550, 2,700, and 2,850 kcal/kg) and 3 CP levels (14.5%, 16.0%, and 17.5%), obtaining that the moderate ME and high CP were optimum for the egg production (EP), egg mass (EM) and feed convention ratio (FCR) of Lohmann Brown laying hens. Junqueira et al. (2006) evaluated laying performance based on ME (2,850, 2,950, and 3,050 kcal/kg) and CP (16%, 18%, and 20%), which showed that diets with 2,850 kcal/kg of ME and 16% CP for semi

* Corresponding author.
E-mail address: xtzou@zju.edu.cn (X. Zou).

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heavy hens were adequate for satisfactory performance and egg quality. Nahashon et al. (2007) conducted an experiment, which suggested that diets composed of 2,800 kcal/kg of ME and 14% CP were utilized with more efficiency by the Pearl Gray guinea fowl laying hens at 26 to 50 wk and 62 to 86 wk of age. Reports about the requirements of dietary ME and CP were inconsistent, which may be due to the difference of experimental conditions, strains, bird age, lay period and evaluation index.

Although many studies have been carried out to evaluate the effects of dietary ME and CP on layers, there is a lack of information in relation to Fengda-1 layers. Therefore, the objective of this study was to determine the effects of dietary ME and CP on laying performance, egg quality, and serum biochemical indices of Fengda-1 layers (33 to 41 wk of age).

2. Materials and methods

The experiment was conducted in accordance with the Chinese guidelines for animal welfare and was approved by the Animal Welfare Committee of Animal Science College of Zhejiang University (Hangzhou, China).

2.1. Experimental diets

Hens were fed the experimental diets based on maize and soybean meal. The contents of calcium (Ca), phytate phosphorus trace minerals, and vitamins were identical among the 6 diets. The ingredients and nutrient composition of the experimental diets are shown in Table 1.

2.2. Birds and housing

Two thousand and four hundred 32-week-old Fengda-1 layers with similar performance were obtained from Anhui Rongda Poultry Development Co., Ltd (Xuancheng, China). They were randomly assigned to 6 dietary treatments with 2 ME levels (2,650 and 2,750 kcal/kg) and 3 CP levels (14.50%, 15.00%, and 15.50%) in a factorial arrangement. Each treatment group contained 400 birds, and each group consisted of 5 replicates of 80 birds (4 birds/cage). Each cage (45 cm × 45 cm × 50 cm) was equipped with 2 nipple drinkers and 1 feeder. Cages were located in a ventilated room with temperature between 18 and 27°C, relatively humidity between 60% and 70% and 16 h/d of illumination (10 to 20 lx). Diets in mash form were offered for *ad libitum* intake. Birds had free access to water throughout the entire experimental period. The feeding trial lasted 10 wk included a 2-wk acclimation period and an 8-wk experimental period.

2.3. Laying performance

Feed intake (FI) was determined weekly by subtracting the ending feed weight of each replicate from the beginning feed weight. Egg production, egg weight, and cracked eggs were recorded daily. Mortality was determined daily so that the feed consumption could be adjusted accordingly. Eight hens from each replicate were weighed individually at the beginning and at the end of the experiment. Based on these data, hen-day egg production, egg weight (EW), EM, ADFI, FCR, broken egg rate (BER) and ADG were calculated. The EM was calculated as: EM = EW × EP. The FCR was the ratio of FI to EM.

2.4. Egg quality

Thirty eggs (6 eggs from each replicate) were randomly collected to assess egg quality parameters. The eggs were weighed and cracked. Albumen height, Haugh unit, yolk color, eggshell were measured with a digital egg tester. Eggshell thickness (without the eggshell membrane) was measured at 3 sites (blunt, middle, and sharp) of the egg, and the mean of the 3 parts was calculated.

Table 1
Ingredients and nutrient composition of the experimental diet (as fed basis).

Item	ME, kcal/kg/CP, %					
	2,650/14.50	2,650/15.00	2,650/15.50	2,750/14.50	2,750/15.00	2,750/15.50
Ingredient, %						
Corn	62.52	62.52	62.45	66.50	64.75	63.35
Soybean meal	18.90	21.10	22.70	20.10	21.92	23.35
Wheat bran	6.70	5.20	3.75	1.15	1.00	0.76
Limestone	9.40	8.93	9.20	9.40	9.40	9.40
Soybean oil	0.99	0.74	0.54	1.30	1.50	1.70
$CaHPO_4$	0.55	0.68	0.58	0.63	0.59	0.66
NaCl	0.35	0.35	0.35	0.35	0.35	0.35
Trace elements premix[1]	0.20	0.20	0.20	0.20	0.20	0.20
Vitamin premix[2]	0.03	0.03	0.03	0.03	0.03	0.03
DL-Met	0.12	0.10	0.10	0.12	0.11	0.10
L-Lys·HCl	0.20	0.14	0.10	0.19	0.15	0.10
L-Thr	0.04	0.01	–	0.03	–	–
Nutrient level, %[3]						
ME, kcal/kg[4]	2,650 (2,645)	2,650 (2,652)	2,650 (2,642)	2,750 (2,751)	2,750 (2,755)	2,750 (2,760)
CP[4]	14.50 (14.52)	15.00 (15.13)	15.50 (15.59)	14.50 (14.47)	15.00 (15.10)	15.51 (15.58)
Calcium	3.50	3.40	3.44	3.51	3.51	3.53
Total P	0.44	0.46	0.44	0.42	0.42	0.43
Linoleic acid	1.58	1.56	1.55	1.58	1.55	1.52
Methionine	0.34	0.33	0.33	0.34	0.33	0.33
Lysine	0.85	0.86	0.85	0.85	0.86	0.85
Threonine	0.58	0.57	0.58	0.57	0.57	0.58
Tryptophan	0.16	0.17	0.17	0.16	0.17	0.17

[1] The trace elements premix provided the following per kg of diet: Fe, 80 mg; Zn, 120 mg; Cu, 10 mg; Mn, 60 mg; I, 1 mg; Se, 0.4 mg; Co, 0.2 mg.
[2] The vitamin premix provided the following per kg of diet: vitamin A, 12,000 IU; vitamin D_3, 3,750 IU; vitamin E, 30 mg; vitamin K_3, 2.4 mg; vitamin B_1, 1.8 mg; vitamin B_2, 7.5 mg; vitamin B_6, 4.5 mg; vitamin B_{12}, 0.02 mg; biotin, 0.15 mg; D-Pantothenic acid, 15 mg; folic acid, 1.35 mg; niacin, 48 mg; antioxidant, 0.15 mg.
[3] Nutrient levels were calculated from data provided by Feed Database in China (2013).
[4] Value in parentheses was the analyzed value.

2.5. Blood samples

At the end of the feeding study, blood samples were collected from 10 hens (2 birds/replicate). The whole blood was put aside for approximately 20 min, and then centrifuged at 3,000 × g for 10 min for serum at room temperature. Pure serum samples were aspirated by pipette and stored in 1.5-mL Eppendorf tubes at −80°C. They were thawed at 4°C before analysis. Serum concentrations of total protein (TP), albumin (ALB), uric acid (UA), triglyceride (TG), and total cholesterol (T-CHO) were measured spectrophotometrically (UV-2000, UNICCO Instruments Co. Ltd., Shanghai, China) using commercial kits (Nanjing Jiancheng Bioengineering Institute, Nanjing, China).

2.6. Statistical analyses

Data were presented as means ± SE and analyzed by using analysis of variance (ANOVA) one-way test SPSS software, version 18.0 (SPSS Inc., Chicago, IL, US), When significant differences were found ($P < 0.05$), Duncan's tests were performed.

3. Results

3.1. Laying performance

As shown in Table 2, feed intake decreased by 3.45% when the ME level of the diet increased from 2,650 to 2,750 kcal/kg ($P < 0.05$). Layers on 2,750 kcal/kg ME and 15.50% CP diets exhibited higher mortality than those on 2,650 kcal/kg ME and 15.00% CP diets ($P < 0.05$). The EP, EW, FCR, EM, BER, or ADG were not affected by the ME levels ($P > 0.05$).

The EP was 2.36% and 2.06% higher in layers fed diets containing 14.50% and 15.00% CP when compared with those fed 15.50% CP diets, respectively ($P < 0.05$). Higher EM in layers fed the 15.00% CP than those fed the 15.50% CP diets ($P < 0.05$). The ADFI, EW, FCR, BER, mortality, or ADG were not affected by the CP levels ($P > 0.05$).

There was no significant interaction among evaluated factors ($P > 0.05$).

3.2. Egg quality

As shown in Table 3, yolk color increased as the ME level of the diet increased from 2,650 to 2,750 kcal/kg ($P < 0.05$). When the ME level of the diet increased from 2,650 to 2,750 kcal/kg, eggshell thickness decreased by 5.41% ($P < 0.05$).

None of parameters related to egg quality was significantly affected by the interaction between the levels of ME and CP or by the CP levels ($P > 0.05$). However, the yolk color tended to increase with the increasing CP levels ($P = 0.087$).

3.3. Serum concentrations of TP, albumin, uric acid, triglyceride and T-CHO

As shown in Table 4, it was found that dietary ME and CP level had no effect on the serum concentrations of TP, albumin, or T-CHO ($P > 0.05$). However, the serum concentrations of uric acid and triglyceride in layers fed with 2,750 kcal/kg ME diets were higher than those fed with 2,650 kcal/kg ME diets ($P < 0.05$).

4. Discussion

According to Golian and Maurice (1992) and Leeson et al. (1993), birds consume feed to primarily meet their energy requirement. In the present study, an increase in the ME level of the diet from 2,650 to 2,750 kcal/kg decreased FI by 3.45%, which was consistent with previous studies (Perez-Bonilla et al., 2012; Sohail et al., 2003; Wu et al., 2005). Contrary to these findings, Grobas et al. (1999a,b) and Nahashon et al. (2006) reported that dietary ME do not affect the FI. These conflicting results may be attributed to the different experimental conditions or lay period. The EP was not affected by ME level in this trial. Similar results have been reported by Han and Thacker (2011), Harms et al. (2000), Wu et al. (2007), Parsons et al. (1993), and Sell et al. (1987). In contrast, in White Leghorn layers fed diets varying from 2,350 to 2,600 kcal/kg ME, and in Brown hens fed diets varying from 2,650 to 2,850 kcal/kg ME, detecting significant differences in EP (Rama Rao et al., 2011; Perez-Bonilla et al., 2012). The lack of significant difference in EP between layers on 2,650 and those on 2,750 kcal of ME/kg of diet in this study may be due to the narrow range of the 2 ME levels (100 kcal of ME/kg of diet) when compared with the dietary ME range of the 2 dietary treatments (250 kcal of ME/kg of diet) in the report of Rama Rao et al. (2011). Nevertheless, EP increased as the ME level increased from 2,650 to 2,850 kcal/kg, but an increase to 2,950 kcal/kg did not result in any further improvement reported by Perez-Bonilla et al. (2012). These data supported the hypothesis that an excess in energy intake results primarily in increases in

Table 2
Effect of dietary ME and CP levels on laying performance of Fengda-1 layers.

Item		EP, %	ADFI, g/d	EW, g	FCR, g/g	EM, g/d	BER, %	Mortality, %	ADG, g/d
ME, kcal/kg	CP, %								
2,650	14.50	81.13 ± 0.93[a]	100.13 ± 0.99[a]	52.11 ± 0.14	2.37 ± 0.04	42.49 ± 0.65[ab]	0.13 ± 0.05	0.90 ± 0.38[ab]	0.75 ± 0.31
2,650	15.00	82.25 ± 1.15[a]	96.82 ± 4.90[ab]	52.27 ± 0.27	2.25 ± 1.20	42.99 ± 0.42[a]	0.11 ± 0.06	0.50 ± 0.47[b]	0.72 ± 0.46
2,650	15.50	80.07 ± 1.04[b]	98.01 ± 4.40[a]	52.14 ± 0.22	2.35 ± 0.09	41.75 ± 0.60[ab]	0.16 ± 0.04	0.70 ± 0.41[ab]	0.99 ± 0.55
2,750	14.50	82.50 ± 1.50[a]	95.86 ± 3.78[ab]	51.87 ± 0.39	2.24 ± 0.10	42.79 ± 1.05[ab]	0.15 ± 0.03	1.10 ± 0.38[ab]	0.85 ± 0.49
2,750	15.00	80.42 ± 1.48[b]	97.20 ± 5.29[a]	52.36 ± 0.28	2.31 ± 0.09	42.11 ± 0.88[ab]	0.15 ± 0.04	0.85 ± 0.49[ab]	0.83 ± 0.55
2,750	15.50	79.34 ± 1.63[b]	91.71 ± 0.79[b]	51.97 ± 0.43	2.23 ± 0.07	41.66 ± 1.27[b]	0.13 ± 0.06	1.15 ± 0.43[a]	0.88 ± 0.54
ME, kcal/kg									
2,650		81.15 ± 1.34	98.32 ± 3.83[a]	52.17 ± 0.21	2.32 ± 0.10	42.41 ± 0.74	0.13 ± 0.05	0.70 ± 0.42[b]	0.82 ± 0.47
2,750		80.53 ± 1.83	94.93 ± 4.25[b]	52.06 ± 0.41	2.26 ± 0.09	42.19 ± 1.11	0.14 ± 0.04	1.03 ± 0.42[a]	0.85 ± 0.48
CP, %									
14.50		81.58 ± 1.24[a]	98.00 ± 3.44	51.99 ± 0.30	2.31 ± 0.10	42.64 ± 0.84[a]	0.14 ± 0.04	1.00 ± 0.37	0.80 ± 0.39
15.00		81.34 ± 1.58[a]	97.01 ± 4.81	52.31 ± 0.27	2.28 ± 0.11	42.55 ± 0.80[a]	0.13 ± 0.06	0.68 ± 0.49	0.78 ± 0.48
15.50		79.70 ± 1.34[b]	94.86 ± 4.46	52.05 ± 0.33	2.29 ± 0.10	41.70 ± 0.94[b]	0.14 ± 0.05	0.93 ± 0.46	0.94 ± 0.54
ANOVA (P-value)									
ME		0.300	0.022	0.343	0.060	0.484	0.650	0.042	0.788
CP		0.007	0.192	0.057	0.816	0.042	0.895	0.223	0.492
ME × CP		0.079	0.156	0.441	0.056	0.313	0.279	0.805	0.610

EP = egg production; EW = egg weight; EM = egg mass; BER = broken egg rate.
[a,b]Within the same column, values with no or same superscripts differ not significantly ($P > 0.05$), whereas values with different superscripts differ significantly ($P < 0.05$).

Table 3
Effect of dietary ME and CP levels on egg quality of Fengda-1 layers.

Item		Albumin height, mm	Yolk color	Haugh unit	Eggshell strength, kg/cm^3	Eggshell thickness, mm
ME, kcal/kg	CP, %					
2,650	14.50	5.40 ± 0.85	11.35 ± 0.14^b	75.45 ± 5.84	4.52 ± 0.36	0.36 ± 0.01^a
2,650	15.00	5.12 ± 0.16	11.30 ± 0.45^b	72.17 ± 2.13	4.19 ± 0.31	0.37 ± 0.02^a
2,650	15.50	5.07 ± 0.71	11.25 ± 0.61^b	73.03 ± 4.62	4.47 ± 0.45	0.37 ± 0.01^a
2,750	14.50	4.98 ± 0.53	11.40 ± 0.33^b	70.57 ± 4.07	4.43 ± 0.17	0.36 ± 0.01^a
2,750	15.00	5.25 ± 0.61	12.11 ± 0.19^a	72.99 ± 4.92	4.22 ± 0.51	0.34 ± 0.01^b
2,750	15.50	5.40 ± 0.42	11.35 ± 0.28^b	73.80 ± 2.82	4.40 ± 0.39	0.36 ± 0.01^{ab}
ME, kcal/kg						
2,650		5.20 ± 0.62	11.30 ± 0.39^b	73.44 ± 4.18	4.39 ± 0.38	0.37 ± 0.01^a
2,750		5.21 ± 0.52	11.54 ± 0.42^a	72.46 ± 3.99	4.35 ± 0.37	0.35 ± 0.01^b
CP, %						
14.50		5.19 ± 0.70	11.27 ± 0.27	72.74 ± 5.26	4.48 ± 0.27	0.36 ± 0.01
15.00		5.18 ± 0.42	11.70 ± 0.59	72.58 ± 3.60	4.21 ± 0.40	0.35 ± 0.02
15.50		5.24 ± 0.58	11.27 ± 0.48	73.46 ± 3.48	4.43 ± 0.40	0.36 ± 0.01
ANOVA (*P*-value)						
ME		0.963	0.038	0.495	0.764	0.018
CP		0.977	0.087	0.910	0.259	0.286
ME × CP		0.353	0.090	0.266	0.928	0.218

[a,b]Within the same column, values with no or same superscripts differ not significantly ($P > 0.05$), whereas values with different superscripts differ significantly ($P < 0.05$).

body weight gain rather than in further increases in egg mass production. In addition, low FI (94.93 g/d) also contributed to the result because of the lower intake of other nutrients except ME and CP.

Results of Jalal et al. (2006) and Valkonen et al. (2008) found that differences in EW of the layers fed diets with different ME levels were not significant, which were in agreement with our result. The effects of increasing the ME level of diet on EW might depend on the fat and linoleic acid contents of the diets (Jensen et al., 1958). Our result may be due to the linoleic acid levels in the diets of this trial (above 1.50%), who reported that layers require no more than 1.15% linoleic acid in the diet to maximize EW (Grobas et al., 1999a,b). In this study, increasing dietary ME level had no significant effect on the EM and FCR, but tended to decrease the FCR. This might be due to the layers fed the high ME diet (2750 kcal/kg) had lower FI but higher energy intake than layers fed the other diets, but the excess of energy was not used for improvement in EM. Conflicting results reported by Nahashon et al. (2007) and Gunawardana et al. (2008) may be attributed to the bird age, diet, and different experimental conditions.

High levels of ME (2,750 kcal/kg) resulted in higher mortality in our study, which exceeded our expectation. This result was inconsistent with those of other studies, in which diets with high ME level did not increase the mortality (Gunawardana et al., 2008; Yuan et al., 2009). In the present study, the fatty liver symptom was observed when the dead layers that were fed 2,750 kcal/kg ME diet were necropsied. Therefore, it still suggested that ME should not exceed 2,750 kcal/kg in consideration of health. Excessive caloric consumption may lead to increased ADG associated with fatness, and as a result, reduced EP of layers (Rosenboim et al., 1999). Although not statistically significant, the ADG in the high ME group (2,750 kcal/kg) was 3.7% higher than those in the low ME group (2,650 kcal/kg). We speculated that birds fed diets with much energy may cause more fat deposited, which was reflected in ADG. Negative correlations between fatness and egg production have been reported (Richards et al., 2003). Therefore, the higher ADG of birds could lead to the lower EP.

The EP were 2.36% and 2.06% higher in birds fed with 14.50% and 15.00% CP than those fed 15.50% CP diets, respectively. Because the 14.50% and 15.00% CP diets seem to be adequate for the Fengda-1

Table 4
Effect of dietary ME and CP levels on serum concentrations of TP, albumin, uric acid, triglyceride and T-CHO of Fengda-1 layers.

Item		TP, g/L	ALB, g/L	UA, mg/L	TG, mmol/L	T-CHO, mmol/L
ME, kcal/kg	CP, %					
2,650	14.50	43.73 ± 3.67	29.14 ± 1.89	38.49 ± 9.26^b	3.69 ± 0.42^{ab}	2.54 ± 0.31
2,650	15.00	46.68 ± 6.24	29.87 ± 0.56	43.37 ± 8.32^{ab}	3.58 ± 0.26^b	2.69 ± 0.62
2,650	15.50	46.32 ± 4.40	30.77 ± 1.72	41.38 ± 8.26^b	3.72 ± 0.19^{ab}	2.82 ± 0.43
2,750	14.50	41.53 ± 6.12	29.79 ± 1.32	46.56 ± 4.62^{ab}	3.97 ± 0.47^{ab}	2.89 ± 0.20
2,750	15.00	43.59 ± 4.68	30.12 ± 1.45	47.35 ± 3.98^{ab}	4.08 ± 0.26^a	2.67 ± 0.29
2,750	15.50	43.82 ± 4.03	30.37 ± 1.34	49.43 ± 3.19^a	3.86 ± 0.21^{ab}	2.86 ± 0.13
ME, kcal/kg						
2,650		45.58 ± 4.20	29.90 ± 1.68	41.08 ± 8.18^b	3.66 ± 0.27^b	2.68 ± 0.45
2,750		42.98 ± 4.64	30.38 ± 1.41	47.78 ± 3.05^a	3.97 ± 0.30^a	2.81 ± 0.23
CP, %						
14.50		42.63 ± 5.02	29.44 ± 1.92	42.53 ± 8.90	3.83 ± 0.45	2.72 ± 0.29
15.00		45.13 ± 5.24	30.47 ± 1.30	45.36 ± 7.33	3.83 ± 0.23	2.68 ± 0.33
15.50		45.07 ± 4.89	30.56 ± 1.49	45.41 ± 6.82	3.79 ± 0.20	2.84 ± 0.42
ANOVA (*P*-value)						
ME		0.076	0.391	0.032	0.017	0.372
CP		0.328	0.172	0.415	0.169	0.358
ME × CP		0.403	0.513	0.613	0.732	0.473

TP = total protein; ALB = albumin; UA = uric acid; TG = triglyceride; T-CHO = total cholesterol.
[a,b]Within the same column, values with no or same superscripts differ not significantly ($P > 0.05$), whereas values with different superscripts differ significantly ($P < 0.05$).

layers, and the decreased production performance of the birds on 15.50% CP diets may be due to the increasing expenditure of energy in catabolism of excess dietary amino acids. However, previous studies (Gunawardana et al., 2008; Liu et al., 2005; Keshavarz and Nakajima, 1995) that were contrary to this report have shown that the EP increased due to increasing dietary CP levels although the FI was not affected by these dietary changes. The EM was significantly affected by dietary CP level in the study, which was consistent with the result of Novak et al. (2006). Birds fed the 15.00% CP diet had higher EM compared with those fed 15.50% CP diet, which may be due to the higher EP and EW based on the calculation of EM. Consequently, diets containing 14.50% and 15.00% CP might be more effective in improving egg mass production. Protein utilization, which was essential in EP, would therefore be highly dependent on this notion. In the present study, the 2,650 kcal of ME/kg of diet may have optimal energy to protein ratio for better utilization of dietary ME and CP by the Fengda-1 layers. The energy to protein ratio of the diets composed of 2,650 kcal/kg ME and 14.50% or 15.00% CP was 183 and 177. Although not statistically significant, EP and EM of layers in low ME and medium CP group (2,650 kcal/kg; 15.00%) were higher than those of layers in the low ME and high CP group (2,650 kcal/kg; 15.50%), this may attributed to that birds consuming diets with energy to protein ratio of 177 would consume more protein than those fed diets with energy to protein ration of 183 in this trial. There were no ME $\times$ CP interaction effects on laying performance, which were consistent with the previous studies (Novak et al., 2008; Wu et al., 2005; Gunawardana et al., 2008).

Haugh unit and the albumen height were important indices concerning internal egg characteristics to measure the viscosity of the thick albumen (Roberts, 2004). Based on the calculation of Haugh units, the albumen height was usually converted into Haugh units. However, Haugh units were also influenced by the age, stain of bird or storage (Naber, 1979). The albumen height and Haugh unit were not affected by dietary ME and CP levels in our study. Junqueira et al. (2006) and Sehu et al. (2005) compared diets of different ME and CP levels on the egg quality of layers, and they also did not find any effects on the Haugh units. Yolk color had a considerable influence in egg marketing. The study of factors affecting the intensity of yolk color was therefore of economic significance for egg producers. In the studies of dietary energy and protein requirements of layers, yolk color was commonly an easily changed parameter. Xanthophyll was the key factor that controls the yolk color, and it could be changed in accordance with the corn percentage of the diet (Karunajeewa, 1972). In the present study, yolk color was significantly enhanced in the high dietary ME level group. However, yolk color was not intensified in the high protein group, which were fed the same amount of corn as in the medium protein group. This result was consistent with previous reports (Gunawardana et al., 2008; Karunajeewa, 1972; Wu et al., 2007). Accordingly, we suggested that change of corn percentages in the layers' diet had potential influence on yolk color. Eggshell strength and eggshell thickness were two important indicators for reflecting eggshell quality. Eggshell strength ultimately affected the soundness of the shell, and weaker shelled eggs were more prone to have cracks and breakages followed by subsequent microbial contamination. This study showed that with increasing ME levels of the diet, the eggshell thickness was significantly decreased. The reason for this result may be related to reduce FI (including Ca) with increased supplemented fat.

Total protein and albumin concentrations were indicators for the protein status of the blood. Uric acid was produced as the main end product of N metabolism in poultry, and the level of uric acid in sera directly reflect protein catabolism of the body. Serum uric acid concentration increased with increasing dietary ME levels, indicating that the expenditure of energy was used for the metabolism of protein. Serum triglyceride and T-CHO concentrations could reflect the absorption and metabolism of lipids. Layers fed diet with 15.00% CP and 2,750 kcal/kg ME had higher serum triglyceride concentration compared with those fed diet with 15.00% CP and 2,650 kcal/kg ME in our study. This result may be due to the expenditure of energy that was used for lipid synthesis when the dietary ME met the demand of growth and production of layers.

5. Conclusion

The FI and eggshell thickness decreased and mortality increased with increasing ME level of diet from 2,650 to 2,750 kcal/kg. Diets containing 14.50% and 15.00% CP increased EP and EM, compared with diets containing 15.50% CP.

Based on the data under the experimental conditions, diet containing 2,650 kcal/kg ME and 15.00% CP produced better laying performance and egg quality in Fengda-1 layers from 33 to 41 wk of age.

Conflict of interest statement

The authors declare that they have no competing interests.

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