



Effect of Dietary Metabolic Energy and Crude Protein on Productive Performance, Reproductive Hormones and Biochemical Indices of Blue Peacock (*Pavo Cristatus*)

■ Author(s)

Dongzhi Mⁱ  <https://orcid.org/0000-0002-3679-9892>
Chi Zⁱ  <https://orcid.org/0000-0002-6705-9432>
Ying Wⁱ  <https://orcid.org/0000-0002-8515-5559>
Haiming Yⁱ  <https://orcid.org/0000-0003-0171-4649>
Yongjian Gⁱⁱ  <https://orcid.org/0009-0001-7328-5583>
Yuzhong Yⁱⁱⁱ  <https://orcid.org/0009-0001-7476-3583>
Yafeng Yⁱⁱⁱ  <https://orcid.org/0009-0006-8016-3185>

- ⁱ College of Animal Science and Technology, Yangzhou University, Yangzhou 225009, China.
ⁱⁱ Jiangsu Institute of Agricultural Sciences along the Yangtze River, Nantong 226000, China.
ⁱⁱⁱ Nantong Fengzhiling Agricultural Development Co, Nantong 226000, China.

■ Mail Address

Corresponding author e-mail address
Wang Ying
88 Daxue South Road, Yangzhou City, Jiangsu Province, 225009, China.
Phone: +051487979045
Email: dkwangying@yzu.edu.cn

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ABSTRACT

In Experiment one, a total of 60 blue peacocks were randomly assigned into the control group (12.00 MJ/kg) and treatment group (12.30 MJ/kg). Each group consisted of three repeats and each replicate contained 10 peacocks (2 males and 8 females). In Experiment two, based on better metabolic energy (ME) (12.30 MJ/kg), 90 blue peacocks were randomly assigned to three dietary treatments with 18.00%, 19.00% and 20.00% crude protein (CP) levels. The results of Experiment one revealed that the different dietary ME levels had minor effect on productive performance of peacocks ($p>0.05$). Decreasing dietary ME levels decreased the concentration of estradiol (E_2) and increased the concentrations of follicle stimulating hormone (FSH) and luteinizing hormone (LH) ($p<0.01$). However, 12.00 MJ/kg ME stimulated creatinine levels of blue peacock, which exceeded normal values. Based on this result, blue peacocks fed with 12.30 MJ/kg ME had better performance. Results from Experiment two indicated that egg production of blue peacocks was significantly improved by the 20.00% CP diet. The concentrations of FSH, LH, and E_2 remained at high levels fed with the 20.00% CP diet. Changes in serum biochemical indices such as total cholesterol, creatinine and globulin of peacocks supplied with 18.00% and 19.00% CP diets were abnormal ($p<0.05$). Based on the results of two experiments, the optimal dietary ME and CP levels of blue peacocks were 12.30 MJ/kg ME and 20.00% CP.

INTRODUCTION

Blue peacocks, well known for their exquisite crest, plumage and train, are distributed worldwide, and highly prized as ornamental birds (Kumar *et al.*, 2017). However, apart from the wild, they are usually found as park and zoo exhibits or are raised for breeding and conservation purposes due to their scarcity (Jackson, 2006). Previous researchers reported that dietary metabolic energy (ME) and crude protein (CP) levels had significant influences on the laying performance of laying hens. (Junqueira *et al.*, 2006; Li *et al.*, 2013). Sakurai (1981) showed that diets with 12.55 MJ/kg of ME and 24% CP for Japanese quails were adequate for satisfactory laying performance. Yu *et al.* (2009) concluded that the laying hens fed organic diet of 11.72 MJ ME/kg and 16% CP showed superior egg production than those of other dietary regimens. Reports about the requirements of dietary ME and CP were inconsistent due to the difference of breeds and strains. However, few reports were available on the effects of dietary energy and crude protein concentrations on the productive performance of blue peacocks.

Currently, the nutrient requirements for birds were formulated according to the nutrient recommendation of chickens. Therefore, studies on nutritional requirements and for evaluating practical levels



of nutrients in diets of blue peacock have become indispensable. Thus, the objectives of the experiments were to study the effects of dietary ME and CP levels on the productive performance, reproductive hormones and biochemical indices of Blue Peacock, with a view to being able to increase their numbers and contribute to their conservation. The basis for the conservation of endangered birds like the green peacock would even come from research focused on the blue peacock.

MATERIALS AND METHODS

Animals and diets

The experiment was approved by the Animal Welfare Committee of Yangzhou University (permit number SYXK [Su] 2016-0020) and followed the Chinese Animal Welfare Guidelines.

A total of 150 blue peacocks with similar performance were selected from Nantong Fengzhiling Agricultural Development Co., Ltd. (Nantong, China). The peacocks were raised in accordance with local farming practices.

Each column (5 m × 5 m) was equipped with 2 nipple drinkers and 1 feeder. Columns were located in a ventilated room with temperature between 18 and 27 °C, relatively humidity between 60% and 70%. Blue peacocks were fed once in the morning and once in the evening. Water was provided ad libitum.

Experiment one: a total of 60 blue peacocks were randomly assigned into two groups with different ME (Table 1), control group (C, 12.00 MJ/kg) and treatment group (T, 12.30 MJ/kg). Each group consisted of three repeats and each replicate contained 10 peacocks (two males and eight females).

Experiment two: based on better ME (12.30 MJ/kg), 90 blue peacocks randomly assigned to three dietary treatments with 18.00%, 19.00%, and 20.00% CP levels (T1, T2, T3) in a factorial arrangement (Table 1). Each group consisted of three repeats and each replicate contained 10 peacocks (two males and eight females).

Productive performance

The blue peafowls fasted for 12 hours were weighed before and after the experiment. The intake of the peacocks in each group was recorded, and the eggs were collected every day. After picking up the eggs, the weekly egg production was calculated, and the hatching rate, fertilization rate and egg shape index were calculated and recorded. Poultry embryonic development depended on the nutrients that were

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

Ingredient	Treatment			
	C	T1	T2	T(T3)
Ingredients, %				
Corn	54.00	60.00	58.00	56.80
Soybean meal	25.20	21.00	23.30	25.00
Fish meal	6.50	5.50	5.70	6.50
Rice bran	3.30	4.00	3.50	2.80
Wheat bran	3.50	2.00	2.50	2.20
Stone powder	2.00	2.00	1.50	1.20
Calcium	0.70	0.70	0.70	0.70
Soybean oil	2.20	2.20	2.20	2.20
Salt	0.50	0.50	0.50	0.50
Methionine	0.10	0.10	0.10	0.10
50% Choline	0.50	0.50	0.50	0.50
Trace elements	1.00	1.00	1.00	1.00
Vitamin	0.50	0.50	0.50	0.50
ME, MJ/kg	12.00 (12.08)	12.30 (12.30)	12.30 (12.27)	12.30 (12.29)
CP	20.00 (20.11)	18.00 (18.05)	19.00 (19.00)	20.00 (19.97)
Calcium ¹	1.29	1.24	1.07	1.00
Total P	0.74	0.70	0.71	0.72
N-Phy-P	0.47	0.44	0.45	0.47
Lysine	1.15	1.00	1.06	1.14
Methionine	0.45	0.42	0.43	0.45
Cystine	0.30	0.27	0.29	0.30

¹The contents of calcium (Ca), phosphorus(P), minerals and vitamins were identical among the 4 diets.

available in the egg (Han *et al.* 2023). The eggs must be sterilized and heated before being placed in the brooder. The temperature was kept between 37.5 and 38 °C, while the relative humidity was between 60% and 70%.

Hormones analysis

At 10 a.m. on the final day of the feeding study (Experiment one and two), 4 mL blood samples were collected from female peacocks, and each set of blood was taken simultaneously. Blood was centrifuged at 3000 r/min for 10 min and stored at -20°C. The concentrations of luteinizing hormone (LH), follicle stimulating hormone (FSH) and estradiol (E₂) of peacocks were determined by enzyme linked immunosorbent assay (ELISA) according to the manufacturer's protocol. The ELISA kits of LH, FSH and E₂ were purchased from Nanjing Jiancheng Bioengineering Institute (Nanjing, China), and each concentration is represented as ng/L, IU/L, µg/L in plasma, respectively. The lowest limits of LH, FSH and E₂ detections were 0.625 ng/L, 0.1875 IU/L and 18.75 µg/L. Intra-assay and inter-assay coefficients of variation were lower than 9.00% and 15.00% in turn.



Biochemical indexes

Glucose (GLU), creatinine (Cr), total protein (TP), albumin (ALB), urea, triglyceride (TG), and total cholesterol (TC) of peafowl serum were measured by spectrophotometrically (UV-2000, UNICCO Instruments Co. Ltd., Shanghai, China) using commercial kits (Nanjing Jiancheng Bioengineering Institute, Nanjing, China) and strictly following the instructions. The normal biochemical indexes range specified by the respective kits were used as reference (Ding *et al.*, 2016).

Statistical analysis

Data were analyzed by SPSS 13.0 version. The differences between groups were determined by One-way ANOVA. When the differences were significant,

LSD test was performed. Data were expressed as means \pm SD.

RESULTS

The results of Experiment One

Productive performance and biochemical indices of blue peacocks fed with different ME levels

As shown in Table 2, when the ME levels of the diet increased from 12.00 to 12.30 MJ/kg, the egg production, fertilization rate and hatching rate of peacocks tended to increase, however, there were no significant differences ($p>0.05$). The dietary ME had minor effect on FI, BW, ESI ($p>0.05$). As shown in

Table 2 – Effect of dietary ME levels on productive performance of blue peacocks².

Item ¹	FI(kg)	BW (kg)	ESI(mm)	Egg production (pcs)	Fertilization rate (%)	Hatching rate (%)
C	7.097 \pm 0.011	3.042 \pm 0.025	1.419 \pm 0.002	110.333 \pm 0.577	81.570 \pm 0.006	94.452 \pm 0.010
T	7.100 \pm 0.003	2.999 \pm 0.024	1.415 \pm 0.004	110.667 \pm 2.517	82.235 \pm 0.007	95.241 \pm 0.006
<i>p</i> -value	0.659	0.104	0.179	0.834	0.290	0.320

¹FI = feed intake; BW = body weight; ESI = egg shape index, ESI = vertical diameter / horizontal diameter; pcs = pieces; Fertilization rate = number of fertilized eggs / number of hatched eggs \times 100%; Hatching rate = number of hatched eggs / number of hatched eggs \times 100%.

²Values marked with different letters on the bars are significantly different ($p<0.05$), values marked with capital letters on the bars are extremely significantly different ($p<0.01$). The same as below.

Table 3, serum Urea, TG, TC and Cr decreased as the ME levels of the diet increased from 12.00 to 12.30 MJ/kg ($p<0.01$). However, when the ME levels of the diet increased from 12.00 MJ/kg to 12.30 MJ/kg, contents of serum TP, ALB, GLb and GLU increased ($p<0.01$).

Reproductive hormones of blue peacocks fed with different ME levels

The comparative analysis of the effect of dietary ME levels on reproductive hormones of blue peacock was shown in Figure 1. The serum FSH and LH concentrations were higher in peacocks fed with 12.00 MJ/kg ME than

those fed with 12.30 MJ/kg ME, respectively ($p<0.01$). On the contrary, higher E_2 concentrations were detected in peacocks fed with 12.30 MJ/kg ME than those fed with 12.00 MJ/kg ME ($p<0.01$).

As the results showed, the egg production tended to increase with the increasing ME levels. 12.00 MJ/kg ME diet caused Cr to exceed normal levels (Brugere-Picoux *et al.* 2015). Furthermore, the E_2 level in peacocks fed with 12.30 MJ/kg were higher than those fed with 12.00 MJ/kg, which was closely related to follicular development. In conclusion, 12.30 MJ/kg diet improved the productive performance of blue peacocks.

Table 3 – Effect of dietary ME levels on biochemical indices of blue peacocks.

Item ¹	Urea (mmol/L)	TG (mmol/L)	TC (mmol/L)	GLU (mmol/L)	Cr (mmol/L)	TP (g/L)	ALB (g/L)	GLb (g/L)
C	0.64 \pm 0.09 ^A	8.60 \pm 1.04 ^A	3.60 \pm 0.50 ^A	5.04 \pm 1.04 ^B	45.98 \pm 4.39 ^A	41.29 \pm 3.72 ^B	11.01 \pm 2.28 ^B	30.28 \pm 4.01 ^B
T	0.41 \pm 0.08 ^B	5.74 \pm 0.95 ^B	2.30 \pm 0.53 ^B	7.61 \pm 1.32 ^A	36.98 \pm 3.11 ^B	54.96 \pm 4.56 ^A	16.94 \pm 2.05 ^A	38.01 \pm 3.62 ^A
<i>p</i> -value	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.01

¹TG=triglyceride; TC = total cholesterol; GLU = Glucose; Cr = creatinine; TP = total protein; ALB = albumin; GLb = TP – ALB.

The results of Experiment Two

Productive performance and biochemical indices of blue peacocks fed with different CP levels

As shown in Table 4, there were no significant differences in FI, BW, or incubation rate under different

CP levels. ($p>0.05$). ESI and egg production were significantly affected by dietary CP levels ($p<0.05$). Egg production in 20.00% CP group was significantly higher than that in 19.00% and 18.00% groups ($p<0.05$). As shown in Table 5, the concentrations of Urea, TG, TC and Cr fed with 18.00% CP were higher than those fed with 19.00% and 20.00% CP



Table 4 – Effect of dietary CP levels on productive performance of blue peacocks.

Item	FI (kg)	BW (kg)	ESI (mm)	Egg production (pcs)	Fertilization rate (%)	Hatching rate (%)
T1	7.105±0.006	2.981±0.027	1.408±0.001 ^b	119.670±0.577 ^b	80.497±0.018	93.787±0.009
T2	7.109±0.005	3.013±0.017	1.423±0.006 ^a	120.330±3.215 ^b	83.962±0.017	94.696±0.038
T3	7.100±0.003	2.998±0.024	1.415±0.004 ^b	126.000±2.646 ^a	83.872±0.009	94.962±0.010
p-value	0.162	0.323	0.014	0.035	0.051	0.815

Table 5 – Effect of dietary CP levels on biochemical indexes of blue peacocks.

Item	Urea mmol/L	TG mmol/L	TC mmol/L	GLU mmol/L	Cr mmol/L	TP g/L	ALB g/L	GLb g/L
T1	0.53±0.07 ^a	7.07±1.35 ^a	2.90±0.61 ^a	6.37±1.27 ^c	41.17±4.35 ^a	48.36±6.07 ^c	14.13±2.19 ^c	34.23±4.96 ^b
T2	0.31±0.12 ^c	4.54±1.19 ^c	1.81±0.32 ^c	8.92±0.78 ^a	33.54±5.25 ^b	62.97±3.80 ^a	20.03±3.33 ^a	42.93±3.60 ^a
T3	0.41±0.08 ^b	5.74±0.95 ^b	2.30±0.52 ^b	7.61±1.32 ^b	36.98±3.10 ^b	54.96±4.56 ^b	16.94±2.05 ^b	38.01±3.63 ^b
p-value	0.00	0.00	0.00	0.00	0.03	0.00	0.00	0.01

diets ($p < 0.05$), on the contrary, TP, GLU, ALB and GLb fed with 19.00% CP were lower than those fed with 18.00% and 20.00% CP diets ($p < 0.05$).

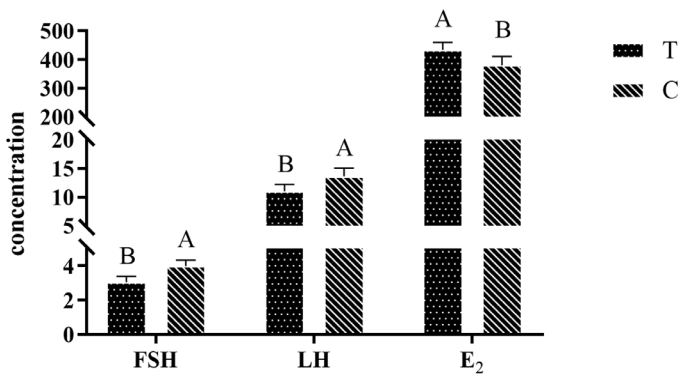


Figure 1 – Effect of dietary ME levels on hormones of blue peacocks.

Reproductive hormones of blue peacocks fed with different CP levels

As shown in Figure 2, the concentrations of FSH ($p < 0.01$) and LH ($p < 0.05$) in peacocks were higher with 18.00% CP than with 19.00% and 20.00% CP diets. The E₂ level increased when the CP levels of the diet increased from 18.00% to 19.00% ($p < 0.01$) and decreased when the CP levels of the diet increased from 19.00% to 20.00%, but there was no significant difference ($p > 0.05$).

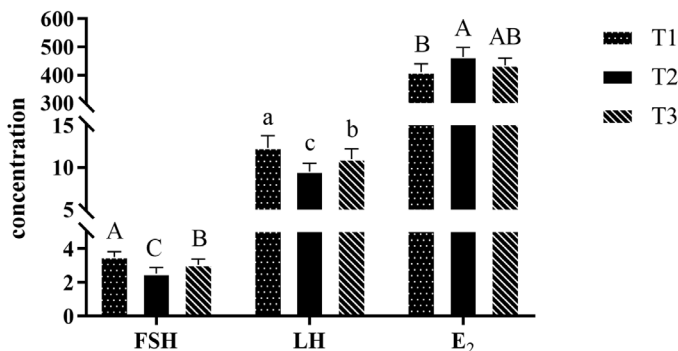


Figure 2 – Effect of dietary CP levels on reproductive hormones of blue peacocks.

DISCUSSION

Birds consume feed to meet their requirements (Golian & Maurice, 1992), dietary ME or CP had minor effect on FI and BW of chickens and ducks (Grobas *et al.*, 1999; Nahashon *et al.*, 2006; Rama Rao *et al.*, 2011; Zeng *et al.*, 2015), which is in accordance with our results. Furthermore, the hatching rate of peacock eggs didn't affect by the ME and CP levels in this study, which was consistent with previous studies in broilers (Attia *et al.*, 1995; Lopez & Leeson, 1995). Gunawardana *et al.* (2008) also found that even if FI was not influenced by dietary changes, egg production increased due to an increase in dietary CP levels (Liu *et al.*, 2005), furthermore, increasing CP levels increased the egg production of peacocks in this study. Different protein-energy ratios showed significant interactions with egg production (Heijmans *et al.*, 2021). When the protein level in the diet increased, so did the protein-energy ratio, and more energy was required to maintain the proper protein-to-energy level. In Experiment one, we found that 12.3 MJ/kg was a better energy level, and based on this, we believed that 12.3 MJ/kg and 20% CP were a better combination than 18% and 19% CP. Retes *et al.* (2019) reported that dietary CP, when deficient, might limit the synthesis of egg components, reducing the number of eggs, suggesting 20.00% CP was optimal to meet the needs of peacocks.

In this study, most parameters were within the range of recommendations except for creatinine, TC and GLb (Brugère-Picoux *et al.*, 2015). We found that high concentration of creatinine in peacocks fed with 12.00 MJ/kg ME and 18.00% CP diets suggested decreased glomerular filtration capacity for creatinine. Serum TC levels reflected the absorption and metabolism of lipids, the TC concentration decreased as CP levels rose, improving the quality of ketone body (Lin *et al.*, 2014), however, 19.00% CP level resulted in abnormal TC



levels in peacocks, which may be due to inappropriate energy-to-protein ratio. Serum GLb level was indicator of the immune function and protein metabolism of the poultry, 19.00% CP significantly increased the serum GLb of hens compared to 14.00% CP (Poosuwan *et al.* 2010), in our study, 19.00% CP also induced the serum GLb concentration in peacock. However, the serum GLb concentration in peacock fed with 20.00% CP was normal, indicating irrational CP might lead to chronic or acute inflammatory reaction (Walton & Siegel, 2022).

Egg production was controlled by the development of ovarian follicles, which in turn were regulated by reproductive hormones, including LH, FSH and E_2 (Wilson, 1978; Wang & Johnson, 1993). Plasma E_2 was significantly lower in 12.00 MJ/kg ME diet than 12.30 MJ/kg ME diet, which was probably responsible for the earlier following follicle appearance due to follicular atresia caused by a shortage of nutrition. The E_2 peak occurred after the peak in gonadotropins, indicating that FSH and LH drive E_2 secretion in follicle development (Imai & Nalbandov, 1978; Renama *et al.*, 1999). Meanwhile, low ME diet could repress the declaration of LH receptor in the ovary, while high energy level diet could advance the outflow of FSH and LH receptor in the ovary (León *et al.*, 2014), which was in accordance with our results, lower ME diet led to higher concentrations of FSH and LH.

In Experiment two, E_2 concentrations were significantly lower in peacocks fed with 18.00% CP diet than other diets, serum E_2 concentration showed a consistent trend with egg production, Reddy *et al.* (2002) also indicated that higher egg production had high serum E_2 concentration. FSH and LH stimulated gonadal development and were regulated in a negative feedback manner by gonadal steroids (Yang *et al.* 2005), the negative trend between the concentrations of FSH, LH and E_2 of peacocks fed with different CP levels also confirmed that E_2 was more effective than other steroids in inhibiting LH secretion (Sharp 1975). Furthermore, small follicles were the main sources of estrogen in domestic fowl (Yu *et al.*, 1992), the higher E_2 levels indicated that the ovaries of the peacocks might have more small follicles in 19.00% and 20.00% CP diets.

CONCLUSION

There is a noticeable trend where higher levels of dietary energy, ranging from 12.00 to 12.30 MJ/kg, lead to an increase in egg production. Diets containing 20.00% CP increased egg production and maintained

better FSH, LH and E_2 levels, compared with diets containing 18.00% and 19.00% CP. Based on the data under the experimental conditions, blue peacocks fed with diet containing 12.30 MJ/kg ME and 20.00% CP had better productive performance.

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DECLARATION OF COMPETING INTERESTS

The authors declare no conflict of interest.

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