Effects of dietary protein and energy levels on growth performances and reproductive system development in female Betong chicken (*Gallus domesticus*) during growing-pullet period

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ABSTRACT: The objective of this study was to investigate the effect of dietary protein and energy levels on growth performances and reproductive system development in female Betong chicken during growing to pullet period (12-20 weeks of age). Total of 180 chickens, 12 weeks of age, were assigned to the 2 x 3 factorials in completely randomized design. This study consisted of two factors i.e. dietary energy levels (2,850 and 3,000 metabolizable energy (ME) kcal/kg) and dietary protein levels (14, 16 and 18% crude protein, CP). The experiment consisted of 6 treatments with 3 replications. All chickens were fed *ad libitum*. Varying dietary protein levels had no significant effect ($P > 0.05$) on growth performances and reproduction system development. However, it was found that chickens received 2,850 ME kcal/kg had significantly higher ($P < 0.05$) body weight gains (581.11 g/bird) than those of 3,000 ME kcal/kg (519.44 g/bird) during 12-20 weeks of age. Moreover, chickens fed with 2,850 ME kcal/kg had significantly better ($P < 0.05$) feed conversion ratios than did 3,000 ME kcal/kg (6.942 and 7.757, respectively). There was no interaction ($P > 0.05$) between protein and energy levels on average body weight, body weight gain, feed intake, feed conversion ratio, ovary weight, oviduct length and oviduct weight of female Betong chicken. There was a significant interaction ($P < 0.05$) between protein and energy levels on number of small size (diameter < 2 mm) of ovarian follicles. Chickens fed with 16% CP and 2,850 ME kcal/kg had a significantly higher ($P < 0.05$) number of small size of ovarian follicles than those of other treatments. There was no significant difference ($P > 0.05$) among other treatments. In conclusion, 14-16% CP and 2,850 ME kcal/kg are recommended for the female Betong chickens during 12-20 weeks of age.
Keywords: Betong chicken, protein, energy, growth performance, reproductive system

Introduction

Betong chicken is the native chicken in the southern region of Thailand, especially Pattani, Yala and Narathiwat provinces. Recently, Betong chickens are increasing in demand and accepted from consumers due to tender meat, good taste, good aroma, high meat quality, low carcass fat, high lean and yellow meat when compared with other Thai native chickens (Gongruttananun and Chotesangasa, 1996; Gongruttananun and Chotesangasa, 2001; Chanjula et al., 2004; Chatreewong and Waree, 2006). Moreover, Gongruttananun and Chotesangasa (1996) reported that the Betong chicken had higher productive performances (body weight and feed conversion ratio) than other Thai native chickens. Although growth performances directly related to the reproductive system development but female Betong chicken is also a disadvantage in low egg production about 13 eggs/bird/hatch or 60 eggs/bird/year and age at first egg is 23 weeks of age (Chanjula et al., 2004). As a result the low eggs hatch rate that affect to a small number of chicken produced.

In general, the nutrient requirements of birds are influenced by genotype and body size (Miah et al., 2014). The dietary protein and energy levels

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improved growth performance meat yield and reproductive system development for chicken. Sompie et al. (2015) reported that the native chickens who were fed varying dietary protein levels in diet had no significant effect ($P > 0.05$) on feed intake, body weight gain and feed conversion ratio, whereas varying dietary energy levels had significant effect ($P < 0.05$) on feed efficiency. However, there was no interaction between dietary protein and energy levels. Magala et al. (2012) suggested that 18% CP and 2,800 ME kcal/kg diet was adequate for growing Ugandan local chicken cockerels. Mohammad and Sohail (2008) demonstrated that 14% CP and 2,750 ME kcal/kg optimized their performances during a growing stage of Pakistan *desi* native chickens.

Nguyen and Bunchasak (2005) found that 19% CP with energy contents between 3,000-3,200 ME kcal/kg were suitable for early growth stage (0-42 days of age). After that Nguyen et al. (2010) reported that 19% CP with energy content of 3,000 ME kcal/kg were suitable for growing period (42-82 days old). Nevertheless, the two studies found that there were no significant interaction between protein and energy levels in diet on growth performance.

In terms of reproductive system, Alli and Ayorinde (2013) suggested that 20% CP and 2,750 ME kcal/kg diet improved reproductive performances such as age at the first egg, egg number, egg mass and hen day production of guinea hen during 20-52 weeks of age. While Bunchasak and Silapasorn (2005) found that increasing methionine in low protein diet improved ovary and oviduct weights of commercial laying hen (Isa-brown) during 24-44 weeks of age.

However, information of nutrient requirements, particularly CP and ME of Betong chicken are limited. Thus, the objective of this study was to investigate the effect of dietary protein and energy levels on growth performance and reproductive system development in the female Betong chicken during growing to pullet period.

Materials and Methods

Animals and managements

The experiment was conducted in accordance with the principles and guidelines approved by the Songkhla Rajabhat University (SKRU) Animal Care and Use committee which followed Guidelines for the Care and Use of Agricultural Animals in Agricultural Research and Teaching, SKRU, Songkhla. The 180 female from Yala Livestock Research and Breeding Center (12 weeks of age) were randomly divided into 6 treatments groups. According to the treatment groups arranged as 2 x 3 factorials in completely randomized design (2 energy levels and 3 protein levels). Each treatment group consisted of 3 replicates of 10 female Betong chickens. The chickens in each treatment were raised in a 1.8 x 3 m^2 pen on a concrete floor, in the indoor house roofed with rice hulls. The photoperiod was 12 h and the temperature was maintained according to conventional rearing practice. Birds were vaccinated for infectious bronchitis and Newcastle disease every 4 weeks throughout the experimental period. The experiment was conducted for a period of 12 weeks during the months of November 2011 to January 2012.

Experimental diets

Six experimental diets were formulated to meet the requirements recommended by NRC (1994), except protein and energy levels. The chemical analysis of the diets was made for CP, crude fiber, ether extract and ash (AOAC, 1998). Feed ingredients and chemical compositions of 6 experimental diets are presented in **Table 1**. The diets consisted of 2 energy levels (2,850 and 3,000 ME kcal/kg) and 3 protein levels (14, 16 and 18% CP). The female Betong chickens were fed *ad libitum* and had access to water throughout the experimental period. The experimental diets (chemical analysis) were a slightly lower CP when compared with the calculated results.

Table 1 Feed ingredients and compositions of the experimental diets (12-20 weeks of age)

Ingredients	----------Treatment Diets (kg) ----------					
	T1	T2	T3	T4	T5	T6
Corn	47	47	47	46	43.2	42
Rice bran	34	31	28	27	28	28
Soybean meal (44% CP)	10.2	11	16.5	16.8	21	21
Fish meal (55% CP)	3	3	3	3	3.5	3.7
Palm oil	0	2.4	0	2.3	0	2.1
Dicalcium phosphate	3.8	3.6	3.6	3	2.3	2
Salt	1	1	0.9	0.9	1	0.6
Premix[1/]	1	1	1	1	1	0.6
Total	**100**	**100**	**100**	**100**	**100**	**100**
Calculated Compositions						
Crude Protein (%)	14	14	16	16	18	18
ME (kcal/kg)	2,850	3,000	2,850	3,000	2,850	3,000
Ca (%)	1.17	1.12	1.13	0.99	0.87	0.81
Available Phosphorus (%)	0.99	0.94	0.94	0.83	0.73	0.68
Methionine + Cystine (%)	0.50	0.53	0.58	0.58	0.58	0.58
Lysine (%)	0.70	0.70	0.83	0.83	0.96	0.97
Proximate Analysis						
Crude Protein (%)	13.70	13.67	15.78	16.10	17.58	18.21
Fat (%)	4.87	5.25	6.36	8.72	6.87	8.87
Fiber (%)	4.39	4.30	4.55	5.11	5.32	5.08
Ash (%)	9.34	9.27	9.19	8.75	8.93	8.30
Moisture (%)	10.08	9.87	9.38	9.30	9.68	9.14

[1/] Premix (/kilogram diet): vitamin A, 12,000 IU; vitamin D3, 3,500 IU; vitamin E, 30 mg; vitamin K3 2.5 mg; vitamin B1 3 mg; vitamin B2 7 mg; vitamin B6 4 mg; vitamin B12 0.015 mg; nicotinic acid 50 mg; biotin 0.5 mg; folic acid 1 mg; pantothenic acid 10 mg; choline chloride 50 mg; Mn 80 mg; Fe 80 mg; Zn 60 mg; Cu 8 mg; I 0.5 mg; Si 0.15 mg

Measurements

Body weight, body weight gain, feed intake, feed conversion ratio were recorded every 2 weeks throughout the experimental period (12-20 weeks of age). At 16 and 20 weeks of age, 1 female Betong chicken per replication of each treatment group was stunned and slaughtered by exsanguination (Chotesangasa et al., 1993). Ovary weight, number of ovarian follicles (size classification according to Waddington et al., 1985); small : diameter < 2 mm, medium : diameter 2-8 mm and large : diameter > 8 mm), oviduct weight and oviduct length were recorded.

Statistical analysis

The data were analyzed by Analysis of Variance (ANOVA) in a 2 x 3 factorial completely randomized design. Significant treatment means were analyzed by using Duncan's New Multiple Range Test (DMRT) according to the method previously described by SAS (1998).

Results and Discussion

Effects of dietary protein and metabolizable energy levels on growth performances

The effects of protein and energy levels on growth performances of female Betong chickens are shown in **Tables 2, 3**. There was not a significant difference (P > 0.05) between chickens fed with different dietary protein levels or different energy levels in body weight at weeks 12, 14, 16, 18 and 20. There was no significant interaction (P > 0.05) between protein and energy levels on average body weight **(Table 2)**. In this study, there was not a significant difference (P > 0.05) between chickens fed with different dietary protein levels in body weight gain, feed intake and FCR **(Table 3)**. Chicken fed with low protein diet (18% CP) tended to have better FCR (7.109) compared with those of 16% CP (7.211) and 14% CP (7.730). These results are in agreement with Nguyen and Bunchasak (2005) who studied in early growing period (0-42 days of age) of Betong chicken (KU line) fed with 17, 19, 21 and 23% CP diets.

However, Nguyen et al. (2010) reported the growing period (42-84 days of age) of Betong chicken (KU line) fed with 21% CP had significantly higher (P<0.05) weight gain and better feed conversion ratios than 15 and 17% CP although the chicken fed with 19% CP was not significant.

Although the difference between feed intake of female Betong chickens fed diets containing 2,850 and 3,000 ME Kcal/kg was not significant (P>0.05), however body weight gain and feed conversion ratio of female Betong chicken fed diet containing 2,850 ME Kcal/kg were better than those fed diet containing 3,000 ME Kcal/kg (P<0.05). Theses result were supported by Dozier et al. (2006) who point that feed conversion decreased linearly with gradient increases in AME content. The levels of dietary energy were negatively correlated with feed intake and feed conversion ratio (Larbier and Leclercq, 1992; Summer and Leeson, 1993). In this study, there was no significant interaction (P > 0.05) between protein and energy levels on average body weight, body weight gain, feed intake and FCR of female Betong chickens during 12-20 weeks of age. According Mohammad and Sohail (2008) who reported there was no interaction between different energy protein ratio on the performances of Desi native chicken during growing phase.

Similarly, Magala et al. (2012) reported there was no significant interaction (P>0.05) between dietary protein and energy on growth performances of Ugandan Local chicken. And agreement with Putsakul et al. (2010) who found that there was no interaction between protein (16, 18 and 20% CP) and energy levels (2,800 and 3,000 kcal/kg) on growth performance during 12-20 weeks of age in female Betong chickens (KU line). They also reported that there was not a significant difference in body weight. The body weight of these chickens which were fed 3 levels of dietary protein were 1,699, 1,774 and 1,725 g/bird, respectively and the 2 levels of dietary energy were 1,741 and 1,724 g/bird, respectively. Moreover, feed conversion ratio of the chickens during 4-20 weeks of age was 4.66.

Nguyen and Bunchasak (2005) found that energy contents (3,000 and 3,200 ME kcal/kg) had no effect on body weight, weight gain, feed intake and FCR of early growing chicken. Moreover, Nguyen et al. (2010) reported that in growing period dietary energy content did not affect feed intake and feed conversion ratio while increasing dietary energy content which led to improved body weight (P < 0.05).

Table 2 Effect of protein and energy levels on body weight (kg) of female Betong chickens at different weeks old

Items		12 wks.	14 wks.	16 wks.	18 wks.	20 wks.
CP (%)						
14		0.969	1.101	1.280	1.431	1.510
16		0.974	1.070	1.260	1.425	1.538
18		0.968	1.117	1.265	1.453	1.513
P-value		0.9391	0.1573	0.8073	0.5902	0.6257
ME (kcal/kg)						
2,850		0.959	1.092	1.267	1.447	1.540
3,000		0.981	1.100	1.270	1.427	1.501
P-value		0.1785	0.6847	0.8991	0.8026	0.1547
ME (kcal/kg) X CP (%)						
2,850	14	0.96 ± 0.03	1.09 ± 0.03	1.28 ± 0.02	1.45 ± 0.03	1.56 ± 0.06
	16	0.96 ± 0.01	1.06 ± 0.06	1.26 ± 0.05	1.46 ± 0.11	1.52 ± 0.02
	18	0.96 ± 0.05	1.12 ± 0.05	1.27 ± 0.08	1.43 ± 0.06	1.53 ± 0.04
3,000	14	0.98 ± 0.03	1.11 ± 0.03	1.28 ± 0.02	1.41 ± 0.04	1.46 ± 0.06
	16	0.99 ± 0.04	1.08 ± 0.05	1.26 ± 0.08	1.39 ± 0.10	1.55 ± 0.08
	18	0.97 ± 0.01	1.11 ± 0.02	1.26 ± 0.04	1.47 ± 0.08	1.49 ± 0.05
P-value		0.781	0.717	0.983	0.500	0.135
SEM[1]		0.020	0.023	0.032	0.044	0.031

[1/] SEM = standard error of the mean

Table 3 Average body weight gain, feed intake and feed conversion ratio (FCR) of Betong chicken fed with different protein and metabolizable energy levels

Items		BWG (g/bird)	Feed Intake (g/bird/d)	FCR
CP (%)				
14		540.83	70.19	7.730
16		564.17	72.62	7.211
18		545.83	69.01	7.109
P-value		0.647	0.609	0.236
ME (kcal/kg)				
2,850		581.11[a]	71.95	6.942[b]
3,000		519.44[b]	69.26	7.757[a]
P-value		0.013	0.380	0.019
ME (kcal/kg) X CP (%)				
2,850	14	0.60 ± 0.02	74.19 ± 1.16	6.89 ± 0.26
	16	0.57 ± 0.01	73.11 ± 3.52	7.21 ± 048
	18	0.57 ± 0.03	68.56 ± 3.67	6.73 ± 0.40
3,000	14	0.48 ± 0.06	66.20 ± 10.45	8.57 ± 1.35
	16	0.56 ± 0.06	72.13 ± 8.76	7.21 ± 0.21
	18	0.52 ± 0.46	69.46 ± 4.80	7.49 ± 0.34
P-value		0.115	0.457	0.116
SEM[1]		0.026	0.002	0.368

[a, b] Means within the same columns between treatments with different superscripts were significantly different ($P < 0.05$)

[1/] SEM = standard error of the mean

Effects of dietary protein and metabolizable energy levels on reproductive system development

The effect of protein and energy levels on reproductive tract development (ovary weight, oviduct weight and oviduct length) of female Betong chicken at 16 and 20 weeks of age are shown in **Table 4**. In fact, study on the effect of protein and energy on poultry reproductive system especially in Thailand was rare. In the present study, the result showed that there were no interactions ($P > 0.05$) between protein and energy levels on ovary weight, oviduct weight or oviduct length. Additionally, there were not a significant difference ($P > 0.05$) between protein levels and energy levels on ovary weight, oviduct weight and oviduct length at 16 and 20 week of age. Except, at 16 weeks old, birds fed with 3,000 ME kcal/kg had significantly higher ($P < 0.05$) ovary weight (0.899 g) than those of 2,850 ME kcal/kg (0.704 g). This result suggested that high energy in diet for female Betong chickens could increase the ovary weight when compared with the low energy. Chotesangasa et al. (1993) studied native chickens and reported that at 20 weeks of age the ovary and oviduct weights were 0.6±0.1 and 0.9±0.4 g/kg body weight, respectively while the average oviduct length was 15.1±2.6 cm. In male chicken, Tadondjou et al. (2013) reported that level of energy had affected on testicular development in local barred chicken of western highlands of Cameroon. The birds fed high energy intake led to precocious testicular

development and testicular hypertrophy. Both male and female Betong chickens should be studied for the effects of environments and other nutrients such as amino acids, vitamins and minerals on growth performances and reproduction.

Table 4 Ovary weight, oviduct weight and oviduct length at 16 and 20 weeks old of Betong chicken fed with different protein and metabolizable energy levels

Items		At 16 weeks old			At 20 weeks old		
		OW (g)	ODW (g)	OL (cm)	OW (g)	ODW (g)	OL (cm)
CP (%)							
14		0.900	4.086	16.000	7.050	8.900	25.300
16		0.717	0.690	9.160	7.590	3.203	16.367
18		0.822	3.842	17.550	30.340	16.765	33.417
p-value		0.216	0.390	0.2169	0.168	0.090	0.158
ME (kcal/kg)							
2,850		0.704[a]	1.630	12.071	6.105	8.862	25.278
3,000		0.899[b]	3.947	16.289	21.190	10.383	24.778
P-value		0.014	0.215	0.1991	0.158	0.745	0.942
ME (kcal/kg) X CP (%)							
2,850	14	0.67 ± 0.04	0.36 ± 0.16	7.05 ± 0.92	3.53 ± 3.70	5.83 ± 5.37	23.03 ± 11.00
	16	0.63 ± 0.10	0.72 ± 0.53	9.70 ± 0.71	1.64 ± 0.79	3.68 ± 3.59	18.97 ± 6.94
	18	0.77 ± 0.19	3.09 ± 2.44	17.00 ± 9.07	11.11 ± 10.11	17.08 ± 8.39	33.83 ± 11.25
3,000	14	1.05 ± 0.15	6.57 ± 7.08	21.97 ± 7.88	10.56 ± 16.49	11.97 ± 15.62	27.57 ± 19.96
	16	0.77 ± 0.12	0.67 ± 0.38	8.80 ± 2.12	13.55 ± 21.98	2.73 ± 2.01	13.77 ± 3.57
	18	0.87 ± 0.12	4.60 ± 3.95	18.10 ± 10.46	39.46 ± 33.83	16.45 ± 14.20	33.00 ± 22.49
P-value		0.237	0.439	0.2213	0.788	0.777	0.840
SEM[1]		0.078	2.190	4.154	10.796	5.587	8.208

[a, b] Means within the same columns between treatments with different superscripts were significantly different ($P < 0.05$)

OW = Ovary weight, ODW = oviduct weight, OL = oviduct length

[1/] SEM = standard error of the mean

The effect of protein and energy levels on ovarian follicle development of Betong chicken at 16 and 20 weeks of age are shown in **Table 5**. The result showed that the large size (>8 mm) of ovarian follicle was not found at week 16. This result suggested that at a young age chicken did not have well-developed ovarian follicles when compared with those at week 20. At both weeks 16 and 20, there were not significant differences ($P < 0.05$) between protein and energy levels on number of ovarian follicles of any sizes. Similarly, birds which received high energy tended to have higher big follicles than those of low energy level. There was interaction ($P < 0.05$) between protein and energy levels on number of small (< 2 mm) ovarian follicles at 20 week of age. The result showed that birds fed with 2,850 ME kcal/kg and 16% CP had significantly higher ($P < 0.05$) number of small ovarian follicles (5.67 ± 3.21) than did other treatments. There were no significant differences ($P > 0.05$) among other treatments. This result suggests that protein and energy levels

could have an influence on ovarian follicle development. Buakeeree and Nualhnuplong (2013) found that at 24 weeks of age Betong chickens fed 16% CP with 2,850 ME kcal/kg raised under photoperiod 8L:16D had significantly higher ($P < 0.05$) number of small (<2 mm) ovarian follicles than those of 12L:12D and 14L:10D, respectively. Moreover, Alli and Ayorinde (2013) reported that there were significantly different ($P < 0.05$) among protein levels (16, 18, 20, 22 and 24% CP) in age at first egg, egg number, egg mass and hen day production for 20-52 weeks of age of guinea hen. However there were not significant differences between dietary energy contents (2,750 and 2,850 ME kcal/kg) on those. There was significant interaction ($P < 0.05$) between protein and energy levels on age at first egg, egg number, egg mass and hen day production.

Table 5 Average number of ovarian follicles (n) at 16 and 20 weeks old of Betong chicken fed with different protein and metabolizable energy levels

Items		At 16 weeks old		At 20 weeks old		
		< 2 mm (n)	2- 8 mm (n)	< 2 mm (n)	2- 8 mm (n)	> 8 mm (n)
CP (%)						
14		2.500	2.833	1.833	10.833	1.500
16		1.167	0.833	2.833	11.5	0.667
18		0.833	1.500	0.167	17.167	4.667
p-value		0.742	0.517	0.114	0.695	0.152
ME (kcal/kg)						
2,850		0.667	1.222	2.556	11.778	1.222
3,000		2.333	2.222	0.667	14.556	3.333
p-value		0.384	0.557	0.073	0.680	0.221
ME (kcal/kg) X CP (%)						
2,850	14	0	0	2.00 ± 3.46^{b}	8.67 ± 3.79	0.67 ± 1.15
	16	0.33 ± 0.58	0.67 ± 1.15	5.67 ± 3.21^{a}	16.33 ± 13.80	0
	18	1.67 ± 2.08	3.00 ± 2.00	0^{b}	10.33 ± 10.50	3.00 ± 4.36
3,000	14	5.00 ± 8.66	5.67 ± 7.23	1.67 ± 1.53^{b}	13.00 ± 16.52	2.33 ± 4.04
	16	2.00 ± 3.46	1.00 ± 1.73	0^{b}	6.67 ± 11.55	1.33 ± 2.31
	18	0	0	0.33 ± 0.58^{b}	24.00 ± 21.00	6.33 ± 5.51
P-value		0.367	0.098	0.050	0.374	0.073
SEM[1]		2.257	1.836	1.179	8.036	2.005

[a, b] Means within the same columns with different superscripts were significantly different ($P < 0.05$).

[1/] SEM = standard error of the mean

Conclusions

The efficiency of protein and energy utilization for female Betong chicken during 12-20 weeks of age is best with 14-16% CP and 2,850 ME kcal/kg. Varying dietary protein did not affect growth performances and reproductive system. While female Betong chicken received 2,850 ME kcal/kg had significantly higher body weight gain and better FCR than 3,000 ME kcal/kg although at 16 weeks old, chickens fed with 3,000 ME kcal/kg had significantly higher ($P<0.05$) ovary weight than 2,850 ME kcal/kg. However, there was interaction ($P<0.05$) between protein and energy levels on number of small (<2 mm) ovarian follicles at 20 weeks of age.

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