Songklanakarin J. Sci. Technol.
41 (6), 1211-1218, Nov. - Dec. 2019

http://www.sjst.psu.ac.th

Original Article

Effect of low-protein diets supplemented with methionine on growth performance, carcass characteristics, and expression of *ANT* and *UCP* genes in the breast muscle of Betong chickens (KU Line)

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Received: 23 February 2018; Revised: 7 June 2018; Accepted: 29 June 2018

Abstract

We investigated the effects of low-protein diets supplemented with methionine on the growth performance of Betong chickens (KU-Line) from 4 to 18 weeks. A total of 192 four-week-old male Betong chickens were divided into three groups: high-protein diet group (high crude protein [CP]), low protein diet group (low CP), and low-protein diet group supplemented with methionine (low CP+Met). We found no significant effect of protein levels on the body weight gain and average daily gain. Betong chickens fed a low-CP diet showed the highest feed intake ($P<0.01$) and protein intake ($P<0.05$), while birds fed the low CP+Met diet had the highest methionine intake ($P<0.05$). The addition of methionine to the low CP diet resulted in a significantly improved feed conversion ratio compared to the low CP and the high CP diet groups ($P<0.01$). Moreover, the feed cost was reduced in the low CP+Met diet compared to the high CP diet ($P>0.05$). At the end of the experiment, two birds per replicate were randomly selected and slaughtered. The carcass weights were not significantly different between the treatments. The abdominal fat yield decreased in the treatment with methionine supplementation ($P<0.05$) at 12 weeks. Breast muscle samples were collected for total RNA extraction. The cDNA was amplified using primers specific for *ANT* and *UCP* gene expression and analyzed using real-time PCR. At 12 weeks, mRNA *ANT* expression was significantly higher in the muscle of birds fed diets supplemented with methionine. The highest *UCP* expression was obtained from the low CP diet group, which also resulted in the worst feed conversion ratio at 18 weeks. Our results indicated that methionine supplementation in low CP diets improved growth performance, reduced feed costs, and influenced the expression of *ANT* and *UCP* in the muscle.

Keywords: adenine nucleotide translocase protein, uncoupling protein, Betong Chicken (KU line), low protein diets, methionine supplementation

1. Introduction

The Betong chicken (KU Line) is a slow-growing chicken and the meat-type strain is popular in the southern region of Thailand. A previous study reported that the Betong chicken required a diet with 17% crude protein (CP) and an energy level of 3,000–3,200 metabolizable energy (ME) Kcal/kg, while high levels of dietary protein (23, 21, and 19% CP) did not alter growth performance at 0–42 days of age (Nguyen & Bunchasak, 2005). Putsakul, Bunchasak, Chomtee, Kao-ian and Sopannarath (2010) also reported that suitable protein and energy levels in the diets for these chickens were 18% CP and 3,000 ME Kcal/ kg at 4–16 weeks of age and 16% CP and 2,800 ME Kcal/ kg at 16–20 weeks of age with males having a higher performance than females. However, the market price per kg of Betong meat is still relatively high due to the low growth rate. Generally, the animals reach the slaughtering phase at around 16−20 weeks.

A low-protein diet is one approach to reduce feed costs, which account for more than 70% of the total pro-

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duction costs. However, reducing the protein concentration in the diet generally decreases growth performance and breast muscle proportion and increases body fat accumulation because of amino acid imbalances. Studies have shown that insufficient amounts of amino acids in diets resulted in imbalanced amino acids in the blood, causing reduced feed intake (Kumar, Gloridoss, Singh, Prabhu, & Suresh, 2016). Thus, amino acid supplementation in a low protein diet is more effective at improving the amino acid balance and the growth performance of chickens (Iyayi, Aderemi, Ladele, & Popoola, 2014).

Methionine is used mainly for protein synthesis for muscle growth and broiler chicken performance (Noori, Farhoomand, & Ebrahzadehim, 2011) and plays a major role in cellular antioxidant mechanisms which require methionine for glutathione (GSH) synthesis (Griffith & Meister, 1985). Moreover, Bottje and Carstens (2009) found that amino acid supplementation in low protein diets improved the feed efficiency (FE) of broiler chickens. Since the growth rate of chickens depends on the FE and muscle accretion, in situations where broilers produce less adenosine triphosphate (ATP) because of a lower efficiency of their mitochondria, the FE and growth are generally low (Bottje, Pumford, Ojano-Dirain, & Iqbal, 2006). Therefore, it is essential to gain a better understanding of how amino acid supplementation, especially with methionine, influences the expression of genes involved in energy production of a slow growing Betong chicken.

In ATP synthesis, adenine nucleotide translocase (ANT) protein and uncoupling protein (UCP) are transporters in the inner mitochondrial membrane. The function of the ANT protein is to transport adenosine diphosphate (ADP) from the cytosol to the mitochondria and move ATP through the inner mitochondrial membrane (Ojano-Dirain, Toyomizu, Wing, Cooper, & Bottje, 2007). Additionally, the UCP decreases ATP synthesis energy to heat production by a leak of protons from the electron transport chain system through the inner membrane, resulting in uncoupled substrate oxidation from phosphorylation of ADP to ATP which leads to the production of heat (Ledesma, Lacoba, & Rial, 2002). Gasparino *et al.* (2014) found that gene expression of ANT proteins is involved in energy production, which is probably associated with the feed efficiency of broiler chickens. Recent studies have also shown that methionine supplementation resulted in a better performance of broiler chickens, and the *UCP* mRNA expression in the breast muscle was suppressed compared to basal diets (Del Vesco, Gasparino, & Grieser, 2014).

In this context, we examined the effects of supplementation of methionine to low protein diets on the performance of male and female Betong chickens and feed cost. In particular, we investigated how the availability of an amino acid (methionine) in the diet affected the expression of *ANT* and *UCP* genes and, consequently, the energy production in the mitochondria.

2. Materials and Methods

2.1 Experimental design and diets

One-day-old Betong chicks from the same hatch were obtained from the Suvanvajokasikit Animal Research Farm, Kasetsart University, Thailand. Feather-sexing the chicks was not done at 1 or 2 days old. The chicks were then raised conventionally over a period of four weeks under the same conditions and fed a basal diet with 18% CP. Subsequently, the animals were sexed based on feather, comb morphology, and body composition. A total of 288 male birds were completely randomized to the treatments (six replicates, with 16 birds per treatment).

The compositions of the experimental diets are shown in Table 1. The diets were based on corn and soybean meal and formulated according to the recommendations of Nguyen and Bunchasak, (2005) and Putsakul *et al.* (2010). Since Betong chickens do not have specifically established amino acid requirements, the amount of methionine supplementation was based on the requirements of broiler chickens according to the National Research Council (1994). The treatments consisted of a basal diet with 18% protein (high CP), a diet with 16% protein (low CP) and a diet with 16% protein and methionine supplementation (low CP+Met) for 4-12 weeks. The subsequent diets were a basal diet with 16% protein (high CP), a diet with 14% protein (low CP) and a diet with 14% protein and methionine supplementation (low CP+Met) at for 12-18 weeks.

2.2 Growth performance and carcass characteristics

To determine growth performance, all birds were weighed every two weeks from the beginning of the experiment. At the end of the 12-week and 18-week periods, 12 chickens (two chickens of average body weight (BW) were selected from each pen) from each treatment were sacrificed. At the slaughterhouse, the chickens were slaughtered and scalded in a hot water bath (60 °C for 45 s). Carcass yield and organs were reported as a percentage of live weight.

2.3 Gene expression analysis with real-time PCR

For the mRNA analysis, 0.2 g of the breast muscle were rapidly excised and stored in an RNA stabilization reagent (RNAlater Solution, Ambion, USA) at –80 °C until extraction of the total RNA (two birds per treatment). All materials were previously treated with RNase inhibitor (DEPC, Sigma-Aldrich, USA). Total RNA was extracted using an RNeasy® Fibrous Tissue Mini Kit (QIAGEN, Germany) according to the manufacturer's protocol. Four hundred nanograms of total RNA obtained from the muscle samples from each treatment were used as a template in the reverse transcription reaction. Thermal cycling was performed in the CFX Connect Real-Time System (BIO-RAD, USA) according to the manufacturer's instructions. The specific primers for *ANT* and *UCP* genes used in the reactions are shown in Table 2. The *GAPDH* gene was determined for normalisation of quantitative real-time PCR analysis and simultaneously quantified during each real-time PCR run, including negative controls (containing no template cDNA). Each sample was analyzed in 4 replicates. The mRNA expression levels of the *ANT* and *UCP* genes were acquired from the value of the threshold cycle (Ct) of real-time PCR relative to *GAPDH* using the comparative Ct method (Livak & Schmittgen, 2001).

2.4 Statistical analysis

Data were evaluated with ANOVA in a completely randomised design. Computations employed the general linear

Table 1. Ingredients and chemical composition (percent on an as fed basis) of experimental diets.

Feed ingredients	4–12 weeks			12–18 weeks		
	High-CP[1]	Low-CP[2]	Low-CP+Met[3]	High-CP[4]	Low-CP[5]	Low-CP+Met[6]
Experimental diets. (g/100g)						
Corn	63.95	70.99	71.52	72.34	77.25	77.26
Soybean 46%	29.73	23.83	23.20	23.49	18.06	17.56
Rice bran oil	2.41	1.23	1.05	0.78	0.30	0.30
Monodicalciumphosphate 21%	1.92	1.95	1.95	1.36	2.76	3.17
Calcium carbonate	1.54	1.57	1.57	1.55	1.27	1.10
Premix, Broiler[7]	0.25	0.25	0.25	0.25	0.25	0.25
Salt	0.18	0.18	0.18	0.14	0.13	0.13
L-Lysine			0.20			0.19
DL-Methionine	0.02		0.08			0.06
Total	100	100	100	100	100	100
Composition analysis (%)						
Metabolisable energy (kcal/kg)	3000	3000	3000	3000	3000	3000
Crude protein	18.00	16.00	16.00	16.00	14.00	14.00
Methionine	0.317	0.276	0.349	0.276	0.251	0.309
Methionine+cysteine	0.600	0.530	0.600	0.531	0.477	0.531
Lysine	0.994	0.804	0.944	0.801	0.666	0.801
Fiber	3.41	3.23	3.20	3.247	3.033	3.003
Fat	5.20	4.18	4.01	3.777	3.385	3.377
Calcium	1.00	1.00	1.00	0.900	1.00	1.00
Total P	0.757	0.742	0.740	0.619	0.889	0.972
Available P	0.450	0.450	0.450	0.350	0.582	0.651
Sodium	0.200	0.200	0.200	0.150	0.150	0.150

[1]18% protein diets. [2]16% protein diets. [3]16% protein with methionine supplementation. [4]16% protein diets. [5]14% protein diets. [6]14% protein with methionine supplementation. [7]Vitamin & mineral premix content; Composition per kg: Vitamin A 4,800 IU, Vitamin D3 1,200 IU, Vitamin E 60 IU, Vitamin K3 0.6 g., Vitamin B1 0.6 g, Vitamin B2 2.2 g, Vitamin B6 0.8 g, Vitamin B12 0.04 g, Nicotinic acid 10 g, Pantothenic acid 4.8 g, Folic acid 0.2 g, Biotin,0.048 g, Mn 32 g, Zn 24 g, Fe 16 g, Cu, 32 g, I 0.2 g, Se 0.04 g, Co 0.04 g.

Table 2. Primer sequences for real-time PCR.

Gene	Sense primer (5'-3')	Antisense primer (5'-3')	Amplicon (bp)	Annealing (°C)	GenBank Accession number
ANT[1]	TATCAGCTGGATGATTGCACAGA	ACATGATATCAGCTCCTTTGCGT	119	65	AB088686
UCP[2]	GGAGTGCGTGGGCGTTGGAGA	CGGTGGATGTGGTGAAGACGC	221	60	AB088685
GAPDH[3]	GAGGGTAGTGAAGGCTGCTG	GGGAAGCAGGACCCTTTGTT	225	65	NM_204305

[1]Adenine nucleotide translocase protein. [2]Uncoupling protein.[3]Glyceraldehyde 3-phosphate dehydrogenase.

models procedure of the Statistical Analysis System (SAS: version 9; SAS Institute Inc., Cary, NC, USA). Pen means were used as the experimental unit for the analysis. Differences among treatments were tested for significance using Duncan's multiple range tests at a significance level of 5%.

3. Results

3.1 Growth performance

Table 3 shows the growth performance of the Betong chickens in all three treatment groups. Food intake (FI) and ME were the highest ($P<0.01$) in the low CP group. Moreover, the crude protein intake (CPI) was the highest ($P<0.05$) in the chickens fed a low CP diet throughout the experiment. Although the FI and CPI were low ($P<0.05$), the chickens fed a low CP+Met diet received the highest level of methionine ($P<0.05$). Based on our results, the low CP+Met diets led to better feed conversion ratio (FCR) (high FE) compared to the low CP diet (low FE) ($P<0.01$). Betong chickens fed the low CP diets had the highest feed cost per gain (FCG) ($P<0.05$) at 12 and 18 weeks. The FCG of birds fed the low CP+Met was lower than the high CP group, but did not quite achieve a significant difference ($P>0.05$).

3.2 Carcass and relative weights of organs

Dressing percentages were calculated by comparisons to the live weights of the chicken (Table 4). There were no significant differences between the groups in the total percentage carcass yield ($P>0.05$). The highest values of abdominal fat percentages were found in the group with low CP diets at 12 weeks ($P<0.05$). The relative weights of breast, wings, drumsticks, tenderloin, and liver were similar between the treatments.

Table 3. Performance of Betong chickens (KU line) at 4-12 and 12-18 weeks.

Items	BW[7]	BWG[8]	ADG[9]	FI[10]	ME/day[11]	CPI[12]	Met-I[13]	FCR[14]	FE[15]	FCG[16]
4-12 weeks										
High-CP[1]	1,461.08	1,108.51	20.16	3,230.94^{a}	2,851.53^{a}	581.57^{ab}	10.34^{a}	2.93^{a}	0.344^{b}	35.57^{a}
Low-CP[2]	1,362.02	1,012.27	18.41	3,956.31^{b}	3,440.62^{b}	633.00^{b}	10.41^{a}	3.97^{b}	0.256^{a}	41.88^{b}
Low-CP+Met[3]	1,445.82	1,092.18	19.86	3,367.61^{a}	2,954.85^{a}	538.82^{a}	11.82^{b}	3.12^{a}	0.325^{b}	33.35^{a}
SEM[17]	26.87	26.18	0.476	108.37	90.53	14.82	0.266	0.160	0.014	2.42
P value	0.2928	0.3003	0.2998	<0.01	<0.01	0.0138	0.0165	<0.01	<0.01	0.0171
12-18 weeks										
High-CP[4]	2,125.87	1,773.30	18.10	7,046.32^{a}	3,766.89^{a}	1192.03^{b}	20.49^{a}	3.97^{a}	0.252^{b}	45.30^{a}
Low-CP[5]	2,012.53	1,662.78	16.97	8,467.01^{b}	4,469.57^{b}	1264.51^{b}	21.14^{a}	5.10^{b}	0.197^{a}	50.29^{b}
Low-CP+Met[6]	2,053.83	1,700.19	17.35	7,281.68^{a}	3,753.59^{a}	1086.79^{a}	23.41^{b}	4.29^{a}	0.234^{b}	43.07^{a}
SEM[17]	20.91	20.35	0.208	216.90	126.10	27.58	0.474	0.163	0.008	1.20
P value	0.0645	0.0613	0.0618	<0.01	<0.01	0.0106	0.0111	<0.01	<0.01	0.0212

[1] 18% protein diets. [2] 16% protein diets. [3] 16% protein with methionine supplementation. [4] 16% protein diets. [5] 14% protein diets. [6] 14% protein with methionine supplementation. [7] body weight (g). [8] body weight gain (g). [9] averaged daily gain (g/day). [10] feed intake (g). [11] metabolizable energy (kcal). [12] crude protein intake (g). [13] methionine intake (g). [14] feed conversion ratio. [15] feed efficiency. [16] feed cost per gain (1,000 g live weight). [17] standard error of the mean ($n = 6$). $^{a, b, c}$ Within each row, means with different superscript letters are different ($p < 0.05$).

Table 4. Carcass characteristics of Betong chickens (KU Line) at 12 and 18 weeks (Percentage of live weight).

Item	Carcass	Breast muscle	Abdominal fat	Wings	Drumstick	Tenderloin	Liver
12 weeks (%)							
High-CP[1]	93.42	8.97	2.44^{ab}	9.35	22.21	3.03	1.99
Low-CP[2]	85.26	6.87	2.75^{b}	8.77	19.07	2.70	1.76
Low-CP+Met[3]	94.64	8.76	2.14^{a}	9.36	22.38	3.22	1.83
SEM[7]	2.28	0.420	0.104	0.277	0.850	0.135	0.042
P value	0.198	0.062	0.038	0.654	0.212	0.309	0.052
18 weeks (%)							
High-CP[4]	94.21	9.08	0.918	8.91	24.90	3.41	1.40
Low-CP[5]	94.53	8.40	0.545	8.90	24.98	3.44	1.49
Low-CP+Met[6]	91.82	8.38	0.339	8.69	24.20	3.46	1.35
SEM[7]	0.652	0.248	0.317	0.104	0.285	0.066	0.044
P value	0.187	0.458	0.301	0.666	0.520	0.953	0.434

[1] 18% protein diets. [2] 16% protein diets. [3] 16% protein with methionine supplementation. [4] 16% protein diets. [5] 14% protein diets. [6] 14% protein with methionine supplementation. [7] SEM, standard error of the mean ($n = 6$). $^{a, b, c}$ Within each row, means with different superscript letters are significantly different ($p < 0.05$).

3.3 Gene expression

ANT gene transcripts were significantly increased in the low CP+Met group compared to other groups (P<0.05) at 12 weeks of age (Figure 1). The expression of *UCP* in the breast muscle of the chickens fed the different diets did not differ significantly at 12 weeks. However, at 18 weeks the *UCP* gene transcripts of the high CP and low CP+Met group were significantly lower than the low CP groups (P<0.05).

4. Discussion

The growth performance parameters including BW, body weight gain, and average daily gain of the Betong chickens did not differ between the diets with different protein levels and methionine supplementation. These results are in agreement with the findings of Putsakul *et al.* (2010), who reported a similar growth performance of animals fed diets with different protein levels. In a previous study on broiler chickens, Jianlin, Fritts, Burnham, & Waldroup, (2004) found no significant effect on BW (P>0.05) when dietary protein was reduced by 10%. Moreover, the addition of methionine had no significant effect on BW in 21- and 49-day-old broilers.

In our study, FI of the chickens fed a low CP diet was significantly higher than the other groups (P<0.01), and FI did not differ significantly between the high CP and the low CP+Met groups throughout the experiment. Aletor, Hamid, Neib, and Pfeffer, (2000) also reported that broilers fed a low protein diet also had low FI compared to broilers fed a high CP diet. In chickens, energy and protein levels of the diet significantly impact growth performance, based on the assumption that chickens can maintain their energy intake by adjusting FI to match the energy in the diet for maintenance and growth purposes (Classen, 2017). Therefore, the chickens could compensate to maintain energy levels under reduced protein levels (low CP group) by increasing the amount of feed. In this case, the response of the birds fed low CP diets

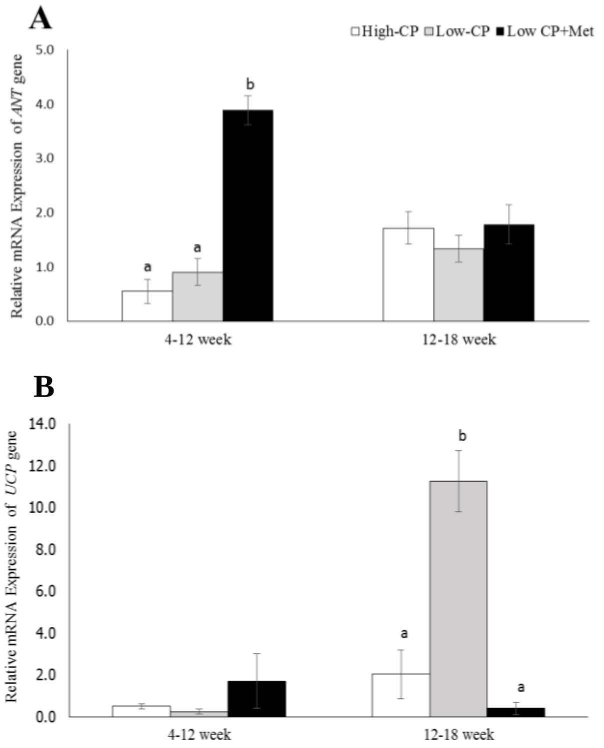

Figure 1. Relative mRNA expression of *ANT* (A) and *UCP* (B) gene in the muscle of Betong chickens (KU line) fed high CP, low CP, and low CP+Met diets at 12 and 18 weeks. Results are averages *a posteriori* and standard deviation represented by vertical bars. Different superscript letters (a, b) represent statistical differences with 95% credibility interval.

was therefore affected by the increased FI as shown in the level of ME (Table 3). The ME level in the low CP group was the highest (P<0.01) for the FI at all ages.

CPI calculated from the FI, was the highest in the low CP diet group (P<0.05), but the methionine intake was not significantly different from that of the high CP group. Betong chickens that received the methionine supplement in the low CP group, thereby received the highest methionine levels and showed improved FCR (high FE) (P<0.01) throughout the experiment. This finding is in agreement with Nguyen, Bunchasak, and Chantsavang (2010), who observed that the protein level (15–21% CP) of the high protein diet group had a FCR that was greater (P<0.05) than the low-protein diet group of Betong chickens, which was in agreement to the findings of Putsakul *et al.* (2010). Although birds fed a low CP and low ME diet showed increased FI levels to maintain their energy intake, the energy requirements of the chickens were not fulfilled. Therefore, low CP diets negatively impact FI and FCR, causing an amino acid imbalance, which was also reported by Yang and Yang (2015), mainly because amino acid requirements are essential for maximum growth and performance of chickens (Pesti, 2009).

Carcass weight did not differ significantly between the treatment groups. Nguyen *et al.* (2010) reported that different levels of CP in diets did not influence the percentage weights of carcass, breast muscle, and wings which supported our results. Similarly, several studies have found that the carcass yields of broilers were not affected by low CP diets (Aletor *et al.,* 2000; Bregendahl, Sell, & Zimmerman, 2002). In this study, abdominal fat deposition had increased significantly in the low CP group at 12 weeks. Similar results were found in Betong chickens with diets of 15% and 17% CP (Nguyen *et al.,* 2010). Additionally, reduced dietary protein levels increased abdominal fat in broiler chickens (Kamran *et al.,* 2008). Based on these results, we infer that the level of protein in a diet regulates fat metabolism in chickens. Previous reports found that reducing dietary protein affected the expression of malic enzyme in the liver of chickens (Rosebrough, Poch, Russell, & Richards 2002). Moreover, the reports found that the expression of acetyl-coenzyme carboxylase and fatty acid synthase in the liver were also suppressed at high CP diets (Rosebrough, Russell, & Richards, 2008; Rosebrough, Russell, & Richards, 2011) and regulated fat deposition by increasing fat in chickens (Zhau, Ma, Zou, Chen, & Zhao, 2007). Interestingly, when adding methionine to low CP diets, the negative effects of a low-CP diet were mitigated. The Betong chickens fed a low CP+Met diet showed the lowest yield of abdominal fat in this study. Conde-Aguilera *et al.* (2013) also found that feeding broilers with a low CP diet supplemented with methionine (20% CP) caused a significant reduction in fat accumulation.

However, in the present study, protein and amino content did not affect the fat accumulation of Betong chickens at the end of experiment (week 18). Abdominal fat weight did not differ significantly between the treatments (P>0.05). Other studies found that fat deposition was not reduced in broilers fed low CP diets supplemented with methionine (Yao *et al.,* 2006; Zhan, Li, Xu, & Zhao, 2006). It was possibly due to other factors, such as hormonal and age factors, that affected fat deposition in the chickens (Nikolova, Pavlovski, Milosevic, & Peric, 2007; Zerhdaran, Vereijken, Arendonk, & Van Der Waaij, 2005).

Moreover, Collin *et al.* (2003) reported that methionine supplementation led to improved FCR in broiler chickens. Feed efficiency is impacted by many factors of which mitochondrial function is the most important one. Studies reported that an inefficient electron transport chain (ETC) may affect the FCR and growth performance of broilers (Bottje *et al.,* 2002; Havenstein, Ferket, & Qureshi, 2003). In broiler chickens, Bottje & Carstens (2009) indicated that the birds produced low ATP levels because of a lower mitochondrial efficiency which negatively impacted feed efficiency. Additionally, recent reports demonstrated the impacts of mitochondrial genes and proteins on nutrient use and growth performance in birds (Ojano-Dirain *et al*., 2007; Voltolini *et al.,* 2014). One of main proteins for ATP production by the ETC complex is the ANT protein. The ANT protein increases the quantity of ADP for transformation into ATP by ATP synthase. Therefore, it is possible there is an association between the expression of the *ANT* gene and the feed efficiency phenotype (Bottje *et al.,* 2006). In our study, *ANT* mRNA expression in the breast muscle of the Betong chickens was the highest in the low CP+Met diets (P<0.05) at the beginning of the 12-week period, which showed the best FCR (high FE) (Table 3). However, *ANT* mRNA expression was not affected by different levels of crude protein or methionine supplementation of low CP diets at 18 weeks which was probably because the methionine intake was not sufficient to promote growth of the chickens. Similar to the findings of our study, increased expression of *ANT* transcripts in the breast muscle was found in high FE broilers (Bottje *et al.,* 2006), and the highest *ANT* mRNA expression was observed for high FE quails (Gasparino *et al.*, 2014) which indicated that the ETC may produce more ATP for growth in high FE chickens. In addition, other previous reports found that methionine supplementation in low CP diets improved the FE of broiler chickens which resulted in increased ATP synthesis (Bottje *et al.,* 2006; Bottje & Carstens, 2009).

In this case, methionine possibly controls the oxidation of mitochondrial proteins by antioxidants which require methionine for GSH synthesis. Del Vesco *et al.* (2014) reported that methionine alleviated the reactive oxygen species (ROS)-induced damage by increasing the activity of GSH, resulting in the reduction of oxidant stress. Since, S-adenosylmethionine is the direct product of methionine, it inhibits oxidative stress by increasing the activities of glutathione peroxidase, glutathione-S-transferase, and superoxide dismutase (Li, Cui, & Fang, 2017). Overproduction of ROS inside the cells during energy production negatively affect cell function and increase mitochondrial dysfunction over time (Nicoletti, Marino, Cuppari, & Licciardello, 2005; Simon, 2000). Kemp, Causton, and Clerk (2003) reported that the production of ROS led to oxidative damage and suppressed *ANT* and *COXII* gene expression in mitochondria, thereby influencing the FE of broilers (Bottje & Carstens, 2009; Ojano-Dirain *et al.,* 20 07). In addition, Bottje and Kong (2013) also found that mitochondrial gene expression was influenced in low FE broilers by the modulation of oxidative stress. However, we found no influence of methionine supplementation on *ANT* gene expression at 18 weeks. This was possibly due to the low CP+Met diets that provided insufficient methionine to control ROS production and the reduced mitochondrial proteins in the ETC complex due to age (Beyer, Starnes, Edington, & Lipton, 1984; Kerner, Turkaly, Minkler, & Hoppel, 2001).

The *UCP* gene transcripts resulted in the significantly high gene expression that was observed in the low CP diet groups at 18 weeks (low FE Betong chickens), with no significant difference at 12 weeks. This is consistent with the findings of a previous study by Del Vesco *et al.* (2013), who evaluated the effect of methionine supplementation and found the highest mRNA of the *UCP* gene ($P<0.05$) in the muscle of the basal diet group that resulted in low FE broilers. Similar to a study by Ojano-Dirain *et al.* (2007), the *UCP* gene was found to increase ($P<0.05$) in the breast muscle of low FE broilers, with higher levels of *UCP* mRNA in the muscle of low FE quails compared to high FE quails (Gasparino *et al.*, 2014). Echtay *et al.* (2002) observed that ROS can regulate ATP production by activating the *UCP* gene, which is a transporter in the inner mitochondrial membrane that provides a new route for protons from ATP synthase and diverts ATP synthesis energy to heat production (Vidal-Puig, 2000). Therefore, high expression of the *UCP* gene may affect FE because it reduces ATP production (Voltolini *et al.,* 2014). Moreover, the level of *UCP* mRNA expression was not significantly different at 12 weeks, which may the increasing of heat production was lower than 18 week. Additionally, Mujahid *et al.* (2006) reported that the levels of *UCP* and *ANT* mRNA significantly decreased which were accompanied by a significant decrease in the levels of the protein in muscle.

We conclude that methionine supplementation significantly influences the expression of *ANT* and *UCP* genes and the effective control of ROS production. Ojano-Dirain *et al.* (2007) reported that the high production of ROS and protein oxidation in cells are often found in low FE birds as a result of an insufficient function of the mitochondrial gene in the mitochondria. Moreover, in our study, the methionine supplementation in the low CP diet reduced feed costs and lead to improved production efficiency of Betong chickens.

5. Conclusions

Betong chickens fed with low-protein diets supplemented with methionine showed significantly improved growth performance and reduced abdominal fat accumulation. The expression of *ANT* and *UCP* mRNA in the breast muscle differed between treatments and the mitochondrial mRNA expressions were affected by different levels of methionine supplementation. We also observed the reduction of feed costs in the methionine supplementation to low protein diet.

Acknowledgements

The authors gratefully acknowledge Animal Research Farm, Department of Animal Science, Faculty of Agriculture, Kasetsart University, Bangkok, Thailand, as well as the Graduate School, Kasetsart University, Thailand for funding support.

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