Effect of dietary crude protein and metabolizable energy on growth performance and backfat thickness of Lanyu miniature pigs during cool and hot seasons ⁽¹⁾

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Abstract

Experiments were conducted to determine the dietary crude protein (CP) and metabolizable energy (ME) levels for growing Lanyu miniature pigs from 7 to 17 weeks of age. Experimental arrangement was $2 \times 2 \times 2$ factorial design with 2 levels of crude protein (13% and 16%), 2 levels of ME (2,800 kcal/kg and 3,100 kcal/kg) and 2 seasons (hot and cool season). Average feed intake, body weight gain, feed conversion ratio, backfat thickness and blood constituents of pigs were used as criteria for the establishment of optimal dietary CP and ME of Lanyu miniature pig. Results showed that Lanyu miniature pigs provided the diet with CP 16% have higher (P < 0.05) average feed intake and average daily gain during 7 - 17 wks. There was no significant difference on feed conversion ratio among all experimental groups. Pigs fed with ME 3,100 kcal/kg regardless of CP in the diet had higher (P < 0.05) increment of backfat thickness during 7 - 12 wks and 7 - 17 wks of age. For the season effect, higher feed intake and average daily gain were obtained when pigs were reared during the cool season (November-April) compared to the hot season (May-October). There were no season effect on feed conversion ratio and increment of backfat thickness. In conclusion, Lanyu miniature pigs fed the diet with CP 16% and ME 2,800 kcal/kg had higher feed intake and average daily gain.

Key words: Lanyu miniature pigs, Crude protein, Metabolizable energy, Growth performance, Backfat thickness.

Introduction

Laboratory animals play a crucial role in biomedical research and biotechnology development. The application of high-quality laboratory animals for research will increase the accuracy and reliability of experimental results. The quality of laboratory animals can be affected by feeding and management, breeding program, specific nutrition and pathogenic monitoring. Lanyu miniature pigs were introduced from Lanyu Islet to Taitung Animal Propagation Station (TAPS) on 1980 (Lee *et al.*, 1994). Currently, Lanyu miniature pigs have been used for biomedical research since then. Nevertheless, no specific recommendation for nutrient requirements for Lanyu miniature pigs are available so far. Lee *et al.* (2003) showed that no significance difference on growth, feed conversion ratio and carcass characteristics of 30 - 50 kg body weight (BW) Lanyu miniature pigs when daily provided 1 kg diet with CP 17.5% and DE 3,500 kcal/kg and allowed to approach pasture compared with pigs fed feed *ad libitum*. Then, calculating the minimal maintenance CP and DE requirement for Lanyu miniature pigs per day were respectively 94.7 g and 1,499 kcal. Backfat thickness of Lanyu miniature pigs (2.7 cm) was higher than that of LYD hybrid pigs (2.15 cm) at 50 kg of body weight. Laboratory pigs for medical research are required to have minimum fat deposition in order to facilitate the surgical procedure. The purpose of this study was to evaluate the effect

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of dietary CP and ME levels on the growth performance, backfat thickness and blood constituents for growing Lanyu miniature pigs.

Materials and Methods

I. Experimental diets

The experimental diets were formulated with corn-soybean meal and wheat bran. Feed composition and their calculated value were listed as Table 1.

Ingredients, %	СР, %	13	16	13	16
-	ME, kcal/kg	2,800	2,800	3,100	3,100
Yellow corn		48.4	40.6	68.9	61.9
Soybean meal		5.0	14.5	11.1	20.0
Wheat bran		40.5	38.8	13.9	12.0
Limestone		1.0	1.0	1.0	1.0
Calcium phosphate		1.5	1.5	1.5	1.5
Salt		0.4	0.4	0.4	0.4
Beet, Sugar, pulp, dried		3.0	3.0	3.0	3.0
Vitamin premix ^a		0.1	0.1	0.1	0.1
Mineral premix ^b		0.1	0.1	0.1	0.1
Total		100.0	100.0	100.0	100.0
Calculated values					
Crude protein, %		12.82	16.07	13.02	16.04
Lys, %		0.54	0.78	0.60	0.82
Met + cys, %		0.48	0.57	0.48	0.56
ME, kcal/kg		2,811.00	2,807.00	3,100.00	3,101.00
Calcium, %		0.8	0.83	0.79	0.81
Non-phytate phosphorus, %		0.93	0.95	0.71	0.73

Table 1. The composition and calculated nutrient values of experimental diet

^a Supplied per kilogram of diet: Fe (FeSO₄ • 7H₂O), 140 mg; Cu (CuSO₄ • 5H₂O), 7 mg; Mn (MnSO₄), 20 mg; Zn (ZnO), 70 mg; I (KI), 0.45 mg.

^b Supplied per kilogram of diet: Vitamin A, 6,000 IU; Vitamin D₃, 800 IU; Vitamin B₁₂, 0.02 mg; Vitamin E, 20 IU; Vitamin K₃, 4 mg; Vitamin B₁, 4 mg; Pantothenic acid, 16 mg; Niacin, 30 mg; Pyridoxine, 1mg; Folic acid, 0.5 mg; Biotin, 0.1 mg.

II. Animals and experiment of design

Experiment was a $2 \times 2 \times 2$ factorial design with two levels of CP, 13% and 16%, two levels of ME, 2,800 kcal/kg and 3,100 kcal/kg and 2 seasons, hot and cool season. A total of 80 Lanyu miniature pigs from Taitung Animal Propagation Station (TAPS) were allocated into 5 replicates. Each replicate had 16 pigs (8 castrated male and 8 female) with an initial BW of 7 kg and were assigned to 4 dietary treatments (CP 13%, ME 2,800 kcal/kg; CP 13%, ME 3,100 kcal/kg; CP 16%, ME 2,800 kcal/kg and CP 16%, ME 3,100 kcal/kg) (Table 1). Body weight of Lanyu miniature pigs in each pen was balanced at the start of the experiment. The 16 pigs were allotted to 8 pens with 2 pigs in each pen. Each pen had a space of 9.12 m² (Length 3.8 m × Width 2.4 m). Water and feed were provided *ad libitum*. Body weight, feed intake and backfat thickness (first rib, last rib and last lumbrae) were measured at 7 weeks, 12 weeks and 17 weeks of age. Besides, blood samples were collected for blood constituent analysis which includes glucose, blood urinary nitrogen, creatinine, total protein and triglyceride. The experimental procedures were complied the Guide for Care and Use of Agricultural Animals of Livestock Research Institute, Council of Agriculture.

III. Statistical analysis

All data were analyzed by SAS analysis software (SAS, 2008) as a completely randomized design with $2 \times 2 \times 2$

factorial arrangement of the treatments. Main effects of CP, ME, season and their interaction were tested. The statistical model included 1 df each for CP, ME, season and their interaction.

Results and Discussions

There was an interaction effect between CP, ME and season on the feed intake and average daily gain. No interactive effect on feed conversion ratio, backfat thickness and blood constituents of Lanyu miniature pigs were observed. Therefore, the main effect and interactive effect was discussed respectively.

- I. Dietary CP and ME effect
 - (i) Daily feed intake

Lanyu miniature pigs fed diet with CP 16%, regardless of ME had higher (P < 0.01) daily feed intake (Table 3), crude protein and ME intake (Table 2) than that fed with CP 13%. The dietary ME levels did not affect daily feed intake (Table 3). Lanyu miniature pigs fed diet with ME 3,100 kcal/kg had larger daily metabolizable energy intake than pigs fed with the ME 2,800 kcal/kg during 7-17 wks of age (Table 2).

 Table 2.
 Main effect of crude protein and metabolizable energy on daily crude protein and metabolizable energy intake of Lanyu miniature pigs

	Season (S) ^a		Crude (C	Crude protein (C) %		ME (M) kcal/kg		Significance		
	Hot	Cool	13	16	2,800	3,100		S	С	М
Daily crude protein intake, kg/d										
7-12 wk	0.09	0.08	0.07	0.10	0.09	0.09	0.001	NS	**	NS
12-17 wk	0.11	0.13	0.10	0.14	0.12	0.12	0.004	*	**	NS
7-17 wk	0.11	0.12	0.10	0.13	0.12	0.11	0.003	NS	**	NS
Daily ME intak, kcal/d										
7-12 wk	1762.4	1711.6	1650.5	1823.4	1663.9	1810.0	26.4	NS	**	**
12-17 wk	2294.6	2552.7	2281.4	2565.8	2344.3	2503.0	89.1	*	*	NS
7-17 wk	2245.4	2389.2	2167.8	2466.8	2232.4	2402.2	56.2	NS	**	*

^a From April/22/2014 to November/6/2014 was classified as hot season. From November/7/2014 to April/9/2015 was regarded as cool season.

*: P < 0.05, **: P < 0.01

(ii) Average daily gain

Lanyu miniature pigs fed diet with CP 16% had significantly larger (P < 0.05) average daily gain than those fed with CP 13% and significantly (P < 0.01) larger during 7 - 12, 12 - 17, and 7 - 17 wks of age (Table 3). No difference on average daily gain was observed for Lanyu miniature pigs fed either ME 3,100 kcal/kg or ME 2,800 kcal/kg.

II. Dietary CP and ME effect on different season

There was an interactive effect among CP, ME and season (Table 4). During cool season, Lanyu miniature pigs fed diet with CP 16%, ME 3,100 kcal/kg, had higher feed intake and average daily gain than in hot season. Nevertheless, Lanyu miniature pigs fed diet with CP 16%, ME 2,800 kcal/kg in cool season, did not have difference on feed intake and average daily gain when compared to those pigs in hot season. As for pigs fed diets with CP 13%, ME 3,100 kcal/kg during cool season, no difference on feed intake and average daily gain when compared to those pigs in hot season. As for pigs fed diets with CP 13%, ME 3,100 kcal/kg during cool season, no difference on feed intake and average daily gain when compared to those pigs fed CP 13%, ME 3,100 kcal/kg diet in hot season was observed. The interactive effect indicated that the growth enhancing effect on Lanyu miniature pigs by higher dietary protein during the cool season was observed when feed was formulated with high ME level. On the contrary, the effect was obtained at lower ME level during the hot season. Animals utilized more energy to regulate the body temperature homeostasis during lower temperature and less energy for the homeostasis purpose during the hot temperature.

	Season (S) ^a		Crude (C	Crude protein (C) %		ME (M) kcal/kg		Significance		
	Hot	Cool	13	16	2,800	3,100		S	С	М
Body weight, kg										
7 wk	6.59	7.50	7.05	7.04	7.07	7.01	0.15	**	NS	NS
12 wk	12.85	12.16	11.68	13.33	12.55	12.47	0.29	NS	**	NS
17 wk	22.30	22.02	20.76	23.56	21.99	22.33	0.50	NS	**	NS
Average daily gain, kg/d										
7-12 wk	0.22	0.20	0.20	0.22	0.21	0.21	0.01	0.06	*	NS
12-17 wk	0.29	0.33	0.29	0.33	0.31	0.31	0.01	**	*	NS
7-17 wk	0.25	0.29	0.24	0.29	0.26	0.27	0.01	**	**	NS
Average daily feed intake, kg/d										
7-12 wk	0.60	0.59	0.56	0.62	0.59	0.58	0.01	NS	**	NS
12-17 wk	0.79	0.87	0.77	0.87	0.84	0.81	0.03	*	*	NS
7-17 wk	0.76	0.81	0.74	0.84	0.80	0.77	0.02	0.06	**	NS
Feed conversion ratio, G/F										
7-12 wk	0.37	0.35	0.36	0.36	0.36	0.36	0.01	NS	NS	NS
12-17 wk	0.38	0.41	0.40	0.39	0.38	0.41	0.02	NS	NS	NS
7-17 wk	0.33	0.35	0.33	0.35	0.33	0.35	0.01	NS	NS	NS
Backfat thickness, mm										
7 wk	6.65	7.37	6.91	7.10	7.36	6.65	0.22	*	NS	*
12 wk	10.00	9.21	9.41	9.80	9.42	9.79	0.26	*	NS	NS
17 wk	13.94	13.98	13.64	14.28	13.51	14.40	0.40	NS	NS	NS
Increment of backfat thickness										
7-12 wk	3.35	1.84	2.50	2.70	2.05	3.15	0.27	**	NS	**
12-17 wk	3.94	4.77	4.23	4.48	4.10	4.61	0.36	NS	NS	NS
7-17 wk	7.29	6.61	6.73	7.18	6.15	7.76	0.41	NS	NS	**

 Table 3. Main effect of crude protein and metabolizable energy on growth performance and backfat thickness of Lanyu miniature pigs

^a From April/22/2014 to November/6/2014 was classified as hot season. From November/7/2014 to April/9/2015 was regarded as cool season.

*: P < 0.05, **: P < 0.01.

Table 4. Interactive effect of CP, ME and season on feed intake and average daily gain for Lanyu miniature pig

Season	Cool Season			Hot Season					
ME, kcal/kg	2,800		3,100		2,800		3,100		SEM
СР, %	13	16	13	16	13	16	13	16	
Average daily gain, kg/d	0.28 ^{bc}	0.29 ^{bc}	0.25 ^{ac}	0.32 ^b	0.21 ^a	0.27 ^{bc}	0.24 ^{ac}	0.26 ^{bc}	0.019
Average daily feed intake, kg/d	0.84 ^b	0.83 ^b	0.70 ^a	0.88 ^b	0.67 ^a	0.85 ^b	0.73 ^{ac}	0.79 ^{bc}	0.037

^{a, b, c} P < 0.05.

III. Feed conversion ratio (G/F)

The dietary CP and ME levels had no effect on feed conversion ratio (Table 3). No seasonal effect on feed conversion ratio was found either.

IV. Backfat thickness

Lanyu miniature pigs fed diet with CP 16% had numerically thicker backfat thickness and higher increment of

backfat thickness than pigs fed with CP 13%. Lanyu miniature pigs fed diet with ME 3,100 kcal/kg had higher (P < 0.01) increment on backfat thickness than that fed ME 2,800 kcal/kg during the 7 - 12 wks of age and 7 - 17 wks of age, due to Lanyu miniature pigs fed diet with ME 3,100 kcal/kg had higher daily metabolizable energy intake than that fed with ME 2,800 kcal/kg (Table 2).

V. Blood constituents

Lanyu miniature pigs, provided diet with high concentration of CP16%, resulted in increasing total serum protein and blood urea nitrogen (Table 5). The total blood protein and blood urea nitrogen content for Gottingen minipigs were elevated when fed diet with CP 20% compared to the pigs fed with CP 12% (Bollen *et al.*, 2005). Other blood constituents of pigs measured, including glucose, creatinine and triglyceride were within normal ranges.

	Crude protein, (C) %		ME (M)	, kcal/kg	Significance		
	13	16	2,800	3,100	С	М	
Glucose, mg/dl							
7 wk	136.13	134.65	142.03	128.75	NS	NS	
12 wk	130.18	117.13	118.28	129.03	0.06	NS	
17 wk	105.60	102.25	99.50	108.35	NS	NS	
Blood urea nitrogen, mg/dl							
7 wk	10.78	10.36	10.56	10.58	NS	NS	
12 wk	9.95	9.92	9.73	10.14	NS	NS	
17 wk	9.23	11.44	10.89	9.78	**	NS	
Creatinine, mg/dl							
7 wk	1.01	0.93	0.99	0.95	NS	NS	
12 wk	0.79	0.76	0.77	0.78	NS	NS	
17 wk	0.97	0.99	0.99	0.97	NS	NS	
Total protein, g/dl							
7 wk	6.14	6.09	6.19	6.04	NS	NS	
12 wk	6.77	7.02	6.85	6.94	0.06	NS	
17 wk	7.38	7.87	7.70	7.55	**	NS	
Triglyceride, mg/dl							
7 wk	43.00	38.38	43.13	38.25	NS	NS	
12 wk	50.70	45.50	43.50	52.70	NS	NS	
17 wk	43.08	46.32	43.90	45.50	NS	NS	

Table 5.	Main effect of cru	ide protein and	d metabolizable	energy on bloo	d constituents	of Lanvu	miniature pigs
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*: P < 0.05, **: P < 0.01

VI. Seasonal effect

During cool season in Taitung area, Lanyu miniature pigs had higher (P < 0.05) daily feed intake (Table 3), daily CP and ME intake (Table 2) than during the hot season from 12 to 17 wks of age (P < 0.05) and had significantly higher (P < 0.01) average daily gain during the 7 - 17 wks of age. Although Lanyu miniature pigs consumed higher amount of CP and ME during cold season than hot season, there were no difference in the increment of backfat thickness from 7 wks to 17 wks of age. Noteworthy, there were no differences in the average daily feed intake (Table 3), daily CP and ME intake (Table 2) during the 7 - 12 wks of age. Lanyu miniature pigs raised under hot season had significantly (P < 0.01) higher increment of backfat thickness during the 7 - 12 wks of age. In our experiment, pigs were moved from farrowing barn to nursery building at the age of 7 wks when the experiment started. Younger, smaller body size pigs are more sensitive to stressful challenge, including moving, unfamiliar environment and lower temperature, would contribute to reduced feed intake on the following few day. Nevertheless, more energy was mobilized from growth to maintenance requirement,

resulting in less increment of backfat thickness during cool season from the 7 - 12 wks of age (McConnel *et al.*, 1987, Renaudeau *et al.*, 2012).

Regarding to animal welfare, the feeding of laboratory animals need to maintain their health and physical condition and avoid hunger or excessive accumulation of body fat. The National Research Council (NRC, 1998) gives detailed scientific information concerning the nutrient requirements of swine under ad libitum conditions. The data are based on achieving maximal growth for economic purposes. Ad libitum feeding for Lanyu miniature pigs is not regarded as optimal because animals will become obese. In 1993, the working group on nutrition of the German Society for Laboratory Animal Science (GV-SOLAS, 1993) issued guidelines for the composition of Göttingen miniature pig diets for breeding and maintenance. Body weight of 9 - 10 kg Germany Göttingen miniature pigs required less crude protein and lysine but higher minerals and vitamins than commercial production of pigs (Bollen et al., 1997). Lee et al. (2003) indicated that there was no significant difference on growth performance between daily provision of 1kg of feed with roughage fed ad libitum and fed complete feed ad libitum for Lanyu miniature pigs. However, feeding on ad libitum basis had thicker backfat. The difficulty encountered on surgical operation was mainly caused by thicker backfat. Ad libitum feeding for female Lanyu miniature pigs caused obesity, but not in male. It grows slower on male Lanyu miniature pigs compared to female pigs (Lee et al., 2003). Therefore, Lanyu miniature pigs for biomedical applications should restrict its feed intake to reduce the fat accumulation. During the finishing period, grazing pasture and supplementing the complete diet increased lean and decreased fat content of Lanyu miniature pigs (Lee et al., 2003). Grazing pasture for Lanyu miniature pigs increase physical activity, resulting in energy consumption and reduce fat accumulation. However, grazing pasture has the concern for the spreading of animal diseases.

Our results showed that Lanyu miniature pigs fed diet with CP 13% and ME 2,800 kcal/kg had minimal backfat thickness, lighter weight, lower average daily gain and acceptable blood constituents. Lanyu miniature pigs fed diet with ME 3,100 kcal/kg had higher increment of backfat thickness than that fed ME 2,800 kcal/kg during 7-17 wks of age. Lanyu miniature pigs fed *ad libitum* had accumulated backfat than restricted feeding (Lee *et al.*, 2003). The present results indicated that the strategy of restricted feeding for Lanyu miniature pigs warrant further research in order to reduce its backfat thickness.

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飼糧不同粗蛋白質及代謝能含量在涼季和熱季對蘭嶼豬 生長性能及背脂厚度的影響⁽¹⁾

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摘 要

本研究目的為測定 7 - 17 週齡生長期蘭嶼豬於涼季及熱季,飼糧中不同粗蛋白質 (CP) 和代謝能 (ME) 含量, 對生長的影響。選取 7 週齡蘭嶼豬共 80 頭,分 5 批,每批 16 頭,2 頭關在一欄。飲水充分供應及飼料任飼,試驗 採 2 × 2 × 2 複因子,即 2 個等級粗蛋白質 CP 13% 及 16%,2 等級代謝能含量 ME 2,800 kcal/kg 及 ME 3,100 kcal/kg 和冷熱二季區分,形成 8 種試驗組合,試驗期間含括一年。測定項目包括豬隻飼料攝食量、每日增重、飼料轉換率、 背脂厚度及血液性狀等。結果顯示,蘭嶼豬餵飼 CP 16% 飼糧,其攝食量及每日增重皆顯著地 (P < 0.05) 高於餵飼 CP 13% 飼糧者;而對飼料轉換率及背脂厚度無影響。蘭嶼豬餵飼 ME 3,100 kcal/kg 者,於試驗前期 (7 - 12 週)以 及試驗全期 (7 - 17 週),其背脂厚度的增加量顯著地 (P < 0.05) 高於餵飼 ME 2,800 kcal/kg 者。蘭嶼豬於涼季 (11 月至 4 月份) 之飼料攝食量及每日增重,皆較熱季顯著增加 (5 月至 10 月份);飼料轉換率及背脂厚度於涼熱季並無 影響。蘭嶼豬餵飼 CP 16% 及 ME 2,800 kcal/kg 者,有較佳的每日攝食量及增重。

關鍵詞:蘭嶼豬、粗蛋白質、代謝能、生長性能、背脂厚度。

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