Original Article

Effects of Dietary Protein Deficiency on Plasma Amino Acids and Physical – Histological Conformation of the Mammary Glands in Lactating Sows

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Abstract

Eleven multiparous crossbred lactating sows were used to determine the influence of deficient protein diet containing 8.2% crude protein and normal protein diet containing 18.2% crude protein on plasma amino acid concentrations of sows and their piglets, physical mammary gland and histological changes of mammary cells. They were provided sufficient water and feed access to one of two isocaloric diets, either deficient (6 sows) or normal protein diets (5 sows). The experimental period started from farrowing (day 0) until weaning day (day 28). In this study, sows fed deficient protein diet had significant increase in body weight losses and their piglets had significant decrease in body weight gain and average daily gain (p<0.05) throughout lactation period. On day 16 of lactation, plasma amino acid concentrations, notably for some neutral and cationic amino acids, had significant decrease in sows and only for proline increment in their piglets of deficient protein group. During peak lactation (day 18), appearance of visible physical change of mammary glands in each group was observed to be smaller in protein-deficient sows than in the control sows. In addition, histology of anterior functional mammary glands in sows fed deficient protein diet demonstrated that alveolus lumen of mammary cells was smaller and connective tissues surrounding them were thicker than those fed normal protein diet, respectively. In conclusion, the present study demonstrates that dietary protein deficiency has an important negative effect on body weight loss, alteration of some plasma amino acid concentrations of lactating sows and their litters, and impairment of mammary gland conformation or function during lactation period. These important causes lead to the retarded growth performance of their piglets.

Keywords: amino acid, dietary protein, lactation, mammary, piglet

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บทคัดย่อ

ผลของระดับโปรตีนในอาหารที่ไม่เพียงพอต่อกรดอะมิโนในพลาสมาและลักษณะทั้งทางด้านกายภาพ และเซลล์เนื้อเยื่อของเต้านมในแม่สุกรระยะให้นม

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การศึกษาครั้งนี้ใช้แม่สุกรระยะให้นมพันธุ์ผสมที่ผ่านการตั้งท้องแล้วอย่างน้อย 1 ครั้ง จำนวน 11 ตัว เพื่อศึกษาผลของโปรตีนในอาหารที่ไม่เพียงพอซึ่งมีระดับโปรตีนรวมเท่ากับร้อยละ 8.2 เปรียบเทียบกับอาหารที่มีโปรตีนเพียงพอ ซึ่งมีระดับโปรตีนรวมเท่ากับร้อยละ 18.2 ต่อระดับกรดอะมิโนในพลาสมาของแม่และลูกสุกร การเปลี่ยนแปลงทั้งทางด้านกายภาพและเซลล์เนื้อเยื่อของเต้านมแม่สุกร แม่สุกรถูกแบ่งออกเป็น 2 กลุ่มคือ สุกรที่ได้รับอาหารที่มีโปรตีนไม่เพียงพอจำนวน 6 ตัว และสุกรกลุ่มที่ได้รับอาหารที่มีระดับโปรตีนเพียงพอจำนวน 5 ตัว ใช้ระยะเวลาในการทดลอง 28 วัน เริ่มตั้งแต่วันคลอดจนถึงวันที่หย่านม ผลการศึกษาครั้งนี้พบว่า แม่สุกรที่ได้รับอาหารที่ระดับโปรตีนไม่เพียงพอ มีการสูญเสียน้ำหนักตัวมากขึ้น ส่วนในลูกสุกรมีผลต่อการเพิ่มน้ำหนักตัวและอัตราการเจริญเติบโตต่อวันลดลงอย่างมีนัยสำคัญทางสถิติ (p<0.05) ตลอดระยะเวลาการให้นม ในวันที่ 16 ของการให้นม ระดับความเข้มข้นของกรดอะมิโนส่วนใหญ่ โดยเฉพาะกรดอะมิโนบางตัวที่เป็นกลางและที่มีประจุบวกในพลาสมาแม่สุกรลดลงอย่างมีนัยสำคัญทางสถิติ (p<0.05) แต่ไม่พบการเปลี่ยนแปลงความเข้มข้นของกรดอะมิโน ยกเว้นโพรลีนที่เพิ่มขึ้นในพลาสมาของลูกสุกร นอกจากนี้ช่วงให้น้ำนมสูงในวันที่ 18 ของระยะให้นม สังเกตพบว่าเต้านมของแม่สุกรที่ได้รับอาหารที่ระดับโปรตีนไม่เพียงพอมีขนาดเล็กกว่าแม่สุกรกลุ่มที่ได้รับอาหารที่มีระดับโปรตีนปกติ และเมื่อศึกษาลักษณะทางด้านเซลล์ของเนื้อเยื่อเต้านมแม่สุกรที่ยังคงทำงานทั้ง 2 กลุ่มการทดลองในช่วงดังกล่าว พบว่าช่องว่างในถุงเก็บน้ำนมฝอยมีขนาดเล็กลง และเนื้อเยื่อเกี่ยวพันรอบๆถุงเก็บน้ำนมฝอยหนาขึ้น ในกลุ่มแม่สุกรที่ได้รับอาหารที่มีระดับโปรตีนไม่เพียงพอ เมื่อเปรียบเทียบกับกลุ่มที่ได้รับอาหารที่มีระดับโปรตีนปกติ จากผลการศึกษาในครั้งนี้สรุปได้ว่า แม่สุกรในระยะให้นมที่ได้รับอาหารที่มีระดับโปรตีนไม่เพียงพอ แสดงผลในด้านลบ ได้แก่ การสูญเสียน้ำหนักตัว การเปลี่ยนระดับความเข้มข้นของกรดอะมิโนส่วนใหญ่ในพลาสมา รวมทั้งความเปลี่ยนแปลงในหน้าที่และลักษณะทางกายภาพของเต้านม ซึ่งสาเหตุที่สำคัญเหล่านี้ส่งผลต่อสมรรถนะการเจริญเติบโตที่ลดลงของลูกสุกร

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Introduction

Growth and health of newborn piglets depend upon protein content in sows' milk. Therefore, dietary management of lactating sows ensuring a sufficient protein diet is an important factor to achieve the ability to produce adequate milk for the rapid growth of their litters and to maintain mammary gland growth. The main source of amino acids or protein of suckling piglets is found in sow's milk. Thus, diet of lactating sows needs to be formulated to maximize milk production and maintain body condition throughout lactation. Insufficient amino acid requirements of the sows during lactation can lead to decrease in milk production and litters' weights after being weaned (King et al., 1993). The studies by Trottier et al. (1997) and Nielsen et al. (2002) reported that there were two different physiological mechanisms responsible for the appearance of amino acids in milk, namely mammary plasma flow and mammary arteriovenous difference. The level of dietary protein is an important factor affecting amino acid concentration in sow's milk. As dietary crude protein (CP) increased from deficient protein (7.8% CP) to normal protein (18.2% CP) level, all essential amino acids taken up across mammary tissues increased and reached maximum in sows fed the normal protein diet, and decreased in those fed the excess CP diet. The study by Trottier et al. (1995) found that the mammary arteriovenous difference of amino acids increased with increasing dietary CP concentration while the mammary plasma flow did not change (p>0.05). Consequently, the mechanisms responsible for directly increasing amino acids which enter and leave the mammary glands play important roles in amino acid transport for milk composition and for sow's milk production, which finally affect the performances of piglets.

Free amino acids are taken up by lactating mammary gland from blood in large quantities not only to satisfy the need for protein synthesis (Guan et al., 2004[b]) and synthesis of nonessential amino acids in mammary tissue protein (Trottier et al., 1997), but also to regulate metabolic pathways critical to milk production (Kim and Wu., 2009). The requirements for sow milk production are nowadays based solely on the amino acid composition in secreted milk protein, which may be inaccurate (Nielsen et al., 2002). Thus, the estimation of the amino acid requirements should be assessed using the amino acid uptake across mammary tissues (Trottier et al., 1997). Generally, the essential amino acids are taken up across mammary tissues in excess of their quantitative excretion in milk. However, many factors that can affect amino acid uptake of mammary tissues involve nutritional factors such as fasting, deficiency or malnutrition, hormonal factor, and physiological stage of sows (Ferraris and Carey, 2000; Guan et al., 2004[a]).

Based on their functions, there are two types of sow mammary tissues: parenchymal tissues such as alveoli and alveolar lumen which involve the milk synthesis, and stromal tissues consisted of adipose tissues and connective tissues which support the mammary gland. Mammary tissues grow by elongation and branching of ducts into mammary fat pad as day of lactation progresses (Tucker, 1987). Furthermore, the secretory cells increase as day of lactation increase. For lactating sows fed sufficient protein diet, amino acids which are derived from the digestive system can be transferred to maintain peripheral tissues including mammary gland growth.

A few information has been published to explain the effects of dietary protein level on performance of lactating sows and their piglets raised in a tropical farm. Therefore, the objectives of this study were to determine the effects of dietary protein levels as deficient and normal crude protein requirement on plasma amino acid concentrations of sows and their piglets, both physical mammary gland and histological changes of sow mammary cells at peak lactation.

Materials and Methods

Experimental design and diets: Eleven multiparous crossbred lactating sows were randomly selected based on their genetic backgrounds, mammary gland conformation and farrowing day. The sows were provided feed and sufficient free drinking water and were allocated into 2 groups: 6 sows for deficient protein diet group and 5 sows for normal protein diet group (8.2% and 18.2% CP, respectively). The diet provided were isocaloric (3.2 ME, Mcal/kg) and balance of their nutrients, following the recommendation of NRC (1998) (Table 1). Feed samples of two experimental groups were randomly collected and used as a representative for each group. Nutrient composition of both experimental diets was analyzed by proximate analysis method (AOAC, 1990). Amino acid concentrations in feed samples were analyzed by reverse-phase high-performance liquid chromatography (RP-HPLC) method as described by Reverter et al. (1997). Chemical analysis was conducted in duplicate.

Table 1 Composition and calculated nutrient contents of experimental diets (as fed basis).

Ingredients	Dietary CP, (%)	
	Deficient (8.2)	Normal (18.2)
Broken rice	20.00	18.00
Corn	15.00	17.00
Cassava chip	25.00	10.00
Palm oil	3.35	3.00
Fat powder	1.60	2.00
Raw bran rice	18.50	7.00
Rice solvent bran	10.00	10.00
Soybean meal (48% CP)	0.70	21.00
Full fat soybean meal	1.00	7.00
L-lysine	0.35	0.30
DL-methionine	0.10	0.30
Calcium carbonate	1.50	1.50
Monocalcium phosphate (P_{21})	2.30	2.30
Salt	0.35	0.35
Vitamin and mineral premix [1]	0.25	0.25
Total	100.00	100.00
Calculated nutrient contents (as dry matter basis)		
ME, Mcal/kg	3.25	3.23
Crude protein, %	8.15	18.24
Fat, %	8.98	8.47
Fiber, %	4.59	4.47
Calcium, %	1.05	1.08
Available phosphorous, %	0.47	0.48
Lysine, %	0.58	1.18
Methionine + cysteine, %	0.35	0.85
Methionine, %	0.25	0.58
Threonine, %	0.25	0.66
Tryptophan, %	0.07	0.22

[1]Each kilogram of the trace minerals and vitamins contained the following: vitamin A, 6.0 IU; vitamin D3, 0.8 IU; vitamin E, 12,000 IU; vitamin K3 , 0.8 g; vitamin B1, 0.6 g; vitamin B2, 1.4 g; vitamin B6, 0.8 g; vitamin B12, 0.008 g; pantothenic acid, 6.0 g; niacin, 8.0 g; folic acid, 0.12 g; biotin, 0.12 g; choline, 120.0 g; selenium, 0.12 g; iron, 40.0 g; manganese, 12.0 g; zinc, 24.0 g; copper, 8.0 g; cobalt, 0.08 g; iodine, 0.20 g.

Animals: Animal procedures used in this study were approved by the Animal Care and Use committee, Faculty of Veterinary Science, Chulalongkorn University. The sows were moved to farrowing create 2 weeks prior to farrowing and housed individually in farrowing creates. After farrowing (day 0), piglets were cross-fostered after birth to achieve equal number of piglet per sow. The piglets received sow's milk throughout lactation period and given creep feed *ad libitum* from day 18 of lactation until weaning day on day 28 of lactation. The sows were weighed individually on day 1 postpartum, day 14, and day 28 of lactation. The piglets were weighed individually on day 1 postpartum and every three days until weaning day. Feed intakes of the sows and piglets were recorded daily during day 0 until day 28 and day 18 until day 28 of lactation, respectively.

Blood sampling: Blood samples were obtained from each sow on day 16 of lactation. Four milliliters of blood were collected from each sow 4 hours after feeding via anterior vena cava puncture and put into tubes and heparinized tubes. All blood samples were kept on ice until centrifugation. Approximately 0.5 ml of blood was automatically counted (Coulter T890, Diamond Diagnostics Inc., Holliston, MA, USA) for red blood cell, hemoglobin, hematocrit, white blood cells and blood parasite as an indicator of the sows' health. Plasma was separated by centrifugation at 3,000g for 15 min at 4°C, then transferred to new tube and stored at -20°C until analysis of amino acid concentration according to method documented by Reverter et al. (1997).

Mammary tissue sampling: On day 18 of lactation, mammary tissue samples were obtained by incisional biopsy in five anterior glands of the udder 4 hours after the feed was withdrawn in the morning. The sows were anesthetized by intramuscular administration of Azaperone. The udders was locally infused by 2% xylocaine (OLIC, Thailand) at the incision site, performing approximately at midpoint (4 to 5 cm) between the teat and the upper line of the udder. The incision was continued through the subcutaneous tissues and fascia layers to expose the underlining mammary tissues. Approximately 15-mm elliptical incision at a depth of 5-10 mm, mammary tissues (1-5 g) were collected and immediately placed in 10% buffered formalin solution.

Histological procedure and determination: Visualization of udders of sows fed deficient and normal protein diet was performed and captured using 3.0 megapixel digital camera on day 14, 18 and 28 of lactation. Sow mammary tissues designated for histological examination were fixed in 10% buffered neutral formalin for 48 hours. Subsequently, the samples were routinely automated tissue process and embedded in paraffin. After sections of tissue samples (4-6 μm) were made, thick sections were stained with hematoxylin and eosin for histological study of the presence of mammary parenchymal tissues. (Kensinger et al., 1982).

Statistical analyses: All assays were conducted in duplicate or triplicate. Statistic significance of the parameters was presented as means ± SD. Differences between groups were analyzed by unpair *t*-test using commercial computer program GraphPad Prism (Prism3) at p-value ≤ 0.05.

Results

Nutrient composition and amino acid concentrations of two experimental diets: The experimental diets were similar in nutrient composition, except for crude protein contained in both diets. Deficient and normal protein diets are composed of 8.48% and 19.43% crude protein, respectively, as presented in Table 2. The amino acid concentrations in dietary protein deficiency were lower than those in normal protein diet. The analysis results of individual amino acid composition in two experimental diets are presented in Table 3.

Table 2 Analyzed nutrient contents of experimental diets.

Analyzed contents	Dietary CP	
	Deficient group	Normal group
Crude protein, %	8.48	19.43
Fat, %	9.51	9.98
Fiber, %	2.85	2.89
Ash, %	8.31	8.05
Calcium, %	1.02	1.09
Phosphorous, %	1.18	1.34
Moisture, %	9.27	9.08

Table 3 Analyzed amino acid composition of experimental diets (mg/100 mg).

Amino acids	Dietary CP, (%)	
	Deficient group (8.2)	Normal group (18.2)
Lysine	0.76	1.32
Threonine	0.30 (0.39)[a]	0.61 (0.46)
Arginine	0.47 (0.62)	1.11 (0.84)
Histidine	0.14 (0.18)	0.36 (0.27)
Isoleucine	0.26 (0.34)	0.62 (0.47)
Leucine	0.60 (0.79)	1.27 (0.96)
Phynylalanine	0.29 (0.38)	0.65 (0.49)
Valine	0.38 (0.50)	0.74 (0.56)
Alanine	0.50 (0.66)	0.82 (0.62)
Aspartic	0.77 (1.01)	1.93 (1.46)
Serine	0.34 (0.45)	0.81(0.61)
Glutamic	1.39 (1.83)	3.23 (2.45)
Glycine	0.35 (0.46)	0.68 (0.52)
Proline	0.24 (0.32)	0.65 (0.49)
Tyrosine	0.18 (0.24)	0.43 (0.33)

[a]*Values in parenthesis represent the analyzed amino acid:lysine concentration ratio*

Table 4 Hematological values of lactating sows in two experimental groups[1]

Hematological values	Normal values[2]	Deficient group	Normal group
RBC (x 10^6/μl)	4.00 - 8.00	5.90	4.90
Hemoglobin (g/dl)	10.00 - 16.00	10.40	9.70
Hematocrit (%)	30.00 - 50.00	37.10	33.60
WBC (x 10^3/μl)	7.00 - 20.00	11.40	14.00
Neutrophils (%)	20.00 - 70.00	25.50	31.30
Eosinophils (%)	5.00 - 11.00	8.00	4.30
Lymphocytes (%)	35.00 - 75.00	62.50	59.70
Monocytes (%)	0.00 - 5.00	4.00	4.70
Blood parasite	not found	not found	not found

[1]*Data are presented as means. (n = 3 in each group)*
[2]*Veterinary services "Normal hematological values" Lab Animals (1993)*

Health monitoring of sows: Six randomly selected sows were used as the representatives of both experimental groups, which were healthy. The average of each hematological value in sows' blood of both experimental groups was in normal value, as shown in Table 4.

Growth performance: There was no significant effect on daily feed intake of sows throughout lactation and of piglets during the last 10 day of lactation. Sows fed deficient protein diet lost 37.60±9.80 kg of their body weights which was greater than those fed normal protein diet, which lost 18.3±4.6 kg. The number of piglets at birth until weaning day was not different between the deficient and control groups (p>0.05). Even though the body weight of the piglets at birth was not different between the two groups, the body weight gain and average daily gain (ADG) of piglets in deficient protein diet group was significantly lower than those of piglets in normal protein diet group throughout lactation ($p \leq 0.05$), especially during day 18-28 of lactation. However, the average daily feed intake of the piglets did not differ between the two experimental groups (p>0.05). Data are presented in Table 5.

Table 5 Effects of dietary protein deficiency on performance of lactating sows and litters[1] over 28 day lactation

	Parameters		Dietary crude protein (%)		*P*-value[2]
			Deficient (8.2)	Normal (18.2)	
Sow	Number of sows		6	5	
	Parity number		3.17 ± 1.50	2.80 ± 1.30	0.676
	Body weight at d 0, kg		208.00 ± 40.80	206.00 ± 16.50	0.952
	Body weight at d 28, kg		170.00 ± 40.00	188.00 ± 18.00	0.438
	Body weight change, kg		-37.60 ± 9.80	-18.30 ± 4.60	0.010
	Daily feed intake, kg				
		wk 1	2.67 ± 1.09	2.99 ± 0.91	0.617
		wk 2	3.50 ± 0.70	3.29 ± 0.58	0.595
		wk 3	3.27 ± 0.78	3.00 ± 0.71	0.567
		wk 4	3.42 ± 0.89	3.30 ± 0.55	0.792
Litter	Piglet number at birth		9.83 ± 0.98	9.00 ± 1.00	0.198
	Piglet number at weaning		9.00 ± 1.55	8.40 ± 2.07	0.596
	Piglet survivability, %		91.00 ± 9.17	92.50 ± 16.80	0.853
	Daily feed intake, g		21.70 ± 7.30	27.30 ± 9.90	0.305
	Body weight, kg				
		d 0	1.40 ± 0.25	1.46 ± 0.24	0.709
		d 6	2.20 ± 0.51	2.41 ± 0.24	0.429
		d 12	3.20 ± 0.79	3.19 ± 0.28	0.975
		d 18	4.27 ± 0.65	4.26 ± 0.29	0.972
		d 24	5.14 ± 0.79	5.46 ± 0.39	0.429
		d 28	5.75 ± 0.70	6.58 ± 0.46	0.047
	Body weight gain, kg				
		d 0-6	0.80 ± 0.34	0.95 ± 0.19	0.403
		d 6-12	1.00 ± 0.30	0.78 ± 0.22	0.206
		d 12-18	1.08 ± 0.20	1.08 ± 0.17	0.993
		d 18-24	0.86 ± 0.21	1.20 ± 0.27	0.045
		d 24-28	0.61 ± 0.27	1.12 ± 0.24	0.001
		Overall (d 0-28)	4.35 ± 0.52	5.12 ± 0.63	0.053
	Average daily gain, g				
		d 0-6	133.3 ± 56.58	158.3 ± 31.11	0.403
		d 6-12	166.4 ± 49.70	129.7 ± 36.81	0.206
		d 12-18	179.2 ± 33.84	179.3 ± 27.65	0.993
		d 18-24	143.9 ± 34.49	199.7 ± 45.34	0.045
		d 24-28	153.3 ± 66.38	280.5 ± 60.58	0.009
		Overall (d 0-28)	155.4 ± 18.64	183.0 ± 22.58	0.053

[1] Data are presented as means ± SD. 2 Data are determined as statistical significant at $p<0.05$.

Plasma amino acid concentrations of sows and piglets: In sows, plasma concentrations for most amino acids, notably the essential amino acids, i.e. arginine, histidine, threonine, tyrosine, phenylalanine, and branched-chain amino acids (valine, isoleucine, and leucine) decreased significantly in sows fed dietary protein deficiency ($p\leq0.05$). Similar to essential amino acid, in sows fed dietary protein deficiency, the plasma concentration of nonessential amino acid such as serine was decreased significantly ($p\leq0.05$). Plasma concentrations of other amino acids seemed to be unaffected ($p>0.05$). However, plasma alanine increased significantly in sows fed deficient protein diet ($p\leq0.05$). Data of plasma amino acid concentrations of sows are given in Table 6. In piglets, only proline concentration in plasma increased significantly ($p\leq0.05$) in the group of sows fed dietary protein deficiency compared to those fed normal protein diet. Other plasma amino acid concentrations of piglets and of sows fed with normal and deficient protein diet did not differ ($p>0.05$). The plasma amino acid concentrations of piglets are shown in Table 7.

Physical changes of sow mammary glands: The mammary glands of sows fed deficient and normal protein diet were observed on day 14, 18, and 28 of lactation. The udders of sows fed deficient protein diet seemed to be smaller when compared to those fed normal protein diet, especially on day 18 of lactation. The illustrative pictures of physical changes are shown in Fig 1.

Histological changes of sow mammary tissues: In sow mammary tissues of the two groups, functional cell types were present, including alveolar lumens and connective tissues (Fig 2). Alveoli of sow mammary tissues in both groups were different. The alveolar lumens in mammary tissues of sows fed deficient protein diet were smaller than of those fed normal protein diet. Additionally, the connective tissues surrounding the alveolus of mammary tissues of sows fed deficient protein diet were thicker than those of sows fed normal protein diet.

Table 6 Effect of dietary protein deficiency on individual plasma amino acid concentration in lactating sows1 (pmol/µl).

Amino acids	Dietary crude protein (%)		P-value[2]
	Deficient (8.2)	Normal (18.2)	
Aspartic	9.78 ± 0.22	15.20 ± 4.90	0.132
Serine	130.00 ± 11.00	237.00 ± 18.00	0.001
Glutamic	88.60 ± 9.60	96.30 ± 26.00	0.654
Glycine	917.00 ± 91.00	983.00 ± 180.00	0.605
Histidine	352.00 ± 23.00	449.00 ± 8.10	0.003
Taurine	30.70 ± 3.70	33.90 ± 0.31	0.327
Arginine	156.00 ± 34.00	244.00 ± 25.00	0.023
Threonine	129.00 ± 28.00	254.00 ± 59.00	0.045
Alanine	697.00 ± 80.00	502.00 ± 53.00	0.025
Proline	256.00 ± 11.00	288.00 ± 28.00	0.139
Tyrosine	65.80 ± 9.30	134.00 ± 19.00	0.005
Valine	228.00 ± 12.00	310.00 ± 40.00	0.028
Methionine	110.00 ± 0.52	147.00 ± 58.00	0.921
Lysine	441.00 ± 42.00	329.00 ± 62.00	0.061
Isoleucine	73.40 ± 8.30	120.00 ± 9.30	0.003
Leucine	96.90 ± 3.20	162.00 ± 25.00	0.011
Phenylalanine	65.50 ± 7.20	91.50 ± 11.00	0.026
Tryptophan	114.00 ± 7.20	142.00 ± 19.00	0.082

[1] *Data are presented as means ± SD. (n = 3 in each group)*
[2] *Data are determined as statistical significance at P≤0.05 by Unpair T-test.*

Table 7 Effect of dietary protein deficiency on individual plasma amino acid concentration in piglets1 (pmol/µl).

Amino acids	Dietary crude protein (%)		P-value[2]
	Deficient (8.2)	Normal (18.2)	
Aspartic	17.2 ± 5.6	15.4 ± 1.8	0.621
Serine	244.0 ± 21.0	240.0 ± 19.0	0.805
Glutamic	161.0 ± 50.0	144.0 ± 25.0	0.627
Glycine	966.0 ± 112.0	840.0 ± 107.0	0.231
Histidine	516.0 ± 17.0	489.0 ± 69.0	0.548
Taurine	107.0 ± 20.0	113.0 ± 30.0	0.810
Arginine	156.0 ± 51.0	163.0 ± 47.0	0.858
Threonine	188.0 ± 14.0	237.0 ± 110.0	0.486
Alanine	636.0 ± 47.0	513.0 ± 100.0	0.125
Proline	621.0 ± 38.0	473.0 ± 62.0	0.024
Tyrosine	146.0 ± 22.0	160.0 ± 39.0	0.616
Valine	230.0 ± 29.0	210.0 ± 36.0	0.499
Methionine	67.2 ± 14.0	62.0 ± 23.0	0.755
Lysine	255.0 ± 37.0	227.0 ± 24.0	0.333
Isoleucine	129.0 ± 17.0	102.0 ± 27.0	0.215
Leucine	187.0 ± 25.0	175.0 ± 28.0	0.629
Phenylalanine	73.7 ± 14.0	74.3 ± 13.0	0.962
Tryptophan	77.7 ± 5.9	105.0 ± 24.0	0.138

[1] *Data are presented as means ± SD. (n = 3 in each group)*
[2] *Data are determined as statistical significance at P<0.05 by Unpair T-test.*

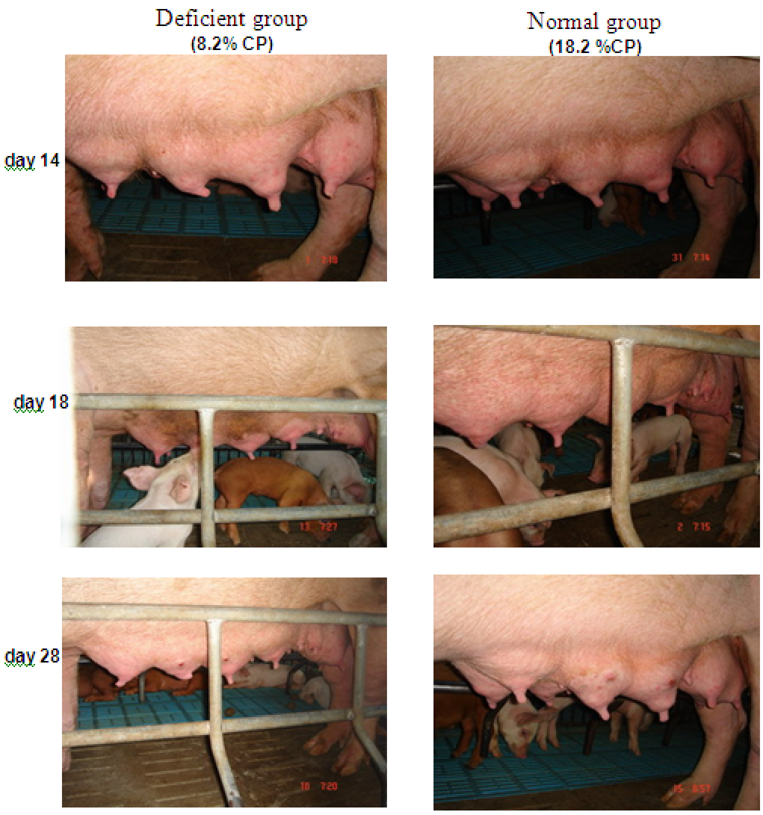

Figure 1 Comparison of physical changes of mammary glands between sows fed deficient protein diet (8.2% CP) and normal protein diet (18.2% CP) on day 14, 18, and 28 of lactation. The photographs of sow-udders fed deficient protein diet have smaller size than those fed normal protein diet, especially on day 18 of lactation.

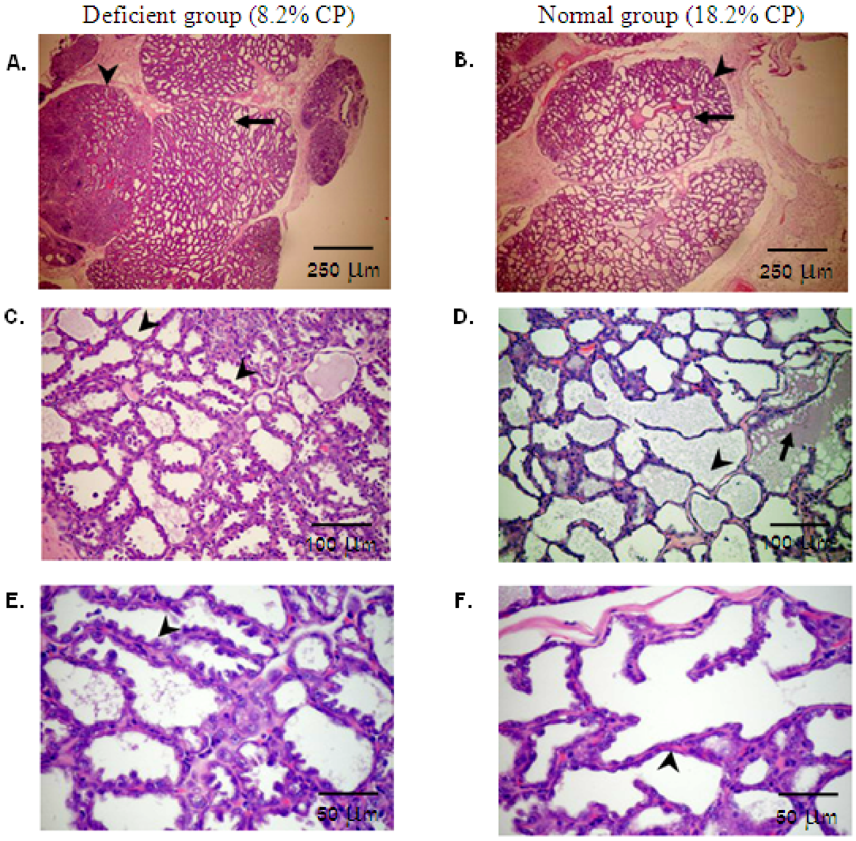

Figure 2 Comparison of histological changes of mammary tissues between sows fed deficient (8.2% CP) and normal protein diet (18.2% CP) on day 18 of lactation.

A and B: The density of alveoli (arrows) in lobules (arrow heads) of mammary tissues in deficient group (A) is greater than those appeared in normal group (B).

C and D: Arrow heads indicate alveolar lumen and arrow indicates milk droplet. The alveolar lumens in mammary tissues in sows fed deficient protein diet (C) are smaller than those fed normal protein diet (D).

E and F: The connective tissues (arrow heads) surrounding the alveolus of mammary tissues in sows fed deficient protein diet (E) are thicker than those in sows fed normal protein diet (F).

Discussion

This present study provided the results of the effects of dietary protein deficiency on plasma amino acid concentrations of sows and their piglets, physical mammary gland and histological change of mammary cells in peak lactating sows which lead to the result in growth performance of piglets. Deficient protein diet had negative effects on body weight change of lactating sows which consequently affected growth performance of their piglets by decreasing the piglets' body weight and average daily gain, especially at the last period of lactation. These results agree with previous study of Guan et al. (2004[a]), in that sows fed deficient protein diet (7.8% CP) lost 25.8 kg of their weight. Lactating sows often lose of their body proteins to support milk production. Nutritional deficiency can cause an excessive body protein loss and consequently retards litter growth rate. One of main effects of reduction in piglet growth is sow's milk production. Severe protein restriction during lactation decreased milk production of sow (Jones and Stahly, 1999) and also decreases proteins contained in sow's milk (Guan et al., 2004[a]). Consequently, piglets do not receive sufficient nutrients including amino acids for body protein synthesis to support rapid growth at early stage of life. However, milk production of sow is relatively unaffected by short term of dietary protein deficiency because sows have remarkable capacity to buffer by mobilizing their body proteins to support amino acid needs for milk synthesis (Revell et al., 1998). Therefore, in this experimental period during day 0 to 18 of lactation, both body weight and average daily gain of piglets did not significantly change between the 2 experimental groups (p>0.05). On the other hand, during day 18 to 24 of lactation, it is assumed as peak lactation in sows (Trottier et al., 1997). An average daily gain of piglets in sows fed deficient protein diet decreased significantly (p<0.05). According to Renaudeau and Noblet (2001), piglets compensated for decreased milk production by increasing their creep feed intake. In our study, piglets consumed similar amounts of creep feed during day 18 to 28 of lactation. However, two case studies of dietary crude protein reduction from 16.5 to 13.7% and from 17.6 to 14.2% by Johnston et al (1999) and Renaudeau and Noblet (2001), respectively, exhibited no change in body weight gain of litters nursed by heat-stressed sows. This present study implied that sows could not buffer sufficient amino acids needed for high milk yield synthesis by mobilizing their body protein reserves. Interestingly, the deficient protein diet was not likely to affect health of these sows as partially confirmed by the hematological values. There was no significant difference between the experimental groups and the values were in normal range. Furthermore, the result of reduction in piglets' growth can be evidenced by the level of plasma amino acid concentration found in the plasma of sows.

The quantity of the essential amino acids taken up across sow mammary tissues depends upon the lactating period. They are increasingly taken up by mammary gland as day of lactation increases (Trottier et al., 1997). The investigation of plasma amino acid concentrations may be used as an indicator of available plasma amino acid supply to mammary gland at peak lactation accounting from day 15-21 of lactation (Trottier et al., 1997). The plasma essential amino acid concentration in sows fed dietary protein deficiency, such as arginine, threonine, tyrosine, branched-chain amino acids (valine, isoleucine, and leucine), and phenylalanine decreased compared to those fed dietary protein sufficiency ($p \leq 0.05$) whereas most of the plasma nonessential amino acid concentrations constantly remained unchanged. The nonessential amino acids can be mobilized from the body protein, particularly skeletal muscle (Jones and Stahly, 1999). In some essential amino acids, uptake of branched-chain amino acids from blood substantially exceeds their output in milk and Li et al., (2009) found that an enrichment of glutamine and aspartate in sow milk was the major nitrogenous products of branched-chain amino acids catabolism in lactating sow mammary gland. Further supported by Guan et al. (2004[a]), they found that the arterial plasma essential amino acid concentrations were lower in sows fed deficient protein diet (7.8% CP) compared with those fed normal protein diet (18.2% CP). Consequently, the uptake of these amino acids across mammary tissues dramatically decreased. In addition, the protein and amino acid composition in sows' milk were lower in the sows fed deficient protein diet (7.8% CP) compared with those fed normal protein diet (18.2% CP). Change of plasma amino acid concentrations in piglets was not affected by dietary protein deficiency, except proline that significantly increased ($p<0.05$), despite the fact that the piglets received lower milk protein composition (Guan et al., 2004[a]). Therefore, this shows that the piglets had enough capacity to compensate for the plasma amino acid concentration so it did not affect their body weights and average daily gain as confirmed at day 0 until day 18 of lactation ($p>0.05$). According to the increasing amount of plasma proline in piglets, proline can be synthesized from arginine catabolism in mammary gland. In addition, it is considered as a conditionally essential amino acid for young pig because of the necessary requirement in this period of life (Ball et al., 1986) and used for synthesis of collagen matrix necessary for muscle development and mineralization of bone to support rapid growth of young pig (Bengtsson and Hakkarainen, 1975). However, the reason why body weight and average daily gain of piglets significantly decreased after day 18 until day 28 ($p<0.05$) could be the amino acids supplied in sow milk may still not be adequate to support the rapid growth of piglets. There needs to be further studies of milk protein or amino acid concentrations during lactation in sows fed normal and deficient protein diet. Then, this information can help to confirm the piglet changes of body weight and average daily gain.

Moreover, visible physical observation of udders of sows fed deficient protein diet showed smaller size than those fed normal protein diet, especially at day 18 of lactation. One possible explanation is the insufficient amount of amino acids and/or protein which are partially needed for mammary gland growth such as mammary tissue synthesis (Trottier et al., 1997). An additional investigation on histological change of porcine mammary tissues between the two groups showed that mammary tissues of sows fed deficient protein diet had less function than of those fed protein diet. Noticeably, smaller size of alveolar lumens, characteristic of alveoli cells, and less appearance of milk constituent in alveolar lumens within mammary tissues of sows fed deficient protein diet were found. Protein and other contents in parenchymal tissues of individual lactating glands are good indicators of mammary gland growth and lactogenesis (Kim et al., 2000). The mammary gland is a functional major tissue for lactating sows because of its metabolic importance in synthesizing and secreting milk. In addition, the mammary gland growth can be affected by dietary amino acid intake during lactation. Therefore, dietary management of the lactating sow ensuring a sufficient diet in protein is important to achieve the ability of the sows not only to produce adequate milk but also maintain the mammary gland growth (Kim et al., 1999). Consequently, these findings can explain the retarded growth of piglets in group of sows fed protein diet deficiency.

Our study showed significant effect of dietary protein deficiency on negative mobilization of body reserves of lactating sows, alteration in plasma concentrations of some essential amino acids and impairment of mammary gland conformation or capability of milk production at peak lactation. Consequently, these effects are the important causes to retard growth performance of piglets such as body weight at weaning day. Future studies with swine model are necessary to elucidate the underlying mechanisms at molecular, cellular, and tissue levels through manipulation of nutrient transporter gene-protein expressions under sows fed deficient protein. To the best of our knowledge, it is useful for further studies of amino acid transporter gene-protein expressions in sow mammary tissues and/or dietary amino acid supplementation to optimize amino acid composition in sows' milk.

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