CHAPTER IV

RESULTS

Growth performance of sows and piglets

Sows and litter performance were shown in Table 4.1. Even though dietary crude protein concentration had no significant effect on daily feed intake of sows throughout lactation (P>0.05), dietary crude protein concentration affected sow body weight change during lactation ($P\leq$0.05). Sows fed with deficient protein diet loss their weight for 37.60 ± 9.80 kg that was greater than those fed with normal protein diet for 19.3 ± 5.2 kg. A number of piglets at birth till weaning day were not different between deficient and control group (P>0.05). The body weight of piglets at birth was not different between two groups, but at weaning day the body weight of deficient protein diet group was significantly lower than those of piglets in normal protein diet group ($P\leq$0.05). In addition to body weight of piglets, ADG of piglets in deficient protein diet group was lower than those of piglets in normal protein diet group throughout lactation ($P\leq$0.05), especially during d 18-28 of lactation. However, the average daily feed intake of piglets did not differ between two experimental groups (P>0.05).

Table 4.1 Effect of dietary protein deficiency on performance of lactating sow and litter [1]

	Parameters	Dietary crude protein (%)		P-value[2]
		8.2	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	0.428
	d 28	5.75 ± 0.70	6.58 ± 0.46	0.047
	Average daily gain, g			
	d 0-6	134.0 ± 56.10	158.0 ± 31.00	0.405
	d 6-12	168.0 ± 46.90	129.0 ± 36.70	0.172
	d 12-18	179.0 ± 33.26	180.0 ± 27.98	0.964
	d 18-24	145.0 ± 33.60	200.0 ± 45.10	0.045
	d 24-28	134.0 ± 45.68	256.0 ± 38.84	0.001

[1] Data are presented as means ± SD. [2] Data are determined as statistical significant at $P \leq 0.05$.

Health monitoring of sows used in the experiment

Five randomly selected sows were used as the representative of both experimental groups, which were healthy. The average of each hematological value in sow's blood of both experimental groups was in normal value, as shown in Table 4.2.

Table 4.2 Hematological values of sows in two experimental groups[1]

Hematological values	Normal values[2]	Deficiency group (n=2)	Normal group (n=3)
R.B.C. ($10^6/\mu l$)	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
W.B.C. (x $10^3/\mu l$)	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.

[2] Veterinary services "Normal hematological values" Lab Animals (1993)

Nutrient composition in experimental diets

The experimental diets were similar in nutrient composition, except for crude protein composed in both diets. Deficient and normal protein diet composed of 8.48% and 19.43% crude protein, respectively, as presented in Table 4.3.

Table 4.3 Proximate analysis of nutrient composition in the experimental diets, as dry matter basis

Nutrient composition	Dietary CP, %	
	8.2	18.2
Crude protein, %	8.48	19.43
Fat, %	9.51	9.98
Fiber, %	2.85	2.89
Ash, %	8.31	8.05
Calcium, %	1.20	1.09
Phosphorous, %	1.18	1.34
Moisture, %	9.27	9.08

Amino acid concentrations of two experimental diets

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 were presented in Table 4.4.

Table 4.4 Result of amino acid composition in the experimental diets (dry matter basis) using RP-HPLC (mg/100 mg)

Amino acids	Dietary CP, %	
	8.2	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 AA:lysine concentration ratio

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 with dietary protein deficiency ($P \leq 0.05$). Similar to essential amino acid, in sows fed with dietary protein deficiency, the plasma concentration of nonessential amino acid such as serine was decreased significantly ($P \leq 0.05$). Plasma concentrations of other amino acids were seemed to be unaffected ($P > 0.05$). However, plasma alanine was increased significantly in sows fed with deficient protein diet ($P \leq 0.05$). Data of plasma amino acid concentrations of sows were given in Table 4.5.

In piglets, only proline concentration in plasma was increased significantly ($P \leq 0.05$) from the group of sows fed with dietary protein deficiency compared to those fed with normal protein diet. Other plasma amino acid concentrations of piglets in both groups 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 4.6.

Table 4.5 Effect of dietary protein deficiency on individual plasma amino acid concentration in lactating sows[1] (pmol/μl)

Amino acids	Dietary crude protein (%)		P-value[2]
	8.2 (n=3)	18.2 (n=3)	
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 the means ± SD.

[2] Data are determined as statistical significance at $P \leq 0.05$ by Unpair T-test.

Table 4.6 Effect of dietary protein deficiency on individual plasma amino acid concentration in piglets[1] (pmol/μl)

Amino acids	Dietary crude protein (%)		P-value[2]
	8.2 (n=3)	18.2 (n=3)	
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 the means ± SD.

[2] Data are determined as statistical significance at $P \leq 0.05$ by Unpair T-test.

Effect of dietary protein deficiency on physical changes of sow mammary glands

The mammary glands of sows fed with deficient and normal protein diet were observed on d 14, d 18, and d 28 of lactation. The mammary glands of sows fed with deficient protein diet seemed to be smaller when compared to those fed with normal protein diet, especially on d 18 of lactation. The illustrative pictures of physical changes were shown in figure 4.1.

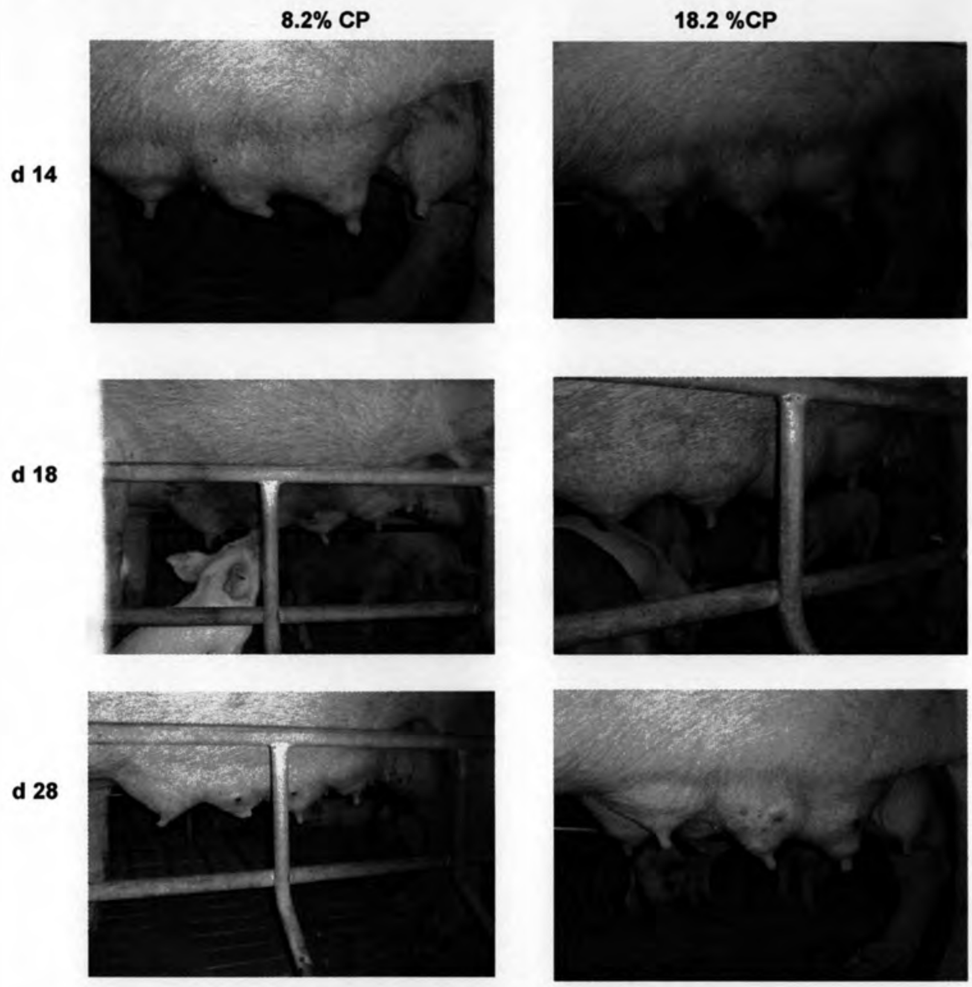


Figure 4.1 Comparison of physical changes of sow mammary glands between sows fed with deficient protein diet (8.2% CP) and normal protein diet (18.2% CP) on d 14, 18, and 28 of lactation. The mammary glands of sows fed deficient protein diet seemed to have smaller size than those of sows fed normal protein diet, especially on d 18 of lactation.

Effect of dietary protein deficiency on histological changes of sow mammary tissues

In sow mammary tissues of two groups, functional cell types were present, including connective tissues and secretory cells, comprising large and small alveolar lumens (Figure 4.2). Alveoli in sow mammary tissues in 2 groups were different. The connective tissues surrounded the alveolus of mammary tissues from sows fed with deficient protein diet were thicker than those from sows fed with normal protein diet. Additionally, the alveolar lumens in mammary tissues of sows fed deficient protein diet were smaller than those fed with normal protein diet.

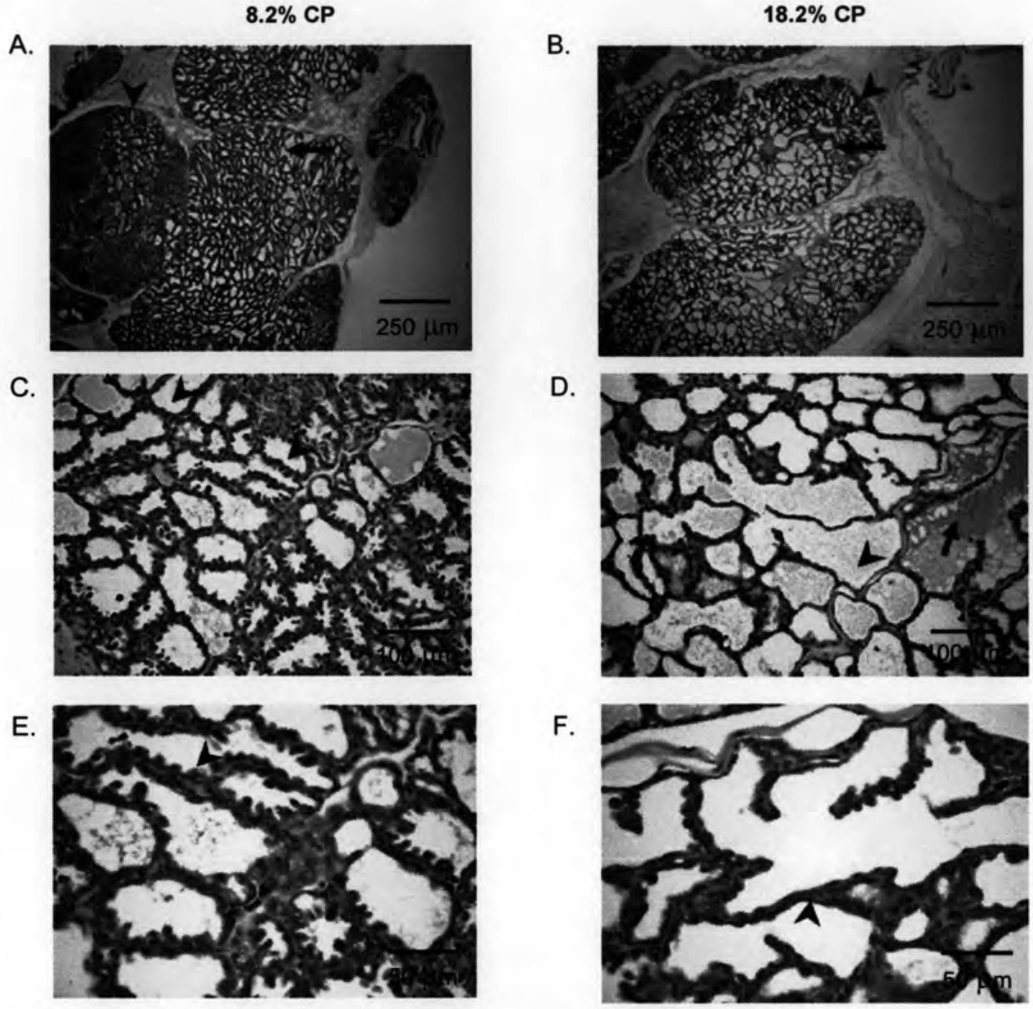


Figure 4.2 Comparison of histological changes of mammary tissues between sows fed with deficient (8.2% CP) and normal protein diet (18.2% CP)

A and B: The density of alveoli (arrows) composed in lobules (arrow heads) of mammary tissues in deficient group (A) seemed to have greater than those appeared in normal group (B). (Magnification X 40)

C and D: Arrow heads indicate alveolar lumen and arrow indicates milk droplet. The alveolar lumens in mammary tissues in sows fed with deficient protein diet (C) were smaller than those fed with normal protein diet (D). (Magnification X 100)

E and F: The connective tissues (arrow heads) surrounded the alveolus of mammary tissues in sows fed with deficient protein diet (E) were thicker than those in sows fed with normal protein diet (F). (Magnification X 200)

Expressions of mRNA amino acid transporters in porcine mammary tissues at peak lactation

Figure 4.3 was a representative picture of mRNA expression of amino acid transporter gene in porcine mammary tissues at peak lactation (d 18 of lactation). The porcine mammary tissues expressed mRNA of 18S rRNA (positive control), ATA2, $ATB^{0,+}$, LAT2, 4F2hc, and CAT2B, shown specific one band of 399, 164, 86, 200, 183, and 121 bp, respectively. Even though CAT2A which was used as negative control was not detected in porcine mammary tissues, it was expressed in pig liver tissues. The DNA product sizes of amino acid transporters were detected as expected size, according to GenBank information.

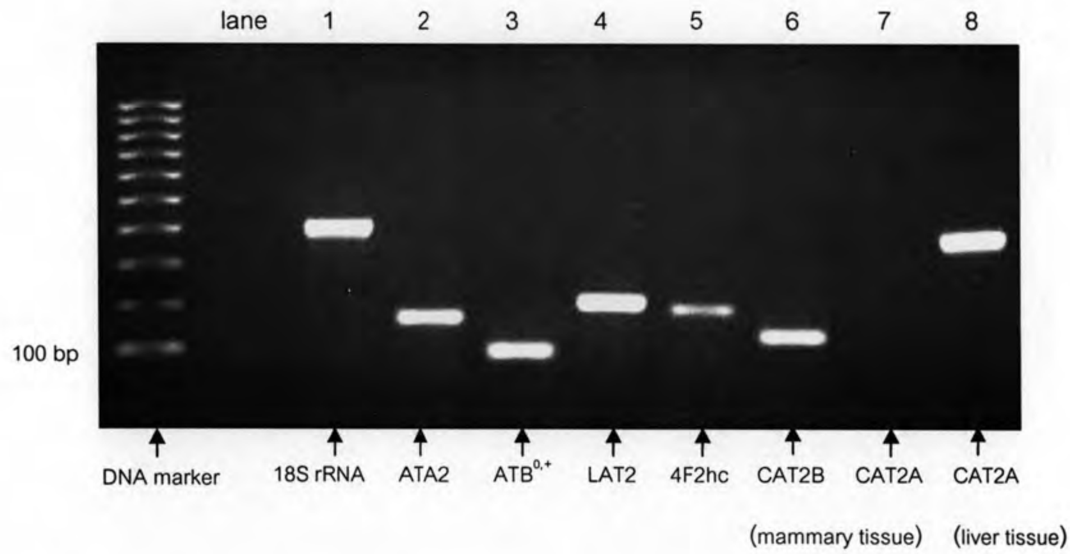


Figure 4.3 PCR amplification of sequences comprising cDNA from porcine mammary tissues by RT-PCR. Amplification products by 40 cycles were subjected to electrophoresis on a 1.8% agarose gel and visualized with ethidium bromide staining. The PCR amplified products were loaded at 18 µl/well. The mRNA of 18S rRNAs, ATA2, $ATB^{0,+}$, LAT2, 4F2hc, CAT2B expressed in porcine mammary tissues (lane 1 to 6) and CAT2A expressed in pig liver tissues (lane 8). CAT2A was used as negative control which did not expressed in porcine mammary tissues (lane 7). The figure was representative of 3 independent experiments.

Optimum cycles for semiquantitative RT-PCR

The PCR amplifications of 2 sample groups were conducted to determine the optimal number of PCR cycles of internal control and interested gene ratio that would enable detected PCR amplification within the linear range of the PCR reaction for both groups of sows fed deficient and normal protein diet. The data of preliminary were shown in Figure 4.4. In both deficient and normal protein diet groups, the optimum cycles of PCR reaction for ATA2, $ATB^{0,+}$, LAT2, 4F2hc, and CAT2B were 33 cycles of PCR reaction for these genes.

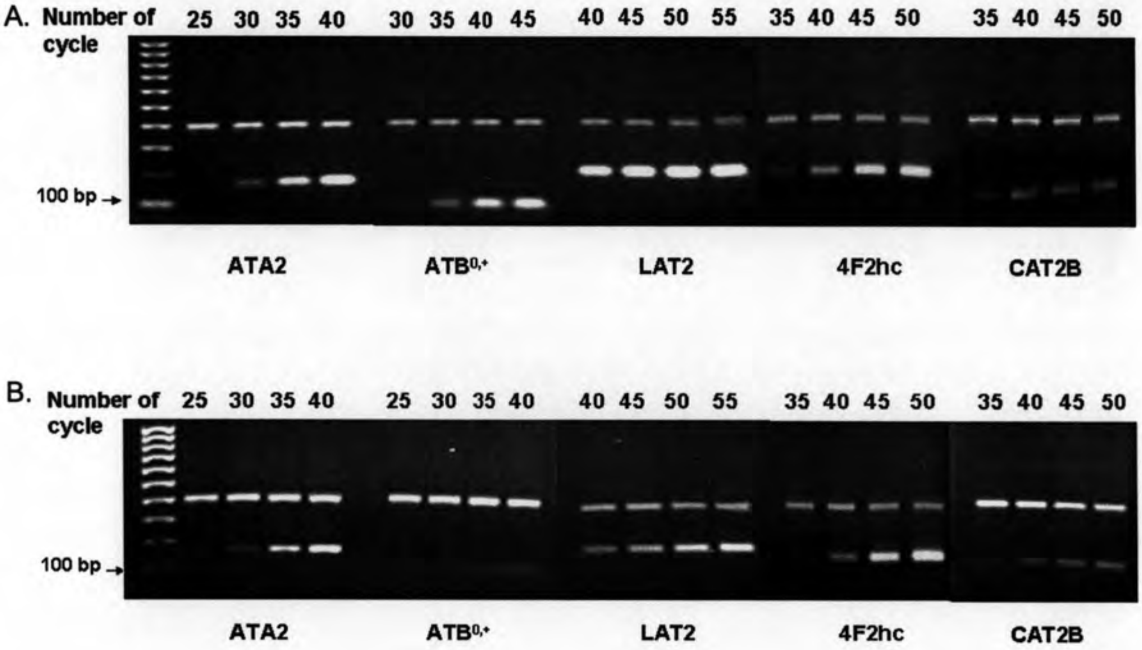
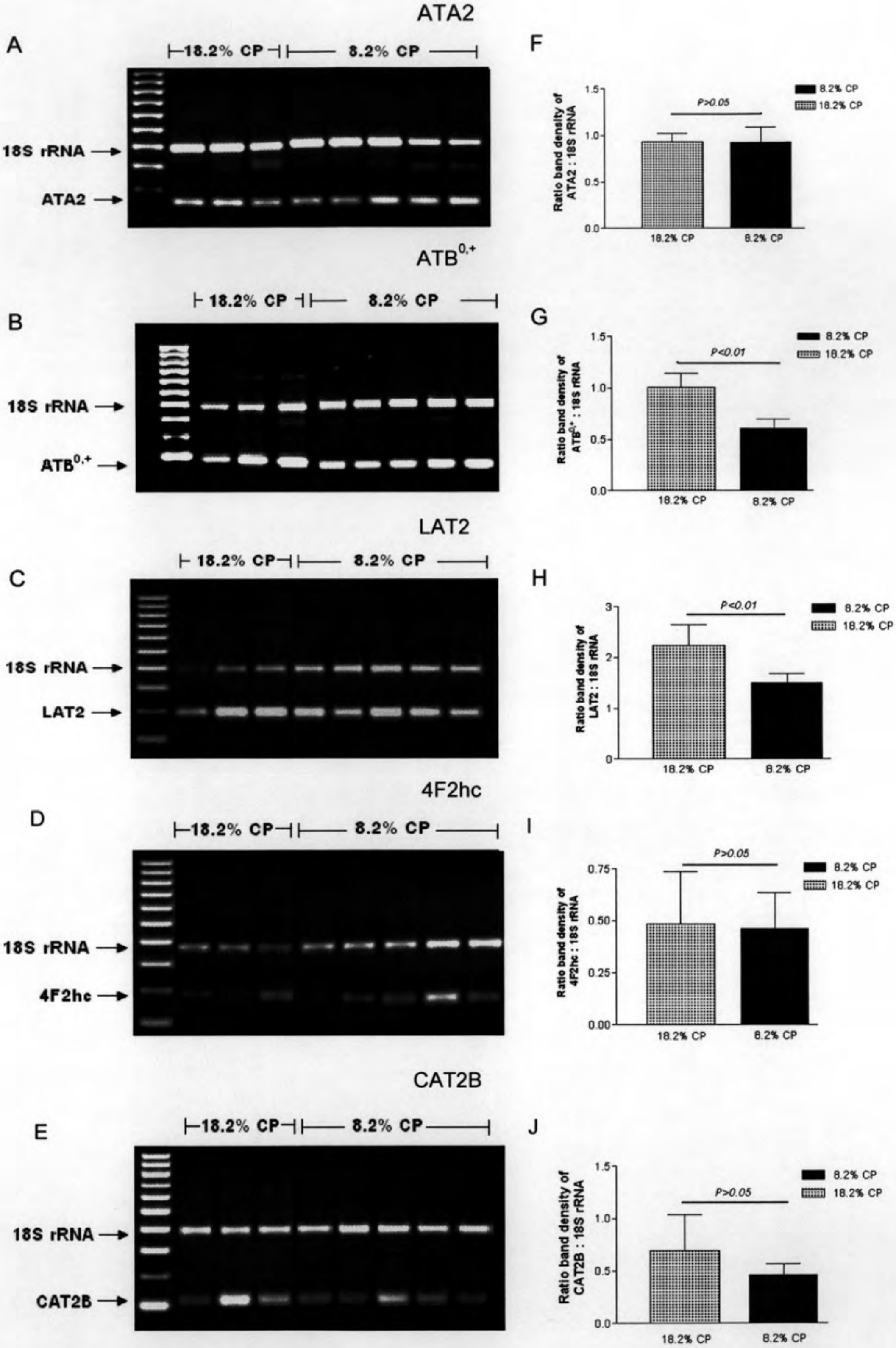


Figure 4.4 Determination of optimum cycles for PCR amplification of ATA2, $ATB^{0,+}$, LAT2, 4F2hc, and CAT2B gene in each experimental group. 18S rRNA was used as internal control. Amplification products were subjected to electrophoresis on 1.6% agarose gel in TEA buffer for 67 min at 70 volt. DNA bands were visualized with ethidium bromide. The PCR amplified products were loaded for 12 μl/well. The figure A and B showed the optimum cycles for PCR amplification of sow's mammary tissues in deficient and normal protein diet group, respectively.

The effect of dietary protein deficiency on relative mRNA abundance of some amino acid transporters in porcine mammary tissues at peak lactation

The mRNA isolated from mammary tissues of sows fed with deficient protein diet and those fed with normal protein diet was subjected to semiquantitative RT-PCR analyses to determine the effect of dietary protein deficiency on the mRNA expression level of amino acid transporters. Figure 4.5 showed the relative mRNA abundance of ATA2, $ATB^{0,+}$, LAT2, 4F2hc, and CAT2B which was representative of each experimental group. For sows fed with deficient protein diet, the relative mRNA abundance of $ATB^{0,+}$; system $B^{0,+}$ and LAT2; system L was decreased significant (P<0.01) when compared to sows fed with normal protein diet. In contrast, no difference in the amount of other amino acid transporters: ATA2; system A, 4F2hc; heterodimer of system L, and CAT2B; system y^{+} was observed in lactating mammary tissues between sows fed with two different protein diet groups (P>0.05).



ATA2
A
├18.2% CP ┤├ 8.2% CP ┤
18S rRNA
ATA2
F
Ratio band density of ATA2 : 18S rRNA
P>0.05
8.2% CP
18.2% CP
ATB0,+
B
├ 18.2% CP ┤├ 8.2% CP ┤
18S rRNA
ATB0,+
G
Ratio band density of ATB0,+ : 18S rRNA
P<0.01
8.2% CP
18.2% CP
LAT2
C
├ 18.2% CP ┤├ 8.2% CP ┤
18S rRNA
LAT2
H
Ratio band density of LAT2 : 18S rRNA
P<0.01
8.2% CP
18.2% CP
4F2hc
D
├18.2% CP ┤├ 8.2% CP ┤
18S rRNA
4F2hc
I
Ratio band density of 4F2hc : 18S rRNA
P>0.05
8.2% CP
18.2% CP
CAT2B
E
├18.2% CP ┤├ 8.2% CP ┤
18S rRNA
CAT2B
J
Ratio band density of CAT2B : 18S rRNA
P>0.05
8.2% CP
18.2% CP

Figure 4.5 Analysis of relative quantification of mRNA amino acid transporter by semiquantitative RT-PCR. Total RNA were isolated from sow mammary tissues of normal protein diet group (18.2% CP) and deficient protein diet group (8.2% CP). Semiquantitative RT-PCR reaction for amino acid transporter gene; ATA2, $ATB^{0,+}$, LAT2, 4F2hc, and CAT2B were performed using 18S rRNA as internal control. The gel figures were representative of 3 independent experiments. The amplification products were size-fractionated by 33 cycles on 1.8% agarose gels and the DNA bands were visualized by ethidium bromide staining. The PCR amplified products were loaded for 18 µl/well. The relative abundance of each transporter gene in both groups was showed as figure and bar chart, respectively. Data represent as the means ± SE. (n=8).

A and F: The relative mRNA abundance of ATA2; system A did not differ between two groups of sows fed with deficient and normal protein diet ($P>0.05$).

B and G: The relative mRNA abundance of $ATB^{0,+}$; system $B^{0,+}$ was decreased significant when compared to sows fed with normal protein diet ($P<0.01$).

C and H: The relative mRNA abundance of LAT2; system L was decreased significant when compared to sows fed with normal protein diet ($P<0.01$).

D and I: The relative mRNA abundance of 4F2hc; heterodimer of system L did not differ between two groups of sows fed with deficient and normal protein diet ($P>0.05$).

E and J: The relative mRNA abundance of CAT2B; system y^+ did not differ between two groups of sows fed with deficient and normal protein diet ($P>0.05$).