CHAPTER V

DISCUSSION

This present study provided the results on the effect of dietary protein deficiency on growth performance and individual plasma amino acid concentration in sows and their piglets as well as physical and histological changes and the relative abundance of some amino acid transporter gene expression in sow mammary tissues at peak lactation. The body weight losses throughout lactation period of sows were significantly increased ($P \leq 0.05$) in response to dietary protein deficiency. Piglets showed significant lower ($P \leq 0.05$) body weight at weaning day and average daily gain during late lactation period in group of sows fed with deficient protein diet (8.2% CP) when compared with those in group of sows fed with normal protein diet (18.2% CP). These results agree with previous study of Kirkwood et al. (1987) in that lactating sows often lost their body proteins to support milk production. An excessive protein loss results from a nutritional deficiency and consequently ultimate reduction of litter growth. Similarly, Guan et al. (2004b) found that sows fed with deficient protein diet (7.8% CP) lost their weight for 25.8 kg. Main effects of reduction in piglet growth including body weight at weaning day and average daily gain are sow's milk production. Severe protein restriction during lactation decreased milk production of sow (Jones and Stahly, 1999) and also decreased proteins composed in sow's milk (Guan et al., 2004b). However, milk production of sow is relative unaffected by short term of dietary protein restriction 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 d 0–18 of lactation, both body weight and average daily gain of piglets did not significantly change between 2 experimental groups ($P>0.05$). On the other hand, during d 18-24 of lactation, it is assumed as peak lactation (Trottier et al., 1997) in sows. An average daily gain of piglets in sows fed with deficient protein diet was decreased significantly ($P \leq 0.05$). This finding implied that sows can not buffer sufficient amino acids needed for high milk yield synthesis by mobilizing their body

proteins. Consequently, piglets did not receive sufficient nutrients including amino acids for body protein synthesis to support rapid growth.

However, the deficient protein diet was not likely to affect health of these sows as partially confirmed by the hematological values. There was no difference between experimental groups and the values were in the normal range.

The investigation of plasma amino acid concentrations may be used as an indicator to estimate plasma amino acid supply to mammary gland at peak lactation accounting from d 15-21 of lactation (Trottier et al., 1997). The plasma amino acid concentrations in sows fed with dietary protein deficiency, most essential amino acids were significantly decreased such as arginine, threonine, tyrosine, branched-chain amino acids (valine, isoleucine, and leucine), and phenylalanine compared to those fed with dietary protein sufficiency ($P \leq 0.05$). In contrast, most of plasma nonessential amino acid concentrations were remained constant in response to dietary protein deficiency. To explain this, it could be that the nonessential amino acids can be mobilized from the body protein, particularly skeletal muscle (Jones and Stahly, 1999). Further supported by Guan et al. (2004b), they found that the arterial plasma essential amino acid concentrations were lower in sows fed with deficient protein diet (7.8% CP) compared with those fed with normal protein diet (18.2% CP). Consequently, the uptake of these amino acids across mammary tissues was dramatically decreased. Thus, regulatory mechanism responsible for the uptake of amino acids across mammary tissues is two different physiological mechanisms; mammary plasma flow and amino acid transport systems in mammary tissues (Trottier et al., 1997). Dietary protein deficiency does not affect mammary plasma flow whereas arteriovenous differences of amino acid concentrations were decreased (Guan et al., 2004b) and may directly affect some amino acid transporters. Finally, they also found that protein composition in sow's milk were lower in sows fed with deficient protein diet (7.8% CP) compared with those fed with normal protein diet (18.2% CP).

Change of plasma amino acid concentrations in piglets was not affected by dietary protein deficiency, except proline that was significantly increased ($P \leq 0.05$), despite the fact that the piglets were received lower milk protein composition (Guan et al., 2004b). Therefore, this shows that piglets had enough capacity to compensate the plasma amino acid concentration so it did not affect their body weights and average daily gain as confirmed at d 0 until d 18 of lactation ($P > 0.05$) (Table 4.1). In according to increasing amount of plasma proline in piglets, proline can be synthesized from arginine catabolism in mammary gland. In addition, proline was considered as a conditional essential amino acid for young pig because of the necessary requirement in this period of life (Ball et al., 1986). Proline was 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 reasons explained why body weight and average daily gain of piglets were significantly decreased after d 18 until d 28 ($P \leq 0.05$) since the amino acids supplied in sow milk may be still not adequate to support rapid growth of piglets. It needs to be studied further on milk protein or amino acid concentrations during lactation in sows fed with normal and deficient protein diet. Then, this information will help to be completely confirmed the changes of body weight and average daily gain.

Moreover, visible physical change of sow's mammary glands fed with deficient protein diet showed smaller size than those fed with normal protein diet, especially at d 18 of lactation. One possible explanation is the insufficient amount 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 with deficient protein diet had less function than those fed with normal 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 seen in Figure 4.2. Therefore, these findings can explain the retarded growth of piglets in group of sows fed with protein diet deficiency. 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 (Kim et al., 1999).

The research study of gene expression in lactating porcine mammary tissue was done only by Laspiur et al (2004) who found that mRNA of CAT2B and $B^{0,+}$ expressed in porcine mammary tissues whereas mRNA of CAT2A did not. To explain the results of *in vivo* experiment, study on the molecular level was conducted to elucidate the mRNA expressions of some important amino acid transporters in porcine mammary tissues at peak lactation. In this present study, the porcine mammary tissues expressed mRNA of LAT2, 4F2hc, ATA2, $ATB^{0,+}$, CAT2B and 18S rRNA but not CAT2A. Since the mRNA of CAT2A expressed in pig liver tissues, this result confirmed that tissue samples used in the experiment were absolutely mammary tissues and the CAT2A primer designed was indeed corrected. Therefore, there is high possibility that lactating sow mammary tissues express these amino acid transporters which play an important role in amino acid uptake across mammary tissue. Christensen (1990) reported that amino acids do not permeate cell membranes and therefore require specialized transport proteins in order to cross the plasma membrane.

In relation to the significantly decrease of plasma concentrations of some essential amino acids in sows fed with deficient protein diet, the quantitative mRNA expressions of some amino acid transporters were also affected by dietary protein deficiency. The quantitative mRNA expressions of $ATB^{0,+}$; system $B^{0,+}$ and LAT2; system L were down regulated significantly ($P<0.01$) in response to dietary protein deficiency whereas the quantitative mRNA expressions of the other amino acid transporters did not differ between two experimental groups ($P>0.05$). The possible explanation why dietary protein deficiency could down regulate mRNA expressions of these two amino acid transporters; amino acid substrates of $ATB^{0,+}$ such as serine, histidine, threonine, alanine, tyrosine, phenylalanine and arginine; and LAT2 such as branched-chain amino acids (valine, isoleucine, leucine), it could be that amino acid substrates were significantly decreased in plasma of sows fed with deficient protein diet. Consequently, substrate availability of sow's mammary gland was decreased, which then may affect on

the decreasing of those amino acid transporter gene expressions. In contrast to mRNA expression of $ATB^{0,+}$ and LAT2, ATA2 of system A was not change in response to dietary protein deficiency because: 1) plasma amino acid concentrations of most substrates of system A such as methionine, glycine, proline were not significantly decreased in sows fed with deficient protein diet ($P>0.05$); 2) the substrates of system A can be transported by other amino acid transport systems such as system ASC (Baumrucker, 1985) that has not been studied in the present study and system $ATB^{0,+}$; 3) mammary secretory cells may need to remain the uptake of amino acids that are substrate of this system into cells to use in milk production metabolism. The same as quantitative mRNA expression of ATA2, CAT2B of system y^+ was not changed in response to dietary protein deficiency because mammary secretory cells may need to remain the uptake of amino acids that are substrate of this system such as arginine and lysine into cells to use in milk production metabolism. Trottier et al. (1997) reported that arginine and lysine were second and third greatest taken up by sow mammary tissues. Correspondingly, mRNA expression of 4F2hc; heterodimeric compound of system L substrates was not change in response to dietary protein deficiency because it may need to remain the expression level in mammary tissues to support activity of other amino acid transporters such as LAT1 (L-type amino acid transporter 1) known to be associated with 4F2hc for transport activity (Yanagida et al., 2001).

To summarize the results of this present study, dietary protein deficiency has the effects on greater body weight losses of sows, impairment of mammary gland conformation during lactation, decrease in plasma concentrations of essential amino acids, and down regulation of quantitative mRNA transporter gene expressions. Consequently, these effects are the important causes to retard growth performance of piglets such as body weight at weaned day. Additionally, this present study conducted on both *in vitro* and *in vivo*, is the first study on the effect of dietary protein deficiency on the expression level of amino acid transporter genes. Furthermore, the regulatory mechanism through manipulation of amino acid transporter gene expressions under sows fed with deficient protein diet can be approached. To the best of our knowledge, it is useful for further studies on amino acid transporter protein expressions in porcine

mammary tissues and/or dietary amino acid supplementation to optimize amino acid composition in sow's milk.