CHAPTER II

BACKGROUND INFORMATION

Anatomy and physiology of sow mammary gland

The number of teats in sow can vary between 6 and 20 (average=14). Sow's mammary tissues have two types of tissues based on its function; the parenchymal and stromal tissues. Mammary gland structure is illustrated in Figure 2.1.

1. Parenchymal tissues

The function of these tissues involves the milk synthesis such as;

- Alveoli

Alveoli exist at termination of lactiferous ducts. Milk is synthesized by the epithelial cells bordering the alveoli; these cells must perform at least three major functions in the synthesis of milk. First, it breaks down substrates by oxidation to provide energy for the synthetic reactions in the mammary gland. The second function is to synthesize the components of milk that are not found in blood, such as lipid, most of protein, and lactose. The third function is selectively absorbed blood precursors of milk constituents from the bloodstream (Tucker, 1987). Once synthesized, the milk is secreted into the alveolar lumen. The unique characteristic of sow's mammary gland is that the secretory tissue of each teat is separate and independent of the secretory tissue of each teat (Turner, 1952).

- Alveolar lumen

Alveolar lumen is an area in which synthesized milk is stored until suckling by piglets.

- Lactiferous duct

In each teat, there are two lactiferous ducts that branch into smaller ducts and ductules, which in turn terminate in the alveoli.

- Teat

The teat of sows contains two streak canals and two teat cisterns. Each teat cistern is continuous with a gland cistern. The contraction of muscle cells surrounding the alveoli enable the milk to pass from alveolar lumen into the ducts and then onto the tip of the teat.

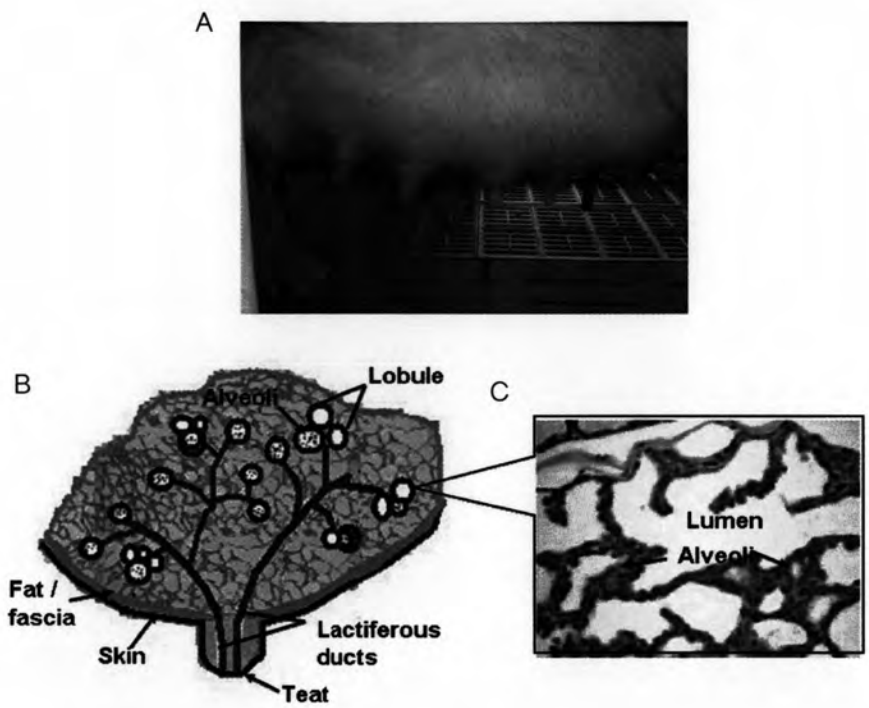

Figure 2.1 (A) The physical appearance of sow's mammary gland. (B) Sow's mammary gland structure composes of parenchymal and stromal tissues. (C) The histological structure of alveoli during milk synthesis.

2. Stromal tissues

The function of these tissues is to support mammary gland. Stromal tissues consist of adipose tissues and connective tissues.

Blood circulatory system of sow mammary gland

The anterior mammary glands of the sows receive blood from the caudal superficial epigastric arteries and the posterior mammary glands receive blood from the external pudendal arteries (Turner, 1952), as shown in Figure 2.2.

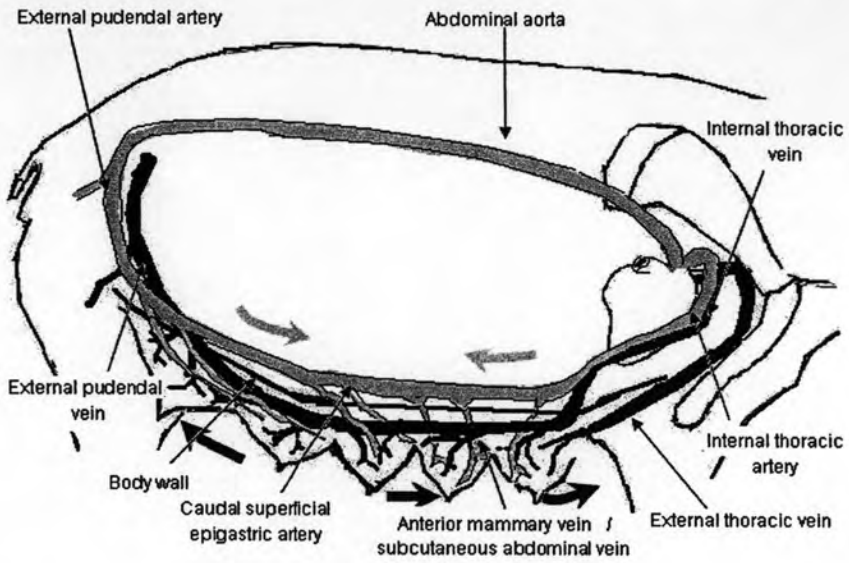

Figure 2.2 Blood supply of sow's mammary gland

(modified from Trottier et al., 1995)

Development of the mammary gland

1. Prepuberty

The mammary gland of newborn piglets is mainly composed of stromal tissue as the duct system is not well developed. Mammary gland growth does not really begin until after three months of age (Sorensen et al., 2000), when the rate of new mammary tissues accumulation increases five-fold and that of DNA four-fold. Thus, mammary growth during this period is rapid (Sejrsen et al., 1982).

2. During gestation and lactation

At puberty, the mammary gland comprises a major system of ducts with a number of terminal end buds (Turner, 1952). The mammary development is

slow during the first two third of pregnancy since almost all of the mammary tissue and DNA accumulation takes place during the last trimester (Hacker and Hill, 1972; Kensinger et al., 1982). Major changes in the mammary gland cells occur during the late pregnancy; adipose tissue is replaced by alveolar tissue, which will become the milk secretory tissue (Hacker and Hill, 1972). At day 75 of pregnancy, there is still no apparent secretion in the alveolar lumen, while between day 90 and 105 there is significant increase in secretion, indicating the start of lactogenesis (Kensinger et al., 1982). The amount of total DNA in mammary tissues increased significantly during gestation until day 4^{th} of lactation (Kensinger et al., 1982; Kim et al., 1999) and the secretory cells increased as day of lactation progressed. In addition, the amounts of total DNA in each suckled mammary gland increased 100% from day 5^{th} of lactation (728 ± 99 mg.) to a peak mean on day 21 of lactation (1,489 ± 100 mg.). These informations provide evidence that fat, both interlobular fat and cellular lipid, is replaced by parenchymal tissues and that the mass of mammary parenchymal tissues continue to increase during lactation. Furthermore, mammary tissues grow by elongation and branching of ducts into the mammary fat pad as day of lactation increased (Tucker, 1987). At the end of each lactation period, the mammary glands involute and alveolar development must therefore be reinitiated during the subsequent pregnancy.

Amino acid utilization in sow mammary tissues during lactation period

Dietary management of the lactating sow ensuring a sufficient diet in protein is important to achieve the ability of the sows to produce adequate milk for supporting the rapid growth of their litters and maintain the mammary gland growth (Kim et al., 1999). Although amino acids used in metabolic process to produce milk are derived largely from diets, a paucity of these amino acids is also derived from metabolized body protein (Jones and Stahly, 1999), especially from skeletal muscle (Guan et al., 2004b). Muscle protein mobilization is an adaptive response that enables a high level of milk production under conditions of dietary protein deficiency (Emma et al., 2005). For lactating sows fed with sufficient amino acid supplement, amino acids which are derived from the

digestive system, can be transferred to maintain peripheral tissues including mammary gland growth.

The availability of amino acids in the blood circulation to the mammary system is critical for optimizing milk production; however, factors yet to be studied, such as plasma flow and regulation of amino acid transport systems across mammary cells, may also influence the uptake rate. Trottier et al. (1997) suggested that approximately 188.5 g of essential amino acids were daily taken up by mammary glands, then 139.5 g was secreted as milk proteins, and the rest (49.0 g) was retained in mammary gland. About 14.3% (7.0 g/d) of these retained essential amino acids was used for structural protein synthesis and mammary cellular remodeling (Kim et al., 1999) in which the remaining 85.7% (36 g/d) may be transformed to other nonessential amino acids, or oxidized as energy, or other metabolic pathways (Richert et al., 1998). The essential amino acids were taken up across mammary tissues in excess of their quantitative excretion in milk. The order of uptake, from the greatest to the least, of the essential amino acids was leucine, arginine, lysine, valine, isoleucine, threonine, phenylalanine, tryptophan, histidine, and methionine (Trottier et al., 1997). However, some amino acids, especially for arginine and branched-chain amino acids, were significantly retained in mammary tissues at 16 and 11 g/d, consecutively. Therefore, it was apparent that the output of these amino acids appeared in milk would be less than quantitative uptake (Nielsen et al., 1997; Trottier et al., 1997). Interestingly, some nonessential amino acids, like alanine, proline, and glutamate, appeared in milk more than their quantitative uptake, indicating that the mammary gland may synthesize these specific amino acids in excess of cellular utilization. For example, arginine catabolism in mammary gland can be synthesized proline, glutamate, polyamine, and nitric oxide (NO). Since, proline is a major precursor for citrulline and arginine synthesis in enterocytes of neonatal pigs (Wu, 1997; Dillon et al., 1999), it may then imply that intestinal synthesis of citrulline and arginine can partially compensate for an arginine deficiency in sow's milk (O'Quinn et al., 2002). The nitric oxide, a major product of arginine catabolism, may play a crucial role in the regulation of mammary plasma flow and thus the uptake of plasma nutrients by the lactating mammary gland (Lacasse et al., 1996). Likewise, polyamines produced by mammary tissues regulate lactogenesis (Oka and Perry, 1974) and greatly contribute to

their relative abundance in sow's milk (Motyl et al., 1995). In addition, polyamines are essential for cell proliferation and differentiation. Milk-borne polyamine may be of nutritional importance for the growth and development of neonatal intestine (Reed et al., 2000). Neonatal pig has a low ability to synthesize proline (an essential amino acid for young pigs) (Ball et al., 1986; Wu et al., 1994). Similarly, branched-chain amino acids (BCAA) such as isoleucine, leucine, and valine were oxidized to produce energy in the lactating sow mammary gland. The greatest oxidation rate of the branched-chain amino acids is valine (Richert et al., 1998).

However, the quantity of the essential amino acids taken up across sow's mammary tissues was depended upon the lactating period. The essential amino acids were increasingly taken up by mammary gland as day of lactation increased (Trottier et al., 1997). The plasma amino acid uptake was calculated by multiplying arteriovenous difference of amino acid concentration by the daily mammary plasma flow. The daily mammary plasma flow was not different among lactation periods (Trottier et al., 1997). The average conversion coefficient was calculated to be 541.41 ± 35.72 L of plasma to produce 1 L of milk. The average daily plasma flow was calculated to be 4,275 ± 386 L. Conversely, amino acid arteriovenous differences were increased from day 11 to 20 of lactation and appeared to reach maximum during day 15 until 20 of lactation (peak lactation). The study by Trottier et al. (1997) indicated that increasing amino acid ateriovenous differences were affected by manipulation of amino acid transport systems in mammary tissues. Similarly, Neilsen et al. (2002) studied the effect of day of lactation on amino acid uptake by porcine mammary tissues, they suggested that milk production increased quadratically as day of lactation increased and appeared to reach maximum between day 15 and 21 of lactation. It was in agreement with results reported by Jones and Stahly (1999) and Kim et al. (1999). Therefore, it may conclude that the amino acid transporters in mammary tissues are affected by lactating period.

The 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., 2004b).

Effect of dietary protein concentration on amino acid uptake across the porcine mammary tissues at peak lactation

The level of dietary protein concentration is now recognized as an important factor that affects amino acid uptake across mammary tissues. The previous study by Guan et al. (2004a) reported that arterial plasma amino acid (except branched-chain amino acid) concentration quadratically increased as crude protein concentration was increased ($P<0.05 - P<0.01$) but reaching a maximum when the normal crude protein diet was used (18.2% CP); while the branched-chain amino acids increased linearly with increasing crude protein concentration ($P<0.05 - P<0.01$). Correspondingly, net uptake of essential amino acids also increased with increasing dietary crude protein. As dietary crude protein increased, arteriovenous difference increased for essential amino acids, reaching a minimum on the deficient crude protein diet (7.8% CP) but a maximum on the normal crude protein diet (18.2% CP), and decreased thereafter for sow fed the excess crude protein diet (23.5% CP). However, dietary crude protein concentration has no effect on mammary plasma flow. Therefore, it may imply that the porcine mammary gland responds to increase in crude protein concentration through changes in amino acid transport which may be caused by amino acid transport systems in mammary tissues. However, the mechanisms involved in modulating the transport of amino acids by mammary tissues are not known. Each mammary gland extracts amino acids from blood by specific amino acid transporters situated at blood-facing aspect of mammary epithelial cells, as reflected in mammary arteriovenous difference (Shennen et al., 1997). Substrate-induced uptake in mammalian cells has been demonstrated where increasing amino acid availability increases amino acid transport activity via transstimulation mechanism (Munir et al., 2000; Pan et al., 2002). Nevertheless, there are several mechanisms by which amino acid influx into cells and/or tissues can be inhibited such as: 1) high concentrations of intracellular amino acids can inhibit the additional uptake of extracellular amino acids, a process referred as transinhibition (Hyde et al., 2003; Palii et al., 2004); 2) high concentrations of extracellular amino acids can inhibit further uptake of those amino acids for which transport systems exhibit a low Michaelis constant (K_m) relative to the amino acid concentration (high affinity, low capacity), a process known as

saturation; and finally 3) high extracellular concentrations of certain amino acids can inhibit the uptake of other amino acids (Meier et al., 2002).

Effect of dietary protein concentration on amino acid transporters and amino acid transporter gene expressions in porcine mammary tissues at peak lactation

Ferraris and Carey (2000) studied the effect of amino acid available in intestine on the transport of amino acids across intestinal epithelium. Their results suggested that rates of amino acid transport were usually vary with dietary protein, decreasing rates with lower dietary protein, until minimum requirements of dietary protein concentrations were reached. Further decrease in concentration of dietary protein below minimum requirements was accompanied by increases in essential but not nonessential amino acid transport. The mechanism underlying changes in this nutrient absorption is specific the increased in site density of transporters in order to increase transport rates of their substrates. Additionally, dietary nutrients may act as signal elements and interact with promoter region of amino acid transporter gene. Consequently, the change in substrate affinity (K_m) or transport velocity (V_{max}), or turn over number of specific transporter, or synthesis of a new transporter type, or increasing abundance of amino acid transporter gene expression was found. During starvation or nutrient deficiency, the intracellular metabolic activity is stimulated thereby increasing the driving force for facilitative transport of the nutrients from the lumen or blood into the enterocyte. The results was further supported by the study of Gazzola et al. (2001) who studied on the effect of amino acid starvation on adaptive regulation of amino acid transport system A in cultured human fibroblasts. This unique characteristic of amino acid transport system A is named 'adaptive regulation' that means the capability to adapt its activity to the extracellular levels of the substrates.

From past to present, not many study on the effects of dietary protein deficiency on amino acid transporters and their gene expressions in porcine mammary tissues at peak lactation is available. Only one study had been conducted on amino acid transporter gene expressions in porcine mammary tissues during lactation period, using Northern blot analysis (Laspiur et al., 2004). The result showed that the mRNA of CAT1, CAT2B, $B^{0,+}$, and ASCT1 were expressed in porcine mammary tissues but not CAT2A.

Amino acid transport across mammary epithelial cells

All cells require a continuous supply of amino acids to meet their metabolic demands. A primary concern of animal nutritionists is the need to understand what the capacity for amino acid absorption is, in order that diets can be formulated to provide adequate, but not excessive, amino acids for a given production state including lactating production state. The literature is repleted with the characterization of amino acid transport systems that are expressed by laboratory animals and humans. By comparison, a few research works were conducted to identify the presumably analogous transport systems in farm animal species. The lack of knowledge regarding specific farm animal transporter physiology may be limiting the ability to formulate the diets and to design feeding strategies that optimize amino/protein requirement.

Although amino acid transport systems can mediate the passage of substrate across the membranes of mammary secretory cells, the routes of amino acids transport from the blood across mammary epithelial cells are generated by diffusion through membrane-spanning channels and paracellular pathway; facilitative transportation, endocytosis. The transporter-mediated process can transport substrates against substrate concentration gradients, in the term of 'active transportation' (Ganapathy et al., 1994). Active transportation derives the energy to translocate substrates across membranes by harnessing the difference in the transmembrane electrical and chemical gradients of substrates and (sometime) co-transported ions (Na^+, K^+, Cl^-, H^+) (Matthew, 2000). The transport process occurring at basolateral membrane (blood-facing membrane) is to translocate the substrates from blood to mammary secretory cells and the transportation occurring at apical membrane is to translocate the substrates from alveolar lumen to milk secretion.

Transporter theory

Transport systems generally are defined for protein which recognizes and transfers a selective group of substrates across cellular membranes, whether acting alone or in combination with other proteins as shown in Figure 2.3. Transport proteins allow the cells (or organelles) to selectively bind and acquire compounds from a milieu of other substrates. The physiological importance of transporters is usually discussed in

terms of their relative abilities to recognize and bind a substrate molecule (affinity), and the amount and rate of substrate translocation through membranes (capacity / velocity).

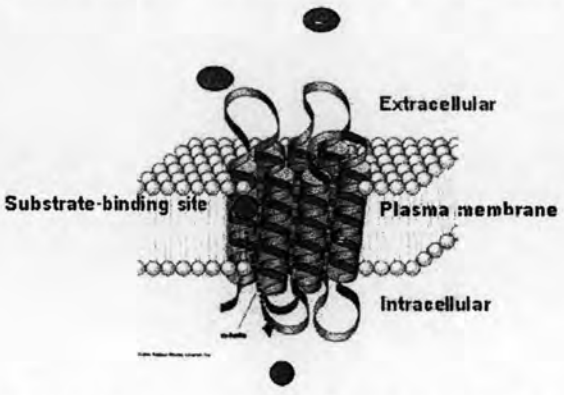

Figure 2.3 Illustrative picture of transporter protein locates on cellular membrane (modified from Yanagida et al., 2001)

Typically, transporters that demonstrate relatively low affinities for substrates have large capacities for transport, whereas those that display high affinities have low capacities. The general process of transporter-mediated passages through membranes is: 1) the substrates bind to the recognition domain of the transporter; 2) the substrates are translocated through the membrane into the cytoplasm; 3) the substrates dissociate into the cytosol; and 4) the substrates binding and translocation domains of the transporter are reoriented for further substrate binding (Ganapathy et al., 1994).

However, transportation of amino acids across cellular membranes occurs by multiple transport activities, often with overlapping substrate specificities for amino acids of the same and/or different class. For example, lysine and leucine are each recognized by at least four amino acid transport systems (D'Mello, 2003). For general transport activities in cells, more than one system may available to any amino acid then substrate can be competitively inhibited to other amino acids that are transported via the same transport system. This phenomenon could also be found in porcine mammary tissue (Jackson et al., 2000; Sarah et al., 2005).

Identification of amino acid transport systems in mammary tissue

The amino acid transport systems typically are categorized according to their substrate specificities, kinetics of absorption, and ion dependence (Christensen, 1990).

The systems that have been characterized based on ion dependence can be classified into 2 systems, namely Na^+–dependent and Na^+–independent transport system.

1. **Na^+–dependent transport systems**; that have been identified in mammalian mammary tissues are summarized in Table 2.1.

Table 2.1 The summary of Na^+–dependent transport systems recognized in mammary tissues

AA transport system	AA transporter gene	Substrate specific	Species	References
A	ATA1, ATA2, ATA3	Met, Ala, Gly, Pro, MeAIB	cattle, human, mouse, and rat	Baumrucker, 1985; Shennan et al., 1994; Sarah et al., 2005
ASC	ASCT1, ASCT2	Thr, Cys, Ser, Ala	cattle, swine, and guinea pig	Baumrucker, 1985; Mepham et al., 1985; Laspiur et al., 2004;
X^-_{AG}	EAAC1, GLAST, GLT1	L-Glu, L-Asp	rat	Millar et al., 1996
N	N	His, Gly, Asp	cattle and rat	Baumrucker, 1985; Carvert and Shennan, 1998
GLY	GLY	Gly	cattle	Baumrucker,1985
β	β	β-Ala, β-Tau	swine and rat	Shennan et al., 1994; Bryson et al., 2001
$B^{0,+}$	$ATB^{0,+}$	neutral and basic amino acids	swine	Laspiur et al., 2004

2. **Na^+–independent transport systems**; that have been identified in mammalian mammary tissues are summarized in Table 2.2.

Table 2.2 The summary of Na^+–independent transport systems recognized in mammary tissues

AA transport system	AA transporter gene	Substrate specific	Species	Reference
L	LAT1, LAT2, LAT3, LAT4, 4F2hc	neutral amino acids such as Met, Trp, Tyr, Phe, and BHC	cattle and guinea pig	Baumrucker, 1985; Shennan et al, 1997
y^+	CAT1, CAT2 (CAT-2B), CAT3, CAT-2a	Arg, Lys, Ort, His	cattle and rat	Baumrucker, 1985; Shennan et al., 1994
$b^{0,+}$	$b^{0,+}$	neutral and basic amino acids	rat	Shennan and Peaker, 2000
y^+L	y^+L	neutral and basic amino acids	rat	Shennan and Peaker, 2000

Functional characteristic of amino acid transport systems

1. **System A** (ATA; amino acid transporter A)

Principle amino acids reported to be transported by this system are short-chain neutral amino acids such as alanine, serine, glutamine, glycine, proline, and methionine (Baumrucker, 1985). Methionine is an important substrate for system A because it is often considered to be one of the limiting amino acid of maize-based rations, particularly when heated soybeans are the main protein source. In addition, methionine is involved in multiple pathways leading to synthesis of phospholipids, carnitine, creatine,

and polyamine (D'Mello, 2003). At the same time, methionine provides the methyl groups for a number of transmethylation reaction involved in regulation of DNA activity and it provides sulphur for cysteine synthesis that can occur in mammary gland (Guan et al., 2004a). Mouse and rat mammary tissue explants transport aminoisobutyric acid (AIB) via a pathway that is both Na^+-dependent and sensitive to methylamino-isobutyric acid (MeAIB), a characteristic of system A (Shennen and McNeillie, 1994). Similarly, the lactating bovine mammary gland also appears to express system A (Baumrucker, 1985). An important feature of system A is its activities regulated by a variety of factors such as amino acid starvation, hormones, and growth factors in many cell types (McGivan and Pastor-Anglada, 1994). For example, activities of system A increased from pregnancy to lactation in mouse mammary tissues (Sarah et al., 2005).

Recently, three subtypes of amino acid transport systems A, namely ATA1, ATA2, and ATA3 were reported (Sugawara et al., 2000). ATA1 expressed primarily in the rat brain and predominant expressed in the human placenta, heart, and brain (Wang et al., 2000). ATA3 expressed abundantly in the liver and smaller amount in the skeletal muscle. The interesting transporter that should be studied is ATA2. Unlike ATA1 and ATA3, ATA2 expressed ubiquitously in rat and human tissues (as shown in Figure 2.4) and ATA2 is represented as the classical system A (Sugawara et al., 2000). Moreover, expression of ATA2 has not been yet studied in porcine mammary tissues.

Figure 2.4 Northern blot analysis of ATA2-specific mRNA in human tissues

(Hatanaka et al., 2000)

2. **System L** (LAT; L-type amino acid transporter)

System L is a major nutrient transport system responsible for the Na^+-independent transport of neutral amino acids including several essential amino acids such as branch-chained amino acids; isoleucine, leucine, and valine (Yanagida et al., 2001). 2-aminobicycle-[2, 2, 1]-heptane-2-carboxylic acid (BCH) is used as a specific substrate test of system L (Shennen and Peaker, 2000). It has been demonstrated that LAT requires an additional protein, the heavy chain of 4F2 antigen (4F2hc), which forms a heterodimeric functional complex with the LAT protein (Yanagida et al., 2001) as shown in Figure 2.5. The branched-chain amino acids that are substrates of this system are very important for lactating sows. The previous study reported that the excess amount of branched-chain amino acids, about 30-80% above the requirement for milk protein synthesis was taken up by mammary gland because these amino acids are used as an energy source (Richert et al., 1996). In which valine had high oxidation rate of any amino acid in the mammary gland as evident in lactating sow (Trottier et al., 1997) and goat (Bequette et al., 1994). However, only a small fraction of the branched-chain amino acids uptake appears to be utilized strictly as an energy source because branched-chain amino acids play a large part as carbon and nitrogen donors for synthesis of nonessential amino acids, lactose, and/or lipid (Richert et al., 1998).

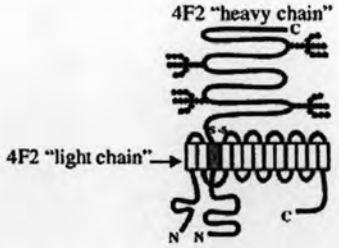

Figure 2.5 Model of 4F2hc

(modified from Bode, 2001)

Recently, the L-type amino acid transporter 1, 2, 3, and 4 (LAT1, LAT2, LAT3, and LAT4) were isolated (Kim et al., 2004; Bodoy et al., 2005). LAT1 mRNA is expressed only in restricted organs such as the brain, spleen, placenta, and testis (Kanai et al., 1998; Nakamura et al., 1999; Prasad et al., 1999; Yanagida et al., 2001). In contrast, the mRNA of LAT2 and 4F2hc are ubiquitously expressed in both embryonic and adult tissues (Kanai et al., 1998; Segawa et al., 1999; Yanagida et al., 2001). LAT1 prefers large neutral amino acids as its substrate whereas LAT2 transports not only large neutral amino acids but also small neutral amino acids. Thus LAT2 appears to have broader substrate selectivity than LAT1. For these reasons, LAT2 was chosen to conduct on this present study. LAT3, however, is expressed less widely and narrower substrate selectivity than LAT1 and LAT2. LAT4 was newly isolated from the epithelial cells of the distal tubule and the collecting duct in kidney (Kim et al., 2004).

3. **System $B^{0,+}$** ($ATB^{0,+}$; Amino acid transporter $B^{0,+}$)

System $B^{0,+}$ accepts both cationic and neutral amino acids as substrates. It functions as the exchanger for cationic and neutral amino acids in a Na^+ and Cl^- –dependent manner (Sloan and Mager, 1999). This system has been reported in mouse blastocysts, *Xenopus* oocytes, a human intestinal cell line, rabbit small intestine, rat pituitary gland, human lung, and porcine mammary tissues (Sloan and Mager, 1999; Laspiur et al., 2004). $ATB^{0,+}$ (amino acid transport $B^{0,+}$) was isolated as a member of system $B^{0,+}$. It is expressed in the lung, the salivary gland, the stomach, the pituitary gland, and the mammary gland (Closs, 2002). Similarly, Laspiur et al. (2004) found that $B^{0,+}$ mRNA expressed in porcine mammary tissues during lactation period as shown in Figure 2.6.

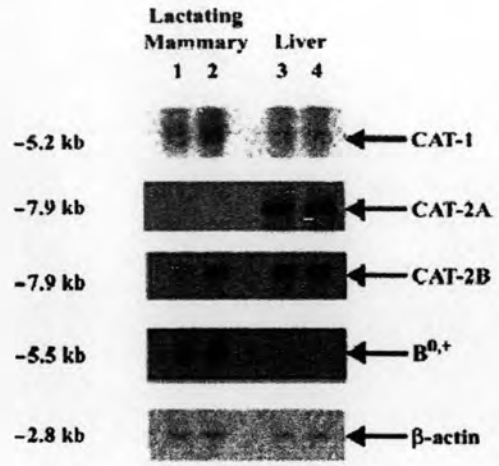

Figure 2.6 Northern blots of human CAT1, CAT-2A, CAT-2B, and porcine $B^{0,+}$ in porcine lactating mammary (lanes 1 and 2) or liver tissues (lanes 3 and 4)

(modified from Laspiur et al., 2004)

4. **System y^+** (CAT; cationic amino acid transporter)

System y^+ is known as the Na+-independent transport of the cationic amino acids arginine, lysine, ornithine and portion of histidine molecule that is positively charge. Its transport is not couple directly to transmembrane-driving ion gradients. The substrates mediated through this system can be accumulated against its concentration gradients because of difference between positive charge and the relatively negative charge on the cytosolic side of the membrane (D'Mello, 2003). However, lysine is the most important than other amino acid substrates of system y^+. Lysine is often the first limiting amino acid for lactating sows, particularly when corn and soybean meal are the main protein diet sources (Guan et al., 2002). In addition to lysine, arginine is also an important amino acid as a precursor for synthesis of proline (Ball et al., 1986), polyamine, nitric oxide, and other nonessential amino acid such as glutamate and glutamine (O'Quinn et al., 2002). Nevertheless, Baumrucker (1985) and Shennen et al. (1994) concluded that the cationic amino acids transport in bovine and rat mammary tissues was mediated via system y^+.

Currently, four cDNAs have been identified that encoded system y^+ activity, namely CAT1, CAT2A, CAT2B, and CAT3. Of these, CAT1, CAT2A, and CAT3 cDNAs encode high affinity cationic amino acid transporter (Closs et al., 1997). CAT1 mRNA is ubiquitously expressed in being present in all tissues tested except liver (Deves and Boyd, 1998). However, CAT3 expressed only significant amounts in the brain. Tissue expression of CAT2A was found in porcine liver but did not express in porcine mammary tissues (Laspiur et al., 2004). Therefore, CAT2A was used as a negative control in this present study. On the other hand, CAT2B is expressed in porcine mammary tissues at lactation period (Laspiur et al., 2004) as shown in Figure 2.6.