

Nutritional control of amino acid supply to the mammary gland during lactation in the pig

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Much of the progress in understanding the physiology of lactation and the control of nutrient supply to the mammary gland has been achieved in the dairy cow. This is partly due to the combined importance of efficient milk production and its use as a major dietary component for man. Another reason is the relative ease of using the bovine model compared with the pig with regard to handling and milking. The availability of equipment that permits accurate, easy and direct measurement of total milk output in the cow certainly facilitates lactational metabolism studies in that species. Unfortunately, it is still difficult to accurately estimate milk output in the sow. Consequently, there is a dearth of information relating physiological and metabolic mechanisms to lactational performance as a basis for advancing the study of sow nutrition. It is increasingly apparent that refinements in nutritional strategies are not keeping pace with production improvements made by geneticists.

Free amino acids in blood constitute the major precursors of milk proteins, whether provided from the dietary or body protein pool, although the mechanism of delivery of these amino acids to the milk-protein synthetic machinery remains a puzzling and challenging question. Recent advances in amino acid nutrition of the lactating sow suggest that an optimum dietary ratio of the essential amino acid valine relative to lysine maximizes milk production in sows nursing large litters (Pettigrew, 1993; Richert *et al.* 1996). While the availability of these amino acids in the blood to the mammary system is critical for optimizing milk production, factors yet to be studied, such as blood flow and regulation of amino acid transporter systems across mammary cells, may also influence the rate of uptake. The present paper will discuss factors involved in protein metabolism as they pertain to amino acid nutrition, and a new approach to dietary control of amino acid supply for milk synthesis. It is suggested that a better understanding of whole-body protein metabolism and the control of amino acid availability to, and utilization by, the mammary gland would provide an improved basis for estimation of amino acid requirements of the lactating sow.

PROTEIN AND AMINO ACID METABOLISM DURING LACTATION

Plasma amino acid dynamics

Little importance has been given to protein degradation and its importance in sustaining milk synthesis in the sow. Amino acid metabolism and inter-organ exchange dynamics are virtually unknown in the lactating sow and there is little information relating to the concentrations of circulating amino acids during lactation. Lactation causes a substantial increase in the rate of amino acid uptake from the blood by the mammary gland to support milk synthesis (Spincer *et al.* 1969). Consequently, total plasma α -NH₂-N concentrations decrease with day of lactation (Weldon *et al.* 1994) and this is particularly significant for the branched-chain amino acids and alanine (Tokach *et al.* 1992; Trottier & Easter, 1995). With advancing lactation, glutamine concentrations rise steadily (Trottier & Easter, 1995) along with urea-N (Tokach *et al.* 1992; Hultén *et al.* 1993; Rojkittikhum *et al.* 1993),

possibly reflecting a net release from muscle tissues and a concurrent increase in protein degradation rate respectively. This is supported by a recent study showing that the myofibrillar-protein degradation rate, as measured through 3-[²H₃]methylhistidine kinetics, increases from approximately 2.6 to 3.1% during the first and third week of lactation respectively (Trottier, 1995). Body composition at parturition, as influenced by gestation feeding levels, also affects the dynamics of protein degradation during lactation, where myofibrillar-protein breakdown rates vary between 3.3 and 3.0% in fat sows compared with lean sows respectively. Although the hormonal control of protein metabolism is essentially unknown, we have some evidence to suggest that the dynamics of protein metabolism vary with the stage of lactation, and body compositional changes may be influenced by insulin and glucagon. However, we can only speculate on the role of insulin and/or glucagon in the control of dietary amino acid delivery to the mammary system.

The role of insulin and glucagon

While insulin promotes the uptake of amino acids, in particular the branched-chain amino acids, by peripheral tissues, it has no effect on the mammary gland. Several studies have shown that the uptake of amino acids, glucose, β -hydroxybutyrate and triacylglycerols is independent of insulin in the bovine mammary gland (Laarveld *et al.* 1985; Brockman & Laarveld, 1986). The role of insulin during lactation, therefore, is unclear. Advancement of lactation in the sow is characterized by an increase in milk production capacity coupled with a decrease in circulating insulin concentrations (Trottier, 1995). Insulin concentration has been shown to be negatively correlated with sow lean tissue loss and litter-weight gain (White *et al.* 1984; Tokach *et al.* 1992). This was demonstrated previously by Reynolds & Rook (1977) who found that insulin concentration was inversely proportional to milk yield and lactose content. Administration of insulin decreases milk production in the dairy cow (Kronfeld *et al.* 1963), the ewe (Leenanuruksa *et al.* 1988) and the sow (Goldobin, 1976). The decrease in plasma insulin concentration with advancing lactation may be the result of a combination of factors, such as: (1) lower blood glucose, as observed in the lactating sow, (2) decreased β -cell mass (Marynissen *et al.* 1983), (3) decreased glycaemic stimulus to the pancreas (Madon *et al.* 1990), (4) increased removal of the hormone by the mammary tissue (Jones *et al.* 1984), as observed in the lactating rat. This latter factor suggests a role for insulin in mammary tissue.

A role for insulin in mammary gland function may be substantiated by the presence of specific receptors for insulin in the mouse, rat and bovine mammary tissue (Oscar *et al.* 1986). This has yet to be studied, however, in the porcine mammary gland. Alternatively, since insulin has been shown to up-regulate prolactin receptors in mammary tissue, the presence of insulin receptors may be specific for this function only. Interestingly, Rojkittikhun *et al.* (1993) have shown that heavier sows losing more weight tend to have higher plasma prolactin concentrations. It is also possible that high concentrations of insulin may down-regulate its own receptor in mammary tissue. In the rat, insulin receptor numbers on adipocytes remain unchanged (Flint *et al.* 1979). In addition, no difference in insulin-binding activity on adipocytes, as measured by tyrosine kinase (*EC* 2.7.1.112) activity, was observed between lactating and non-lactating rats (Burnol *et al.* 1990). Therefore, since receptor number and binding activity in peripheral tissues remain unchanged during lactation and circulating insulin concentrations decrease, this may allow a shift of nutrients towards mammary tissues rather than peripheral tissues. In sheep, the ability of adipose tissue to respond to insulin decreases during lactation, as shown by a decreased utilization of glucose and acetate in response to an insulin surge (Vernon &

Taylor, 1988). Similarly, lean tissue response to insulin, as measured by arterio-venous (A-V) difference of glucose across the hindlimb after an insulin infusion, appears to be lower in the lactating rat than in the non-lactating rat (Vernon, 1989).

Regression analysis by Pettigrew *et al.* (1993) showed that insulin concentration was related to milk protein (r 0.42), fat (r 0.39) and lactose (r 0.36) in the sow, indicating that insulin may account for a significant proportion of the plasma substrate uptake by the mammary gland during milk production. High circulating glucagon, coupled with decreased circulating insulin concentrations stimulates glycogenolysis and gluconeogenesis. In contrast to the increase in high-affinity insulin receptor numbers (Oscar *et al.* 1986), mammary tissue in the rat lacks glucagon receptors (Robson *et al.* 1984). This is of great significance in keeping the mammary gland in an 'anabolic status'. Both plasma glucagon and insulin concentrations are elevated during the early post-partum period, and their concentrations may be affected by the body composition of the sow (Trottier, 1995). While insulin decreases with advancing lactation, glucagon concentration decreases in lean sows and increases in fat sows, although the molar ratio, insulin : glucagon decreases in both fat and lean sows, thus contributing to the increase in body protein breakdown. Lower insulin concentrations are also associated with high cortisol concentrations (Baidoo *et al.* 1992), contributing further to protein degradation rate.

AMINO ACID DELIVERY TO THE MAMMARY GLAND

Blood flow and milk production

While amino acids are supplied to the arterial circulation through either dietary intake or body protein mobilization, the efficiency of delivery to the mammary system is dependent on blood flow and the efficiency of amino acid carrier systems across the mammary cellular membranes. As lactation advances, the A-V difference for lysine remains constant, with an increase in extraction rate and a parallel linear decrease in arterial lysine concentration (Trottier *et al.* 1997). The increased extraction rate is indicative of increased blood flow and/or an increased rate of uptake at the mammary cellular level. The effect of nutrition or genetics on blood flow and receptor regulation is unknown.

For many years it has been known (Linzell, 1960) that there is a close correlation between udder blood flow and milk yield in the goat in which blood flow rises at parturition and increases steadily as lactation advances. The relationship between direct blood flow and milk yield in the lactating sow has not been studied. In using an indirect approach, the Fick method for estimating blood flow in the lactating sow, Trottier *et al.* (1997) found that blood flow was correlated with milk yield. In turn, milk yield was positively correlated with A-V differences of all essential amino acids. This supports the earlier suggestion by Pettigrew *et al.* (1993) that total nutrient supply to the mammary gland (nutrients concentration \times blood flow), rather than only metabolite concentrations, would be more closely related to milk yield and lactational performance, since milk protein, fat and lactose are poorly related to their concentrations in plasma.

Blood flow and methodological difficulties in the lactating sow

Accurate measurement of blood flow to the mammary gland would contribute immensely to the understanding of lactational physiology and nutritional control of amino acid supply for milk synthesis in the pig. The biggest impediment to direct blood flow measurement in the porcine mammary system is the anatomical and vascular complexity. While the mammary circulatory system of the ruminant is localized in the inguinal region, the pig

circulatory system is multiple, extending from the xiphisternum to the groin (Linzell, 1974). The porcine mammary system can be divided into three regions, i.e. inguinal, abdominal and thoracic (Bourdelle & Bressou, 1964). In the sow, the external pudendal artery runs downward and descends through the inguinal canal. At this point, in contrast to the bovine mammary system, it divides into three main branches, i.e. the lateral, middle cranial and medial cranial of the external pudendal artery. These branches are also called lateral, middle cranial and medial cranial branches of the caudal superficial epigastric artery. These branches extend from the inguinal to the mid-abdominal region, carrying blood for up to four pairs of posterior mammary glands rather than the whole mammary system. In the cow, the external pudendal artery becomes the main mammary artery, running from the posterior to anterior gland regions, hence supplying nutrients to the whole mammary system. In contrast to the sow, the pudendal artery branches off into the lateral basal and the medial basal arteries just before leaving the anterior mammary glands and entering the abdominal region, and the lateral basal branch of the main mammary (pudendal) artery anastomoses with deep branches of the cranial epigastric artery. In the sow, despite multiple dissections of the vascular system, it is still unclear as to how, where and which of the branches of the pudendal artery anastomose with the cranial epigastric artery.

The venous drainage is also puzzling and does not offer an alternative approach to direct measurement of blood flow, since anatomical evaluation of the circulatory system suggests that blood leaves the mammary glands using two different pathways (Trottier *et al.* 1995). The anterior glands are drained through two large longitudinal mammary veins, also referred to as the subcutaneous abdominal veins. These veins run parallel on each side of the line of mammary glands. Dissection of the mammary system showed that they are readily identifiable, running along in the *plica lateralis* (Trottier *et al.* 1995). These vessels drain into the internal thoracic vein, while some other branches drain into the external thoracic vein. It has been suggested that blood leaves the posterior mammary glands by way of the same subcutaneous abdominal vein found in the anterior region and drains into the external pudic vein (Bourdelle & Bressou, 1964; Linzell, 1974), thus implying two different regulatory flow systems. In ruminants, because the mammary glands are inguinal, the vasculature is simplified and only the caudal superficial epigastric vessels drain the mammary tissue. Virgin female and male ruminants resemble the lactating sow, since blood drains forwards into the cranial vein in the anterior and abdominal region of the abdomen and backward (cranial to caudal) into the caudal vein in the inguinal region. During mammary gland development and lactatogenesis, the posterior vein enlarges progressively upon the increase in flow of blood entering the inguinal region, and consequently valves become incompetent. As a result, the blood flows entirely from the inguinal region toward the cranial superficial epigastric vein. This facilitates both measurement of nutrient output and flow rate in both the lactating goat and the cow. This developmental process does not seem to occur in the pig.

SUPPLY OF AMINO ACIDS TO THE MAMMARY SYSTEM: DIETARY CONSIDERATION

Practical dietary approach

Pettigrew (1993) has described extensively a factorial approach to the estimation of dietary requirements based on litter growth rate and the pattern of amino acids relative to lysine contained in milk, thus aiming at an 'ideal' protein estimation. Current production trends imposed on lactating sows and the resulting lactation response to feeding crystalline amino acids indicate that key amino acids other than lysine may be critically needed during

lactation. In a recent cooperative experiment, Knabe *et al.* (1996) suggested that the addition of crystalline lysine alone, more than 7.5 g lysine/kg, to a 135 g crude protein ($N \times 6.25$)/kg diet, would not improve litter performance, possibly due to other limiting amino acids. This strengthens the importance of formulating lactating-sow diets based on an ideal profile. In a recent study by Coma *et al.* (1996), adult sows nursing ten-pig litters with an average total growth of 2.2 kg/d required 55 g lysine/d to minimize plasma urea-N (PUN) concentrations, with sows consuming 66 g valine/d. The method used by Coma *et al.* (1996) assumes that N balance is maximized and body protein catabolism minimized when PUN reaches a constant minimum concentration. They estimated that 55 g lysine was required daily to minimize protein catabolism and maximize lactational performance. This value is extremely close to the modelling estimate by Pettigrew (1993). In another recent study, incremental amounts of dietary valine resulting in valine : lysine values from 83.3 to 127.8 (7.5–11.5 g valine/kg) resulted in a positive linear litter-growth-rate response (Richert *et al.* 1996). This may not indicate that valine is a limiting amino acid since parallel increases in PUN were observed, suggesting that valine was being oxidized rather than incorporated into proteins. Increased performance response to increased valine may thus be related to some property of valine itself other than as a 'limiting' amino acid.

Higher dietary ratios of amino acids relative to lysine are now proposed, as opposed to using the values initially observed in milk. This is particularly the case with valine and to some extent leucine. The role of the mammary gland in terms of physiological and nutritional demand *per se* has not been previously considered. It is becoming more apparent that an understanding of this missing link is essential for devising a biological basis for dietary recommendations and in building mathematical models to predict amino acid requirements.

Effect of nutrition on mammary amino acid uptake

Amino acids are made available to the mammary gland via the arterial supply, mostly as free entities in plasma and erythrocytes. However, evidence from the goat suggests that peptide-bound amino acids may contribute to the pool of amino acids found in milk casein (Backwell *et al.* 1994). In addition, L. Hoffmann, N. L. Trotter, B. J. Bequette, T. T. Nielsen and R. A. Easter (unpublished results) have demonstrated that plasma free leucine uptake by the mammary gland is insufficient to account for all leucine present in milk casein.

The relationship between A-V difference of essential amino acids across the mammary gland and arterial concentration of these amino acids (Fig. 1) demonstrates that the relative amino acid A-V differences are a function of their relative concentration in the arterial plasma. For example, A-V difference for leucine is the highest, as reflected by leucine concentration in the arterial plasma. The question of whether a similar relationship would be obtained through dietary alteration of each individual amino acid remains. We have found that the A-V difference for lysine increases quadratically with an increase in arterial lysine concentration (Fig. 2). The inflexion point may correspond with the requirement necessary for milk protein synthesis, and a further increase in arterial lysine concentration, hence from the diet, does not result in a further increase in mammary uptake. The region of linear increase would correspond with the limiting lysine status. On the other hand, no relationships between arterial concentration and A-V difference were observed for the other essential amino acids such as leucine (Fig. 3) and valine (Fig. 4), suggesting that these amino acids are not readily limiting in milk synthesis and that mammary gland uptake rapidly reaches saturation.

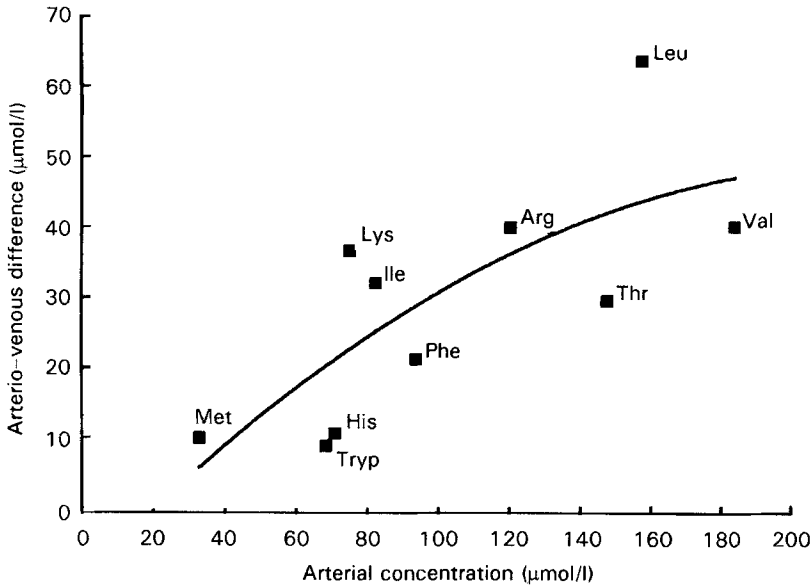


Fig. 1. Relationship between amino acid arterial concentration and arterio-venous difference (r^2 0.57). Data are overall average lactation arterial concentration and arterio-venous difference for each essential amino acid. (From Trottier *et al.* 1997.)

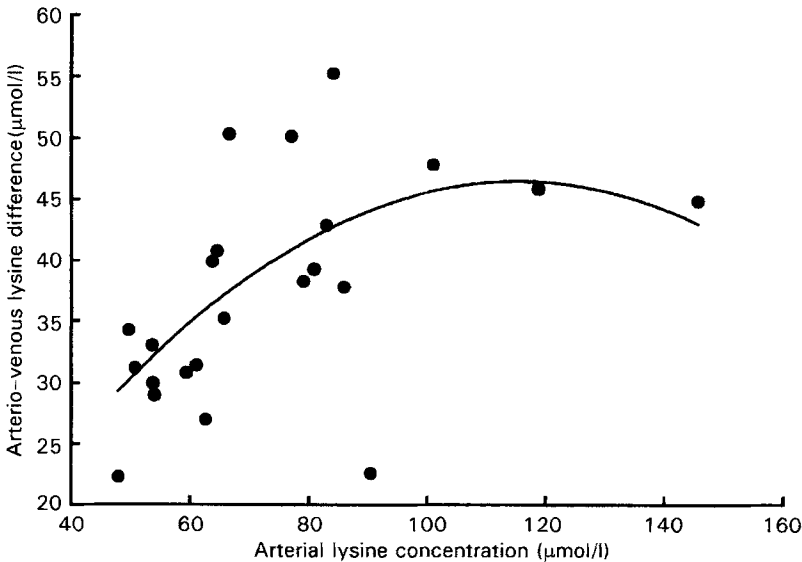


Fig. 2. Relationship between lysine arterial concentration and arterio-venous lysine difference (r^2 0.32). Data from an individual sow sampled at day 11, 14, 17 and 20 of lactation. Arterio-venous lysine difference represents arterial concentration of lysine minus mammary venous concentration of lysine. (From Trottier, 1995.)

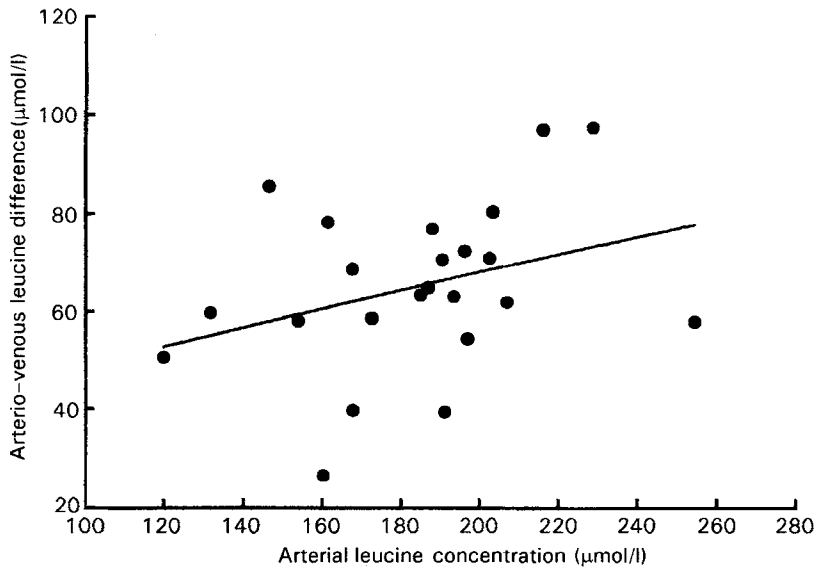


Fig. 3. Relationship between leucine arterial concentration and arterio-venous leucine difference (r^2 0.10). Data from an individual sow sampled at day 11, 14, 17 and 20 of lactation. Arterio-venous difference represents arterial concentration of leucine minus mammary venous concentration of leucine. (From Trotter, 1995.)

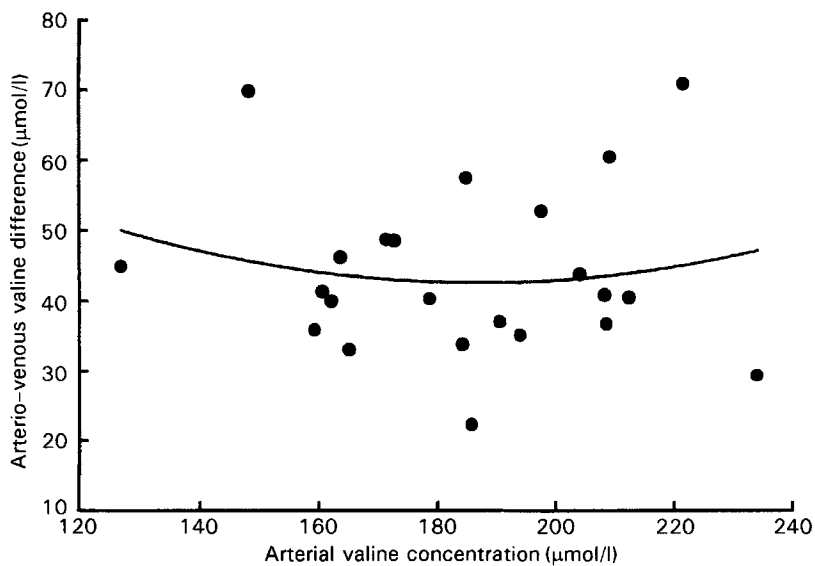


Fig. 4. Relationship between valine arterial concentration and arterio-venous valine difference (r^2 0.02). Data from an individual sow sampled at day 11, 14, 17 and 20 of lactation. Arterio-venous difference represents arterial concentration of valine minus mammary venous concentration of valine. (From Trotter, 1995.)

Table 1. *Amino acid (AA) pattern relative to lysine in sow milk and in mammary uptake pool*

AA	Milk AA : lysine	Mammary AA uptake : lysine	
		Trottier <i>et al.</i> (1997)	Nielsen <i>et al.</i> (1997)
Arginine	0.66	1.25	1.09
Histidine	0.40	0.30	0.28
Isoleucine	0.55	0.76	0.79
Leucine	1.15	1.50	1.48
Lysine	1.00	1.00	1.00
Methionine	0.26	0.28	0.26
Phenylalanine	0.55	0.65	0.61
Threonine	0.58	0.68	0.64
Valine	0.73	0.88	0.89

Table 1 shows that amino acids are taken up in very similar proportion relative to lysine, whether dietary lysine is 6.3 g/kg (Trottier *et al.* 1997) or 9.0 g/kg (Nielsen *et al.* 1997), with the exception of arginine. Higher lysine levels may decrease mammary arginine uptake through an increase in mammary arginase (*EC* 3.5.3.1) activity, although the presence of an arginase enzyme in porcine mammary tissue has never been investigated. The discrepancy observed between the amino acid pattern in milk and amino acid uptake pattern by mammary tissue relative to lysine gives rise to new thoughts on the estimation of dietary amino acid requirements. The mammary amino acid uptake pattern represents a combination of both independent mammary metabolism needs or functions and milk amino acid output. Thus, it is likely that the required amino acid profile for lactating sow would better resemble the amino acid pattern of mammary uptake rather than the amino acid pattern of milk.

Mammary amino acid retention

Amino acid uptake exceeds quantitative excretion in milk, especially for branched-chain amino acids and arginine (Trottier *et al.* 1997). Of the total 188.5 g essential amino acids taken up by the sow mammary gland daily, 49 g are retained, accounting for about 25 % of the total uptake (Trottier *et al.* 1997). This significant 'mammary retention' may indicate specific requirements at the mammary cellular level *per se*, although excess leucine uptake in the bovine mammary gland has been suggested to be the result of a counter-current uptake mechanism between the erythrocytes and the mammary cells (Hanigan *et al.* 1991). Amino acids that 'appear' to be taken up in excess of their output in milk may be 'biologically required' by the mammary gland. This assumption would imply that the mammary gland is not in a steady-state with regard to protein synthesis.

Retention may be divided into two distinct components: maintenance and metabolic functions. For example, mammary amino acid maintenance requirement may be estimated from regression of mammary amino acid uptake *v.* daily milk production (Fig. 5) by fitting the ordinate to zero. The difference between retention and maintenance values may indicate the potential use of amino acids for other metabolic functions (Table 2). It has been suggested that the accumulation of amino acids by the mammary gland in the cow and goat may stimulate the synthesis of non-essential amino acids, functional and structural proteins, and provide energy available for lactose and fatty acid synthesis (Spire *et al.*

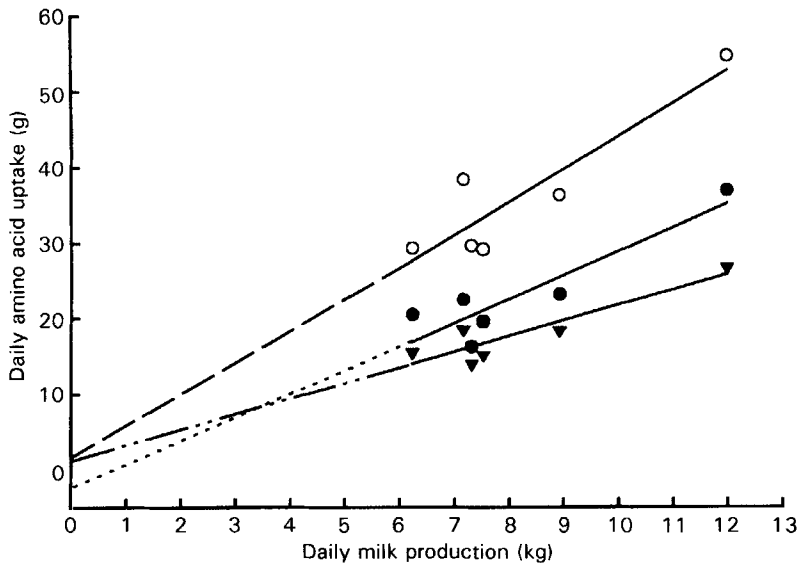


Fig. 5. Relationship between daily milk production and daily uptake of leucine (○), lysine (●) and isoleucine (▼) by the porcine mammary gland. (---), Leucine; (.....), lysine; (-·-·-·-), isoleucine; each indicates extrapolation to the y intercept and estimate of mammary maintenance requirement of leucine, lysine and isoleucine respectively, when daily milk production equals zero. Regression equations are: mammary leucine uptake (g/d) = $4.41x + 0.34$, r^2 0.83; mammary lysine uptake (g/d) = $3.22x - 3.00$, r^2 0.82; mammary isoleucine uptake (g/d) = $2.10x + 1.18$, r^2 0.83. Maintenance requirement for leucine, lysine, and isoleucine is estimated at 0.34, -3.00, and 1.18 g/d respectively.

1975). Isoleucine is a putative source of energy required for milk biosynthesis (Hanigan *et al.* 1991). Leucine metabolites such as α -ketoisocaproate and isovalerate, and β -hydroxy- β -methyl butyrate have been shown to increase milk fat secretion when fed to sows (Nissen *et al.* 1994). Valine, which is a strictly glucogenic amino acid, cannot be incorporated into lipids. Because gluconeogenesis occurs predominantly in the liver and kidney tissues, and glucagon receptors are absent in mammary tissue, at least in the rat, the use of valine as a C source for *de novo* mammary glucose synthesis is unlikely. Biologically speaking, the mammary gland is an 'anabolic' organ, while gluconeogenesis occurs in a catabolic environment.

Although the fate of essential amino acids in the lactating mammary gland has stimulated much interest, the actual control of their uptake and their metabolic fate remain uncertain. Because the mammary gland is a unique entity in which active cellular

Table 2. Daily mammary retention, maintenance requirement estimates, and utilization for metabolic functions of selected amino acids

Amino acid	Retention* (g/d)	Maintenance† (g/d)	Metabolic functions‡
Leucine	10.71	0.34	10.37
Lysine	0	-3.00	3.00
Isoleucine	6.24	1.18	5.06
Threonine	3.40	0.96	2.44
Valine	5.42	5.81	-0.39

* Data from Trotter *et al.* (1997).

† Obtained from regression of mammary amino acid uptake *v.* daily milk production.

‡ Obtained by difference between retention and maintenance requirement estimates.

breakdown and remodelling occur continuously, research is needed to assess the energy costs and control of amino acid requirements associated with mammary gland maintenance during lactation.

SUMMARY

The paucity of data relating to lactation physiology of the sow has frustrated researchers in estimating nutrient needs for production and mammary maintenance functions. The nutritional control of amino acid supply for milk synthesis is influenced by factors that have yet to be measured, such as blood flow and amino acid contribution from the body protein pool. The interaction or role of hormones such as insulin, glucagon or prolactin in amino acid dynamics and inter-organ exchange during lactation in the sow, are not well understood. The discrepancy existing between milk and mammary amino acid uptake profiles relative to lysine may be indicative of mammary metabolism and possibly maintenance requirements for specific amino acids. Hence, amino acid metabolism in the mammary gland, regardless of arterial blood substrate supply, may play an important role in a factorial approach to determining requirements. Mammary amino acid uptake ratios rather than milk amino acid ratios should provide a better tool to estimate amino acid requirements relative to lysine. Although lysine has typically been limiting in maize-soyabean-meal-based diets fed to lactating sows, current production trends are bringing a new dimension to the formulation of lactating-sow diets. Other amino acids may become limiting if dietary crystalline lysine is added without concern for the whole essential amino acid profile. Formulations based on an ideal amino acid profile for the lactating sow will, therefore, become critical.

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