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► **To cite this version:**

Chantal Farmer, N.L. Trottier, Jean-Yves Dourmad. Mammary blood flow and nutrient uptake. The gestating and lactating sow, Wageningen Academic Publishers, 2015, 978-90-8686-253-5. 10.3920/978-90-8686-803-2_14 . hal-01210733

HAL Id: hal-01210733

<https://hal.science/hal-01210733>

Submitted on 5 Jun 2020

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14. Mammary blood flow and nutrient uptake

C. Farmer^{1*}, N.L. Trottier² and J.Y. Dourmad³

¹Agriculture and Agri-Food Canada, Dairy and Swine R & D Centre, 2000 College St., Sherbrooke, QC, J1M 0C8, Canada; chantal.farmer@agr.gc.ca

²Michigan State University, Department of Animal Science, 2209 Anthony Hall, East Lansing, MI 48824, USA

³INRA-Agrocampus Ovest, UMR1348 PEGASE, 35590 Saint-Gilles, France

Abstract

Sow milk is the major source of nutrients for suckling piglets and taking into account the large litter sizes of our current sow genotypes, it is imperative to maximize nutrient use by the mammary gland. The amount of nutrients available to mammary tissue is dependent upon the concentrations of nutrients in blood and the rate of its flow to the lactating glands. Nutrient availability to the udder may be estimated by measuring mammary arteriovenous differences, and mammary blood flow can be measured either directly or via indirect calculations. For all these measures, mammary venous and arterial blood samplings are required and catheters must be inserted in mammary veins. Blood flow to the udder is influenced by numerous factors, such as litter size, time since feeding, postural behaviour, vasoactive substances, and ambient temperature; yet, the single most important effector of mammary blood flow is milk removal. Glucose represents 40 to 60% of the total carbon mass taken up by sow mammary tissue and its uptake by the mammary gland appears to be mediated by glucose transporter proteins. Other energetic precursors which are used by the mammary gland are triglycerides, phospholipids, acetate, propionate and lactate. The uptake of amino acids by sow mammary glands has been extensively studied and is affected by diet, stage of lactation, and milk demand. Recent data also showed that amino acid uptake is controlled by transporter proteins that channel amino acids intracellularly, and that these proteins are in turn affected by diet and physiological status. Data on mammary uptake of hormones are contradictory; yet, the endocrine involvement in the regulation of milk synthetic processes is evidenced by the presence of specific hormonal receptors in mammary tissue.

Keywords: dietary nutrients, lactation, mammary gland, mammary uptake, sow

14.1 Introduction

Sow milk yield is the major determinant of growth of suckling piglets and, in turn, weight of piglets at weaning has a major impact on their post-weaning gain (Klindt, 2003). Yet, primiparous sows cannot produce optimal milk yields because of underdeveloped mammary tissue, low voluntary feed intake during lactation, and their prolonged catabolic state present during late-gestation and lactation (see Chapter 4; Farmer and Hurley, 2015 and Chapter 7; Theil, 2015 from this book). It is therefore imperative to find ways to

improve sow milk yield and a better understanding of the mechanisms involved in the control of milk production is needed to achieve that goal. Nutrients available to mammary tissue may be enhanced by increasing the concentrations of nutrients in blood and the blood flow to the glands (Renaudeau *et al.*, 2002). The present chapter therefore focusses on mammary blood flow and the uptake of nutrients and hormones by mammary tissue in swine. The direct and indirect methods of estimating blood flow will be described and an update on the current knowledge of the factors involved in its regulation will be provided. Mammary uptake of major nutrients, such as glucose and amino acids, and of other energetic precursors will be covered, with some emphasis on novel findings pertaining to glucose and amino acid transporters. Finally, the limited information available on mammary uptake of hormones will be summarized and the potential involvement of specific hormones for nutrient uptake by the sow's udder discussed.

14.2 Blood flow

14.2.1 Anatomy of the mammary circulatory system

The arterial, venous and lymphatic circulation of the sow mammary glands are provided on each side of the ventral midline by a network that extends longitudinally from the axillary to the inguinal regions (Schummer *et al.*, 1981), as depicted in Figure 14.1. In contrast to ruminants where only one artery (pudendal artery) supplies blood to both sides of the udder (Barone, 1996), several arteries supply each side of the sow's udder (Trottier *et al.*, 1995a). The external pudendal artery descends through the inguinal canal where it divides into the lateral cranial, middle cranial and medial cranial branches

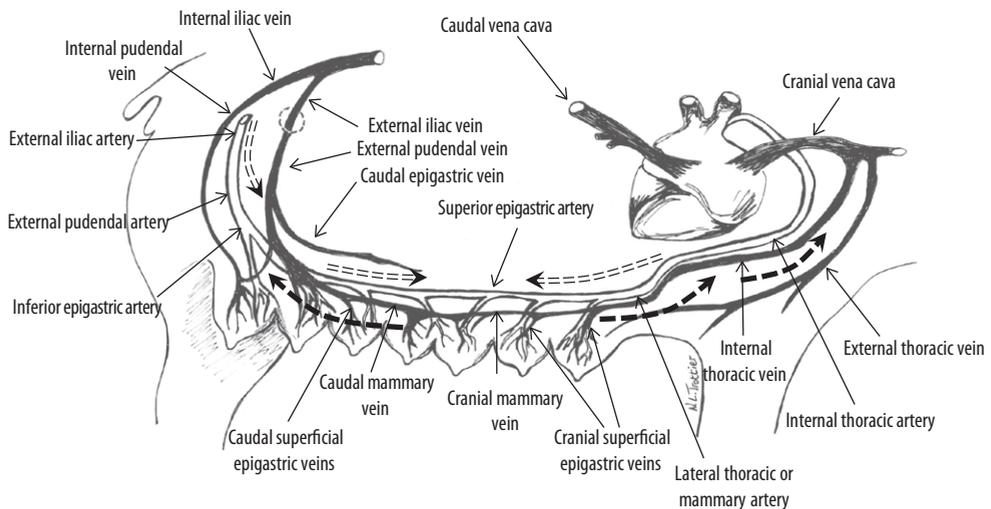


Figure 14.1. Illustration of the lactating sow mammary circulatory system. The oval line represents the inguinal canal. Single dash arrows indicate direction of venous blood flow and double dash arrows indicate direction of arterial blood flow. Arteries are opened and veins are filled. Drawing not to scale.

supplying the posterior mammary glands (Ghoshal, 1975). The anterior mammary glands are mainly supplied by the cranial epigastric artery originating from the internal thoracic artery (Ghoshal, 1975; Trottier *et al.*, 1995a). Blood leaving the mammary glands uses two distinct pathways (Turner, 1952); the anterior glands are drained cranially through two subcutaneous abdominal veins which run parallel on each side of the mammary system to reach the internal thoracic vein (Trottier *et al.*, 1995a). Blood that exits the posterior glands in the inguinal region does so caudally by way of the same subcutaneous abdominal vein thereby draining into the external pudic vein (Turner, 1952). In contrast to other litter bearing species studied to date, superficial veins arising from the mammary parenchyma and branching from the epigastric veins are joined between the right and left side of each pair of glands through venous anastomosis (Lignereux *et al.*, 1996).

14.2.2 Estimating mammary blood flow

Mammary venous and arterial blood samplings are required to measure blood flow and to estimate mammary nutrient uptake. Mammary blood flow in lactating sows has been estimated both directly and indirectly. All porcine mammary blood flow values obtained by indirect methods (Guan *et al.*, 2002, 2004a,b; Linzell *et al.*, 1969a; Nielsen *et al.*, 2002a,b; Trottier *et al.*, 1997) are based on Fick's original principle of diffusion (Fick, 1870). Kety and Schmidt (1945) adapted the Fick's principle to measure cerebral blood flow using nitrous oxide as an inert marker. Linzell *et al.* (1969a) used the Kety-Schmidt method (Kety and Schmidt, 1945) and DO as an inert marker to estimate blood flow across one single mammary gland. This value was then used to estimate blood flow across the entire udder (Table 14.1). In that same study, Linzell *et al.* (1969a) also measured for the first time, the arteriovenous difference in nutrient concentrations by sampling mammary venous and arterial blood following catheterization of the external pudic artery and of a mammary vein draining a single functional gland (Figure 14.1). Milk yield of the gland was measured over a 5-h period and total mammary tissue was weighed at the end of the study to allow estimation of milk yield and blood flow per 100 g of mammary tissue. For the purpose of comparison with other studies, milk yield and blood flow values in this chapter were estimated for an entire udder based on an udder weight of 770 g (Table 14.1).

When applying the Fick's principle to measure blood flow in lactating sows, catheter placement for the arterial infusion of an external inert marker and blood sampling may be done using any arterial sites. The Fick approach, however, is more feasible for determination of long-term changes in blood flow (i.e. daily) as opposed to changes occurring in the short term (i.e. minutes). More recent studies (Guan *et al.*, 2002, 2004a,b; Manjarín *et al.*, 2012b; Nielsen *et al.*, 2002a,b; Trottier *et al.*, 1997) applied the Fick's principle to estimate mammary blood flow using an internal marker. The advantage of an internal marker is that there is no need for arterial infusion of an external marker. In those studies, lysine or phenylalanine + tyrosine were used with the major premise that lysine and phenylalanine are negligibly catabolized or utilized by the porcine mammary gland, other than their incorporation into milk proteins (Guan *et al.*, 2002, 2004a). Such assumption may however have led to an underestimation of mammary plasma flow. For instance, Lapierre *et al.* (2009) reported lysine to be utilized and oxidized through pathways for non-milk protein

Table 14.1. Mammary plasma flow through the entire mammary gland, milk yield and amount of plasma required to produce 1 kg of milk estimated from data of various studies in sows fed diets providing the minimum nutrient requirements (Farmer *et al.*, 2008b).

Study	Method	Litter size	Plasma flow (l/min)	Milk yield (l/d)	Plasma (l per l milk)
Guan <i>et al.</i> (2002)	Fick (phe + tyr) ¹	12	4.9	12.1	581
Guan <i>et al.</i> (2004a)	Fick (lysine)	11	4.5	11.4	560
Guan <i>et al.</i> (2004b)	Fick (lysine)	11	3.6	10.7	490
Linzell <i>et al.</i> (1969a) ²	Fick (³ HO)	6	1.9 ³	6.2	441 ³
Nielsen <i>et al.</i> (2002b)	Fick (lysine)	8	3.2	10	550
Nielsen <i>et al.</i> (2002a)	Fick (lysine)	12	5.5	7.4	1,050
Nielsen <i>et al.</i> (2002a)	Fick (methionine)	12	4.4	7.4	858
Nielsen <i>et al.</i> (2002a)	Fick (calcium)	12	2.8	7.4	548
Renaudeau <i>et al.</i> (2002)	Flow probe	12	3.6 ³	11	471 ³
Renaudeau <i>et al.</i> (2002)	Fick (lysine)	12	3.9 ⁴	11	511
Trottier <i>et al.</i> (1997)	Fick (lysine)	11	3.0	8.1	541

¹ Phenylalanine + tyrosine.

² A weight of 770 g for the gland subjected to blood flow measurement was derived from the reported milk yield of 43 ml/h or 134 ml/d per 100 g of tissue, and blood flow of 41 ml/min per 100 g of tissue. Blood flow through that gland was recalculated as l/gland/day where 41 ml/min/100 g tissue represents 316 ml/min/gland or 455 l/day/gland. The number of functional glands was based on the number of suckling piglets, to calculate mammary blood flow through all functional glands. This value was verified by dividing the total mammary tissue weight of 4.62 kg by the single gland weight of 770 g (as estimated above). Thus, total mammary blood flow was estimated as 2,728 l/day (455 l/day/gland × 6 glands) and total milk yield as 6.19 l/day (1,032 ml/day × 6 glands). Milk yield per gland was estimated as 1,032 ml/day where 134 ml/100 g tissue per day × 770 g/gland.

³ Values are for blood flow and liter of blood per liter of milk.

⁴ Estimated from reported blood to milk ratio of 511 and average milk yield of 11 l/d.

synthesis in lactating dairy cows. Also, the NRC (2012) recently estimated dietary lysine efficiency of utilization into milk protein synthesis to be 67% in sows; albeit this value appears to be overestimated, the findings of Lapierre *et al.* (2009) in dairy cows together with this NRC (2012) estimate suggest that lysine utilization by the porcine mammary gland may be of greater significance than previously suggested.

Most of the reported plasma flow values from studies using Fick's principle range from 1.9 to 4.9 l/min with corresponding plasma:milk (vol:vol) and milk yield, respectively ranging from 441 to 581 and 6.2 to 12.1 l/d (Table 14.1). Plasma:milk values as high as 1,050 and 858 were also reported when lysine and methionine, respectively, were used as internal markers (Nielsen *et al.*, 2002a, Table 14.1). These values raise questions when considering the reported low average daily milk production of 7.4 kg for a litter size of 12 (Nielsen *et al.*, 2002a). In that same study, when calcium was used as marker, the average plasma flow was 2.8 l/min, a value falling within the reported range. Because no other studies are available to compare the plasma flow estimated with calcium, it is unknown whether calcium is a valid marker. Unlike amino acids, calcium is not metabolized hence

it offers the advantage of falling under the definition of ‘inert’ marker. On the other hand, given the low arteriovenous difference and extraction rate of Ca (Nielsen *et al.*, 2002a) its validity as an internal marker is questionable.

Renaudeau *et al.* (2002) obtained direct measurements of blood flow through the mammary glands of lactating sows by implanting an ultrasonic blood flow probe around the right external pudic artery. The average pudic mammary blood flow was 910 ± 283 ml/min, corresponding to 3.6 l/min or 4,984 l/day. These values compare to those obtained from previously-cited authors using the Fick principle (Table 14.1). This approach offers the advantage of evaluating short-term changes (i.e. minutes) in mammary blood flow

14.2.3 Regulation of mammary blood flow

Regulation of blood flow to the sow udder and each of the respective mammary glands is poorly understood. One major factor that appears to play a significant role in modulating blood flow is milk removal. Renaudeau *et al.* (2002) reported that mammary blood flow decreased markedly shortly after intravenous administration of oxytocin in sows and returned to basal values as quickly as 12 min following initiation of nursing. Thereafter, blood flow increased and reached a maximum value 22 min after the onset of milking. It was suggested that the post-suckling increase in mammary blood flow may be in response to the nursing event or to the decrease in milk volume. Olsson *et al.* (2003) showed that a jugular vein infusion of oxytocin caused a short-term increase in mammary blood flow in lactating goats. It was also demonstrated that there is an increase in intra-mammary pressure during natural milk ejection or following an intravenous injection of oxytocin in sows and that the timing of milk ejection between teats may be influenced by blood flow (Kent *et al.*, 2003). The link between nursing and mammary blood flow is also apparent at weaning, whereby blood flow decreases by 40 and 60%, respectively, 8 and 16 h after weaning in pigs (Renaudeau *et al.*, 2002). A positive relationship between litter size and mammary plasma flow was reported by Nielsen *et al.* (2002b); as litter size increased from 3 to 13, mammary plasma flow increased linearly from approximately 2,000 l/d to over 5,000 l/d, reflecting an increase in the number of functional glands and total milk demand. Minor changes in mammary blood flow in sows are also seen with postural changes and time relative to feeding. Renaudeau *et al.* (2002) reported a 6% decrease in mammary blood flow when sows were standing compared with lying, and a 7.7% increase after a meal reaching a peak 65 min post-prandially. The effect of ambient temperature on mammary blood flow (Renaudeau *et al.*, 2003) was also examined. While exposure of sows to an ambient temperature of 28 °C did not affect milk yield, mammary blood flow increased by approximately 8%. The authors suggested that this small increase was consequent to an increase in blood flow to subcutaneous capillaries for dissipating heat.

Nitric oxide, also known as the endothelium-derived factor, stimulates vascular smooth muscle relaxation resulting in vasodilation and increase in blood flow. Lacasse *et al.* (1996) demonstrated that intra-mammary infusion of a nitric oxide donor in lactating goats rapidly increased mammary blood flow. Yet, a causal relationship between mammary blood flow and milk yield remains to be demonstrated. Mammary blood flow of goats was affected unilaterally and milk yield was measured.

Responses of the milk vein and the cranial mammary artery to potential other vasoactive substances were studied in isolated blood vessels of lactating sows. Noradrenaline, serotonin, prostaglandin F, prostacyclin, histamine and potassium had vasoconstrictive effects on both these vessels whereas acetylcholine had a relaxing effect on both arterial and vein and β -adenosine had a relaxing effect on the milk vein only (Busk *et al.*, 1999). In rodents and humans, adrenergic nerve fibers cause constriction of blood vessels in the mammary gland thereby reducing blood flow through the glands (Erikson *et al.*, 1996). Franke-Radowiecka and Wasowicz (2002) showed that, in swine, the majority of adrenergic and acetylcholinesterase-positive nerve fibers are localized in the subcutaneous tissue of the nipple and mammary glands. Franke-Radowiecka and Wasowicz (2002) reported that cholinergic innervation is considerably less abundant than adrenergic innervation in porcine mammary glands. Nerve supply to the cranial mammary glands of sows differs from that to the inguinal glands. Cranial mammary glands receive their innervation from thoracic nerves whereas inguinal mammary glands receive their innervation mainly from the pudendal nerve (Klopfenstein *et al.*, 2006).

14.3 Uptakes of nutrients

14.3.1 Measure of mammary arteriovenous difference

Measurements of mammary nutrient arteriovenous difference (AVD) and uptake necessitate cannulation of an artery and of the major venous system draining the mammary parenchyma. In these *in vivo* studies, arterial blood was collected following cannulation of the carotid and venous blood following cannulation of the abdominal vein (Trottier *et al.*, 1995a; Figure 14.1). The cranial end of the anterior portion of the mammary system is the preferred cannulation site for representative measurement of nutrient concentrations contributed by glands located in both the thoracic and abdominal regions. In addition, mammary involution appears to be initiated in the inguinal region in smaller litters, whereas the abdominal and thoracic glands usually remain functional until the end of the lactation period (Kim *et al.*, 2001).

14.3.2 Uptake of glucose

Blood glucose is the major precursor for the synthesis of lactose. Glucose represents 40 to 60% of the total carbon mass taken up by sow mammary tissue (Dourmad *et al.*, 2000; Linzell *et al.*, 1969b; Renaudeau *et al.*, 2003; Spincer *et al.*, 1969). Glucose uptake by the mammary gland makes up a considerable proportion of the total body glucose requirement. Once in the mammary gland, glucose is used as the main substrate for lactose, glycerol and fatty acid synthesis (Linzell *et al.*, 1969b) and may also provide energy for metabolic processes associated with maintenance of the mammary gland. Linzell *et al.* (1969a) reported an extraction rate of glucose by the mammary gland of about 26%. This is consistent with the estimates of 31% obtained by Spincer *et al.* (1969) and 26% by Renaudeau *et al.* (2003), whereas Dourmad *et al.* (2000) and Trottier *et al.* (1995b) reported lower values of 20 and 20.7%, respectively. Using labeled-carbon glucose, Linzell *et al.* (1969b) estimated that 53% of glucose was used for lactose synthesis,

34% was oxidized to CO and the remaining 13% was used for fatty acid or amino acids synthesis. For instance, Dourmad *et al.* (2000) showed that free fatty acids AVD decreased to negative values post-feeding during the peak of glucose AVD, indicating that glucose utilization for fatty acid synthesis increases under absorptive state. The same authors also reported lactate AVD to increase post-feeding compared with pre-feeding, indicative of increased glucose oxidation under absorptive state. The mammary gland appears to respond to dietary glucose availability by altering the AVD; in the study by Dourmad *et al.* (2000), AVD for glucose markedly decreased 16 h post-prandially from 24.2 to 12.2 mg/dl, a decrease that was directly related to that of arterial glucose concentrations from 126.8 to 62.4 mg/dl. Albeit based on only one study, given that the extraction rate of glucose by the sow mammary gland remained constant over a relatively wide range of arterial glucose concentrations (i.e. 126.8 to 62.4 mg/dl), glucose uptake by the mammary gland is likely mediated by high capacity, low affinity glucose transporter proteins.

Transcript abundance for the genes *SLC2A1*, *SLC2A4*, and *INSR*, respectively encoding for the non-insulin dependent glucose transporter GLUT1, the insulin-dependent glucose transporter GLUT4, and the insulin receptor proteins, was measured in mammary tissue at the end of pregnancy, throughout lactation, and post-weaning (Manjarin *et al.*, 2012a). Relative abundance of all 3 genes was relatively high across all stages, with GLUT1 being two-fold greater than GLUT4 and insulin receptor, with no change in expression occurring between early and peak lactation. Both GLUT1 and GLUT4 are high capacity, low affinity and high Km transporter proteins, and as such, monosaccharide uptake by the mammary gland would be expected to increase with an increase in arterial glucose concentrations. This is not surprising given the importance of glucose as a substrate for lactose synthesis in sow milk. Renaudeau *et al.* (2003) estimated that 1,300 g of glucose was needed to support a milk yield of 11 kg. From the work of Guan *et al.* (2004b), it was estimated that the porcine udder removes approximately 2,000 g of glucose per day to support a daily milk yield of 11.4 kg (Farmer *et al.*, 2008a). The ratio between lactose output and glucose uptake ranges from 0.35 to 0.68 across studies (Dourmad *et al.*, 2000; Linzell *et al.*, 1969b; Spincer *et al.*, 1969).

14.3.3 Uptake of other energetic precursors

Three classic studies, namely those of Spincer *et al.* (1969), Linzell *et al.* (1969b) and Spincer and Rook (1971), have reported mammary uptake of triglycerides, phospholipids, acetate, propionate and lactate in sows (Table 14.2). Other than glucose, the major sources of non-amino acid carbon uptake are triglycerides and lactate (Table 14.2). Linzell *et al.* (1969b) used five sows, ranging from 12 to 61 d of lactation to estimate mammary AVD. Lactate and triglycerides AVD were considerable whereas AVD for phospholipids, free fatty acids, volatile fatty acids and β -hydroxybutyrate were small and/or variable. Spincer *et al.* (1969) reported that of the total recorded mammary uptake of plasma constituents, 11% was accounted for by plasma triglycerides and only 2% by acetate. Of the plasma triglycerides, oleate (23%), linoleate (21%), palmitate (19%) and stearate (16%) were the most prominent and when radioactive triglycerides were infused intravenously, more than 60% of palmitic acid and 70% of stearic acid in milk fat were accounted for by uptake from plasma triglycerides (Spincer and Rook, 1971). Recent findings by Dourmad *et al.*

Table 14.2. Arteriovenous differences (mg/100 ml for all variables except volatile fatty acids and free fatty acids which are in Meq/l) of plasma carbohydrates and lipids in the lactating sow (Farmer et al., 2008b).

	Linzell <i>et al.</i> , 1969b	Spincer <i>et al.</i> , 1969	Spincer and Rook, 1971
Glucose	19.8 ¹	37.5	28.7
Triglycerides	7.6	7.2	10.2
Lactate	7.4	1.4	ND ²
Phospholipids	0.3	4.1	ND
B-hydroxybutyrate	0.2	0.2	ND
Volatile fatty acids	0.2 ^{1,3}	0.6 ⁴	ND
Free fatty acids	0.1 ³	ND	ND
Citrate	ND	0	ND

¹ Adjusted from whole blood to plasma using the average hematocrit value of 31.7%.

² Not determined.

³ Both negative and positive values were observed.

⁴ Value for acetate only.

(2000) corroborated these earlier reports on the relative uptakes of glucose, triglycerides and amino acids by the sow mammary glands.

There is some recent information on the uptake of minerals and vitamins across the mammary glands of lactating sows. Both Nielsen *et al.* (2002a) and Dourmad *et al.* (2000) showed a positive mammary AVD for calcium. Dourmad *et al.* (2000) also noted a positive uptake of phosphorus, with its AVD across mammary tissue increasing after a meal, whereas calcium uptake remained constant post-prandially. The extraction rates of calcium and phosphorus from arterial blood are typically lower (4.1 and 3.1%, respectively) than that of other major nutrients (ranging from 20 to 35%). In addition, there was very little to no uptakes of riboflavin (3.4 pmol/ml), vitamin B (0 pg/ml) or folic acid (-1.2 ng/ml) by the sow mammary glands (Dourmad *et al.*, 2000).

14.3.4 Uptake of energy and energetic efficiency of milk synthesis

The uptake of energy by the mammary glands can be calculated from the uptake of glucose, amino acids and other milk precursors and their energy content. From the studies of Linzell *et al.* (1969b) and Renaudeau *et al.* (2003), energy AVD of 9.1 and 8.8 kJ/l plasma, respectively, can be calculated (Figure 14.2). A similar value of 8.9 kJ/l was measured in postprandial animals (Dourmad *et al.*, 2000), whereas after 16 h of fasting the AVD of energy tended to decrease (7.6 kJ/l). In fed sows, the contribution of glucose and lactate to total energy uptake by the mammary glands averaged 45%, whereas in fasted sows this value dropped to 28% (Dourmad *et al.*, 2000). The contribution of free fatty acids to energy uptake was highly dependent on nutritional status of the sows. In fasted sows, free fatty acids contributed approximately 35% of the energy uptake whereas no uptake was detected after a meal. The situation in restrictively-fed sows was

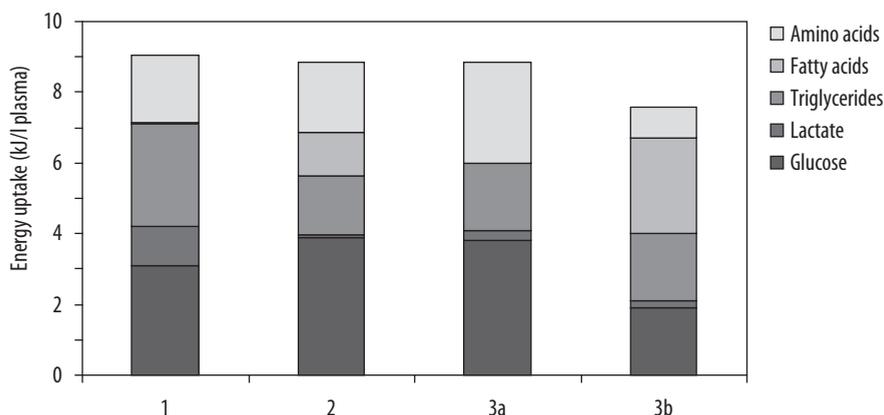


Figure 14.2. Partition of energy uptake by the mammary gland in different studies (Farmer *et al.*, 2008b). 1 = Linzell *et al.* (1969b); 2 = Renaudeau *et al.* (2003); 3 = Dourmad *et al.* (2000). 3a = post-prandial; 3b = fasted.

intermediate between that of fed and fasted sows (Renaudeau *et al.*, 2003), with free fatty acids contributing 15% of total mammary energy uptake.

The energetic efficiency of milk synthesis from plasma precursors can be calculated (Table 14.3) using mammary AVD measured by Renaudeau *et al.* (2003) and assuming a plasma flow of 550 l/l of milk. For a daily milk production of 10 kg, the amount of energy present in precursors and products was 48.8 and 43.6 MJ/d, respectively, corresponding to an energy efficiency of 0.89. This value is greater than the usual estimate of ME efficiency for milk production (0.72) derived from energy metabolism studies but is very close to that reported for the efficiency of energy use from body reserves (0.88; Noblet and Etienne, 1987). This discrepancy between the estimates of efficiency obtained from energy metabolism studies, on one hand, and mammary balance studies, on the other hand, could be partially related to other metabolic processes associated with milk production but occurring outside the mammary gland. It may however also originate from an under-estimation of either the uptake of nutrient or blood flow. Indeed, using a biochemical model describing the stoichiometry of nutrient transformation in the mammary gland, Van Milgen *et al.* (2003) showed that with the data from Renaudeau *et al.* (2003) blood flow or AVD had to be increased by about 10% to ensure correspondence between observed and stoichiometric balances.

14.3.5 Uptake of amino acids

In contrast to other nutrients, the AVD and net uptake of amino acids by the sow mammary gland has been extensively measured in response to the diet (Guan *et al.*, 2002, 2004a,b; Manjarín *et al.*, 2012b), to stage of lactation, and to milk demand (Guan *et al.*, 2004a,b; Nielsen *et al.*, 2002b). Guan *et al.* (2004a,b) showed that net mammary uptake of amino acids increases with advancing lactation from approximately 6 d up to peak milk (i.e. d 14 to 18) demand and decreases thereafter. This increase in uptake was associated to an increase in AVD. In contrast, the increase in net amino acid uptake

Table 14.3. Estimation of energetic efficiency of milk synthesis from precursors (Farmer et al., 2008b).

	g/d	kJ/d
Precursors (uptake for 10 l milk/d) ¹		
Glucose	1,359	21,328
Lactate	26	388
Glycerol	246	9,363
Triglycerides	172	6,714
Fatty acids	8	149
Amino acids	561	10,883
Total	2,373	48,825
Products (output for 10 l milk/d) ²		
Lactose	560	8,960
Fat	580	22,040
Protein	530	12,561
Total	1,670	43,561
Efficiency (%)		89.2

¹ Precursors were calculated from AVD measured by Renaudeau *et al.* (2002) assuming that a blood flow equivalent to 550 l plasma was required to produce 1 kg of milk (Trottier *et al.* 1997).

² Products were estimated from Renaudeau *et al.* (2002).

observed with increasing litter size was associated with an increase in blood flow rather than an increase in AVD (Nielsen *et al.*, 2002b). The change in AVD associated with day of lactation raises the question as to whether this change is related to cellular transporter protein abundance or to amino acid competitive transport inhibition. Amino acid uptake by all vertebrate cells is controlled by a coordinated activity of protein-carriers located in the cellular membrane that channel amino acids intracellularly (Broër *et al.*, 2008; Palacín *et al.*, 1998; Shennan *et al.*, 2000). A review of the current knowledge on amino acid transporter proteins and their regulation in the mammary gland including that of the sow is beyond the scope of the current book chapter. Briefly, transcripts for the genes encoding for amino acid transporter proteins that fall under two sodium-dependent and two sodium-independent systems have been measured in sow mammary tissue. Of the sodium dependent systems, B^{0,+} and y^{+L}, the respective transcripts of the genes encoding for ATB^{0,+}, y⁺LAT1/4F2hc and y⁺LAT2/4F2hc were reported by Pérez-Laspiur *et al.* (2009) (ATB^{0,+}) and Manjarín *et al.* (2011, 2012b) (ATB^{0,+}, y⁺LAT1/4F2hc and y⁺LAT2/4F2hc). For the sodium independent system, y⁺ and b^{0,+}, the respective transcripts of their genes encoding for CAT-1, CAT-2b, and b^{0,+}AT1/rBAT were also reported by Pérez-Laspiur *et al.* (2009) (CAT-1, CAT-2b) and Manjarín *et al.* (2011, 2012b) (CAT-1, CAT-2b, and b^{0,+}AT1).

Transcript abundance of several of these genes was shown to increase in response to milk demand (Manjarín *et al.*, 2011), and to dietary amino acid availability (Pérez-Laspiur *et al.*, 2009). In contrast, in the recent work by Manjarín *et al.* (2012b), no changes were

found in transcript abundance of these same genes in response to dietary crude protein levels and amino acid availability. Manjarín *et al.* (2012b) therefore suggested that the changes in AVD of amino acids in response to dietary amino acid availability are likely a result of competitive inhibitory processes at the blood mammary cell interface rather than changes in gene expression. Continued characterization of amino acid transporter systems and their respective proteins, particularly in response to dietary and physiological challenges, will allow to better understand how milk production in sows is governed. For instance, in the study by Manjarín *et al.* (2011), transcript abundance of CAT-1, ATB^{0,+}, and y⁺LAT2 increased between day 112 of gestation and day 17 of lactation. Expression of these three genes was positively correlated with expression for genes encoding for β -casein and α -lactalbumin, which are two mammary synthesized proteins. These authors (Manjarín *et al.*, 2011) proposed that CAT-1, ATB^{0,+}, and y⁺LAT2 may become molecular targets for improving sow milk production since these proteins are responsible for transport of the typically limiting amino acid lysine in the lactating sow's diet.

14.3.6 Hormones and mammary uptake of nutrients

Numerous hormones play a major role in the regulation of milk synthetic processes and the direct involvement of specific hormones is evidenced by the presence of their receptors in mammary tissue. Receptors for growth hormone (Manjarín *et al.*, 2012a), IGF-I (Lee *et al.*, 1993; Manjarín *et al.*, 2012a; Theil *et al.*, 2006), leptin (Palin *et al.*, 2004), prolactin (Manjarín *et al.*, 2012a; Palin *et al.*, 2004; Plaut *et al.*, 1989; Theil *et al.*, 2005; Trott *et al.*, 2009), insulin (Manjarín *et al.*, 2012a), glucocorticoids (Manjarín *et al.*, 2012a), and oxytocin (Lundin-Schiller *et al.*, 1996) are present in porcine mammary tissue. These receptors are under complex regulation, which will not be discussed because it is beyond the scope of the present chapter.

There is a scarcity of information on the role of lactogenic hormones in the regulation of mammary nutrient uptake during lactation in swine. Pettigrew *et al.* (1993) showed that circulating insulin concentrations were related to protein ($r=0.42$), fat ($r=0.39$) and lactose ($r=0.36$) contents in milk, thereby indicating that insulin may be involved in uptake of plasma substrates by the mammary gland during lactation. The transcript of the gene encoding for the insulin receptor protein is relatively abundant in porcine mammary tissue and remains invariant between the end of gestation, throughout lactation and post-weaning (Manjarín *et al.*, 2012a).

An *in vitro* study showed that exogenous insulin, in combination with prolactin and cortisol, can lead to significant increases in rates of lipid synthesis and glucose oxidation by porcine mammary explants (Jerry *et al.*, 1989). Furthermore, a close relationship between concentrations of prolactin and rate of glucose metabolism in porcine mammary tissue was reported (Jerry *et al.*, 1989) and prolactin binding to its receptor on sow mammary tissue was postulated as being a major effector of mammary metabolic rate (Plaut *et al.*, 1989). Such an involvement of prolactin in nutrient metabolism within mammary tissue would not be surprising in light of its essential role for both the initiation and the maintenance of lactation in sows (Farmer *et al.*, 1998).

An *in vivo* study showed that when sows were fed different levels of crude protein, arterial insulin and prolactin concentrations were strongly correlated with mammary amino acid AVD, thereby providing evidence of a link between insulin and prolactin concentrations and amino acid uptake by the mammary gland. It may therefore be that amino acid utilization by the sow mammary gland could be regulated via circulating concentrations of insulin and prolactin binding to porcine mammary cells (Farmer *et al.*, 2008). The mRNA abundance, however, for the prolactin receptor encoding gene in sow mammary tissue was greater on d 17 than on d 5 of lactation, but was not correlated to the expression of genes encoding the milk proteins α -lactalbumin and β -casein (Manjarín *et al.*, 2012a). A recent study also showed that increasing circulating concentrations of prolactin in late gestation led to greater piglet growth rate, and also augmented the postpartum increase in mRNA expression of genes encoding for β -casein, α -lactalbumin and glucose transporter 1 (VanKlombenberg *et al.*, 2013). In the study by Farmer *et al.* (2008) however glucose AVD was poorly correlated with circulating concentrations of prolactin. In that same study, glucose AVD was also poorly correlated with circulating concentrations of insulin. The extent of insulin dependence for glucose transport across the porcine mammary gland is unknown. The bulk of glucose transport is likely mediated via an insulin independent glucose transporter. As mentioned earlier, transcript for the genes encoding GLUT1, an insulin independent glucose transporter was twice as abundant as that of GLUT4, the insulin dependent glucose transporter. Furthermore, transport per se of neither glucose nor amino acids appeared to be under IGF-I regulation (Farmer *et al.*, 2008). Transgenic sows over-expressing mammary IGF-I also had similar milk composition than control sows (Monaco *et al.*, 2005), further suggesting little role of IGF-I on nutrient uptake by mammary tissue.

Finally, there is little information on the role of glucocorticoids in sows. In one study, the expression of the gene encoding for the glucocorticoid receptor during the prepartum until the post-weaning period was positively correlated with expression of α -lactalbumin and β -casein (Manjarín *et al.*, 2012a), indicating a likely role in amino acid uptake.

14.4 Conclusions

The amount of milk produced by lactating sows is ultimately dependent on the availability of nutrients to the mammary gland (Boyd *et al.*, 1995). This availability is in turn affected by mammary blood flow and nutrient uptake. Estimation of mammary blood flow is quite sophisticated and requires catheterization of blood vessels. Most reported plasma flow values range between 1.9 and 4.9 l/min, and the one major factor involved in modulating mammary blood flow is milk removal. Studies based on mammary AVD showed that there is major uptake of glucose and amino acids by the sow mammary gland and, to a lesser extent, there is also uptake of other energetic precursors such as triglycerides, phospholipids, acetate, propionate and lactate. Nutrient uptake by the sow udder is largely regulated by the diet and is also affected by factors such as stage of lactation and nursing intensity. In the case of glucose and amino acids, there are transporters located on the mammary glands that are needed for their uptake, yet the nutritional and molecular regulations of these are not yet elucidated. It is evident that the mechanisms involved

in controlling the processes related to milk production in sows are still not completely understood. This knowledge is essential to develop best-adapted management strategies that will optimize sow milk yield.

References

- Barone, R., 1996. Anatomie comparée des mammifères domestiques. Tome cinquième angiologie, Ed. Vigot, Paris, France, pp. 372-373.
- Boyd, R.D., Kensinger, R.S., Harrell, R.J. and Bauman, D.E., 1995. Nutrient uptake and endocrine regulation of milk synthesis by mammary tissue of lactating sows. *Journal of Animal Science* 73(2): 36-56.
- Broër, S., 2008. Amino acid transport across mammalian intestinal and renal epithelia. *Physiological Reviews* 88: 249-286.
- Busk, H., Sorensen, M.T., Mikkelsen, E.O., Nielsen, M.O. and Jakobsen, K., 1999. Responses to potential vasoactive substances of isolated mammary blood vessels from lactating sows. *Comparative Biochemistry and Physiology Part C: Toxicology & Pharmacology* 124: 57-64.
- Dourmad, J.Y., Matte, J.J., Lebreton, Y. and Fontin, M.L., 2000. Influence du repas sur l'utilisation des nutriments et des vitamines par la mamelle chez la truie en lactation. *Journées de Recherche Porcine en France* 32: 265-273.
- Eriksson, M., Lind, B., Uvnas-Moberg, K. and Hokfelt, T., 1996. Distribution and origin of peptide-containing nerve fibres in the rat and human mammary gland. *Neuroscience* 70: 227-245.
- Farmer, C., Guan, X. and Trottier, N.L., 2008a. Mammary arteriovenous differences of glucose, insulin, prolactin and IGF-I in lactating sows under different protein intake levels. *Domestic Animal Endocrinology* 34: 54-62.
- Farmer, C. and Hurley, W.L., 2015. Mammary development. Chapter 4. In: Farmer, C. (ed.) *The gestating and lactating sow*. Wageningen Academic Publishers, Wageningen, the Netherlands, pp. 73-94.
- Farmer, C., Robert, S and Rushen, J., 1998. Bromocriptine given orally to periparturient sows inhibits milk production. *Journal of Animal Science* 76: 750-757.
- Farmer, C., Trottier, N.L. and Dourmad, J.Y., 2008b. Review: current knowledge on mammary blood flow, mammary uptake of energetic precursors and their effects on sow milk yield. *Canadian Journal of Animal Science* 88: 195-204.
- Fick, A., 1870. Ueber die Messung des Blutquantums in den Herzventrikeln. *Physikalisch-Medizinische Gesellschaft zu Wiirzburg* 2: 16-28.
- Franke-Radowiecka, A. and Wasowicz, K., 2002. Adrenergic and cholinergic innervation of the mammary gland in the pig. *Anatomy Histology and Embryology* 31: 3-7.
- Ghoshal, N.G., 1975. Porcine heart and arteries. In: Sisson, S., Grossman, J.D. and Getty, R. (eds.) *The anatomy of the Domestic Animals*, volume 2. W.B. Saunders Company, Philadelphia, PA, USA, pp. 1306-1342.
- Guan, X., Bequette, B.J., Calder, G., Ku, P.K., Ames, K.N. and Trottier, N.L., 2002. Amino acid availability affects amino acid transport and protein metabolism in the porcine mammary gland. *Journal of Nutrition* 132: 1224-1234.
- Guan, X., Bequette, B.J., Ku, P.K., Tempelman, R.J. and Trottier, N.L., 2004a. The amino acid need for milk synthesis is defined by the maximal uptake of plasma amino acids by porcine mammary glands. *Journal of Nutrition* 134: 2182-2190.

- Guan, X., Pettigrew, J.E., Ku, P.K., Ames, N.K., Bequette, B.J. and Trottier, N.L., 2004b. Dietary protein concentration affects plasma arterio-venous difference of amino acids across the porcine mammary gland. *Journal of Animal Science* 82: 2953-2963.
- Jerry, J., Stover, R.K. and Kensing, R.S., 1989. Quantitation of prolactin-dependent responses in porcine mammary explants. *Journal of Animal Science* 67: 1013-1019.
- Kent, J.C., Kennaugh, L.M. and Hartmann, P., 2003. Intramammary pressure in the lactating sow in response to oxytocin and during natural milk ejections throughout lactation. *Journal of Dairy Research* 70: 131-138.
- Kety, S.S. and Schmidt, C.F., 1945. The determination of cerebral blood flow in man by the use of nitrous oxide in low concentrations. *American Journal of Physiology* 143: 53-66.
- Kim, S.W., Easter, R.A. and Hurley, W.L., 2001. The regression of unsuckled mammary glands during lactation in sows: the influence of lactation stage, dietary nutrients, and litter size. *Journal of Animal Science* 79: 2659-2668.
- Klindt, J., 2003. Influence of litter size and creep feeding on preweaning gain and influence of preweaning growth on growth to slaughter in barrows. *Journal of Animal Science* 81: 2434-2439.
- Klopfenstein, C., Farmer, C. and Martineau, G.P., 2006. Diseases of the mammary glands. In: Straw, B.E., Zimmerman, J.J., D'Allaire, S. and Taylor, D.J. (eds.) *Diseases of swine*, 9th edition. Blackwell Publishing, Oxford, UK, pp. 57-85.
- Lacasse, P., Farr, V.C., Davis, S.R. and Prosser, C.G., 1996. Local secretion of nitric oxide and the control of mammary blood flow. *Journal of Dairy Science* 79: 1369-1374.
- Lapierre, H., Doepel, L., Milne, E. and Lobley, G.E., 2009. Responses in mammary and splanchnic metabolism to altered lysine supply in dairy cows. *Animal* 3: 360-371.
- Lee, C.Y., Bazer, F.W. and Simmen, F.A., 1993. Expression of components of the insulin-like growth factor system in pig mammary glands and serum during pregnancy and pseudopregnancy: effects of oestrogen. *Journal of Endocrinology* 137: 473-483.
- Lignereux, Y., Rossel, R. and Jouglard, J.Y., 1996. Note sur la vascularisation veineuse des mamelles chez la truie. *Revue de Médecine Vétérinaire* 3: 191-194.
- Linzell, J.L., Mephan, T.B., Annison, E.F. and West, C.E., 1969a. Mammary metabolism in lactating sows: arteriovenous differences of milk precursors of milk. In: Larson, B.L. and Smith, V.R. (eds.) *Lactation*, volume 1. Academic Press, New York, NY, USA, 143.
- Linzell, J.L., Mephan, T.B., Annison, E.F. and West, C.E., 1969b. Mammary metabolism in lactating sows: arteriovenous differences of milk precursors and the mammary metabolism of [¹⁴C] glucose and [¹⁴C]acetate. *British Journal of Nutrition* 23: 319-332.
- Lundin-Schiller, S., Kreider, D.L., Rorie, R.W., Haresty, D., Mitchell, M.D. and Koike, T.I., 1996. Characterization of porcine endometrial, myometrial, and mammary oxytocin binding sites during gestation and labor. *Biology of Reproduction* 55: 575-581.
- Manjarín, R., Steibel, J.P., Kirkwood, R.N., Taylor, N.P. and Trottier, N.L., 2012a. Transcript abundance of hormone receptors, mammalian target of rapamycin pathway-related kinases, insulin-like growth factor I, and milk proteins in porcine mammary tissue. *Journal of Animal Science* 90: 221-230.
- Manjarín, R., Steibel, J.P., Zamora, V., Am-in, N., Kirkwood, R.N., Ernst, C.W., Weber, P.S., Taylor, N.P. and Trottier, N.L., 2011. Transcript abundance of amino acid transporters, β -casein, and α -lactalbumin in mammary tissue of periparturient, lactating, and postweaned sows. *Journal of Dairy Science* 94: 3467-3476.

- Manjarín, R., Zamora, V., Wu, G., Steibel, J.P., Kirkwood, R.N., Taylor, N.P., Wils-Plotz, E., Trifilo, K. and Trottier, N.L., 2012b. Effect of amino acids supply in reduced crude protein diets on performance, efficiency of mammary uptake, and transporter gene expression in lactating sows. *Journal of Animal Science* 90: 3088-3100.
- Monaco, M.H., Gronlund, D.E., Bleck, G.T., Hurley, W.L., Wheeler, M.B. and Donovan, S.M., 2005. Mammary specific transgenic over-expression of insulin-like growth factor-I (IGF-I) increases pig milk IGF-I and IGF binding proteins, with no effect on milk composition or yield. *Transgenic Research* 14: 761-773.
- National Research Council (NRC), 2012. Nutrient requirements of swine, 11th revised edition. National Research Council of the National Academies. Washington, DC, USA.
- Nielsen, T.T., Pierzynowski, S.G., Borsting C.F., Nielsen, M.O. and Jakobsen, K., 2002a. Catheterization of arteria epigastrica cranialis, measurement of nutrient arteriovenous differences and evaluation of daily plasma flow across the mammary gland of lactating sows. *Acta Agricultura Scandinavia Section A: Animal Science* 42: 113-120.
- Nielsen, T.T., Trottier, N.L., Stein, H.H., Bellavers, C. and Easter, R.A., 2002b. The effect of litter size and day of lactation on amino acid uptake by the porcine mammary glands. *Journal of Animal Science* 80: 2402-2411.
- Noblet, J. and Etienne, M., 1987. Metabolic utilisation of energy and maintenance requirements of lactating sows. *Journal of Animal Science* 64: 774-781.
- Olsson, K., Malmgren, C., Olsson, K.K., Hansson, K. and Häggström, J., 2003. Vasopressin increases milk flow and milk fat concentration in the goat. *Acta Physiologica Scandinavia* 177: 177-184.
- Palacin, M., Estevez, R., Bertran, J. and Zorzano, A., 1998. Molecular biology of mammalian amino acid transporters. *Physiological Reviews* 78: 969-1054.
- Palin, M.F., Beaudry, D. and Farmer, C., 2004. Gene expression of leptin, leptin receptor, prolactin receptor and whey acidic protein in mammary glands of late-pregnant gilts from two breeds. *Canadian Journal of Animal Science* 84: 621-629.
- Pérez Laspiur, J., Burton, J.L., Weber, P.S.D., Moore, J., Kirkwood, R.N. and Trottier, N.L., 2009. Dietary protein intake and stage of lactation differentially modulate amino acid transporter mRNA abundance in porcine mammary tissue. *Journal of Nutrition* 139: 1677-1684.
- Pettigrew, J.E., McNamara, J.O., Tokach, M.D., King, R.H. and Crooker, B.A., 1993. Metabolic connections between nutrient intake and lactational performance in the sow. *Livestock Production Science* 35: 137-152.
- Plaut, K.I., Kensinger, R.S., Griel, Jr., L.C. and Kavanaugh, J.F., 1989. Relationships among prolactin binding, prolactin concentrations in plasma and metabolic activity of the porcine mammary gland. *Journal of Animal Science* 67: 1509-1519.
- Renaudeau, D., Lebreton, Y., Noblet, J. and Dourmad, J.Y., 2002. Measurement of blood flow through the mammary gland in lactating sows: methodological aspects. *Journal of Animal Science* 80: 196-201.
- Renaudeau, D., Noblet, J. and Dourmad, J.Y., 2003. Effect of ambient temperature on mammary gland metabolism in lactating sows. *Journal of Animal Science* 81: 217-231.
- Schummer, A., Wilkens, H., Vollmerhaus, B. and Habermehl, K.H., 1981. Skin and cutaneous organs. In: Nickel, R., Schummer, A. and Seiferle, E. (eds.) *The anatomy of the domestic animals*, volume 3. The circulatory system, the skin, and the cutaneous organs of the domestic mammals. Springer-Verlag, New York, NY, USA, pp. 473-557.

- Shennan, D.B. and Peaker, M., 2000. Transport of milk constituents by the mammary gland. *Physiological Reviews* 80: 925-951.
- Spincer, J. and Rook, J.A.F., 1971. The metabolism of [U-¹⁴C]glucose, [1-¹⁴C]palmitic acid and [1-¹⁴C]stearic acid by the lactating mammary gland of the sow. *Journal of Dairy Research* 38: 315-322.
- Spincer, J., Rook, J.A.F. and Towers, K.G., 1969. The uptake of plasma constituents by the mammary gland of the sow. *Biochemistry Journal* 111: 727-732.
- Theil, P.K., 2015. Transition feeding of sows. Chapter 7. In: Farmer, C. (ed.) *The gestating and lactating sow*. Wageningen Academic Publishers, Wageningen, the Netherlands, pp. 147-172.
- Theil, P.K., Labouriau, R., Sejrsen, K., Thomsen, B. and Sorensen, M.T., 2005. Expression of genes involved in regulation of cell turnover during milk stasis and lactation rescue in sow mammary glands. *Journal of Animal Science* 83: 2349-2356.
- Theil, P.K., Sejrsen, K., Hurley, W.L., Labouriau, R., Thomsen, B. and Sorensen, M.T., 2006. Role of suckling in regulating cell turnover and onset and maintenance of lactation in individual mammary glands of sows. *Journal of Animal Science* 84: 1691-1698.
- Trott, J.F., Horigan, K.C., Gloviczki, J.M., Costa, K.M., Freking, B.A., Farmer, C., Hayashi, K., Spencer, T., Morabito, J.E. and Hovey, R.C., 2009. Tissue-specific regulation of porcine prolactin receptor expression by estrogen, progesterone and prolactin. *Journal of Endocrinology* 202: 153-166.
- Trottier, N.L., Shipley, C.F. and Easter, R.A., 1995a. A technique for the venous cannulation of the mammary gland in the lactating sow. *Journal of Animal Science* 73: 1390-1395.
- Trottier, N.L., Shipley, C.F. and Easter, R.A., 1995b. Arteriovenous differences for amino acids, urea nitrogen, ammonia, and glucose across the mammary gland of the lactating sow. *Journal of Animal Science* 73(2): 57-58.
- Trottier, N.L., Shipley, C.F. and Easter, R.A., 1997. Plasma amino acid uptake by the mammary gland of the lactating sow. *Journal of Animal Science* 75: 1266-1278.
- Turner, C.W., 1952. The anatomy of the mammary gland of swine. In: *The mammary gland. I. The anatomy of the udder of cattle and domestic animals*. Lucas Brothers, Columbia, MO, USA, pp. 279-314.
- Van Milgen, J., Gondret, F. and Renaudeau, D., 2003. The use of nutritional models as a tool in basic research. In: Souffrant, W.B. and Metges, C.C. (eds.) *Progress in research on energy and protein metabolism*. European Federation of Animal Science (EAAP) publication no. 109, pp. 259-263.
- VanKlompbergen, M.K., Manjarín, R., Trot, J.F., McMicking, H.F. and Hovey, R.C., 2013. Late-gestational hyperprolactinemia accelerates mammary epithelial cell differentiation that leads to increased milk yield. *Journal of Animal Science* 91: 1102-1111.