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Effect of nutritional status on nutrient and gas utilization by the mammary gland of lactating sows

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ABSTRACT

Milk synthesis being a continuous process in lactating sows, the mammary gland has to adapt its metabolism in response to extreme short-term changes in nutrient availability in the arterial bloodstream, due to the feeding pattern. The objective of the present study was to better quantify and understand these adaptations. The effect of morning refeeding after an overnight 16-h feed withdrawal was measured on the uptake of energy-supplying nutrients, amino acids (**AA**), and some vitamins and minerals. After farrowing, catheters were fitted in the right anterior mammary vein and in the carotid artery of six sows. Blood samples were drawn on days 7, 14, and 21 of lactation, every 30 from 60 min before the morning meal to 300 min after the morning meal. Plasma concentrations of glucose, lactate, triglycerides (**TG**), non-esterified fatty acids (**NEFA**), glycerol, α-amino nitrogen **(N)**, vitamins B_{12}, and folates were determined on all samples. Riboflavin and AA concentrations were only measured 30 min before the meal and 120 min after the meal. Arterial and venous plasma concentrations of glucose, lactate, and α-amino N increased after the meal ($P < 0.01$), and concentrations of NEFA, glycerol, and TG decreased ($P < 0.01$). Mammary arteriovenous concentration difference increased after the meal for glucose, lactate, and α-amino N ($P < 0.01$), remained constant for TG, and decreased for NEFA ($P < 0.01$) and glycerol ($P < 0.05$). Arterial concentrations of all AA increased after the meal, but changes of arteriovenous difference with the meal differed among AA. Arteriovenous difference of energy (7.6 kJ/l plasma) concentration was similar in feed-deprived and fed sows, but the contribution of the various nutrients differed, and the respiratory quotient was lower ($P < 0.01$) before the meal (0.95) than after the meal (1.54). The relative contributions of glucose, lactate, TG, NEFA, and AA to arteriovenous difference in energy concentration were 50.2, 3.8, 25.1, 0, and 20.8% in fed and 24.6, 2.2, 24.9, 32.9, and 15.0% in feed-deprived sows, respectively. The daily mammary extraction of vitamin B_{12}, estimated from arteriovenous differences was higher than the amount of this vitamin bioavailable from the diet, probably contributing to the 50% decrease in plasma concentration between day 7 and day 21 of lactation. For both riboflavin and folates, arteriovenous differences in plasma concentrations were small or not different from zero. These results indicate that the mammary gland has a great capacity to adapt nutrient uptake very rapidly and modify its metabolism according to the nutrients available in the bloodstream.

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Implications

The mammary gland has a great capacity to adapt its metabolism very rapidly to the nutrients available in the bloodstream, be they energy-supplying nutrients or amino acids. These results contribute to better understanding and consider in practice the effects of feed supplies or appetite on sow mammary gland metabolism, and they provide original quantified data for improving the dynamic modeling of metabolism of sow's mammary gland.

Introduction

In mammals during lactation, the bulk of absorbed nutrients is taken by the mammary gland for milk synthesis. In lactating sows, milk production represents about 75% of total energy requirement (Noblet et al., 1990) and 90% of amino acid **(AA)** requirement (Dourmad et al., 1998; National Research Council NRC, 2012). As reviewed by Farmer et al. (2008), compared to ruminants, only limited research has been conducted on the metabolism of the mammary gland in the pig species, except for AA metabolism, which has been studied more extensively (Trottier et al., 1997; Guan et al., 2002; Nielsen et al., 2002). The pioneering studies on this subject were conducted by Linzell et al. (1969) and Spincer et al. (1969). Using measurements of arteriovenous concentration differences, they showed that glucose, triglycerides (**TG**),

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and, to a lesser extent, lactate, were the main non-AA sources of carbon for milk synthesis. More recently, Renaudeau et al. (2003) and Krogh et al. (2017) showed that in some situations non-esterified fatty acids (**NEFA**) and glycerol might also contribute to the energy supply. To the best of our knowledge, none of these studies has dealt with vitamin uptake for milk synthesis.

Milk synthesis in sows being a continuous process, the mammary gland has to adapt its metabolism in response to rather extreme changes in nutrient availability in arterial bloodstream occurring in the short term, over the course of the day, due to the feeding pattern (Père and Etienne, 2007), or in the longer term, due to the changes in feed intake and milk production over the course of lactation. Since most of the studies on mammary uptake in sows were conducted on fed animals, close to steady-state conditions, this adaptation has not been well evaluated.

The objective of the present study was therefore to quantify the effect of changes in the nutritional status of sows, obtained by overnight feed withdrawal followed by morning refeeding, on the utilization by the mammary gland of energy supplying nutrient, essential and non-essential AA, and some vitamins and minerals. This will also provide interesting information for the dynamic modeling of sow mammary gland metabolism.

Material and methods

Animal management

Six multiparous large white sows, parity 3 to 5, were used in the experiment. At day 110 of pregnancy, the sows were moved to farrowing crates (2.4 × 2.0 m) with slatted floors, and the ambient temperature was set at 24 °C. Sows had free access to water from a low-pressure nipple. Litter size was standardized to 11 piglets within 48 h after farrowing. A heating zone was provided for the piglets using an IR lamp. Piglets were weaned at 22 days of age on average.

During lactation, sows were fed twice a day with a standard lactation diet (13.2 MJ/kg ME, 9.6 g/kg total lysine, Supplementary Table S1). The feeding level was set at 3.5 kg/d on day 1 of lactation and was increased by 0.5 kg each day until day 6. From day 6 to day 21, the feeding level was set at 6 kg/d to avoid feed refusal and limit the variability of feed intake between sows.

Surgery

About 4 days after farrowing, sows were transferred to the surgery room, while their litters remained in their farrowing. Anesthesia and surgery followed the same protocol as described by Renaudeau et al. (2003). For the arterial cannulation, an indwelling Tygon catheter (Tygon Tubing®, Cole Parmer Inst. Company, IL, USA; 2.29 mm o.d., 1.27 mm i.d.) was inserted into the carotid artery up to a distance of 40 cm. The mammary vein cannulation was performed according to Trottier et al. (1995). A 4-cm incision was made between the first and the second gland on the right side, and a Tygon catheter (1.78 mm o. d., 1.02 mm i.d.) was implanted up to a distance of 18 cm in the anterior mammary vein. Postsurgical monitoring followed the same protocol as described by Renaudeau et al. (2003).

Blood sampling

Blood sampling procedure was the same as described in Renaudeau et al. (2003). Before the blood sample was taken, 5 ml of blood was drawn and discarded to eliminate dilution from the heparin block, and then 10 ml of blood was collected in heparinized syringes from the artery and the vein simultaneously and subsequently transferred to heparinized tubes. In addition, 2 ml of blood was collected in heparinized syringes for blood gas analysis.

On the day preceding blood sampling, the remaining feed was removed from the trough at 1730 and no feed was distributed until 0930 the next morning, when sows received 2.5 kg of feed. The arterial (**A**) and venous (**V**) samples were obtained simultaneously every 30 min between 0830 and 1400 on days 7, 14, and 21 of lactation. A total of 11 arterial and 11 venous samples were obtained per sow daily. Immediately after sampling, blood packed-cell volume and blood gases were measured and the remaining blood was centrifuged for 3 min at 8 500 × g at 4 °C (Renaudeau et al., 2003). The supernatant fluid was divided into subsamples and stored at −20 °C for further analysis.

Milk sampling

On days 7, 14, and 21 piglets were separated from the dam after suckling. Forty minutes later, sows were injected 20 IU of oxytocin (Intervet) in the arterial catheter and hand milked. A total of about 350 ml of milk was collected and stored at −20 °C for further analysis.

Chemical analyses

Chemical analyses were performed in the same way as described in Renaudeau et al. (2003). Glucose, insulin, lactate, TG, glycerol, free fatty acid (**FFA**), α-amino acid N, urea, calcium, and phosphorus were measured on all plasma samples as well as blood concentrations of O_2, CO_2, and pH. Oxygen concentration was measured on a Ciba Corning 270 co-oximeter (Ciba, Cergy, France). Carbon dioxide concentration and pH were analyzed simultaneously using a Ciba Corning 768 blood gas system. Plasma glucose, FFA, lactate, TG, glycerol, urea, α-amino acids N, calcium, and phosphorus were analyzed using enzymatic methods adapted to a Cobas Mira multianalyzer apparatus (Roche, Basel, Switzerland). Insulin concentration was measured using radioimmunoassay commercial kits (GIS Bio Int., Gif, France; Pharmacia, St. Quentin, France; Linco Research Inc., St. Louis, MO, USA, respectively).

The AA content in feed and milk was determined by ion-exchange liquid chromatography (Biochrom 20, Pharmacia, Saclay, France) after 24-h hydrolysis in HCl (6 mol/l). For sulfur AA, the hydrolysis was performed by performic acid oxidation. Free amino acid content in plasma was determined in the samples collected 30 min before and 120 min after the meal. The diluted samples (1:10) were analyzed by chromatography on a cation-exchange resin column (Beckman 6300 analyzer, Global Medical Instrumentation Inc., Albertville, MN, USA), as previously described by Guan et al. (2002).

Three B-vitamins (folate, vitamin B_{12}, and riboflavin) were chosen to explore the relevance of the arteriovenous balance for assessing vitamin uptake by the mammary gland in sows and its eventual impact on vitamin requirements of lactating sows. Folates and vitamin B_{12} determinations were done on all serum samples according to Tremblay et al. (1986) and Simard et al. (2007), respectively. Riboflavin was analyzed in plasma samples collected 60 min before and 150 min after the meal according to an HPLC method that included all active forms of this vitamin: flavin mononucleotide, flavin adenine dinucleotide, and riboflavin (Giguère et al., 2002).

AOAC (1990) methods were used for analyzing feed and milk for moisture, ash, N, and fat, and feed for crude fiber. Gross energy in feed and milk was measured using an adiabatic bomb calorimeter.

Calculations and statistical analysis

Average milk production over 21 days of lactation was calculated from piglet BW gain and litter size (Hansen et al., 2012). The extraction rates of nutrients were calculated as the ratio between A–V difference and arterial concentration, and expressed as a percentage. The respiratory quotient **(RQ)** at the mammary gland level was calculated as the ratio between the volume of CO_2 eliminated and the volume of O_2 consumed.

Arterial concentrations, A–V differences, and extraction rates of nutrients were analyzed with an ANOVA for repeated measurements (GLM procedure, SAS Inst. Inc., Cary, NC, USA), including the effect of sows, lactation stage, and sampling time. The effect of the interaction between sampling time and lactation stage was also tested but was removed from the model because it was not significant. Variance homogeneity was validated using Levene's test. A paired *t*-test was performed for testing the hypothesis that A–V differences were significantly different from zero. Linear regressions (SAS Inst. Inc., Cary, NC, USA) between A–V differences and arterial concentrations were calculated.

Results

Sow and litter performance

The performance of sows and their litter is presented in Supplementary Table S2. Average feed consumption during lactation was 5.18 ± 0.34 kg/d. It increased from 4.34 ± 0.38 kg/d in the first week to 5.38 ± 0.35 kg/d in the second week, and 5.81 ± 0.65 kg/d in the third week. The average litter weight gain over lactation was 2 042 ± 302 g/d with 10.2 ± 1.3 suckling piglets on average. The average milk production over lactation was 8.37 kg per day. The detailed composition of milk is given in Supplementary Table S2.

Arterial and venous concentrations and arterio-venous difference in nutrient concentrations

Hematocrit was greater ($P < 0.05$) in arterial than in venous blood (Table 1). Blood pH was lower ($P < 0.05$) in venous blood because of the higher CO_2 content ($P < 0.05$). The average O_2 extraction rate was 34.8%. Arteriovenous difference and extraction rate of O_2 were lower ($P < 0.01$) after the meal, whereas CO_2 production was not affected (Table 1). The RQ averaged 1.10, and was lower ($P < 0.01$) before the meal (0.95) than after the meal (1.54).

The effect of sow on arterial and venous concentration and A–V difference was significant for most nutrients and vitamins. Plasma arterial and venous concentrations of glucose increased ($P < 0.001$) after feeding (Fig. 1, Table 2). Glucose A–V difference differed from zero ($P < 0.05$) at all sampling times. It was at its lowest before the meal. It increased 60 min after feeding and subsequently remained at a high level. On average, glucose plasma arterial and venous concentrations and A–V difference were about two times greater ($P < 0.001$) after feeding (sampling time +60 to +180 min) than before feeding (sampling time −60 to 0 min), whereas the extraction rate (19.3% on average) did not differ ($P > 0.10$, Table 2). Like for glucose, lactate plasma arterial and venous concentrations and A–V difference increased ($P < 0.001$) drastically after feeding (by almost 80%), whereas the extraction rate did not change (16.5% on average). Plasma insulin concentration increased ($P < 0.001$) after the meal (Table 1), peaking at 60 min postprandial. Insulin A–V difference never differed from zero ($P > 0.10$).

Arterial and venous concentrations of NEFA, glycerol, and TG decreased ($P < 0.001$) after feeding (Fig. 1, Table 2). That reduction was particularly marked for NEFA, which dropped from 1 100 before to 120 μmol/l after the meal. In the case of NEFA, these changes resulted in a pronounced reduction of A–V difference ($P < 0.01$), which even became negative 2 to 3 h after the meal ($P < 0.05$), indicating an apparent production of NEFA by the mammary gland. A similar pattern was observed for glycerol. Although the arterial concentration of TG decreased after the meal, its A–V difference was not affected ($P > 0.10$) by the meal, as a result of the increase ($P < 0.05$) in the extraction rate from 24.2 to 33.3% between before and after feeding (Fig. 2, Table 2).

Arterial and venous concentrations and A–V difference of α-amino N increased ($P < 0.001$) after the meal, with the highest concentration measured 2 h after feeding (Fig. 1, Table 3). The large increase ($P < 0.001$) in A–V difference of α-amino N after the meal resulted from both an increase in arterial concentration and extraction rate. The A–V difference of urea concentration was low with an average extraction rate of only about 3%. A significant uptake by the mammary gland was measured for all essential AA ($P < 0.05$) before as well as after the

Table 1
Effect of stage of lactation of sows and meal on arterial (A) and venous (V) pH, concentration of O_2, CO_2, insulin, and some vitamins, and their arteriovenous (A–V) concentration difference.

		Day of lactation[1]			Nutritional status/meal		*P*-value[2]			
		7	14	21	Before	After	RSD	Day	Time	Meal[3]
Red cells, %	A	26.1	25.2	26.2	26.0	24.9	2.4		***	**
	V	26.7	25.8	26.7	26.3	25.6	2.1		***	*
	A–V[4]	*−0.63**	*−0.55**	*−0.47*t	*−0.30*	*−0.70*	*1.8*			*
pH	A	7.47	7.47	7.47	7.46	7.49	0.03		*	***
	V	7.43	7.42	7.41	7.46	7.49	0.02			*
	A–V	*0.05*	*0.05*	*0.06*	*0.04*	*0.07*	*0.03*		*	*
Insulin, μUI/ml	A	65.0	65.8	62.7	5.1	116	46.2		***	***
	V	64.8	69.9	59.1	4.3	116	49.3		***	***
	A–V	*0.2*	*−0.9*	*2.6*	*0.8*	*1.3*	*20.3*			*
O_2 total, ml/l	A	118	112	115	108	115	14.6		***	*
	V	79	72	74	64	80	12.0		***	***
	A–V	*38* (32%)*	*40* (36%)*	*41* (35%)*	*44* (47%)*	*35* (30%)*	*15.8*		**	**
CO_2 total, ml/l	A	752[a]	768[a]	732[b]	757	764	50.6	**	***	*
	V	811[a]	810[ab]	779[b]	799	818	53.2	*	***	*
	A–V	*−59* (−8%)*	*−42* (−5%)*	*−48* (−7%)*	*−42* (−6%)*	*−54* (−7%)*	*29.0*		*	*
Folates, ng/ml	A	68.7	61.1	52.6	55.2	63.2	4.5		***	***
	V	69.5	60.5	53.7	55.9	64.6	4.6		***	***
	A–V	*−0.8*	*0.6*	*−1.1*	*−0.7*	*−1.3*	*3.3*		**	
Vitamin B_{12}, pg/ml	A	506	391	249	426	412	52.5			
	V	502	385	237	424	410	53.2			
	A–V	*3.6*	*5.6*	*12.3* (4%)*	*1.6*	*2.1*	*28.0*			
Riboflavine, pmole/ml	A	288	282	316	292	298	11.9			
	V	283	273	327	286	302	13.1		*	*
	A–V	*5*	*9* (3%)*	*−11*	*5.0*	*−3.5*	*9.4*			*

[1] Means with different superscripts differ significantly ($P < 0.05$).
[2] *P*-value. ***: $P < 0.001$, **: $P < 0.01$, *: $P < 0.05$. The effect of sow was significant for all criteria ($P < 0.05$).
[3] Effect of meal: value measured on fasted (sampling time −60 to 0 min) versus fed animals (sampling time +60 to +180 min).
[4] A–V: From a Student's paired *t*-test, different from zero: *t*: $P < 0.10$, *: $P < 0.05$. Between brackets: extraction rate (%).

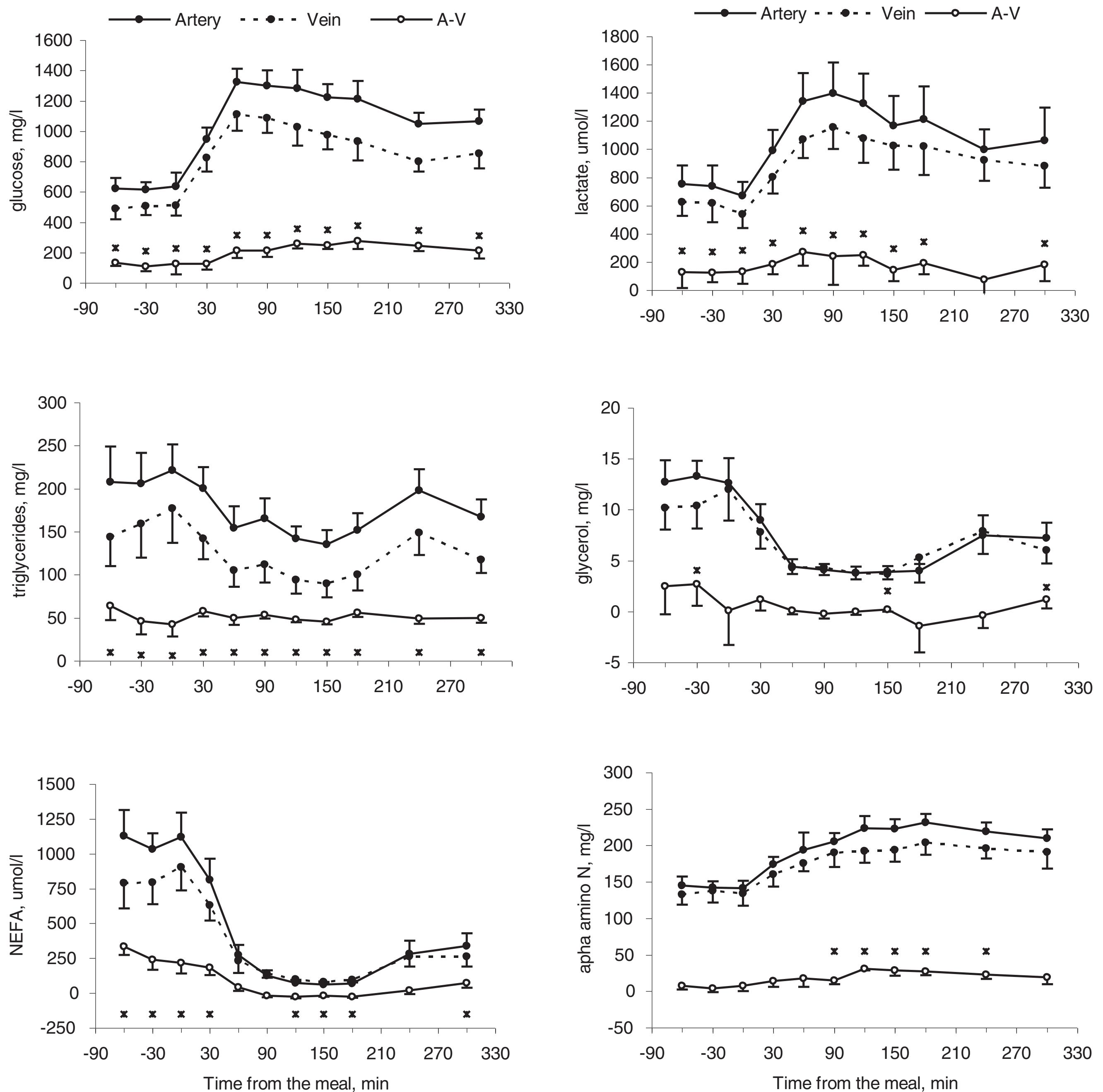

Fig. 1. Changes in sows' arterial (A) and venous (V) concentrations, and arteriovenous concentration difference (A–V) of glucose, lactate, triglycerides, glycerol, non-etherified fatty acids (NEFA), and α-amino nitrogen, with sampling time (0 = feed distribution).

meal (Table 3). Arterial concentration of all essential AA was greater ($P < 0.01$) after the meal (+120 min) than before the meal (−60 min, see Table 3), along with an increase in mammary A–V difference that was significant ($P < 0.05$) for methionine, leucine, isoleucine, and phenylalanine (Fig. 3). This was however not the case for valine, histidine, and arginine as their A–V difference was similar or tended to be greater during feed withdrawal (Fig. 3), due to a higher extraction rate. Arterial concentration of non-essential AA was lower before the meal than after the meal ($P < 0.001$, Table 3). On average, extraction rates of essential and non-essential AA were similar (18.4%).

Arterial and venous concentrations of plasma vitamin B_{12} tended to decrease ($P = 0.09$) on days 7 and 21 but were not affected by the meal (Table 1). Arteriovenous differences in vitamin B_{12} concentrations were positive ($P < 0.05$) only on day 21 and were not influenced by day of lactation or meal intake. For riboflavin, there was no effect of day of lactation or meal intake on arterial plasma concentrations but, for venous concentration, postprandial values were higher ($P < 0.05$) than preprandial. Arteriovenous differences in riboflavin concentrations were positive and differed from 0 only on day 14 ($P < 0.05$). None of these values were influenced by day of lactation, but, a trend toward a reduction of mammary extraction after the meal was observed for the meal response ($P = 0.06$), although neither pre- nor postprandial values deviated from zero. For folates concentrations, there was a marked postprandial increase (approximately 15%, $P < 0.01$) for both arterial and venous values. None of the A–V differences for folates deviated from zero nor were affected by day of lactation or meal intake.

Table 2
Effect of stage of lactation of sows and meal on arterial (A) and venous (V) concentration of different nutrients and their arteriovenous (A–V) concentration difference.

		Day of lactation[1]			Nutritional status/meal		P-value[2]			
		9	14	21	Before	After	RSD	Day	Time	Meal[3]
Glucose, mg/l	A	1038	1064	980	624	1268	153		***	***
	V	847	897	769	502	1026	149		***	***
	A–V[4]	191* (18.4)	185* (17.4)	210* (21.4)	122* (19.6)	242* (19.1)	86		***	***
Lactate, μmol/l	A	905^{b}	1080^{a}	1198^{a}	721	1289	334	*	***	***
	V	780	926	955	593	1069	259		***	***
	A–V	125* (13.8)	153* (14.1)	243* (20.2)	128* (17.8)	220* (17.1)	196			**
Triglycerides, mg/l	A	184	179	169	211	150	45.7		***	***
	V	133	134	113	160	100	47.1		***	***
	A–V	50.9^{ab}* (27.7)	45.3^{b}* (25.3)	56.3^{a}* (33.3)	51.0* (24.2)	50.0* (33.3)	37.1	**		
NEFA[5], μmol/l	A	500	422	519	1 094	121	206		***	***
	V	369	370	430	831	130	187		***	***
	A–V	90^{ab}* (18.0)	52^{a}* (12.3)	131^{a}* (25.2)	264* (24.1)	−9	169	*	***	***
Glycerol, mg/l	A	8.1	6.6	7.6	12.8	4.0	2.7		***	***
	V	7.0	7.1	6.5	10.8	4.3	3.3		***	***
	A–V	1.08* (13.3)	−0.5 (− 5.0)	1.03* (13.5)	1.9* (14.8)	−0.3	3.4		*	***
Calcium, mg/l	A	86.4	89.3	90.2	84.3	91.8	4.9		***	***
	V	84.2	85.8	86.6	82.2	88.0	4.1		***	***
	A–V	2.2* (2.5)	3.5* (3.9)	3.6* (4.0)	2.1* (2.5)	3.8* (4.1)	5.7			
Phosphorus, mg/l	A	59.7	59.8	62.9	56.1	63.3	3.5		***	***
	V	58.1	55.7	61.7	55.8	61.3	3.3		***	***
	A–V	1.6* (2.7)	2.0* (3.3)	1.2	0.3ns	2.0* (3.1)	2.8			**
α-amino N[5], mg/l	A	791	805	800	143	214	98		***	***
	V	741	709	712	133	191	95		***	***
	A–V	41.5t (5.2)	96.0* (11.9)	88.4* (11.0)	7.0* (4.9)	23.8* (11.1)	101		***	***
Urea, mg/l	A	402^{a}	404^{a}	358^{b}	338	403	23.4	*	***	***
	V	394	400	360	336	398	14.0		***	***
	A–V	8.1^{a}* (2.0)	4.3^{ab}	−2.2^{b}	2.3	5.1t (1.3)	23.8	*		

[1] Means with different superscripts differ significantly ($P < 0.05$).
[2] P-value. ***: $P < 0.001$, **: $P < 0.01$, *: $P < 0.05$. The effect of sow was significant for all criteria ($P < 0.05$).
[3] Effect of meal: value measured on fasted (sampling time −60 to 0 min) versus fed animals (sampling time +60 to +180 min).
[4] A–V: From a Student's paired t-test, different from zero: t: $P < 0.10$, *: $P < 0.05$. Between brackets: extraction rate (%).
[5] NEFA: non-esterified fatty acids; α-amino N: α-amino nitrogen.

Discussion

Hematocrit was significantly greater by 0.6 percentage points, in venous than in arterial blood, the difference being greater after the meal than before (0.7 vs 0.3 percentage points), in line with the results obtained by Renaudeau et al. (2003). This increased hematocrit value of venous blood can be explained by the water uptake by the mammary gland.

Arterial concentrations of glucose, lactate, AA, urea, and minerals increased after the meal, whereas those of FFA, TG, and glycerol decreased. Similar results were found in various studies in which lactating sows were fed after overnight feed withdrawal (Messias de Bragança and Prunier, 1999).

Average glucose A–V difference (196 mg/l) was similar to the average value measured by Spincer et al. (1969), Renaudeau et al. (2003), and (Krogh et al., 2017, 198, 247, and 178 mg/l, respectively). Lactate A–V difference (175 μmol/l) was within the range of values measured by Linzell et al. (1969), Renaudeau et al. (2003), and (Krogh et al., 2017, 90, 60, and 220 μmol/l, respectively). Like in lactating cows (Rulquin, 1997), we observed a linear relationship, according to nutritional status, between A–V concentration difference and arterial concentration of glucose ($R^2 = 0.72$, Fig. 2). A similar linear relationship ($R^2 = 0.77$, Fig. 2) was found for lactate. These nutrients with a high osmotic gradient are transferred from blood to the lumen of the acinus in the Golgi vesicles. This transfer requires the presence of transporters, among which GLUT1, that is not insulin-dependent, is the primary one for glucose transport to mammary gland (Camps et al., 1994).

For NEFA, average A–V difference (93 μmol/l) was higher than the average values measured by Linzell et al. (1969) and (Krogh et al., 2017, 8 and 25 μmol/l, respectively). These differences between studies may be related to the nutritional status of sows and the duration of feed withdrawal. The elevated preprandial level of arterial NEFA concentration indicates a mobilization of body lipids (Dunshea et al., 1989; Père and Etienne, 2007) that contributes to the supply of energy to the mammary gland, as indicated by the increased A–V difference in NEFA concentration during feed withdrawal, in keeping with the results of Krogh et al. (2017). After the meal, NEFA plasma concentration decreased drastically, concomitantly with increasing levels of glucose and insulin that inhibit lipolysis, as already shown by Père and Etienne (2007). This resulted in a transient negative A–V difference of NEFA concentration, in line with the results of Krogh et al. (2017) who suggested that this release of NEFA after the meal could be related to the mobilization of body fat from the mammary gland or the hydrolysis of TG, as shown in goat mammary gland (West et al., 1972). Plasma arterial concentration of glycerol was about three times higher before than after the meal, with an average value of 7.5 mg/l, which is close to the 6.4 mg/l value measured by Renaudeau et al. (2003). Like with NEFA, we observed a release of glycerol by the mammary gland after the meal which may also be related to TG hydrolysis as observed in the goat mammary gland (West et al., 1972). The absorption of NEFA and glycerol by the mammary gland depends on their arterial concentrations, with a closer relationship for NEFA than for glycerol. The greater A–V difference of these nutrients during feed withdrawal results from both the increase in their arterial concentration and their extraction rate. According to Veerkamp (1995), the extraction rate of NEFA depends on their metabolic utilization. This would explain the much lower and even negative uptake of NEFA by the mammary gland when the availability of glucose is high after feeding.

Average A–V difference in TG concentration (51 mg/l) was similar to the values measured by Spincer et al. (1969), Renaudeau et al. (2003), and (Krogh et al., 2017, 72, 47, and 51 mg/l, respectively). From our results, it appears that A–V difference in TG concentration is not affected by the nutritional status of the sow, since the decreased arterial concentration is offset by the increased extraction rate.

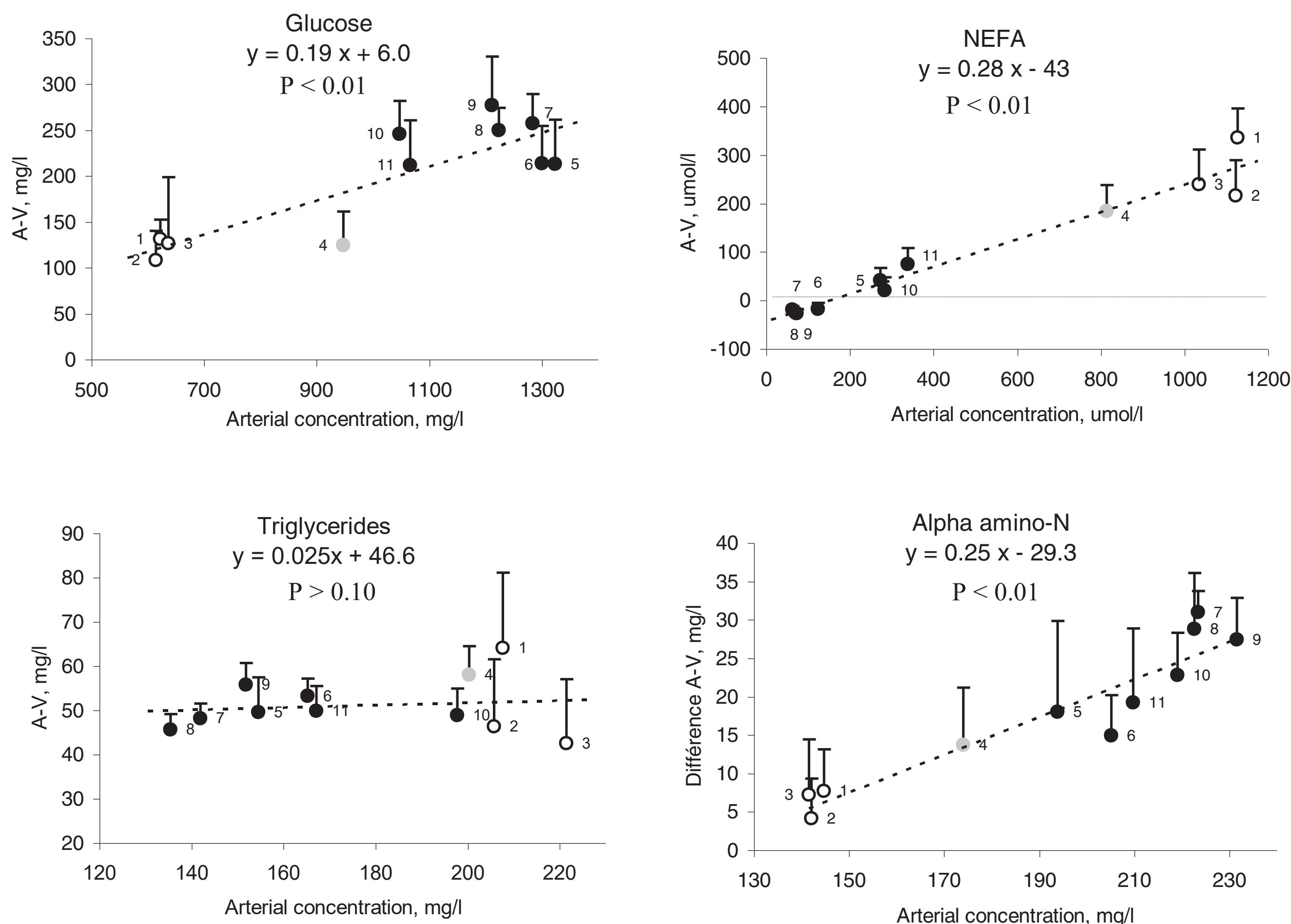

Fig. 2. Effect of sows' arterial concentration of glucose, non-esterified fatty acid (NEFA), triglycerides, and α-amino nitrogen on arteriovenous concentration difference (A–V). (1 to 11: from −60 to +300 min after meal distribution; ○ before the meal, ● after the meal).

Average α-amino N A–V difference (18 mg/l) was similar to the values measured by Linzell et al. (1969), Spincer et al. (1969), and (Renaudeau et al., 2003, 16, 24, and 21 mg/l, respectively). The increase in α-amino N concentration after the meal resulted in a drastic increase (× 3.4) of A–V difference which originated from increases in both arterial concentration (Fig. 2) and extraction rate.

The average extraction rate of total essential AA did not differ significantly between 30 min before (20.9%) and 120 min after the meal (16.7%). These values are similar to those measured by Renaudeau et al. (2003), Guan et al. (2002), and (Krogh et al., 2017, 23, 21, and 20%, respectively). The change after the meal in A–V differences of essential AA concentrations depends on the AA. It increased significantly for methionine, leucine, isoleucine, and phenylalanine, but decreased significantly for arginine, which might thus play a specific role during fasting.

The respiratory coefficient calculated from A–V difference in O_2 and CO_2 concentrations was lower before the meal (0.95) than after the meal (1.54). This is consistent with the high A–V difference in glucose concentration across the mammary gland after the meal. Conversely, before the meal, the A–V difference in glucose concentration decreased and the A–V difference of NEFA and TG concentrations increased, resulting in a lower RQ.

The A–V difference in energy concentration can be calculated from A–V difference in nutrients concentrations and their energy content, assuming these energy-providing nutrients are all oxidized for ATP production or transferred directly into the milk. From the results in the present study, A–V energy concentration difference was 7.6 kJ/l plasma on average. This value is similar to the values that can be calculated from the studies of Renaudeau et al. (2003) and (Krogh et al., 2017, 8.3 and 7.9 kJ/l plasma, respectively, Fig. 4). Although nutritional status had no significant effect on energy A–V difference (7.8 and 7.5 kJ/l plasma for feed-deprived and fed sows, respectively), it had a large effect on the relative contribution of the different nutrients to energy supply. In fed sows, the contribution of glucose to total energy uptake averaged 50.2%, compared to 24.6% in feed-deprived sows. Conversely, the contribution of NEFA to energy supply was much higher in fasted (32.9%) than in fed sows (0%). The contribution of TG was similar in feed-deprived and fed sows with 24.9 and 25.1%, respectively. In the study from Renaudeau et al. (2003) with restrictedly fed sows the results were intermediate, with NEFA and glucose contributing to 14 and 45% of energy intake, respectively. In the study of Krogh et al. (2017), with sows fed more liberally, the relative contribution of the different nutrients was closer to the results obtained in the present study with fed sows.

These results indicate that the mammary gland has a great capacity to adapt its metabolism to the nutrients available in the bloodstream. This adaptation may occur in the very short term with, for instance, a very rapid switch of energy uptake from NEFA to glucose after the meal, over a period of less than 1 hour. In ruminants, because of the buffering effect of the rumen on artery nutrient content such rapid and extreme changes have not been observed, although some adaptations also exist in the longer term.

These results are consistent with the results on whole-body energy metabolism (Noblet and Etienne, 1986), which indicate that energy restriction in lactating sows results in increased mobilization of body fat

Table 3
Effect of the meal on sows' arterial concentration (A, mg/l) arteriovenous concentration difference (A–V, mg/l), and mammary extraction rate (E, %)[1] of amino acids.

		Average	−30 mn	+120 mn	Statistical significance		
					RSD	Day	Meal[2]
Lysine	A	39.5	29.0	50.1	9.4	0.68	<0.001
	A–V[3]	6.8*	6.2*	7.3*	5.1	0.36	0.51
	E	*17.1*	*21.3*	*14.7*			
Methionine	A	4.0	3.3	4.7	1.1	0.79	0.001
	A–V	1.0*	0.7*	1.3*	0.7	0.05	0.02
	E	*24.0*	*19.5*	*27.1*			
Threonine	A	15.1	11.8	18.3	3.9	0.70	<0.001
	A–V	3.4*	2.8*	4.1*	3.0	0.23	0.23
	E	*22.8*	*23.8*	*22.2*			
Leucine	A	33.3	28.2	38.4	6.6	0.39	<0.001
	A–V	7.1*	5.2*	9.1*	4.1	0.05	0.01
	E	*21.4*	*18.4*	*23.7*			
Isoleucine	A	22.5	18.8	26.2	4.2	0.81	<0.001
	A–V	4.5*	3.5*	5.6*	2.4	0.03	0.03
	E	*20.2*	*18.8*	*21.3*			
Valine	A	45.8	40.0	51.6	5.6	0.20	<0.001
	A–V	6.5*	6.9*	6.1*	4.4	0.31	0.59
	E	*14.2*	*17.2*	*11.7*			
Arginine	A	21.2	15.5	26.9	8.3	0.41	0.001
	A–V	5.5*	6.4*	4.5*	5.1	0.22	0.29
	E	*25.9*	*41.0*	*16.7*			
Histidine	A	17.9	15.6	20.2	3.8	0.86	0.002
	A–V	1.9*	2.1t	1.7t	3.7	0.83	0.75
	E	*10.8*	*13.6*	*8.6*			
Phenylalanine	A	14.5	10.0	19.1	4.0	0.98	<0.001
	A–V	3.7*	2.7*	4.6*	2.4	0.83	0.04
	E	*25.3*	*27.3*	*24.2*			
Tyrosine	A	17.4	12.0	22.9	4.9	0.70	<0.001
	A–V	3.6*	3.2*	4.0*	3.0	0.22	0.42
	E	*20.6*	*26.5*	*17.5*			
Total essential	A	213.8	172.1	255.5	40.6	0.75	<0.001
	A–V	40.4*	36.5*	44.3*	18.9	0.15	0.25
	E	*18.9*	*21.2*	*17.3*			
Total non-essential	A	180.3	142.2	218.5	30.6	0.01	<0.001
	A–V	32.1*	28.7*	35.6*	20.0	0.04	0.33
	E	*17.8*	*20.2*	*16.3*			

[1] Extraction rate was calculated as (A–V)/A × 100 when A–V was significantly different from zero.
[2] Effect of sampling time (−30 min vs +120 min after meal distribution).
[3] From a Student's *t*-paired test procedure, A–V significantly different from zero: *t*: $P < 0.10$, *: $P < 0.05$.

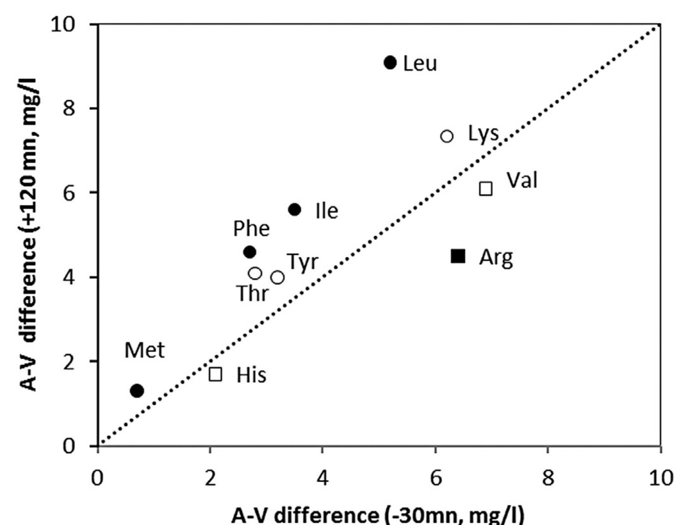

Fig. 3. Relationship between sows' arteriovenous concentration difference (A–V) of essential amino acids (mg/l plasma) measured 30 min before or 120 min after the distribution of the meal (● significant increase ○ non-significant increase ■ significant decrease □ non-significant decrease).

reserves and increased fat content in milk, with limited effects on total energy production in milk. In humans, Mahmoud et al. (2014) also showed that the nutritional status of women (fasted or fed) and the composition of the diet (high carbohydrate or high fat) affected the lipid content and composition of human milk.

For vitamins, mammary extraction was generally very small, with some A–V values slightly positive (≤ 4.5%) for riboflavin and vitamin B_{12} during the second half of lactation whereas those for folates never differed significantly from zero. Nevertheless, although it is apparently small, such extraction, depending on the vitamin, might be sufficient to account for amounts that are transferred toward milk synthesis. For vitamin B_{12}, using daily mammary blood plasma flows between approximately 4 000 (Renaudeau et al., 2003) to 7 000 l/d (Krogh et al., 2017), the daily total extraction may be estimated within a range of 50 to 86 µg toward the end of lactation. This estimated amount is probably a major contributor to the strong tendency of arterial and venous plasma concentrations of vitamin B_{12} to decline by as much as 50% during lactation, an observation also reported by Simard et al. (2007). During the last third of lactation, the estimated extraction by the mammary gland may be twice as high as the net amount of vitamin B_{12} available from the diet, given a dietary supply of vitamin B_{12} of approximately 100 µg/d and an intestinal bioavailability between 10 and 38% (Matte et al., 2010). The present estimation of daily mammary extraction for vitamin B_{12} is consistent with the total content of vitamin B_{12} in sow milk, given the present milk yield (Supplementary Table S2) and a concentration of vitamin B_{12} of approximately 6 µg/l of milk, as reported earlier by Audet et al. (2015).

For riboflavin, the daily total extraction, as estimated for vitamin B_{12}, ranged between 36 and 63 µmole (13.6 to 23.8 mg) at mid-lactation. There was apparently no impact of the mammary extraction of riboflavin on changes in plasma arterial or venous concentrations of riboflavin during lactation. With a dietary supply of riboflavin of roughly 62 mg/d and intestinal bioavailability of approximately 80%, the net amount of riboflavin available for the whole body during mid-lactation was apparently sufficient for the transfer of this vitamin to the mammary gland. The present estimation of mammary extraction for riboflavin is in line with the total riboflavin content in sow milk, given the present milk yield (Supplementary Table S2) and a riboflavin concentration of 3.5 (range of 1.3 to 8.2) mg/l of milk as reported by Pond and Houpt (1978).

For folates, it appears that the present approach was not sensitive enough to detect significant A–V differences. Using the present daily milk production and concentrations of folates in sow milk as reported by Barkow et al. (2001), the total amount of folates in sow milk can be estimated at 90 µg. If this is equivalent to the daily mammary extraction, it could correspond to A–V differences within a range of 0.01 to 0.02 ng/ml of plasma folate. Such values are likely much too small to be detected by the current analytical procedure and are in line with the present statistical results, where none of A–V differences for folates deviate from 0. Therefore, the mammary extraction is apparently negligible as compared to the overall metabolic folates status and then did not affect profiles of plasma arterial and venous folates during lactation of sows.

Conclusion

The results obtained in this study indicate that the mammary gland has a great capacity to very rapidly adapt its nutrient uptake and modify its metabolism according to the nutrient available in the bloodstream. After feeding, glucose contributed to 50% of energy uptake, compared to 25% before feeding. Conversely, NEFA contributed to 33% of energy uptake before feeding whereas no significant uptake was measured after feeding. The relative contribution of AA to energy uptake increased after the meal whereas contribution of TG was not affected by the nutritional status. The change after the meal in A–V difference of essential AA concentration depended on the AA, indicating that some of them could play a specific role during fasting. Concerning the vitamins, the present exploratory study with three B-vitamins showed that the arteriovenous

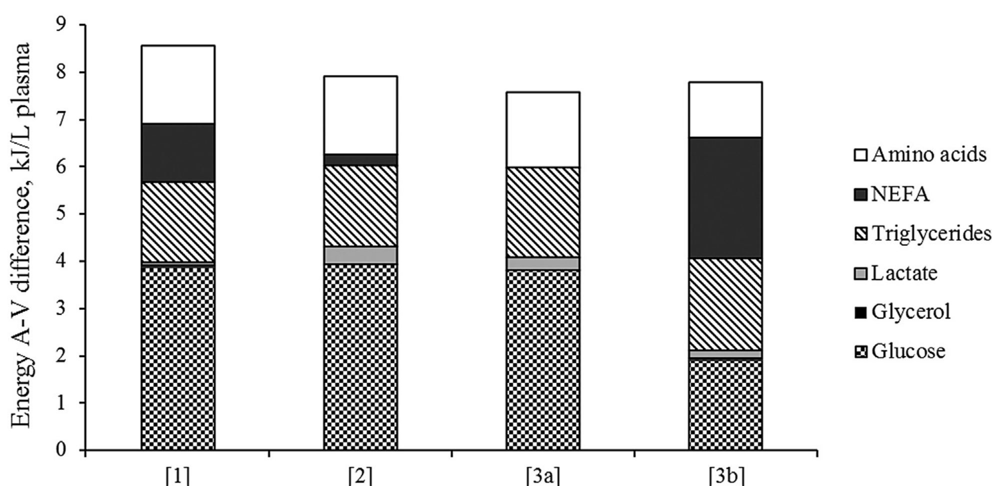

Fig. 4. Partition of energy uptake from various nutrients by the sow mammary gland in different studies. [1] Renaudeau et al. (2003), [2] Krogh et al. (2017), [3] present study, a: postprandial, b: feed-deprived. A–V: arteriovenous concentration difference, NEFA: non-esterified fatty acids.

balance may be used for assessing vitamin uptake by the mammary gland in sows but it might not apply to all vitamins.

Supplementary materials

Supplementary data to this article can be found online at https://doi.org/10.1016/j.animal.2020.100116.

Ethics approval

The experiment was conducted in accordance with the French guidelines for animal care and use drafted by the French Ministries of High Education and Research, and of Agriculture and Fisheries (https://www.recherche-animale.org/charte-nationale-sur-lethique-de-lexperimentation-animale). The experimental unit was a holder of a pig experimentation authorization (No. C-35-275-32) and all the technical and scientific staff involved in the experiment had an individual agreement for experimentation on living animals.

Data and model availability statement

Data are not deposited in any official repository.

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Author contributions

J.Y. Dourmad: Conceptualization, methodology, validation, investigation, writing – review & editing, supervision, project administration. J.J. Matte: Conceptualization, methodology, validation, writing – review & editing.

Declaration of interest

None of the authors had a financial or personal interest in relation to the present study.

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References

AOAC, 1990. Official methods of analysis, 15th edition. Association of Analytical Chemists, Washington, DC, USA.

Audet, I., Girard, C.L., Lessard, M., Lo Verso, L., Beaudoin, F., Matte, J.J., 2015. Homocysteine metabolism, growth performance, and immune responses in suckling and weanling piglets. Journal of Animal Science 93, 147–157.

Barkow, B., Matte, J.J., Bohme, H., Flachowsky, G., 2001. Influence of folic acid supplements on the carry-over of folates from the sow to the piglet. British Journal of Nutrition 85, 179–184.

Camps, M., Vilaro, S., Testar, X.M., Palacín, M., Zorzano, A., 1994. High and polarized expression of GLUT1 glucose transporters in epithelial cells from mammary gland: acute down-regulation of GLUT1 carriers by weaning. Endocrinology 134, 924–934.

Dourmad, J.Y., Noblet, J., Etienne, M., 1998. Effect of protein and lysine supply on performance, nitrogen balance, and body composition changes of sows during lactation. Journal of Animal Science 76, 542–550.

Dunshea, F.R., Bell, A.W., Trigg, T.E., 1989. Relations between plasma non-esterified fatty acid metabolism and body fat mobilization in primiparous lactating goats. British Journal of Nutrition 62, 51–61.

Farmer, C., Trottier, N.L., Dourmad, J.Y., 2008. 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.

Giguère, A., Girard, C.L., Matte, J.J., 2002. Erythrocyte glutathione reductase activity and riboflavin nutritional status in early-weaned piglets. International Journal for Vitamin and Nutrition Research 72, 383–387.

Guan, X., Bequette, B.J., Calder, G., Ky, P.K., Ames, K.N., Trottier, N.L., 2002. Amino acid availability affects amino acid flux and protein metabolism in porcine mammary gland. Journal of Nutrition 132, 1224–1234.

Hansen, A.V., Strathe, A.B., Kebreab, E., France, J., Theil, P.K., 2012. Predicting milk yield and composition in lactating sows: a Bayesian approach. Journal of Animal Science 90, 2285–2298. https://doi.org/10.2527/jas.2011-4788.

Krogh, U., Oksbjerg, N., Storm, A.C., Feyera, T., Theil, P.K., 2017. Mammary nutrient uptake in multiparous sows fed supplementary arginine during gestation and lactation. Journal of Animal Science 95, 2517–2532.

Linzell, J.L., Mephan, T.B., Annison, E.F., West, C.E., 1969. Mammary metabolism in lactating sows: arteriovenous differences of milk precursors and the mammary metabolism of [^{14}C]glucose and [^{14}C]acetate. British Journal of Nutrition 23, 319–332.

Mahmoud, A.M., Sunehag, A.L., Haymond, M.W., 2014. De novo synthesis of milk triglycerides in humans. American Journal of Physiology Endocrinology and Metabolism 306, E838–E847.

Matte, J.J., Guay, F., Le Floc'h, N., Girard, C.L., 2010. Bioavailability of dietary cyanocobalamin (vitamin B_{12}) in growing pigs. Journal of Animal Science 88, 3936–3944.

Messias de Bragança, M., Prunier, A., 1999. Effects of low feed intake and hot environment on plasma profiles of glucose, non-esterified fatty acids, insulin, glucagon, and IGF-I in lactating sows. Domestic Animal Endocrinology 16, 89–101.

National Research Council (NRC), 2012. Nutrient requirements of swine, 11th revised edition. National Academy Press, Washington, DC, USA.

Nielsen, T.T., Trottier, N.L., Stein, H.H., Bellaver, C., Easter, R.A., 2002. 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., Etienne, M., 1986. Effect of energy level in lactating sows on yield and composition of milk and nutrient balance of piglets. Journal of Animal Science 63, 1888–1896.

Noblet, J., Dourmad, J.Y., Etienne, M., 1990. Energy utilization in pregnant and lactating sows: modeling of energy requirements. Journal of Animal Science 68, 562–572.

Père, M.C., Etienne, M., 2007. Insulin sensitivity during pregnancy, lactation, and postweaning in primiparous gilts. Journal of Animal Science 85, 101–110.

Pond, W.G., Houpt, K.A., 1978. The Biology of the Pig. Cornell University Press, Ithaca, NY, USA.

Renaudeau, D., Noblet, J., Dourmad, J.Y., 2003. Effect of ambient temperature on mammary gland metabolism in lactating sows. Journal of Animal Science 81, 217–231.

Rulquin, H., 1997. Régulation de la synthèse et de la sécrétion des constituants du lait chez les ruminants. Rencontres Recherches Ruminants 4, 327–338.

Simard, F., Guay, F., Girard, C.L., Giguère, A., Laforest, J.P., Matte, J.J., 2007. Effects of concentrations of cyanocobalamin in the gestation diet on some criteria of vitamin B-12 metabolism in first-parity sows. Journal of Animal Science 85, 3294–3302.

Spincer, J., Rook, J.A.F., Towers, K.G., 1969. The uptake of plasma constituents by the mammary gland of the sow. Biochemistry Journal 111, 727–732.

Tremblay, G.F., Matte, J.J., Lemieux, L., Brisson, G.J., 1986. Serum folates in gestating swine after folic acid addition to diet. Journal of Animal Science 63, 1173–1178.

Trottier, N.L., Shipley, C.F., Easter, R.A., 1995. 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., Easter, R.A., 1997. Plasma amino acid uptake by the mammary gland of the lactating sow. Journal of Animal Science 75, 1266–1278.

Veerkamp, J., 1995. Fatty acid transport and fatty acid-binding proteins. Proceedings of the Nutrition Society 54, 23–37.

West, C.E., Bickerstafee, R., Annison, E.F., Linzell, J.L., 1972. Studies on the mode of uptake of blood triglycerides by the mammary gland of the lactating goat. Biochemistry Journal 126, 477–490.