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U. Krogh, Hélène Quesnel, Nathalie Le Floc'H, A Simongiovanni, Jaap J. van Milgen

Animal 15 (2021) 100049

Contents lists available at ScienceDirect

Animal
The international journal of animal biosciences

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U. Krogh [a], H. Quesnel [a], N. Le Floc'h [a], A. Simongiovanni [b], J. van Milgen [a,*]

[a] PEGASE, INRAE, Institut Agro, 35590 Saint-Gilles, France
[b] Ajinomoto Animal Nutrition Europe, 32 rue Guersant, 75017 Cedex 17 Paris, France

ARTICLE INFO

Article history:
Received 11 December 2019
Received in revised form 12 August 2020
Accepted 13 August 2020
Available online 10 December 2020

Keywords:
Amino acids
Energy metabolites
Mammary blood flow
Milk
Pigs

ABSTRACT

Quantitative estimates of mammary nutrient inputs, outputs and metabolism in sows are scarce, despite being critical elements to identify parameters controlling milk synthesis central for the feeding of lactating sows. The objective of this study was to quantify the mammary gland input and output of nutrients as well as the intramammary partitioning of carbon and nitrogen with the purpose to identify mechanisms controlling mammary nutrient inputs, metabolism and milk production in lactating sows. A data set was assembled by integration of results from four studies. The data set included data on litter performance, mammary arterial-venous concentration differences (**AV-difference**) of energy metabolites and amino acids, and the contents of lactose, fat and amino acids in milk. Milk yield was estimated based on average litter size and litter gain, and mammary plasma flow (**MPF**) was estimated using the sum of phenylalanine and tyrosine as internal flow markers. The yield and composition of milk were used to estimate mammary nutrient output in milk, and MPF and AV-difference were used to estimate net mammary input of carbon and nitrogen and output of CO_2. Carbon and nitrogen used for the synthesis of lactose, fat and protein in milk and CO_2-yielding processes were represented in a static nutrient partitioning model. The origin of mammary CO_2 output was calculated using theoretical estimates of carbon released in processes supporting mammary synthesis of *de novo* fat, protein and lactose in milk, mammary tissue protein turnover and transport of glucose and amino acids. Results indicated that total input of carbon from glucose and lactate was partitioned into lactose (36%), fat (31%) and CO_2-yielding processes (34%). Theoretical CO_2 estimates indicated that *de novo* fat synthesis, milk protein synthesis and mammary tissue protein turnover were the main processes related to mammary CO_2 production. More than 90% of mammary gland amino acid input was used for milk protein. The quadratic relationship between AV-difference and mammary input of essential amino acids indicated that both changes in AV-difference and MPF contributed to the regulation of mammary input of essential amino acids. The impact of the arterial supply of amino acids on mammary input may be greater for the branched-chain amino acids, arginine and phenylalanine than for other essential amino acids. In conclusion, relationships between input and output parameters indicate that AV-difference and MPF regulate mammary nutrient input to match the supply and demand of nutrients for the mammary gland.

Implications

These results contribute to the existing knowledge about the regulation of milk synthesis in lactating sows and how nutrients are utilized for milk production. An integrated understanding of mechanisms regulating milk synthesis must be established to quantify the impact of nutrient supply and animal characteristics on mammary nutrient metabolism. Quantification of the use of nutrients by the mammary gland combined with a fundamental understanding of factors controlling milk production is important to develop nutritional and other management strategies to improve nutrient utilization and the milk synthesis capacity in lactating sows.

Introduction

The sow mammary gland exerts considerable flexibility to accommodate changes in nutrient supply and/or demand for milk synthesis during lactation (Farmer et al., 2008). Numerous factors including feed composition (Guan et al., 2004), nutritional state of the sow (Dourmad et al., 2000) and litter size (Nielsen et al., 2002) influence nutrient supply to the mammary gland, nutrient demand for milk synthesis and, consequently, nutrient input and metabolism by the mammary gland. It is important to identify and understand the factors driving the control of mammary nutrient inputs and partitioning to optimize dietary nutrient supply for lactating sows. However, important traits for the quantification of mammary gland metabolism, such as milk yield and mammary plasma flow (**MPF**), have been estimated by various different approaches across studies (Renaudeau et al., 2003; Farmer et al.,

* Corresponding author.
E-mail address: jaap.vanmilgen@inrae.fr (J. van Milgen).

https://doi.org/10.1016/j.animal.2020.100049

2008; Krogh et al., 2017). Integration of data from available studies into a single data set and standardization of these MPF and milk yield estimations may be an approach to investigate and identify driving forces controlling mammary nutrient input, metabolism and milk synthesis in lactating sows. The objective of the present study was to quantify mammary gland input and output of nutrients by integrating and standardizing data from existing studies and to build a static model describing mammary gland carbon and nitrogen partitioning to identify mechanisms controlling mammary nutrient inputs, metabolism and milk production in lactating sows.

Material and methods

Data set for model development

Available results from studies examining mammary inputs of major milk precursors in lactating sows were integrated in a single data set. The inclusion criteria were the availability of the following data: 1. arterial and mammary vein concentrations of red blood cells (hematocrit), CO_2 in blood and major energy metabolites in plasma (i.e., glucose, lactate, non-esterified fatty acids [**NEFA**] and triglycerides) and free individual essential amino acids (**EAA**) and non-essential amino acids (**NEAA**) in plasma for the estimation of mammary nutrient inputs; 2. litter performance (i.e., average litter size and litter gain) for the estimation of milk yield and 3. milk composition of lactose, fat and individual amino acids for the estimation of output in milk. It was required that all inclusion criteria be completely available in order for a study to be included in the data set. An overview of the four studies included in the final data set is given in Table 1. The final data set contained 13 group means (i.e., data points) for each variable in the data set and covered a time period between day 3 and 21 in lactation. The sows in the four studies were fed diets mainly based on wheat and soybean meal as well as barley and/or corn. The diets contained between 14.7 and 15.2 MJ metabolizable energy/kg DM and between 150 and 196 g CP/kg DM. Average feed intake was 5.0 kg/day and varied between 2.8 and 8.1 kg/day (see Table S1 of the Supplementary material for further details).

Variable calculations

Sow milk yield

In contrast to dairy species, milk yield is not easily measured in sows. In the four studies contributing to the analysis, milk yield was estimated indirectly using different methods. To standardize the estimation of milk yield across studies, five prediction methods to estimate milk yield were applied to compare the methods and select the most appropriate method. The comparison of the five methods is described in Supplementary Material S2. The method of Hansen et al. (2012) based on data from studies that applied the deuterium oxide dilution technique was selected to estimate milk yield. Average litter gain and litter size during lactation were the two input traits used to estimate milk yield for each day during lactation.

Output of carbon and nitrogen in milk

Output of lactose, fat and individual amino acids in milk was calculated based on the analyzed concentration multiplied by the estimated milk yield on the actual day of sampling. Output of carbon and nitrogen in milk from lactose, fat and individual amino acids was then calculated based on the content of carbon and nitrogen in these components. Sow milk consists mainly of fatty acids with 14-, 16- and 18-carbon chain lengths constituting approximately 5, 40 and 55% of total fatty acids in milk, respectively (Hurley, 2015). The weighted average of this composition was assumed to characterize the average chain length of fatty acids in milk, resulting in an average of 17 carbon atoms/fatty acid and a corresponding molecular weight of 849 g/mol for triglycerides in milk fat. Lysine, methionine, threonine, tryptophan, isoleucine, leucine, valine, histidine, phenylalanine and arginine were considered EAA, whereas alanine, tyrosine, asparagine, aspartate, glutamine, glutamate, glycine, cysteine, serine and proline were considered NEAA according to the ideal amino acid profile proposed for gestating and lactating sows (Dourmad et al., 2008). Milk concentrations of tryptophan, tyrosine and proline were not analyzed in all studies, and missing data values were consequently estimated based on the milk protein content according to the review by Hurley (2015). Details of estimation of missing amino acid data in milk and the separation of analyzed milk aspartate + asparagine and glutamate + glutamine into the individual amino acids are described in Supplementary Material S3.

Mammary plasma flow and blood flow

To standardize MPF across studies, 10 different amino acid marker candidates were compared to select the most appropriate method. The sum of phenylalanine + tyrosine was selected as the most appropriate marker to estimate MPF, and details of this selection method are described in Supplementary Material S4. Daily MPF (l/day) was estimated as the output of phenylalanine + tyrosine in milk (mmol/day) divided by the mammary arterial-venous concentration difference (**AV-difference**) of phenylalanine + tyrosine (mmol/l) according to Fick's principles. The daily mammary blood flow (**MBF**, l/day) was estimated as MPF/(1-hematocrit/100).

Table 1
Overview of the experimental setup of the four sow studies contributing data for the present study.

Reference	Number of sows	Number of treatments	Sampling days in lactation	Groups[1]	Blood samplings			Milk samplings[2]
					#/day	Relative to feeding		
						Interval, h	Average sampling time, min	
Dourmad et al. (2000)	6	1	9, 14, 21	3	11[3]	−1.0; +4.5	120[6]	1
Krogh et al. (2017)	8	2	3, 17	4	8[4]	−0.5; +6.5	180	1
Krogh U. (Unpublished results)	8	2	10	2	8[4]	−0.5; +6.5	180	1
Renaudeau et al. (2003)	6	2	12, 19	4	14[5]	−0.3; +1.8	105	1
Total	28	7	8	13	–	–	–	–

[1] Combination of treatments and sampling days.
[2] Milk samples were collected within 1 h after the last blood sampling in all studies.
[3] Amino acids were analyzed in two of the 11 samples (30 min. before and 120 min. after feeding). Other nutrients were analyzed for all samples.
[4] All samples were analyzed for all nutrients.
[5] Amino acids were analyzed in one pooled sample originating from the 14 collected samples. Other nutrients were analyzed for all samples.
[6] An average of 90 min after feeding for the amino acid analyses and 120 for other analyses.

Mammary input and output of carbon and nitrogen from and into the circulatory system

Mammary inputs of carbon and nitrogen (mol/day) from plasma were calculated by multiplying MPF (l/day) with the AV-difference of carbon and nitrogen (mol/l) originating from individual amino acids, glucose, lactate, triglycerides and NEFA. Mammary output of CO_2 into the circulatory system was estimated by multiplying the absolute values of the AV-difference of CO_2 with MBF (l/day) because CO_2 was measured in blood. Plasma fatty acid composition was not measured in any of the studies. Thus, the average chain length of fatty acid input from triglycerides and NEFA was assumed to be identical to the average chain length of milk fatty acids (i.e., 17 carbon atoms/fatty acid).

Plasma concentrations of tryptophan, asparagine, glutamine, arginine and proline were not measured in all studies. To predict these missing values, linear relationships between AV-differences of these amino acids and lysine were estimated using results from other studies measuring the mammary AV-difference and the arterial concentration of individual amino acids. The R-square of the linear relationships between AV-differences of lysine and the amino acids of interest was 0.78 for tryptophan, 0.53 for asparagine, 0.50 for glutamine, 0.38 for arginine and 0.80 for proline (see Supplementary Material S5). Similarly, arterial concentrations of tryptophan, asparagine, glutamine, arginine and proline were predicted by the arterial concentration of phenylalanine, glycine, histidine, methionine and isoleucine, respectively. The R-square of these linear relationships was 0.63 for tryptophan, 0.71 for asparagine, 0.79 for glutamine, 0.42 for arginine and 0.63 for proline (see Supplementary Material S5).

Partitioning of carbon and nitrogen from blood to milk

A static nutrient partitioning model from blood via the mammary gland to milk was constructed based on the mammary input and output of carbon and nitrogen. Mammary input of carbon and nitrogen from glucose, lactate, NEFA, triglycerides, EAA and NEAA from arterial circulation was partitioned into carbon and nitrogen in lactose, fat, EAA and NEAA in the milk or as CO_2. Carbon from glucose and lactate was combined and characterized as "lactogenic carbon", while carbon from NEFA and triglycerides was combined and further referred to as "lipogenic carbon". The carbon and nitrogen fluxes followed the structure illustrated in Fig. 1 and was estimated by the procedure described below:

1.0 Mammary NEAA carbon input is used for:

A. Milk NEAA carbon output
B. Mammary CO_2 output, when the mammary NEAA carbon input is greater than milk NEAA carbon output

1.1 Mammary NEAA nitrogen input is used for:

A. Milk NEAA nitrogen output
B. "not accounted for", when the mammary NEAA nitrogen input is greater than milk NEAA nitrogen output

2.0 Mammary EAA carbon input is used for:

A. Milk EAA carbon output
B. Milk NEAA carbon output, when the mammary EAA carbon input is greater than the milk EAA carbon output, but only if the mammary NEAA carbon input is smaller than the milk NEAA carbon output
C. Mammary CO_2 output, when the mammary EAA carbon input is greater than the carbon flux in 2.0.A plus the carbon flux in 2.0.B

2.1 Mammary EAA nitrogen input is used for:

A. Milk EAA nitrogen output
B. Milk NEAA nitrogen output, when the mammary EAA nitrogen input is greater than the milk EAA nitrogen output, but only if the mammary NEAA nitrogen input is smaller than the milk NEAA nitrogen output
C. "not accounted for", when the mammary EAA nitrogen input is greater than the nitrogen flux in 2.1.A plus the nitrogen flux in 2.1.B

3.0 Mammary lipogenic carbon (i.e., NEFA + triglyceride) input is used for:

A. Milk fat carbon output
B. "not accounted for", when the mammary lipogenic carbon input is greater than the milk fat carbon output

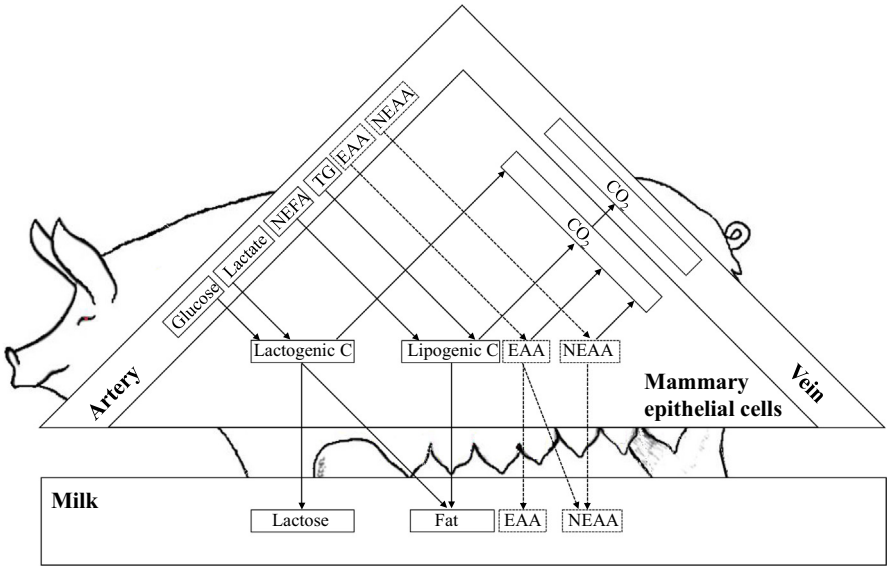

Fig. 1. Structure of the static sow mammary gland model describing the partitioning of carbon and nitrogen from blood to milk. Dashed lines indicate the existence of carbon and nitrogen estimates, and solid lines indicate the existence of carbon estimates only. NEFA: Non-esterified fatty acids, TG: Triglycerides, EAA: Essential amino acids, NEAA: Non-essential amino acids.

4.0 Mammary lactogenic carbon (i.e., glucose + lactate) input is used for:

A. Milk lactose carbon output
B. Mammary CO_2 output
C. Milk fat carbon output, when the milk fat carbon output is greater than the mammary lipogenic carbon input

Origin of the mammary output of carbon dioxide

The mammary output of CO_2 into the blood stream was compared with theoretical estimations of carbon released as a result of the synthesis of energy and co-factors needed to support main CO_2-producing processes associated with milk synthesis (i.e., *de novo* fat synthesis, milk protein synthesis, mammary tissue protein turnover, glucose and amino acid transport, and lactose synthesis). Estimations of the amount of CO_2 related to each of these processes are described below. For all processes, an energy yield of 31 mol ATP per mol glucose oxidized was assumed according to van Milgen (2002).

De novo milk fat

The release of CO_2 associated with the requirement of energy and co-factors to support the *de novo* fat synthesis was estimated based on the carbon needed for the carbon skeletons of the *de novo* synthesized fat. It was assumed that for each mol of carbon in the carbon skeleton of fat, an additional 0.638 mol carbon was required to support *de novo* fat synthesis according to the stoichiometry model by van Milgen (2002).

Milk protein

Carbon to support the energy demand associated with the formation and degradation of peptide bonds linked to milk protein secretion was estimated assuming that 1.27 peptide bonds were formed for each peptide bond found in milk protein (Huber et al., 2018). Accordingly, it was assumed that 0.27 peptide bonds were degraded for each peptide bond in milk protein. The energy cost of peptide bond formation and degradation was assumed to be 4 and 2 ATP/bond, respectively (Wu, 2013).

Mammary tissue protein turnover

Carbon needed to support the energy demand for peptide bond formation and degradation in mammary tissue protein turnover was based on the mammary tissue protein pool estimated on the day of sampling and the turnover rate of the mammary tissue protein pool. Data presented by Kim et al. (1999b) were used to estimate the mammary tissue protein pool on the day of sampling (Supplementary Material S6). The daily turnover rate of mammary protein was assumed to be 61% (Huber et al., 2018) and the average molecular weight of amino acids in mammary tissue protein during lactation to be 131 g/mol (Kim et al., 1999a). The energy cost of peptide bond formation and degradation was assumed to be identical to that of milk protein synthesis (i.e., 4 and 2 ATP/bond; Wu, 2013).

Lactose synthesis and transport of glucose and amino acids

Carbon to support the synthesis of lactose was calculated assuming a cost of 3 ATP per lactose molecule (Kuhn et al., 1980). Carbon needed to support the energy cost for transport of amino acids and glucose across cell membranes was assumed to be 0.33 ATP per molecule transported (Pettigrew et al., 1992). The number of transported glucose molecules was assumed to be equal to the mammary input of glucose. The total number of transported amino acids was calculated as the output of amino acids in milk multiplied by 2.7, based on the average inward transport rates of lysine, methionine and valine relative to their respective outputs in milk protein from sows fed amino acids balanced diets (Guan et al., 2002).

Statistical analyses

Estimated milk yield, MPF, MBF, mammary input of carbon and nitrogen containing nutrients, mammary output of carbon and nitrogen in CO_2 and milk components, and estimations of mammary CO_2 contributing processes were analyzed using the lmer function of the lme4 package (Bates et al., 2015) in R 3.5.1. The origin of the study was included as a random effect in the statistical analysis. The same statistical model was used to analyze the results of the static model partitioning carbon and nitrogen input. Linear and quadratic relationships between arterial concentration, AV-differences, MPF, mammary input and mammary output were estimated using the lm function of R 3.5.1. Statistical significance was accepted at $P \leq 0.05$, and $P \leq 0.10$ was considered as a tendency.

Results

The average milk yield across the data set was 11.1 l/day (corresponding to 910 ml/piglet), and MPF was 7684 l/day (Table 2).

Mammary carbon input and partitioning

Mammary input of carbon from the lactogenic substrates (59 mol/day; 50% of total carbon input) was greater than the output of carbon in lactose (21 mol/day; 18% of total carbon input; Table 2). In contrast, mammary input of the lipogenic substrates (36 mol/day; 31% of total carbon input) was lower than carbon output in fat (50 mol/day; 43% of total carbon input). Mammary carbon input from total amino acids (23 mol/day; 19% of total carbon input) balanced the output of carbon in amino acids (22 mol/day; 19% of total carbon output).

The partitioning of carbon and nitrogen fluxes from blood into milk is shown in Table 3. Almost all lipogenic carbon was sufficient to

Table 2
Plasma flow, blood flow, milk yield and carbon and nitrogen balance (input–output) of the sow mammary gland.

	Mean	[Min; max][1]	SEM
Mammary plasma flow, l/day[2]	7 684	[4 015; 11 472]	571
Mammary plasma flow, l/l milk[2]	723	[435; 1 116]	68
Mammary blood flow, l/day[3]	10 520	[6 670; 14 682]	827
Mammary blood flow, l/l milk[3]	986	[621; 1 533]	92
Milk yield, l/day[4]	11.1	[6.9; 14.8]	1
Mammary carbon input, mol/day[5]			
Glucose + lactate	59	[27; 84]	6
Non-esterified fatty acids + triglycerides	36	[16; 57]	5
Essential amino acids	12	[7; 16]	1
Non-essential amino acids	11	[6; 19]	1
Total	119	[64; 154]	11
Mammary carbon output, mol/day[6]			
Carbon dioxide	23	[16; 46]	3
Lactose	21	[11; 27]	1
Fat	50	[40; 61]	2
Essential amino acids	11	[8; 13]	1
Non-essential amino acids	11	[8; 14]	1
Total	115	[84; 156]	6
Mammary carbon balance, mol/day[7]	4	[−32; 46]	12

[1] Minimum and maximum values included in the data set.
[2] Estimated using phenylalanine + tyrosine as flow marker (Fick's principles).
[3] Estimated as mammary plasma flow/(1 − hematocrit, %/100).
[4] Estimated according to Hansen et al. (2012), based on litter size and gain.
[5] Estimated as arterial venous concentration difference × Mammary plasma flow.
[6] Estimated as milk yield × concentration in milk.
[7] Not different from zero ($P = 0.76$).

Table 3
Model output of the partitioning of mammary carbon and nitrogen input and estimation of processes contributing to mammary output of CO_2 in sows.

	Mean	[Min; Max][1]	SEM	*P*-value[2]
Partitioning of carbon input				
Carbon from glucose and lactate, %				
Milk lactose	36	[29; 49]	3	0.001
Mammary net release of CO_2	34	[4; 58]	8	0.02
Milk fat (*de novo* synthesized)	31	[0; 99]	13	0.09
Carbon from non-esterified fatty acids + triglycerides, %				
Milk fat	98	[83; 100]	2	<0.001
Not accounted for	2	[0; 17]	2	0.44
Carbon from essential amino acids, %				
Milk essential amino acids	88	[65; 100]	4	<0.001
Milk non-essential amino acids	2	[0; 13]	1	0.31
Mammary net release of CO_2	10	[0; 35]	4	0.09
Carbon from non-essential amino acids, %				
Milk non-essential amino acids	93	[60; 100]	4	<0.001
Mammary net release of CO_2	7	[0; 40]	3	0.11
Partitioning of nitrogen input				
Nitrogen from essential amino acids, %				
Milk essential amino acids	87	[68; 100]	3	<0.001
Milk non-essential amino acids	3	[0; 14]	2	0.22
Not accounted for	10	[0; 31]	4	0.08
Nitrogen from non-essential amino acids, %				
Milk non-essential amino acids	92	[56; 100]	4	<0.001
Not accounted for	8	[0; 44]	4	0.14
Estimation of processes contributing to CO_2, % of mammary output of CO_2				
De novo fat synthesis[3]	45	[0; 133]	13	0.05
Peptide bonds, milk protein[4]	22	[12; 33]	3	0.003
Peptide bonds, mammary tissue protein[5]	15	[9; 23]	2	0.003
Lactose synthesis[6]	5	[3; 7]	1	0.008
Mammary glucose input[7]	3	[2; 5]	1	0.01
Mammary amino acid input[7, 8]	4	[2; 5]	1	0.005
Total	94	[40; 179]	11	<0.001

[1] Minimum and maximum values included in the dataset.
[2] *P*-value testing if the mean differs from zero.
[3] Assuming 0.638 mol carbon per mol carbon in *de novo* fat (van Milgen, 2002).
[4] Assuming a cost of 4 ATP/peptide bond formed and 2 ATP/peptide bond degraded (Wu, 2013). For each peptide bond in milk protein output, the total number of peptide bonds synthesized was assumed to be 1.27 and the total number of bonds degraded was assumed to be 0.27 (Huber et al., 2018).
[5] Assuming a cost of 4 ATP/peptide bond synthesized and 2 ATP/peptide bond degraded (Wu, 2013). Mammary tissue protein turnover was assumed to be 61%/day (Huber et al., 2018). Mammary tissue protein content was assumed to be 11.2% of tissue wet weight (Kim et al., 1999b). Mammary tissue wet weight (g) was estimated based on data from (Kim et al., 1999b).
[6] Assuming a cost of 3 ATP/lactose molecule synthesized (Kuhn et al., 1980).
[7] Assuming a cost of 0.33 ATP/molecule transported across cell membranes (Pettigrew et al., 1992).
[8] Inward transport of amino acids across cell membranes (blood to mammary cells) was assumed to be 2.7 times greater than milk amino acid output (Guan et al., 2002).

account for milk fat carbon (98%), while, respectively, 88 and 93% of EAA and NEAA carbon were directed toward EAA and NEAA carbon in milk. On average, 36% of lactogenic carbon (21 of 59 mol/day) was directed toward lactose carbon. The remaining lactogenic carbon was released in the blood as the mammary output of CO_2 (34%) or secreted in milk as mammary output of *de novo* fat (31%).

The estimation of CO_2 resulting from the oxidation of nutrients to supply energy to support *de novo* fat synthesis, synthesis of milk protein and lactose, mammary protein turnover and the transport of glucose and amino acids constituted 94% of the mammary output of CO_2 (Table 3).

Relationships between mammary carbon output, input and input determinants

The output of carbon in lactose, EAA and fat in milk is presented in relation to MPF and the arterial concentration, AV-difference and input of carbon in Fig. 2. The output of lactose carbon was linearly related to the input of lactogenic precursors ($P < 0.01$; Fig. 2D). The output of EAA carbon was linear and tended to be quadratically related to the input of EAA carbon ($P < 0.01$ and $P = 0.09$, respectively; Fig. 2H). Outputs of lactose and EAA carbon were quadratically related to the AV-difference of lactogenic carbon and EAA, respectively ($P < 0.05$; Fig. 2B and F).

The input of lactogenic, EAA and lipogenic carbon in milk is presented in relation to MPF and to the arterial concentration and AV-difference of carbon in Fig. 3. Positive linear relationships between input and AV-difference of carbon were observed for lactogenic ($P = 0.009$; Fig. 3B) and lipogenic carbon ($P = 0.01$; Fig. 3H). The input of carbon from EAA was positively linearly related to the arterial EAA concentration ($P = 0.04$; Fig. 3D) and quadratically related to the AV-difference of EAA carbon ($P = 0.008$; Fig. 3E).

Mammary nitrogen balance

Mammary input of EAA nitrogen exceeded the output of EAA nitrogen in milk ($P = 0.04$), while total nitrogen from amino acids balanced the output of total amino acid nitrogen in milk ($P = 0.42$; Table 2). The mammary balance (input–output) of nitrogen from phenylalanine ($P = 0.07$) and arginine ($P = 0.02$) tended to or differed positively from zero (Table 4). The mammary balance of nitrogen from tyrosine ($P = 0.07$), proline ($P = 0.03$) and asparagine ($P < 0.001$) tended to or differed negatively from zero. The mammary input of branched-chain amino acids (**BCAA**) tended to exceed the output in milk (183 ± 77 mmol N/day; $P = 0.09$; Table 4). Among BCAA, the mammary balance of isoleucine tended to be positively different from zero (55 mmol N/day; $P = 0.09$), while the balance for leucine and valine was only numerically positive.

Discussion

The mammary input and output of carbon and nitrogen as well as the input determinants (i.e., MPF and arterial concentrations and AV-differences of carbon and nitrogen) were quantified to investigate the partitioning of carbon and nitrogen in lactating sows. Glucose, lactate, NEFA, triglycerides, EAA and NEAA in plasma were considered as the main contributors of carbon and nitrogen inputs to the mammary gland, while other nutrients were assumed to be of minor quantitative importance (Farmer et al., 2008). Indeed, short-chain fatty acids and beta-hydroxybutyrate constitute <2.6% of the total mammary carbon input (Farmer et al., 2008; Krogh et al., 2017) and were not considered explicitly here. The approach of integrating data sets from four independent experiments required standardization of MPF and milk yield estimates, which both have a quantitative impact on mammary input and output estimates.

General regulation of mammary nutrient input

In sows, litter size and litter growth capacity are important drivers controlling milk output (so-called pull elements; Dourmad et al., 2008; Hansen et al., 2012). Simultaneously, nutrients provided by the blood from the diet and from body reserves are potential drivers of milk output (push elements; Strathe et al., 2017a, 2017b; Hojgaard et al., 2019). However, it is not clear to what extent push and pull elements control mammary metabolism. The positive relationships between the output of lactose carbon and the input of lactogenic carbon on the one hand and the output of EAA carbon in milk protein and the input of EAA carbon on the other hand (Fig. 2D and H, respectively) illustrate that the output and input of carbon are a jointly controlled process. In addition, the observation that the input of EAA carbon was positively correlated with the arterial concentrations of EAA carbon (Fig. 3D) suggests that EAA carbon input is partly controlled by the arterial concentration of EAA carbon (i.e., a push element). Such a relation is not observed between arterial concentrations and output of EAA carbon in milk (Fig. 2E), suggesting that EAA carbon input is not necessarily transferred directly into milk protein. Although the mammary gland

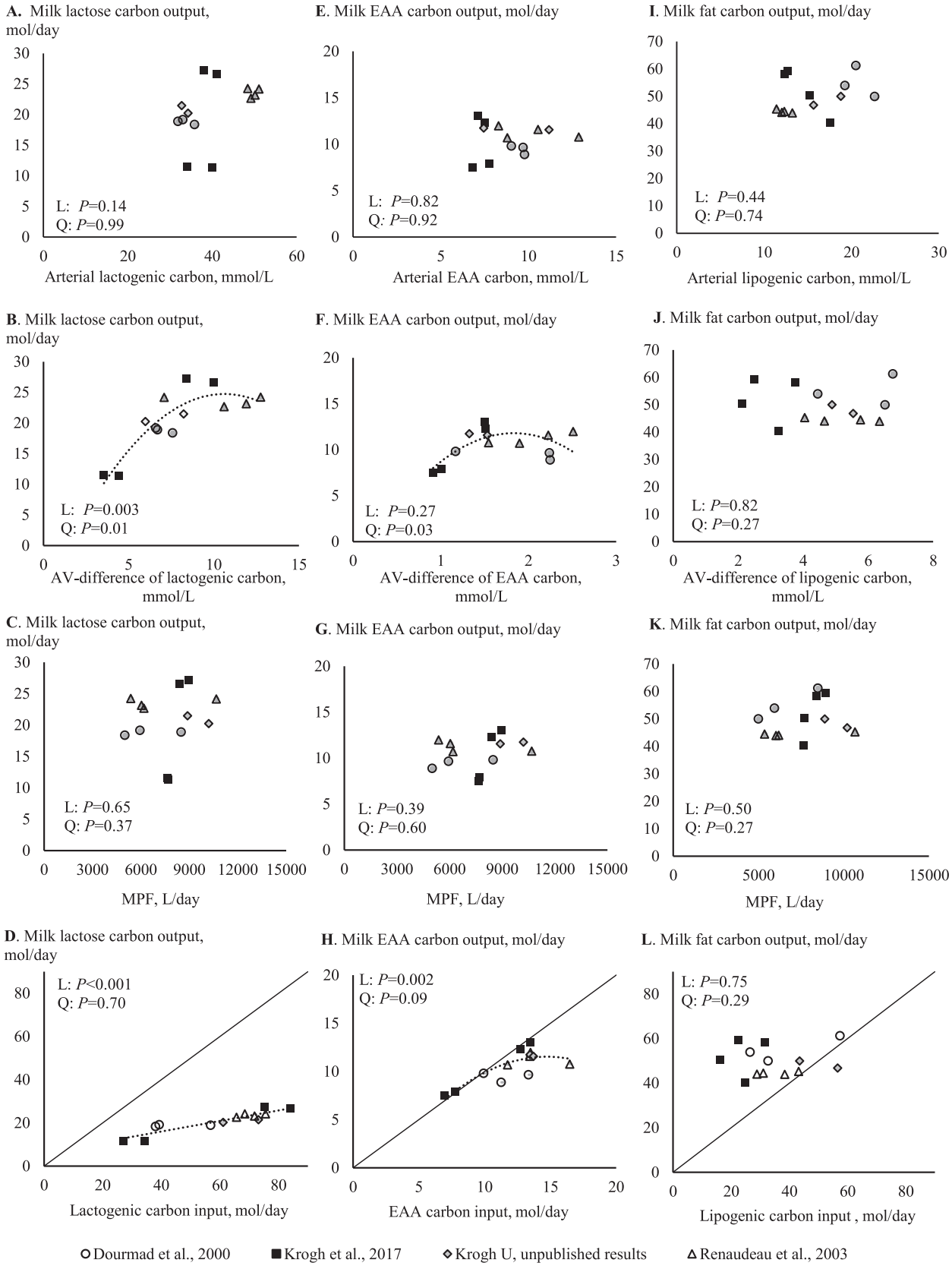

Fig. 2. Sow milk output of lactose carbon in relation to: A. Arterial lactogenic carbon (i.e., carbon from glucose and lactate), B. Arterial-venous concentration difference (AV-difference) of lactogenic carbon, C. Mammary plasma flow (MPF) and D. Lactogenic carbon input. Milk output of essential amino acid (EAA) carbon as a function of: E. Arterial essential amino acid carbon, F. Arterial-venous concentration difference of essential amino acid carbon, G. Mammary plasma flow and H. Essential amino acid carbon input. Milk output of lipogenic carbon (i.e., carbon from non-esterified fatty acids and triglycerides) as a function of: I. Arterial lipogenic carbon, J. Arterial-venous concentration difference of lipogenic carbon, K. Mammary plasma flow and L. Lipogenic carbon input. *P*-value testing for linear (L) and quadratic relationships (Q) is given for each sub-figure.

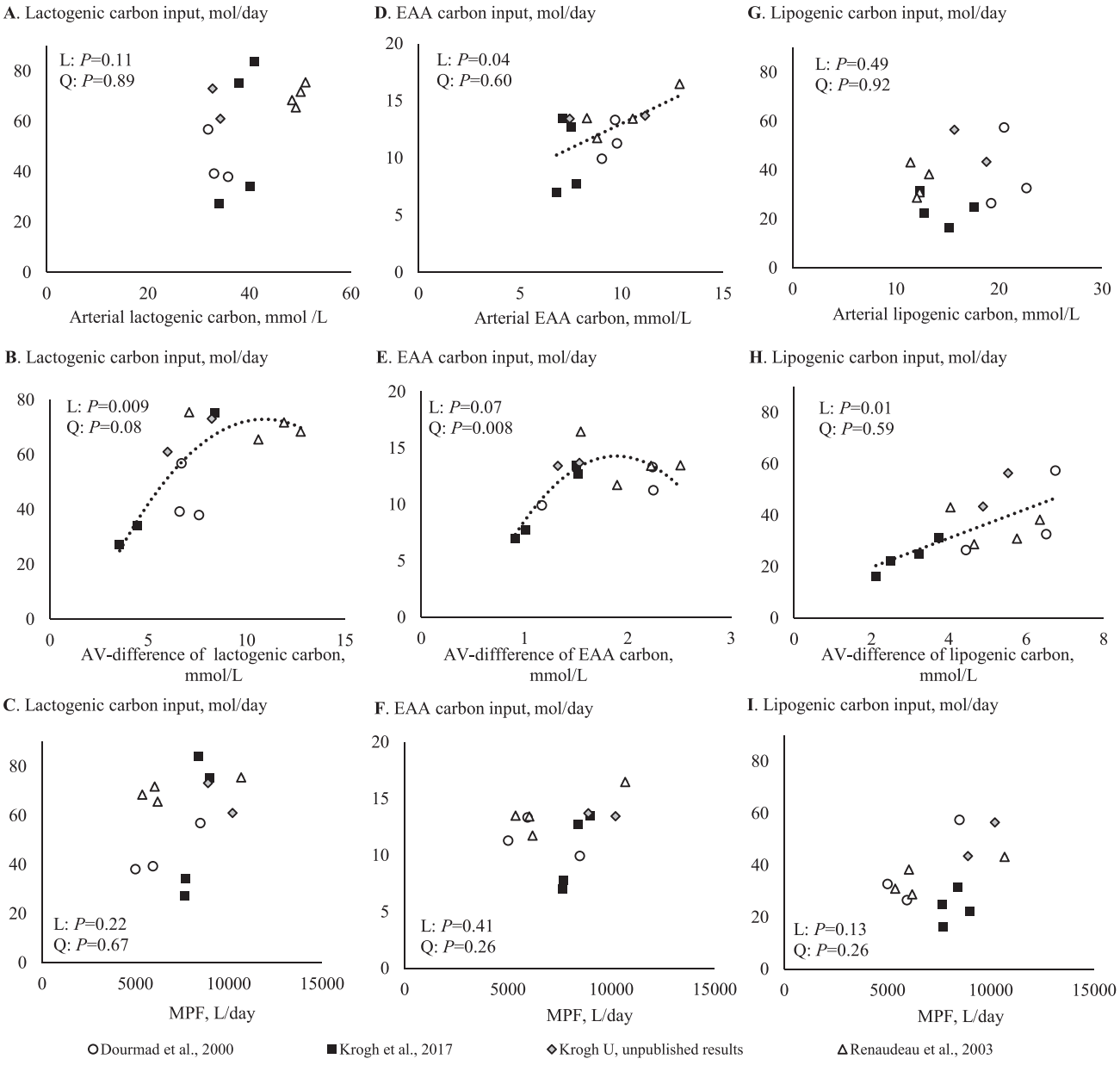

Fig. 3. Sow mammary input of lactogenic carbon in relation to: A. Arterial lactogenic carbon (i.e., carbon from glucose and lactate), B. Arterial-venous concentration difference (AV-difference) of lactogenic carbon and C. Mammary plasma flow (MPF). Mammary input of essential amino acid carbon as a function of: D. Arterial essential amino acid carbon (EAA), E. Arterial-venous concentration difference of essential amino acid carbon, and F. Mammary plasma flow. Mammary input of lipogenic carbon (i.e., carbon from non-esterified fatty acids and triglycerides) as a function of: G. Arterial lipogenic carbon, H. Arterial-venous concentration difference of lipogenic carbon and I. Mammary plasma flow. *P*-value testing for linear (L) and quadratic relationships (Q) is given for each sub-figure.

may not directly affect the arterial concentration of nutrients, it can exert an effect on the AV-difference as illustrated by Trottier et al. (1997), who showed that the AV-difference of EAA decreased dramatically after weaning, which induced a sudden change in the pull element. The quadratic relationship between the input of EAA carbon (estimated as MPF × AV-difference) and the AV-difference suggests that increased demand of EAA by the mammary gland (pull element) is associated with an increase in AV-difference and that mammary input of EAA carbon is saturable or being controlled by another mechanism for EAA inputs >10 mol/day (Fig. 3E). In such situations, changes in MPF, as a push element, may become an increasingly important mechanism adjusting the metabolite supply to the mammary gland. Although MPF is an important regulator of mammary nutrient supply (Bequette et al., 2000), no relationship between AV-difference of EAA and daily MPF was observed in our study. Nevertheless, AV-difference of EAA and MPF was negatively correlated when MPF was expressed per liter milk produced (i.e., liter plasma per liter milk, $P < 0.001$); data not shown). This is in line with the observation that MPF changes with litter size (pull element; Nielsen et al., 2002). In summary, both push and pull elements seem to be involved in the regulation of mammary input of lactogenic and EAA carbon.

Mammary metabolism

Amino acids

The greater proportion of EAA nitrogen in mammary input (50% of total amino acid nitrogen) than in milk output (47% of total amino

Table 4
Mammary input, output and balance (input–output) of amino acid nitrogen in sows.

	Input	SEM	Output	SEM	Balance	SEM	*P*-value[1]
Essential amino acids (EAA), mmol N/day							
Lysine	606	51	565	24	37	46	0.48
Methionine	76	11	75	5	1	7	0.85
Threonine	203	12	195	8	8	9	0.44
Tryptophan	68	7	76	4	−8	8	0.35
Isoleucine	234	20	179	10	55	23	0.09
Leucine	421	46	364	16	55	34	0.20
Valine	332	34	259	12	74	36	0.13
Histidine	314	61	283	14	30	55	0.62
Phenylalanine	152	8	135	6	16	6	0.07
Arginine	810	92	640	60	164	64	0.02
Non-essential amino acids (NEAA), mmol N/day							
Tyrosine	107	6	124	9	−16	6	0.07
Proline	375	56	561	27	−189	50	0.03
Cysteine	43	26	65	2	−22	28	0.49
Alanine	274	71	220	10	52	69	0.51
Asparagine	263	41	286	12	−25	40	0.58
Aspartate	34	9	234	10	−198	13	<0.001
Glutamine	639	53	510	25	130	42	0.44
Glutamate	656	114	649	32	−14	80	0.87
Glycine	254	62	242	13	11	62	0.86
Serine	523	162	280	13	241	165	0.24
Amino acid groups, mmol N/day							
BCAA	986	86	802	37	183	77	0.09
EAA	3218	253	2778	143	438	140	0.04
NEAA	3177	435	3140	147	25	392	0.95
Total amino acids	6403	668	5918	274	471	517	0.42

[1] *P*-value testing if the nitrogen balance (input–output) differs from zero.
Abbreviations: BCC, branched chain amino acids.

acid nitrogen) could be explained by a greater input of BCAA, phenylalanine and arginine than their output in milk as indicated by sow studies (Trottier et al., 1997; Guan et al., 2004) and studies on dairy cows (Lapierre et al., 2012). This excess input suggests that these amino acids were deaminated or transaminated for synthesis of NEAA. In support, mammary input of nitrogen from phenylalanine and arginine in excess of milk output in the present study (16 and 164 mmol N/day, respectively) corresponded well with the negative nitrogen balance of tyrosine and proline (−16 and −189 mmol N/day, respectively; Table 4). The mammary input of BCAA nitrogen in excess of output in milk (184 mmol N/day) was similar to the negative mammary balance of aspartate (198 mmol N/day), suggesting that this excess BCAA nitrogen was used for aspartate synthesis. The excess BCAA carbon was likely partly oxidized as indicated by Li et al. (2009), who studied the metabolic fate of labeled BCAA in sow mammary tissue explants. In comparison, the BCAA carbon input in excess of output across the four studies (1.0 mol/day; data not shown) constituted approximately 80% of the mammary carbon from EAA that was estimated to be oxidized by the model procedure (i.e., 10% of the EAA input).

The amino acid nitrogen balance indicated that 93% of the total amino acid nitrogen input was exported as milk protein. This is in line with the review by Cant et al. (2018), who concluded that mammary input of amino acids in dairy cows is regulated by factors controlling milk protein synthesis and that approximately 90% of mammary amino acid input was accounted for in milk protein. The tendency of a quadratic relationship between input and output of EAA carbon suggests a nonlinear decrease in mammary efficiency with an increased input of amino acids (Fig. 2H). Such a quadratic relationship may be driven by the excess mammary input of BCAA, arginine and phenylalanine nitrogen relative to the output in milk (Guan et al., 2004) or an unbalanced input of carbon and nitrogen causing the use of amino acids for other purposes than milk protein (Lapierre et al., 2012; Omphalius et al., 2019).

Energy metabolites

Lipogenic carbon input was insufficient to cover milk fat secretion (72% of output), while lactogenic carbon was in great excess of milk lactose secretion (281% of output; Table 2). This suggests that lactogenic carbon input contributes substantially to *de novo* fat synthesis and the primary substrate for CO_2-yielding processes. Although lactogenic carbon was in great excess of the demand for milk lactose, mammary input of lactogenic carbon was positively and tightly associated with milk lactose (Fig. 2D), suggesting lactogenic input may be a driver of milk synthesis. In support, Cant et al. (2002) found that arterial infusion of glucose increased mammary glucose input, lactose synthesis, and reduced the input of fatty acids by the mammary gland of dairy cows. Thus, an increase in lactogenic carbon input seems to increase lactose synthesis, the use of lactogenic carbon for *de novo* fat and the proportion of energy and co-factors related to the synthesis of *de novo* fat. In support, the theoretical estimations of the included processes linked to the release of CO_2 suggest that *de novo* fat synthesis (45% of CO_2) was the main contributor of mammary CO_2 release, followed by milk protein synthesis (22% of CO_2) and mammary tissue protein turnover (15% of CO_2; Table 3).

Conclusion

In conclusion, MPF flow and the AV-difference both appear to be involved in the regulation of mammary lactogenic and EAA carbon input. Mammary amino acid nitrogen balances suggested BCAA, arginine and phenylalanine were taken up in excess of their output in milk, suggesting that the arterial supply of these amino acids may have a greater impact on mammary input than other EAA. Mammary input of lactogenic carbon was closely associated with milk lactose synthesis and the primary substrate for CO_2 yielding processes and an essential contributor of *de novo* fat synthesis.

Supplementary materials

Supplementary data to this article can be found online at https://doi.org/10.1016/j.animal.2020.100049.

Ethics approval

Not applicable.

Data and model availability statement

None of the data were deposited in an official repository.

Author ORCIDs

U. Krogh: https://orcid.org/0000-0001-6535-6612
H. Quesnel: https://orcid.org/0000-0002-2053-7126
N. Le Floc'h: https://orcid.org/0000-0001-9858-1584
A. Simongiovanni: https://orcid.org/0000-0002-8870-2343
J. van Milgen: https://orcid.org/0000-0002-6131-5255

Author contributions

U. Krogh: Methodology, validation, formal analysis, data curation, writing - original draft. H. Quesnel: Conceptualization, methodology, validation, writing - review & editing, supervision, project administration, funding acquisition. N. Le Floc'h: Conceptualization, methodology, validation, writing - review & editing, project supervision, administration, funding acquisition. A. Simongiovanni: Conceptualization, methodology, validation, writing - review & editing, supervision, project administration, funding acquisition. J. van Milgen: Conceptualization, methodology, validation, writing - review & editing, supervision, project administration, funding acquisition.

Declaration of interest

None.

Acknowledgements

The authors are grateful to David Renaudeau and Jean-Yves Dourmad (PEGASE, INRAE, Institut Agro, France) and Peter Kappel Theil (Department of Animal Science, Aarhus University Foulum, Denmark) for their contribution of raw data not available in published papers.

Financial support statement

The financial support was provided through a collaborative project between INRAE, Institut Agro, Saint-Gilles, France and Ajinomoto Animal Nutrition Europe, Paris, France.

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