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Modulation of the nutritional, metabolomic and microRNA profile of colostrum and piglet performance via a high-energy, high-lysine transition diet in sows

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Abstract

Background The transition period is a critical phase for the sow due to physiological changes and nutritional requirements. A diet balanced in energy and amino acid (AA) content could improve reproductive performance, colostrum quality and piglets' growth. This study evaluated the efficacy of a transition diet (TRT) with higher energy (12.97 MJ/kg of metabolizable energy (ME)) and SID lysine (Lys; 0.85%), compared to a standard (CO) diet (12.33 MJ/kg of ME and 0.70% SID Lys), on the composition and quality of colostrum and on sow and piglet performance. The AA/SID Lys ratio was maintained in both diets. Sows (50 sows/group) were fed the CO or TRT diet from 6 d prepartum to d 4 postpartum. At farrowing, sow performance (50 sows/group) and piglet vitality (12 sows/group) were recorded, and colostrum (20 sows/group) was collected to analyze its composition and microRNAs. Piglet performances were collected d 6 and weaning (d 24).

Results The diet did not affect sow feed intake, body condition score, backfat and muscle loss, nor farrowing duration and time interval between piglets. The TRT group had fewer stillbirths ($P=0.002$). Piglets of TRT litters had higher body weight at d 24 ($P=0.032$) and tended to have higher average daily gain from d 0 to d 24 ($P=0.080$). Colostrum from the TRT group tended to be higher in somatic cell count ($P=0.07$), higher in fat percentage ($P=0.036$), and higher in C18:2 9cis,12cis, C18:4 6cis,9cis,12cis,15cis, C20:0, UDP-glucuronate and carnitine ($P<0.05$); moreover, it had a lower concentration of citrate ($P<0.05$). The 208 microRNAs were detected in colostrum, 13 of which were differentially expressed ($P<0.05$). The TRT group had a higher ssc-miR-143-3p expression, which is associated with increased phagocytosis and reduced inflammation and oxidative stress. This, together with the increase in fat and specific metabolites related to energy metabolism, could potentially benefit piglet performance.

Conclusion These results suggest that the TRT diet improves sow parturition and lactation performance by modifying sow energy metabolism and colostrum quality. This highlights the importance of a properly designed transition diet for sows.

Keywords Energy metabolism, Metabolomics, ssc-miR-143-3p, Stillbirths

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Introduction

In the sow, the transition period is a short interval in the reproductive cycle, defined as the period from 7–10 d before farrowing to 3–5 d after farrowing. Although this is a relatively short period, the correct management of this period is crucial for the reproductive efficiency of the sow, which in turn translates into greater sustainability for the pig sector itself. Piglet mortality is highest during farrowing and the first few days after birth. In addition, the first days of life are crucial for the growth and robustness of the piglets in the following period. Piglet survival and growth during lactation are closely linked to proper mammary development, adequate fetal growth, the farrowing process and adequate production of colostrum and transitional milk [1]. In particular, several physiological and behavioural changes occur during the transition period, such as 1) rapid mammary and fetal growth; 2) increased motor activity of the sow for nest preparation; 3) expulsion of piglets and placenta and rapid uterine regression at the end of farrowing; and 4) production and expulsion of colostrum and, subsequently, transitional milk, which is very rich in fat. These processes influence the energy and protein requirements of the sow [1].

The use of a specific diet during this critical period can greatly influence these physiological processes and thus affect piglet survival and growth. Therefore, in recent years, scientific research has focused on effective feeding strategies during the transition period to better meet the needs of the sow, with the ultimate aim of improving piglet survival and growth [2, 3]. Two main nutritional aspects that need to be evaluated during the transition period are the energy, requirements and protein and amino acid (AA) requirements of the sow.

During the transition period, the energy requirement of the sow appears to increase [4]. This is due to several physiological changes, including uterine contractions, the production of colostrum and transitional milk, and physical activity due to nest-building [4]. This energy deficiency is particularly relevant in genetic lines of highly productive sows, where the metabolic demands of the uterus are very high in order to support the numerous growing fetuses [1]. In addition, the day of farrowing is particularly demanding in terms of energy expenditure in these highly productive lines. Although the energy required to support uterine contractions during parturition is not fully known, it is estimated that under thermoneutral conditions, sows expend 18 kJ net energy/min for parturition (total energy cost of the farrowing process) [5].

It is also known that the uterus contracts four to five times at an intensity of 9.4 mm Hg for a period of 12 s to deliver each piglet [6–8]. In hyperprolific sow lines, premature depletion of available energy during farrowing

occurs more frequently and impairs uterine contractions, delaying the completion of farrowing and increasing the rate of stillborn or hypoxic piglets [9]. Therefore, the provision of adequate energy levels is important to reduce the duration of parturition, as recently observed by Carnevale et al. [10]. In addition to energy, protein/AA requirements also appear to play an important role in the peripartum period, affecting piglet survival and growth [11]. In particular, the AA requirements of sows increase prior to farrowing compared to the gestation period [11, 12]. According to recent studies, the demand for and supply of AAs to the mammary gland is minimal during gestation, while it increases in the days leading up to farrowing and during lactation for the specific production of colostrum and milk [12, 13]. As lysine (Lys) is the major AA limiting in pig diets, several studies have investigated its requirements in periparturient sows. According to a recent study by Farmer et al. [14], the administration of 14.8 or 20.8 g/d of standardized ileal digestible (SID) Lys from d 90 to d 110 of gestation did not alter the productive response of sows in terms of colostrum and milk. Furthermore, increasing the protein content of the diet at the end of gestation and lactation may lead to inefficient energy use and reduced milk production [15, 16]. Conversely, an excessive reduction of protein and Lys can lead to increased mobilisation of body protein [17]. More recently, a dose–response study of SID Lys requirements estimated the optimal requirement of a sow near farrowing to be 22 g/d [2].

A change in energy and AA availability during the transition period could affect the piglet survival also by altering colostrum composition. For example, a more energetic colostrum/milk could help piglet thermoregulation. Moreover, increasing energy and AAs in the sow diet could also affect the concentration of metabolites and fatty acids in colostrum, consequently affecting litter performance [18, 19]. In addition, the alterations of sow metabolism due to different diets could change the profile of microRNAs (miRNAs; small non-coding RNA long approximately 22 nucleotides that can regulate the immune system) present in milk exosomes and consequently affect the development of the piglet immunity [20], as suggested for calves and human infants [21–23].

As a result of the above, studies on the energy and AA requirements of the transition period are rather scarce, and there is still no clear indication for application in breeding practice, partly due to the fact that most of the mentioned studies have been conducted in experimental units and, therefore, under optimal conditions. Moreover, insufficient attention has been paid to the potential changes in colostrum composition induced by the transition diet, despite the fact that colostrum quality is crucial for the survival and early development of piglets. In

this context, emerging compounds such as metabolites, bioactive molecules, and miRNAs may play a key role in supporting piglet survival and growth and thus deserve further investigation.

Therefore, the aim of the present study is to verify the efficacy of a diet designed to improve the coverage of the energy and Lys requirements of the sow during the transition period, compared to a standard diet, on colostrum composition and quality, on the number of piglets born alive or poorly viable, and on productive performance in terms of survival and litter growth until weaning.

Material and methods

Experimental design and animals

The study involved 112 sows and their litters from two consecutive batches housed in the same room (56 sows per batch, balanced between the two treatments). During the trial, the sows were fed the same gestation diet throughout pregnancy (until 6 d before the predicted farrowing date) and the same lactation diet (from 5 d post-partum onwards).

The management of the sows in the farm required that they were housed in groups immediately after service until one week before the expected farrowing date, after which they were moved to the farrowing pen. At the entry into the farrowing unit, the sows were divided into two groups (56 sows/group) balanced for parity order (PO), body condition score (BCS), muscle and backfat thickness and historical litter size at birth and were fed two different transition diets from 6 d before the planned farrowing day until 4 d after farrowing. Farrowing was induced by an intramuscular injection of 2 mg (corresponding to 1 mL per animal) of alfaprostol (Gabbrostim, FATRO S.p.A., Ozzano dell'Emilia, Italy), an analogue of PGF $_{2\alpha}$, on d 114 of gestation. Due to the uncertainty of the farrowing process the actual days of dietary consumption of the transition diets before farrowing were: 5.54 ± 1.06 d (mean $\pm$ standard error, SE) for the control group (CO) and 5.43 ± 0.91 d for the treated (TRT) diet.

The two diets were defined as follows: CO diet: the transition diet normally used on the farm, characterised by an energy content of 12.33 MJ/kg of metabolizable energy (ME) and 0.70% of SID Lys; a TRT diet: a transition diet based on recent study by Johannsen et al. [2], for hyper prolific sows, characterised by a higher energy content of 12.97 MJ/kg ME and 0.85% SID Lys. The composition of the diets and the levels of protein and total and free AAs analyzed are reported in Table 1. During the transition period, feed distribution followed a rationed plan based on the sow BCS, providing approximately 2.5 kg/sow/d; whereas, during the post-farrowing period, feed was provided ad libitum. Feed was administered manually and the individual feed consumption of the

Table 1 Composition of the diets and the levels of total and free protein and amino acids

Item	Diet ^a	
	CO	TRT
Ingredients, %		
Barley	23.33	23.13
Wheat bran	14	8
Corn	13	13
Wheat	10	10
Sunflower meal	5.5	6
Beet pulp	5	5
Dried distillery grains	5	5
Expanded corn	4	4
Roasted whole-grain soybeans	4	4
Cookie byproduct	4	4
Soya bean husks	2	3
Flax seed	2	2
Soybean protein concentrate	0	2
Soy oil	1.8	4
Sugar cane molasses	1.5	1.5
CaCO $_3$	1.2	1.1
PREM.SCROFE 0.8% ^b	0.8	0.8
Natural dietary fibre ^c	0.8	0.8
L-Lysine	0.42	0.57
Liquid organic acids	0.4	0.4
Salt	0.3	0.3
Calcium chloride encapsulated	0.3	0.3
Sodium bicarbonate	0.25	0.25
L-Threonine	0.16	0.25
Choline HCl 75%	0.1	0.1
Probiotic: <i>Clostridium butyricum</i>	0.05	0.05
Vit. C coated	0.03	0.03
L-Valine	0.03	0.12
Vit. E 50%	0.02	0.02
L-Tryptophan	0.015	0.04
Dicalcium phosphate 18%	0	0.15
DL-Methionine	0	0.09
Nutrients (calculated)		
Moisture, %	11.87	11.39
Dry matter, %	88.13	88.61
Net Energy, MJ/kg	9.06	9.67
Metabolized energy, MJ/kg	12.33	12.97
Crude protein, %	13.99	14.69
Crude fat, %	5.7	7.71
Crude fiber, %	7.11	7.05
ADF, % ^d	9.26	9.23
NDF, % ^e	21.18	20.00
ADL, % ^f	2.19	2.10
Crude ash, %	5.33	5.25
Calcium, %	0.71	0.72
Phosphorus, %	0.46	0.44

Table 1 (continued)

Item	Diet ^a	
	CO	TRT
CA/P ^g available, %	2.29	2.21
Lys SID ^h , %	0.70	0.85
Val SID, %	0.51	0.62
Treo SID, %	0.49	0.6
Arg SID, %	0.68	0.71
Trip SID, %	0.15	0.18
Ile SID, %	0.36	0.39
Met/Lis SID, %	0.35	0.4
Met + Cis/Lis SID, %	0.64	0.64
Treo/Lis SID, %	0.70	0.71
Val/Lis SID, %	0.73	0.73
Arg/Lis SID, %	0.98	0.84
Trip/Lis SID, %	0.21	0.21
Fenil/Lis SID, %	0.52	0.45
Tir/Lis SID, %	0.32	0.28
Iso/Lis SID, %	0.52	0.46
Nutrients (analyzed)		
Dry matter, %	90.04	90.34
Total protein, %	14.5	14.3
Total Lys, %	0.83	0.97
Free Lys, %	0.27	0.427

^a CO: Control diet with 12.33 MJ/kg of ME and 0.70% SID Lys; TRT: Treatment diet with 12.97 MJ/kg of ME and 0.85% SID Lys

^b Composition per kg of premix: Dry matter 94.0%, vitamin A 1,500,000.0 UI, vitamin D₃ 250,000.0 UI, vitamin E 10,000.0 mg, vitamin B₁ 250.0 mg, vitamin B₂ 800.0 mg, vitamin B₆ 500.0 mg, vitamin B₁₂ 5.0 mg, vitamin PP 4,500.0 mg, vitamin K₃ 504.0 mg, folic acid 480.0 mg, biotin 70.0 mg, pantothenic acid 4,000.0 mg, zinc chelate of the hydroxylated analogue of methionine 10,000.0 mg, Mn chelate of the hydroxylated analogue of methionine 5,000.0 mg, Cu chelate of the hydroxylated analogue of methionine 1,900.0 mg, iron (iron sulphate (III)) 9,000.0 mg, iodine (calcium iodate anhydrous) 210.0 mg, selenium (sodium selenite) 40.0 mg, 6-Flitase (4a18i) 125,000.0 FYT

^c Commercial product ARBOCEL/OptiCell C5

^d Acid detergent fiber

^e Neutral detergent fiber

^f Acid detergent lignin

^g Calcium/Phosphorus

^h Standardized ileal digestible

sows was recorded daily. Later, during lactation, all sows received the same lactation diet with ad libitum feeding.

During farrowing, when feasible (excluding night-time farrowings), obstetrical assistance provided by the same farm veterinarian was recorded. In cases of prolonged farrowing, sows were first stimulated by gentle teat rubbing to promote natural oxytocin release. If this stimulation was insufficient, the veterinarian administered an intravulvar oxytocin injection (2 mL of Izossitocina, IZO S.r.l. Brescia (BS), Italy). If two injections, administered at least 30 min apart, failed to elicit a response, manual assistance was performed. The total number of injections

and manual examinations was recorded. Sow health status was monitored throughout the study and any abnormalities or drug treatments were promptly recorded.

Sows and piglets were housed in farrowing pens (2.7 m × 1.7 m) with partially slatted floors. Sows were housed in cages throughout lactation. Room temperature was maintained at 22 °C. Lights in the farrowing rooms were turned on from 07:00 to 18:00 and during farrowing. No bedding was provided until d 115 of gestation, after which shredded paper was provided daily. Each farrowing pen was equipped with a piglet nest with a heated floor and infrared lamps to maintain the temperature at 32 °C. Cross-fostering of the piglets was carried out within 12 h after farrowing by sows in the same experimental group to standardise litter size, to match the sow's rearing capacity with litter size and to ensure that all the piglets could access a functional teat. The average litter size after cross fostering was 13.2 ± 0.7 for the CO group and 13.4 ± 0.8 for the TRT group (mean ± SE). The sows included in the trial met the following criteria: 1) PO between 2 and 9; 2) a total litter size of at least 13 piglets after cross fostering; 3) a BCS between 2 and 5 at farrowing; and 4) no drug treatment.

Sampling and data collection

The BCS of the sows included in the trial (50 sow/group) was measured using a caliper (Pig Improvement Company, Tennessee, USA), while the measurements of the backfat and muscle thickness were performed using an ultrasound scanner (ATL, Milan, Italy) at point P2, approximately 65 mm from both sides of the spine to the last rib, as previously described by Luise et al. [24]. Caliper unit was measured at the same backfat point. The BCS and the thickness of backfat and muscle were performed by the same operator (A.Z.) at the start of the experimental diet distribution (d −6) and then repeated at weaning (d 24) to calculate backfat and muscle loss by calculating the difference between initial and final values. Daily feed-intake before (from d −6 to d 0) and after farrowing (from d 0 to d 6) was calculated.

At farrowing (d 0), data were collected on the production parameters of the sows normally recorded on the farm, such as the number of total births, live births, stillbirths and mummies. In addition, data on the time interval between piglet births, as well as their vitality, were recorded for 12 sows per group (only for the sows for which it was possible to monitor the entire farrowing process and record all data on oxytocin injections and obstetrical assistance; sub-groups were balanced for parity order and representative of the group mean litter size). Piglet vitality was assessed immediately after birth using a 4-category scale (0–3) described by Uddin et al. [25]. Vitality scores (VS) were assigned as follows: 0 = no

movement, no respiration after 15 s, stillbirths; 1=no movement within 15 s but the piglet was breathing or attempting to breathe; 2=when the piglet showed little movement within 15 s, breathed or attempted to breathe; 3=when the piglet had good movement, good respiration and attempted to stand within 15 s.

At birth, piglets were individually identified by the application of a unique ear tag and weighed at birth (d 0), on d 6 of life (d 6) and at d 24 (23.65 ± 0.04 d, mean $\pm$ SE). At d 24, piglets were also scored for ear and tail lesions according to the Welfare Quality® protocol [26], which provides a three-point scoring system for lesions. Ear lesions: 0, up to 4 visible lesions; 1, 5 to 10 visible lesions; 2, 11 to 15 visible lesions. Tail lesions: 0, no damage; 1, superficial bite along the tail without swelling; 2, open lesion visible on the tail, presence of scarring, swelling or partial absence of the tail. The results were then expressed as the prevalence of the scores obtained (0, 1, 2) per litter. The injury score index was then calculated, considering both the frequency and severity of the injuries (ranging from 0 to 200, with 0 representing no injuries and 200 representing all animals with severe injuries):

$$\text{Lesion score index (LSI)} = [\% \text{ type 1 lesions} + (2 \times \% \text{ type 2 lesions})].$$

A colostrum sample was collected from 20 sows/group (balanced for PO, liveborn piglets and BCS) after the birth of the first piglet and by the birth of the second piglet. Colostrum was collected into a sterile tube by gently milking at least four mammary glands (including the anterior, middle and posterior glands and pooling at least one teat from each region) [19]. One part of the colostrum sample was stored in the refrigerator and immediately transported to the laboratory for compositional analysis, while a second part of the sample was immediately frozen in dry ice and then stored at -80°C for metabolomic analysis.

Colostrum analysis on proximal composition and immunoglobulins

Colostrum composition (20 sows/group; balanced for parity order and representative of the group mean litter size) was analyzed for total fat, total proteins, caseins, lactose, urea and somatic cells (SCC) in triplicate using Milkoscan (FT2 FOSS A/S, Padua, Italy). Before the analysis, the samples were diluted 1:2 with water.

Quantification of immunoglobulins (Igs) was performed by enzyme-linked immunosorbent assay (ELISA) using porcine specific goat anti-pig IgA/IgM/IgG affinity

purified and goat anti-pig IgA/IgM/IgG HRP conjugate antibodies (BETHYL Laboratories, Montgomery, TX, USA) and porcine Igs specific standards for IgA (Alpha Diagnostic Intl. Inc., San Antonio, TX, USA), IgM (Abcepta, San Diego, CA, USA) and IgG (BETHYL Laboratories, Montgomery, TX, USA). Absorbance was read at 405 nm using a microplate reader (Multiskan™ FC Microplate Photometer, Thermo Fisher Scientific, USA). Concentration values, expressed in $\mu\text{g/mL}$, were calculated using a four-point parametric curve. Colostrum samples were previously defatted as described by Luise et al. [27] and then diluted 1:50,000 for IgA, 1:10,000 for IgM and 1:500,000 for IgG analysis.

Metabolomic analysis of colostrum

Colostrum (20 sows/group; balanced for parity order and representative of the group mean litter size) was thawed, carefully mixed by inversion and 15 mL of each colostrum sample was diluted 1:1 with pure water. To each diluted sample, 0.02% sodium azide was added to inhibit bacterial growth during sample preparation. The sample was then defatted by centrifugation at 4°C for 30 min at $1,500\times g$. The aqueous phase was transferred to a clean Falcon tube, avoiding the outer layer of fat, and centrifuged again; this procedure was repeated three times. A total of 700 μL of supernatant was then mixed with 100 μL of a D_2O solution containing 10 mmol/L 2,2,3,3- D_4 -3-(trimethylsilyl)propionic acid sodium salt (TSP), used as the NMR chemical shift reference. This solution was buffered at $\text{pH } 7.00 \pm 0.02$ using 1 mol/L phosphate buffer. To avoid microbial proliferation, 10 μL of NaN_3 (2 mmol/L) were also added. Each sample was centrifuged again as described before.

Proton nuclear magnetic resonance (^1H -NMR) spectra were acquired at 298 K using an AVANCE™ III spectrometer (Bruker, Milan, Italy) operating at a frequency of 600.13 MHz as described by Brugaletta et al. [28].

Metabolite signals were identified by comparing chemical shifts and multiplicities with those in the Chenomx software library (version 10; Chenomx Inc., Edmonton, Canada) and Human Metabolome Database (HMDB—release 2). The quantification of the molecules in absolute terms was done in one reference sample, by relying on TSP as internal standard. Variations in water content across samples were then accounted for by applying probabilistic quotient normalization (PQN) [29] to the full set of spectra. Signal integration for each molecule was carried out in the MestReNova software (Ricerca Mestrelab S.L. Santiago De Compostela (Spain)—ver 14.2.0–26256). For this, after having applied a line broadening of 0.3 and a baseline adjustment by the Whittaker Smoother procedure, a global spectra deconvolution procedure was used.

Fatty acid analysis

Fatty acids were analyzed both on the sows' transition diet and on the colostrum samples (20 sows/group; balanced for parity order and representative of the group mean litter size). The total lipids of diets were extracted according to Rodriguez-Estrada et al. [30]. Briefly, 7 g of raw sample were mixed with 60 mL of a chloroform/methanol solution (1:1, v/v), homogenized for 30 s, and incubated in an oven at 60 °C for 20 min. Subsequently, 30 mL of chloroform was added, adjusting the solvent ratio to 2:1 (v/v, chloroform/methanol). The mixture was then homogenized again for 1 min and filtered to remove the solid residue, mainly composed of proteins. The filtrate was combined with 30 mL of 1 mol/L KCl and kept at 4 °C overnight to allow phase separation. The chloroform layer was collected, evaporated with a rotary evaporator, and the lipid extract was weighed.

Colostrum fat extraction was carried out using the protocol by Serra et al. [31]. In brief, 0.4 mL of ammonia (25%), 1 mL of ethyl alcohol (95%), and 5 mL of hexane were added to 2 g of raw colostrum. After vortexing, the samples were centrifuged at $1,200\times g$ and 2 °C for 15 min. Following phase separation, the upper layer was carefully collected. The extraction process was repeated a second time with 1 mL of ethyl alcohol 95% and 5 mL of hexane, with the samples being centrifuged at $1,200\times g$ for 15 min and the upper layer collected again. A third extraction was performed using 5 mL of hexane, after which the samples were centrifuged at $1,200\times g$ for 15 min and the upper layer was collected. The extracted fat was then dried at 35 °C using a rotary evaporator, weighed, and finally dissolved in hexane.

The derivatisation of total lipids of diets and colostrum was performed according to Christie [32], 10 mg of total lipids were combined with 0.5 mg of nonadecanoic acid (C19:0) methyl ester (Sigma Chemical Co., St. Louis, MO, USA) as the internal standard. The mixture was then transesterified using a cool base-catalysed transesterification method, employing a 0.5 mol/L methanolic solution of sodium methoxide. The transmethylation process was completed in 5 min at room temperature.

Fatty acid methyl esters were identified and quantified by gas chromatography (GC), using a GC-FID apparatus (GC 2000 plus, Shimadzu, Columbia, MD, USA) equipped with a high-polarity fused silica capillary column (Chrompack CP-Sil 88 Varian, Middelburg, Netherlands; 100 m $\times$ 0.25 mm i.d.; film thickness 0.20 μ m). Helium was used as the carrier gas at a flow rate of 1 mL/min, with a split ratio of 1:100. A 1 μ L sample was injected under the following GC conditions: the oven temperature was initially set to 120 °C and held for 1 min, then increased to 180 °C at a rate of 5 °C/min and held

for 18 min. It was then raised to 200 °C at 2 °C/min, held for 1 min, increased to 230 °C at 2 °C/min, and held for 19 min. The injector temperature was set to 270 °C, while the detector temperature was set to 300 °C.

Individual fatty acid methyl esters (FAMES) were identified by comparison with a 37-component FAME mix standard (Supelco, Bellefonte, PA, USA). The identification of isomers of C18:1 was based on commercial standard mixtures (Supelco, Bellefonte, PA, USA) and published isomeric profiles.

Results for fatty acid composition in feed were expressed in g/100g as feed, while fatty acids in colostrum were expressed in g/100g of colostrum.

MiRNA analysis on colostrum

For miRNA analysis, total RNA extraction was carried out from defatted colostrum samples (10 sows/group; balanced for parity order and representative of the group mean litter size). RNAs were extracted from 200 μ L of colostrum using 1 mL TRIzol® Reagent (Life Technologies, Carlsbad, CA, USA) applying an adapted protocol from Chen et al. [33]. Samples were treated with DNAase (TURBO-DNASE, Thermo Fisher Scientific, Milan, Italy) to remove DNA contaminants. The quantity and purity were analyzed using 6000 Nanodrop (Agilent, CA, USA). In addition, the quantification of miRNA was carried out using Qubit™ microRNA Assay Kits following the manufacturer's procedure (Thermo Fisher, USA). After the quality check, the QIAseq miRNA Library Kit (QIAGEN, Hilden, Germany) was used for library preparation according to the manufacturer's guidelines. RNA samples were quantified and assessed for quality using the Agilent 2100 Bioanalyzer RNA assay or TapeStation RNA assay (Agilent Technologies, Santa Clara, CA, USA). Final libraries were verified using the Qubit 3.0 Fluorometer (Invitrogen, Carlsbad, CA, USA) and the Agilent Bioanalyzer DNA assay. The libraries were then prepared for sequencing and sequenced in single-end 75 bp mode on the Aviti platform (Element Biosciences, San Diego, CA, USA). Raw sequencing reads were processed using the Illumina Bcl2Fastq v2.20 pipeline [34] for base calling, format conversion, and demultiplexing. Subsequent analysis was performed using sRNAbench, a component of the sRNAtoolbox suite [35], designed for small RNA expression profiling, prediction of novel miRNAs, isomiR analysis, genome alignment, and read length distribution. Reads were mapped to the Sscrofa11.1_mp using a seed alignment strategy. Mapping parameters included a seed length of 20 nucleotides (seed=20), and a minimum read length of 15 nt. No upper limit was set on read length. Only reads with a count ≥ 2 were retained for downstream analysis.

Statistical analysis

All statistical analyses were performed using RStudio v4.3 (RStudio, PBC, Boston, MA, USA) with the packages *jmv* [36], *car* [37], and *emmeans* [38].

For the analyses of sow performance and colostrum traits, the sow was considered the experimental unit. Data were analyzed using a general linear model (GLM), followed by ANOVA and Tukey post-hoc tests. For sow performance (BCS, backfat and muscle thickness, days of gestation, feed intake, and farrowing performance) and colostrum composition (proximate analysis and Igs), the model included the experimental diet (CO vs. TRT) and batch (two batches) as fixed factors, with PO and litter size at d 0 were included as a covariate. In addition, for sow performance at weaning and for the change between prenatal measurements and weaning, the number of piglets at weaning and the average piglet body weight (BW) per litter were included as additional covariates. For farrowing duration, average interval between piglet births, and piglet vitality at birth, the model also included the number of oxytocin injections, and the number of veterinary interventions as covariates.

For piglet performance (BW and average daily gain: ADG) and the percentage of exclusions or deaths, the litter was considered the experimental unit. Data were analyzed using a GLM followed by ANOVA and Tukey post-hoc tests. The model included the experimental diet (CO vs. TRT) and batch (two batches) as fixed factors, while PO and the number of piglets per litter at the relevant timepoint were included as covariates (i.e., number of cross-fostered piglets for performance at d 0 and periods d 0–6 and d 0–24; litter size at d 6 for performance at d 6 and for d 0–6; litter size at d 24 and for performance at d 24). Average piglet weight per litter at d 0 was also included as a covariate. Finally, for performance at d 24 and for the interval d 0–24, the age at weaning was added as an additional covariate.

The possible interaction between diet and batch was also tested but it was never significant therefore it was removed. Before running the model, the distribution of data was tested with the Shapiro-test and corrected when it was not normal. When data were not possible to be normalized, a Poisson distribution in the GLM was used. The difference in SCC number in colostrum was tested with Kruskal–Wallis test. The distribution of model residuals was examined visually using a quantile–quantile (Q–Q) plot and an overlaid reference line (*qqnorm* and *qqline*), to evaluate the assumption of normality. In addition, residual versus fitted value plots, obtained via the “plot” function, were inspected to verify the assumption of homoscedasticity and to detect any potential model misspecification or influential data points.

Metabolomics data on colostrum were analyzed using a multivariate approach using MetaboAnalyst 6.0 [39], then, the metabolites were analyzed using the same GLM and ANOVA model of sows’ performance on RStudio. For the multivariate approach, data were normalized by sum and log transformed and then analyzed using the orthogonal partial least squares-discriminant analysis (OPLS-DA) approach. The variable importance projection (VIP) scores were determined for diet.

Fatty acid profile of colostrum was analyzed using a multivariate sparse partial least squares discriminant analysis (PLS-DA) [40]. Data were initially filtered to exclude fatty acids showing zero variance or with more than 60% missing values and then were normalised using total sum scaling normalisation coupled with the centred log-ratio (CLR) transformation. The optimal number of components and the optimal number of variables selected for each component included in the PLS-DA model were selected based on the average balanced classification error rate with Mahalanobis distance over 100 repeats of the loo cross-validation of the PLS-DA model. The stability of frequency scores of the selected fatty acid was calculated (“perf” function) with loo cross-validation and 100 repetitions; the fatty acid showing an importance > 1 were retained. In addition, each fatty acid and class of fatty acid were analyzed using an ANOVA model on RStudio using the same GLM model used for sows’ performance.

The miRNA profile of the CO and TRT groups was analyzed using a PLS-DA of the first four components and the “perf” function with “Mfold” cross-validation and 10 repetitions was used to obtain the stability of frequency scores. The four principal components and Mahalanobis distance were chosen for the tuning process to determine the optimal number of variables, in order to obtain the discriminant miRNAs per diet from the first two components. Results were visualised using the score plot of the first two components. In addition, the differential expression analysis of miRNAs was conducted using sRNAde, a module within the sRNAtoolbox. Multiple statistical methods, including DESeq2, edgeR, and NOISeq were applied to perform pairwise comparisons between the diets. *P*-values were adjusted using the False Discovery Rate.

Results were expressed as least-square means and SE. A difference was declared significant when $P \leq 0.05$ and marginally significant when $0.05 < P \leq 0.10$.

Results

Performance

One hundred sows were included (50 sow/group) according to the inclusion criteria. All sows included in the

study were healthy and they were not treated or excluded during the lactation period.

Table 2 shows the results for BCS, backfat thickness, muscle thickness, gestation length, and sow feed intake during the experimental diet supplementation. There were no significant differences between CO and TRT sows in prenatal BCS (CO: 3.58 ± 0.13 vs. TRT: 3.74 ± 0.13), backfat (CO: 10.9 ± 0.39 vs. TRT: 11.6 ± 0.39) and dorsal muscle thickness (CO: 52.3 ± 0.75 vs. TRT: 51.8 ± 0.75) at the entry in the farrowing room (d -6). The BCS (CO: 1.78 ± 0.20 vs. TRT: 2.20 ± 0.20), backfat (CO: 8.82 ± 0.43 vs. TRT: 9.40 ± 0.43) and dorsal muscle thickness (CO: 47.1 ± 0.86 vs. TRT: 47.9 ± 0.86) at weaning (d 24) were not influenced by the different transition diets, nor was the difference between d -6 and d 24 BCS (CO: 1.76 ± 0.14 vs. TRT: 1.57 ± 0.14), backfat (CO: -2.10 ± 0.27 vs. TRT: -2.31 ± 0.27) and dorsal muscle thickness (CO: -5.31 ± 0.76 vs. TRT: -4.11 ± 0.76). The number of days of gestation was not affected by the diet (CO: 115.6 ± 0.15 vs. TRT: 115.8 ± 0.15). The daily feed intake of the sows pre (CO: 2.31 ± 0.22 vs. TRT: 2.32 ± 0.22) and post farrowing (CO: 5.32 ± 0.33 vs. TRT: 5.4 ± 0.33) were not affected by the diet.

Table 3 shows the results of sow performance at farrowing (50 sows/group). The number of total births and mummified piglets were not influenced by the transition diet. However, the percentage of mummified piglets was significantly lower in the TRT group (CO: 2.73 ± 0.22 vs. TRT: 2.02 ± 0.22 ; $P=0.018$). The TRT group tended to have a higher number (CO: 14.2 ± 0.49 vs. TRT: 15.5 ± 0.49 ; $P=0.06$) and percentage of live births (CO: 89.3 ± 1.36 vs. TRT: 93.8 ± 1.36 ; $P=0.018$), and a significantly lower number (CO: 1.28 ± 0.14 vs. TRT: 0.68 ± 0.14 ; $P=0.002$) and percentage of stillbirths (CO: 7.83 ± 0.34 vs. TRT: 4.00 ± 0.34 ; $P<0.0001$). In addition, it was observed that the percentage of mummified piglets was significantly influenced by sow PO ($P<0.001$).

Table 4 shows the results for average farrowing duration, interval between piglets' births, number of oxytocin injections and veterinary inspections per sows during farrowing, and the piglet vitality score (VS) at farrowing (12 sows/group). The average farrowing duration did not differ between the CO and TRT group (CO: 308 ± 40 vs. TRT: 283 ± 40 min) and neither the average time interval between piglets' birth was significantly different (CO: 21.3 ± 2.44 vs. TRT: 19.3 ± 2.44). The number of oxytocin

Table 2 Effects of diet on the sows BCS, backfat and muscle depth and daily feed intake

Item ^a	Diet ^b		SE	P value			
	CO	TRT		Diet	PO ^c	Number of piglets ^d	Piglet weight ^e
BCS ^f Prenatal (d -6)	3.58	3.74	0.13	0.375	0.948	-	-
BCS Weaning (d 24)	1.78	2.20	0.20	0.157	0.216	0.084	0.050
BCS difference ^g	1.76	1.57	0.14	0.323	0.041	0.196	0.002
Prenatal backfat thickness, mm (d -6)	10.9	11.6	0.39	0.194	0.843	-	-
Weaning backfat thickness, mm (d 24)	8.82	9.40	0.43	0.353	0.597	0.037	0.451
Dorsal fat thickness difference ^g , mm	-2.10	-2.31	0.27	0.594	0.368	0.020	0.022
Prenatal dorsal muscle thickness, mm (d -6)	52.3	51.8	0.75	0.646	0.555	-	-
Weaning dorsal muscle thickness, mm (d 24)	47.1	47.9	0.86	0.497	0.633	< 0.001	0.040
Dorsal muscle difference ^g , mm	-5.13	-4.11	0.76	0.352	0.230	0.008	0.036
Days of gestation	115.6	115.8	0.15	0.279	0.102	-	-
Daily feed intake pre-farrowing, kg/d	2.31	2.32	0.22	0.983	0.820	-	-
Daily feed intake post-farrowing, kg/d	5.32	5.40	0.33	0.860	0.580	0.89	0.42

^a The analysis was carried out on 50 sows per group

^b CO: Control diet with 12.33 MJ/kg of ME and 0.70% SID Lys; TRT: Treatment diet with 12.97 MJ/kg of ME and 0.85% SID Lys

^c Parity order. Parity order affected the BCS difference ($P=0.041$; coeff = -0.085)

^d Number of piglets: The number of piglets per litter at d 0 after cross-fostering was used for the parameters collected before farrowing, whereas the number of piglets at weaning (d 24) was used for the parameters collected at weaning. It tended to be significant for the BCS Weaning ($P=0.084$; coeff = -0.008) and it was significant for the Weaning backfat thickness ($P=0.037$; coeff = -0.004), Dorsal fat thickness difference ($P=0.020$; coeff = -0.29), the Weaning dorsal muscle thickness ($P<0.001$; coeff = -1.42) and Dorsal muscle difference ($P=0.008$; coeff = -0.92),

^e Piglets weight: The average piglet weight per litter at d 0 after cross-fostering was used for the parameters collected before farrowing, whereas the average piglet weight per litter at weaning was used for the parameters collected at weaning. It was significant for BCS Weaning (d 24) ($P=0.050$; coeff = -0.0002), BCS difference ($P=0.002$; coeff = 0.0004), Dorsal fat thickness difference ($P=0.022$; coeff = -0.0005), Weaning dorsal muscle thickness ($P=0.040$; coeff = -0.002), and Dorsal muscle difference ($P=0.036$; coeff = -0.001)

^f Body condition score

^g The difference was calculated subtracting the value at weaning to the prenatal measure

Table 3 Effects of the transition diet on the sow performance at farrowing

Item ^a	Diet ^b		SE	P value	
	CO	TRT		Diet	PO ^c
Total births, n	15.9	16.6	0.53	0.416	0.332
Number of live births, n	14.2	15.5	0.49	0.066	0.302
Number of stillbirths, n	1.28	0.68	0.14	0.002	0.505
Number of mummified, n	0.44	0.37	0.09	0.614	0.346
Live births, %	89.3	93.8	1.36	0.018	0.585
Stillbirths, %	7.83	4.00	0.34	<0.0001	0.394
Mummified, %	2.73	2.02	0.22	0.018	<0.0001
Litter size post-cross fostering	13.1	13.4	0.17	0.27	0.96

^a The analysis was carried out on 50 sows per group^b CO: Control diet with 12.33 MJ/kg of ME and 0.70% SID Lys; TRT: Treatment diet with 12.97 MJ/kg of ME and 0.85% SID Lys^c Parity order. Parity order affected the Mummified ($P < 0.0001$; coeff = -0.13)

injections did not differ between the CO and TRT group (CO: 0.64 ± 0.24 vs. TRT: 0.34 ± 0.18), while the number of veterinary inspections tended to be lower in the TRT group compared to the CO group ($P = 0.067$; CO: 0.28 ± 0.17 vs. TRT: 0.05 ± 0.06).

The percentage of piglets with VS zero was significantly lower in sows from the TRT group (CO: 7.85 ± 0.6 vs. TRT: 3.16 ± 0.6 ; $P < 0.0001$). The experimental diet did not affect the percentage of piglets that showed a VS of one (CO: 34.1 ± 5.30 vs. TRT: 41.3 ± 5.30), two (CO: 50.5 ± 5.35 vs. TRT: 49.7 ± 5.35) or three (CO: 3.64 ± 0.57 vs. TRT: 3.90 ± 0.57) at farrowing.

Table 5 reports the results of piglet growth and survival during the trial (50 litters/group). Diet had no effect on piglet weight at birth (d 0; CO: $1,516 \pm 42.2$ vs. TRT: $1,559 \pm 42.1$ g), but the piglets of the TRT litters tended to be heavier at d 6 (CO: $2,576 \pm 28.5$ vs. TRT: $2,653 \pm 28.5$ g; $P = 0.057$) and were significantly heavier at d 24 (CO: $7,730 \pm 85.8$ vs. TRT: $8,022 \pm 85.7$ g; $P = 0.017$) than piglets from the CO group. ADG was not affected by the diet from d 0 to d 6 (CO: 183 ± 4.86 vs. TRT: 191 ± 4.86 g/d). However, ADG of piglets from the TRT litters tended to be higher in the period from d 6 to d 24 (CO: 285 ± 3.98 vs. TRT: 294 ± 3.97 g/d; $P = 0.093$) and was significantly higher from d 0 to d 24 (CO: 261 ± 3.69 vs. TRT: 272 ± 3.69 g/d; $P = 0.045$). No significant differences in litter size were observed at d 0 (post cross-fostering, CO: 13.10 ± 0.17 vs. TRT: 13.40 ± 0.17), d 6 (CO: 12.50 ± 0.14 vs. TRT: 12.50 ± 0.14) and d 24 (CO: 11.40 ± 0.20 vs. TRT: 11.60 ± 0.20). Piglets were moved due to management decisions or died. It was observed that the TRT group

Table 4 Farrowing duration, time interval between piglet births, and vitality of piglets

Item ^a	Diet ^b		SE	P value		Number of born piglets ^d	Number oxytocin injections ^e	Number of inspections ^f
	CO	TRT		Diet	PO ^c			
Farrowing duration, min	308	283	40	0.675	0.600	0.720	0.455	0.542
Average time interval between piglets, min	21.3	19.3	2.44	0.509	0.574	0.325	0.767	0.199
Number of oxytocin injections ^e	0.64	0.34	0.20	0.304	0.806	0.211	-	-
Number of inspections ^f	0.28	0.05	0.13	0.067	0.660	0.061	-	-
Vitality score zero, %	7.85	3.16	0.6	<0.0001	<0.0001	0.005	0.069	0.900
Vitality score one, %	34.1	41.3	5.30	0.322	0.570	0.955	0.544	0.993
Vitality score two, %	50.5	49.7	5.35	0.916	0.247	0.860	0.965	0.704
Vitality score three, %	3.64	3.90	0.57	0.744	0.003	0.001	0.003	<0.0001

^a The analysis was carried out on 12 sows per group. Vitality score were assigned according to Uddin et al. [25], protocol: 0 = no movement, no respiration after 15 s, stillbirths; 1 = no movement within 15 s but the piglet was breathing or attempting to breathe; 2 = when the piglet showed little movement within 15 s, breathed or attempted to breathe; 3 = when the piglet had good movement, good respiration and attempted to stand within 15 s^b CO: Control diet with 12.33 MJ/kg of ME and 0.70% SID Lys; TRT: Treatment diet with 12.97 MJ/kg of ME and 0.85% SID Lys^c Parity order. It was significant for Vitality score zero ($P < 0.0001$, coeff: 0.24) and for Vitality score three ($P = 0.003$, coeff: -0.10)^d Total number of piglets born. It was significant for Vitality score zero ($P = 0.005$, coeff: 0.11) and for Vitality score three ($P = 0.001$, coeff: -0.13) and tended to be significant for the Number of inspections ($P = 0.061$; coeff: -0.26)^e Number of interventions that included an oxytocin injection. It tended to be significant for Vitality score zero ($P = 0.069$, coeff: -0.27) and it was significant for Vitality score three ($P = 0.003$, coeff: -0.63)^f Number of interventions that included operator assistance. It was significant for Vitality score three ($P < 0.0001$, coeff: -1.27)

Table 5 Effects of the transition diet on the piglets' performance during lactation period

Item ^a	Diet ^b		SE	P value				
	CO	TRT		Diet	PO ^c	Number of piglets ^d	Weight ^e	Age d 24 ^f
Body weight, g								
d 0	1,516	1,559	42.1	0.473	0.366	0.202	-	-
d 6	2,576	2,653	29.0	0.057	0.052	0.054	< 0.0001	-
d 24	7,730	8,022	85.8	0.017	0.533	0.003	< 0.0001	0.009
Average daily gain, g/d								
d 0–6	183	191	4.86	0.262	0.083	0.171	0.002	-
d 6–24	285	294	3.98	0.093	0.102	0.004	< 0.0001	-
d 0–24	261	272	3.69	0.045	0.696	0.002	< 0.0001	-
Number of piglets, n								
d 0	13.10	13.40	0.17	0.268	0.854	-	0.202	-
d 6	12.50	12.50	0.14	0.988	0.344	< 0.0001	0.043	-
d 24	11.40	11.60	0.20	0.723	0.595	< 0.0001	0.088	0.888
Percentage of excluded piglets								
d 0–6	2.28	2.91	0.23	0.044	0.002	< 0.0001	< 0.0001	-
d 6–24	5.82	5.10	0.37	0.086	0.0001	< 0.0001	< 0.0001	-
d 0–24	9.30	8.75	0.42	0.350	0.055	< 0.0001	< 0.0001	0.230
Percentage of dead piglets								
d 0–6	2.95	2.26	0.23	0.032	0.893	0.900	< 0.0001	-
d 6–24	0.65	0.99	0.13	0.035	0.0001	0.879	< 0.0001	-
d 0–24	3.83	3.64	0.281	0.625	0.389	0.828	0.133	0.800

^a The analysis was carried out on 50 litters per group^b CO: Control diet with 12.33 MJ/kg of ME and 0.70% SID Lys; TRT: Treatment diet with 12.97 MJ/kg of ME and 0.85% SID Lys^c Parity order. It tended to be significant for the body weight at d 6 ($P=0.06$; coeff: -17.31) and the Average daily gain d 0–6 ($P=0.06$; coeff: -2.61); it was significant for the percentage of excluded piglets at d 0–6 ($P=0.002$; coeff: -0.091), at d 6–24 ($P=0.0001$; coeff: 0.069) and d 0–24 ($P=0.055$; coeff: 0.029) and for the Percentage of dead piglets at d 6–24 ($P=0.0001$; coeff: 0.140)^d Number of piglets per litter at the matching timepoint. The number of piglets at d 0 represent the litter size post cross fostering. In case of a time range, it was used the number of piglets at the beginning of the time range. It tended to be significant for the body weight at d 6 ($P=0.054$; coeff: -27.2) and it was significant for the body weight at d 24 ($P=0.003$; coeff: -128), for the average daily gain at d 6–24 ($P=0.004$; coeff: -5.63) and at d 0–24 ($P=0.002$; coeff: -5.31); for the number of piglets at d 6 ($P<0.0001$; coeff: 0.87) and d 24 ($P<0.0001$; coeff: 0.68) for the percentage of excluded piglets at d 0–6 ($P<0.0001$; coeff: 0.20), d 6–24 ($P<0.0001$; coeff: 0.21), d 0–24 ($P<0.0001$; coeff: 0.14)^e Average piglet weight per litter at d 0 after cross-fostering. It was significant for piglet body weight at d 6 ($P<0.0001$; coeff: 1.24) and d 24 ($P<0.0001$; coeff: 1.64); for the average daily gain at d 0–6 ($P=0.002$; coeff: 0.041), d 6–24 ($P<0.0001$; coeff: 0.026) and at d 0–24 ($P<0.0001$; coeff: 0.03); for the number of piglets at d 6 ($P=0.043$; coeff: 0.0007); for the percentage of excluded piglets at d 0–6 ($P<0.0001$; coeff: -0.0007), d 6–24 ($P<0.0001$; coeff: -0.001), d 0–24 ($P<0.0001$; coeff: -0.001); for the percentage of dead piglets at d 0–6 ($P<0.0001$; coeff: -0.001) and d 6–24 ($P<0.0001$; coeff: 0.001). It tended to be significant for the number of piglets at d 24 ($P=0.088$; coeff: 0.001)^f The age of all piglets was the same at d 0 and d 6. At d 24, piglets had an average age of 23.65 ± 0.04 (mean $\pm$ SE). It was significant for the piglets' body weight at d 24 ($P=0.009$; coeff: 157.7)

had a higher percentage of piglets excluded during the period d 0–6 than the CO group (CO: 2.28 ± 0.23 vs. TRT: 2.91 ± 0.23 ; $P=0.044$), whereas the CO group had a significantly higher percentage of dead piglets during the same period (CO: 2.95 ± 0.23 vs. TRT: 2.26 ± 0.23 ; $P=0.032$). On the contrary, from d 6 to d 24, the TRT group tended to have a lower percentage of excluded piglets (CO: 5.82 ± 0.37 vs. TRT: 5.1 ± 0.37 ; $P=0.086$) and a significantly higher percentage of dead piglets (CO: 0.65 ± 0.13 vs. TRT: 0.99 ± 0.13 ; $P=0.035$). However, over the entire period d 0–24, the CO and TRT group did not show any difference in percentage of excluded piglets (CO: 9.3 ± 0.42 vs. TRT: 8.75 ± 0.42) and dead piglets (CO: 3.83 ± 0.28 vs. TRT: 3.64 ± 0.28).

At d 24, piglets were scored for ear lesions (Fig. 1A) and tail lesions (Fig. 1B). The TRT group exhibited a significantly lower ear LSI than the CO group (CO: 17.7 ± 0.6 vs. TRT: 12.3 ± 0.6 ; $P<0.0001$), whereas their tail LSI was higher (CO: 0.46 ± 0.2 vs. TRT: 1.78 ± 0.2 ; $P<0.0001$).

Colostrum proximal composition and immunoglobulins quantity

Colostrum composition and Igs concentration are shown in Table 6 (20 sows/group). The colostrum of sows from the CO and TRT groups did not show differences in terms of protein (CO: $15.4\% \pm 0.45\%$ vs. TRT: $15.3\% \pm 0.45\%$ m/m), casein (CO: $11.3\% \pm 0.34\%$ vs. TRT: $11.3\% \pm 0.34\%$ m/m), lactose (CO: $2.92\% \pm 0.11\%$ vs. TRT: $2.70\% \pm 0.11\%$ m/m) and urea (CO:

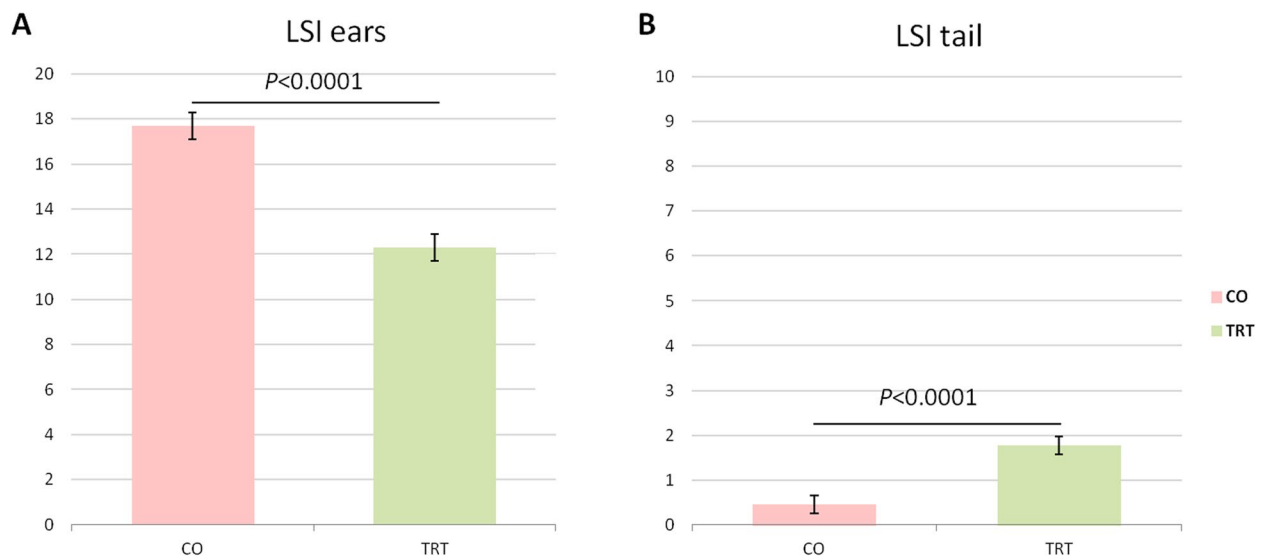


Fig. 1 Effects of the transition diet on the ear and tail lesions of piglets. The analysis was carried out on 50 litters per group. **A** Lesion score index (LSI) of piglets' ears at d 24. **B** LSI of piglet tail at d 24. CO: Control diet with 12.33 MJ/kg of ME and 0.70% SID Lys; TRT: Treated diet with 12.97 MJ/kg of ME and 0.85% SID Lys

Table 6 Composition and Igs concentration of colostrum

Colostrum composition ^a	Diet ^b		SE	P value	
	CO	TRT		Diet	PO ^c
Fat, % m/m	4.22	4.83	0.20	0.037	<0.001
Protein, % m/m	15.4	15.3	0.45	0.911	0.665
Casein, % m/m	11.3	11.3	0.34	0.983	0.612
Lactose, % m/m	2.92	2.70	0.11	0.156	0.266
Urea, mg/100 mL	112	110	3.30	0.661	0.239
IgG, mg/mL	63.5	64.1	8.16	0.962	0.694
IgA, mg/mL	28.1	28.7	1.76	0.817	0.204
IgM, mg/mL	0.47	0.46	0.17	0.966	0.814

^a The analysis was carried out on 20 sows per group

^b CO: Control diet with 12.33 MJ/kg of ME and 0.70% SID Lys; TRT: Treatment diet with 12.97 MJ/kg of ME and 0.85% SID Lys

^c Parity order. It was significant for the Fat ($P < 0.001$; coeff: -0.21)

112 ± 3.30 vs. TRT: 110 ± 3.30 mg/100mL). However, the TRT diet had a significant effect on fat concentration in colostrum (CO: 4.22% ± 0.20% vs. TRT: 4.83% ± 0.20% m/m; $P = 0.037$). Concentration of IgG (CO: 63.5 ± 8.16 vs. TRT: 64.1 ± 8.16 mg/mL), IgA (CO: 28.1 ± 1.76 vs. TRT: 28.7 ± 1.76 mg/mL) and IgM (CO: 0.47 ± 0.17 vs. TRT: 0.46 ± 0.17 mg/mL) did not differ between groups. Finally, colostrum from TRT sows tended to have a higher number of SCC (CO: 108,350 ± 42,901 vs. TRT: 623,250 ± 227,395 cells/mL; $P = 0.08$) (Fig. 2).

Colostrum metabolomics

Figure 3 shows the multivariate (OPLS-DA) and univariate (ANOVA) results of the effect of the transition diet

on the metabolomic composition of colostrum (20 sows/group). The first two main components allowed a classification of colostrum samples according to diet to be observed, explaining relatively 6% and 14.6% of the variability (Fig. 3A). UDP-glucuronate, taurine, citrate, creatine phosphate, uridine monophosphate (UMP), ribose and carnitine were the most discriminant metabolites for principal component (PC) 1 and PC2 (Fig. 3B and C, respectively). ANOVA analysis showed that the TRT diet increased the concentration of UDP-glucuronate (CO: 352 ± 39 vs. TRT: 501 ± 39; $P = 0.008$) and carnitine (CO: 184 ± 3.25 vs. TRT: 226 ± 3.25; $P < 0.0001$) while it reduced the concentration of citrate (CO: 1,437 ± 8.2 vs. TRT: 1,126 ± 8.2; $P < 0.0001$) compared to the CO diet (Fig. 3D).

Fatty acid profile of diet and colostrum

The fatty acid profile of the two transition diets is reported in Table S1 (Additional file 1). A total of 29 fatty acids were detected in the diets, and the most abundant were C18:2 *9cis,12cis* (CO: 7,581.28 vs. TRT: 7,647.59 g/100g), C18:1 *9cis* (CO: 3,843.96 vs. TRT: 3,888.37 g/100g), C16:0 (CO: 2,191.45 vs. TRT: 2,076.24 g/100g) and C18:3 *9cis,12cis,15cis* (CO: 1,728.6 vs. TRT: 1,580.92 g/100g).

Figure 4 shows the multivariate PLS-DA and the significant results of the ANOVA model regarding the effect of the transition diet on the fatty acids composition of colostrum (20 sows/group). The first two main components showed a classification of colostrum samples according to diet, explaining relatively 11% and 4% of the variability (Fig. 4A). According to the biplot

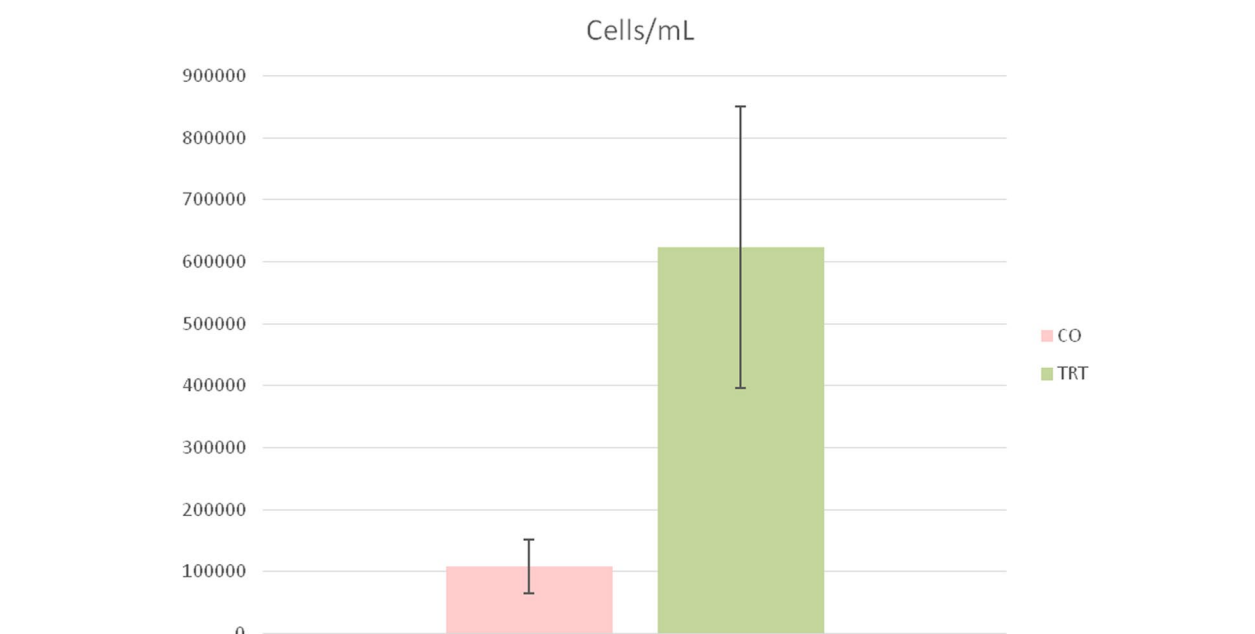


Fig. 2 Effects of the transition diet on the number of somatic cells in colostrum. The analysis was carried out on 20 sows per group. CO: Control diet with 12.33 MJ/kg of ME and 0.70% SID Lys; TRT: Treated diet with 12.97 MJ/kg of ME and 0.85% SID Lys

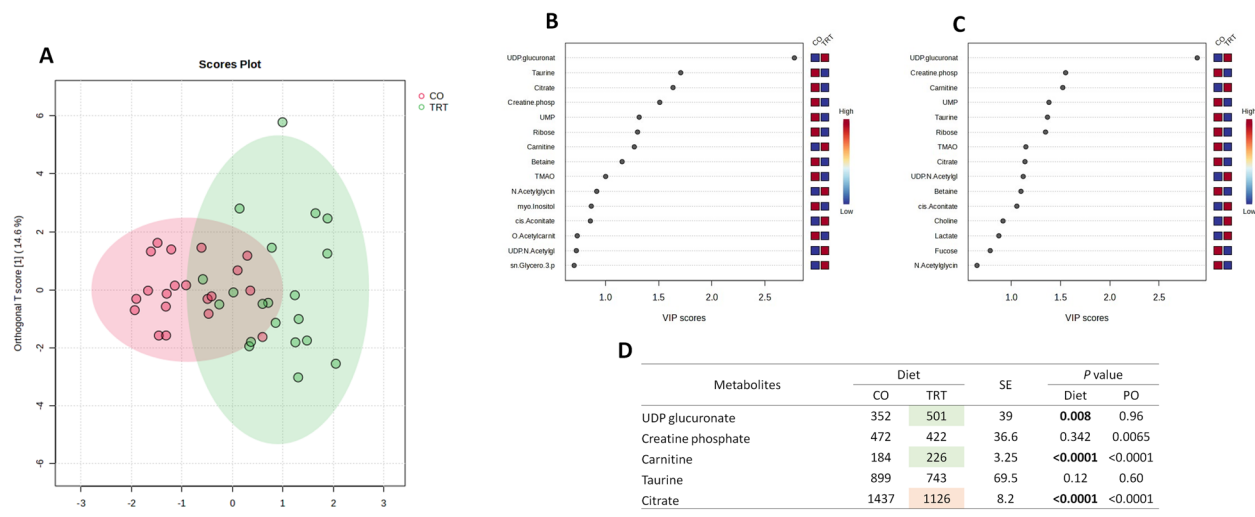


Fig. 3 Effects of the transition diet on the colostrum metabolomics profile. The analysis was carried out on 20 sows per group. **A** OPLS-DA analysis showing the first two PC (principal components). **B** VIP scores of the most discriminant metabolites for PC1. **C** VIP scores of the most discriminant metabolites for PC2. **D** ANOVA analysis of colostrum metabolites. CO: Control diet with 12.33 MJ/kg of ME and 0.70% SID Lys; TRT: Treated diet with 12.97 MJ/kg of ME and 0.85% SID Lys; PO: Parity order of the sows

for the PC1 and PC2 (Fig. 4B), the main variables distinguishing the colostrum samples of the two experimental groups were C12:0 and the polyunsaturated fatty acids (PUFA) n-6/n-3 ratio (Fig. 4C). ANOVA analysis showed no differences between the CO and TRT groups for most fatty acids measured. However, the TRT group showed significantly higher levels of

C18:2 9cis,12cis (CO: 1,301 ± 94.6 vs. TRT: 1,625 ± 94.6 g/100g; $P=0.018$), C18:4 6cis,9cis,12cis,15cis (CO: 2.47 ± 0.40 vs. TRT: 3.70 ± 0.40 g/100g; $P=0.033$) and C20:0 (CO: 5.75 ± 0.65 vs. TRT: 8.38 ± 0.65 g/100g; $P=0.006$). Moreover, colostrum from TRT sows was characterised by a significantly higher abundance of total PUFA (CO: 1,646 ± 116 vs. TRT: 2,010 ± 116

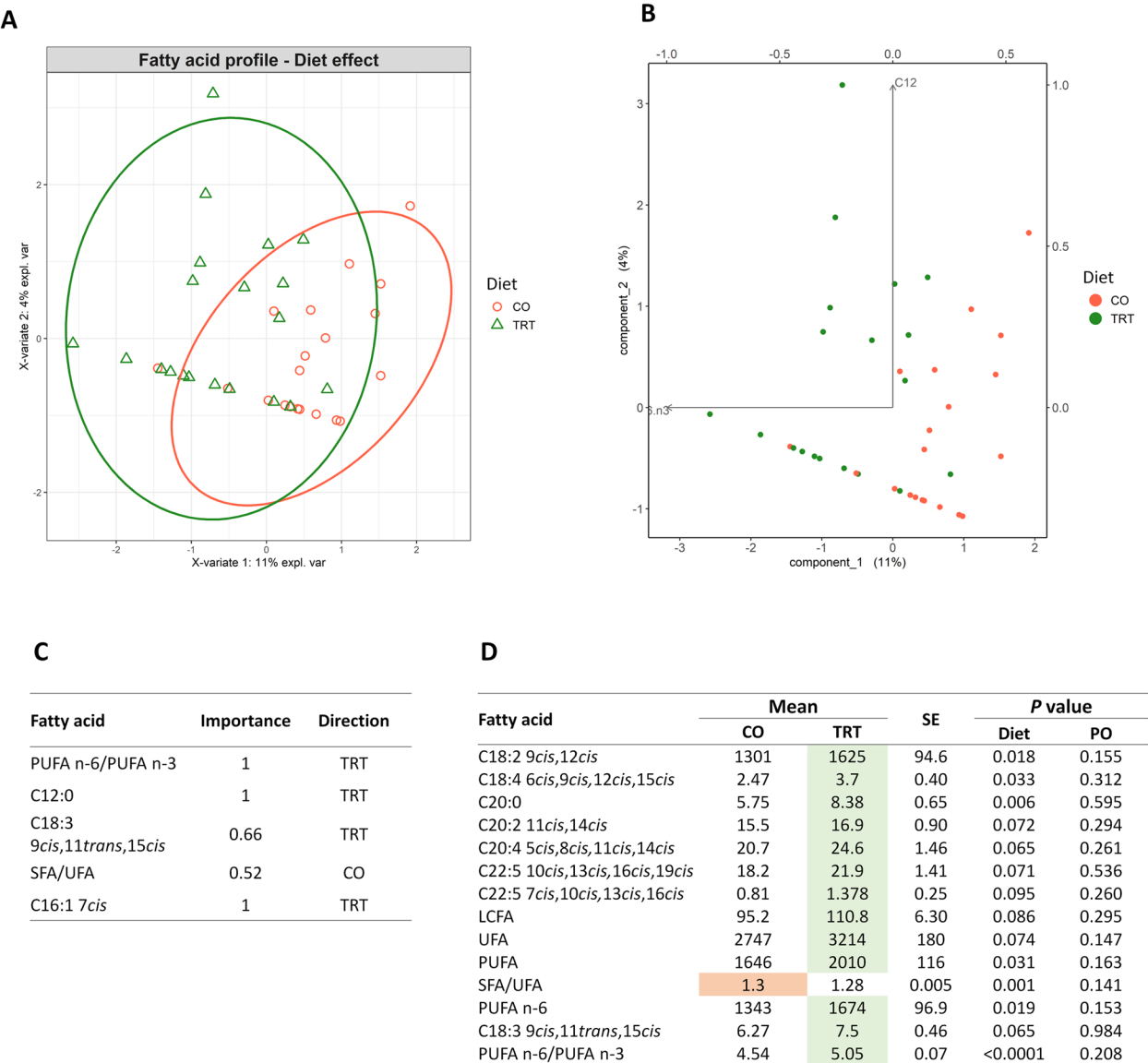


Fig. 4 Effects of the transition diet on the colostrum fatty acid profile. The analysis was carried out on 20 sows per group. **A** PLS-DA plot along the first two PC (principal components) based on fatty acids profile of colostrum. **B** Biplot for PC1 and PC2. **C** Table reporting the most discriminant fatty acids and ratios for the PC1 and PC2. Importance is an indicator of the absolute contribution of fatty acids to class separation. **D** Table reporting the fatty acids (mg/100 g of colostrum) that differentiate between the CO and TRT group ($P < 0.1$, ANOVA). Diet: CO = the group fed the transitional diet normally used on the farm with 12.33 MJ/kg of ME and 0.70% SID Lys; TRT = the group fed a transitional diet characterised by a higher energy content of 12.97 MJ/kg of ME and 0.85% of SID Lys. LCFA: Long chain fatty acids; UFA: Unsaturated fatty acids; PUFA: Polyunsaturated fatty acids; SFA: Saturated fatty acids; PO: Parity order of the sows

g/100g; $P = 0.031$), PUFA n-6 (CO: $1,343 \pm 96.9$ vs. TRT: $1,674 \pm 96.9$ g/100g; $P = 0.019$) and PUFA n-6/PUFA n-3 ratio was significantly higher (CO: 4.54 ± 0.07 vs. TRT: 5.05 ± 0.07 ; $P < 0.001$). In contrast, the ratio of saturated/unsaturated fatty acids (SFA/UFA) was significantly lower in the TRT group compared to CO group (CO: 1.3 ± 0.005 vs. TRT: 1.28 ± 0.005 ; $P = 0.001$) (Fig. 4D).

Colostrum miRNAs

A total of 208 miRNAs were identified in the colostrum samples (10 sows/group). A PLS-DA analysis was carried out to identify the discriminant miRNAs in the colostrum of sows belonging to each dietary group (Fig. 5A). The TRT group was discriminated by 11 miRNAs, and the CO group was discriminated by 10 miRNAs, as reported in Fig. 5B. The CO group was discriminated by

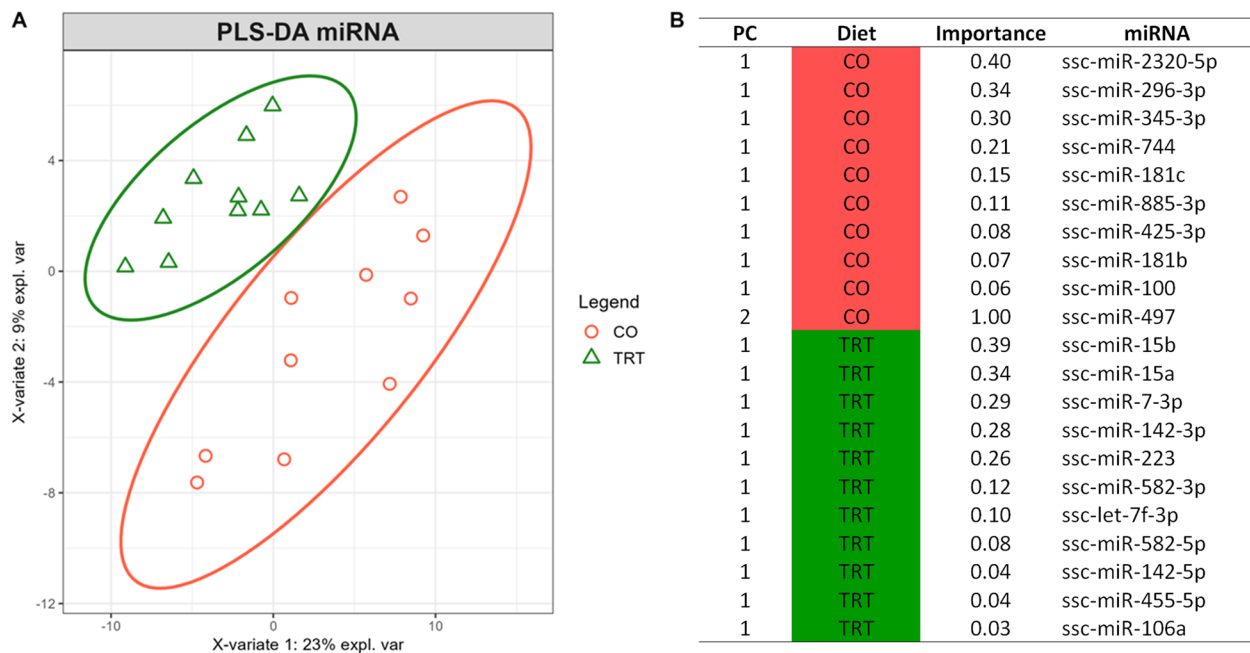


Fig. 5 Effects of transition diet on miRNA expression in sows' colostrum (10 sows/group). **A** PLS-DA plot along the first two principal components (PC) based on miRNA expression profiles. **B** Table reporting the most discriminant miRNAs per diet. Diet: CO = the group fed the transitional diet normally used on the farm with 12.33 MJ/kg of ME and 0.70% SID Lys; TRT = the group fed a transitional diet characterised by a higher energy content of 12.97 MJ/kg of ME and 0.85% of SID Lys; Importance is an indicator of the absolute contribution of miRNA to class separation

ssc-miR-497 (importance 1.00), ssc-miR-2320-5p (importance 0.40), ssc-miR-296-3p (importance 0.34) and ssc-miR-345-3p (importance 0.30), while the TRT group by ssc-miR-15b (importance 0.39), ssc-miR-15a (importance 0.34) and ssc-miR-7-3p (importance 0.29).

Thirteen miRNAs were differentially expressed in the colostrum from sows from the TRT group compared to the CO group according to the univariate analysis (Table 7). However, after False Discovery Rate correction, only ssc-miR-142-3p showed a trend

Table 7 Effect of the transition diet on miRNAs expression in colostrum

miRNA ^a	Diet ^b		Fold change	log ₂ (Fold change)	P value	
	CO	TRT			P value	P _{adj}
ssc-miR-142-3p	24.0	73.1	0.34	-1.56	< 0.0001	0.077
ssc-miR-223	8,176.58	27,890.55	0.29	-1.79	0.003	0.23
ssc-miR-15a	317.88	489.03	0.65	-0.62	0.003	0.23
ssc-miR-100	26.86	12.12	2.12	1.08	0.006	0.292
ssc-miR-885-3p	69.04	32.51	2.09	1.06	0.011	0.401
ssc-miR-15b	6,106.83	7,403.17	0.82	-0.29	0.012	0.401
ssc-miR-2320-5p	744.04	590.46	1.26	0.33	0.014	0.42
ssc-miR-345-3p	1,182.92	928.87	1.27	0.34	0.016	0.427
ssc-miR-296-3p	1,932.44	1,566.32	1.23	0.30	0.02	0.459
ssc-miR-181c	104.38	73.02	1.42	0.51	0.024	0.502
ssc-miR-486	135.02	61.88	2.16	1.11	0.037	0.703
ssc-miR-744	4,664.41	3,949.56	1.18	0.24	0.045	0.759
ssc-miR-582-3p	21.85	33.54	0.66	-0.60	0.047	0.759

^a miRNA analysis was carried out on 10 sows per group

^b CO: Control diet with 12.33 MJ/kg of ME and 0.70% SID Lys; TRT: Treatment diet with 12.97 MJ/kg of ME and 0.85% SID Lys

towards a differential expression between the two groups ($P_{adj}=0.077$), with a higher expression in the TRT group.

Discussion

In recent years, interest has grown in introducing a transition diet into sow feeding programmes, in line with precision-feeding principles, to better support the substantial physiological adaptations and the highly variable energy and nutrient requirements of the peri-partum period [1]. The underlying assumption is that targeted modulation of energy and AA supply may induce rapid changes in colostrum and transition milk composition, with potential benefits for neonatal immunocompetence and piglet survival [1].

Supporting this hypothesis, a recent study demonstrated that a transition diet specifically formulated to optimize the colostrogenic AA requirements of the sow and provided for only 4 d before farrowing was able to increase colostrum IgA concentrations and reduce pre-weaning mortality [41]. Although current evidence is still limited and the specific peri-partum nutrient requirements of the sow are not yet fully defined, the present study aimed to evaluate the effects of a nutrient-dense transition diet on sow performance and colostrogenesis. The transition diet formulated in the present study resulted in sows having a higher percentage of piglets born alive and fewer stillbirths at farrowing. These results are consistent with the existing bibliography comparing recent studies. Both the higher intake of Lys and ME may have contributed to improving the farrowing process by facilitating the expulsion of piglets. Indeed, as reported by Johannsen et al. [2], using a daily SID Lys dose of 22 g/d can improve the number of piglets born alive. It is also important to emphasise that the dose of SID Lys cannot be excessive and/or unbalanced in relation to other nutrients and energy; in fact, when the amount of SID Lys is excessive, the sow's farrowing performance deteriorates. The positive energy balance of the sow at farrowing combined with excess of protein could favour maternal protein deposition, favouring energy consumption for muscle deposition. Therefore, sows fed a protein surplus relative to their requirements would have less energy available for contractions at farrowing, which could be linked to an increase in the number of stillborn piglets [2]. It is therefore crucial to maintain a correct ratio of energy to Lys in the diet. This was achieved in the present study for both diets under investigation (SID Lys/ME=6.70 MJ). An increase in total energy and Lys intake could potentially reduce feed intake in sows, particularly in the days following farrowing when feed is offered ad libitum [42]. However, no reduction in feed intake was observed in the TRT group in this study. The lack of reduction in feed intake by sows in the TRT group could

be due to the correct maintenance of the energy-protein ratio and suggests the actual need of sows for an increase in energy and protein share. The lack of differences in BCS, lard and muscle thickness or their difference in the time interval analyzed suggests that the TRT diet did not influence the negative energy balance of the sows in the lactation period. This could suggest a preference on the part of the sows to direct energy and resources towards colostrum and milk production.

According to the literature, increasing the energy concentration and dietary density, particularly through the inclusion of oils and fats, can modify mammary gland functionality, colostrum and milk composition, immune-related components and, although less frequently reported, may also influence their overall yield and piglet performance, with the magnitude and direction of the effects depending on both the type and the inclusion level of the oil used [43, 44]. Most of the existing evidence, however, derives from studies in which oils were supplemented over longer feeding periods, typically spanning late gestation and lactation. In particular, soybean oil, which was incorporated into the TRT diet of the present study to increase energy density, has been reported to increase milk fat content, enhance the concentrations of essential fatty acids (e.g., linoleic acid, α -linolenic acid) and Igs levels compared with alternative fat sources such as coconut oil and palm oil [45].

In the present study, chemical composition, Igs, metabolites and miRNAs of colostrum were analyzed. The increase in fat concentration and SCC in colostrum of the TRT group may confirm the ability of dietary soybean oil to increase total fat in swine colostrum as reported by Bai et al. [45]. In addition, the present findings suggest that this effect was achieved even if the enriched diet is provided for a shorter period. The results also suggest a more concentrated composition, potentially reflecting a lower water content in TRT colostrum compared to CO colostrum. Furthermore, a previous study suggests that there is a positive correlation between fat and SCC and a negative correlation of these with lactose concentration in sow colostrum that needs further investigation [46].

Metabolomic analysis of the colostrum showed a clear separation of the samples as a result of the transition diet used. In particular, colostrum from the TRT group was characterised by a higher abundance of UDP-glucuronate, a nucleotide sugars that acts as glycosyl donors in glycosylation reactions (catalysed by glycosyl-transferase enzymes) [47]. The possible reason behind UDP-glucuronate increase is that a higher abundance of ME could rise its precursor abundance (UDP-glucose) by incrementing glucose and uridine availability [48]. UDP-glucuronate is a form of glucuronic acid that can be incorporated into proteoglycans or conjugated with

exogenous or endogenous compounds to facilitate their excretion [49]. Therefore, an increased availability of UDP-glucuronate could help piglets to dispose of potentially toxic compounds more effectively. In particular, it has been noted in other species (humans and cattle) that a brain disorder (Kernikterus) can occur in newborns due to an increase in unconjugated bilirubin in the blood as a result of catabolism of fetal haemoglobin and a transient deficiency in the liver's ability to conjugate UDP-glucuronyltransferase, which can be cured by the administration of orotate. The orotate mechanism of action involves precisely the increased formation of UDP-glucuronate that increases transferase saturation, resulting in more efficient bilirubin disposal [50]. With this in mind, it appears that the TRT diet may have benefited the piglets through increased UDP-glucuronate. The TRT group was also characterised by increased level of carnitine and a decrease level of citrate compared to the CO group. The higher carnitine concentration could be related to the increase of Lys, which is necessary to synthesize carnitine. Both carnitine and citrate are also involved in energy metabolism. Carnitine is essential to use fatty acids for the production of Acetyl-CoA, which is used as a substrate for the Krebs cycle (TCA) in which citrate is also involved. Furthermore, carnitine appears to be very abundant in colostrum (370 nmol/mL) and important for the health of piglets that are deficient in it at birth [51]. Citrate has an indirect role in the de novo promotion of fatty acids; particularly in the udder as de novo synthesis of fatty acids increases there is a reduction in citrate [52]. These results are in line with the higher abundance of fat in TRT colostrum, thus suggesting that the increase of energy and Lys in the diet altered sow energy metabolism, especially in colostrum production. However, the present study did not investigate the expression of enzyme involved these metabolic pathways. This poses a limitation and it should be addressed in future research.

The colostrum of TRT sows presents a higher abundance of C18:2 *9cis,12cis* (linoleic acid), C18:4 *6cis,9cis,12cis,15cis*, C20:0 and C20:2 *11cis,14cis* compared to the CO group. These fatty acids were more abundant in the TRT feed compared to the CO diet, which is possibly connected to their higher concentration in the TRT colostrum. In fact, to reach a higher ME content in the TRT diet, the feed was enriched with 4% of soybean oil (vs. 1.8% in the CO diet) which is rich in linoleic acid (54%) oleic acid (C18:1 *9cis*; 23%) and α -linolenic acid (C18:3 *9cis,12cis,15cis*, 7.2%) [53]. On the other hand, C20:4 *5cis,8cis,11cis,14cis*, C22:5 *10cis,13cis,16cis,19cis* and C22:5 *7cis,10cis,13cis,16cis* were absent from both transition diets but were later synthesised endogenously by the sow, and in a greater amount by the TRT group. Even if PUFA n-6, play many

essential roles in physiological processes, the quantity of PUFA n-6 and PUFA n-3 in the diet should be balanced. The optimal PUFA n-6/PUFA n-3 ratio for human is around 1–4:1 and a higher ratio was associated to several health disorders [54]. The optimal PUFA n-6/PUFA n-3 ratio for pigs is not yet defined, but Duan et al. [55] observed that a ratio of 5:1 (close to the ratio of TRT sows' colostrum) produced the best growth performance in growing-finishing pigs.

Preliminary evidence suggests that sows' diet can affect miRNA expression in colostrum and milk [56, 57]. However, to the authors' knowledge, this is the first study investigating the modulation of miRNAs in sow colostrum in response to a transition diet enriched in ME and SID Lys. There are strong empirical suggestions that milk miRNAs can be absorbed by piglets [58]. Chen et al. [59] observed that porcine milk-derived miRNAs were absorbed by a porcine intestinal cell line (IPEC-J2). Moreover, the results of Baier et al. [60] provide evidence that milk miRNAs can be absorbed by humans and modulate gene expression. This suggests that milk miRNAs could have a regulatory effect on piglet genes and that the diet could directly modulate one of the mechanisms involved in epigenetic. Among the identified miRNAs, ssc-miR-142-3p was the only one with a marginally significant higher expression in the TRT group compared to the CO group. Ssc-miR-142-3p presence in pigs was reported in myocardial [61] and ventricular tissue [62]; however, it was previously identified in colostrum and milk of several species, including cattle [63], mice [64] and humans [65]. An overexpression of miR-142-3p in human myeloid and granulocytic cells significantly attenuated the secretion of inflammatory mediators, including as well as the phagocytosis of pathogenic bacteria [66]. The reduction of the phagocytic capacity may have resulted in a compensatory immune response, which could potentially explain the increase in SCC observed in the TRT group. Other discriminant miRNAs identified in the colostrum of sows fed the TRT diet play a role in regulating the immune system. For example, the ssc-miR-7-3p is reported to stimulates the maturation of the immune system [67], ssc-miR-223 targets genes are involved in activating defence mechanisms of innate and adaptive immunity [68], and, in humans, the miR-142-5p is known to play a role in immune response to virus infection [69]. Ssc-miR-15a, ssc-miR-15b, and ssc-miR-106a may have a role in immune response, besides cell growth, migration, adhesion, differentiation, apoptosis [70, 71]. Other miRNAs detected, such as miR-582-3p and miR-582-5p may have antitumoral effect in humans [72], while miR-455-5p regulates the growth and development of adipose tissue [73]. Overall, these findings show that the dietary change altered the miRNA profile of sow colostrum,

meaning that the TRT diet influenced the metabolism of sows, including pathways that regulate the immune system. However, the systemic effects of these alterations on sows and piglets are difficult to predict.

The increase of fat content, specific fatty acids, metabolites as carnitine and UDP-glucuronate, and the modification of the miRNA profile in colostrum, could explain the positive effects observed in the growth performance of piglets from the TRT group. During the lactation period, the TRT transition diet was found to improve piglet body weight at d 6 and ADG in the d 0–6 period. A higher body weight was also observed in piglets in the TRT group at d 24 compared to the CO group. It is well known that ingesting good-quality colostrum (at least 200 mL within 24 h) improves thermoregulation and immunity in the short term, contributing to piglet survival [74], and improve growth in the long term [75]. In the present trial, colostrum from the TRT group may have contributed to the intestinal maturation of the piglets. Consequently, it could be hypothesis that piglets in the TRT group, having achieved a greater degree of intestinal maturation, may have been better able than those in the CO group to digest and absorb the creep feed provided from d 6 onwards. This, coupled with the provision of creep feed from d 6 onwards, improved their long-term growth. The LSI values indicate only slight aggressive behaviour in the piglets at ear and tail level, which is likely because no additional piglets were introduced to the pen, only removed from it. This may have reduced the manifestation of aggressive hierarchical behaviour within the litter.

Conclusion

In conclusion, the results of this study suggest that a transition diet consisting of 12.97 MJ/kg of ME and 0.85% SID Lys can improve birth performance of the sow (by reducing stillbirths), and lactation performance by modifying sow energy metabolism and colostrum quality. At weaning, piglets showed an increase in their weight, which could improve their response to a stressful event such as weaning and thus reduce post-weaning mortality. The increase in sow productive performance was also achieved without increasing feed ingestion. Overall, these findings demonstrate the importance of a properly designed transitional diet to meet the needs of the sow during a phase characterised by rapid physiological changes.

Abbreviations

AA	Amino acid
ADG	Average daily gain
BCS	Body condition score
IgG	Immunoglobulins
Lys	Lysine
LSI	Lesion score index

ME	Metabolizable energy
miRNA	MicroRNA
OPLS-DA	Orthogonal partial least squares-discriminant analysis
PLS-DA	Partial least squares discriminant analysis
PO	Parity order
PUFA	Polyunsaturated fatty acids
SCC	Somatic cells count
SE	Standard error
SFA	Saturated fatty acids
SID	Standardized ileal digestible
UFA	Unsaturated fatty acids
VIP	Variables importance projection
VS	Vitality score

Supplementary Information

The online version contains supplementary material available at <https://doi.org/10.1186/s40104-026-01355-5>.

Additional file 1: Table S1. Fatty acid (FA) profile of the two transition diets.

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Authors' contributions

Conceptualization, DL, PT; methodology, SB, AS, LL, FC; collection and preparation of samples, SB, AZ, FP; formal analysis, DL, SB, AS, LL, FC; investigation, DL, SB, FP; resources, DL, AS, LL, PT; data curation, AS, LL, FC; writing – original draft preparation, DL, SB; writing – review and editing, DL, SB, AZ, AS, LL, FC, FP, PT; supervision, DL, PT; funding acquisition, DL. All authors read and approved the final manuscript.

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Data availability

The raw sequence reads for miRNAs have been deposited on NCBI under The BioProject ID PRJNA1334581.

Declarations

Ethics approval and consent to participate

The in vivo trial was conducted in a commercial multiplication unit located in the so-called ‘Italian Food Valley’. The animals enrolled in the present study were sows and piglets raised under conventional farm-rearing conditions in Europe according to Dir. 120/2008 EC. The Italian Ministry of Health approved the experimental procedures of the trial with protocol No. 218342 of 30/07/2024.

Consent for publication

All authors have read and agreed to the published version of the manuscript.

Competing interests

The authors declare no competing interests.

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