NON RUMINANT NUTRITION

Phytogenic actives supplemented in hyperprolific sows: effects on maternal transfer of phytogenic compounds, colostrum and milk features, performance and antioxidant status of sows and their offspring, and piglet intestinal gene expression

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Abstract

Phytogenic actives (PA) are plant-derived natural bioactive compounds that may promote livestock health and well-being, as well as improve growth performance and production efficiency. The current study aims to evaluate their effects on sows and their offspring. Eighty-one hyperprolific sows (up to parity 7) were assigned to 3 experimental treatments. Control sows were offered a nonsupplemented diet during gestation and lactation, and treated sows were fed the control diet supplemented with 1 g/kg of a blend of PA (BPA) in lactation (L) or during gestation and lactation (GL). An evaluation was made of placental and milk maternal transfer of these BPA and colostrum–milk features, sows and piglets antioxidant status, reproductive performance (litter size), body weight (BW) changes, weaning-estrus interval, and litter performance. Finally, piglet's jejunum gene expression was measured. The BPA supplementation during gestation (GL) increased the number of piglets born alive ($P = 0.020$) and reduced ($P < 0.05$) the newborn piglets BW, while there were no differences among treatments on the suckling (day 20) and weaned (day 7) piglets BW ($P > 0.05$). Dietary phytogenic volatile compounds reached GL placental fluid, and milk of L and GL sows ($P < 0.05$). Moreover, colostrum protein in GL and milk fat content in L and GL were increased ($P < 0.05$). Milk of GL showed inhibitory activity against *Bacillus subtilis* and *Staphylococcus aureus* ($P < 0.05$). Antioxidant status of GL sows showed an enhanced ($P < 0.05$) of catalase (CAT) and total antioxidant capacity levels at early gestation (day 35), whereas higher levels of superoxide dismutase (SOD) and glutathione peroxidase (GSH-Px) enzymes at late gestation (day 110). Likewise, GL newborn piglets showed higher CAT levels, whereas both CAT and SOD

levels in suckling piglets, as well as CAT, SOD, and GSH-Px in weaned piglets, were increased in L and GL ($P < 0.05$). Jejunum messenger ribonucleic acid abundance of suckling piglets in L and GL groups showed overexpression of barrier function MUC2, digestive enzyme IDO, and immune response PPARGC- α , TNF- α , TGF- β 1, and IL-10 genes ($P < 0.05$). In conclusion, dietary BPA supplementation in hyperprolific sows increased the litter size (born alive) and improved the composition and bioactivity of colostrum and milk, besides, modified the antioxidant status of sows and their offspring, as well as the suckling piglets gut health gene expression. Several BPA volatile compounds were prenatal and postnatal maternally transferred (placental fluid and milk).

Key words: antioxidant status, colostrum–milk, hyperprolific sows, maternal transfer, phytogetic actives, piglet gene expression

Abbreviations

ANPEP	aminopeptidase-N
BFT	back-fat thickness
BPA	blend of phytogetic actives
CAT	catalase
CLDN4	claudin-4
FEDNA	Spanish Federation for the Development of Animal Nutrition
GSH-Px	glutathione peroxidase
IDO	indoleamine 2, 3- dioxygenase
IL-10	interleukin 10
MUC2	mucin 2
NO	nitric oxide
PA	phytogetic actives
PPARGC1- α	peroxisome proliferative activated receptor gamma coactivator 1 alpha
ROS	reactive oxygen species
SOD	superoxide dismutase
TAC	total antioxidant capacity
TBARS	thiobarbituric acid-reactive substances
TGF- β 1	transforming growth factor beta 1
TNF- α	tumor necrosis factor alpha
WOI	weaning-to-estrus interval

Introduction

Genetic selection for commercial hyperprolific sows during last decades has stimulated average litter size (total born) but at the expense of an increased number of stillborn piglets, a decreased mean piglet birth weight, reduced preweaning survival (Foxcroft et al., 2006), and increased within-litter weight variability at birth. Moreover, a high prevalence of low birth weight piglets may lead to long-term effects, with some animals showing poor growth performance and detrimental effects on carcass and meat quality at slaughter (López-Vergé et al., 2018). Major constraints on birth weight are due to a limited uterine capacity, which leads to the combined effects on uterine, placental, and embryo/fetal functions (Burton and Fowden, 2012). During their first days of life, piglets rely on colostrum and milk, which not only contain macronutrients but also various types of bioactive substances, including immune compounds, enzymes, hormones, and growth factors. However, colostrum and milk composition of sows have not significantly changed during the last 30 yr (Zhang et al., 2018). Additionally, litter size is positively related to the colostrum and milk yield, while the milk composition has been negatively correlated with milk yield (Vadmand et al., 2015). This may suggest that milk composition in high-yielding sows may be affected, restricting the nutrient intake and, consequently, the early development of piglets from large

litters. It is well accepted that high fetal development during late pregnancy, and the synthesis of colostrum and milk during lactation lead to the catabolic status of sows (Berchieri-Ronchi et al., 2011), the production of reactive oxygen species (ROS), and the induction of oxidative stress (Kim et al., 2013). Different studies have described that mothers may experience increased oxidative stress and inflammation during these periods, which probably determine reproductive disorders, such as embryonic reabsorption, or intrauterine growth retardation, and fetal death (Agarwal et al., 2005). Oxidative stress indicators and cytokines can also be transferred from mothers to the colostrum and milk, significantly affecting the oxidative stress and the health of their offspring (Wang et al., 2010).

Appropriate dosage of dietary antioxidant nutrients or feed additives may be considered during pregnancy and lactation. Phytogetic actives (PA) is a term used to describe plant-derived natural bioactive compounds that promote livestock health and well-being and improve growth and production efficiency. These actives represent a source of various phytochemical compound groups, such as terpenes, phenols, glycosides, saccharides, aldehydes, esters, and alcohols. Although the modes of action of many phytogetic compounds are not fully understood, their benefits to the overall health of animals have been noted. The literature describes some of their effects, such as stimulation of digestive secretions, immune stimulation and anti-inflammatory activities, intestinal microflora modulation and antioxidant effects (Durmic and Blache, 2012), as well as estrogenic and hyperprolactinaemic properties (Farmer, 2018), and effects on colostrum and milk porcine sensory profiles (Val-Laillet et al., 2018). They represent interesting antibiotic alternatives in swine production (Omonijo et al., 2018). There are also references that indicate that herbal extracts may improve intrauterine growth of fetuses in rats by an elevation of fetal blood glucose and growth hormone levels (Takei et al., 2007).

In the present study, we hypothesized that dietary PA supplementation of hyperprolific sows during the lactation, or the whole gestation and lactation period, may influence the reproductive performance and oxidative status of sows and offspring, including changes in bioactivity and composition of colostrum and milk. Therefore, the objectives of this study were to determine the effects of a blend of PA (BPA) supplementation to sow diets during gestation and lactation, or lactation only, on: 1) the reproductive performance and oxidative status of the sows; 2) the maternal transfer of BPA to placental fluid and milk and milk composition; and 3) the piglet performance and jejunal gene expression-related intestinal functions (digestive enzymes, intestinal integrity, and local immune response).

Materials and Methods

The protocol and all experimental procedures used were approved by the Animal Ethics Committee of the Autonomous University of Barcelona and were performed according to the directive of the European Parliament, 2010/63/EU, on the protection of animals used for scientific purposes.

Experimental design, animals, and housing

Eighty-one hyperprolific gilts and sows (up to parity 7) DanBred hybrid line (Landrace × Yorkshire) were randomly distributed by parity number and body weight (BW) into 3 dietary treatment groups ($n = 27$). After breeding, sows were fed unsupplemented control diets (C) during gestation and lactation or the control diets supplemented with 1 kg/MT of a BPA (Delacon Biotechnik GmbH, Steyregg, Austria) either during lactation (L) or during the whole gestation and lactation period (GL). In addition, piglets received experimental treatments in the creep feed and prestarter diets. At the start of the experiment, sows were assigned to individual cages (1.8×0.8 m) and kept in those from mating (0 d) until confirmed gestation (35 d). Thereafter, sows were allocated by parity and BW into group pens (4.5×5.0 m; 9 sows/pen) until day 110 of gestation. On day 110, sows were moved to the farrowing unit, where they were placed in individual farrowing pens (2.6×1.8 m). The number of sows allotted per treatment at farrowing and during lactation was $n = 19$ for C, $n = 19$ for L, and $n = 20$ for GL. Difference with the initial number of sows before breeding corresponds to sows that showed physiological issues (heat failure [gilts], repeated estrous, or abortion). Farrowing pens were mounted over a partially slatted floor with a heated floor pad for piglets and equipped with individual feeders and nipple drinkers for sows and piglets. The temperature in the farrowing room was automatically controlled. Parturitions were monitored as much as possible to interfere opportunely in the farrowing process. Within 24–48 h after birth, piglets were cross-fostered within the respective treatment group of sows (C, L, or GL) in order to standardize litter size to 15 piglets/litter but not among treatments. After weaning, piglets were moved to the nursery unit on the same farm in order to evaluate BW at weaning and growth performance during the prestarter phase (day 7) postweaning.

Experimental diets and feeding system

Control diets for each experimental period were formulated to meet or exceed nutrient requirements for DanBred sows (Tybirk et al., 2015), with adaptations based on Spanish recommendations for gestating and lactating sows (FEDNA, 2013; Table 1) and for prestarter piglets (same specification and formula than for creep feed; Table 2). Experimental diets were control diets plus 1 kg/MT BPA supplement containing a blend of eucalyptol, p-cymene, linalool, anethole, and thymol added as essential oils from the Fabaceae, Laminaceae, Schisandraceae, and Zingiberaceae plant families. Sows were fed 2.1 kg/d from weaning to service, a mean of 2.9 kg/d from service to 35 d of gestation, based on individual body condition, and 2.5 kg/d from 35 to 110 d of gestation of the gestation diet of their corresponding treatments (flat line). Each gestating pen was equipped with enough mechanical free access self-closing semi-cage without pneumatic actuators (Rotecna, Lleida, Spain) to keep animals individually monitored during feeding. From 110 d of gestation and during lactation, sows were fed ad libitum. Control creep feed diet was unsupplemented, whereas experimental diets were creep feed formula than for the control group but supplemented

Table 1. Ingredients and calculated nutrient compositions of gestation and lactation diets (as-fed basis)

Item	Gestation	Lactation
Ingredient composition, %		
Barley	35.00	10.00
Maize	22.70	27.01
Wheat middling's	15.00	7.00
Wheat	9.00	25.55
Sunflower meal	5.65	4.50
Sugar beet pulp	3.10	2.50
Soybean meal, 47 % CP	2.50	13.50
Rapeseed meal	2.50	4.50
Calcium carbonate	0.99	1.25
Lard	1.05	1.00
Dicalcium phosphate	0.99	1.25
Salt	0.40	0.50
L-lysine HCl	0.31	0.63
L-threonine	0.10	0.18
Mycofix plus 3.E	0.10	0.10
Vit-min premix ¹	0.50	0.50
Calculated nutrient composition		
Net energy, kcal/kg	2,261	2,455
CP, %	13.0	16.7
Calcium, %	0.85	0.91
Total phosphorus, %	0.56	0.57
Dig. phosphorus, %	0.35	0.37
SID lysine, %	0.60	1.00

¹Supplied the following per kg of diet: vitamin A (retinyl acetate), 10,000 IU; vitamin D3 (cholecalciferol), 2,000 IU; vitamin E (acetate de tot-rac-3-tocopheryl), 45 mg; vitamin K3 (menadione nicotinamide bisulphite), 3 mg; vitamin B1 (thiamine mononitrate), 3 mg; vitamin B2 (riboflavin), 9 mg; vitamin B6 (pyridoxine hydrochloride), 4.5 mg; vitamin B12 (cyanocobalamin), 0.04 mg; nicotinamide, 51 mg; pantothenic acid (calcium D-pantothenate), 16.5 mg; biotin (D-(+)-biotin), 0.15 mg; folic acid, 1.8 mg; choline chloride, 350 mg; iron (as iron sulphate monohydrate), 54 mg; zinc (as zinc oxide), 66 mg; manganese (as manganese oxide), 90 mg; iodine (as calcium iodine anhydrous), 1.2 mg; selenium (as sodium selenate), 0.18 mg; copper (as copper sulphate pentahydrate), 12 mg; ethoxyquin, 4 mg; D,L-malic acid, 60 mg; fumaric acid, 75 mg; sepiolite, 907 mg; vermiculite 2001 mg; colloidal silica 45 mg.

with 1 g/kg of BPA, which were offered to each respective litter as mash from day 7 of lactation to weaning. At weaning, all piglets from the same experimental treatment were allotted in a single large pen (all together, mixed litters within each treatment); this resulted in 3 large pens (1 per treatment) of 350–400 piglets each. Each pen was equipped with complete slatted floor, 3 ad libitum pan hoppers (Swing Feeder R3 Wet WTF, Rotecna, Spain) in the middle of the pen and free access to fresh water with nipple drinkers on the wall. The corresponding pelleted creep feed fed to the piglets during lactation for each treatment was used as prestarter diet until day 7 postweaning. Weaned piglets were individually weighted, and each piglet was considered as experimental unit for either BW, as well as blood and tissue sampling.

Data recording and sampling

Sow BW and back-fat thickness (BFT) were measured at day 0, day 110 of gestation, and at weaning. BFT was measured by digital B-ultrasound (model WED-3000V, Welld, Shenzhen, China) at P2 position (left side of the midline at last rib and 7.5 cm to the spine). Average daily feed intake (ADFI) was controlled per sow during gestation by weighing the feed

Table 2. Ingredients and nutrient compositions of control basal diet used from lactation day 7 to weaning (as creep feed in mash form) and as prestarter diet (pelleted form) from weaning to postweaning day 7 (as-fed basis)

Item	Creep feed/prestarter diet
Ingredient composition, %	
Maize + extruded barley	44.00
Sweet milk whey	10.26
Wheat	10.00
Barley	15.00
HP 300	5.00
Maize flour	1.01
Extruded soybean	5.13
Soybean meal, 47% CP	5.00
Plasma	1.50
L-lysine	0.81
Dicalcium phosphate	0.87
Methionine	0.23
Salt	0.25
Threonine solid	0.21
L-valine	0.09
L-tryptophan	0.03
Vit-min premix ¹	0.60
Calculated nutrient composition	
Net energy, kcal/kg	2,438
CP, %	17.0
Calcium, %	0.30
Total phosphorus, %	0.40
Dig. phosphorus, %	0.30
SID lysine, %	1.11

¹Supplied the following per kg of diet: vitamin A (retinyl acetate), 10,000 IU; vitamin D3 (cholecalciferol), 2,000 IU; vitamin E (all-rac α -tocopheryl-acetate) 100 ppm; choline chloride, 187 ppm; iron (as iron sulphate monohydrate), 100 ppm; iodine (potassium iodide), 100 ppm; copper (as copper sulphate pentahydrate), 149 ppm; manganese (as manganese oxide), 58 ppm; zinc (as zinc oxide), 120 ppm; selenium (as sodium selenate), 0.30 ppm; selenomethionine (produced by *Saccharomyces cerevisiae*), 0.1 ppm; butyl-hydroxytoluene (BHT), 63 ppm; citric acid, 8 ppm.

offered and checking for refusals (mechanical free access self-closing semi-cage; Rotecna, Spain). During lactation, sows were fed ad libitum by using a feeding ball system (ad libitum pan with ball mechanism for farrowing; Rotecna, Spain) and ADFI was recorded by weighing feed offered and refusals (feeding ball hopper was filled twice a day at 800 and 1600 hours to ensure that ad libitum feed availability and feed refusals were weighed the day after, before the morning hopper filling). Reproductive and production performance parameters included the litter size at farrowing, taking into account the total number of live, dead, or mummified piglets, as well as the individual piglet BW at birth, cross-fostering, day 20 of lactation, weaning, and day 7 postweaning. The number of days from weaning to estrus (weaning-to-estrus interval [WOI]) was also recorded.

Blood samples (6 mL per sow) were collected from sows by caudal venepuncture on day 0 (day of service), day 35, and day 110 of gestation ($n = 27$ per treatment) and day 2 (140 d) postweaning ($n = 12$ per treatment). Placental fluid samples (60 mL per sow) were collected at farrowing from the same subset of sows in order to determine maternal transfer of compounds ($n = 12$ per treatment). Colostrum samples (30 mL per sow) were collected within 12 h of farrowing ($n = 12$ per treatment) and milk samples (30 mL per sow) were collected at day 20 of lactation from all functional mammary glands, after injecting 2 mL oxytocin, both

for chemical composition and maternal transfer of compounds ($n = 12$ per treatment). Samples were not filtered and were immediately chilled on farm and stored at -20°C until analysis. From the same subset of sows, 8 piglets per treatment (1 piglet per litter with a medium BW) were euthanized at farrowing, at day 20 of lactation, and at day 7 postweaning in order to obtain both a blood sample (6 mL per treatment) and jejunum tissue ($n = 8$ per treatment) for gene expression analysis.

The newborn piglets selected to be euthanized at farrowing were removed immediately after birth without sucking colostrum. Piglet blood samples were collected by jugular vein puncture. Before being euthanized, piglets were anesthetized by intramuscular injection of 1 mL/22 kg BW of the final combination containing 100 mg telazol, 50 mg ketamine, and 50 mg xylazine in 1 mL. Piglets were subsequently euthanized with sodium pentobarbital 0.5 mL/kg of BW by jugular vein injection and a sample of jejunum tissue (1 cm²) was collected immediately and preserved into 1 mL of aqueous RNeasy lysis buffer (Applied Biosystems, Foster City, CA) in order to stabilize and protect RNA, with immediate RNase inactivation for messenger RNA (mRNA) analysis. RNA samples were stored for 24 h at room temperature (25°C) and, then, stored at -80°C until analysis. The blood samples were centrifuged at 3,500 rpm for 15 min to obtain plasma or serum and stored at -20°C until analysis.

Determination of maternal transfer of volatile compounds

The volatile profile of PA compounds maternally transferred through the placental fluid and milk were determined by solid-phase microextraction, gas chromatography-mass spectrometry (Servei d'Anàlisi Química, Autonomous University of Barcelona [UAB], Bellaterra, Spain) based on the volatile PAs profile characterized in the tested BPA. Results of the relative peak abundance were estimated based on the ratio (abundance/retention time) for each compound and expressed as proportional increased concentrations in relation to values in control sows.

Colostrum and milk chemical analyses

Colostrum and milk composition were determined by standardized methods as follows: crude protein (CP) by the Kjeldahl method AOAC 991.22; crude fat by the Röse-Gottlieb method AOAC 905.02; lactose by the Luff-Schoorl volumetry method B.O.E. num. 52 R.D. 2257/ 1994, and ash content was measured by difference method B.O.E. num. 52 R.D. 71/250/ CEE.

Evaluation of antimicrobial activity of milk

The antimicrobial growth inhibition of milk against *Bacillus subtilis*, *Escherichia coli*, *Staphylococcus aureus*, *Lactobacillus plantarum*, and *Candida albicans* was determined using a modification of the Kirby-Bauer test (Hudzicki, 2009). In our study, the antibiotic discs were replaced by sterile discs impregnated with each one of the milk samples under study. The inhibition values (IVs) were calculated using the equation ($\text{IV} = [\text{inhibition diameter of zone} - \text{diameter of sterile disc impregnated with milk}]/2$) and were expressed as millimeters of inhibition.

Measurement of oxidative status in sows and offspring

Oxidative status of sows and their offspring was determined by measuring the antioxidant enzyme activities in plasma or serum

of catalase (CAT), superoxide dismutase (SOD), and glutathione peroxidase (GSH-Px). Lipid peroxidation was measured using thiobarbituric acid-reactive substances (TBARS), nitric oxide (NO), and total antioxidant capacity (TAC) values in plasma or serum. Antioxidant enzyme activities were determined using standardized Cayman Chemical Kits (Cayman Chemical, Ann Arbor, USA) according to the manufacturer's protocol applied on a Tecan microplate reader (SunRise, Austria, Tecan, Männedorf, Switzerland). Catalase activity was assayed at 540 nm and results were expressed as nanomoles per minute per milliliter. SOD activity was measured using the cytochrome c and xanthine oxidase coupled assay at 440–460 nm and results expressed as units per milliliter. One unit was defined as the amount of enzyme needed to exhibit 50% dismutation of the superoxide radical. GSH-Px activity was measured every minute at 340 nm for 10 min, and the results were expressed as nanomoles per minute milliliter.

Plasma lipid peroxidation was analyzed based on the measurement of TBARS present in the sample as a byproduct of lipid peroxidation. In short, plasma or serum was mixed with deionized water, 0.5 N HCL, and thiobarbituric acid and incubated at 95 °C for 15 min. The measurement was made in fluorescence mode (maximum excitation 515 nm; emission range 548 nm) using a Tecan Infinite M200 microplate reader. The results were expressed as nanomoles of malondialdehyde per milliliter of plasma using 1,1,3,3-tetramethoxypropane as standard. NO levels were evaluated using the Nitrate/Nitrite Colorimetric Assay Kit (Cayman Chemical, MI) by following the manufacturer's instructions. Absorbance was read at 550 nm using a microplate reader (Tecan Infinite M200), with results measured using a NaNO₂ standard curve ranging from 0 to 100 µM. The TAC in plasma was assayed using a QuantiChrom kit (BioAssay Systems, Hayward, USA) as follows: 20 µL undiluted plasma or serum or Trolox standard solution along with 100 µM working reagent were added to a 96-well microplate, mixed by tapping, and incubated at room temperature for 10 min according to manufacturer's protocol. The absorbance of the reaction was read at 570 nm using a Tecan Infinite M200 microplate reader. Results were expressed as micromolar Trolox equivalents.

Piglet intestinal gene expression reverse transcription polymerase chain reaction

Total RNA was extracted from 50 mg of jejunum tissue using the Ambion RiboPure Kit (Life Technologies, Carlsbad, CA) by following the manufacturer's protocol. RNA concentration was measured using a NanoDrop ND-1000 spectrophotometer (NanoDrop products), and RNA quality was checked using Agilent Bioanalyzer-2100 equipment (Agilent Technologies). Around 1 µg of total RNA in a final volume of 20 µL was used for cDNA synthesis with random primers using the High-Capacity cDNA Reverse Transcription Kit (Applied Biosystems, Foster City, CA). The following temperature profile was applied: 25 °C 10 min; 37 °C 120 min; 85 °C 5 min; 4 °C hold. A 25-ng cDNA sample was preamplified, using a TaqMan PreAmp Master Mix (Life Technologies, Foster City, CA) and a Pooled Taqman Gene Expression custom assay following the manufacturer's protocol. A total of 56 genes were previously selected based on the bibliography, including 4 reference genes. Primers were designed by spanning exon–exon boundaries using PrimerExpress 2.0 software (Applied Biosystems), and genomic DNA amplification and primer dimer formation were controlled. One replicate per sample was run in a Taqman Open Array gene expression

custom plate format for gene expression with 56 assays of 48 samples per plate (OpenArray plate) in a QuantStudio 12K Flex Real-Time PCR System (Applied Biosystems, Foster City, CA). Data was collected and analyzed using the ThermoFisher Cloud software 1.0 (Applied Biosystems) applying the appropriate standard curve method for relative quantification.

Calculations and statistical analyses

Different procedures of the statistical package SAS 9.4 (SAS Inst. Inc., Cary, NC) were used to analyze all of the data. During whole gestation, the sows were individually controlled; therefore, the individual sows were considered as experimental unit at service and gestation period, while pen was included as a random effect. During lactation, the sow with her litter was considered as experimental unit. The performance and oxidative status of sows and piglets, colostrum–milk composition, milk maternal transfer, and gene expression were analyzed with ANOVA by using the GLIMMIX procedure, defining the model:

$$Y_{ij} = \mu + \text{treat}_i + \text{parity}_j + \varepsilon_{ij}$$

where Y_{ij} relates to each observation of the outcome variable, μ is the global mean, treat_i is the main effect of treatment, parity_j is the covariate effect for sow parity number and, finally, ε_{ij} is the experimental error term.

Therefore, all data were analyzed considering the treatment as main effect, and results are presented as least square (LS) means with their corresponding SEM. Regarding placental maternal transfer, data from 2 groups (C vs. GL) were analyzed by using the TTEST procedure. Data corresponding to milk bacteriostatic activity was analyzed using Fisher exact test. All data were checked for outliers before the statistical analysis with outliers defined by a deviation of ≥ 2.5 times the SD of the mean. Normality and equal variances were verified in all continuous variables using the Shapiro–Wilk test by using the UNIVARIATE procedure. Tukey adjust test was considered for all multiple comparisons between treatments. Finally, mean significant differences were declared at $P < 0.05$, while $0.05 < P < 0.10$ were considered near-significant trends.

Results

Reproductive sow and litter performances

Sow BW weight and BFT parameters during gestation and lactation periods (Table 3) were not affected ($P > 0.05$) by the dietary treatments. Likewise, no differences were observed ($P > 0.05$) between treatments either on ADFI or on WOI. Sow reproductive and litter performance during lactation and early postweaning are shown in Table 4. Litter birth weight and litter size were not affected ($P > 0.05$) by BPA supplementation during gestation. However, BPA supplementation (GL group) increased ($P = 0.020$) the number of piglets born alive but reduced ($P = 0.024$) piglet weight at birth. There was a mean of 2.02 piglets per litter below 1 kg birth weight in the GL group and 1.55 in the C and L groups (data not shown). Cross-fostering homogenized the mean number of piglets per litter between 1 and 2 d of life, with no significant differences being observed among treatments ($P > 0.05$) on piglets BW (at lactation day 20 and at 7 d postweaning), as well as the days of lactation length and the preweaning mortality rate.

Table 3. Effects of dietary BPA supplementation during lactation or gestation and lactation on performance of sows

Item	Treatment			SEM	P-value ⁵
	Control	L	GL		
No. of sows ¹	27	27	27	—	—
Parity, n	2.78	2.72	2.70	—	—
Sow BW, kg					
Breeding (day 0)	209.22	214.00	218.62	5.490	0.415
Gestation (day 110)	269.21	267.71	273.17	8.171	0.882
Farrowing standardized ²	246.76	244.94	251.24	7.610	0.826
Weaning	237.87	231.52	245.33	7.501	0.340
Loss lactation	-8.89	-13.42	-5.91	3.871	0.291
Sow BFT, mm					
Breeding (day 0)	14.04	13.89	15.00	0.905	0.341
Gestation (day 110)	13.83	14.17	12.68	1.079	0.584
Weaning	12.14	12.18	11.81	0.892	0.642
Loss lactation	-1.69	-1.99	-0.87	0.498	0.270
Feed intake, kg					
Lactation ADFI (day 20) ³	6.13	5.82	6.40	0.370	0.537
Total feed intake ⁴	428.39	425.92	434.64	5.535	0.491
WOI, d	3.43	4.44	3.84	0.313	0.176

¹Total number of sows allocated per treatment at the beginning of trial.²Sow BW at day 110 less litter birth weight.³Lactation ADFI at day 20.⁴Standardized total feed intake considered both the gestation and lactation at day 20 feed intake.⁵Sow parity was considered as covariable for the statistical analysis.**Table 4.** Effects of dietary BPA supplementation during lactation or gestation and lactation on reproductive sows and litter performances

Item	Treatment			SEM	P-value ⁴
	Control	L	GL		
No. of sows ¹	19	19	20	—	—
Parity, n	2.84	2.83	2.75	—	—
Sow reproductive performance					
Litter birth weight, kg	22.01	21.85	21.90	1.350	0.996
Total born piglets, n	17.01	17.67	19.13	0.941	0.238
Piglets born alive, n	14.47 ^b	15.28 ^b	17.53 ^a	0.811	0.020
Born alive piglet weight, kg	1.41 ^a	1.24 ^{ab}	1.16 ^b	0.070	0.024
Litter performance					
Litter size at cross-fostering (CF), n ²	14.39	14.90	14.93	0.173	0.057
Piglet CF weight, kg	1.48	1.44	1.33	0.072	0.299
Piglet lactation weight day 20, kg	4.94	5.04	4.70	0.247	0.585
Piglet postweaning weight day 7, kg	5.41	5.58	5.51	0.288	0.701
Piglet weight gain from CF to day 7 postweaning, kg	3.93	4.14	4.18	0.203	0.497
Lactation length, d	23.45	22.86	23.91	0.575	0.403
Prewaning mortality rate, ³ %	2.19	1.27	3.68	0.199	0.281

^{a,b}Means within a row with different superscripts indicate significant differences ($P < 0.05$).¹Data from number of sows allotted per treatment at farrowing and during lactation. Difference with the initial number of sows before breeding corresponds to sows that showed physiological issues (heat failure [gilts], repeated estrous, or abortion).²Litter sizes were adjusted by cross-fostering within treatment between days 1 and 2 after farrowing.³Piglets preweaning mortality rate was estimated from cross-fostering to weaning.⁴Sow parity was considered as covariable for the statistical analysis.

Placental and milk maternal transfer of phytogetic compounds

Thymol, anethole, linalool, and p-cymene were detected in placental fluid and milk, while eucalyptol was detected in placental fluid but not in milk. Dietary BPA supplementation during gestation (GL) increased ($P < 0.05$) the concentration of some tested volatile compounds in the placental fluid (Fig. 1), such as thymol, anethole, linalool, and eucalyptol. Supplementation of BPA during the lactation period increased ($P < 0.05$) the concentrations of thymol, anethole and p-cymene

in milk compared to C in both L and GL (Fig. 2). Concentrations of p-cymene in placental fluid and linalool in milk were not significantly changed ($P > 0.05$).

Colostrum and milk composition

The chemical composition of colostrum and milk is described in Table 5. Results showed that BPA supplementation during the gestation period (GL) increased the CP content in colostrum ($P \leq 0.001$). No significant changes ($P > 0.05$)

Figure 1. Proportional increase of PA volatile compounds in placental fluid by maternal transfer of PA dietary supplementation of sows during gestation against not supplemented. Control, not supplemented; GL, PA supplemented during gestation. Values are expressed as percentage of concentration (proportion = 1:100%) $\pm$ SEM, $n = 12$. ^{a,b}Means with different superscripts indicate significant differences ($P < 0.05$). ¹Percentage of concentrations (abundance/retention time) relative increase respect to control. Data were analyzed by t-test procedure.

were observed in colostrum lactose, fat, or ash content. Supplementation of BPA during gestation and lactation (GL) or lactation (L) increased ($P = 0.028$) the crude fat content in milk, but no significant changes ($P > 0.05$) were observed in CP, lactose, or ash content.

Milk bacterial inhibition activity

The antimicrobial activity of milk is shown in Table 6. Milk from the C and L groups did not show any antimicrobial effects against the studied microorganisms, while milk from the GL sows exhibited inhibitory activity against *B. subtilis* ($P = 0.015$) and *S. aureus* ($P = 0.001$) growth in 6 and 7 samples out of 7 analyzed samples, respectively.

Sows and piglets plasma antioxidant status

Oxidation and antioxidant status in the plasma or serum of sows (Fig. 3) showed changes during the gestation and lactation period and also between experimental treatments. GSH-Px increased in sows during both the gestation and lactation period, showing the highest values at the end of the lactation period. In contrast, TAC was clearly reduced during the first weeks of pregnancy and NO was temporarily increased at the end of pregnancy. Among treatments, dietary BPA supplementation during gestation increased ($P < 0.05$) CAT, NO, TBARS, and TAC on day 35, while SOD activity was lower ($P < 0.05$) than C group. At the end of the gestation period (day 110), BPA increased ($P < 0.05$) GSH-Px and SOD activity. During the lactation period, supplementation of BPA increased ($P < 0.05$) CAT activity at day 140 in L, while it also decreased the TAC in blood of L and GL groups.

As regards the antioxidant status of the offspring (Fig. 4), results show that plasma CAT activity and TBARS concentrations were higher ($P < 0.05$) in newborn piglets from GL than from L and C groups. At day 20 of lactation and after weaning, suckling piglets from GL showed higher ($P < 0.05$) CAT and SOD activities than C piglets. In contrast, piglets from L group showed higher ($P < 0.05$) TAC than the GL group at day 20 of lactation. In the

postweaning period, higher ($P < 0.05$) GSH activity was observed in the BPA groups (L and GL) compared to the C group.

Piglets intestinal gene expression

Jejunum gene expression of newborn and postweaned piglets was not significantly affected ($P > 0.05$) by the dietary treatments (data not shown). Jejunum gene expression of suckling piglets at day 20 is described in Fig. 5. As compared to the C group, BPA supplementation decreased ($P < 0.05$) barrier function claudin-4 (CLDN4) and digestive enzyme aminopeptidase-N (ANPEP) genes (L and GL) and increased immune response tumor necrosis factor alpha (TNF α) and transforming growth factor beta 1 (TGF- β 1; L), digestive enzyme indoleamine 2, 3-dioxygenase (IDO; in GL group), and cytokine interleukin 10 (IL-10) genes (L and GL).

Discussion

Effects on sow metabolism and performance

In hyperprolific sows, the high metabolic energy demand during pregnancy and lactation may increase the oxygen requirement and the production of ROS and, consequently, the DNA damage (Berchieri-Ronchi et al., 2011). Oxidative stress refers to the imbalance due to excess ROS or oxidants over the capacity of the cell to mount an effective antioxidant response. TBARS are formed by degradation of the initial products of free radical attacks, and these can be measured as indicators of the damage produced by oxidative stress (Pryor, 1991). In response to oxidants, the organism can counteract their negative impact using a series of antioxidant enzymes, such as those mentioned in the present study, SOD, CAT, and GSH-Px. In contrast to antioxidant enzymes, the TAC predominantly measures the low molecular weight chain-breaking antioxidants, excluding the contribution of the antioxidant enzymes mentioned above, and metal-binding proteins. Consequently, TAC is decreased under oxidative stress (Woodford and Whitehead, 1998).

Figure 2. Proportional increase of PA volatile compounds in milk at 20 d by maternal transfer of PA dietary supplementation of sows during gestation and/or lactation against not supplemented. Control, not supplemented; L, PA supplemented during lactation; GL, PA supplemented during gestation and lactation. Values are expressed as percentage of concentration (proportion = 1:100%) $\pm$ SEM, $n = 12$. ^{a,b}Means with different superscripts indicate significant differences ($P < 0.05$). ¹Percentage of concentrations (abundance/retention time) relative increase respect to control.

Table 5. Effects of dietary BPA supplementation during lactation or gestation and lactation on colostrum and milk composition of sows

	Treatment				
Item	Control	L	GL	SEM	P-value ¹
Analyzed nutrient content, %					
Colostrum					
Protein	15.71 ^b	15.74 ^b	20.80 ^a	0.397	<0.001
Fat	5.57	5.55	6.10	0.200	0.098
Lactose	3.05	3.13	2.48	0.070	0.061
Ash	0.67	0.67	0.71	0.013	0.134
Milk					
Protein	6.85	6.61	6.16	0.240	0.152
Fat	7.21 ^b	8.94 ^a	9.12 ^a	0.414	0.028
Lactose	4.89	4.57	4.75	0.051	0.085
Ash	0.79	0.79	0.78	0.019	0.773

^{a,b}Means within a row with different superscripts indicate significant differences ($P < 0.05$).

¹Sow parity was considered as covariable for the statistical analysis.

Based on our results, the oxidant status in sows were associated with a stepwise increase in the enzymatic antioxidant activities during gestation, with the highest values found at day 35 for SOD and GSH-Px activity at final gestation and lactation, as well a rapid decrease in TAC during the first third of gestation, which confirmed the induced oxidative stress during gestation. Similarly, Meng et al. (2018) described increase in oxidative stress markers during gestation. Among antioxidant enzymes, SOD destroys the free radical superoxide by converting it into peroxide, which, in turn, can be destroyed by CAT or GSH-Px reactions (Matés, 2000). Therefore, increases in SOD enzyme activity corresponds to enhanced resistance to oxidative stress. In the present study, the C and L groups showed higher SOD levels in early gestation (day 35), with simultaneously lowering values of TBARS, and a similar tendency was shown in GL sows at late gestation (day 110).

The catalase is one of the most efficient antioxidant enzymes known; it cannot be saturated by H_2O_2 at any concentration, protecting the cells from endogenous hydrogen peroxide by breaking down H_2O_2 to O_2 and 2 molecules of water (Aruoma et al., 2006). Thereby, it plays an important role in the acquisition of tolerance to oxidative stress in the adaptive response of cells (Hunt et al., 1998). According to our results, short-term responses on CAT were observed with BPA dietary treatments on sows (day 35) and on piglets. Similar to SOD and CAT, GSH-Px is located in the mitochondria and the cytosol, where it serves as an important cellular protectant against low levels of oxidant stress, whereas CAT becomes more significant in protecting against severe oxidant stress (Yan and Harding, 1997). Despite that GSH-Px shares the substrate H_2O_2 with CAT, it alone can react effectively with lipid and other organic hydroperoxides. In this study, BPA supplementation contributed to increase

the GSH-Px levels in gestating sows (day 110) and postweaned piglets (day 7).

Oxidative stress may exert major effects on embryonic development. During early gestation, the embryo is more susceptible to oxidative stress and antioxidant defenses are important in modulating oxidative stress-mediated events (Dennerly, 2007). In the present study, supplementation of BPA during gestation (GL group) increased CAT activity in early gestation (day 35), as well as NO levels, associated with a greater litter size and piglets survival at farrowing. It is known that NO can exert a role in the endothelial cells, resulting in increased blood flow and vasodilation (Kim et al., 2013). Besides, it is known that high ovulation rates (>30) in commercial hyperprolific sows, as well as deficits in fetal or placental NO production, are associated with the number of surviving embryos, resulting in likely uterine crowding in the early postimplantation period (Foxcroft et al., 2006; Takei et al., 2007). Nevertheless, volatile

phytogenic compounds were also detected in amniotic fluid of sows fed BPA supplemented diets, which likely contributed to placental angiogenesis process. Therefore, changes in placental functionality, such as vascularization and nutrient transport due to BPA, as a major mediator and determinant of fetal growth and viability deserves to be studied.

Recently, Su et al. (2017) described that placental antioxidant system of sows may have an adaptive response to oxidative stress, which is normalized by antioxidant supplementation. They also reported that feeding oxidized corn oil to sows markedly decreased the contents of protein and fat in colostrum and milk during 21 d of lactation. Therefore, sow colostrum-milk composition may depend on the oxidation status of the animal; due to higher dietary fat sources, stability may promote higher availability of those energy sources for final milk yield and quality. We described a possible galactagogue (lactogenic activity) effect of BPA, with significant increases in protein and fat content in sow colostrum and milk. There are limited reports about changes promoted by phytochemical compounds on milk composition in sows and none for colostrum. For example, reductions in the fat percentage in milk on day 7 (6.6% vs. 8.3%, $P < 0.05$) and day 14 ($P = 0.07$) were observed in sows supplemented with oregano essential oils during lactation compared with those fed a plain diet (Ariza-Nieto et al., 2011).

It was reported in humans that a mixture of ginger, a spice that is believed to increase blood circulation, and fenugreek, a spice known to enhance prolactin levels by stimulating the anterior pituitary gland, may improve milk yield by around 49% (Bumrungpert et al., 2018). Farmer et al. (2014) have reported that silymarin increased prolactin levels in sows. Not many reports are available on sows that describe changes in mammary secretion (colostrum-milk) composition associated with dietary changes or BPA supplementation. In our study, we observed that PAs, such as thymol, anethole, and p-cymene, but not linalool and eucalyptol, were significantly transferred to supplemented

Table 6. Bactericide capacity in sow milk by dietary BPA supplementation during lactation or gestation and lactation of sows

Item	Control n = 7 ¹	L n = 8	GL n = 7	P-value ³
Bactericide capacity against				
<i>Bacillus subtilis</i> , n ²	0 ^b	0 ^b	6 ^a	0.015
<i>Escherichia coli</i> , n	0	0	0	—
<i>Staphylococcus aureus</i> , n	0 ^b	0 ^b	7 ^a	0.001
<i>Lactobacillus plantarum</i> , n	0	0	0	—
<i>Candida albicans</i> , n	0	0	0	—

^{a,b}Values within a row with different superscripts indicate significant differences ($P < 0.05$).

¹Total number of milk samples analyzed per treatment.

²Number of milk samples with bactericide capacity.

³Data were analyzed using Fisher test.

Figure 3. Antioxidant status in plasma or serum of supplemented sows with BPA during gestation and/or lactation against not supplemented. Control, not supplemented; L, PA supplemented during lactation; GL, PA supplemented during gestation and lactation. G 0, gestation day 0 ($n = 27$); G 35 = gestation day 35 ($n = 22$); G 110, gestation day 110 ($n = 20$); L 140, postlactation day 2 ($n = 19$). Values are expressed as LS means $\pm$ SEM. ^{a,b}Means with different superscripts indicate significant differences ($P < 0.05$).

Figure 4. Antioxidant status in plasma or serum of piglets from supplemented sows with BPA during gestation and/or lactation against not supplemented. Control, not supplemented; L, PA supplemented during lactation; GL, PA supplemented during gestation and lactation. Birth, newborn piglets without suckling; L 20, lactation day 20; Post-w 7, postweaning day 7. Values are expressed as LS means $\pm$ SEM, $n = 8$. ^{a,b}Means with different superscripts indicate significant differences ($P < 0.05$).

Figure 5. Effects of dietary BPA supplementation during gestation and/or lactation of sows on mRNA relative expression of jejunum genes in suckling piglets at 20 d. Control, not supplemented; L, PA supplemented during lactation; GL, PA supplemented during gestation and lactation. (a) Barrier function genes: CLDN4, claudin-4; MUC2, mucin 2. (b) Digestive enzyme genes: ANPEP, aminopeptidase-N; IDO, indoleamine 2, 3-dioxygenase. (c) Immune response genes: PPARGC1-α, peroxisome proliferative activated receptor gamma coactivator 1 alpha; TNF-α, tumor necrosis factor alpha; TGF-β1, transforming growth factor beta 1; IL-10, interleukin 10. All values are expressed as LS means $\pm$ SEM, $n = 8$. ^{a,b}Means with different superscripts indicate significant differences ($P < 0.05$).

sows milk, which may be related to the enhanced composition of colostrum (protein) and milk (fat). Val-Laillet et al. (2018) also referred the transfer of limonene, carvone, and anethole into sow colostrum and milk. Flavor compounds appear to reach the milk differentially from the mother's diet (Hausner et al., 2008), with lipophilic compounds ingested by the mother showing a higher probability of being detected in the milk as compared to hydrophilic compounds.

Our results also showed an inhibitory effect of milk from GL sows against *B. subtilis* and *S. aureus*, which reflect the presence of PA compounds in milk. For example, p-cymene is a naturally occurring aromatic organic compound, which is a constituent of several essential oils, most commonly the oil of cumin and thyme. Different studies have reported an antimicrobial effect of thyme essential oil against foodborne pathogens and spoilage microorganisms, such as *E. coli*, *S. aureus*, *Listeria monocytogenes*,

and *Salmonella typhimurium* (Kang et al., 2018). Thyme essential oil had a marked effect on whole-cell proteins of *Bacillus cereus* by either inhibiting their synthesis or destroying them after synthesis, with evident consequences on the bacterial cell lives. Anethole also has potent antimicrobial properties against bacteria, yeasts, and fungi (De et al., 2002). Likewise, our results indicated that supplementation of BPA was able to influence the total enzymatic activity in milk of sows (data not shown). When a pooled milk sample per treatment was analyzed, there was an increase in enzyme activities, such as alkaline phosphatase, acid phosphatases, and β -glucuronides, among others, in GL (235 nmol) and L (175 nmol) compared to C (20 nmol) sows. In this sense, some reports suggested that adenosine triphosphate dephosphorylation in alkaline phosphatase may reduce intestinal inflammation, regulate calcium absorption, and modulate intestinal bacterial growth (Hashem et al., 2016). However, the likely contribution of essential oils transferred into sow colostrum or milk to control gut microbiota has hardly been explored.

Effects on piglet's metabolism and performance

The relationship between the sow and the fetus during pregnancy is exhibited through the placental function, which could be perceived as an important factor modulating the programming of the progeny. During the lactation period, sows exert their influence on piglets via colostrum and milk. Therefore, early stages in the piglets life (including maternal environment) may play a key role in setting the offspring's short- and long-term metabolism and health status (Chavatte-Palmer et al., 2016). Our results showed that TBARS values and CAT activity in newborn piglets were higher in GL compared to C and L sows, with similar changes to those observed in gestating sows during the early period of BPA supplementation (day 35). The results also demonstrated that L and GL piglets also increased CAT, SOD, and GSH-Px at day 20 of lactation and after weaning (7 d postweaning). Moreover, Hu et al. (2015) described that dietary supplementation with glycitein (soy isoflavone) in sows during late pregnancy and lactation increased the antioxidant factors (CAT, SOD, GSH-Px, and TAC). In addition, these isoflavones decreased the malondialdehyde content in sow's plasma and milk and improved the milk protein and fat contents, resulting in enhanced growth performance of the suckling piglets. Meng et al. (2018) reported that improving the dietary antioxidant intake of sows might prevent or alleviate oxidative stress by increasing the antioxidant status, with beneficial implications for piglets' weight at weaning.

It is interesting to highlight that suckling piglets were also offered creep feed from lactation day 7, including BPA for the GL and L groups. Although it is possible that a confounding effect could be stated with the effect of dietary feed provided to the sows, creep feed intake was very low (<300g/litter during the whole lactation). However, this is not the case for newborn piglets to whom the prenatal maternal effect is clear. Suckling piglets from the BPA treatments showed decreases in genes related to the barrier function group, such as CLDN4, and digestive enzymes, such as the ANPEP involved in the digestion of protein. They also showed higher gene expression for proinflammatory cytokines, such as TNF- α , and their counterpart anti-inflammatory responses, with significant increases in the gene expression for IDO, the peroxisome proliferative activated receptor gamma, coactivator 1 alpha (PPARGC1- α), TGF- β 1, and IL-10. In porcine small intestine, PPARGC1- α and related genes, such as PPARGC1- α , play a critical role in glucose homeostasis and adipocyte differentiation and

modulate inflammation processes by providing protection against proinflammatory signaling pathways NF- κ B (Mach et al., 2014). In addition, anti-inflammatory cytokines, such as TGF- β and IL-10, regulate the intestinal barrier by attenuating defects in tight junction permeability in intestinal morphology of early weaned piglets (Hu et al., 2013). Recently, Meng et al. (2018) also showed that resveratrol, a plant phenol supplemented during pregnancy and lactation, improves the antioxidant status of both sows and piglets and regulates placental antioxidant gene expression by the Keap1-Nrf2 and Sirt1 pathway in placenta. Graugnard et al. (2015) also described that maternal supplementation of a yeast mannan-rich fraction during pregnancy and lactation increased protein and Immunoglobulin G content in milk (at day 20 of lactation), with alterations in the intestinal gene expression in the progeny.

Some studies have described the presence of exosomes in porcine milk, a heterogeneous group of cell-derived membranous structures that are present in biological fluids (Zhang et al., 2018) and contain mRNA, microRNA, DNA, proteins, and lipids. They are transferred from maternal milk to neonates via the digestive tract, participating in the regulation of neonatal immune system (Gu et al., 2012), stimulating gastric and pancreatic digestion, as well as regulating intestinal cell proliferation and digestive tract development (Chen et al., 2017). Present results suggest an early activation of the immune mechanism in piglets, probably due to the transfer of different compounds into colostrum and milk. However, there is hardly any evidence regarding the effects of BPA supplementation of sow diets on the offspring, and further studies are required in order to know and understand their likely long-term effects on piglet performance and resilience against challenging diseases.

In conclusion, the results confirmed the prenatal and postnatal maternal transfer of dietary BPA supplemented to sows, with major effects on sow reproductive performance (litter size born alive), colostrum and milk composition, as well as bacteriostatic effects in milk, and interesting results on piglet oxidative status and gut health gene expression. The relevance of these results and likely changes in the early gut microbiota colonization in piglets and the growth performance of the animals after weaning should be also explored.

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Conflict of interest statement.

None declared.

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