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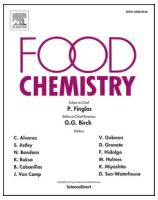

Nutrition during pregnancy and lactation: New evidence for the vertical transmission of extra virgin olive oil phenolic compounds in rats

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ABSTRACT

Maternal breast milk provides the newborn with passive immunity and stimulates the maturation of the infant immune system. The aim of the present study was to evaluate the vertical transmission of phenolic compounds and their metabolites to offspring in rats fed with extra virgin olive oil (EVOO) during pregnancy and lactation. For this purpose, plasma and lactic serum from dams and plasma from offspring were analyzed by LC-ESI-LTQ-Orbitrap-MS. Both enzymatic and microbial metabolites of hydroxytyrosol and tyrosol were detected in dam plasma and lactic serum. In addition, significant levels of phenolic compounds and their metabolites were found in offspring plasma. The concentration and number of hydroxytyrosol derivatives was higher than those of tyrosol and the microbial metabolites were found in the highest concentration. The observed vertical transmission of EVOO phenolic compounds, whose health benefits are widely reported, provides further support for the importance of the maternal diet during pregnancy and lactation.

1. Introduction

Maternal breast milk provides the newborn with passive immunity and stimulates the maturation of the infant immune system. The composition of breast milk is dynamic and can even change during a single feed as well as throughout the lactation period. While 87% of breast milk is water, the remaining 13% contains nutritional and bioactive compounds that benefit infant health, including immune factors, hormones and enzymes (Martin et al., 2016). Breast milk is also an important source of microorganisms that reach the milk through the mammary tract (Martín et al., 2003). Thus, by breastfeeding, the mother provides an initial inoculum for the baby's digestive system and promotes the establishment of a beneficial microbiota. This colonization of the gut has a great influence on health, not only during the early stages of life but also in adulthood, preventing the development of conditions such as allergies, autoimmune diseases, and obesity (Walker, 2017).

Several factors can affect the composition of breast milk, including the maternal diet (Dror & Allen, 2018). Nutritional interventions during pregnancy and lactation can therefore have an impact on the quality of breast milk, and consequently the health of the offspring. Previous studies in animals have observed that probiotic supplements modify the lipid and immunoglobulin profile of breast milk (Azagra-Boronat et al., 2020b), and these changes correlate with the plasma antibodies of the offspring (Azagra-Boronat et al., 2020a). The Mediterranean diet, characterized by high amounts of vegetable, fruits and food rich in unsaturated fats such as those found in extra virgin olive oil (EVOO), also influences the lipid composition of breast milk (Kumar et al., 2016).

EVOO is highly valued for its unique organoleptic attributes and health benefits (Polari et al., 2018). A Mediterranean diet rich in EVOO is reported to help prevent type-2 diabetes, cancer, and neurodegenerative and cardiovascular diseases (Medina-Remón, Estruch, Tresserra-Rimbaua, Vallverdú-Queralt, & Lamuela-Raventosa, 2013; Tresserra-

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Rimbau et al., 2014). The nutritional and health-promoting properties of EVOO are mainly attributed to a high content of monounsaturated fatty acids (oleic acid, C18: 1, between 55 and 83%), and minor components, especially phenolic compounds, such as phenolic alcohols and secoiridoid derivatives (Leporini et al., 2018; Soler et al., 2010). To date, only one study has demonstrated a positive impact of olive oil consumption during pregnancy on offspring health, reporting a protective effect against wheezing during the first year of life (Castro-Rodriguez et al., 2010).

Emerging data suggest that EVOO and its bioactive compounds may positively affect the intestinal microbiota, which is necessary for the development and maintenance of beneficial intestinal and immune functions (Rosignoli et al., 2013) and can prevent certain diseases in the short or long term (van't Land et al., 2010). However, it is unknown if the ingestion of these compounds during pregnancy and lactation can influence the maternal microbiota, reach breast milk, or modify the intestinal microbiota of the infant. Therefore, the aim of the present study was to evaluate vertical transmission of phenolic compounds and their metabolites in EVOO-fed rats to their offspring during pregnancy and lactation. For this purpose, plasma and lactic serum from dams that followed a diet rich in EVOO and plasma from pups were analyzed by LC-ESI-LTQ-Orbitrap-MS.

2. Materials and methodology

2.1. Chemicals and reagents

Oleuropein, luteolin, *m*-coumaric acid, pinoresinol, lariciresinol, isolariciresinol, secoisolariciresinol, homovanillic acid, tyrosol, 3,4-dihydroxyphenylacetic acid, 3-hydroxyphenylacetic acid and 3-(4-hydroxyphenyl)propionic acid were obtained from Sigma-Aldrich (Madrid, Spain). Oleocanthal was purchased from Merck (Darmstadt, Germany), oleacein and oleuropein aglycone from Toronto Research Chemicals (North York, ON, Canada). *p*-Coumaric acid, vanillic acid, ferulic acid, apigenin and 3-hydroxybenzoic acid were obtained from Fluka (Buchs, Switzerland), hydroxytyrosol (OH-TY) from Extrasynthese (Genay, France), and verbascoside from HWI ANALYTIK GmbH (Rülzheim, Germany). Ultrapure water was obtained using a Milli-Q purification system (Millipore, Bedford, MA, USA). The reagents methanol, acetonitrile and formic acid were acquired from AppliChem, Panreac Quimica SLU (Barcelona, Spain).

2.2. EVOo

EVOO of the Picual cultivar was purchased from a supermarket in 2020. The phenolic profile of the EVOO and refined olive oil (ROO) was analyzed by liquid chromatography coupled with high-resolution tandem mass spectrometry in negative ion mode, using a mass spectrometer with an electrospray source coupled to a hybrid high resolution mass analyzer (LC-ESI/LTQ-Orbitrap-MS). The liquid–liquid extraction of polyphenols was performed following previously described procedures (Lozano-Castellón et al., 2021). The chromatographic and mass spectrometer conditions were the same as those used for the identification of metabolites (**Section 2.6**).

2.3. Animals

All experimental procedures were conducted in accordance with the institutional guidelines for the care and use of laboratory animals and were approved by the Ethical Committee for Animal Experimentation of the University of Barcelona and the Catalan Government (CEEA-UB Ref. 240/19 and DAAM10933, respectively), which are in full compliance with national legislation following the EU-Directive 2010/63/EU for the protection of animals used for scientific purposes.

Seven-week-old Lewis adult rats (20 females and 5 males, HanHsd) were obtained from Envigo (Sant Feliu de Codines, Spain) and individually housed in cages with bedding consisting of large fibrous particles. After a seven-day acclimatization period, animals were divided and crossed by placing two randomly selected females inside the cage of each male for 48 h. Afterwards, the females were once again individually housed, monitored daily, and allowed to deliver naturally. The dams were given a standard diet corresponding to the American Institute of Nutrition 93G formulation (Reeves et al., 1993) (Teklad Global Diet 2014, Envigo, Indianapolis, IN, USA) and water *ad libitum*. The day of birth was established as day 1 of life. On day 2, litters were culled to six pups per lactating dam, with free access to maternal milk and rat diet. The pups were separated from their mothers each day to be identified and weighed, and the intervention was orally administered to the dams. To avoid the influence of biological rhythms, handling was done in the same time range. Animals were housed under controlled conditions of temperature and humidity in a 12 h light–12 h dark cycle in the Faculty of Pharmacy and Food Sciences animal facility (University of Barcelona, Spain).

2.4. Experimental design

The rats were randomly divided into three dietary groups, control (REF), ROO, and EVOO, with an initial number of eight rats per group, resulting in a final number of pregnancies of 4, 4 and 7, respectively. During lactation, the dams were individually housed with their respective six pups (n = 20 pups per group). Dams were supplemented daily with water (REF), ROO or EVOO by oral gavage at a dose of 10 mL/kg body weight for six weeks, corresponding to three weeks of gestation and three weeks of lactation. At the end of this period, the dams were intramuscularly anesthetized with 10 mg/100 g ketamine (Merial Laboratories S.A., Lyon, France) and after 30 min, 2 UI of oxytocin (Syntocinón 10 U.I./mL, Alfasigma, Bologna, Italia) was intraperitoneally injected. Milking was carried out as in previous work by our group (Azagra-Boronat et al., 2020a). Briefly, it was initiated after 5 min of oxytocin administration by gentle manual stimulation from the base to the top of the teat. The milk was collected in a sterile pipette tip. Afterward, dams and pups were anesthetized with ketamine (90 mg/kg) and xylazine (10 mg/kg) (Bayer A.G., Leverkusen, Germany) and euthanized before blood was drawn by cardiac puncture. The blood was collected in EDTA tubes and centrifuged to obtain plasma. The milk was centrifuged (800 *g*, 10 min) to obtain lactic serum. All samples were stored at −80 °C until further analysis.

2.5. Sample pre-treatment for phenolic metabolite analysis

For the extraction, all samples were slowly thawed on ice. To avoid phenolic compound oxidation, the samples were extracted and analyzed in a room with filtered light and kept on ice at all times. The clean-up of the biological matrix (protein precipitation) was performed using a method previously developed by our group (López-Yerena, Vallverdú-Queralt, Lamuela-Raventós, & Escribano-Ferrer, 2021; Orrego-Lagarón, Martínez-Huélamo, Vallverdú-Queralt, Lamuela-Raventos, & Escribano-Ferrer, 2015).

Plasma samples were centrifuged for 10 min (3000×*g*, 4 °C). Briefly, 100 μL of samples were deproteinized with ice-cold ACN acidified with formic acid (2%) in a 1:5 (v/v) ratio. After homogenizing the samples for 1 min, they were kept for 20 min at −20 °C to complete the protein precipitation. After centrifugation for 10 min at 4 °C at 11,000×*g*, 100 μL of each organic phase was transferred to vials and analyzed by LC-MS/MS as follows.

Lactic serum samples were centrifuged for 10 min (11,000×*g*, 4 °C). Briefly, 100 μL sample volumes were deproteinized with ice-cold ACN acidified with formic acid (2%) in a 1:3 (v/v) ratio. The samples were then homogenized for 1 min and kept at −20 °C for 20 min. After that, the samples were centrifuged (11,000×*g*, 4 °C, 10 min) and finally, 100 μL of the supernatant was transferred to the chromatography vials for analysis by LC-MS.

2.6. Instruments and analytical conditions

Metabolite profiling was performed by liquid chromatography analysis coupled to high resolution mass spectrometry (Escobar-Avello et al., 2019). An Accela chromatograph (Thermo Scientific, Hemel Hempstead, UK) equipped with a photodiode array detector, a quaternary pump, and a thermostated autosampler was employed. Chromatographic separation was carried out on an Atlantis T3 Column 2.1 × 100 mm, 3 μm (Waters, Milford, MA, USA), maintained at 50 °C. Gradient elution was performed with water/0.05% formic acid (solvent A) and methanol/0.05% formic acid (solvent B) at a constant flow rate of 400 μL/min, and the injection volume was 5 μL. The gradient employed was 0 min, 0% B; 0–2 min, 0% B; 2–10 min, 100% B; 10–11 min, 100% B; 11–11.1 min, 0% B and 11.1–15 min, 0% B.

For accurate mass measurements, the liquid chromatography system was coupled to an LTQ–Orbitrap Velos mass spectrometer (Thermo Scientific, Hemel Hempstead, UK), equipped with an electrospray ionization (ESI) source in negative mode. The main instrumental conditions were set as follows: source voltage, 4 kV; capillary temperature, 275 °C (FT Automatic gain control (AGC) target $5{\cdot}10^5$ for MS mode and $5{\cdot}10^4$ for MSn mode); sheath gas, 20; auxiliary gas, 10; and sweep gas, 2. The plasma and lactic serum samples were analyzed in Fourier transform mass spectrometry (FTMS) mode at a resolving power of 30,000 at *m/z* 600, and data-dependent MS/MS events collected at a resolving power of 15.000 at *m/z* 600. The most intense ions detected in the FTMS spectrum were selected for the data-dependent scan. Parent ions were fragmented by high-energy collisional dissociation with normalized collision energy of 35% and an activation time of 10 min. The mass range in FTMS mode was from *m/z* 100 to 1500. The data analyses and instrument control were performed with Xcalibur 3.0 software (Thermo Fisher Scientific).

The identification of OH-TY, homovanillic acid, tyrosol, dihydroxyphenylacetic acid (diHPAAc), hydroxyphenylacetic acid (HPAAc), hydroxyphenylpropionic acid (HPPAc) and hydroxybenzoic acid was achieved using a commercial standard. The identification of the rest of metabolites was based on chromatographic elution time, chemical composition, and MS/MS fragmentation pattern, also as performed previously. These metabolites, the tentative formula, mass error, retention times, and major fragments are presented in supporting information (Table S1).

Phenolic compounds and their metabolites were semi-quantified using pure standards when available or the most similar compound. The calibration curves were prepared in plasma and lactic serum from rats in concentrations of 0.1 up to 3 μg/mL. Table S2 (supporting information) shows the commercial standards used to quantify each phenolic compound and/or its metabolites. When standard compounds were not available, the phenolic compound was tentatively quantified with its aglycone or a phenolic compound with a similar chemical structure. All calibration curves presented $R^2 > 0.97$.

2.7. Data analysis

Data analysis was performed using Statgraphics Centurion XVI software (Statpoint Technologies Inc.,Warrenton, VA, USA) with a *p*-value < 0.05 considered statistically significant. Data are presented as mean and standard deviation (SD). The assumption of normalization was checked with standardized bias and standardized kurtosis. Statistical differences in the concentration of each compound (EVOO, ROO and REF) were evaluated using a one-way ANOVA, followed by the LSD post-hoc test.

3. Results and discussion

Although EVOO is the main source of fat in the Mediterranean diet, to date it is one of the least studied fats in terms of health impact when consumed during pregnancy and lactation. In the current study, a rat model was used to examine the vertical transmission of phenolic compounds to the offspring following maternal intake of EVOO for six weeks, corresponding to three weeks of gestation and three weeks of lactation. In addition, the metabolite profile in plasma and lactic serum was determined by UPLC coupled with high-resolution mass spectrometry (HRMS), using Orbitrap technology to overcome the limitations of the low-resolution mass spectrometry (LRMS) techniques such as triple quadrupole (López-Yerena, Domínguez-López, et al., 2021) of the previous studies focused on phenolic compounds metabolism (Domínguez-Perles et al., 2017; Khymenets et al., 2011; López de las Hazas et al., 2016; Rubió et al., 2012).

The concentration of total polyphenols and individual phenolic compounds in the administered EVOO is provided in the Table 1. On the other hand, the concentration of phenolic compounds and their metabolites in dam lactic serum and plasma and pup plasma in the EVOO, REF or ROO groups is presented in Fig. 1, Fig. 2 and Fig. 3. In addition, the numerical values of the quantified concentrations in the different matrices are presented in the supporting information (Table S3).

Although the EVOO contained a high concentration of secoiridoids (oleuropein and ligstroside aglycone, oleacein, oleocanthal and elenolic acid), representing more than 90% of the total phenolic content (Table 1), none were detected in the analyzed samples. However, enzymatic and microbial metabolites of both OH-TY and tyrosol were found, which could have been formed by secoiridoid metabolism (López-Yerena, Vallverdú-Queralt, Lamuela-Raventós, & Escribano-Ferrer, 2021; López-Yerena, Vallverdú-Queralt, Lamuela-Raventós, et al., 2021; López de las Hazas et al., 2016). The other minor compounds present in the EVOO (elenolic acid, luteolin, apigenin, pinoresinol, lariciresinol, isolariciresinol, verbascoside, *o*-coumaric and vanillic acid), or their metabolites, were not detected in the lactic serum or dam or pup plasma.

In 2020, Nalewajko-Sieliwoniu et al. (Nalewajko-Sieliwoniuk et al., 2020) confirmed that phenolic compounds can reach breast milk and be potentially available to infants. In the aforementioned study, gallic acid and caffeic acid were identified and quantified in human milk. A study carried out by Song and colleagues (Song et al., 2013) identified and quantified flavonoids in breast milk from 17 mothers at 1, 4, and 13 weeks postpartum. Delivering phenolic compounds via breast milk was also demonstrated in mothers after pomegranate juice consumption. In this study, dimethyl ellagic acid glucuronide and urolithin A-glucuronide were identified in breastmilk on day 14 after consumption of pomegranate juice (Henning et al., 2019). In our study, the low concentration of flavones, lignans and phenolic acids in the administered EVOO (<4% of total polyphenols) could explain the absence of these compounds and their derivatives in the samples.

Table 1
Phenolic compound contents (mg/kg) of EVOO.

Compounds	Concentration (mg/kg)
Total polyphenols	861.54 ± 101.84
Oleuropein aglycone	306.75 ± 30.25
Ligstroside aglycone	5.00 ± 0.54
Oleacein	298.72 ± 26.57
Oleocanthal	109.43 ± 15.05
OH-TY	26.60 ± 1.37
Tyrosol	28.13 ± 1.97
Elenolic acid	56.82 ± 2.82
Luteolin	17.36 ± 1.14
Apigenin	7.29 ± 0.62
Pinoresinol	2.69 ± 0.27
Lariciresinol	0.13 ± 0.00
Isolariciresinol	0.14 ± 0.01
Verbascoside	0.03 ± 0.00
o-coumaric	1.07 ± 0.06
Vanillic acid	1.47 ± 0.03

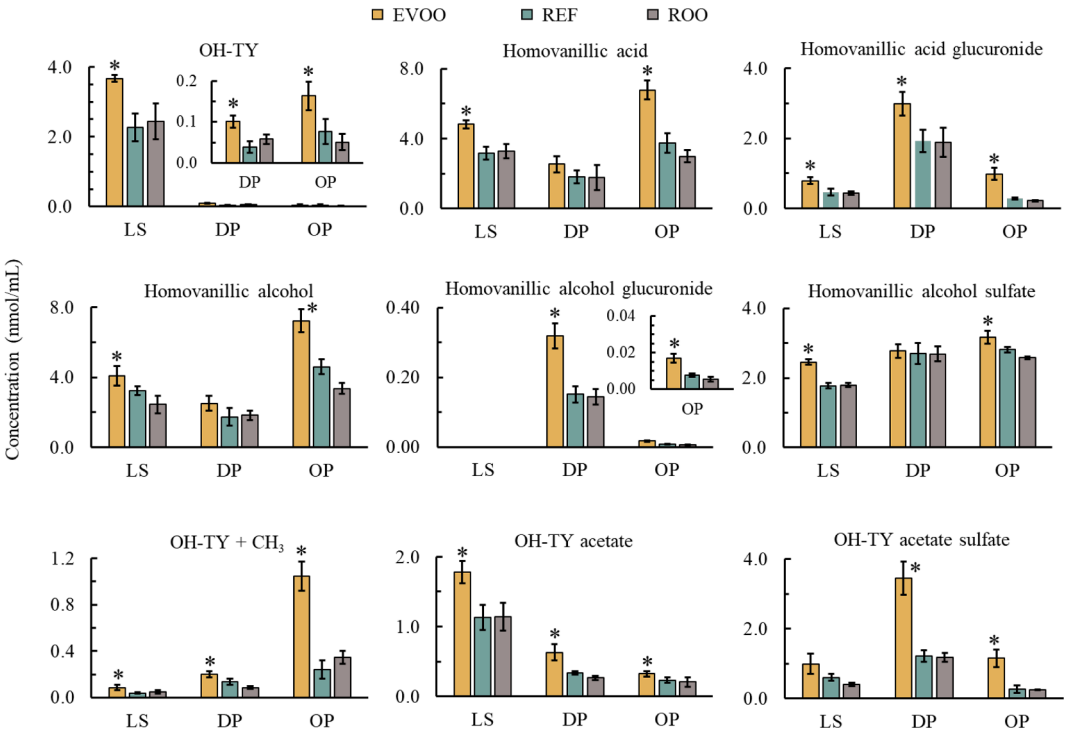

Fig. 1. Concentration of OH-TY and its derivatives in plasma and lactic serum. Results are expressed as the mean ± standard deviation. * Indicates significant differences between EVOO, ROO and REF groups ($p < 0.05$). LS: Lactic serum; DP: Dam plasma and OP: Offspring plasma.

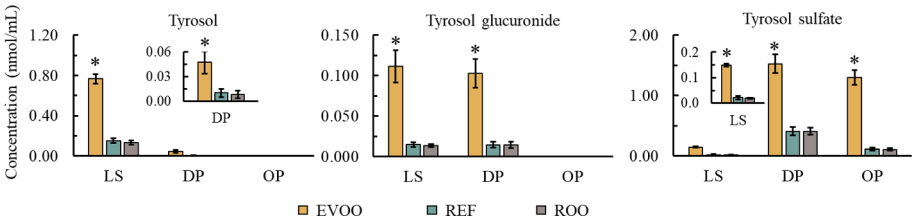

Fig. 2. Concentration of tyrosol and phase II metabolites in plasma and lactic serum. Results are expressed as the mean ± standard deviation. * Indicates significant differences between EVOO, ROO and REF groups (p < 0.05). LS: Lactic serum; DP: Dam plasma and OP: Offspring plasma.

3.1. OH-TY and its derivatives

The metabolites arising from phase I and phase II reactions identified in dam and offspring plasma and lactic serum are presented in Fig. 1. The highest concentrations of OH-TY were found in the three sample types from the EVOO group ($p < 005$). It is noteworthy that the concentration was higher in lactic serum than in dam or pup plasma (Fig. 1). The OH-TY concentration in dam plasma and lactic serum with respect to the initial concentration in the EVOO, as is shown is Table 1, could be explained by the EVOO content of secoiridoids and their hydrolysis by nonlipolytic carboxylester hydrolases and/or the acidic environment of the stomach. Other *in vivo* studies with rats have demonstrated that OH-TY reaches the systemic circulation after the ingestion of oleacein as a dietary supplementation (López de las Hazas et al., 2016) or an acute oral administration (López-Yerena, Vallverdú-Queralt, Lamuela-Raventós, et al., 2021). In the present work, the presence of OH-TY in pup plasma could be attributed to the high concentration of OH-TY detected in lactic serum and its possible vertical transmission from the dams. According to available data, OH-TY from EVOO has anti-inflammatory and antioxidant properties and is only biologically active when unmetabolized. Thus, its presence in maternal milk may enhance the metabolic status of the offspring and provide protection against a variety of diseases (Heras-Molina et al., 2020). A study carried out in rats by Zheng et al. demonstrated that maternal OH-TY improved neurogenesis and cognitive function in prenatally stressed offspring (Zheng et al., 2015). A study with piglets found that supplementation of the maternal diet with OH-TY during pregnancy improved placental gene expression, fetal antioxidant status and glucose metabolism in a sex-dependent manner (males were favored), and prevented the DNA hypomethylation associated with oxidative stress (Garcia-Contreras et al., 2019). Supplementation of sows with OH-TY also enhanced pre- and early post-natal development of offspring in pregnancies at risk of intrauterine growth restriction (IUGR) (Vazquez-Gomez et al., 2017). In a study on the potential role played by dietary polyphenols in protecting fetuses/neonates against growth disturbance caused by environmental pollutants, the presence of OH-TY in dam milk was thought to contribute to protecting pups against bisphenol A-induced hypothyroidism (Mahmoudi et al., 2018).

The highest concentrations of OH-TY derivatives were found mainly in samples corresponding to the EVOO group, as shown in Fig. 1. The detection of derivatives such as homovanillic alcohol and homovanillic acid in the REF and ROO groups can be attributed to their endogenous formation by dopamine oxidative metabolism, which leads to their presence in biological matrices, although at low concentrations (Rodriguez-Morato et al., 2016). *In vitro* studies have shown that, besides having antioxidant and purifying properties, homovanillic alcohol and homovanillic acid can exert beneficial effects by modulating intracellular signaling (Serreli & Deiana, 2018). The higher concentration of

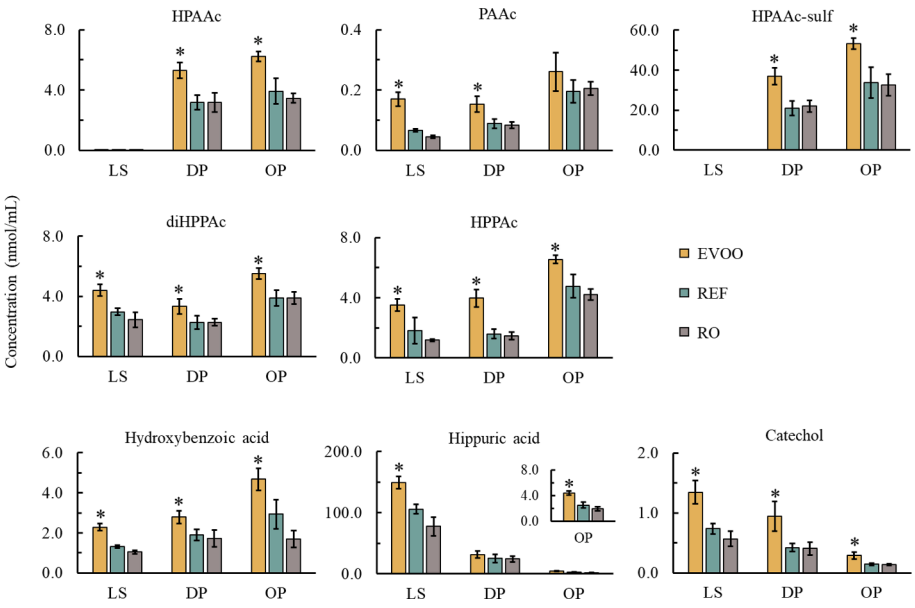

Fig. 3. Microbial metabolite concentrations in plasma and lactic serum. Results are expressed as the mean ± standard deviation. * Indicates significant differences between EVOO, ROO and REF groups ($p < 0.05$). LS: Lactic serum; DP: Dam plasma and OP: Offspring plasma.

OH-TY derivatives in samples from the EVOO group could be attributed to the metabolism of EVOO phenolic compounds, specifically oleuropein (Lin et al., 2013; López de las Hazas et al., 2016) and tyrosol (Mosele et al., 2014). It is striking that higher levels of both homovanillic alcohol and homovanillic acid were detected in pup rather than dam plasma (Fig. 1), which could be explained by the metabolism undergone by OH-TY before reaching the systemic circulation of the offspring. Deconjugation reactions of conjugate metabolites of homovanillic alcohol and homovanillic acid could be another explanation for their presence in pup plasma, as suggested for OH-TY glucuronide (Rubió et al., 2014). The highest concentrations of the glucuronidated form of the two metabolites were detected in dam plasma ($p < 0.05$); in fact, no homovanillic alcohol glucuronide was identified in lactic serum. Finally, higher concentrations of homovanillic alcohol sulfate were found in lactic serum and pup plasma of the EVOO group, whereas levels in dam plasma were not significantly different ($p < 0.05$).

It is well documented that the main phase II metabolic transformations of phenolic compounds are methylation, sulfation and glucuronidation, although the acetylation of OH-TY has also been demonstrated (Rubió et al., 2012). In this study, two OH-TY derivatives arising from acetylation by acyltransferase enzymes were identified: OH-TY acetate, with the highest concentrations in lactic serum ($p < 0.05$), and OH-TY acetate sulfate, whose levels were higher in dam plasma than in lactic serum, reaching up to 3.45 ± 0.48 nmol/mL. In the case of lactic serum, no significant differences in OH-TY acetate sulfate were detected between groups ($p < 0.05$).

3.2. Tyrosol and its derivatives

The concentration of tyrosol and its derivatives in the different groups (EVOO, REF and ROO) is shown in Fig. 2. Unmetabolized tyrosol was unable to reach the pup plasma and was only detected in dam plasma and lactic serum, with the highest concentrations corresponding to the EVOO group ($p < 0.05$). The detection of tyrosol and its derivatives is attributable to the absorption of the initial tyrosol content in the administered EVOO into the systemic circulation. It could also be due to the hydrolysis of EVOO secoiridoids, specifically ligstroside aglycone and oleocanthal by nonlipolytic carboxylester hydrolases and/or the acidic environment of the stomach (López-Yerena, Vallverdú-Queralt, Lamuela-Raventós, & Escribano-Ferrer, 2021). Finally, it has been suggested that tyrosol is a microbial metabolite produced by the dehydroxylation of the OH-TY aromatic ring (Domínguez-Perles et al., 2017). The very low concentrations of tyrosol compared to those of OH-TY and its derivatives could be due to its endogenous bioconversion into OH-TY, as suggested by Boronat et al. (Boronat et al., 2019). The fact that tyrosol is a source of OH-TY is significant, as most of the beneficial health effects associated with the consumption of EVOO in the context of the Mediterranean diet are attributed to its content of monounsaturated fatty acids and OH-TY.

Similarly, the glucuronidated form of tyrosol was only detected in samples obtained from dams (0.09 ± 0.02 and 0.11 ± 0.02 nmol/mL, plasma, and lactic serum, respectively), and its very low concentrations could explain its absence in pup plasma. Finally, tyrosol sulfate was found in all sample types, with the highest concentrations in animals administered EVOO during gestation and lactation. Our results are in agreement with previous studies in which tyrosol in rat urine (Domínguez-Perles et al., 2017), tyrosol glucuronide in human urine (Khymenets et al., 2011) and tyrosol sulfate in human plasma (Suárez et al., 2011) were identified after the intake of EVOO or oral administration of the pure compound.

3.3. Microbial metabolism

It is well known that unabsorbed dietary phenolic compounds reach the large intestine where they undergo catabolic transformations by microbial metabolism. In the gut, phenolic compound transformation into simpler components is mediated by different reactions, such as ring fission, hydrolysis, demethylation, reduction, decarboxylation, dehydroxylation and isomerization (Marhuenda-Muñoz et al., 2019). In 2021, Lu and colleagues demonstrated that dietary intake of polyphenols from plant-based foods was positively correlated with the concentrations of phenolic compounds in breast milk of Asian population, including the relevant microbial-derived phenolic metabolites (equol, 3,4-dyhydroxyphenylacetic acid and 3,4-dihydroxybenzoic acid) (Lu et al., 2021). Although several studies promote that plant-based diets could modify or even enrich the phenolic content of breast milk and thus be more available to infants through breastfeeding (Lu et al., 2021; Nalewajko-Sieliwoniuk et al., 2020), this is the first study to address the impact of EVOO intake during lactation. Fig. 3 shows the concentration of the gut microbial metabolites detected in lactic serum and plasma

from dams and pups.

One of the main microbial metabolic transformations of OH-TY is through oxidation, in which the primary alcohol is converted into diHPAAc, followed by rapid dehydroxylation to HPAAc (López de las Hazas et al., 2016). However, it has also been suggested that HPAAc is a metabolite of tyrosol (Mosele et al., 2014) and OH-TY acetate (Lopez de las Hazas et al., 2017; Mosele et al., 2014). The highest concentrations of HPAAc were found in plasma samples from the EVOO group, being higher in the pups than dams (Fig. 3). Very low concentrations were detected in lactic serum ($\leq$0.3 nmol/mL), without significant differences between groups.

The dihydroxylation of HPAAc leads to the formation of phenylacetic acid (PAAc) (López de las Hazas et al., 2016; Mosele et al., 2014), while its sulfation results in hydroxyphenyl acetic acid sulfate (HPAAc-sulf) (López de las Hazas et al., 2016). As shown in Fig. 3, PAAc was detected in in all sample types but in very low concentrations ($\leq$0.26 nmol/mL), and differences between groups were only significant ($p < 0.05$) for dam plasma and lactic serum, the EVOO group having higher values. To date, PAAc has been identified in human feces, but without significant differences in concentration between pre- and post-EVOO-consumption (Mosele et al., 2014). The same metabolite was identified in the caecum content in rats after a 21-day supplementation with 5 mg/kg rat weight/day of OH-TY (López de las Hazas et al., 2016), without differences between the control and OH-TY groups. On the other hand, we detected HPAAc-sulf in concentrations of up to 53.31 $\pm$ 2.68 in pup plasma and 36.91 $\pm$ 4.26 nmol/mL in dam plasma, but with very low concentrations in lactic serum.

The carboxylation of diHPAAc results in dihydroxyphenylpropionic acid (diHPPAc), which in turn is converted into HPPAc after dihydroxylation. Both compounds were detected in all analyzed samples, with the highest concentrations found in pup plasma in the EVOO group, which had higher values than the REF and ROO groups overall. In the aforementioned study by López de las Hazas, these metabolites were also identified in rat caecum content after 21 days of OH-TY, oleacein or oleuropein supplementation (López de las Hazas et al., 2016). In an *in vivo* study, no differences in HPPAc concentration were found in human fecal samples before and after intake of phenol-enriched olive oil (Mosele et al., 2014). It has been demonstrated *in vitro* that PAAc and HPPAc induce cell cycle arrest and promote apoptosis, thus providing further evidence of the anti-cancer effects of EVOO phenolic compounds and their colonic metabolites (Lopez de las Hazas et al., 2017).

Hydroxybenzoic acid has also been proposed as a microbial derivative of OH-TY and/or tyrosol (López de las Hazas et al., 2016; Mosele et al., 2014). It was identified in all sample types but with higher concentrations in the EVOO group ($p < 0.05$). In another study with rats, this metabolite was detected in feces but not in plasma (López de las Hazas et al., 2016).

Finally, as shown in Fig. 3, hippuric acid and catechol were also identified in all the samples and its concentrations were significantly higher in the EVOO group ($p < 0.05$). The highest values for hippuric acid were found in lactic serum and for catechol, in lactic serum and dam plasma.

Thus, up to eight microbial phenolic metabolites were identified and quantified in the analyzed samples, and their concentration was clearly higher after dietary exposure to phenolic compounds from EVOO, with potential biological significance for the offspring. In this context, more research is necessary to understand the maternal–infant transmission of EVOO phenolic compounds and its health implications.

4. Conclusions

Although EVOO represents the main source of fat in the Mediterranean diet, to our knowledge, this is the first study to report the vertical transmission of EVOO phenolic compounds from mother to offspring fluids during lactation.

Hydroxytyrosol and tyrosol derivatives, from enzymatic and microbial metabolism, were detected in plasma and lactic serum from rats administered EVOO for 6 weeks. Our results support that diets enriched with EVOO could modify or even increase the phenolic content of breast milk, with potential health benefits for the infant. Moreover, promising levels of EVOO phenolic compounds and their metabolites were detected in offspring plasma. Our findings shed fresh light on the importance of the maternal diet during pregnancy and lactation, and provide the basis for future studies on the impact of phenolic compounds on the health of mother and offspring.

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CRediT authorship contribution statement

Anallely López-Yerena: Data curation, Formal analysis, Methodology, Resources, Software, Writing – original draft. **Blanca Grases-Pintó:** Data curation, Formal analysis, Methodology, Writing – review & editing. **Sonia Zhan-Dai:** Data curation, Formal analysis, Methodology, Writing – review & editing. **Francisco J. Pérez-Cano:** Investigation, Supervision, Writing – original draft, Writing – review & editing. **Rosa M. Lamuela-Raventos:** Funding acquisition, Project administration, Visualization, Writing – review & editing. **Maria J. Rodríguez-Lagunas:** Conceptualization, Formal analysis, Funding acquisition, Investigation, Methodology, Project administration, Supervision, Validation, Visualization, Writing – review & editing. **Anna Vallverdú-Queralt:** Conceptualization, Data curation, Data curation, Funding acquisition, Investigation, Project administration, Supervision, Validation, Visualization, Writing – original draft, Writing – review & editing.

Declaration of Competing Interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: R. M.L.-R. reports personal fees from Cerveceros de España, personal fees, and other from Adventia, Wine in Moderation, Ecoveritas S.A., outside the submitted work. The authors declare no conflict of interest. The funders had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript, or in the decision to publish the results.

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Appendix A. Supplementary data

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References

Azagra-Boronat, I., Tres, A., Massot-Cladera, M., Franch, À., Castell, M., Guardiola, F., … Rodríguez-Lagunas, M. J. (2020a). Associations of breast milk microbiota, immune factors, and fatty acids in the rat mother–offspring pair. *Nutrients, 12*(2), 319.

Azagra-Boronat, I., Tres, A., Massot-Cladera, M., Franch, À., Castell, M., Guardiola, F., … Rodríguez-Lagunas, M. J. (2020b). Lactobacillus fermentum CECT5716 supplementation in rats during pregnancy and lactation impacts maternal and offspring lipid profile, immune system and microbiota. *Cells, 9*(3), 575.

Boronat, A., Mateus, J., Soldevila-Domenech, N., Guerra, M., Rodríguez-Morató, J., Varon, C., … Gaedigk, A. (2019). Cardiovascular benefits of tyrosol and its endogenous conversion into hydroxytyrosol in humans. A randomized, controlled trial. *Free Radical Biology and Medicine, 143*, 471–481.

Castro-Rodriguez, J. A., Garcia-Marcos, L., Sanchez-Solis, M., Pérez-Fernández, V., Martinez-Torres, A., & Mallol, J. (2010). Olive oil during pregnancy is associated with reduced wheezing during the first year of life of the offspring. *Pediatric Pulmonology, 45*(4), 395–402.

Domínguez-Perles, R., Auñón, D., Ferreres, F., & Gil-Izquierdo, A. (2017). Gender differences in plasma and urine metabolites from Sprague-Dawley rats after oral administration of normal and high doses of hydroxytyrosol, hydroxytyrosol acetate, and DOPAC. *European Journal of Nutrition, 56*(1), 215–224.

Dror, D. K., & Allen, L. H. (2018). Overview of nutrients in human milk. *Advances Nutrition, 9*(suppl_1), 278S–294S.

Escobar-Avello, D., Lozano-Castellón, J., Mardones, C., Pérez, A. J., Saéz, V., Riquelme, S., Von Baer, D., & Vallverdú-Queralt, A. (2019). Phenolic profile of grape canes: Novel compounds identified by LC-ESI-LTQ-orbitrap-MS. *Molecules, 24*(20), 3763.

Garcia-Contreras, C., Vazquez-Gomez, M., Barbero, A., Pesantez, J. L., Zinellu, A., Berlinguer, F., … Torres-Rovira, L. (2019). Polyphenols and IUGR pregnancies: Effects of maternal hydroxytyrosol supplementation on placental gene expression and fetal antioxidant status, DNA-methylation and phenotype. *International Journal of Molecular Sciences, 20*(5), 1187.

Henning, S. M., Wallenstein, M. B., Weigel, N., Johnson, C., Yang, J., Lee, R. P., … Ben-Nissan, D. (2019). Appearance of ellagic acid metabolites from pomegranate juice in breast milk: A case report. *Ann Clin Case Rep., 2019*(4), 1738.

Heras-Molina, A., Pesantez-Pacheco, J. L., Astiz, S., Garcia-Contreras, C., Vazquez-Gomez, M., Encinas, T., … Gonzalez-Bulnes, A. (2020). Maternal supplementation with polyphenols and omega-3 fatty acids during pregnancy: Effects on growth, metabolism, and body composition of the offspring. *Animals, 10*(11), 1946.

Khymenets, O., Farré, M., Pujadas, M., Ortiz, E., Joglar, J., Covas, M. I., & de la Torre, R. (2011). Direct analysis of glucuronidated metabolites of main olive oil phenols in human urine after dietary consumption of virgin olive oil. *Food Chemistry, 126*(1), 306–314.

Kumar, H., du Toit, E., Kulkarni, A., Aakko, J., Linderborg, K. M., Zhang, Y., … Collado, M. C. (2016). Distinct patterns in human milk microbiota and fatty acid profiles across specific geographic locations. *Frontiers in Microbiology, 7*, 1619.

Leporini, M., Loizzo, M. R., Tenuta, M. C., Falco, T., & Sicari, V. (2018). *Calabrian extra-virgin olive oil from Frantoio cultivar : chemical composition and health properties*. *30*(7), 631–637. https://doi.org/10.9755/ejfa.2018.v30.i7.1743.

Lin, P., Qian, W., Wang, X., Cao, L., Li, S., & Qian, T. (2013). The biotransformation of oleuropein in rats. *Biomedical Chromatography, 27*(9), 1162–1167. https://doi.org/10.1002/bmc.2922

López-Yerena, A., Domínguez-López, I., Vallverdú-Queralt, A., Pérez, M., Jáuregui, O., Escribano-Ferrer, E., & Lamuela-Raventós, R. M. (2021). Metabolomics technologies for the identification and quantification of dietary phenolic compound metabolites: An overview. *Antioxidants, 10*(6), 846.

López-Yerena, A., Vallverdú-Queralt, A., Jáuregui, O., Garcia-Sala, X., Lamuela-Raventós, R. M., & Escribano-Ferrer, E. (2021). Tissue distribution of oleocanthal and its metabolites after oral ingestion in rats. *Antioxidants, 10*(5), 688.

López-Yerena, A., Vallverdú-Queralt, A., Lamuela-Raventós, R. M., & Escribano-Ferrer, E. (2021). LC-ESI-LTQ-Orbitrap-MS for profiling the distribution of oleacein and its metabolites in rat tissues. *Antioxidants, 10*(7), 1083.

Lopez de las Hazas, M.-C., Pinol, C., Macià, A., & Motilva, M.-J. (2017). Hydroxytyrosol and the colonic metabolites derived from virgin olive oil intake induce cell cycle arrest and apoptosis in colon cancer cells. *Journal of Agricultural and Food Chemistry, 65*(31), 6467–6476.

López de las Hazas, M.-C., Piñol, C., Macià, A., Romero, M.-P., Pedret, A., Solà, R., Rubió, L., & Motilva, M.-J. (2016). Differential absorption and metabolism of hydroxytyrosol and its precursors oleuropein and secoiridoids. *Journal of Functional Foods, 22*, 52–63.

Lozano-Castellón, J., López-Yerena, A., Olmo-Cunillera, A., Jáuregui, O., Pérez, M., Lamuela-Raventós, R. M., & Vallverdú-Queralt, A. (2021). Total analysis of the major secoiridoids in extra virgin olive oil: Validation of an uhplc-esi-ms/ms method. *Antioxidants, 10*(4), 1–13. https://doi.org/10.3390/antiox10040540

Lu, Z., Chan, Y.-T., Lo, K.-K.-H., Wong, V.-W.-S., Ng, Y.-F., Li, S.-Y., … Zhao, D. (2021). Levels of polyphenols and phenolic metabolites in breast milk and their association with plant-based food intake in Hong Kong lactating women. *Food & Function, 12*(24), 12683–12695.

Mahmoudi, A., Ghorbel, H., Feki, I., Bouallagui, Z., Guermazi, F., Ayadi, L., & Sayadi, S. (2018). Oleuropein and hydroxytyrosol protect rats' pups against bisphenol A induced hypothyroidism. *Biomedicine & Pharmacotherapy, 103*, 1115–1126.

Marhuenda-Muñoz, M., Laveriano-Santos, E. P., Tresserra-Rimbau, A., Lamuela-Raventós, R. M., Martínez-Huélamo, M., & Vallverdú-Queralt, A. (2019). Microbial phenolic metabolites: Which molecules actually have an effect on human health? *Nutrients, 11*(11), 2725.

Martin, C. R., Ling, P.-R., & Blackburn, G. L. (2016). Review of infant feeding: Key features of breast milk and infant formula. *Nutrients, 8*(5), 279.

Martín, R., Langa, S., Reviriego, C., Jimínez, E., Marín, M. L., Xaus, J., … Rodríguez, J. M. (2003). Human milk is a source of lactic acid bacteria for the infant gut. *The Journal of Pediatrics, 143*(6), 754–758.

Medina-Remón, A., Estruch, R., Tresserra-Rimbaua, A., Vallverdú-Queralt, A., & Lamuela-Raventosa, R. M. (2013). The effect of polyphenol consumption on blood pressure. *Mini-Reviews in Medicinal Chemistry, 13*(8), 1137–1149.

Mosele, J. I., Martín-Peláez, S., Macià, A., Farràs, M., Valls, R.-M., Catalán, U., & Motilva, M.-J. (2014). Faecal microbial metabolism of olive oil phenolic compounds: In vitro and in vivo approaches. *Molecular Nutrition and Food Research, 58*(9), 1809–1819. https://doi.org/10.1002/mnfr.201400124

Nalewajko-Sieliwoniuk, E., Hryniewicka, M., Jankowska, D., Kojło, A., Kamianowska, M., & Szczepański, M. (2020). Dispersive liquid–liquid microextraction coupled to liquid chromatography tandem mass spectrometry for the determination of phenolic compounds in human milk. *Food Chemistry, 327*, Article 126996.

Orrego-Lagarón, N., Martínez-Huélamo, M., Vallverdú-Queralt, A., Lamuela-Raventos, R. M., & Escribano-Ferrer, E. (2015). High gastrointestinal permeability and local metabolism of naringenin: Influence of antibiotic treatment on absorption and metabolism. *British Journal of Nutrition, 114*(2), 169–180.

Polari, J. J., Garcí-Aguirre, D., Olmo-García, L., Carrasco-Pancorbo, A., & Wang, S. C. (2018). Impact of industrial hammer mill rotor speed on extraction efficiency and quality of extra virgin olive oil. *Food Chemistry, 242*, 362–368.

Reeves, P. G., Nielsen, F. H., & Fahey, G. C., Jr. (1993). *AIN-93 purified diets for laboratory rodents: Final report of the American Institute of Nutrition ad hoc writing committee on the reformulation of the AIN-76A rodent diet*. Oxford University Press.

Rodriguez-Morato, J., Boronat, A., Kotronoulas, A., Pujadas, M., Pastor, A., Olesti, E., … Farre, M. (2016). Metabolic disposition and biological significance of simple phenols of dietary origin: Hydroxytyrosol and tyrosol. *Drug Metabolism Reviews, 48*(2), 218–236.

Rosignoli, P., Fuccelli, R., Fabiani, R., Servili, M., & Morozzi, G. (2013). Effect of olive oil phenols on the production of inflammatory mediators in freshly isolated human monocytes. *The Journal of Nutritional Biochemistry, 24*(8), 1513–1519. https://doi.org/10.1016/j.jnutbio.2012.12.011

Rubió, L., Macià, A., Valls, R. M., Pedret, A., Romero, M.-P., Solà, R., & Motilva, M.-J. (2012). A new hydroxytyrosol metabolite identified in human plasma: Hydroxytyrosol acetate sulphate. *Food Chemistry, 134*(2), 1132–1136. https://doi.org/10.1016/j.foodchem.2012.02.192.

Rubió, L., Serra, A., Macià, A., Piñol, C., Romero, M.-P., & Motilva, M.-J. (2014). In vivo distribution and deconjugation of hydroxytyrosol phase II metabolites in red blood cells: A potential new target for hydroxytyrosol. *Journal of Functional Foods, 10*, 139–143.

Serreli, G., & Deiana, M. (2018). Biological relevance of extra virgin olive oil polyphenols metabolites. *Antioxidants, 7*(12), 11–13. https://doi.org/10.3390/antiox7120170

Soler, A., Romero, M. P., Macià, A., Saha, S., Furniss, C. S. M., Kroon, P. A., & Motilva, M. J. (2010). Digestion stability and evaluation of the metabolism and transport of olive oil phenols in the human small-intestinal epithelial Caco-2/TC7 cell line. *Food Chemistry, 119*(2), 703–714.

Song, B. J., Jouni, Z. E., & Ferruzzi, M. G. (2013). Assessment of phytochemical content in human milk during different stages of lactation. *Nutrition, 29*(1), 195–202.

Suárez, M., Valls, R. M., Romero, M. P., Maclà, A., Fernández, S., Giralt, M., … Motilva, M. J. (2011). Bioavailability of phenols from a phenol-enriched olive oil. *British Journal of Nutrition, 106*(11), 1691–1701. https://doi.org/10.1017/S0007114511002200

Tresserra-Rimbau, A., Rimm, E. B., Medina-Remón, A., Martínez-González, M. A., De la Torre, R., Corella, D., … Arós, F. (2014). Inverse association between habitual polyphenol intake and incidence of cardiovascular events in the PREDIMED study. *Nutrition, Metabolism and Cardiovascular Diseases, 24*(6), 639–647.

van't Land, B., Boehm, G., & Garssen, J. (2010). Breast milk: components with immune modulating potential and their possible role in immune mediated disease resistance. In *Dietary components and immune function* (pp. 25–41). Springer.

Vazquez-Gomez, M., Garcia-Contreras, C., Torres-Rovira, L., Pesantez, J. L., Gonzalez-Añover, P., Gomez-Fidalgo, E., … Astiz, S. (2017). Polyphenols and IUGR pregnancies: Maternal hydroxytyrosol supplementation improves prenatal and early-postnatal growth and metabolism of the offspring. *PLoS One, 12*(5), e0177593.

Walker, W. A. (2017). The importance of appropriate initial bacterial colonization of the intestine in newborn, child, and adult health. *Pediatric Research, 82*(3), 387–395.

Zheng, A., Li, H., Cao, K., Xu, J., Zou, X., Li, Y., … Feng, Z. (2015). Maternal hydroxytyrosol administration improves neurogenesis and cognitive function in prenatally stressed offspring. *The Journal of Nutritional Biochemistry, 26*(2), 190–199.