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Introduction of Distillate Rosemary Leaves into the Diet of the Murciano-Granadina Goat: Transfer of Polyphenolic Compounds to Goats' Milk and the Plasma of Suckling Goat Kids

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The effect of the introduction of distilled rosemary leaves into the diet of the Murciano-Granadina goat on the polyphenolic profile of the goats' milk during the physiological stages of gestation and lactation was studied. The inclusion of rosemary leaves into the animal diet modified neither animal productivity (milk yield) nor milk quality. The following components were found in increased concentration ($P < 0.05$) in the goats' milk after the introduction of rosemary leaves into their diet: flavonoids hesperidin, naringin, and genkwanin; gallic acid; and phenolic diterpenes carnosol and carnosic acid. With regard to the transfer of polyphenols to the plasma of the suckling goat kid, a statistically significant increase ($P < 0.05$) in rosmarinic acid, carnosic acid, and carnosol concentrations was detected. From this point of view, distillate rosemary leaves can be proposed as an ingredient in ruminant feed because they both alter neither the yield nor the quality of Murciano-Granadina goats' milk and allow for an increased concentration of polyphenolic components in the goats' milk and in the plasma of the suckling goat kid.

KEYWORDS: Murciano-Granadina goats' milk; *Rosmarinus officinalis*; distillation byproduct; polyphenolic transmission

INTRODUCTION

The region of Murcia is one of the most important in Spain with respect to goat husbandry, based on the Murciano-Granadina breed, which is the most numerous milking breed with the highest milk production in the country (*1*). With an annual yield of 35 million liters, Murciano-Granadina goats' milk is characterized by a fat content of 5.4%, a protein content of 3.6%, and a dry extract content of 14.5% and is highly suited for the production of different varieties of cheeses (*2*, *3*).

The secondary metabolites of plants have, in the past, generally been considered a source of antinutritional factors, owing to the fact that polyphenols reduce the bioavailability of proteins and minerals (*4*). However, according to Wang et al. (*5*), providing a moderate source of proanthocyanidins in the diet of sheep, by supplementing feed with *Lotus corniculatus*, increased milk yield, protein content, and lactose content. The increased amounts of protein and milk may be related to the fact that in the reticulomen (which has a pH of 6.0−7.0), polyphenols interact with proteins, thereby inhibiting the utilization of protein in the rumen by indigenous microorganisms (it has been estimated that the reticulomen microflora degrade up to 75% of ingested protein). However, once the polyphenol−protein complex passes into the abomasum (pH 2.5−3.5), the complex breaks down and the released protein is degraded and utilized by the ruminant (*6*).

The occurrence of polyphenols in milk may be a consequence of several factors, including the consumption of particular fodder crops by ruminants and the catabolism of proteins by bacteria (*7*).

Some of the aromatic plants widespread in the Mediterranean area have already been studied for their richness in polyphenolic components, including sage, rosemary, and thyme (*8*−*12*).

Rosemary (*Rosmarinus officinalis* L.) polyphenolic extract contains antioxidant compounds, the most active being phenolic diterpenes such as carnosol, carnosic acid, rosmanol, epirosmanol, isorosmanol, methyl carnosate, and other phenolic acids, such as rosmarinic acid (*8*). Carnosic acid is the major phenolic constituent present in rosemary leaves, with an antioxidant activity approximately 3 times higher than that of carnosol and 7 times higher than that of the synthetic antioxidants butylated hydroxytoluene and butylated hydroxyanisole (*9*).

Currently, the search for natural antioxidants in the byproduct of aromatic plants has produced an alternative to synthetic antioxidants in the food and pharmaceutical industries (*10*, *11*, *13*). Some authors considered the study of the material remaining after distillation potentially interesting due to the water-soluble properties of phenolic compounds that rarely form part of essential oils (*14*).

Previous studies accomplished by Moñino et al. (*15*) have demonstrated that the incorporation of distilled rosemary leaves

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Table 1. Chemical Composition of the Concentrated Basal Diet

nutrient	concentrate[a]
ash (g/kg)	76.0
crude protein (CP) (g/kg)	175.5
fat (g/kg of dm)	30.9
neutral detergent fiber (NDF) (g/kg)	366.4
disgestible NDF (%)	25.0
acid detergent fiber (g/kg)	190.8
non-proteic nitrogen (g/kg)	0.5
ruminal degradable protein (RDP), % of CP	64.7
non-fiber carbohydrate (g/kg)	267.9
adjusted total starch (g/kg)	90.7
net energy lactation (mcal/kg)	1.5
total soluble RDP (g/kg)	43.3
ruminal undegradable protein (g/kg)	55.6
calcium (g/kg)	10
phosphorus (g/kg)	5.7
vitamin A (IU/g)	14.9
vitamin D (IU/g)	3.1
vitamin E (IU/g)	32.4
magnesium (g/kg)	3.4
selenium (mg/kg)	0.72
zinc (mg/kg)	154.4
total methionine (g/kg)	2.6
total lysine (g/kg)	6.7

[a] Concentrations are referred to weight of dry matter. Formulated using the following ingredients/tonne: wheat bran, 266.5 kg; scale soy, 250 kg; barley, 150 kg; malt comb, 80 kg; sunflower oil (30%), 70 kg; rye, 69.9 kg; honey bean, 41.6 kg; corn flour, 36.7 kg; calcium carbonate, 14.9 kg; molasses-cane, 10 kg; vitamin and mineral feed additives, 10.4 kg.

into the ewe diet at a rate of 10 or 20% does not modify the animal yield and improves the antioxidant status of the lamb meat.

At present, international legislation does not allow for the addition of synthetic antioxidants into fresh milk or its derivatives; their stability should be conferred by components naturally present in them. Taking into account all of these considerations, the inclusion of natural antioxidant compounds in the goats' diet, as an alternative to synthetic antioxidant additives and as a means of improving goat's milk quality, merits investigation. The principal goals of feeding goats with rosemary byproduct are to improve the animals' production characteristics and transfer the antioxidant properties of the byproduct to the milk produced and, consequently, to the plasma of the suckling goat kid.

MATERIALS AND METHODS

Experimental Design. Thirty-six Murciano-Granadina dairy goats were assigned randomly, on the basis of their age and milk yield, into three homogeneous groups. The average values of these parameters were 3.2 years of age and a 462 kg yield (estimated in 210 days), respectively. Goats in the control group were given a basal diet (BD) consisting of 2.3 kg/animal/day. The diets of the other two groups were modified by substituting 10% or 20% of the BD (respectively) with distilled aromatic plant leaves, using pellets consisting of 50% barley and 50% distilled leaves. The basal diet (**Table 1**) was provided by Cargill Animal Nutrition (Torre Pacheco, Murcia, Spain). Steam-distilled rosemary (*R. officinalis*) leaves were obtained from a local grower. Leaves were steam-distilled for 3 h using a distillation system with a stainless steel steam boiler.

Animals were fed on these diets for 224 days, coinciding with the gestation and lactation periods in order to study the transfer of polyphenolic compounds to the goats' milk and consequently to the plasma of the suckling goat kid.

The goats and kids were reared at the CIFEA Research Center (Consejería de Agricultura, Región de Murcia, Spain).

Milk and Plasma Sampling. Milk yield was measured twice a month from April to June (gestation) and from October to November (lactation) 2005. Due to vaccination necessities, goats did not produce milk during the summer (July and August); thus, there are no records for these two months. In addition, due to variations in litter dates, data from September have not been considered in the present work.

Table 2. Dietary Administration of Polyphenols Present in the Concentrate-Based Diet and in the Rosemary Pellet (50% Barley−50% Distilled *Rosmarinus officinalis* L. Leaves)[a]

polyphenol	control diet (mg/kg)	control diet, 10% rosemary leaves (mg/kg)	control diet, 20% rosemary leaves (mg/kg)
gallic acid	178.88 ± 29.67	163.61 ± 26.36	148.34 ± 23.05
caffeic acid	71.21 ± 12.33	78.17 ± 15.72	85.4 ± 19.11
ferulic acid	32.68 ± 2.57	41.87 ± 4.36	51.05 ± 6.17
coumaric acid	18.69 ± 0.84	33.62 ± 1.46	48.54 ± 2.07
naringin	46.29 ± 8.70	51.05 ± 9.24	55.8 2 ± 9.78
hesperidin	111.97 ± 17.82	284.42 ± 30.00	456.88 ± 42.19
luteolin	12.45 ± 1.26	17.49 ± 2.21	22.54 ± 3.17
rosmarinic acid	7.02 ± 0.33	140.44 ± 4.95	273.86 ± 9.58
apigenin	9.27 ± 0.63	12.99 ± 1.23	16.72 ± 1.84
genkwanin	120.06 ± 20.97	755.94 ± 119.79	1391.81 ± 218.60
carnosol	72.26 ± 3.94	374.55 ± 29.06	676.83 ± 62.07
carnosic acid	68.22 ± 1.53	107.71 ± 8.08	147.20 ± 14.62

[a] Data are the mean of three independent replicates from four pellet fabrications (n = 12) ± standard deviation.

To estimate the influence of the introduction of distilled rosemary leaves on animal production, along with the transmission of the polyphenolic components to the goats' milk and consequently to the goat kid plasma at the lactation stage, dams were milked after the kids were fed, and the amount of residual milk was recorded. Goats were milked in a double 12-stall Casse system parallel milking parlor, and individual recorder jars were used to measure the volume of milk extracted.

Blood samples from the suckling goat kids (body weight = 13 kg) were obtained by jugular venipuncture at the end of the lactation period. Samples (10 mL) were collected in heparinized evacuated tubes (Belliver Industrial Estate, Plymouth, U.K.). All tubes were immediately placed on ice and, within 2 h after bleeding, were centrifuged at 2500*g* for 10 min at 4 °C. The plasma was acidified (1:11) with acetic acid (10 mM) and keep at −80 °C until the extraction of the polyphenolic fraction.

Milk Chemical Composition. Samples of milk, collected into sterile vials with preservative (azidiol) and refrigerated at 4 °C, were analyzed (protein, fat, lactose, and total dry extract) using an infrared spectrophotometer (Milko Skan in a Combi-Foss 5000, Foss Electric, Hillerod, Denmark) according to FIL-IDF standard 141B (*16*).

Polyphenolic Extraction. *Basal and Enriched Rosemary Diets*. Basal diet grains and aromatic plant-distillate pellets were dried in a forced-air dryer at 35 °C for 48 h (until they reached a constant weight) and then ground to pass through a 2 mm sieve. Dried samples (0.5 g) were first extracted using 20 mL of petroleum ether, by stirring, and brought to dryness at room temperature. Second, they were extracted using 150 mL of methanol in a Soxhlet extractor (B-811) (Buchi, Flawil, Switzerland) for 2 h under a nitrogen atmosphere. Methanolic extracts were taken to dryness at 40 °C under vacuum conditions in an evaporator system (Syncore Polyvap R-96) (Buchi). The residue was redissolved in methanol and brought up to 5 mL. The extracts were kept in vials at −80 °C until their corresponding analyses. The qualitative and quantitative polyphenolic compositions of the three diets assayed are described in **Table 2**.

Goat Kid Plasma. To determine the presence of polyphenolic components in goat kid plasma, a modified method described by Cerdá et al. (*17*) was performed. In this procedure, 5 mL of acidified plasma was applied to a polymeric resin, Chromabond HR-P (polystyrene−divinylbenzene) (Macherey-Nagel, Düren, Germany), using methanol as an extracting solvent. Methanolic extracts (25 mL) were taken to dryness at 40 °C under vacuum conditions in an evaporator system (Syncore Polyvap R-96) (Büchi). The residue was redissolved in methanol and brought up to 5 mL. The extracts were kept in vials at −80 °C until their corresponding analyses.

Polyphenols in Milk. Polyphenolic components in milk were extracted according to the method developed by Ternes and Schwarz (*18*). According to these authors, in milk, carnosic acid and other phenolic diterpenes show a greater affinity for the casein fraction after lactic acidification,

Table 3. Milk Yield during Pregnancy and Lactation Stages of Murciano-Granadina Goat Breed[a]

animal group	gestation (kg/day)	lactation[b] (kg/day)
control	1.22 ± 0.18	1.39 ± 0.04
10%	1.43 ± 0.06	1.47 ± 0.12
20%	1.35 ± 0.12	1.61 ± 0.11

[a] Data are the mean of 10 goats yield ± standard deviation. [b] Values in lactation were recorded after goat kid suckling.

aggregating into gel form. Accordingly, for the analysis of the polyphenols present in goat's milk, a previous lactic acidification of the milk (pH 4.6) was done. The casein and whey fraction were separated by centrifugation (4000*g*) for 10 min at 4 °C in an Eppendorf 5810R (Hamburg, Germany) system.

Goat's milk caseins were lyophilized (VirTis, 6K BTEL-85 freeze drier, Ucoa-erloss, Madrid, Spain) and kept in a dry atmosphere until analysis. Dried samples (1.5 g) were extracted following the protocol above-described for the basal and rosemary diets.

Recovery Efficiencies. To determine the recovery efficiencies of the polyphenols in goat's milk caseins, methanolic dilutions of the polyphenolic components under assay (gallic acid, caffeic acid, ferulic acid, coumaric acid, naringin, hesperidin, luteolin, rosmarinic acid, apigenin, genkwanin, carnosol, and carnosic acid) were added in different concentrations (40, 20, and 9 mg/kg) to the milk before acidification with lactic acid. Sample preparation for the polyphenolic extraction was done according to the protocol described above.

HPLC Analysis of Polyphenols. For the HPLC analysis, a method adapted from Zheng and Wang (*19*) was performed on a reverse-phase Zorbax SB-C18 column (4.6 × 250 mm, 5 μm pore size, Hewlett-Packard) using a guard column (Zorbax SB-C18 4.6 × 125 mm, 5 μm pore size, Hewlett-Packard) at ambient temperature. Extracts were passed through a 0.45 μm filter (Millipore SAS, Molsheim, France), and 20 μL was injected in a Hewlett-Packard (Germany) system equipped with a G1311A quaternary pump and a G1315A photodiode array UV−vis detector. The mobile phase was acetonitrile (A) and acidified water containing 5% formic acid (B). The gradient was as follows: 0 min, 5% A; 10 min, 15% A; 30 min, 25% A; 35 min, 30% A; 50 min, 55% A; 55 min, 90% A; 57 min, 100% A, held for 10 min before returning to the initial conditions. The flow rate was 1.0 mL/min, and the wavelengths of detection were set at 280 and 330 nm. The phenolic components were identified by comparing the retention times and spectra with those of commercially available standard compounds. For the purpose of quantification, linear regression models were determined using standard dilution techniques.

Statistical Analysis. All samples were prepared and analyzed in triplicate. Results are reported as the mean ± standard deviation for 10 measurements on each animal group. To compare the three groups, analysis of variance (ANOVA) was used.

RESULTS AND DISCUSSION

Animal Production. The introduction of a new product (distilled rosemary leaves) in an animal diet implies the control of the animals' productivity. Thus, monthly milk yield was measured from April to June (gestation) and from October to November (lactation). Changes in milk yield resulting from the diet are shown in **Table 3**. According to these results, the introduction of rosemary leaves into the animal diet in proportions of 10 and 20% does not modify productivity, when compared to the control group, because no statisticallly significant differences were detected between the milk yields of the different goat groups throughout the duration of the experimental assay.

Another important parameter to be controlled is the possible variation in the chemical composition of the milk resulting from the introduction of distillate byproduct into the animal diet. Accordingly, the most common parameters defining milk quality (fat, protein, lactose, and total dry extract content) were checked during the milking period studied. Results shown in **Table 4** correspond, as previously stated by Pérez et al. (*2*) and López et al. (*3*), to the normal values quantified for these parameters in Murciano-Granadina goats' milk. They also reflect the negligible influence of the introduction of rosemary leaves into the goats' diet on milk composition, because no statistically significant differences were detected among the values obtained from the three groups under assay (control, 10%, and 20%).

Table 4. Chemical Milk Composition[a]

	gestation	lactation
	Protein (%)	
control	3.65 ± 0.76	3,51 ± 0.59
10%	3.60 ± 0.55	3.40 ± 0.47
20%	3.51 ± 0.38	3.57 ± 0.52
	Fat (%)	
control	5.35 ± 0.86	6.36 ± 1.21
10%	5.47 ± 0.89	6.02 ± 1.28
20%	4.99 ± 0.88	5.98 ± 1.67
	Lactose (%)	
control	4.46 ± 0.83	4.75 ± 0.32
10%	4.59 ± 0.29	4.65 ± 0.31
20%	4.44 ± 0.41	4.60 ± 0.40
	Total Dry Extract (%)	
control	14.22 ± 1.36	15.27 ± 1.50
10%	14.37 ± 1.09	14.72 ± 1.50
20%	13.66 ± 1.19	14.81 ± 1.86
	pH	
control	6.71 ± 0.09	6.82 ± 0.12
10%	6.70 ± 0.04	6.81 ± 0.05
20%	6.81 ± 0.05	6.80 ± 0.09

[a] Data are the mean of three replicates ± standard deviation.

In all of the studies published to date related to the incidence of polyphenols in animal production, the real effect of these components on milk composition and yield remains unclear. For example, Blauwiekel et al. (*20*) declared that feeding cattle a gossypol-rich diet increases milk yield, fat content, and noncasein content, which runs contrary to the idea that polyphenols are antinutritive due to the fact that they reduce the bioavailability of proteins and minerals (*4*).

However, the results reported in the present work confirm those published by Kowar et al. (*21*) and Baruah (*22*), who reported that the supplementation of dairy cattle feed with up to 20% decaffeinated tea waste, which has a high content of polyphenols, had little effect on milk yield or quality. From this point of view, the waste from rosemary distillation can be proposed as an ingredient in ruminant feed because it alters neither the yield nor the quality of Murciano-Granadina goats' milk.

Milk Polyphenolic Profile. As a consequence of the affinity of phenolic components to the milk casein fraction (*18*), prior to the polyphenolic quantification in goat's milk, the recovery efficiency of these compounds in casein was determined. The polyphenolic profile of the three diets assayed (Table**2**) revealed the presence of 12 major quantified phenolic components. **Table 5** shows the results obtained from this assay along with the limit of detection (LOD). In agreement with the results published by Ternes and Schwartz (*18*), phenolic diterpenes (carnosic acid and carnosol) were mostly extracted in the casein fraction. The efficiency recovery for carnosic acid calculated in the present work (87%) was higher than that published by the above authors (68%).

Table 5. Recovery of Polyphenols in Caseins and Limit of Detection[a]

component	recovery (%)	LOD (mg/kg)
galic acid	31.26 ± 0.67	0.0010
caffeic acid	50.00 ± 1.54	0.0034
ferulic acid	99.21 ± 5.41	0.0047
coumaric acid	56.44 ± 3.66	0.0031
naringin	39.79 ± 0.48	0.0056
hesperidin	27.56 ± 2.29	0.0125
luteolin	48.57 ± 1.85	0.0166
rosmarinic acid	84.93 ± 0.99	0.1051
apigenin	78.85 ± 3.97	0.0056
genkwanin	51.13 ± 0.63	0.0048
carnosol	77.62 ± 1.24	0.0910
carnosic acid	87.25 ± 0.77	0.0584

[a] Data are the mean of three replicates ± standard deviation.

However, flavonoids were the polyphenolic components with the lowest recovery efficiency, probably due to the strong interaction of these components with the casein gel.

To determine the transfer of polyphenolic components from the distillate rosemary leaves to the goats' milk, samples from the three animal groups under assay were studied. As a consequence of different metabolic demands, analysis of the samples was performed at the physiological stages of gestation and lactation.

The results obtained (**Table 6**) show that there was a positive transfer of some polyphenols to the milk. For instance, the following phenolic components were found in increased quantities in the milk from animals fed with a diet containing 10 or 20% aromatic plant byproduct, compared to the control group: the flavonoids hesperidin, naringin, and genkwanin; the lactone carnosol; and carnosic and gallic acids.

According to Reynolds (*23*), lypophilic components with a molecular weight under 500 (apigenin, luteolin, carnosol, and caffeic and carnosic acids) and under 100 for hydrophilic components easily cross lipidic membranes. Above these sizes, permeability is inversely dependent on the component molecular weight. Nevertheless, our results showed that hesperidin, naringin, and genkwanin, molecules with molecular weights of > 500, were the major components transferred to the milk.

Neither rosmarinic acid nor luteolin was detected in the milk analyzed, which demonstrated that these two components are not transferred at detectable levels in their native form to the milk after ruminant metabolism.

Hesperidin, according to Yung-Mei et al. (*24*) and Matsumoto et al. (*25*), is a flavonoid with reduced bioavailabilty in monogastric animals. No literature exists related to the metabolism of this component in ruminants, but in agreement with these authors, our results show that hesperidin could mainly be excreted through urine (personal communication) and milk secretion.

Naringin and genkwanin showed similar behaviors; both components were found in increased quantities in the goats' milk at the physiological stage of lactation. To observe a significant increase in naringin in the milk, a substitution of 20% of the control diet with rosemary leaves was necessary. However, a substitution of 10% was sufficient to obtain similar results for genkwanin.

The amounts of gallic acid administered to the goats were similar in the three groups under assay, due to the low concentration of this component in distillate rosemary leaves. However, an increase in the concentration of this component was detected in milk from goats fed the 20% diet, which was more noticeable during the lactation period. The presence of this component in higher concentrations could be related to the metabolism of other polyphenolic components after rumen metabolism. Studies carried out by Singh et al. (*26*) and Hiura et al. (*27*) on the biodegradation of tannic acid in an in vitro ruminal system confirm that the fermentation products from tannic acid are gallic acid, pyrogallol, and resorcinol.

The lactone carnosol is a component resulting from the oxidative degradation of carnosic acid. For this reason, it is present in higher concentrations than its precursor in distillate rosemary leaves. Nevertheless, the transfer of carnosic acid to the goats' milk was higher than that observed for carnosol. Accordingly, the introduction of rosemary leaves into a goat's diet

Table 6. Murciano-Granadina Goats' Milk Polyphenolic Profile[a]

	gestation	lactation
	Gallic Acid (mg/kg)	
control	0.08 ± 0.02 b**	0.16 ± 0.02 b**
10%	0.11 ± 0.02 bαβ	0.20 ± 0.12 bα
20%	0.17 ± 0.02 aα	0.27 ± 0.02 aβ
	Caffeic Acid (mg/kg)	
control	0.31 ± 0.01	0.30 ± 0.01
10%	0.33 ± 0.01 α	0.30 ± 0.01β
20%	0.30 ± 0.01	0.31 ± 0.01
	Ferulic Acid (mg/kg)	
control	0.15 ± 0.01	0.14 ± 0.00
10%	0.15 ± 0.01 α	0.14 ± 0.00 β
20%	0.16 ± 0.01	0.14 ± 0.00
	Coumaric Acid (mg/kg)	
control	0.29 ± 0.02	0.28 ± 0.05
10%	0.35 ± 0.02 α	0.29 ± 0.06 β
20%	0.34 ± 0.02	0.30 ± 0.05
	Naringin (mg/kg)	
control	0.74 ± 0.01 bα**	0.81 ± 0.03 bαβ**
10%	0.76 ± 0.01 aβ	0.85 ± 0.04 bα
20%	0.77 ± 0.01 aβ	0.97 ± 0.03 aα
	Hesperidin (mg/kg)	
control	2.05 ± 0.14 b**	1.95 ± 0.23 b**
10%	2.19 ± 0.14 b	2.69 ± 0.26 a
20%	2.60 ± 0.14 a	2.85 ± 0.24 a
	Apigenin (mg/kg)	
control	0.19 ± 0.01	0.19 ± 0.00
10%	0.20 ± 0.01	0.18 ± 0.00
20%	0.20 ± 0.01 α	0.19 ± 0.00 β
	Genkwanin (mg/kg)	
control	0.64 ± 0.02	0.64 ± 0.01 b*
10%	0.63 ± 0.02	0.70 ± 0.02 a
20%	0.68 ± 0.02	0.70 ± 0.01 a
	Carnosol (mg/kg)	
control	0.40 ± 0.03 α	0.38 ± 0.04 bα**
10%	0.43 ± 0.02	0.51 ± 0.04 a
20%	0.43 ± 0.03	0.54 ± 0.03 a
	Carnosic Acid (mg/kg)	
control	0.25 ± 0.01 bα**	0.45 ± 0.05 bα**
10%	0.31 ± 0.01 aγ	0.63 ± 0.06 aα
20%	0.33 ± 0.01 aβ	0.77 ± 0.05 aα

[a] Different English letters denote significant differences among treatments; *, $P < 0.05$); **, $P < 0.01$. Different Greek letters denote significant differences among values in the same row ($P < 0.05$) ± standard deviation.

Table 7. Murciano-Granadina Suckling Goat Kid Plasma Polyphenolic Profile[a]

polyphenol	control (mg/L)	10% (mg/L)	20% (mg/L)
gallic acid	nd	nd	nd
caffeic acid	3.92 ± 0.29	3.74 ± 0.11	3.67 ± 0.07
ferulic acid	0.26 ± 0.01	0.28 ± 0.02	0.29 ± 0.02
coumaric acid	5.26 ± 0.23	5.82 ± 0.25	5.84 ± 0.56
naringin	0.52 ± 0.05	0.59 ± 0.05	0.61 ± 0.09
hesperidin	2.82 ± 0.44	2.90 ± 0.6	2.84 ± 0.93
luteolin	0.95 ± 0.06	0.98 ± 0.1	0.95 ± 0.04
rosmarinic acid	nd a	0.78 ± 0.07 b	0.81 ± 0.06 b
apigenin	0.50 ± 0.01	0.51 ± 0.01	0.50 ± 0.01
genkwanin	0.32 ± 0.02	0.35 ± 0.03	0.33 ± 0.02
carnosol	nd a	0.31 ± 0.05 b	0.38 ± 0.17 b
carnosic acid	3.22 ± 0.53 a	6.17 ± 0.62 b	7.83 ± 0.9 b

[a] Different letters denote significant differences among treatments ($P < 0.05$) ± standard deviation.

increases the presence of carnosic acid in its milk, showing statistically significant differences in the 10 and 20% groups with respect to the control group. This fact was observed at both physiological stages (gestation and lactation), although in higher concentrations in the lactation stage, probably due to the transfer of this component to the fetus during gestation. These results are in agreement with those published by Yan et al. (*28*), who found that carnosic acid exhibits high levels of absorption through the diet (65%) of monogastric animals. At the same time, considering the statements published by Reynolds (*23*), this diterpene has an elevated placental permeability, which could justify its higher level of transfer into the milk during lactation as compared to the gestation period.

The different metabolic demands associated with different physiological stages should also be taken into account. Depending on physiological conditions, according to Castillo et al. (*29*), metabolic activity may determine the antioxidant status in cows. These authors evaluated the antioxidant status of healthy cows during late pregnancy and lactation. A negative correlation was detected between the total antioxidant status in plasma and cholesterol. Cholesterol metabolism requires cytochrome P-450, which is a significant source of reactive oxygen metabolites that consume antioxidants (*29*). This could also explain the different concentrations of polyphenols detected in milk during pregnancy and lactation periods. The consumption of components with high antioxidant power is associated with an increase in metabolic demands, occurring to maintain the balance of oxidant/antioxidant status in blood (*30*).

Polyphenols in Goat Kid Plasma. The polyphenolic profile of suckling goat kid plasma is shown in **Table 7**. The presence of polyphenols in the plasma can be associated with the transfer of these components during gestation (*23*) and lactation periods (*7*, *31*).

It is interesting to highlight the detectable presence of rosmarinic acid and luteolin in kid plasma. These components were not detected in the dams' milk, so the possible transfer, according to Thurmon et al. (*32*), could be attributed to maternal blood flow.

The high concentration of carnosic acid in goat kid plasma, showing statistically significant differences in the groups on 10 or 20% substitution diets with respect to the control group, could be justified by the high levels of absorption of this component through the diet (*28*), as well as by its high level of placental permeability and its distribution through fetal blood flow (*23*).

A similar behavior was observed for carnosol. Given the chemical structure of this lactone and considering that it comes from the oxidative degradation of carnosic acid, one would expect the transfer of this diterpene to be close to that of its precursor. Nevertheless, once again, the bioavailability of carnosol seems to be lower than that of carnosic acid.

No statistically significant differences were detected for the remaining polyphenols quantified in goat kid plasma among the three groups studied. Therefore, this fraction of polyphenolic content in goat kid plasma is not modified by the introduction of distillate rosemary leaves into the dams' diet.

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