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CHARACTERIZATION AND POTENTIAL USE OF OLIVE PIES FROM DIFFERENT EXTRACTIONS AND INDUSTRIES IN RUMINANT FEEDING

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Abstract

This work aimed to characterize coproducts from extra virgin olive oil extraction, its chemical composition and nutritive value of two olive cultivars (Arbequina and Koroneiki) in industries in Brazil Southern region. Sampling from cultivar Arbequina was done at two moments (time 0 and 48h post processing) and from cultivar Koroneiki, only at time zero was sampled. The composition of the two cultivars was different, as expected. Higher levels of carbohydrates (CHO) and lower levels of neutral detergent fiber (NDF) contributed to improve in situ degradability (61.0 vs 43.5%) of Koroneiki cultivar. Both co-products obtained on the day of processing showed high levels of lignin, which led to a lower level of in situ degradability of the NDF (21.1%). High levels of EE (19.4% DM), total phenols (FT; 1.8% DM), total tannins (TT; 1.11% DM) and TDN (72.7% DM) were also observed. These results demonstrate that the byproduct represents a potential source of lipids and phenolic compounds that can be used to adjust diets as well as the aggregation of functional molecules in meat and milk products. The care in obtaining and preserving the product is important because the material obtained 48 hours after the processing showed losses in the contents of CHO, CNF, FT and TT, which can impair its nutritional value and the potential of use.

Keywords carbohydrate fractions, co-products, lipids, *Olea europaea* L., phenolic compounds

CARACTERIZAÇÃO E POTENCIAL DE UTILIZAÇÃO DE TORTAS DE OLIVA PROVENIENTES DE DIFERENTES EXTRAÇÕES E INDÚSTRIAS NA ALIMENTAÇÃO DE RUMINANTES

Resumo

O objetivo foi caracterizar coprodutos da extração do azeite de oliva extravirgem, sua composição química e valor nutritivo de duas cultivares de azeitona (Arbequina e Koroneiki) de indústrias do sul do Brasil. As amostras do cultivar Arbequina foram realizadas em dois momentos (tempo 0 e 48h pós-processamento) e da cultivar Koroneki somente no tempo 0. Níveis superiores de carboidratos (CHO) e os menores de fibra em detergente neutro (FDN) contribuíram para melhorar degradabilidade *in situ* (61,0 vs 43,5%) da cultivar Koroneiki. Ambos os subprodutos obtidos no dia do processamento apresentaram altos teores de lignina, o que ocasionou um menor teor da degradabilidade *in situ* da FDN (21,1%). Também foram observados altos teores de EE (19,4% MS), fenóis totais (FT; 1,8% MS), taninos totais (TT; 1,11% MS) e NDT (72,7% MS). Esses resultados demonstram que o subproduto representa uma fonte potencial de lipídios e compostos fenólicos que podem ser utilizados para adequação de dietas, bem como agregação de moléculas funcionais em carnes e produtos lácteos. O cuidado na obtenção e conservação do produto é importante, pois o material obtido 48 horas após o processamento apresentou perdas nos teores de CHO, CNF, FT e TT, o que pode prejudicar seu valor nutricional e o potencial de aproveitamento.

Palavras-chave compostos fenólicos, coprodutos, frações de carboidratos, lipídios, *Olea europaea* L.

INTRODUCTION

According to the International Olive Council (2013), Brazil is the third largest importer of olive oil. The country has regions with adequate soil and climatic conditions for the cultivation of olive trees and for this reason, olive growing has been arousing interest in recent years, and in terms of commercial production is still an emerging but expanding agricultural activity (CHAVES et al., 2021).

The processing of olives to obtain extra virgin olive oil, obtained by cold, either by pressing and decanting or by continuous system followed by centrifugation, presents low yield, resulting in a co-product (olive cake) consisting of pulp, skin and water (FILODA *et al*., 2021). Thus, olive oil production is associated with the production of large quantities of olive cake, which is difficult to eliminate and can generate environmental liabilities if not correctly destined (VARGAS-BELO-PÉREZ *et al*., 2013).

The cold extraction has the objective of preserving the quality of olive oil, which promotes a partial extraction, which makes the industrial waste a potential alternative lipid source for animal feed. In addition, when used for this purpose, this co-product can transfer important molecules for the improvement of meat or milk quality, with potential beneficial effects on animal health (TZAMALOUKAS et al., 2021).

Among the compounds present in olives, polyphenol levels are highly significant due to their wide range of biochemical and pharmaceutical effects, including anti-carcinogenic, anti-atherogenic and antimicrobial properties reported in humans (HABEEB et al., 2017; MORALEs and UNGERFELD, 2015). However, the occurrence of these compounds in olive cake depends on several factors, such as the degree of fruit maturity, cultivar, soil-climatic conditions, and method of olive extraction (FILODA et al., 2021).

The production chain of the olive tree of Brazil, is still in the structuring phase, does not explore the possibility of marketing and use of olive cake for animal feed, perhaps due to the scarce information in the literature regarding its chemical composition and nutritional values for the different olive cultivars implanted here.

So, we aimed to characterize the nutritional parameters, phenolic compounds and in situ degradability of two olive cakes of different cultivars

("Arbequina" and "Koroneiki"), each from different industries in addition to the evaluation of two storage times for Arbequina cultivar after obtaining it in the industry (0 and 48 hours) to evaluate its chemical composition.

MATERIAL AND METHODS

Olive cake sampling

The olive cakes were obtained from the olive oil extraction, in two different industries. The Arbequina cultivar was collected at Batalha olive oil industry, at Pinheiro Machado-RS (Latitude: 31° 34' 37'' S, Longitude: 53° 23' 6'' W), with the same extraction process for both storage times. Eight barrels were collected for each storage time (0 and 48 hours), considering the collection time from the industry to arrival at the laboratory as time 0 and, from that time on, 48 hours. The Koroneiki cultivar was collected at Verde Louro olive oil industry, in the municipality of Canguçu-RS (latitude 31º23'42" S and longitude 52º40'32" W), with the entire sample resulting from the same batch of extraction (8 barrels). The barrels were hermetically sealed and transported to the Bromatological and Animal Nutrition LAB of Brazilian Agricultural Research Corporation (EMBRAPA-Clima Temperado Station), to carry out the analyzes and evaluations.

Experimental management

Each barrel was considered an experimental unit, with 8 barrels for each treatment. Immediately upon arrival at the laboratory, the olive cake contained in each barrel was homogenized inside the barrels by centrifugation and retropulsion movements using a large ladle and a filling for approximately 3 minutes each. These samples were homogenized again, and approximately 30% of this sample was removed, resulting in a single sample/barrel. This same procedure was repeated four more times, resulting in five replicates for each collection time. The representative samples were frozen at -20 ° C for further analyzes, others lyophilized and dried at 55 °C for 72 h in a forced-air oven the bromatological evaluations. This collection procedure for the cultivar Arbequina was repeated after 48 hours of storage, and samples of the material conditioned in the barrels were collected.

Assessments

Partially dried samples were ground in a 1-mm and 2-mm sieve of a Wiley-type mill™ (Thomas Scientific). The chemical analyses for nutritional compounds were performed in 1mm pre-dried samples for dry matter (DM) and ash (mineral matter MM) by Method 967.03; AOAC, 1998, organic matter (OM) estimated by the equation 100-% MM. Crude protein (CP) was estimated from the total nitrogen value (N), using the Kjeldahl method (AOAC, 2001), and ether extract according to AOAC (1996), and neutral detergent fiber (NDF) with addition of thermostable α-amylase but without the use of sodium sulfite, acid detergent insoluble fiber (ADF), acid detergent lignin (ADL) (VAN SOEST *et al*. 1991), using autoclave (SENGER *et al*. 2008). The contents of Hemicellulose and Cellulose were determined by the difference of NDF and ADF + ADL were performed sequentially. Neutral detergent insoluble nitrogen (NDIN), acid detergent insoluble nitrogen (ADIN), non-protein nitrogen (NPN), followed the methodology described by Licitra *et al*. (1996). In the frozen samples, the pH and buffer capacity (BC) were determined according to Playne & Mcdonald (1966), ammonia nitrogen ($N\text{-}NH_3$) according to techniques proposed by Bolsen (1992) and Vieira (1980) and water-soluble carbohydrates according to methodology described by Dubois *et al*. (1956).

The total phenols and total tannins were determined using the lyophilized samples (MAKKAR, 2000), keeping the samples immersed in ice water throughout the process.

The total digestible nutrients (TDN) were calculated using the Weiss equation (1999): TDN = PBD + (2.25 x EED) + NDFcpD + CNFcpD, where PBD = digestible crude protein; EED = digestible ether extract; NDFcpD = neutral detergent fiber, corrected for ash and protein, digestible; CNFcpD =non-fiber carbohydrates, corrected for ash and protein, digestible. To convert TDN values to net energy of lactation (NEl), it was used the equation described by the NRC (2001): EL 3x (mcal/kg) = 0.0245*NDT (%) - 0.12

In situ degradability was determined using four ruminally fistulated Jersey cows (DETMANN et al., 2001). All surgical and animal care protocols were approved by the Ethics Committee on Animal Research from Universidade Federal de Pelotas (CEEA n°5076-2013). These females were kept on pasture and received concentrated supplementation containing 20% of olive cake and mineral salt at will. For incubation, 1 g of partially dried sample, 2 mm ground ground, were weighed into polyester bags

(5 x 5 cm and 50 µm porosity). The post-incubation residues were washed in running water until the water flowed clear and kept in solution for bacterial dissociation for 15 minutes (Whitehouse *et al*. 1994) and neutral detergent solubilization according to Goering & Van Soest (1970) to predict total digestibility.

Experimental design and statistical analysis

The experimental design was completely randomized, with two treatments (zero and 48 hours) and three replications. The values found for the different cultivars were not statistically evaluated since, in this case, the objective was solely to characterize the co-product.

The results for Arbequina cultivar cake collected at different periods after being obtained in the industry (0 and 48 hours) were analyzed using the General Linear Models procedure (PROC GLM), according to the statistical model:

$$Yij = \mu + \alpha i + \varepsilon ij,$$

where Yij is the value observed for the i-th storage time of the olive cake (0 and 48 hours) and j-th repetition; µ + αi is the mean for the storage time in repetition i; εij is the random error associated with the measurement in the different storage times in the ith repetition. The measurements were estimated using the LSMEANS (Least Squares Means) command and compared by the Tukey test at the 5% level of significance. Statistical analyzes were performed on the SAS® System for Windows ™ version 9.0 application.

RESULTS AND DISCUSSION

DM content differed significantly ($p < 0.05$) for the same cultivar at different storage times (0 and 48h), probably due to evaporation of water in the material stored for a longer time (Table 1). Regarding the cultivars, both underwent the same industrial processing of two phases to obtain extra virgin olive oil, justifying this similarity.

The dry matter contents of the co-product can be influenced by the type of extraction, by the type of the equipment used in the oil extraction, as the cultivar and the climatic conditions. The values of DM found are lower than those described in the literature (CHEBAIBI et al., 2019; CIBIK and KELES, 2016) who related DM value above than 320g/kg from wet material. This type of difference is pretty common when

Table 1. Dry matter (DM), hydrogenation potential (pH), buffer capacity (BC), soluble carbohydrates (CHO's), total phenols (TP) and total tannins (TT) of different co-products from olive oil extraction.

	DM (% of IM*)	pH	CT (mEq NaOH) **	CHO's (%of DM)	TP (% of DM)	TT (%of DM)
Koroneiki[1]	28.22±0.14	5.42±0.01	21.27±0.28	13.53±0.39	1.65±0.019	0.94±0.019
Arbequina[2]	29.83±0.34b	5.40±0.01	21.16±0.34a	8.81±0.62a	2.02±0.001a	1.28±0.003a
Arbequina[3] (48h)	32.06±0.66a	5.44±0.04	19.05±0.21b	5.07±0.17b	1.77±0.036b	1.07±0.026b
p-value*	0.0064	0.3295	0.0008	0.0006	0.0002	0.0002
SEM	0.3040	0.0169	0.1648	0.2630	0.0148	0.0107

[1] Olive Cake (*Olea europaea*) cv. Koroneiki; [2] Olive Cake (*Olea europaea*) cv. Arbequina; [3] Olive Cake (*Olea europaea*) cv. Arbequina after 48 hours of storage; * IM= Integral matter;** Milliequivalent gram of sodium hydroxide needed to elevate the pH of 4 for 6;
a, b Means followed by different letters in the same column differ from each other; * P values greater than 0.05 do not differ from each other by the 5% Tukey test; SEM - standard error of the mean.

the material is considered as an alternative feeding, in this case, as a function of the different forms of olive oil extraction (pressing or centrifugation), as well as by the product used by these authors have been previously dried for their conservation. This is necessary insofar as the average time of deterioration of the fresh olive cake obtained by centrifugation is 4 to 5 days post extraction (ROSELLÓ-SOTO *et al*., 2015) compared to about 15 days for the same coproduct when obtained by pressing. However, considering its *in natura* use, the DM content does not seem to be limiting, since it resembles that of other forages used in ruminant diets and especially the one recommended for grass silage.

No effect of the storage time was observed for pH, and the values found for the different cultivars were similar. The buffer capacity presented a significant difference ($P<0.05$) for the effect of storage time being higher in the fresh material, fact that occurs due to the low variability of the analysis even with such small differences. The cultivars Arbequina and Koroneiki showed similar values. The results found are very close to the upper limit of 20 mEq of NaOH / 100 g of DM described by Fernandes, et al. (2008) as favorable to fermentation.

The values of water-soluble carbohydrates (CHO's) were significantly higher ($P<0.05$) for fresh olive cake, when compared to the 48-hour stocking in the industry. This difference is probably due to the fact that these carbohydrates have been consumed by microorganisms present in the olive cake, or due to the continuity of plant cell metabolism. However, the contents found for CHO in this cultivar are lower

than those considered optimal (10% of DM) by Kung Jr. et al. (2018) for a successful fermentation process in the form of silage. In this aspect, the Koroneiki had higher soluble CHO values than the Arbequina, and above the value considered optimal as previously mentioned.

According to Wróbel et al. (2023) and Kung et al. (2018), the knowledge of the soluble CHO content present in the food, together with data from its BC, and DM content are good indicators to predict the fermentative capacity of the food when destined to silage. When we observed the values found for CHOs in the different co-products of olive oil extraction, we assumed that they are similar to those found by Cibik and Keles (2016), which reported 121 g/kg of DM for fresh olive cake.

Regarding the total phenol content, there was a significant reduction ($P<0.05$) in the olive cake stored for 48 hours when compared to that obtained soon after processing, when higher concentrations were observed for Arbequina cultivar. This reduction was probably due to the oxidation of phenolic compounds present in the olive cake (CHEBAIBI et al., 2019) in an attempt to avoid oxidation of the fatty acids present in the lipid fraction; as these compounds help in maintaining the oxidative stability of olive oil, although its presence is much lower than that found in olive cake. In our study, total phenol contents were similar to those described by Hamdi *et al*. (2018). Chebaibi et al. (2019), evaluating fresh olive cake from four different cultivars in Morocco, found contents of total phenols varying from 41.6 g/kg to 70.4 g/kg DM, higher than both cultivars evaluated.

Among the phenolic compounds present in the olive cake a large quantity of tannins were found, which are defined as a heterogeneous complex of polyphenols of vegetable origin with high molecular weight (500 to 3000 Da) (BESHARATI et al., 2022; SMERIGLIO et al., 2017), which differ from and other polyphenols due to their ability to precipitate proteins (Adamczyk et al., 2017; LORENZ et al., 2014), metal ions, amino acids and polysaccharides, among which polymers such as cellulose, hemicellulose and pectin, thereby decreasing their digestibility (NAUMANN et al., 2017; LORENZ et al., 2014).

However, these compounds do not alter the availability of these constituents while maintaining plant cell integrity, since they are stored in vacuoles in plant cells, occurring only at the time of cell disruption (BESHARATI et al., 2022), as in olive grinding for extraction of olive oil. In the present study, the reduction in the total

phenol content was also accompanied by a significant reduction in the total tannin content for the olive cake stocked for 48 hours, remaining the values found above those described by Chebaibi et al. (2019).

The expressive levels of total phenols found in the olive cakes analyzed in the present study, from those duly preserved, when offered in the ruminant diet, can serve as a source of antioxidant compounds, acting beneficially on animal health with the potential for aggregation in animal tissues and their products. Scientific studies with natural phenolic compounds have been developed in order to try to transfer these substances to the animal tissues and their final products (meat and milk) (HAMDI et al., 2018), improving, for example, the oxidative stability of the meat, increasing the product image and shelf life.

The analyzed materials did not differ (P> 0.05) regarding the mineral content and concentration of the analyzed minerals (Table 2). Even if not statistically different, the cultivar Koroneiki showed higher mineral matter content than the cultivar Arbequina, probably due to differences between the properties of origin of the materials with respect to the fertility of the soil.

Table 2. Contents of mineral matter (MM), ether extract (EE), Calciun (Ca), Magnesium (Mg) e Potassium (K) of different co-products from the extraction of olive cake.

	MM (% of DM)	EE (% of DM)	Ca (% of DM)	Mg (% of DM)	K (% of DM)
Koroneiki[1]	3.79±0.05	19.90±1.14	1.63±0.04	1.60±0.01	0.98±0.06
Arbequina[2]	3.27±0.07	18.85±0.44a	1.60±0.07	1.42±0.03	0.91±0.03
Arbequina[3] (48h)	3.21±0.04	19.69±0.08b	1.55±0.09	1.47±0.10	0.97±0.03
p-value*	0.0634	0.0295	0.4716	0.4221	0.4221
SEM	0.0349	0.1798	0.0474	0.0448	0.0159

[1] Olive Cake (*Olea europaea*) cv. Koroneiki; [2] Olive Cake (*Olea europaea*) cv. Arbequina; [3] Olive Cake (*Olea europaea*) cv. Arbequina after 48 hours of storage; a, b Means followed by different letters in the same column differ from each other; * P values greater than 0.05, do not differ from each other by the 5% Tukey test; SEM - standard error of the mean.

The content of ether extract showed significant variation (P<0.05) in storage time, being higher values found in the olive cake stored for 48 hours (Table 2). It is probable that this finding is due to dilution factors, that is, the decrease in other fractions (NFC and CHO's, for example) ends up increasing the content of the other nutrient fractions. The values of EE (lipids) found for the different co-products of olive oil extraction analyzed in the present study are higher than those reported in the literature by Vargas-Bello-Pérez *et al*. (2013) (13.9% EE in DM), reflecting the

variability in the efficiency of olive oil extraction by industry, or differences between cultivars evaluated, since the great majority of studies evaluating the inclusion of olive cake in ruminants diet do not disclose this information.

The content of ether extract present in olive cake resembles values as described in the NRC (2001) for cotton seed (19.3 ± 1.4% of DM), being superior, for example, to the bran of brown rice (15.2 ± 4.2% of DM), a food traditionally used in the region. The EE content in olive cake demonstrates that this co-product may represent a potential source of mono- and polyunsaturated fatty acids to be exploited in the ruminant diet.

Samples of the olive cake from Arbequina, collected in two times after extraction of olive oil, showed significant differences ($p < 0.05$) in NDF and ADF levels (Table 3). These differences probably have the same explanation given to the ether extract content, that is, dilution effect.

Table 3. Contents of the fiber components and non-fibrous carbohydrate (NFC) of different co-products from the extraction of olive oil.

	NDF (% of DM)	ADF (% of DM)	CEL (% of DM)	HEM (% of DM)	LIG (% of DM)	SIL (% of DM)	NFC (% of DM)
Koroneiki[1]	46.91±1.56	41.00±0.33	22.36± 0.59	5.90±1.22	16.68±2.04	1.08±0.07	21.63±0.08
Arbequina[2]	53.80±0.87b	40.48±1.86b	27.08±2.13	13.31±1.45	14.32±1.58	0.91±0.14	16,61±0.25a
Arbequina[3] (48h)	58.10±0.65a	46.10±0.85a	28.70±0.90	12.00±0.78	16.27±0.90	0.87±0.05	11.27±0.53b
p-value*	0.0024	0.0089	0.2929	0.2374	0.1370	0.5976	<0.0001
SEM	0.4426	0.8348	0.9427	0.6707	0.7415	0.0576	0.2519

[1] Olive Cake (*Olea europaea*) cv. Koroneiki; [2] Olive Cake (*Olea europaea*) cv. Arbequina; [3] Olive Cake (*Olea europaea*) cv. Arbequina after 48 hours of storage; NDF - neutral detergent fiber; ADF - acid detergent fiber; CELL - cellulose; HEM - hemicellulose; LIG - lignin; Silica; NFC - non-fibrous carbohydrates;
a, b Means followed by different letters in the same column differ from each other; * P values greater than 0.05 do not differ from each other by the 5% Tukey test; SEM - standard error of the mean.

The NDF and ADF values found for olive cake stored for 48 hours are similar to those reported by Vargas-Bello-Pérez et al. (2013) and Awawdeh & Obeidat (2013) but higher than reported by Cibik and Keles (2016), with 96 g/kg DM for NDF content. When compared both cultivars, NDF contents are higher in the Arbequina, due to the higher levels of cellulose and hemicellulose. Cellulose, hemicellulose, lignin and silica were not affected by the storage time (Table 3). The lignin content observed was higher than that found in other co-produtcs, and its similar that reported by Ferrer et al. (2020), evaluating the efficiency of olive cake in piglet diets. According to De Blas et al. (2015), olive cake presents lignin content higher than the most of

alternative foods, as an expressive variable content, with means between 160 to 557 g lignin/kg DM.

The CP, NDIN and ADIN contents did not differ in olive cake of Arbequina cultivar at different storage times, these fractions being numerically lower in Koroneiki cultivar (Table 4). The CP contents found in the present study are supported by reports from the literature for olive cake, with average values around 6-7% of DM (HADHOUD et al., 2020), however, it is noteworthy known that approximately 30% of the total nitrogen is bound to the fiber lignocellulosic fraction (ADIN), making it unavailable for ruminal microbiota.

Table 4. Mean crude protein (CP), non-protein nitrogen (NPN), neutral detergent insoluble nitrogen (NDIN) and acid detergent insoluble nitrogen (ADIN) of different co-products from the extraction of olive oil.

	CP (% of DM)	NPN (% of TN	NDIN (% TN)	ADIN (% TN)
Koroneiki[1]	7.77±0.29	35.14±1.52	32.03±0.12	24.83±0.51
Arbequina[2]	8.14±0.15	72.83±2.80a	51.90±3.58	31.70±0.63
Arbequina[3] (48h)	8.56±0.22	47.07±3.53b	45.39±1.32	31.50±0.88
p-value*	0.0510	0.0006	0.1238	0.8647
SEM	0.1094	1.8433	2.3675	0.7396

[1] Olive Cake (*Olea europaea*) cv. Koroneiki; [2] Olive Cake (*Olea europaea*) cv. Arbequina; [3] Olive Cake (*Olea europaea*) cv. Arbequina after 48 hours of storage; TN= Total Nitrogen; a, b Means followed by different letters in the same column differ from each other; * P values greater than 0.05, do not differ from each other by the 5% Tuckey test; SEM - standard error of the mean.

The storage for 48 hours reduced the NPN contents without affecting the CP content, this variation is probably due to the use of part of this nitrogen fraction by the microorganisms present in the olive cake, which corroborates with the differences observed in the CHO and NFC values, assuming a rapid consumption of these compounds would potentially occur as a source of energy and protein for microbial growth. It should be noted that no ammoniacal nitrogen was detected in the samples under study, which justifies the use of other nitrogen compounds.

The estimated values of total digestible nutrients (TDN) and net lactation energy (NEl) were shown to be close, and there were no significant differences between the samples of the olive cake collected in two times after extraction of the olive oil (Table 5), being also similar to the values determined for the cultivar Koroneiki. The estimated values of TDN and NEl of lactation were higher than those estimated by Fadel & El-Ghonemy (2015) for TDN (34% of DM), and NEl reported by Vargas-Belo-Pérez *et al*. (2013) and Cibik & Keles (2016) (1.01 and 1.23 Mcal / kg DM,

respectively), probably due to the higher EE participation in the olive cakes analyzed in this study.

The *in situ* degradability values of the fiber fraction were lower after storage of the olive cake for 48 hours, in the two incubation times evaluated, differing significantly (P <0.05). The lower degradability of this nutrient fraction may be associated with the complexation of its constituent fractions with tannins present in the olive cake (TZAMALOUKAS et al., 2021; EL OTMANI et al., 2019). These lower digestibility values of the fiber fraction seem to explain the significant (P<0.05) reduction also observed in DM digestibility in the first 24 h of incubation.

It was observed higher dry matter degradability values for the Koroneiki in relation to the Arbequina samples, probably due to the higher CHO and NFC participation in this cultivar, as well as numerically inferior values of total nitrogen bound to the fiber lignocellulosic fraction (ADIN).

Table 5. Total digestible nutrients (TDN), Liquide energy of lactation (LEl), *in situ* degradability of dry matter (ISDMD) and fiber fraction (ISNDFD) of different olive cake

	TDN (% of DM)	LE l (Mcal/kg of DM)	ISDMD (24h) (% of DM)	ISDMD (48h) (% of DM)	ISNDFD (24h) (% of NDF)	ISNDFD (48h) (% of NDF)
Koroneiki[1]	73.34±3.70	1.86±0.12	55.83±0.21	61.01±0.49	18.46±0.62	22.26±0.32
Arbequina[2]	72.07±2.17	1.81±0.07	39.13±1.17a	43.46±1.62	15.16±0.56a	20.02±1.90a
Arbequina[3] (48h)	69.26±1.33	1.73±0.05	33.65±0.34b	40.58±2.09	5.93±0.89b	10.32±3.29b
p-value*	0.1277	0.1705	0.0015	0.1210	<0.0001	0.0118
SEM	1.0129	0.0324	0.4973	1.0813	0.4313	1.5614

[1] Olive Cake (*Olea europaea*) cv. Koroneiki; [2] Olive Cake (*Olea europaea*) cv. Arbequina; [3] Olive Cake (*Olea europaea*) cv. Arbequina after 48 hours of storage; a, b Means followed by different letters in the same column differ from each other; * P values greater than 0.05 do not differ from each other by the 5% Tukey test; SEM - standard error of the mean.

All the samples evaluated showed a ISDMD lower when compared to study carried out by El Otomani et al. (2019), which describe values above 900 g/kg DM for this parameter. In general, the alternative food, for not having a standard for its use in animal feeding, not only show great variability in their chemical composition between samples, but compared to the same type of food (PRACHE et al., 2022; MAKAR, 2018), but their impact is direct on digestibility. In addition to the anti-nutritional factors, it is necessary to know the nutritional composition of these foods before supplying any category, aiming not only to optimize production, but not to cause future problems in the production system.

CONCLUSION

Despite the low digestibility of the fiber fraction, the high content of ether extract in the olive cake makes its use as an energy source.

It can also serve as an important source of interesting phenolic compounds from the nutraceutical point of view, both for animal health, as human.

Further studies are needed in order to determine the conservation of this co-product and levels of inclusion in the diet of ruminant species, given its seasonal availability.

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REFERENCES

ADAMCZYK B.; SIMON J.; KITUNEN V.; ADAMCZYK S.; SMOLANDER A. Tannins and Their Complex Interaction with Different Organic Nitrogen Compounds and Enzymes: Old Paradigms versus Recent Advances. **Chemistry Open**. v.16; n.6, p.610-614, 2017. https://doi.org/10.1002/open.201700113.

A.O.A.C. ASSOCIATION OF OFFICIAL ANALYTICAL CHEMISTS. **Official Methods of Analysis of the Association of Official Analytical Chemistry**, 17th Edition Property, 2001.

A.O.A.C. ASSOCIATION OF OFFICIAL ANALYTICAL CHEMISTS **Official methods of analysis of the Association of Official Analytical Chemistry**.16a 2nd ed. Maryland, 1998.

A.O.A.C. ASSOCIATION OF OFFICIAL ANALYTICAL CHEMISTS **Official methods of analysis of the Association of Official Analytical Chemistry**, 16th ed. AOAC, Washington, DC, USA.

AWAWDEH, M. S.; OBEIDAT, B. S. Treated olive cake as a non-forage fiber source for growing awassi lambs: effects on nutrient intake, rumen and urine ph, performance, and carcass yield. **Asian-Australasian Journal of Animal Sciences**, v.26, n.5, p.661-667, 2013. https://doi.org/10.5713/ajas.2012.12513

BESHARATI M.; MAGGIOLINO A.; PALANGI V.; KAYA A.; JABBAR M.; ESECELI H.; DE PALO P.; LORENZO J.M. Tannin in Ruminant Nutrition: Review. **Molecules**, v.27, n.23, p.8273, 2022. https://doi.org/10.3390/molecules27238273

CHAVES, B.W.; VALLES, GUSTAVO, A.F.; SCHEIBLER, R.B.; SCHAFHÄUSER

JÚNIOR, J.; NÖRNBERG, J.L. Milk yield of cows submitted to different levels of olive pomace in the diet. **Acta Scientiarum:** Animal Sciences, v.*43*, Article e51158, 2021. https://doi.org/10.4025/actascianimsci.v43i1.51158

CHEBAIBI, S.; LERICHE GRANDCHAMP, M.; BURGÉ, G.; CLÉMENT, T.; ALLAIS, F.; LAZIRI, F. Improvement of protein content and decrease of anti-nutritional factors in olive cake by solid-state fermentation: A way to valorize this industrial by -product in animal feed. **Journal of Bioscience and Bioengineering**, v.128, n.3, p.384–390, 2019. https://doi.org/10.1016/j.jbiosc.2019.03.010

CIBIK M.; KELES, G. Effect of stoned olive cake on milk yield and composition of dairy cows. **Revue de Médecine Vétérinaire**, v.167, n.5-6, p.154-158, 2016.

DE BLAS C.; RODRIGUEZ C.A.; BACHA F.; FERNANDEZ R.; ABAD-GUAMÁN R. Nutritive value of co-products derived from olive cake in rabbit feeding. **World Rabbit Science**, v.23, p.255–262, 2015.

DETMANN, E., PAULINO, M. F., ZERVOUDAKIS, J. T., VALADARES FILHO, S. C., EUCLYDES, R. F., LANA, R. P. & QUEIROZ, D. S. Cromo e indicadores internos na determinação do consumo de novilhos mestiços, suplementados, a pasto. **Revista Brasileira de Zootecnia**, v.30, n.5, 1600-1609, 2001.

DUBOIS, M., GILLES, K. A., HAMILTON, J. K., REBERS, P. T., SMITH, F. Colorimetric method for determination of sugars and related substances. **Analytical Chemistry**, v.28, n.3, p.350-356, 1956.

EL OTMANI, S.; CHENTOUF, M.; HORNICK, J.; CABARAUX, J. Chemical composition and in vitro digestibility of alternative feed resources for ruminants in Mediterranean climates: Olive cake and cactus cladodes. **The Journal of Agricultural Science**, v.157, n.3, p. 260-271, 2019. https://doi.org/10.1017/S0021859619000558

FADEL, M.; EL-GHONEMY, D. H. Biological fungal treatment of olive cake for better utilization in ruminants nutrition in Egypt. **International Journal of Recycling of Organic Waste in Agriculture**, v.4, n.4, p.261-271, 2015.

FERNANDES, F. E. P; CARVALHO, G. G. P. D.; GARCIA, R.; PEREIRA, O. G.; PIRES, A. J. V.; OLIVINDO, C. D. S. Ensilagem de sorgo forrageiro com adição de ureia em dois períodos de armazenamento. **Revista Brasileira de Zootecnia**, v.38, n.11, p.2111- 2115, 2008.

FERRER, P.; CALVET, S.; GARCÍA-REBOLLAR, P.; DE BLAS, C.; JIMÉNEZ-BELENGUER, A. I.; HERNÁNDEZ, P.; CERISUELO, A. Partially defatted olive cake in finishing pig diets: implications on performance, faecal microbiota, carcass quality, slurry composition and gas emission. **Animal**, v.14, n.2, p.426–434, 2020. https://doi.org/10.1017/s17517311190020

FILODA, P.F.; CHAVES, FC; HOFFMANN, J.F.; ROMBALDI, C.V. Olive oil: a review on the identity and quality of olive oils produced in Brazil. **Revista Brasileira de Fruticultura**, v.43, n.3, Article e-847, 2021. https://doi.org/10.1590/0100-29452021847

GOERING, H. K.; VAN SOEST, P. J. **Forage Fiber Analysis**. USDA Agricultural Research Service. Handbook number 379. US Department of Agriculture.

Superintendent of Documents, US Government Printing Office, Washington, DC, 1970.

HABEEB, A.A.M.; GAD, A.E.; EL-TARABANY, A.A.; MUSTAFA, M.M.; ATTA, M. A.A. Using of olive oil co-products in farm animals feeding. **International Journal of Scientific Research in Science and Technology**, v.3, n.6, p.57-68, 2017.

HADHOUD, F.I.; SHAABAN, M.M.; ABD EL TAWAB, A.M.; KHATTAB, M.S.A.; EBEID, H.M.; GOUDA, G.A., ABDO, M.M. Olive cake silage as alternative roughage for ruminant: Effect on rumen degradability and *in vitro* gas production. **Egyptian Journal of Nutrition and Feeds**, v.23, n.2, p.265-272. 2020.

HAMDI, H.; MAJDOUB-MATHLOUTHI, L.; DURAND, D.; THOMAS, A., KRAIEM, K. Effects of olive-cake supplementation on fatty acid composition, antioxidant status and lipid and meat-colour stability of Barbarine lambs reared on improved rangeland plus concentrates or indoors with oat hay plus concentrates. **Animal Production Science**, Article FR20210167096, 2018. https://doi.org/10.1071/AN16352

KUNG JR., L., SHAVER, R.D., GRANT, R.J., SCHMIDT, R.J. Silage review: Interpretation of chemical, microbial, and organoleptic components of silages. **Journal of Dairy Science**, v.101, n.5, Article P4020-4033, 2018. https://doi.org/10.3168/jds.2017-13909

LICITRA, G.; HERNANDEZ, T. M.; VAN SOEST, P. J. Standardization of procedures for nitrogen fractionation of ruminant feeds. **Animal Feed Science and Technology**, v.57, n.4, p.347-358, 1996.

LORENZ MM, ALKHAFADJI L, STRINGANO E, NILSSON S, MUELLER-HARVEY I, UDÉN P. Relationship between condensed tannin structures and their ability to precipitate feed proteins in the rumen. **Journal of Science and Food Agriculture**, v.94, n.5, p.963-968, 2015. https://doi.org/10.1002/jsfa.6344.

MAKKAR, H. P. S. Review: Feed demand landscape and implications of food-not feed strategy for food security and climate change. **Animal**, v.12, n.8, p.1744–1754, 2017. https://doi.org/10.1017/s175173111700324x

MAKKAR, H. P. S. (2000). Quantification of tannins in tree foliage - a laboratory manual, a Joint FAO. **IAEA working document**, Vienna, Austria, 2000.

MORALES, R; UNGERFELD, E.M. Use of tannins to improve fatty acids profile of meat and milk quality in ruminants: A review. **Chilean Journal OF Agricultural Research**, v.75, n.2, p.239-248, 2015. https://dx.doi.org/10.4067/S0718-58392015000200014

NATIONAL RESEARCH COUNCIL – NRC. **Nutrient requirements of dairy cattle**. 7.ed. Washington, D.C. National Academic Press, p.381, 2001. https: // doi.org/10.17226/9825.

NAUMANN, H.D.; TEDESCHI, L.O.; ZELLER, W.E.; HUNTLEY, N. Invited review: The role of condensed tannins in ruminant animal production: advances, limitations and future directions. **Revista Brasileira de Zootecnia**., v.46, n.12, p.929-949, 2017.

PRACHE, S.; ADAMIEC, C.; ASTRUC,T.; BAÉZA-CAMPONE, E.; BOUILLOT, P.E.;

CLINQUART, A.; FEIDT, C.; FOURAT, E.; GAUTRON, J.; GIRARD,A.; GUILLIER, L.; KESSE-GUYOT, E.; LEBRET,B.; LEFÈVRE, F.; LE PERCHEC, S.; MARTIN, B.; MIRADE, P.S.; PIERRE, F.; RAULET, M.; RÉMOND, D.; SANS, P.; SOUCHON, I.; DONNARS, C.; SANTÉ-LHOUTELLIER,V. Review: Quality of animal-source foods. **Animal**, v.16, supp.1, Article 100376, 2022. https://doi.org/10.1016/j.animal.2021.100376

ROSELLÓ-SOTO, E.; KOUBAA, M.; MOUBARIK, A.; LOPES, R. P.; SARAIVA, J. A.; BOUSSETTA, N.; BARBA, F. J. Emerging opportunities for the effective valorization of wastes and by-products generated during olive oil production process: non-conventional methods for the recovery of high-added value compounds. **Trends in Food Science & Technology**, v.45, n.2, p.296-310, 2015. https://doi.org/10.1016/j.tifs.2015.07.003

SENGER, C. C.; KOZLOSKI, G. V.; SANCHEZ, L. M. B.; MESQUITA, F. R.; ALVES, T. P.; CASTAGNINO, D. S. Evaluation of autoclave procedures for fiber analysis in forage and concentrate feedstuffs. **Animal Feed Science and Technology**, v.146, n.1 -2., p.169-174, 2008.

SMERIGLIO A.; BARRECA D.; BELLOCCO E.; TROMBETTA D. Proanthocyanidins and hydrolysable tannins: occurrence, dietary intake and pharmacological effects. **Brazilian Journal of Pharmacology**, v.174, n.11, p.1244-1262, 2017. https://doi.org/10.1111/bph.13630

STATISTICAL ANALYSIS SYSTEM - SAS. (2002) The SAS system for windows. v.9.0 Cary. SAS Institute Inc.

TZAMALOUKAS O.; NEOFYTOU M.C.; SIMITZIS P, E. Application of Olive Co-products in Livestock with Emphasis on Small Ruminants: Implications on Rumen Function, Growth Performance, Milk and Meat Quality. ***Animals***, v.11, n.2, p.531, 2021. https://doi.org/10.3390/ani11020531

VAN SOEST, P. V.; ROBERTSON, J. B.; LEWIS, B. A. Methods for dietary fiber, neutral detergent fiber, and nonstarch polysaccharides in relation to animal nutrition. **Journal of Dairy Science**, v.74, n.10, p.3583-3597, 1991. https://doi.org/10.3168/jds.S0022-0302(91)78551-2

VARGAS-BELLO-PÉREZ, E., VERA, R. R., AGUILAR, C., LIRA, R., PEÑA, I., & FERNÁNDEZ, J. Feeding olive cake to ewes improves fatty acid profile of milk and cheese. **Animal Feed Science and Technology**, v.184, n.1-4, p.94-99, 2013. https://doi.org/10.1016/j.anifeedsci.2013.05.016

WEISS, W.P. Energy prediction equations for ruminant feeds. In: CORNELL NUTRITION CONFERENCE FOR FEED MANUFACTURERS, 61., 1999, **Proceedings...** Ithaca: Cornell University, 1999. p.176-185.

WHITEHOUSE, N. L.; OLSON, V. M.; SCHWAB, C. G.; CHESBRO, W. R.; CUNNINGHAM, K. D.; LYKOS, T. Improved techniques for dissociating particle-associated mixed ruminal microorganisms from ruminal digesta solids. **Journal of Animal Science**, v.72, n.5, p.1335-1343, 1994. https://doi.org/10.2527/1994.7251335x