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Fermentation parameters and nutritional value of olive cake silage

Parâmetros fermentativos e valor nutricional da silagem de torta de oliva

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Highlights

Adding corn to olive cake silage improves pH.
Adding corn to olive cake silage reduces the fibrous carbohydrates and lignin levels.
When the cake is stored for 48 hours, there is a loss of non-fibrous carbohydrates.

Abstract

The aim of this research was to improve the fermentation parameters and the nutritional value of olive cake silage (*Olea europaea* "Arbequina") *in natura* through two periods of rest of the material after the extraction of olive oil and/or with the addition of levels of ground corn. The matter was ensiled in two times after extraction (zero and 48 hours), using ground corn grain on dry matter basis, as an additive in the levels: 0, 5 and 10% of ensiled olive cake wet matter. The design was completely randomized, and the silages tested for fermentation characteristics, conservation, concentration of phenolic compounds, chemical composition and nutritive value. The silages did not differ in non-protein nitrogen, silica and

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NDF digestibility. At the zero time, it presented higher values of buffering capacity, total phenols, total tannins (TT) and lower dry matter compared to silages made from silage after 48 h. Silages containing contents 10% of corn compared with non-corn add, had higher digestibility, NDT content and lower pH and EE. The inclusion of corn improved the fermentation characteristics and the nutritional value of the fresh-olive cake silages. The ensilage technique has been shown to be an alternative to preserve the important characteristics of olive cake.
Key words: *In situ* digestibility. *Olea europea.* Olive oil. Nutritional value. Total phenols.

Resumo

O objetivo desta pesquisa foi melhorar parâmetros fermentativos e o valor nutricional da silagem da torta de oliva (*Olea europaea* "Arbequina") *in natura* através de dois tempos de repouso do material após a extração do azeite e/ou com adição de níveis de milho moído. O material foi ensilado em dois tempos após a extração (0 e 48 horas), incluindo como aditivo grão de milho moído nos níveis: 0, 5 e 10% de matéria fresca de torta de oliva a ser ensilada. O delineamento foi inteiramente casualisado de forma randômica e avaliados os parâmetros fermentativos, conservação, concentração de compostos fenólicos, composição química e valor nutritivo. As silagens não diferiram quanto à digestibilidade de nitrogênio não proteico, sílica e FDN. No tempo zero foram observados valores superiores para capacidade tampão, fenóis totais e taninos totais e inferior para matéria seca em relação a torta de oliva após 48 horas de repouso pós-extração do azeite. As silagens contendo inclusão de 10% de milho, em comparação sem a inclusão, apresentaram maior digestibilidade, NDT e menor pH e EE. A inclusão de milho moído melhora as características fermentativas e o valor nutritivo das silagens de torta de oliva fresca. A técnica de ensilagem tem se mostrado uma alternativa para preservar as características importantes da torta de oliva.
Palavras-chave: Azeite de oliva. *Digestibilidade in situ.* Fenóis totais. *Olea europea.* Valor nutricional.

Introduction

Agroindustry's byproducts have been ensiled or tested as additives (Dentinho et al., 2023; Pang et al., 2018), aiming not only to reduce production costs, but to disposal of production waste and environmental impact. However, the use of alternative foods, either for silage or direct supply in the trough itself, presents great nutritional variability, in addition to anti-nutritional factors.

The olive production system in South of Brazil is in the structuring phase, yet it does not make use of olive cake for animal feed, in part, due to lack of information regarding their chemical characteristics, nutritional value and forms of conservation. It's a byproduct with a mixture of skin, pulp and seeds after the extraction and difficult to dispose off, thus being a potential contaminant to the environment (Khwaldia et al., 2022). Cardoso et al. (2010) reported that for each 1 L of olive oil it is necessary do extract the oil from 5 kg of olive fruits, resulting in a large amount of product to be discarded. The treatment or disposal of this waste is currently a serious environmental problem in Mediterranean countries, such as Spain, Italy and Greece, countries that traditionally stand out in the production of olive oil. In these countries, mainly Italy, there is the largest concentration of studies on the disposal of this waste, known as olive cake.

Studies have been carried out showing the potential use of the olive cake in animal performance and, meat and milk composition (Russo et al., 2023; Bionda et al., 2022; Chiofalo et al., 2020), besides, the addition of phenols to final products, as well as on performance and animal health (Habeeb et al., 2017; Luciano et al. 2013). Therefore, it is studied preserving forms of olive cake like silage, that, until now, have a lack of information about it, in addition to the lack of research that associates the silage of this food with corn silage, only with molasses (Weinberg et al., 2008). Furthermore, the matter nutritional composition will vary with the processing, the contents of core, pulp, skin, as the cultivar used for extraction and the year of harvest (Selim et al., 2022).

Silage is a conservation method widely used in the world, allowing the conservation of most feeds through microbial fermentation, into organic acids, mainly lactic acid. As a result, there is a reduction in pH and the material, preserving the nutritional value of the product (Kung et al., 2018). To provide and potentiate the fermentation process in the silages several additives have been used over the years, to provide a rapid development of lactic acid bacteria and pH decrease, rendering undesirable and unfeasible microorganisms, such as enterobacteria, molds and yeasts (Bezerra et al., 2015; Weinberg et al., 2008). However, there are still no reports of the association of this cake with the use of corn as a silage additive, a feed that presents a great fermentation process due to its composition, it is easy to acquire and is a usual feed used in the diet of ruminants. In addition to having the potential to improve the fermentation process of olive cake, corn can also add nutritional value to silage.

Another important point is that to produce silage from olive cake it is necessary, for this technique, to be quickly applied, due to the large variation in the ether extract content, which can reach around 14% of DM (Hadhoud et al., 2020; Vargas-Bello-Pérez et al., 2013), and can cause an increase in biomass oxidative strength when the silage is not well-stored. In this way, knowing the ensiling potential when this byproduct cannot be ensiled immediately becomes interesting. Also, as the main matter comes from industries and may have high levels of ether extract, evaluating the time in which this material can be stored before being ensiled is essential, since in experiments on this silage, silage is not citrated, and the time between extraction and ensiling are not even mentioned.

In this sense, it was aimed to evaluate the fermentative characteristics, chemical composition, nutritional value, and the efficiency of the process in conserving its original characteristics from olive cake silage (Olea europaea "Arbequina") ensilage into two times after extraction (zero and 48 hours), using ground corn grain as an additive.

Material and Methods

Local, treatments and experimental management

The olive cake was obtained from the *Guarda Velha* Farm (31°30'03.6"S and 53°30'42.7"W), in the region called *Serra do Sudeste* or *Escudo Sul-Rio-Grandense,* in the city of *Pinheiro Machado* during 2016 crop season. The material used came from the milling, for extraction of olive oil in a two-phase system by centrifugation of fruit of *Olea europaea* "Arbequina". The fruit was harvested manually and taken to the processing plant where they were stored in plastic boxes until processing began on the same day or were stored for up to 24 hours in a low-temperature chamber. For the oil-processing, the fruits were dumped onto a lifting conveyor passing through a forced air system to eventually separate leaves and branches. Afterwards, the olives were washed in drinking water to eliminate physical contamination, and ground. After grinding and beating the fruits, the formed mass went to a centrifuge system to separate the liquid phases (vegetation water and olive oil) and solid phases (olive cake) and the olive cake was discarded in open-air tanks, the same location where the material was collected (Figure 1).

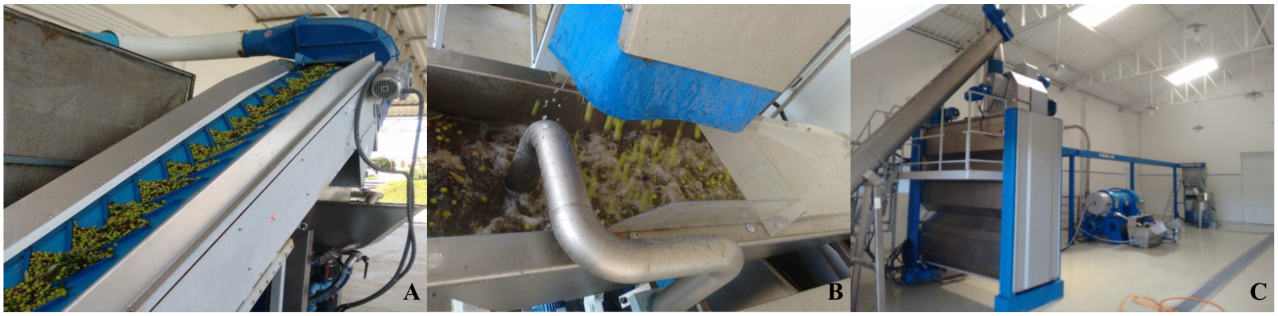

Figure 1. A- Reception of olive fruits for olive oil production; B- Fruit washing; C- Grinding and centrifuge equipment for extracting olive oil. Escudo Sul-Rio-Grandense, Pinheiro Machado – RS, Brazil.

The olive cake was collected on March 31th, 2016, immediately after processing and packed in plastic barrels with the capacity of 200L. The barrels were hermetically sealed and transported to the Animal Bromatology and Nutrition Laboratory-*Embrapa Clima Temperado.* The silages were in a PVC experimental microsilos, with 10 cm-diameter and 40 cm-length, in quadruplicate for each treatment. The treatments were identified as: S00 – fresh olive cake silage without ground corn grains; S50 - fresh olive cake silage with 5% of ground corn grains inclusion; S100 - fresh olive cake silage with 10% of ground corn grains inclusion; S480 - olive cake silage after 48 hours of storage without ground corn grains; S548 - olive cake silage after 48 hours of storage with 5% of ground corn grains inclusion; S1048 - olive cake silage after 48 hours of storage with 10% of ground corn grains inclusion. The corn grains used were pre-ground in a hammer mill equipped with a 3-millimeter sieve. For this, at the time of ensiling, the olive cake was manually homogenized with ground corn grain (Figure 2) and the estimate of the percentage to be included in each treatment was made based on the DM of the corn grain. Table 1 presents the chemical composition of olive cake and ground corn grain used for ensiling.

Figure 2. Pre-ensiled: homogenization of olive cake with ground corn grain for ensilage.

Table 1
Chemical compounds of olive cake and corn grain before ensilage

Variable*	0 hs after processing[1]	48 hours after processing[2]	Ground corn grain
Dry Matter g kg^{-1} wet	298.0	321.2	859.3
pH	5.40	5.44	...
Buffering capacity[3]	21.1	19.1	...
WSC g kg^{-1} DM	87.7	51.3	47.0
NDF g kg^{-1} DM	538,4	581.0	7.5
ADF g kg^{-1} DM	405.3	461.4	11.2
CP g kg^{-1} DM	81.2	86.2	88.1
EE g kg^{-1} DM	188.4	197.3	44.2
TP g kg^{-1} DM	20.3	17.7	...
TT g kg^{-1} DM	12.8	10.7	...
FFM	421.0±3.60	422.4±6.18	419.6±3.77

[1] - Sample collected and processed immediately after the extraction industry; [2] - The sample was collected in the industry during the extraction process vats and kept for 48 hours prior to analysis; [3] - Milliequivalent of sodium hydroxide required to raise the pH from 4 to 6- (mEq-g NaOH).
*g kg^{-1} of DM. WSC: water-soluble carbohydrates; NDF: neutral detergent fiber; ADF: acid detergent fiber; CP: crude protein; EE: ether extract; TP: total phenols; TT: total tannin.

The PVC microsilos were kept in a vertical position and after 100 days silos were opened, the upper portion of the silage was discarded (approximately 200g), with the aim of discarding material that had some type of mould, the rest of the material was homogenized, and samples were immediately drawn for pH and BC according to Playne and McDonald (1966). Furthermore, the total losses of ensiled mass and gas losses were estimated following methodologies proposed by Jobim et al. (2007).

Sample Evaluations

Approximately 1.0 kg of ensiled material was pre-dried in a 55 °C forced-air oven for 72 h for later bromatological and digestibility analyzes. Likewise, to estimate the total phenols (TP) and tannins (TT), representative samples from each silo were lyophilized and stored in a freezer (-20°C). The remaining material of each silage was preserved by freezing (-20°C) in properly identified plastic bags.

The partially dried samples were ground to pass a 1-mm and 2-mm sieve of a Wiley-type mill™ (Thomas Scientific). Samples of 1 mm sieve-grounded were analyzed for: Dry matter (DM) by Method 967.03, and mineral matter by method 942.05 (Association of Official Analytical Chemistry [AOAC], 2019). Organic matter (OM) was estimated by subtracting the mineral matter (MM) content (1000 – MM) obtained after burning the material in a muffle at 550°C. Crude protein content (CP) was assayed indirectly by N content according to methods 984.13 and 2001.11 (AOAC, 2019), and neutral detergent insoluble fiber (NDF), with the addition of thermostable α-amylase but without sodium sulfite, acid detergent insoluble fiber (FDA), acid detergent lignin (LDA) according Van Soest et al. (1991), and autoclave adaptations as described by Senger et al. (2008). The contents of Hemicellulose and Cellulose were determined by difference and the analyzes of NDF, FDA and LDA were performed sequentially. Neutral detergent insoluble nitrogen (NIDN), acid detergent insoluble nitrogen (NIDA) and non-protein nitrogen (NNP) followed the methodology described by Licitra et al. (1996). The non-fiber carbohydrates (NFC) were corrected for ash and protein were calculated using the equation proposed by Hall (2003). In the frozen samples, the values of water-soluble carbohydrates were determined according to the methodology described by Dubois et al. (1956). Total phenols (TP) and total tannins (TT) were determined according to the methodology described by Makkar (2000), using the lyophilized samples, and by keeping the samples in ice water throughout all the process.

The Total Digestible Nutrients (TDN) was estimate from the equation proposed by the NRC (2001):

$$TDN = CPd + NFCd + NDFd + Fad\text{-}2{,}25 - 7$$

Where: CPd = [1 - (0,4*ADIP/CP)]*CP; NFCd = 0,98*NFC; NDFd = 0,75* (NDFp - Lignin)*[1-(Lignin/NDFp)0,667]; NDFp = NDF - NDIP; FAd = EE -1; e 7 refer to fecal metabolic TDN, Where CPd = CP truly digestible; NFCd = truly digestible non-fiber carbohydrate; NDFd = NDF digestible; FAd = truly fatty acids digestibles.

As for the conversion of TDN values to net lactation energy (NeL), it was used the prediction equation proposed by the NRC (2001):

$$NeL3x\ (mcal/kg) = 0{,}0245 * NDT\ (\%) - 0{,}12$$

In situ degradability was performed using four three-years-old healthy cannulated Jersey cows (all the surgical and animal care protocols were approved by the Ethics Committee on Animal Research from *Universidade Federal de Pelotas*, case n°5076-2013), ±350 kg body weight, kept on pasture and additional feeding with concentrated supplementation containing 20% of olive cake and mineral salt during 14 days before the *in situ* procedure. For incubation, it was weighed 1 g of sample partially dried in a forced-air oven at 55°C until constant weight is achieved, ground to 2 mm, into polyester' bags (5 x 5 cm and 50 μm porosity). The post-incubation residues were washed until the water flowed clear and kept in solution for bacterial dissociation for 15 minutes (1994). After it was oven dried 110°C for 8 hours and weighed. After that, it was analyzed to neutral detergent fiber according to Goering and Van Soest (1975) to predict total digestibility.

Statistical procedures

All data were tested for residual normality by the Shapiro-Wilk test and for homoscedasticity by the Levene test. The data were submitted to univariate analysis of variance (ANOVA) and the means were adjusted by the least squares method using the LSMEANS (Least Squares Means) command and compared to each other by

Tukey test at a significance level of 5% using GLM procedure (Statistical Analysis System Institute [SAS Institute], 2013).

Results and Discussion

The storage time and the addition of dry ground corn increased the dry matter content of the silage. This is attributed to the hygroscopic capacity of the corn, which likely absorbed the moisture present in the olive cake during the ensiling process. The inclusion of corn, which has a high dry matter content, contributed to the overall increase in dry matter in the silage (Table 2). The soluble carbohydrate content was significantly different ($p < 0.05$), with higher levels observed in treatments without corn addition. With the inclusion of corn, the soluble carbohydrate content decreased, possibly due to moisture absorption and the diluting effect of the corn. Residual values of CHO is lower than those found in the present study described by Abarghoei et al. (2011), 0.78% of the DM silages were made with fresh olive cake and without the inclusion of additives, and 1.62% of the DM silages made with 0.5% molasses olive cake. The decline in pH with the corn addition suggests the oxidation of carbohydrates, such as starch, during the constant fermentation process, providing the decrease in pH in the silages made with material stored with a greater quantity of corn. There was a greater loss of ensiled mass when corn was added (Table 2). Furthermore, gas losses showed a similar behavior, with greater losses in treatments with corn inclusion. Still, the storage time of olive cake reduced material losses due to gases. Moreover, the pH values indicate that this important substrate does not seem to have been limiting for the fermentation process.

The addition of corn promoted a decline in pH, being the lowest value for S100 treatment. These results occurred due to a higher WSC content in fresh cake, that is better to fermentation (Kung et al., 2018). The effect of corn addition appears to be higher for wet cake, probably due to the reduction in CHO's during the 48-hour storage of the olive cake prior to ensiling.

The buffering capacity is an indicator of the intensity with which the ensiled material resists the change in pH during the fermentation process, that is, the higher its value, the greater amount of lactic acid must be formed to reduce the pH of the silage, the longer the fermentation process with greater carbohydrate consumption (Matias et al., 2020). The main responsible for the buffering power of forages are anionic compounds such as salts of organic acids, nitrates and chlorides, and proteins, which account for 10 to 20% of the total buffering power.

Table 2
Least squares mean olive cake silage fermentative parameters and nutritional compounds

	Fresh Olive Cake			48 hours of storage						
Fermentative Parameters	S00	S50	S100	S048	S548	S1048	SEM	Time of storage	Corn Inclusion	C*T
WSC#, g kg^{-1} DM	246.3§	220.1	196.3	209.1§	189.4	190.5	10.83	0.013	0.016	0.327
pH	5.17a	4.89b	4.50c	5.15a	4.96b	4.86b	0.278	0.001	0.001	0.001
BC (mEq-g NaOH)[1]	142.2	139.4	149.8	86.2	92.4	91.1	25.77	0.001	0.067	0.083
Total DM losses, g 100g^{-1} DM	7.81§	8.90	9.67	8.11§	8.84	8.80	0.478	0.340	0.001	0.099
Gas losses, g 100g^{-1} DM	0.410§	0.402	0.440	0.361§	0.386	0.411	0.015	0.001	0.001	0.143
Nutritional Compounds										
Dry Matter, g kg^{-1} Wet	260.3e	291.1d	300.8d	317.9c	335.9b	360.5a	2.773	0.001	0.001	0.030
Ash, g kg^{-1} DM	4.0	3.0	3.5	2.6	4.0	3.9	0.402	0.001	0.001	0.002
Crude protein, g kg^{-1} DM	87.0§	90.2	94.0§	87.3§	89.4	87.7§	1.40	0.060	0.045	0.059
Non-protein nitrogen, g kg^{-1} CP	273.0	306.8	350.8	250.0	272.8	195.5	53.3	0.121	0.870	0.406
NDIP#, g kg^{-1} CP	408.3a	308.0b	265.2b	373.9a	387.0a	393.1a	12.91	0.001	0.001	0.001
ADIP#, g kg^{-1} CP	396.4a	276.8c	241.5c	335.4b	347.8ab	342.5b	11.44	0.001	0.001	0.001
Ether extract, g kg^{-1} DM	197.3§	181.5	170.3§	196.8§	188.0	173.8§	4.76	0.431	0.001	0.771
NDF#, g kg^{-1} DM	558.5a	468.2b	435.4b	562.0a	534.6a	466.9b	9.39	0.001	0.001	0.013
ADF#, g kg^{-1} DM	513.9a	415.7b	387.8b	510.4a	476.5a	411.5b	8.78	0.001	0.001	0.006
Lignin, g kg^{-1} DM	237.4§	176.3	161.2§	245.3§	204.7	169.1§	6.66	0.014	0.001	0.235
Cellulose, g kg^{-1} DM	276.5a	239.4c	226.6c	265.0ab	271.8a	242.4bc	5.35	0.012	0.001	0.003
Hemicellulose, g kg^{-1} DM	50.4	50.7	51.6	56.9	63.7	60.9	3.26	0.018	0.105	0.945
NFC#, g kg^{-1} DM	105.1c	219.2a	260.7a	122.6bc	158.9b	244.5a	11.53	0.052	0.001	0.012
Total phenols, g kg^{-1} DM	19.1	20.9§	19.5	12.5	13.7§	11.6	7.17	0.001	0.046	0.639
Total Tanin, g kg^{-1} DM	11.0§	14.0§	13.0	6.7§	8.0§	6.6	6.34	0.001	0.012	0.283

#WSC: water-soluble carbohydrate; BC: buffering capacity; NFD: neutral detergent fiber; ADF: acid detergent fiber; NFC: Non-fiber carbohydrate; NDIP: neutral detergent insoluble-protein; ADIP: acid detergent insoluble-protein
S00 - silage olive cake to cool without inclusion of corn; S50 - fresh olive cake silage with 5% corn inclusion; S100 - fresh olive cake silage with 10% corn inclusion; S048 - silage of olive cake after 48 hours of storage without corn inclusion; S548 - olive cake silage after 48 hours of storage with 5% corn inclusion; S1048 - olive cake silage after 48 hours of storage with 10% corn inclusion; [1] Milliequivalent gram of sodium hydroxide required to raise the pH from 4 to 6; Means followed by different letters, for interaction, or "§" for de inclusion effect, in the same line differ at P=0.05 by Tukey test; SEM - standard error of the means.

Furthermore, during the ensiling process, the WSC content is directly influenced by this fermentative parameter (Ali & Tahir, 2021). When we observed the buffering capacity of the olive cake before ensiling (Table 1), even after 48 hours of storage the value remained practically the same. However, after ensiling, the buffering capacity for treatments in which the silage was made on the same day as oil extraction were higher than those stored for 48 hours, due to the higher WSC for fermentation. This higher availability seems to be led to a higher formation of organic acids, since 68 to 80% of the buffering capacity is by the anion fraction of the forage, like orthophosphates, sulfates, nitrates and chlorides (Kung et al., 2018), furthermore, the increase in starch availability and the decrease in moisture content promote an improvement in the fermentation environment (Muck et al., 2018). Still, the possible oxidation of fats can influence the buffering capacity, without influencing the total ether extract content. The increase in fermentative activity also influences the loss of volatile compounds and the increase in the formation of metabolic water, which is reflected in the results of total losses and the loss of material in the form of gases (Verbeke et al., 2015; Kung et al., 2018; Wang et al., 2023).

The higher values in fresh cake can be related to a lower DM content and a slow decrease of pH at the beginning of fermentation, generating a higher concentration of organic acids such as acetic and butyric, that represent the largest influence in the buffering capacity, 80% and 84%, respectively. According to Suong et al. (2022), a superior buffering capacity is desirable for silage fermentation process, because it is related to higher stability of the material to pH changes.

The EE content (Table 2) was lower in treatments with higher corn participation, as expected. The total lipid content was not affected by the storage time; however, further evaluations are required to determine the composition of fatty acids in the products resulting from the fermentation process. Our results for this fraction are higher than those describe by Hadhoud et al. (2020) that observed 14,79% of EE when olive cake silage was untreated with enzymes and 12,36% for those treated with 5000 IU/g of cellulase produced from anaerobic bacteria (Clostridium butyricum).

Total phenols and total tannins content (Table 2) differed significantly for both storage time (p=0.001) and corn inclusion (TP p=0.046; TT p=0.012), with higher levels of these compounds for treatments without storage time, regardless of the addition of corn. The storage time also caused a decrease in the content of tannins and phenolic compounds, possibly due to the oxidation of these compounds and transformation into other chemical groups. The corn addition affected the content of these compounds, with higher levels for treatments S50 and S100 The importance of preserving these phenolic contents during ensiling is owing to the bioactive potential attributed to them, as well as antioxidant and therapeutic properties that produce anticancer, anti-viral, anti-inflammatory, hypolipidemic and hypoglycemic effects (Lopez-Corona, et al., 2022), with possible animal health benefits and the addition of these nutraceutical compounds to their products (meat and milk), with benefits to human health.

A high content of total tannin and total phenols (such as lignin) in animal feeding has unfavorable impacts on the nutritive value and cellulolytic activity of microorganisms in the rumen. The silage of olive cake was effective in preserving the levels of TT and TP, especially when ensiled on time zero, maintaining approximately 98% of TP and 99.5% of the TT. It was preserved when ensiled after 48 hours, 92.6% of TP and only 66% of TT. These results contradict those obtained by Abarghoei et al. (2011) who observed a significant reduction in polyphenols content during silage, as well as the results of Weinberg et al. (2008) and the latter authors found a reduction of 30% to 40% in relation to fresh olive cake during silage.

Although there are differences in the literature, the presence of moderate tannin content in the rumen is can be related to the protection of the protein against degradation by rumen microorganisms, thus increasing the protein flow for absorption at intestinal level (Besharati et al., 2022). It is speculated that this reduction in ruminal degradation rate by tannin effect can be exploited to modulate the availability of nutrients (energy and protein) for microbial multiplication, reducing nitrogen excretion and energy expenditure in the elimination of excess of ammonia, for example, in animals grazing temperate grasses.

It is important to consider the potential of these fractions for new research, which would be impossible if conservation was not possible. Also, they indicate that even without having such a significant pH reduction (Table 2), the anaerobic medium was efficient in preserving the TP and TT, which, in general, oxidize quite easily, and lead one to believe that at least by oxidation the lipid profile has not been altered maintaining the potential of use of the product under study.

The NDF and ADF content (Table 2) showed an interaction between storage time and the addition of corn levels to silage (NDF p=0.013; ADF p=0.006) and was influenced for both the addition of corn (p=0.001) and storage time (p=0.001), with decrease in the contents of fibrous fractions in silage with greater inclusion of corn, as expected. In the material stored for 48 hours prior to ensiling, despite the same behavior, numerically higher values may be related to a lower participation of CHOs, which proportionally should increase the values of the other constituents. NDF and ADF was lower than those found of Hadhoud et al. (2020), even when used cellulolytic enzyme, before describing. This result is one of those that most reinforces the great variability of the nutritional contents of olive cake, and the high need for constant evaluation of non-conventional feeds in ruminant feeding. High fiber contents, as observed in both reported experiments, can cause a decrease in rumen fermentation, impairing the absorption of other components, such as nitrogenous ones.

In turn, cellulose and lignin contents followed the same trend of NDF and ADF levels. As regards the hemicellulose content, this did not differ (P> 0.05) between the olive cake silages evaluated. Lignin content was higher than those observed in several studies that describes values between 4 and 8% (Hadhoud et al., 2020; Luciano et al., 2013; Abarghoei et al., 2011).

NFC levels (Table 2) were higher in silages with greater corn participation (S50, S100, and S1048). These results are linked to the higher carbohydrate content in the fresh olive cake (obtained immediately after the extraction of olive oil), combined with the high starch content of the corn. Although the literature reports variable NFC content for olive cake silage, the values determined in this study are within the range estimated by Abarghoei et al. (2011).

The CP, NIDN and ADIN (Table 2) were affected by the treatments (p <0.05), and in the silages T0 the CP contents showed an increasing behavior with the inclusion of ground corn grains and consequently NIDN and ADIN decreasing behavior. In T48 hours, this behavior was not observed, presenting no clear differences due to the inclusion of ground corn. The lack of default behavior is likely to be related to losses occurring during this period. The silages analyzed in this study presented levels of CP higher than reports that evaluate de crude olive cake silage (Symeou et al., 2021; Hadhoud et al., 2020).

NPN contents were not affected by storage time prior to ensiling or inclusion of grain corn (p> 0.05). However, the observed values were relatively low considering the values found for grass silages, which indicates that there was little proteolysis during the process under the conditions of the study, the high moisture content and high pH are the main factors predisposing to the occurrence of proteolysis. It is likely that the presence of tannins in olive cake silages has conferred some degree of protection on this fraction (Castellani et al., 2017) for the inhibition of plant and microbial enzymes, or for the ability of complexes with the protein fraction (Das et al., 2020).

The means of TDN, Nel, DM in situ degradability (DMISD) and NDF in situ degradability (NDFISD) can be observed in table 3. The storage did not influence in any of the variables mentioned, however, there was a significant difference (p=0.001) when corn was included in the olive cake silage for all treatments.

Table 3
Least mean values of total digestible nutrients (TDN), net energy of lactation (NEl), dry matter in situ degradability (DMISD) and neutral detergent fiber in situ degradability (NDFISD) in olive cake silages

	Fresh Olive Cake			48 hours of storage						
Variables	S00	S50	S100	S048	S548	S1048	SEM	Time of storage	Corn Inclusion	C*T
TDN, g kg^{-1} DM	613.3§	700.8	716.4§	629.8§	662.2	706.2§	10.39	0.219	0.001	0.052
NEl, g kg^{-1} DM	14.9§	17.6	18.0§	15.5§	16.4	17.6§	0.33	0.213	0.001	0.056
DMISD 24 hs, g kg^{-1} DM	441.1§	477.8	543.5§	420.6§	485.9	515.7§	10.71	0.143	0.001	0.237
DMISD 48 hs, g kg^{-1} DM	463.3§	484.8	547.8§	446.5§	487.9	550.5§	10.49	0.592	0.001	0.609
NDFISD 24 hs, g kg^{-1} NDF	43.1	38.7	36.6	38.6	37.8	35.4	8.92	0.535	0.228	0.958
NDFISD 48 hs, g kg^{-1} NDF	65.9	58.8	56.4	84.0	95.0	95.1	13.11	0.771	0.165	0.378

S00 - silage olive cake to cool without inclusion of corn; S50 - fresh olive cake silage with 5% corn inclusion; S100 - fresh olive cake silage with 10% corn inclusion; S048 - silage of olive cake after 48 hours of storage without corn inclusion; S548 - olive cake silage after 48 hours of storage with 5% corn inclusion; S1048 - olive cake silage after 48 hours of storage with 10% corn inclusion;
Means followed by different letters, for interaction, or "§" for de inclusion effect, in the same line differ at P=0.05 by Tukey test; SEM - standard error of the means.

The DM *in situ* degradability increased with the levels of corn inclusion, while the NDF *in situ* degradability showed the opposite behavior, decreasing according to the inclusion levels, a fact that corroborates the chemical composition of the silage, since the inclusion of corn reduced NDF levels. The degradability values of DM found in our study resemble those determined *in vitro* by Hadhoud et al. (2020) with means values around 55% among treatments. Furthermore, the NDF degradability was similar from that observed in this study. Tahseen et al. (2014), evaluating the *in situ* degradability of fresh olive cake, reported values similar to those found in this study, both for degradability within 24 hours and for degradability within 48 hours. Despite these studies, there are no reports in the literature evaluating the in situ degradability of olive cake silage with the addition of ground starchy grains, such as corn.

One of the aspects linked to the lower degradability of olive cake without the addition of corn is linked to the configuration of its cell wall. This low quality of the fibrous fraction reflects the high lignin content (Table 2) is related to the nutritive value of the feed and directly affects the availability of cellulose, the main constituent with the possibility of ruminal digestion observed in co-products of olive oil extraction. However, even though the contribution of the fiber fraction as an energy source and presenting low digestive potential, it is believed that this fraction is not a limiting factor for the use of the product, since the possible negative effect expected would be to decrease the consumption capacity, however, by the structural and composition characteristics of the material, containing particles with sizes smaller than three millimeters and high amounts of oil, a higher ruminal passage rate is expected, thus not adversely affecting consumption.

Several authors suggest that the critical size for ruminal particle output in cattle appears to be around 3.6 mm, but the numerous factors involved and the interaction between them are still poorly understood (Dijkstra et al., 2020; Li & Hanigan, 2020; White et al., 2017). In this sense, more studies evaluating the supply to cattle are necessary to map the possible effects of the inclusion of this co-product.

Conclusion

In this study, silage has proven to be an effective alternative for preserving and maintaining the key characteristics of olive cake, including its lipid, total phenol, and tannin content. The inclusion of 10% ground corn, relative to the natural matter of the olive cake, it enhances both the fermentative properties and the nutritional value of the olive cake silage. However, further studies are required to characterize these fractions and assess the benefits of incorporating this product into ruminant diets.

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