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Fermentation times and feed additives improve the quality of olive bagasse silage

Aditivos alimentares e tempos de fermentação melhoram a qualidade de silagens de bagaço de azeitona

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Abstract

This research aimed to measure the microbiological, chemical composition profile and fermentative characteristics during storage of fresh olive bagasse, *in natura* and incorporated with corn, soybean and rice brans, respectively. The experimental design was completely randomized, with the plots constituting the four main treatments (olive bagasse *in natura* or with added corn, soybean and rice brans, respectively), and the subplots allocated the three sampling times, which corresponded to time zero (at the ensilage moment) and 28 and 56 days of ensilage. The fermentative characteristics (dry matter content [DM], pH and ammoniacal nitrogen [NH_3-N], microbiological profile (populations of filamentous fungi, lactic acid bacteria, enterobacteria and *Clostridia*) and chemical composition profile (mineral matter, organic matter, crude protein [CP], ether extract, neutral detergent fiber, acid detergent fiber, lignin, cellulose and hemicellulose) were determined. The corn grain and rice meal treatments, which demonstrated pH 4.08 and 3.96 at 28 days of fermentation, respectively, provided the best fermentation profile. After storage for 56 days, the samples with added soybean and rice meal reached the highest levels of CP (166.15 and 93.78 g kg^{-1} DM), respectively. Increasing the storage period reduced the pH of the obtained silages but increased the losses of DM, NH_3-N and contributed to the losses of some nutrients. Rice meal and corn grain have been recommended to be used as additives in olive bagasse storage. However, the choice of additives studied is dependent on their commercial availability in each region.

Key words: Cellulose. Crude protein. Microbiology. Nutritional value. *Olea europaea.*

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Resumo

Objetivou-se estudar a composição bromatológica, perfil microbiológico e características fermentativas da silagem de bagaço de azeitona *in natura* e aditivada com farelos de milho, soja e arroz em diferentes tempos de amostragem. O delineamento experimental utilizado foi completamente casualizado em arranjo de parcelas subdivididas no tempo 4x3 com cinco repetições. Nas parcelas foram alocados os tratamentos principais, constituídos do bagaço de azeitona *in natura* ou adicionado com farelo de milho, soja e arroz, e nas subparcelas os tempos de amostragem, que correspondem ao tempo zero (momento da ensilagem) e aos 28 e 56 dias de fermentação. As características fermentativas foram estudadas pela determinação dos conteúdos de matéria seca (MS), pH e nitrogênio amoniacal ($N\text{-}NH_3$). Para determinação do perfil microbiológico foram estudadas as populações de fungos filamentosos, bactérias ácido láticas, enterobactérias e clostrídeos. Na avaliação do perfil bromatológico determinou-se os conteúdos de matéria mineral (MM), matéria orgânica (MO), proteína bruta (PB), extrato etéreo (EE), fibra em detergente neutro (FDN), fibra em detergente ácido (FDA), lignina, celulose e hemicelulose. O uso dos farelos de milho e arroz proporcionou melhores características fermentativas as silagens, estes tratamentos apresentaram pH de 4,08 e 3,96 aos 28 dias de fermentação, respectivamente. Aos 56 dias de armazenamento os tratamentos adicionados com farelos de soja e arroz demostrataram os melhores níveis de PB (116,15 g/kg e 93,78 g/kg MS), respectivamente. O aumento do período de armazenamento reduziu os teores de pH das silagens obtidas, no entanto, aumentou as perdas de matéria seca, teores de nitrogênio amoniacal e contribuiu com perdas de alguns nutrientes. Os farelos de arroz e milho são recomendados para serem utilizados como aditivos na silagem de bagaço de azeitona, porém, a escolha dos aditivos estudados está condicionada a disponibilidade comercial de cada região.

Palavras-chave: Celulose. Microbiologia. *Olea europaea*. Proteína bruta. Valor nutricional.

Introduction

Olive (*Olea europaea*) cultivation is ideally adapted to regions having a Mediterranean type of climate, typified by hot, dry summers, relatively cool winters with seldom frost and average annual precipitation of around 800 mm (ABAZI et al., 2013). During two-stage olive processing, a large volume of residues is produced, amounting to about 800 kg of semi-solid waste (termed *alpeorujo*) per 1000 kg of processed olives (MOLINA-ALCAIDE; YAÑEZ-RUIZ, 2008). Owing to its environmental contamination potential, the industry incurs high costs associated with the treatment and adequate disposal of this residue (WEINBERG et al., 2008). Approximately 300 kg of the waste generated is equivalent to a semi-solid waste, called olive bagasse (NIAOUNAKIS; HALVADAKIS, 2006), which may have the potential for use in animal feed, especially for ruminants (WEINBERG et al., 2008).

The production of olive bagasse is seasonal, corroborating with the time of fruit production and olive oil extraction. Though olive cake presents a high amount of energy and fiber (CHIOFALO et al., 2004), its high polyphenol and ether extract (EE) contents and low digestibility justify the use of alternative foods that improve the composition of this material. The proportional dilution of the polyphenols and EE may favor the formation of microbial protein and a better utilization by the animals at the digestive level.

The possibility of agro-industrial co-products in animal feeding use reduces the demand for optimal feed and contributes to global food security (NASOPOULOU; ZABETAKIS, 2013). However, as mentioned above, olive bagasse presents seasonal production and, besides, has already been considered of low nutritional value, regarding energy and protein, compared with that of other culture residues (NASOPOULOU; ZABETAKIS, 2013).

Among the options for the conservation of these co-products used as animal feed, is ensiling. The main principles of this preservation technique are an anaerobic environment and a low pH, with lactic

acid bacteria dominating the fermentation. Silage is accessible and economical (SANSOUCY et al., 1985) when used for conservation of agro-industry by-products.

However, for the efficient fermentative process, the material to be ensiled needs to have some characteristics, such as an adequate amount of soluble carbohydrates, low buffering capacity and a dry matter (DM) content between 300 to 350 g kg^{-1} (McDONALD et al., 1991). These characteristics, which ensure the achievement of desirable fermentation and conservation standards throughout storage, are not inherent in olive bagasse (SANSOUCY et al., 1985) but can result from the use of food additives, such as brans (NERES et al., 2013). While some by-products have the potential for use in ruminant feed, the nutritional value of these by-products is dependent on the biochemical and dynamic processes of interaction that occur during the storing conditions (AZEVÊDO et al., 2011).

This paper aimed to measure the fermentative, microbiological and bromatological profile of the silage from fresh olive bagasse incorporated with additives (corn, soybean and rice brans, respectively), at different storage periods.

Material and Methods

The experiment was carried out at Tecnolivas® Indústria/Pomares, located in the municipality of Caçapava do Sul, Rio Grande do Sul, Brazil, and at the Animal Nutrition Laboratory of Unipampa, Uruguaiana Campus (latitude: 29°45'17" S, longitude: 57°05'18" W, 66 m altitude), Rio Grande do Sul, Brazil.

The design was completely randomized, with the plots constituting the four main treatments (olive bagasse *in natura* or with added corn grain, soybean meal and rice meal, respectively), and the subplots allocated the three sampling times, which corresponded to time zero (at the ensilage moment) and 28 and 56 days of storage.

To achieve silages with a DM of 330 g kg^{-1}, the mixtures were prepared based on the natural matter in the proportion of 93 parts of olive bagasse *in natura* to seven parts of additives, considering the DM contents determined in an air-forced oven (Table 1). This choice of DM content lies within the range recommended by McDonald et al. (1991) for silage fermentations, which is between 300 and 350 g kg^{-1} DM.

Table 1. Composition of olive bagasse *in natura* and the additives used in the treatments.

Variable	*In natura* bagasse	Additives used in the composition of silages		
		Rice meal	Soybean meal	Corn grain
Dry matter	289.53	862.92	833.50	886.33
Mineral matter	32.60	109.94	67.91	11.56
Organic matter	965.42	890.12	932.13	988.53
Crude protein	50.54	126.33	450.32	88.74
Neutral detergent fiber	602.51	248.22	270.66	99.95
Acid detergent fiber	562.41	137.63	122.86	54.43
Ether extract	242.72	183.21	33.97	41.43
Lignin	355.75	53.87	23.04	29.12
Total carbohydrate	672.26	580.63	447.96	858.45
Non-fibrous carbohydrate	120.93	332.43	177.34	758.52
Fibrous carbohydrate	553.12	248.25	270.68	99.97

Dry matter (g kg^{-1}); all other variables are expressed as g kg^{-1} DM.

The mixtures were homogenized manually and conditioned in experimental silos made with polyvinyl chloride pipes of dimensions 50 cm in height and 10 cm in diameter. Each silo contained 3,900 kg of the blend, equivalent to a silage density of 900 kg m^{-3}. The silos were sealed with caps equipped with Bunsen-type valves, for the free escape of the gases, and fixed with the aid of adhesive tape. For the drainage of the effluent produced, 0.5 kg of dry, autoclaved sand, insulated by a cotton cloth, was placed in the bottom of each silo. Sixty experimental silos were used, due to the number of treatments (four), sampling times (three) and the number of replicates (five) of the experiment.

After the fermentation periods stipulated (28 and 56 days), the silos were opened, and the upper and lower portion of each silo (5 cm) was discarded. The remaining silage was homogenized and sampled, for analysis of the fermentative characteristics, microbiological profile and chemical composition.

The fermentative characteristics included DM, pH and, also, ammoniacal nitrogen, which was determined as the fraction of the total nitrogen (NH_3-N/TN) in the ensilage, at 28 and 56 days of fermentation. The DM was determined gravimetrically on samples of 300 g collected from each repetition, by drying in an oven with forced air circulation at 55 °C for 72 h. Measurements of the pH (SILVA; QUEIROZ, 2009) and NH_3-N (BOLSEN et al., 1992) were taken from 50 g per replicate.

To identify the microbial profiles (SILVA et al., 2007), the collected samples were homogenized. A stock solution of 10 g to 90 mL of peptone water was serially diluted to 10^1–10^8 and inoculated into selective culture medium to determine the colony-forming units (CFU) per gram of DM. The four media used, and the incubation conditions were potato dextrose agar (filamentous fungi and yeasts) at room temperature for 5 to 7 days; de Man–Rogosa–Sharpe (MRS) broth (lactic acid bacteria [LAB]) at 35 °C for 48 h; violet red bile agar (Oxford) (Enterobacteriaceae) at 35 °C for 72 h, and reinforced clostridial agar (*Clostridia*) at 35 °C for 72 h in anaerobic medium. Only dilutions that yielded 30–300 CFU per Petri dish were counted, and the results were expressed as $\log_{10}$ CFU g^{-1} DM (McDONALD et al., 1991).

After oven-drying (55 °C for 72 h) the samples were ground in a Willey Mill (stainless-steel 1 mm mesh sieve), to determine the chemical composition: DM correction at 105 °C, and the contents of mineral matter (MM), crude protein (CP), EE (SILVA; QUEIROZ, 2009), neutral detergent fiber (NDF), acid detergent fiber (ADF), lignin, cellulose and hemicellulose (VAN SOEST et al., 1991).

The data were submitted to analysis of variance. If significant, the mean values were compared by Tukey's test (5%), with the adoption of complex variance, due to subdivided plots. When the interaction of the factors evaluated (treatments × sampling times) was not significant, only the mean of the individual factors was used to compare the results. All statistical analyses were performed using the statistical program Sisvar (FERREIRA, 2011).

Results and Discussion

There were significant differences in the DM, pH and NH_3-N losses of the olive silage with food additives, whereas the DM during storage was not affected by the studied sources of variation (Table 2). At 28 days of fermentation, the highest losses of DM were observed in the storage of olive bagasse with added rice meal while at 56 days of fermentation, the addition of soybean meal resulted in higher losses of DM in the silages (Table 2). The additive-free bagasse presented the least DM loss at 56 days of fermentation, in contrast to the bagasse with added soybean meal. The remaining silages maintained consistent losses at the two fermentation periods (Table 2).

The use of additives increased the DM, thus, favoring the fermentation process (Table 2). Souza et al. (2012) demonstrated the efficiency of ensilage for the conservation of wet brewery residue, which has a moisture content higher than olive bagasse. Likewise, Gonçalves et al. (2014) confirmed the feasibility of the ensilage process in preserving cassava starch residue, thereby supporting the

hypothesis that the method of conservation of the olive bagasse used in the present study offers beneficial conditions for maintaining the quality of different sub-products.

Table 2. Dry matter, dry matter losses, pH and ammoniacal nitrogen (NH_3-N) of olive bagasse silages with feed additives at ensilage and 28 and 56 days of storage.

Treatments	Ensilage	28 days	56 days	Average	Ensilage	28 days	56 days	Average
	Dry matter (g kg^{-1})				Dry matter losses (%)			
Bagasse	299.09	315.35	315.87	310.10d	-	4.70bA	2.50cB	3.60
Bagasse+corn	308.60	375.42	365.65	349.89b	-	4.71bA	5.51bA	5.11
Bagasse+soybean	316.85	338.97	346.31	334.04c	-	5.40abB	11.12aA	8.26
Bagasse+rice	349.90	394.84	382.73	375.82a	-	6.32aA	6.21bA	6.27
Average	318.61B	356.15A	352.64A		-	0.53	0.63	
CV1 (%)	32.40				58.18			
CV2 (%)	6.76				15.46			
Treatments	Times			Average	Times			Average
	Ensilage	28 days	56 days		Ensilage	28 days	56 days	
	pH				NH_3-N (% total nitrogen)			
Bagasse	5.40abA	4.63aB	4.29bC	4.77	1.67cB	2.10cB	10.48bA	4.75
Bagasse+corn	5.27bA	4.08bB	3.79cC	4.38	0.87cB	1.09cB	10.07bA	4.01
Bagasse+soybean	5.61aA	4.85aB	4.80aB	5.09	3.96bB	4.97bAB	5.30cA	4.75
Bagasse+rice	5.68aA	3.96bB	3.77cB	4.47	8.52aC	10.69aB	16.66aA	11.96
Average	5.49	4.38	4.17		3.76	4.71	10.63	
CV1 (%)	26.51				56.91			
CV2 (%)	14.00				42.47			

Averages followed by the same small letter in the column and capital letter in the line do not differ by Tukey's test (5%); CV1 – coefficient of variation of silages; CV2 – coefficient of variation of the times.

The losses of DM are due to the occurrence of gas production during anaerobic fermentation. Particularly, fermentation by clostridia leads to considerable DM losses, attributed to CO_2 formation involving decarboxylation and/or oxidation (McDONALD et al., 1991). To maximize the lactic fermentation, techniques and/or products must be used that avoid the effect of the low DM level of the material to be ensiled.

All treatments presented a pH reduction during fermentation, but only in the bagasse and corn grain silage did the pH reduction persist after fermentation for 28 days (Table 2). The highest NH_3-N levels were observed in the treatments with the addition of rice bran to the bagasse, even before the beginning of the fermentation process.

The silages with added corn grain and rice meal had lower pH values after both fermentation periods, and only the addition of corn bran to bagasse did not exceed the upper limit suggested by McDonald et al. (1991) for the adequate preservation of feed, which is pH 4.2. The pH decline in corn silage (Table 2) is explained by the non-structural carbohydrates present in corn, which can be partially used as a substrate for anaerobic fermentation, contributing to the production of organic acids and the reduction of pH.

The persistent high pH values, meant they did not reach those recommended by McDonald et al. (1991) for a suitable fermentation. This outcome is attributed to the high amount of phenolic compounds present in the olive bagasse (NIAOUNAKIS; HALVADAKIS, 2006), which increase the buffering

capacity of the material to be ensiled (McDONALD et al., 1991) and inhibit the action of LAB during fermentative processes in the silos (RIDWAN et al., 2015). However, the moderate inclusion of olive bagasse in ruminant diets (100 g kg $^{-1}$ DM) may be an advantage for reducing methane gas emissions (KONDO et al., 2014).

The slight decrease in pH values of bagasse silage after 28 days may be related to the increased humidity in the absence of additives, which may have favored the development of microorganisms of the genus *Clostridium*, responsible for the production of acetic acid (McDONALD et al., 1991). The remarkably high NH_3-N values observed in the silage containing rice bran (Table 2) derive from the heat treatment that the rice undergoes during its processing to obtain the bran, which causes denaturation of proteins and accompanying release of non-protein constituents, quantified by NH_3-N analysis.

The increase in the NH_3-N/TN throughout the fermentation for all the silages reveals that protein was degraded inside the silos with the concurrent release of nitrogen compounds. The formation of this alkali (NH_3-N) contributes to the increase in pH, inhibiting the development of undesirable microorganisms, mainly yeasts (ALLI et al., 1983). These alterations result from the action of proteolytic bacteria that act in high humidity conditions, at pH above 4.5, and temperatures between 20 and 45 °C. According to McDonald et al. (1991), protein hydrolysis can increase the non-protein nitrogen by up to approximately 70% of the TN in the silo opening.

Regarding the microbiological profile of the mixtures and influence of storage, the LAB and filamentous fungi populations were significantly changed only as a function of the sampling times, so that it was superior in the silages exposed to 56 days of fermentation (Table 3).

Table 3. Microbiological profile of olive bagasse silages with feed additives in ensilage and at 28 and 56 days of storage. Values expressed as $\log_{10}$ colony-forming units (CFU) per gram of dry matter (DM) of silage.

Treatments	Ensilage	28 days	56 days	Average	Ensilage	28 days	56 days	Average
	Lactic acid bacteria ($\log_{10}$ CFU g^{-1} DM)				Filamentous fungi ($\log_{10}$ CFU g^{-1} DM)			
Bagasse	0.51	8.14	9.39	6.01a	0.50	7.28	9.21	5.66a
Bagasse+corn	0.46	8.15	9.03	5.88a	1.75	7.09	9.11	5.98a
Bagasse+soybean	0.49	8.08	9.47	6.01a	0.49	8.10	9.63	6.07a
Bagasse+rice	0.44	7.89	9.58	5.97a	0.48	7.85	9.09	5.81a
Average	0.48C	8.07B	9.37A		0.80C	7.58B	9.26A	
CV1 (%)	13.08				22.94			
CV2 (%)	8.58				14.60			
Treatments	Times			Average	Times			Average
	Ensilage	28 days	56 days		Ensilage	28 days	56 days	
	Clostridia ($\log_{10}$ CFU g^{-1} DM)				Enterobacteria ($\log_{10}$ CFU g^{-1} DM)			
Bagasse	0.51	7.69	9.41	5.87a	0.51aB	7.79aA	8.13bA	5.48
Bagasse+corn	0.46	8.17	9.05	5.89a	0.46aB	7.75aA	8.37bA	5.53
Bagasse+soybean	1.49	7.61	9.89	6.33a	0.49aC	8.18aB	9.55aA	6.07
Bagasse+rice	0.44	8.21	9.62	6.09a	0.44aC	7.96aB	9.78aA	6.06
Media	0.73C	7.92B	9.49A		0.48	7.92	8.96	
CV1 (%)	20.59				15.78			
CV2 (%)	12.83				9.13			

Averages followed by the same small letter in the column and capital letter in the line do not differ by Tukey's test (5%); CV1 – coefficient of variation of silages; CV2 – coefficient of variation of the times.

The population of clostridia was comparable with that of the LAB, with significance only for the fermentation times, revealing a greater population in the silages with 56 days of anaerobic storage (Table 3). There was a significant interaction between the factors studied when comparing the enterobacteria populations, with a higher population in the mixtures of olive bagasse with rice and soybean bran, and in these blends at 56 days of fermentation. The increased DM of the combinations reduces the possibilities of losses by effluents and the development of bacteria of the genus *Clostridium* (McDONALD et al., 1991) that cause negative impacts on the feeding value (OLADOSU et al., 2016) by promoting undesirable secondary fermentations and the formation of butyric acid (McDONALD et al., 1991). Many species of the *Clostridium* genus are capable of saccharolytic and proteolytic (breaking down carbohydrate and protein, respectively) activities. *Clostridium bifermentans* and *C. sporogenes* are extremely proteolytic. Conversely, *C. butyricum* and *C. tyrobutyricum* are mildly proteolytic, so when these two species predominate in the *Clostridium* population, although the clostridia count may be high, the proteolytic activity and N-NH_3 production may be mild.

At 28 days of fermentation, all silages presented statistically similar populations of enterobacteria, with an average of 7.92 $\log_{10}$ CFU g^{-1} DM. When considering the sampling times, the factors evaluated showed a significant interaction for the enterobacteria population (Table 3). At 56 days of storage, the enterobacteria population decreased when maize was used as the additive, but the use of rice and soybean brans had the opposite trend (Table 3).

The low population of bacteria involved in the homofermentative process, such as LAB, which rapidly reduce the pH of the silage at the beginning of the fermentation process, avoids the development and undesirable fermentations caused by clostridia microorganisms and enterobacteria. Therefore, the low growth of microorganisms of the genus *Clostridium* is desirable since they are the main microorganisms that deteriorate the silages (MOTA et al., 2011). The DM contents of the treatments and the high content of polyphenols present in the olive bagasse were important for this low development because polyphenols act as a barrier to the microbial development of clostridia (NIAOUNAKIS; HALVADAKIS, 2006). However, the maintenance of a saccharolytic clostridia population is owed to their ability to ferment sugar and lactic acid, producing hydrogen, CO_2 and butyric acid (OLADOSU et al., 2016). This process may not drastically alter the pH, but it maintains the total clostridia count of the silage.

The LAB, which exhibited similar populations in all silages, use amino acids as a source of energy for growth and release ammonia inside the silos, consequently increasing the amount of NH_3-N during fermentation (BERNARDES et al., 2005).

The enterobacteria belong to the group of epiphytic microbiota found on feeds, mainly forages (OLADOSU et al., 2016). These bacteria maintained their population throughout the fermentation, in all silages studied (Table 3) because they are facultative anaerobes (MUCK, 2010). Larger populations were observed in the silages with added soybean and rice brans than the other treatments (Table 3), as these two additives increased the CP of the silages. Enterobacteria have weak proteolytic activities and can decarboxylate and deaminate some amino acids (OLADOSU et al., 2016). Also, various species of enterobacteria can use nitrate as an electron acceptor in place of oxygen, reducing nitrate to nitrite or nitrogen oxide (MUCK, 2010).

The MM content of the mixtures and the storage time were affected by the interaction of the studied factors, revealing a higher content of MM in the mixtures with rice bran (Table 4). Whereas the MM content was not altered by the corn and soybean bran additives, it was reduced in the absence of additives, during fermentation. Throughout the

storage process, the production of effluents causes a proportional increase in organic matter and a reduction in MM. The treatment with corn grain presented the lowest levels of MM when compared to the other treatments (Table 4), owing to its chemical composition (Table 1).

Table 4. Chemical composition profile of olive bagasse silages with feed additives at ensilage and 28 and 56 days of storage.

Treatments	Ensilage	28 days	56 days	Average	Ensilage	28 days	56 days	Average
	Mineral matter (g kg^{-1} DM)				Ether extract (g kg^{-1} DM)			
Bagasse	54.61bA	39.45bB	35.69cB	43.25	235.72aB	251.55aB	301.28aA	262.85
Bagasse+corn	31.62dA	30.73cA	29.98cA	30.78	188.96bB	207.24bAB	224.97bA	207.06
Bagasse+soybean	47.03cA	46.78aA	43.66bA	45.83	213.36abA	197.20bA	220.99bA	210.52
Bagasse+rice	70.76aA	47.70aC	56.50aB	58.32	238.05aA	198.28bB	194.48bB	210.27
Average	51.01	41.17	41.46		219.02	213.57	235.43	
CV1 (%)	8.55				6.87			
CV2 (%)	9.38				9.16			
	Times			Average	Times			Average
Treatments	Ensilage	28 days	56 days		Ensilage	28 days	56 days	
	Crude protein (g kg^{-1} DM)				Neutral detergent fiber (g kg^{-1} DM)			
Bagasse	111.70bA	65.60bcB	63.14cB	80.15	593.15	640.84	687.20	640.40ab
Bagasse+corn	56.50cA	55.68cA	60.45cA	57.55	590.17	659.39	673.08	640.88ab
Bagasse+soybean	166.72aA	170.34aA	166.15aA	167.74	564.93	655.67	671.97	630.86b
Bagasse+rice	117.46bA	85.97bB	93.78bB	99.07	575.72	694.36	729.08	666.39a
Average	113.10	94.40	95.88		580.99C	662.57B	690.33A	
CV1 (%)	14.69				5.59			
CV2 (%)	12.21				5.33			
Treatments	Ensilage	28 days	56 days	Average	Ensilage	28 days	56 days	Average
	Acid detergent fiber (g kg^{-1} DM)				Hemicellulose (g kg^{-1} DM)			
Bagasse	405.87aB	597.77aA	599.41aA	534.35	187.28aA	43.07bB	87.79bB	106.05
Bagasse+corn	425.91aC	498.33bB	569.28abA	497.84	164.26aA	161.06aA	103.80abA	143.04
Bagasse+soybean	343.06bB	483.49bA	513.13cA	446.56	221.86aA	172.18aA	158.84abA	184.29
Bagasse+rice	401.64aC	497.94bB	553.61bcA	484.40	174.08aA	196.42aA	175.47aA	181.99
Average	394.12	519.38	558.86		186.87	143.18	131.48	
CV1 (%)	5.16				31.63			
CV2 (%)	4.85				29.31			
	Times			Average	Times			Average
Treatments	Ensilage	28 days	56 days		Ensilage	28 days	56 days	
	Cellulose (g kg^{-1} DM)				Lignin (g kg^{-1} DM)			
Bagasse	114.00bB	159.21bA	163.51bA	145.58	316.43aC	364.57aB	408.91aA	363.30
Bagasse+corn	155.67aA	100.10cB	155.82bA	137.20	271.67bB	354.75abA	354.34bcA	326.92
Bagasse+soybean	76.62cB	242.64aA	234.72aA	184.66	234.68bC	320.40bB	385.41abA	313.50
Bagasse+rice	80.45cB	213.68aA	226.35aA	173.50	266.48bB	341.84abA	323.59cA	310.64
Average	106.69	178.91	195.10		272.31	345.39	368.06	
CV1 (%)	16.29				5.85			
CV2 (%)	12.22				6.87			

Averages followed by the same small letter in the column and capital letter in the line do not differ by Tukey's test (5%); DM – dry matter; CV1 – coefficient of variation of silages; CV2 – coefficient of variation of the times.

The content of MM obtained in the silage with added rice bran is justified by its composition, which traditionally comprises high levels of MM and silica (VALADARES FILHO et al., 2008). The MM levels of *in natura* bagasse silage are consistent with those reported by Nefzaoui (1991). When rice bran was used as an additive, the MM quantity was lowered at 28 days of fermentation and intermediate at 56 days relative to the amount before fermentation. This result is expected, given the production of effluents, which contain minerals present in the ensilaged material (McDONALD et al., 1991), and to the fermentative losses, which favor the consumption of carbohydrates, especially structural carbohydrates, altering the concentration of the other nutrients in the total DM stored in the silos.

The EE of the bagasse decreased significantly when corn and soybean brans were added on the day of ensiling, although, only rice bran treatment significantly reduced the EE content during fermentation. Olive bagasse showed the highest EE content at 56 days of fermentation (Table 4). High levels of EE are directly related to the total digestible nutrient values of feed; however, it is necessary to evaluate and account for digestible EE. The high ADF in olive bagasse (Table 4) ultimately confers a low digestibility to this sub-product. Thus, the high EE and ADF contents of olive bagasse are the two main factors limiting its use at high inclusion levels in ruminant feed.

The use of the additives favored the dilution of EE in relation to *in natura* silage (Table 4). Niaounakis and Halvadakis (2006) cited the EE content of olive bagasse (essentially lipids and polyphenols) as a positive attribute for the preservation of the ensiled mass, by acting to impede the anaerobic degradation of the substrate. The fatty substances in bagasse are very rich in saturated C_{16} and C_{18} fatty acids, contributing to 96% of the total fatty acids, and can be a significant source of energy (SANSOUCY et al., 1985).

The CP was reduced with the addition of corn bran to the olive bagasse while the addition of soybean and rice bran increased the CP contents of the silages (Table 4). The NDF was significantly altered only by the sampling times studied, presenting an increase over the ensiling period (Table 4). There was an interaction effect of the factors examined in regards to the ADF content, which was decreased by the addition of soybean bran to the bagasse (Table 4).

The decrease in CP by the inclusion of corn bran and the increase observed when adding soybean bran and rice reflect the CP present in these brans, which have an average CP content of 87, 450 and 126 g kg^{-1} respectively (Table 1). After fermentation, only the silages with soybean and rice reached the minimum CP content of 70 g kg^{-1} proposed by Van Soest (1994) as the lower limit for survival and multiplication of microorganisms in the ruminal environment. These results suggest the addition of these brans as a promising option for improving the CP contents of the olive bagasse silage.

As the fermentation evolved, the CP contents remained unchanged (corn and soybean bran silages) or decreased (*in natura* bagasse and rice bran silage) (Table 4). These changes characterize the occurrence of fermentative processes inside the silos that caused losses of nitrogenous compounds, by leaching as effluent or volatilization of the volatile compounds formed (McDONALD et al., 1991).

The NDF comprises lignin, hemicellulose and cellulose, and is the most common parameter used for balancing of ruminant diets. Lignin is insoluble in the absence of chemical treatments and is intricately complexed with hemicellulose and cellulose in the plant cell walls (SILVA; QUEIROZ, 2009), so these constituents are largely maintained during fermentation processes. The increase NDF observed in this study is related to the fermentation time, as the fermentation degradative reactions, production of effluents and losses of nitrogen

compounds, cause a proportional increase in the fractions of the fibrous carbohydrates in the ensiled DM (NERES et al., 2013).

The ADF is composed by cellulose and lignin and indicates the amount of indigestible material (lignin) or slow digestion at the ruminal (cellulose) level (VAN SOEST, 1994). The ADF fraction of the silages increased (Table 4), due to the losses that occurred during the fermentation, having a non-beneficial impact on the nutritional quality of the olive bagasse.

The hemicellulose was lower in the olive bagasse silage at 28 and 56 days of fermentation than the mixtures with additives, in which the hemicellulose content largely remained constant (Table 4). Xylan is the most common hemicellulosic material in the plant cell walls (SARATALE et al., 2012). Owing to its structurally mixed composition, degradation of the substrate to its monomer, xylose, requires specialized xylanolytic enzyme systems, possessing different modes of action and specificities. These enzymes are secreted by ruminal microorganisms (VAN SOEST, 1994) and by filamentous fungi (BISWAS et al., 2010), and their action is nutritionally positive, as they promote xylan hydrolysis, facilitating subsequent microbial digestion in the rumen (MARTINS et al., 2007). Hence, the action of xylanases accounts for the observed changes in the hemicellulose contents of the silages.

There was a significant effect of the interaction of the studied factors on the cellulose contents (Table 4). Cellulose is the most abundant, homogenous and recalcitrant carbohydrate fraction of the plant cell wall, and is composed of long linear chains of high molecular weight D-glucopyranoses and a high degree of polymerization (GIGER-REVERDIN, 1995). The recalcitrance of cellulose to enzymatic hydrolysis by ensilage and ruminal fermentation microorganisms is due to the extensive hydrogen bonding interactions of individual cellulose strands, forming cellulose microfibrils with an inner crystalline core that is impenetrable to water. Furthermore, in the plant cell wall, the cellulose microfibrils are embedded in a matrix of hemicellulose and lignin (VAN SOEST, 1994).

Olive bagasse is characterized by high contents of lignin and lignocellulosic compounds (DERMECHE et al., 2013), and its cellulose is highly associated with the xylans and other polysaccharides, such as arabinose and galactose (NIAOUNAKIS; HALVADAKIS, 2006). Hence, the biochemical processes occurring inside the silos with additives are critical to providing a rational approach to using the olive bagasse co-product at the ruminal level.

The lignin contents were reduced in treatments with additives (Table 4). Therefore, the decrease in this phenolic-containing compound, which is considered to be the most harmful to the fermentation processes inside the rumen, suggests the possibility of better utilization of the fermentable carbohydrates present in the olive bagasse (DERMECHE et al., 2013).

Conclusions

The use of corn grain improved the fermentation characteristics of the olive bagasse silage. Corn, rice and soybean meal can be used as additives in the ensilage of olive bagasse to increase the DM content, but only the rice and soybean meal benefited the chemical composition profile of the silages. The meals used are recommended for use in olive bagasse silage, although, the choice of the additives studied will depend on the commercial availability in each region.

Increasing the storage period reduced the pH of the silages, but increased the losses of DM and NH_3-N, and contributed to the losses of some nutrients. Hence, 28 days of storage was recommended for the studied silages. Ensilaged olive bagasse can be used in the diet of ruminants, considering its chemical composition. The inclusion levels in the

feed offered to ruminants, should consider the animal's requirement, limits of different nutrients and, particularly, the EE and ADF contents of the olive bagasse co-products.

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