



# Milk yield of cows submitted to different levels of olive pomace in the diet

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**ABSTRACT.** The cultivation of olive trees is expanding in Brazil, mainly in Rio Grande do Sul in order to meet the demand for olive oil, the main product of the industrialization of olives. However, from the extraction, there is a significant generation of waste. This residue has high moisture and an appropriate destination is necessary given its environmental and economic importance. The chemical composition of the residue is similar to other products used in animal feed, such as corn silage or sorghum, in relation to DM, CP and NDF, the differential being high levels of EE and ADL. In order to evaluate the replacement of corn silage by the extraction residue of olive oil (olive pomace), eight Holstein dairy cow, between 90 and 100 days of lactation, were used, making a double Latin square (4x4). Analyzes were made regarding animals, feed and milk product in order to verify the feasibility of the substitution. The inclusion of olive pomace, conserved as silage, to replace corn silage, in the diet of lactating cows up to 15% (dry basis) does not alter milk production, as well as its composition and feed efficiency.

**Keywords:** unconventional feed; *Olea europaea* L.; agro-industrial residue; ruminants; nutritional value.

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## Introduction

The olive trees cultivation (*Olea europaea* L.) and the olive oil industry play an important social and economic role in several counties, especially in the Mediterranean ones and in countries with similar weather (Berbel & Posadillo, 2018), and it has been growing in Brazil, especially in the State of Rio Grande do Sul. Although the main objective of the olive farming is to obtain olive oil, the separation efficiency is low, resulting an estimate worldwide volume in 4 million tons per year (Lanfranchi, Giannetto, & De Pascale, 2016). In the European Union (EU) the applications given to residue, including the production of pomace oil, animal feed, fertilizers, pesticides, organic compounds and absorbents for contaminated water treatment by heavy metals (Lanfranchi et al., 2016). One of the important aspects that characterize the residue is its high moisture, which makes it difficult to store it for use in animal nutrition (Vlyssides, Lizides, & Karlis, 2004; Federici et al., 2011). The development of technologies that allow the use and valuation of this residue, as a co-product of noble applications, is fundamental for the sustainability of the productive chain especially in the perspective of this activity growth in Brazil.

The use of agro-industrial by-products has assumed considerable importance for animal feed as a strategy to solve the problem of waste disposal and reduce production costs (Vasta, Nudda, Cannas, Lanza, & Priolo, 2008). The possibility of using by-products from the olive oil industry, such as olive pomace, represents an interesting opportunity from an economic and environmental point of view, since about 80 to 90 kg of waste per 100 kg of olive can be collected (Valta, Kosanovic, Malamis, Moustakas, & Loizidou, 2015). This residue has been considered an environmental problem, since it presents high biochemical oxygen demand, thus requiring treatment for proper disposal (Roséillo-Soto et al., 2015).

The physicochemical characteristics of olive pomace may vary according to the method of oil extraction, subsequent treatment and to a lesser extent, olive variety and environmental conditions (Vera et al., 2009). These residues consist of a mixture composed of a high percentage of water, organic compounds such as

residual oil, sugars, fibers, polyphenols, nitrogen compounds, volatile acids, besides tocopherols, pigments such as chlorophyll and carotenoids and inorganic compounds, mainly salts and phosphates (Lafka, Lazou, Sinanoglou, & Lazos, 2011).

Studies carried out with residue from the olive oil extraction in the feeding of lactating ruminants are scarce and show different ways of obtaining (pressing, centrifuging with two or three phases) and processing (partial or total presence or absence of stone, wet or dehydrated), with variable results. Thus, it is observed in the experiments performed with sheep (Chiofalo, Liotta, Zumbo, & Chiofalo, 2004; Abbeddou, Rischkowsky, Richter, Hess, & Kreuzer, 2011), goats (Molina-Alcaide & Yañez-Ruiz, 2008; Keles, Yildiz-Akgul, & Kocaman, 2017), buffaloes (Terramoccia et al., 2013), camels (Faye et al., 2013), and with cows (Castellani et al., 2017; Vargas-Bello-Pérez et al., 2018). The controversial results are, probably, derived from the animal species and the characteristics and level of the residue used.

In this context, we aim evaluate the effect of different levels of olive pomace, obtained by two-stage centrifugal extraction, conserved in the form of silage, replacing corn silage, in lactation cow diets on performance production and chemical composition of the milk produced.

## Material and methods

A commercial milk production unit, located in the municipality of Santa Margarida do Sul, Rio Grande do Sul State (latitude 30°21'56.8" and longitude 54°11'02.6"), was used for animal experiments and the chemical and biological analyzes were carried out at the Núcleo Integrado de Desenvolvimento de Análises Laboratoriais (NIDAL), Departamento de Ciência e Tecnologia de Alimentos (DTCA) of the Universidade Federal de Santa Maria (UFSM), located in Santa Maria, Rio Grande do Sul State, Brazil.

The olive residue, called olive pomace (OP), was obtained from the company Tecnolivas, owner of the brand Prosperato, one of the companies that currently invests the most in this segment in Rio Grande do Sul. It is in the Caçapava do Sul municipality, region that has a microclimate mesothermal humid subtropical, called pampa biome in Rio Grande do Sul. Soon after harvest, the fruits are brought to the processing, the leaves and branches are removed and later the fruits are taken for the washing, removing any impurity that could compromise the hygiene, after that they are sent to the grinder, where grinding is done with stainless steel hammers. After grinding, the slurry is homogenized at a temperature below 28 °C (cold extraction) and taken to the horizontal centrifugation, to begin the process of separation of the liquid phase (oil and water) from the solid phase (dry matter of fruits).

Thus, OP is composed of fruit mass coming from horizontal centrifugation (solid fraction) and water separated from olive oil during vertical centrifugation, the extraction process being known as two-stage centrifugation. The residue, with a pasty appearance and high moisture content, is like the characteristics of forage feed such as corn silage.

The residue used in the experiment originated from the production of extra virgin olive oil during the month of March 2017. The OP was collected and transported to the dairy production unit, being stored in nylon-bag silo, remaining for about 60 days until the beginning of the experiment with the animals.

Eight multiparous (between second and fourth lactation) Holstein dairy cows, with approximate calving dates (between 90 and 100 days of lactation, with record of artificial insemination but without confirmation of pregnancy during the experimental period), with an average weight of 550 kg, body condition score 3 and initial average production of 19 liters of milk, were distributed in two Latin squares (4 x 4), kept in individual stalls. The experimental periods were of 21 days, 14 days of adaptation and the remaining seven days of each period, used for data collection (voluntary intake, live weight, milk production) and samples (feed offered, orts and milk).

The diets were formulated based on National Research Council (NRC, 2001) recommendations, considering the animals weight and estimates of their production potential (20 kg day<sup>-1</sup> with 3.5% fat), to be similar in neutral detergent fiber (NDF), crude protein (CP) and total digestible nutrients (TDN).

Treatments consisted of a control diet, with corn silage (CS) as forage and a commercial concentrated mixture (24% CP), supplied separately, with an estimated ratio of 50:50 (dry basis). The other treatments consisted in the inclusion of increasing levels (10, 20 and 30%) of OP, in the forage fraction, replacing the CS, resulting in levels of OP in the diet of 0, 5, 10 and 15%, making four experimental treatments, denominated, respectively of OP0, OP5, OP10 and OP15.

The concentrate was supplied twice a day, after the milking, and the forage was offered ad libitum, through manual mixing between the CS and the OP in their due proportions for each treatment, allowing at least 10% of orts (dry basis) of the one offered the previous day, and thus guarantee freely consumption.

The cows were mechanically milked twice a day, with an interval of 11 hours, between the milking of the morning (7 a.m.) and the afternoon (6 p.m.), and the individual milk yield were measured at each milking for experimental control. Milk samples, proportional to the milking, of the last two days of each period per cow, were taken for somatic cells count (SCC) assessments, chemical composition, including casein and ureic nitrogen.

Feed intake was obtained by the difference between the quantity offered and the daily refusals, during the seven days of collection of each period. The ingredients and respective diets were chemically analyzed to determine the consumption of each nutritional constituent.

The ingredients used in the diets (corn silage, olive pomace and concentrated) and the refusals were submitted to chemical analysis to determine dry matter (DM), mineral matter (MM), crude protein (CP), and ether extract (EE) according to Association of Official Analytical Chemists (AOAC, 1996), (Methods 967.03, 942.05, 954.05 and 920.39 respectively), neutral detergent fiber using thermostable  $\alpha$ -amylase, but without the use of sodium sulfite, corrected for ash (aNDFom) and acid detergent lignin (ADL) according to Van Soest, Robertson and Lewis (1991). The levels of neutral detergent insoluble nitrogen (NDIN), acid detergent insoluble nitrogen (ADIN) followed the methodology described by Licitra, Hernandez and Van Soest (1996). Non-fiber carbohydrates (NFC) were obtained according to Hall (2003) and total digestible nutrients values (TDN) were estimated according to NRC (2001). The chemical composition and total digestible nutrients (TDN) data of the ingredients diets can be seen in Table 1.

**Table 1.** Chemical composition (% dry matter) of the ingredients used in the experimental diets.

Composition	Concentrate	Olive pomace	Corn silage
DM	89,02	25,86	28,42
MM	9,51	4,15	6,57
CP	25,65	11,45	7,74
EE	4,24	19,05	2,54
aNDFom	15,64	47,74	50,56
NFC	44,96	17,61	32,59
ADIN	0,19	0,74	0,12
NDIN	0,39	1,64	0,28
ADL	2,18	34,15	4,82
TDN	76,83	62,51	55,14

DM = dry matter (% Natural Matter); MM = mineral matter; CP = crude protein; EE = ether extract; aNDFom = neutral detergent fiber corrected for ash; NFC = non-fiber carbohydrates; ADIN = acid detergent insoluble nitrogen; NDIN = neutral detergent insoluble nitrogen; ADL = acid detergent lignin and TDN = total digestible nutrients.

The centesimal composition of the experimental diets can be observed in Table 2, evidencing the increasing substitution of the CS for the OP, increasing levels of EE. The numerical difference observed in the EE is directly related to the greater substitution of the CS for the OP, considering the individual differences in the content of EE of the feed (19.05 for OP and 4.54 for CS, both in  $g\ 100g^{-1}$  DM).

**Table 2.** Centesimal and TDN composition of the experimental diets offered in relation to the different treatments (% DM).

Diets	OM	CP	EE	aNDFom	NFC	TDN
OP0	91,96	16,70	3,39	33,10	38,77	65,99
OP5	92,20	17,07	5,04	32,82	37,27	66,72
OP10	92,44	17,44	6,69	32,54	35,77	67,46
OP15	92,69	17,81	8,34	32,25	34,27	68,20

OM= organic matter, CP= crude protein, EE= ether extract, aNDFom = neutral detergent fiber corrected for ash, NFC= non-fiber carbohydrates, TDN= total digestible nutrients.

The analysis of milk for fat, protein, lactose, total solids, casein and urea nitrogen were performed by infrared spectroscopy (AOAC, 1996) (method 972.16), and somatic cells count (determined by flow cytometry) in Serviço de Análise de Rebanhos Leiteiros (SARLE), Universidade de Passo Fundo (UPF), Passo Fundo, Rio Grande do Sul State, accredited by the Rede Brasileira de Laboratórios (RBQL) of Ministério da Agricultura, Pecuária e Abastecimento (MAPA). Data were submitted to analysis of variance (ANOVA) and regression (linear and quadratic) using the MIXED procedure and considering animal as random effect in

Statistical Analysis System [SAS] (2012). The following causes of variation were evaluated: treatment, period, square, cow within square and the interaction square  $\times$  treatment. The model applied for ANOVA was:

$$Y_{ijkl} = \mu + Q_i + T_j + P_k + QT_{ij} + A(i)l + e_{ijk}$$

where:  $Y_{ijkl}$  = mean value obtained for each observation;  $\mu$  = general mean of the variable in the experiment;  $Q_i$  = effect of the square, where  $i = 1$  and  $2$ ;  $T_j$  = effect of treatment  $j$ , with  $j = 1, 2, 3$  and  $4$ ;  $P_k$  = effect of period, with  $k = 1, 2, 3$  and  $4$ ;  $QT_{ij}$  = interaction between square  $i$  and treatment  $j$ ;  $A(i)l$  = effect of cow  $l$  within square  $i$ ;  $e_{ijk}$  = experimental error.

## Results and discussion

The chemical composition results of the extraction residue of olive oil available were variable, ranging from 28.78 to 49.0% DM, 3.5 to 5.2% MM, 57.1 to 74.0% of NDF, 22.2 to 30.4% of ADL, 5.0 to 8.13% of CP and 10.2 to 12.62% of EE (Martín Garcia, Moumen, Yáñez-Ruiz, & Molina-Alcaide, 2003; Faye et al., 2013; Keles, 2015).

Hadjipanayiotou (1999), in animal research carried out in Cyprus, used olive pomace silage with higher levels of DM (49%) and NDF (74.0%), but lower lignin (24.8%), CP (5.0%) and EE (10.2%). Faye et al. (2013) describes more similar values to those ones found in the present study, with values of OM (94.8%), NDF (67.1%), ADL (30.4%), and CP (7.3%).

It should be noted that, even with differences in chemical composition, it was possible to formulate similar diets in CP, NDF and TDN in the different levels of inclusion of OP in substitution to CS (Table 2). At the same time, when the contents were calculated through the voluntary intake (offered less orts), it is verified that the estimates were reproduced (Table 3).

**Table 3.** Chemical composition and value energetic of the diets calculated based on voluntary intake in the different experimental treatments expressed in g 100g<sup>-1</sup> DM.

Diets	OM	CP	EE	aNDFom	NFC	TDN
OP0	91,75	17,64	3,47	33,44	39,25	65,73
OP5	91,85	17,86	4,14	33,60	38,68	66,12
OP10	91,95	18,11	4,85	33,76	38,08	66,59
OP15	92,01	18,46	5,58	33,85	37,34	67,11

OM= organic matter, CP= crude protein, EE= ether extract, aNDFom=neutral detergent fiber corrected for ash, NFC= non-fiber carbohydrates, TDN= total digestible nutrients.

It is possible to observe that the substitution of olive pomace at the 15% level in the total diet represented an increase of approximately 60% of the EE in relation to the control treatment. A similar result was observed by Hadjipanayiotou (1999), who evaluated the supply of OP to cattle, goats and dairy sheep, observed a 65% increase in EE from the use of 15% OP in the diet.

Faye et al. (2013) evaluated the supply of OP up to the level of 17% in camels diet, so it was observed that the proportion of fat in the total diet increased in the treated group. The comparison with other ingredients with low EE value like alfalfa (2.5 g 100 g<sup>-1</sup> DM) and barley (1.9 g 100g<sup>-1</sup> DM), the OP increased the fat content of the diet by 115%, the fat content in the diet went from 2% in the control group to 4.2% in the treated group. Despite this high increase in fat consumption, no change in milk fat content was observed in the animals.

The differences observed in DMI and OMI can be explained by variations in the live weight of the animals, because when expressed as percentage of LW, statistical analysis showed no difference between treatments (Table 4). In lactating dairy cows, peak milk production (highest energy demand) usually occurs 4 to 8 weeks after calving, and peak intake dry matter (highest energy intake) occurs 10 to 14 weeks after calving. The energy balance in the cow's organism is obtained by the difference between the energy intake by the animal, defined by intake, and the energy the animal needs to meet its daily requirements for different functions. If the balance is positive, or in other words, energy intake is greater than the requirements, the cows store the excess energy consumed through the diet, in the form of fat and LWG. (NRC, 2001). The cows used in this experiment were between 90-100 days of lactation, thus after the peak of lactation and close to the peak of DM intake. This way, it is possible to verify a positive energy balance demonstrated by the observed LWG values, as well as the DMI values observed, it is possible to affirm an adequate fed supply to the animals.

**Table 4.** Effect of different levels of olive pomace (OP) inclusion in the diets on live weight (LW), live weight gain (LWG) and intake of dry matter (DMI), organic matter (OMI), crude protein (CPI), ether extract (EEI), neutral detergent fiber (aNDFomI), non-fiber carbohydrates (NFCI) and of total digestible nutrients (TDNI).

Item	Treatments				MSE	P-value	
	OP0	OP5	OP10	OP15		Linear	Quadratic
LW (kg)	574,06	567,56	573,62	577,13	13,6543	0,8526	0,7853
LWG (kg)	21,87	14,88	15,50	14,00	9,8923	0,4445	0,6815
DMI (kg day <sup>-1</sup> )	18.72 <sup>a</sup>	18.51 <sup>b</sup>	18.45 <sup>c</sup>	18.13 <sup>d</sup>	0.1303	< 0.0001	0.5744
DMI (% LW)	3.28	3.30	3.24	3.16	0.0756	0.3718	0.6331
OMI (kg day <sup>-1</sup> )	17.18 <sup>a</sup>	17.00 <sup>b</sup>	16.96 <sup>c</sup>	16.68 <sup>d</sup>	0.1217	0.0003	0.5570
OMI (% LW)	3.00	3.03	2.97	2.91	0.0687	0.4056	0.6310
aNDFomI (kg day <sup>-1</sup> )	6.26	6.22	6.23	6.14	0.0665	0.0851	0.6262
aNDFomI (%LW)	1.10	1.11	1.09	1.07	0.0270	0.5371	0.6031
CPI (kg day <sup>-1</sup> )	3.30 <sup>a</sup>	3.31 <sup>b</sup>	3.34 <sup>c</sup>	3.35 <sup>d</sup>	0.0097	< 0.0001	0.8345
EEI (kg day <sup>-1</sup> )	0.649 <sup>a</sup>	0.766 <sup>b</sup>	0.895 <sup>c</sup>	1.012 <sup>d</sup>	0.0113	< 0.0001	0.9843
NFCI (kg day <sup>-1</sup> )	7.35 <sup>a</sup>	7.16 <sup>b</sup>	7.02 <sup>c</sup>	6.77 <sup>d</sup>	0.0607	< 0.0001	0.4407
TDNI (kg day <sup>-1</sup> )	12.31	12.24	12.28	12.17	0.0781	0.1238	0.7099

DMI (kg day<sup>-1</sup>) = 18.73 - 0.037 \* % of substitution (R<sup>2</sup> = 0.9404); OM (kg day<sup>-1</sup>) = 17.19 - 0.0306 \* % of substitution (R<sup>2</sup> = 0.9247); CPI (kg day<sup>-1</sup>) = 3.30 + 0.0033 \* % of substitution (R<sup>2</sup> = 0.8915); EEI (kg day<sup>-1</sup>) = 0.648 + 0.024 \* % of substitution (R<sup>2</sup> = 0.9996); NFCI (kg day<sup>-1</sup>) = 7.356 - 0.037 \* % of substitution (R<sup>2</sup> = 0.9857). MSE = mean standard error.

The differences observed for EEI and NFCI were expected, due to the chemical characteristics of the OP, which presented high EE content and low NFC content in comparison to CS. Similarly, Hadjipanayiotou (1999) evaluating the use of OP in cattle, goats and dairy sheep found higher intake of EE fraction with the inclusion of OP. This fact was also observed in a study developed by Obeidat (2017) who evaluated through supplementation with Olive Pomace and *Saccharomyces cerevisiae*, the performance and blood metabolites in lambs and noted the inclusion of OP in the diets of lambs increased the EE content compared to the control diet due to the greater content of EE in OP compared to the EE content in wheat straw and soybean meal. Despite the levels of EE were higher in diets with OP, apparently these did not have a negative effect on the digestibility of roughage, and, consequently, on voluntary intake.

Sadeghi, Yansari and Ansari-Pirsarai (2009) in order to evaluate the effects of different olive residues: crude, the exhausted (usually obtained from crude olive pomace by a solvent extraction process that removes most of its residual oil content), partly destoned and partly destoned exhausted) on the voluntary intake of dry matter, digestibility of nutrients and performance of growing sheep, observed that low degradable fraction of DM, CP and NDF of olives by products are the most important limiting factors in ruminant nutrition. However, destoning of olive residue significantly increased its digestible content and nutritive value, thus, destoned olive pomace may have a higher nutritive value than the other olive by-products (as in this study where crude waste was used) and it has improved body weight gain, growth rate and feed conversions.

The possible effects of the treatments on performance, yield and composition of milk according to the increasing levels of olive pomace (OP0, OP5, OP10 and OP15) are presented in Table 5. The results did not show a statistically significant difference in parameters, even at higher levels of substitution. Hadjipanayiotou (1999) evaluating the consumption of olive pomace silage in cattle, goats and dairy sheep, did not observe significant differences in milk yield, milk yield corrected to fat, milk protein content and weight gain of dairy sheep in different diets. The same author also did not find significant differences for milk yield, yield corrected for fat and milk protein content for cattle and dairy goats, however, there were significant differences for milk fat content which was not observed in this study.

Faye et al. (2013) using OP in the proportion of 17% in the diet of lactating she-camels did not observe any negative or positive effects on milk production.

Cibik and Keles (2016) evaluating the inclusion of OP in dairy cows feed in both the concentrated and bulky fraction observed an increase in dry matter intake. However, no significant variation was observed in milk yield, including yield corrected to 3.5% fat, which is in line with the results observed in this study.

Cabiddu, Canu, Decandia, Moll and Pompel (2004) evaluated olive pomace (EE content: 115 g kg<sup>-1</sup> DM), in two inclusion levels (100 and 200 g kg<sup>-1</sup> DM), as substitute for equivalent amounts of forage, in rations fed to sheep for five weeks before and five weeks after calving it did not obtain significant effects on milk yield and composition, although the higher inclusion level tended to lower milk yield (-8%) and lower milk protein concentration (-7.5%).

**Table 5.** Effect of different levels of olive pomace (OP) inclusion in the diets on milk yield, feed efficiency, composition and yield of chemical components and somatic cells count (SCC).

Item	Treatments				MSE	P-value	
	OP0	OP5	OP10	OP15		Linear	Quadratic
Milk Yield (kg day <sup>-1</sup> )	19,08	18,51	18,35	19,16	0,7593	0,9826	0,5046
Milk 3,5%Fat(kg day <sup>-1</sup> )	19,49	19,44	19,41	19,65	1,1287	0,9362	0,9067
NE (Mcal day <sup>-1</sup> )	0,702	0,717	0,722	0,700	0,0167	0,9994	0,2399
FCE	1,05	1,05	1,05	1,08	0,0594	0,7410	0,8200
Milk, kg kg <sup>-1</sup> MS	1,02	1,00	0,99	1,06	0,441	0,6593	0,4605
Fat (%)	3,62	3,78	3,84	3,63	0,1838	0,8683	0,1912
Fat (kg day <sup>-1</sup> )	0,692	0,704	0,707	0,700	0,0524	0,9149	0,8635
Protein (%)	3,43	3,39	3,43	3,34	0,5775	0,3971	0,6740
Protein (kg day <sup>-1</sup> )	0,656	0,629	0,631	0,640	0,0310	0,9398	0,7343
Lactose (%)	4,37	4,42	4,36	4,42	0,0764	0,8109	0,9109
Lactose (kg day <sup>-1</sup> )	0,834	0,821	0,802	0,849	0,0365	0,9099	0,5611
Total solids (%)	12,43	12,57	12,67	12,43	0,2541	0,9229	0,4220
Total solids (kg day <sup>-1</sup> )	2,375	2,334	2,331	2,394	0,1272	0,9398	0,7343
Casein (%)	2,69	2,67	2,66	2,69	0,0679	0,9604	0,5636
Casein (kg day <sup>-1</sup> )	0,513	0,496	0,489	0,517	0,0265	0,9838	0,4762
MUN, mg dL <sup>-1</sup>	11,16	11,50	12,20	13,89	2,2068	0,2030	0,6611
Casein/Protein ratio	78,37	78,65	77,43	80,68	1,6538	0,3024	0,2297
SCC *	403,4	449,7	493,8	530,1	0,2948	0,1494	0,9384

OP0 = without inclusion of olive pomace; OP5= 5% inclusion of olive pomace; OP10= 10% inclusion of olive pomace; OP15= 15% inclusion of olive pomace. MSE= mean standard error; NE = net energy; FCE= fed conversion efficiency = (milk 3,5% Fat kg<sup>day-1</sup>)/(DMI kg day<sup>-1</sup>); MUN= milk ureic nitrogen; \*SCC= somatic cells count = \*1000 mL<sup>-1</sup>.

In Table 5 it can be observed that there was no significant difference for feed efficiency (FCE), a different situation was observed by Cibik and Keles (2016), while evaluating the inclusion of OP in the diet of dairy cows observed decrease in feed efficiency compared to the control group, attributing such difference to the smaller particle size of the OP and increasing the rate of passage impairing the digestibility.

Castellani et al. (2017) evaluating the dietary supplementation of dairy cows with 10% of OP in the dry matter, verified that the milk production registered during the experimental period was not affected by the diets. Regarding milk composition, significant differences were observed only for protein content that was higher in the experimental group than in the control one. Vargas-Bello-Pérez et al. (2018) evaluating the supplementation with 30g kg<sup>-1</sup> in DM of olive oil (OO) residue and hydrogenated vegetable oil (HVO), reaching the levels of ether extract (%DM) in the diets of 4.6 (control), 7.7 (OO) and 7.1 (HVO) observed that fat supplementation through olive oil resulted in a 10.9% increase in milk yield compared to control and hydrogenated vegetable oil. Regarding milk composition, the authors observed a reduction of 14.6% in the content and yield of milk fat.

Concerning the values of milk urea nitrogen (MUN), the statistical analysis did not identify significant effects, standing in the range of 11.16 to 13.89 mg dL<sup>-1</sup> considered normal and adequate. The usual levels of MUN are between 12 and 17 mg dL<sup>-1</sup> (Broderick, 1995; Fatehi et al., 2012; Aguilar et al., 2012; Huhtanen, Cabezas-Garcia, Krizsan, & Shingfield, 2015), equivalent to 25.7 to 36.4 mg dL<sup>-1</sup> of urea (1 mol of MUN = 2.14 mols of urea). At the same time, evaluating the efficiency of the use of nitrogen in the diet in relation to protein in milk (nitrogen in milk / nitrogen in the diet x 100), values of 18.94, 18.49, 18.54 and 18.66%, respectively, were verified for the increasing levels of inclusion of OP in the diets. Therefore, it can be inferred that the experimental diets were balanced and adequate in protein and energy. These results confirm those found by Cibik and Keles (2016) evaluating the inclusion of OP in the dairy cow diet with a 13% substitution level in the total diet, in addition to cotton seed exclusion in comparison to the control diet, statistically significant differences were not found in relation to the use of OP and the values were within the reference parameters.

Terramocchia et al. (2013) evaluated the use of dried stoned olive pomace in the feeding of lactating buffaloes and noted the fat, protein, lactose and urea content of the milk of the two groups (control and experimental diet) is similar, respectively 7.16 and 7.36%, 4.51 and 4.45%, 4.88 and 4.90%, 32.58 and 33.13 mg 100 ml<sup>-1</sup>. Thus the results obtained demonstrate, as found by Chiofalo et al. (2004), that the experimental diet did not alter the level of the considered parameters.

The colonization of the bovine mammary pathogenic bacteria results in a series of events that lead to changes in milk composition. Initially, high levels of pathogenic bacteria occur, followed by a marked

increase in the number of somatic cells (Kitchen, 1981). According Auldish, Coats, Rogers and McDowell (1995), during mastitis, the percentage of lactose in milk is reduced due to less synthesis caused by the destruction of secretory tissue, the loss of lactose from the gland into the bloodstream due to the increased permeability of the membrane that separates milk from blood and the use of lactose by intramammary pathogens.

The values observed for somatic cell count (SCC) are in accordance with Normative Instruction No. 31, of the Ministério da Agricultura, Pecuária e Abastecimento (MAPA) (Instrução Normativa nº 31, 2018), which determined the limits for SCC Count of  $500 \times 10^3$  cls mL<sup>-1</sup>, the exception of the value referring to OP15. The high values observed for SCC may be linked to the occurrence of subclinical mastitis, however, there were no significant variations in the constituents of milk that could be linked to an inflammatory condition of the mammary gland.

According Machado, Pereira, Silva and Sarries (2000), the activity of microorganisms within the mammary gland releases substances that stimulate leukocyte migration in order to combat aggressive agents thus increasing SCC, in addition SCC can be considered as a general indicator of health of the mammary gland. Also, SCC can be considered an important parameter for the evaluation of milk quality due to direct interference with their microbiological quality, as well as their physicochemical characteristics (Vargas et al., 2014, 2018). Research done by Vargas-Bello-Pérez et al. (2018) showed reduction in the SCC values in the milk of cows supplemented with 10% of olive pomace, however in the present study, as well as in a study carried out by Castellani et al. (2017), it was not possible to verify the effect of OP use on the SCC of the milk produced. The possible reduction in SCC in milk could be partially explained by the presence of polyphenols in olive oil, as reported by Serreli and Deiana (2020), the extra virgin olive oil polyphenols are known to be principally antioxidants in the broadest sense of the word, being able to directly scavenge oxidant species and to increase cellular endogenous antioxidant defenses.

## Conclusion

The olive pomace stored as silage can be used as an ingredient in the diet of cows in the middle of lactation at levels of up to 15% on a dry basis without compromising milk yield, feed efficiency, and centesimal composition, constituting an alternative to the destination of a material that needs to be recycled.

## References

- Abbeddou, S., Rischkowsky, B., Richter, E. K., Hess, H. D., & Kreuzer, M. (2011). Modification of milk fatty acid composition by feeding forages and agro-industrial byproducts from dry areas to Awassi sheep. *Journal of Dairy Science*, *94*(9), 4657-4668. doi: 10.3168/jds.2011-4154
- Aguilar, M., Hanigan, M. D., Tucker, H. A., Jones, B. L., Garbade, S. K., McGilliard, M. L., & James, R. E. (2012). Cow and herd variation in milk urea nitrogen concentrations in lactating dairy cattle. *Journal of Dairy Science*, *95*(12), 7261-7268. doi: 10.3168/jds.2012-5582
- Association of Official Analytical Chemists [AOAC]. (1996). *Official Methods of Analysis of the Association of Official Analysis Chemists* (16th ed.). Arlington, VA: AOAC.
- Auldish, M. J., Coats, S., Rogers, G. L., & McDowell, G. H. (1995). Changes in the composition of milk from healthy and mastitic dairy cows during the lactation cycle. *Australian Journal of Experimental Agriculture*, *35*(4), 427-36. doi: 10.1071/EA9950427
- Berbel, J., & Posadillo, A. (2018). Review and analysis of alternatives for the valorization of agro-industrial olive oil by-products. *Water, Environmental and Agricultural Resources Economics (WEARE) Research Group Sustainability*, *10*(1), 237. doi: 10.3390/su10010237
- Broderick, G. A. (1995). Performance of lactating dairy cows fed either alfalfa silage or alfalfa hay as the sole forage. *Journal of Dairy Science*, *78*(2), 320-329. doi: 10.3168/jds.S0022-0302(95)76640-1
- Cabiddu, A., Canu, M., Decandia, M., Moll, E. G., & Pompel, R. (2004). The intake and performance of dairy ewes fed with different levels of olive cake silage in late pregnancy and suckling periods. In H. Ben Salem, A. Nefzaoui, & P. Morand-Fehr (Eds.), *Nutrition and Feeding Strategies of Sheep and Goats under Harsh Climates* (p. 197-201). Zaragoza, ES: CIHEAM-IAMZ.

- Castellani, F., Vitali, A., Bernardi, N., Marone, E., Palazzo, F., Grotta, L., & Martino, G. (2017). Dietary supplementation with dried olive pomace in dairy cows modifies the composition of fatty acids and the aromatic profile in milk and related cheese. *Journal of Dairy Science*, *100*(11), 8658-8669. doi: 10.3168/jds.2017-12899
- Chiofalo, B., Liotta, L., Zumbo, A., & Chiofalo, V. (2004). Administration of olive cake for ewe feeding: effect on milk yield and composition. *Small Ruminant Research*, *55*(1-3), 169-176. doi: 10.1016/j.smallrumres.2003.12.011
- Cibik, M., & Keles, G. (2016). Effect of stoned olive cake on milk yield and composition of dairy cows. *Revue de médecine vétérinaire*, *167*(5-6), 154-158. Recovered from [https://www.researchgate.net/publication/303910958\\_Effect\\_of\\_stoned\\_olive\\_cake\\_on\\_milk\\_yield\\_and\\_composition\\_of\\_dairy\\_cows](https://www.researchgate.net/publication/303910958_Effect_of_stoned_olive_cake_on_milk_yield_and_composition_of_dairy_cows)
- Fatehi, F., Zali, A., Honarvar, M., Dehghan-banadaky, M., Young, A. J., Ghiasvand, M., & Eftekhari, M. (2012). Review of the relationship between milk urea nitrogen and days in milk, parity, and monthly temperature mean in Iranian Holstein cows. *Journal of dairy science*, *95*(9), 5156-5163. doi: 10.3168/jds.2011-4349
- Faye, B., Konuspayeva, G., Narmuratova, M., Serikbaeva, A., Mussad, A. M. & Mehri, H. (2013). Effect of crude olive cake supplementation on camel milk production and fatty acid composition. *Dairy Science & Technology*, *93*, 225-239. doi: 10.1007/s13594-013-0117-6
- Federici, E., Pepi, M., Esposito, A., Scargetta, S., Fidati, L., Gasperini, S., ... Artieri, R. (2011). Two -phase olive mill waste composting: community dynamics and functional role of the resident microbiota. *Bioresource Technology*, *102*(23), 10965-10972. doi: 10.1016/j.biortech.2011.09.062
- Hadjipanayiotou, M. (1999). Feeding ensiled crude olive cake to lactating Chios ewes, Damascus goats and Friesian cows. *Livestock Production Science*, *59*(1), 61-66. doi: 10.1016/S0301-6226(99)00005-6
- Hall, M. B. (2003). Challenges with non fiber carbohydrate methods. *Journal of Animal Science*, *81*(12), 3226-3232. doi: 10.2527/2003.81123226x
- Huhtanen, P., Cabezas-García, E. H., Krizsan, S. J., & Shingfield, K. J. (2015). Evaluation of between-cow variation in milk urea and rumen ammonia nitrogen concentrations and the association with nitrogen utilization and diet digestibility in lactating cows. *Journal of Dairy Science*, *98*(5), 3182-3196. doi: 10.3168/jds.2014-8215
- Instrução Normativa nº 31, de 29 de junho de 2018 (2018). Altera redação tabela 2 do item 3.1.3.1 do Anexo II da IN nº 62 de 29 de dezembro de 2011. *Diário Oficial da União*, Edição 125, Seção 1, p. 2.
- Keles, G. (2015). Zeytin posasinin ruminantlar için besinve besleme değeri .*Türk Tarım. Gıda Bilimve Teknoloji Dergisi*, *3*(10), 780-789. doi: 10.24925/turjaf.v3i10.780-789.435
- Keles, G., Yildiz-Akgul, F., & Kocaman, V. (2017). Performance and milk composition of dairy goats as affected by the dietary level of stoned olive cake silages. *Asian-Australasian Journal of Animal Sciences*, *30*(3), 363-369. doi: 10.5713/ajas.16.0482
- Kitchen, B. J. (1981). Bovine mastitis: milk compositional changes and related diagnostic tests. *Journal of Dairy Research*, *48*(1), 167-88. doi: 10.1017/S0022029900021580
- Lafka, T. I., Lazou, A. E., Sinanoglou, V. J., & Lazos, E. (2011). Phenolic and antioxidante potential of olive oil mill wastes. *Food Chemistry*, *125*(1), 92-98. doi: 10.1016/j.foodchem.2010.08.041Get rights and content
- Lanfranchi, M., Giannetto, C., & De Pascale, A. (2016). Economic analysis and energy valorization of by-products of the olive oil process: “Valdemone DOP” extra virgin olive oil. *Renewable and Sustainable Energy Reviews*, *57*, 1227-1236. doi: 10.1016/j.rser.2015.12.196
- Licitra, G., Hernandez, T. M., & Van Soest, P. J. (1996). Standardization of procedures for nitrogen fractionation of ruminant feeds. *Animal Feed Science and Technology*, *57*(4), 347-358. doi: 10.1016/0377-8401(95)00837-3
- Machado, P. F., Pereira, A. R., Silva, L. F. P., & Sarries, G. A. (2000). Células somáticas no leite em rebanhos brasileiros. *Scientia Agricola*, *57*(2), 359-361. doi: 10.1590/S0103-90162000000200026
- Martín García, A. I., Moumen, A., Yañez-Ruiz, D. R., & Molina-Alcaide, E. (2003). Chemical composition and nutrients availability for goats and sheep of two-stage olive cake and olive leaves. *Animal Feed Science and Technology*, *107*(1-4), 61-74. doi: 10.1016/S0377-8401(03)00066-X



- Molina-Alcaide, E., & Yañez-Ruiz, D. R. (2008). Potential use of olive by-products in ruminant feeding: a review. *Animal Feed Science Technology*, 147(13), 247-264. doi: 10.1016/j.anifeedsci.2007.09.021
- National Research Council [NRC]. (2001). *Nutrient requirements of dairy cattle*. Washington, DC: NRC.
- Obeidat, B. S. (2017). The effects of feeding olive cake and *Saccharomyces cerevisiae* supplementation on performance, nutrient digestibility and blood metabolites of Awassi lambs. *Animal Feed Science and Technology*, 231, 131-137. doi: 10.1016/j.anifeedsci.2017.07.006
- Rosello-Soto, E., Kobaa, M., Moubarik, A., Lopes, R. P., Saraiva, J. A., Boussetta, ... & Barba, F. J. (2015). 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*, 45(2), 296-310. doi: 10.1016/j.tifs.2015.07.003
- Sadeghi, H., Yansari, A. T., & Ansari-Pirsarai, Z. (2009). Effects of different olive cake by products on dry matter intake, nutrient digestibility and performance of Zel sheep. *International Journal of Agriculture & Biology*, 11, 39-43. doi: 07-336/AWB/2009/11-1-39-43
- Serreli, G., & Deiana, M. (2020). Extra virgin olive oil polyphenols: modulation of cellular pathways related to oxidant species and inflammation in aging. *Cells*, 9(2), 478. doi: 10.3390/cells9020478
- Statistical Analysis System [SAS]. (2012). *System for Windows™ statistical package, version 9.0*. Cary, NC: SAS Institute Inc.
- Terramoccia, S., Bartocci, S., Taticchi, A., Di Giovanni, S., Pauselli, M., Mourvaki, E., ... Servili, M. (2013). Use of dried stoned olive pomace in the feeding of lactating buffaloes: effect on the quantity and quality of the milk produced. *Asian- Australasian Journal of Animal Sciences*, 26(7), 971-980. doi: 10.5713/ajas.2012.12627
- Valta, K., Kosanovic, T., Malamis, D., Moustakas, K., & Loizidou, M. (2015). Overview of water usage and wastewater management in the food and beverage industry. *Desalination and Water Treatment*, 53(12), 3335-3347. doi: 10.1080/19443994.2014.934100
- Van Soest, P. J., Robertson, J. B., & Lewis, B. A. (1991). Methods of dietary fiber, neutral detergent fiber and nonstarch polysaccharides in relation to animal nutrition. *Journal of Dairy Science*, 74(10), 3583-3597. doi: 10.3168/jds.S0022-0302(91)78551-2
- Vargas, D. P., Nornberg, J. L., Mello, R. O., Scheibler, R. O., Milani, M. P., & Mello, F. C. (2014). Correlações entre contagem bacteriana total e parâmetros de qualidade do leite. *Revista Brasileira de Ciência Veterinária*, 20(4), 241-247. doi: 10.4322/rbcv.2014.009
- Vargas, D. P., Nornberg, J. L., Mello, R. O., Scheibler, R. B., Mello, F. C. B., Rizzo, F. A., & Bellato, T. K. B. (2018). Formation of homogeneous groups of bovine milk production units via multivariate statistical techniques. *Ciência Rural*, 48(4), e20170055. doi: 10.1590/0103-8478cr20170055
- Vargas-Bello-Pérez, E., Geldsetzer-Mendoza, C., Morales, M. S., Toro-Mujica, P., Fellenberg, M. A., Ibáñez, R. A., & Garnsworthy, P. C. (2018). Effect of olive oil in dairy cow diets on the fatty acid profile and sensory characteristics of cheese. *International Dairy Journal*, 85, 8-15. doi: 10.1016/j.idairyj.2018.04.006
- Vasta V., Nudda, A., Cannas, A., Lanza, M., & Priolo, A. (2008). Alternative feed resources and their effects on the quality of meat and milk from small ruminants. *Animal Feed Science and Technology*, 147(1-3), 223-246. doi: 10.1016/j.anifeedsci.2007.09.020
- Vera, R., Aguilar, C., Lira, R., Toro, P., Barrales, L., Peña, I., ... Brione, I. (2009). Feeding dry olive cake on modifies subcutaneous fat composition in lambs, nothing cake resistance to degradation and peroxidation. *Chilean Journal of Agricultural Research*, 69(4), 548-559. doi: 10.4067/s0718-58392009000400010
- Vlyssides, A. G., Loizides, M., & Karlis, P. K. (2004). Integrated strategic approach for reusing olive oil extraction by-products. *Journal of Cleaner Production*, 12(6), 603-611. doi: 10.1016/S0959-6526(03)00078-7