Nutritive Value and Silage Characteristics of Whole and Partly Stoned Olive Cakes Treated with Molasses

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ABSTRACT

This study was conducted to determine the nutritive value of fresh and ensiled whole and partly stoned olive cake (OC) with or without molasses *i.e.,* 0 and 50 gkg^{-1} on fresh basis. Dry matter (DM), organic matter (OM), crude protein (CP), neutral detergent fiber (NDF), acid detergent fiber (ADF), lignin(sa), ether extract (EE), water soluble carbohydrates (WSC), and total phenols (TPH) of all treatments were determined by laboratory analysis. Additionally, pH and the concentration of ammonia-N, lactic acid, and volatile fatty acids were measured in the ensiled treatments. An *in vitro* gas production for 24 h was used to estimate organic matter digestibility (OMD) and metabolizable energy content. Ensiling OC decreased ($P<0.05$) DM, pH, EE, WSC, TPH and OMD, and increased ($P<0.05$) NDF, ADF and lignin(sa) contents. Addition of molasses decreased ($P<0.05$) OM, NDF, ADF, lignin(sa) and pH, but increased ($P<0.05$) DM, WSC, OMD and lactic acid. In conclusion, based on these results, the potential to use of OC as a feed in diets of ruminants is limited.

Keywords: Ensiling, Molasses, Nutritive value, Olive cake.

INTRODUCTION

Climatic conditions and shortage of water resources have increased the costs of animal feeds in Iran. Olive by-products are available in large quantities in Asian countries (Ben Salem *et al.*, 2004). For 1000 kg of olives, the three-phase procedure generates 550 kg of OC while the two-phase procedure produces 800 kg. Extracted olive cake provides cheap energy and fiber to the animal and high-fat olive cake may be used to improve the quality of the fat in the animal products (Molina-Alcaide and Yanez-Ruiz, 2008). Moreover, Nik-Khah and Ghorbani (1997) and Rahro-Mehrbani (1998) claimed that olive by-products could be used as feed for ruminants up to 24% in the ration of dairy cow, and 30% of concentrate in the ration of fattening lamb, respectively. Crude OC is the residue of the first extraction of oil from the whole olive by pressure. Partly stoned OC is the result of partly separating the stone from the pulp by screening or ventilation (Sansoucy, 1985). Using this by-product for animal feeding is a means of recycling that, otherwise, if accumulated, might cause environmental pollution (Huber, 1980). However, the storage of this by-product is difficult due to its high moisture content, which is above 600 g kg^{-1} (Ben Salem and Znaidi, 2008). Feed blocks have been used as a way of conserving OC (Ben Salem and Znaidi, 2008; Molina-Alcaide and Yáñez-Ruiz, 2008). Also, ensiling is a suggested technique for long-term preservation of OC (Hadjipanayiotou, 1994). Due to the low sugar content in OC (Al-Masri, 2005), molasses treatment has been suggested for improving fermentation quality of silage (Hadjipanayiotou, 1994). Olive cake contains polyphenol compounds that can have an

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adverse effect on nutrient utilization by animals (Al-Masri, 2005; Molina-Alcaide and Yáñez-Ruiz, 2008). Several methods, such as adding PEG (Ben Salem *et al.*, 2003), alkalis (Nefzaoui, 1985), and ensiling (Weinberg *et al.*, 2008), have been used to deactivate polyphenol and improve the nutritional value of OC. The aim of this study was to evaluate the effects of ensiling OC with or without molasses on its nutritive value and fermentation quality.

MATERIALS AND METHODS

Sample Preparation

Fresh samples of whole and partly stoned OC (from two-stage procedure) were collected from Golden olive factory in the north of Iran and Felahat Dar Feraghat factory in Shiraz (Iran), respectively. From each factory, five 50-kg samples were collected by hand at several locations. The OC was treated with 50 g kg^{-1} sugar cane beet molasses (syrup without diluting with water) on a fresh basis, without any additive. For each treatment, five 3-kg polyethylene bags were used, which were inserted into a plastic bucket, pressed, and sealed. After 80 days, the representative samples were taken from each silo and stored frozen at −20 $^{\circ}$C. A total number of 20 samples (four treatments with five replicates), was used for chemical analysis and *in vitro* gas production of either whole or partly stoned OC.

Chemical Analyses

Dry matter (DM), organic matter (OM) and crude protein (CP) were determined by the procedures of AOAC (1990). Neutral detergent fiber (NDF) and acid detergent fiber (ADF) were determined according to Van Soest *et al.* (1991). Lignin(sa) (lignin determined by solubilisation of cellulose with sulfuric acid) was determined using the method of Robertson and Van Soest (1981). Silage pH was determined on expressed juice

obtained by thorough mixing of 50 g of fresh silage with 125 ml of distilled water and allowing the juice to stand at 25 $^{\circ}$C for 1 h. After decanting the silage extract into a small beaker, the pH was measured (MAFF/ADAS, 1986) using a portable digital pH meter. Water soluble carbohydrates (WSC) were determined by using the anthrone method on lyophilized samples (MAFF, 1982). Ammonia in silages was determined using phenol-hypochlorite (Broderick and Kang, 1980). Lactic acid and volatile fatty acids (VFA) were determined by high performance liquid chromatography (Faithfull, 2002). To determine the total phenols (TPH), the dried material (200 mg) was extracted with acetone: water (10 ml; 70:30, v/v) in ultrasonic bath for 20 min. The contents were centrifuged (4 $^{\circ}$C, 10 min, 3000×*g*) and the supernatant was kept on ice until analysis. The TPH was determined with the Folin–Ciocalteau reagent and detected at 725 nm (Makkar, 2000). A calibration curve was prepared using tannic acid (Merck GmbH, Darmstadt, Germany). The TPH were calculated as tannic acid equivalents and expressed as g kg^{-1} DM.

In Vitro Digestibility

An *in vitro* method was used to estimate the digestibility (Menke and Steingass, 1988). The rumen fluid was collected from three rumen-cannulated ghezel sheep, fed twice daily a diet containing lucerne hay (650 g kg^{-1}) plus a concentrate mixture (350 g kg^{-1}) in the morning, strained, pooled and mixed (1:2, v/v) with an anaerobic mineral buffer solution. The reduced buffer medium composition, per liter, was NaHCO$_3$, 35.0 g; NH$_4$HCO$_3$, 4.0 g; Na$_2$HPO$_4$, 5.7 g; KH$_2$PO$_4$, 6.2 g; MgSO$_4$·7H$_2$O, 0.6 g; Na$_2$S, 0.52 g; CaCl$_2$·H$_2$O, 13.2 g; MnCl$_2$·4H$_2$O, 10.0 g; CoCl$_2$·6H$_2$O, 1.0 g; FeCl$_2$.6H$_2$O, 8.0 g and sodium resazurin, 0.01 g and, 60 ml freshly prepared reduction solution containing 580 mg Na$_2$S·9H$_2$O and 3.7 ml 1M NaOH. The mixture was stirred under CO$_2$ flushing at 39 $^{\circ}$C, using a magnetic stirrer fitted on a hot plate. Approximately 200 mg (DM) samples

were incubated in 100 ml glass syringes (three syringes per sample). The incubation procedure was repeated three times. Analyses were done after incubation for 0, 2, 4, 6, 8, 12, 16, 24, 72 and 96 h. Cumulative gas production data were fitted to the exponential equation, $Y = b (1-e^{-ct})$, where Y is the gas produced at time t, b is the gas production from the insoluble but fermentable fraction (ml g^{-1} OM), c is the gas production rate constant for b and t the incubation time. The ME and OMD were calculated using equations of Menke *et al.* (1979) as:

ME (MJ kg^{-1} DM) = 2.20 + 0.1357 × Gp + 0.0057 × CP + 0.00002859 × CP^2

OMD (g/100 g DM) = 14.88 + 0.8893 × Gp + 0.0448 × CP + 0.0651× XA

Where, CP is crude protein in g kg^{-1} DM, XA is ash in g kg^{-1} DM, and Gp is the net gas production (ml) from 200 mg after 24 h of incubation.

Statistical Analysis

Data on DM, OM, CP, NDF, ADF, lignin(sa), EE, WSC, pH and *in vitro* gas production and digestibility estimates (in five replication) were subjected to analysis using the GLM procedure of SAS (2001) using 2×2×2 factorial design based on the following statistical model; $Y_{ijkl}=\mu+T_i+S_j+M_k+TS_{ij}+TM_{ik}+SM_{jk}+TSM_{ijk}+e_{ijkl}$,

Where, Y_{ijkl} is the measured parameter, μ is the general mean, T_i is the effect of OC type (whole or partly stoned), S_j is the effect of ensilage, M_k is the effect of molasses level, TSM_{ijk} is the interaction between factors and e_{ijkl} is the error term. The obtained data on ammonia-N, lactic acid and VFA were subjected to analysis using 2×2 factorial design based on the statistical model; $Y_{ikl}=\mu+T_i+M_k+TM_{ik}+e_{ikl}$, in which all experimental factors are similar to those in the above-mentioned equation. Means were tested using Duncan's multiple range tests at p<0.05 (Steel and Torrie, 1980).

RESULTS AND DISCUSSION

Chemical Composition

Composition of OC is shown in Table 1. Chemical composition was affected by oil extraction method, extent of de-stoning, and kind of this by-product (Molina-Alcaide *et al.,* 2003). The DM content of whole OC was higher than that of the partly stoned cake, because of higher proportion of stone in the former. Silage DM content increased with addition of molasses (p<0.05), which is due to high DM content of molasses. Similar results have been reported by Donmez *et al.* (2003) and Aksu *et al.* (2006) in maize silage. However, the addition of molasses decreased OM content of partly stoned silage (p<0.05), probably due to fermentation of WSC by lactic acid bacteria during ensiling (Kenskin *et al.,* 2005). The CP content varied according to the type of OC (Table 1). The CP content in the fresh partly stoned OC (not in stoned OC) was more than 80 g kg^{-1} DM which, according to Norton (1998), should provide ruminal ammonia levels above the minimum required by rumen microorganism to support optimum growth. The CP content in partly stoned OC silage was decreased in comparison to fresh OC. This is because some of CP content in partly stoned OC silage was converted into ammonia during ensiling (Table 1). Adding molasses to the partly stoned OC silage led to a more stable fermentation in comparison to those without molasses (Table 1), resulting in less protein degradation *i.e.* the molassed partly stoned OC silage had more CP content than the unmolassed partly stoned OC silage (McDonald *et al.*, 1991). Analysis of fibers by the Van Soest *et al.* (1991) method showed that OC had high cell wall constituents, although, cell wall contents of partly stoned OC were less than stoned OC. The lower NDF in ensiled OC was probably due to cell wall degradation by cellulolytic clostridia or acid hydrolysis (McDonald *et al.,* 1991). Silage NDF and ADF decreased (*P*<0.05) with the addition of molasses, because enhancement of cell wall degradation due to increased silage fermentation caused by the sugars in molasses (Baytok *et al.,* 2005), and the lower NDF and ADF content of the molasses. Lignin(sa)

Table 1. Chemical composition of fresh and ensiled olive cakes (OC) with or without molasses (g kg^{-1} DM).

	Whole OC				Partly stoned OC				factorial analysis							SEM
	fresh		ensiled		fresh		ensiled		T	S	M	TS	TM	SM	TSM	
L	0	50	0	50	0	50	0	50								
DM	572^b	593^a	537^d	554^c	428^g	445^e	389^h	403^f	*	*	*	Ns	Ns	Ns	Ns	1.18
OM	970^a	958^c	970^a	964^{bc}	961^{bc}	957^c	964^{bc}	942^d	*	*	*	Ns	*	Ns	*	1.80
CP	55^c	58^c	55^c	54^c	87^a	83^a	76^a	85^b	*	*	Ns	Ns	*	Ns	*	3.26
NDF	720^{bc}	698^c	767^a	741^b	648^d	582^e	733^b	650^d	*	*	*	*	Ns	*	Ns	4.79
ADF	568^a	507^c	575^a	535^b	468^d	425^e	532^b	472^d	*	*	*	Ns	*	Ns	*	4.27
Lignin (sa)	295^b	283^c	306^a	289^{bc}	236^d	202^f	241^d	225^e	*	*	*	*	*	*	*	2.59
WSC	15.4^e	38.3^b	7.8^g	16.2^e	36.3^c	69.9^a	12.6^f	23.5^d	*	*	*	*	*	*	*	2.34
EE	166^d	163^d	141^e	135^e	200^a	196^{ab}	186^{bc}	176^{cd}	*	*	Ns	*	*	Ns	*	2.76
TPH	17.6^b	9.1^f	13.1^d	8.3^g	19.2^a	13.9^d	15.1^c	12.3^e	*	*	*	*	*	*	*	0.94
Bu	-	-	0.8^c	0.3^d	-	-	2.7^a	2.3^b	*	-	*	-	*	-	-	0.06
Pr	-	-	0.23^b	0.26^b	-	-	0.30^a	0.34^a	*	-	*	-	Ns	-	-	0.09
Ac	-	-	6.0^b	3.7^c	-	-	26.9^a	6.9^b	*	-	*	-	*	-	-	0.83
La	-	-	2.8^d	18.1^b	-	-	16.2^c	27.5^a	*	-	*	-	*	-	-	0.12
NH$_3$-N	-	-	38.9^b	35.7^c	-	-	46.0^a	35.8^c	*	-	*	-	*	-	-	2.1
pH	5.85^a	5.93^a	4.91^c	4.36^d	5.25^b	5.16^b	4.92^c	4.37^b	*	*	*	*	Ns	*	Ns	0.125

L: level of molasses (g kg^{-1} fresh weight.); T: type of OC; S: effect of ensilage; M: effect of addition of molasses; DM: dry matter (g kg^{-1} fresh weight); OM: organic matter; CP: crude protein; NDF: neutral detergent fiber; ADF: acid detergent fiber; lignin(sa): lignin determined by solubilization of cellulose with sulfuric acid; WSC: water soluble carbohydrates; EE: ether extract; TPH: total phenols; Bu: butyric acid; Pr: propionic acid; Ac: acetic acid; La: lactic acid; NH$_3$-N (g kg^{-1} total nitrogen); SEM: standard error of the means. Means in the same row with different letters differ (P<0.05). *: P<0.05; Ns: no significant.

contents of fresh and ensiled OC (202-306 g kg^{-1} DM) were higher than those suitable as a ruminant feed. Seemingly, the phenomenon of "protection" of carbohydrates related to lignin was active, as in the case of straw (Nefzaoui, 1983). The molasses mixed silages had lower lignin(sa), probably due to the lower lignin content of molasses because lignin is relatively stable to hydrolysis during silo fermentation (Hilla *et al.*, 2001). Olive cake had a high fat content that could alter ruminal digestibility and appetite, considering ruminants sensitivity to intake of fat above 5 percent of DM in the ration (Buysse, 1962). The WSC content of OC was less than optimum level (100 g kg^{-1} DM) for successful ensiling (Church, 1991). Also, contents of WSC in fresh and ensiled OC were less than the optimum level required for stimulation of microbial activity in rumen (Chamberlain and Wilkinson, 2000). Consistent with McDonald *et al*. (1991), when molasses was mixed with OC, the WSC contents were increased to improve silage preservation. The TPH content in OC was low *i.e.*, 19.2 g kg^{-1} DM. The reduction in the level of polyphenol content during ensilage could be due to oxidation of tannins in OC (Ben Salem *et al.*, 2005). According to Weinberg *et al.* (2008), excess amount of polyphenol reduce the CP content in OC. Addition of molasses decreased TPH in ensiled OC, which corroborates the results of the latter researchers.

Fermentation Characteristics

Ensiling caused a significant decrease in pH (p<0.05), due to the production of lactic acid and VFAs during fermentation (McDonald *et al.,* 1991). Addition of molasses decreased pH of ensiled samples (p<0.05), which is in agreement with other studies (Evers and Carroll, 1998; Islam *et al.*, 2001). Ammonia-N in ensiling OC was less than 50 g kg^{-1} total N indicating good fermentation quality of silage (Chamberlain and Wilkinson, 2000). Molasses mixed silages produced less (P<0.05) ammonia-N because higher sugar led to a rapid decline of pH to inhibit deamination and decarboxylation of amino acids (McDonald *et al.*, 1991). This indicates that molasses treatment improved fermentation quality of

ensiled OC. Lactic acid concentrations in silages were lower than the normal range of 80–120 g kg^{-1} DM reported by McDonald *et al.* (1991). Lactic acid concentration in whole silage was lower (P<0.05) than partly stoned silages, because of higher moisture in partly stoned OC samples (Yahaya *et al.,* 2002). Lactic acid production was higher in the silages treated with molasses, probably caused by an initial increase in the number of lactic acid bacteria with addition of a WSC source (McDonald *et al.*, 1991). Values for acetic, propionic and butyric acids were all low, suggesting good fermentation. The concentration of butyric acid is indicative of degraded sugars and lactic acid by saccharolytic clostridia. Increasing the molasses levels decreased acetic acid concentration, since addition of molasses leads to homo-fermentative fermentation in which most of WSC convert into lactic acid (McDonald *et al.,* 1991). However, the concentrations of lactic acid and VFAs in ensiled OC showed that addition of molasses had improved profile of produced acids and fermentation quality of the silages.

In Vitro Organic Matter Digestibility

The results of studies on OC digestibility are very heterogeneous (Sansoucy, 1985). On the average, whole OC, as ruminant feed, had a low digestibility. As shown in Table 2, the OMD and ME of OC were higher than the 190 g kg^{-1} DM reported by Martin Garcia *et al.* (2004), probably due to lower lignin content in our OC than that used by them. The higher contents of WSC and CP with lower lignin content in partly stoned OC had improved its OMD and ME in comparison to the whole OC (McDonald *et al.,* 1991). However, ensiling reduced the OMD and ME of OC by decreasing WSC, which is a source of energy for microorganisms in the rumen (Van Soest, 1994). The *b* decreased after ensiling, showing that the process had reduced the extent of *in vitro* gas production. However, the same process increased the *c* (fermentation rate), probably by increasing the contents of soluble fractions during fermentation in silo

Table 2. Results of *in vitro* gas production of fresh and ensiled olive cakes (OC) with or without molasses.

	Whole OC				Partly stoned OC				factorial analysis							SEM
	fresh		ensiled		fresh		ensiled									
L	0	50	0	50	0	50	0	50	T	S	M	TS	TM	SM	TSM	
IVGP	7.9^e	10.0^d	6.5^f	8.3^e	20.7^b	23.5^a	19.2^c	19.5^c	*	*	*	*	*	*	Ns	0.18
b	11.96^d	12.06^c	10.13^f	10.97^e	21.10^b	23.18^a	21.06^b	23.15^a	*	*	*	*	*	*	*	0.03
c	0.030^f	0.046^c	0.034^e	0.039^d	0.054^b	0.053^b	0.063^a	0.047^c	*	*	Ns	*	*	*	*	0.0001
OMD	263^e	291^d	251^f	269^e	397^b	420^a	385^c	398^b	*	*	*	*	*	*	Ns	1.55
ME	3.7^f	4.0^e	3.5^g	3.7^f	5.7^b	6.1^a	5.4^{cd}	5.5^{bc}	*	*	*	*	*	Ns	*	0.03

L: level of molasses (g kg^{-1} fresh weight); T: type of OC; S: effect of ensilage; M: effect of addition of molasses; IVGP: *in vitro* gas production for 24 h (ml/200mg DM); *b*: the gas production from the insoluble but fermentable fractions (ml); *c*: rate constant of gas production during incubation (/h); OMD: OM digestibility (g kg^{-1} DM); ME: metabolisable energy (MJ kg^{-1} DM); SEM: standard error of the means. Means in the same row with different letters differ (P<0.05). *: P<0.05; Ns: no significant.

(McDonald *et al.*, 1991). On the other hand, addition of molasses improved OMD, ME, *b* and *c* because it stimulated microbial activity (Moore and Kennedy, 1994). This result was in agreement with a study on corn silage (Aksu *et al.,* 2006).

CONCLUSION

Chemical composition and rumen fermentability results indicate that the OMD value of OC is low, even with the addition of molasses. Therefore, the potential to use OC as a feed in diets of ruminants is limited.

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ارزش غذایی و خصوصیات سیلویی تفاله زیتون کامل و کم هسته، تیمار شده با ملاس

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چکیده

تحقیق حاضر به منظور تعیین ارزش غذایی تفاله زیتون کامل و کم‌هسته تازه و سیلو شده، با و بدون ملاس (صفر و ۵۰ گرم ملاس در هر کیلوگرم وزن تازه) انجام شد. مقادیر ماده خشک (DM)، ماده آلی (OM)، الیاف نامحلول در شوینده خنثی (NDF)، الیاف نامحلول در شوینده اسیدی (ADF)، لیگنین، چربی خام (EE)، کربوهیدرات‌های محلول در آب (WSC) و ترکیبات فنولیک (TPH) در تمامی تیمارها با استفاده از روش‌های آزمایشگاهی تعیین شدند. به علاوه، pH، غلظت آمونیاک، اسید لاکتیک و اسیدهای چرب فرار در نمونه‌های سیلو شده اندازه‌گیری شد. روش آزمایشگاهی تولید گاز، در ۲۴ ساعت، برای تعیین قابلیت هضم ماده آلی (OMD) و انرژی قابل متابولیسم استفاده گردید. سیلو کردن تفاله زیتون، مقادیر DM، pH، EE، WSC، TPH و OMD را کاهش (P<۰/۰۵) و مقادیر NDF، ADF و لیگنین را افزایش داد (P<۰/۰۵). افزودن ملاس، موجب کاهش (P<۰/۰۵) مقادیر OM، NDF، ADF، لیگنین و pH شد، اما مقادیر DM، WSC، OMD، ME و اسید لاکتیک افزایش یافت (P<۰/۰۵). در مجموع، بر اساس نتایج این پژوهش, پتانسیل استفاده از تفاله زیتون به عنوان خوراک نشخوارکنندگان کاملاً محدود است.