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Ruminal Dry Matter and Nutrient Degradability of Different Olive Cake By–products after Incubation in the Rumen Using Nylon Bag Technique

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ABSTRACT

The experiment was conducted to evaluate the ruminal dry matter, crude protein and neutral detergent fiber degradability of different olive cake by-products including crude, exhausted, partly-destoned and partly-destined-exhausted olive cake using nylon bag technique. Chemical composition of different types of olive cake was significantly different. Crude olive cake had a low crude protein, high crude fiber, neutral and acid detergent fiber and relatively high fat content. Destoning and exhausting significantly increased crude protein content of olive cake; the latter decreased fat content and relatively increased its other contents. In addition, partial destoning lowered its crude fiber, neutral and acid detergent fiber. About 3 g of dry matter (DM) equivalent of samples were weighed in sealed nylon bags (6 cm × 7.5 cm, polyamide, 26% porosity, 40 ± 10 µ pore size), and incubated in the rumen of two cannulated Zel ewes (approximately 1 yr old, BW = 32.2 kg) that fed alfalfa hay and ground barely in a ratio of about 75:25 (DM basis) at 0, 3, 6, 12, 18, 24, 36, 48, 72 and 96 h. The DM degradation showed significant differences in soluble for soluble fraction (7.83, 8.85, 11.05 & 14.40%), slowly digestible fraction (24.33, 25.45, 29.80 & 31.45%), potentially degradable fraction (32.16, 34.30, 40.85 & 45.85%), fractional rate of disappearance (2.8, 3.0, 4.1 & 4.2% h^{-1}) and effective degradability in crude, exhausted, partly-destined and partly destoned exhausted olive cake, respectively. The protein in different olive byproducts had the lowest degradable fraction and rate; however, the NDF and crude protein had similar trend to DM. The results revealed that low degradable fraction of DM, crude protein and neutral detergent fiber of olive byproducts are the most important limiting factors in ruminant nutrition.

Key Words: Ruminant feed; Ruminal degradability; Olive cake; Nylon bag technique

INTRODUCTION

For a long time, the main problem in preserving crude olive cake was relatively high water content and the large quantity of retained oil that when exposed to air, therefore the olive cake quickly became rancid and unfit for animal consumption. Applying a new system in olive oil extraction, generates large amounts of a new byproduct, called two-stage olive cake, which includes the remainders of pulp, stones, skin and vegetable waters (Hermoso *et al*., 1995). These by-products are often extracted and dried and the stones are partially removed. Therefore, there are different types of olive by-products as crude, the exhausted, partly destoned and partly destoned exhausted olive cake for using in ruminant nutrition. However, their use is limited, because of their low nutritive value (René Sansoucy *et al*., 1985), high ADF (Nefzaoui, 1983), condensed tannins (Martin Garcia *et al*., 2003) and seasonality (René Sansoucy *et al*., 1985). The main factor that influences nutritive value in ruminants is the ruminal digestible fraction of feed. Using different methods by researchers, the dry matter (DM) and nutrients digestibilities of olive by-products are measured (Ørskov, 1977). Many experiences have shown poor digestive utilization of olive cake. Reviewing the results of *in vivo* digestibility obtained with different types of olive cake, René Sansoucy *et al*. (1985) concluded that: (1). There are large variations between chemical composition and nutrients digestibility, (2), regardless of the type of olive cake, DM and OM digestibility are low (20 to 50%), (3) fat always has a high digestibility (60 to 90%), (4), with wide variation, CP has a low digestibility (20 to 25%) and (5) the digestibility of CF varied from 0 to 40%. However, little information exists about nutrient utilization of the by-products in ruminants. This study aimed at evaluating kinetics of ruminal digestion of olive by-products including crude, the exhausted, partly destoned and partly destoned exhausted olive cake, over time of incubation in Zel ewes with ruminal cannulas using *in situ* method.

MATERIALS AND METHODS

Fours types of olive cake including crude olive cake, partly destoned olive cake, exhausted olive cake and partly destoned exhausted olive cake were used in this experiment. Samples were dried at 55°C, ground through a Wiley mill (1 mm screen pore size), analyzed for DM, OM, Kjeldahl N, ether extract (AOAC, 2002), NDF, ADF (Van Soest *et al*., 1991, using heat stable amylase and sodium sulfite) and ash at 605°C at 3 h. NFC was calculated by 100- (% CP + % NDF + % Ash + % EE) (Table I).

The ruminal nutrients degradation was determined with *in situ* method, using two cannulated Zel sheep (approximately 1 yr old, average body weight 32.3 ± 1.3 kg). Sheep were given access to water at all times and were housed in an open front shed. The sheep were fed corn silage and concentrates in a ratio of about 65:35 (dry matter basis) according to their requirements. The diet was fed in two equal meals at 0800 and 2000 h. About 3 g of DM equivalent were weighed in sealed nylon bags (6 cm × 7.5 cm, polyamide, 26% porosity, 40 ± 10 μ pore size), that was closed using a heat sealer. Three bags were incubated in the rumen for each of the following periods 0, 3, 6, 12, 18, 24, 36, 48, 72 and 96 h (Ørskov & McDonald, 1979). All incubations started after the morning feeding. Bags were attached to a plastic tube (5 mm diameter) that was fixed to the outside of the fistula with a string. The bags and the tubes had free movement inside the rumen and reticulum. On removal, bags were washed using cold water until the effluent ran clear. The bags were dried in an oven at 60°C for 48 h and weighed. Following the weighing, bags were opened and residues from the three bags for each period were homogenized and placed in tightly capped plastic bottles. Samples were analyzed for Kjeldahl N and NDF (Van Soest *et al*., 1991). All analyses were made on combined residues of three bags, run in duplicate and rerun when differences were greater than 3% and sufficient residue was available. The potentially degradable fraction was calculated as 100 minus the 0 h fraction.

Kinetics of DM, crude protein and NDF disappearance *in situ* was estimated by the non-linear regression procedure of SAS (1998). For each sample, following model was fitted to the disappearance of DM, crude protein and NDF (Ørskov & McDonald, 1979):

$$Y = a + b\,(1 - \exp^{(-Kd\,(t-L))} \text{ for } t > L,$$

Where

a, percent soluble fraction; b, percent slowly digestible fraction; Kd, fractional rate of disappearance (per h); L, lag time (h) and t, = time of incubation (hours). The equation $ED = [a + b \times Kd/(Kd + kp)]$ was used to calculate effective degradability (ED). In this equation, *kp* represents the flow rate of particles out of the rumen that we theoretically consider equal to 0.02 (maintenance level), 0.04 and 0.06% h^{-1}.

The data were analyzed by a completely randomize design as four types of olive cake were considered as treatment. Data were analyzed by using the GLM procedure of SAS® (1998). Means were separated using Duncan's multiple range test ($P < 0.05$).

RESULTS AND DISCUSSION

Chemical compositions of different types of olive cake were significantly different (Table I). Destoning and exhausting significantly increased CP content of olive cake, therefore partly destoned exhausted olive cake had the highest CP content. Crude olive cake had a low crude protein, high crude fiber, NDF, ADF and a relatively high fat content. Exhaustion decreased fat content and proportionately increased crude protein and other contents. In addition, partial destoning lowered its crude fiber content (Table I). All olive by-products had high NDF (68.9, 71.3, 50.3 and 54.3%) ADF (51.2, 56.5, 30.5 and 36.3%) and lignin (31.3, 32.3, 22.5 and 27.1%) on dry matter basis in crude olive cake, partly destoned olive cake, exhausted olive cake and partly destoned exhausted olive cake, respectively. Destoning and exhaustion of olive cake significantly changed cell wall content. In contrast, NFC content of olive cake significantly decreased by destoning, without any effect from exhaustion. The chemical composition of different types of olive by-products has similar trend that reported by René Sansoucy *et al*. (1985). Maymone *et al*. (1961) found that pulp or mesocarp had the highest oil content and stone or endocarp had the highest crude fiber, NDF and ADF content in of ripe olive. Therefore, chemical compositions of different types of olive cake are function of proportion of that pulp or mesocarp to stone or endocarp. René Sansoucy *et al*. (1985) reported that crude olive cake has a low crude protein, high crude fiber and a relatively high fat content. Exhaustion by solvent extraction decreased fat content and relatively increased the other contents. Partial destoning lowers its crude fiber content. As mentioned above, the crude fiber content in destoned olive cake was high. Partial destoning decreased the content considerably but even pure pulp contained about 20% crude fiber. Using Van Soest method, Alibes and Berge (1983) and Ohlde and Becker (1982) found that olive cake has high NDF, ADF and lignin contents. In addition, a large proportion of the protein (80 to 90%) is linked to the ADF (Nefzaoui, 1983) and only 1.5 to 3% of total nitrogen has particularly low solubility (René Sansoucy *et al*., 1985). Martin Garcia *et al*. (2003) found that the olive cake and leaves were rich in NDF (62.4 & 41.3% of DM respectively) and gross energy (18.7 & 16.8 MJ kg^{-1} DM respectively) and poor in CP (7.9 & 7.0% of DM respectively) that a great part of the N attached to the ADF fraction (70.6 & 53.3% of crude protein respectively). The tannin content was higher in olive cake than in olive leaves (1.38 & 0.83% of DM respectively).

Ruminal DM, NDF and CP degradability and effective degradability of different olive cake by-products are presented in (Table II, III & IV), respectively. The fractions of a, b, degradable and un-degradable fraction, fractional rate of disappearance, lag time and effective degradability on different Kp for DM, NDF and CP were significantly different. Destoning and exhaustion significantly increased the a, b, degradable fractions, Kd, lag time and effective degradability on different Kp, but decreased un-degradable fraction for DM, crude protein and NDF (Table II, III & IV). Highest and lowest degradable fractions were observed for DM and crud protein in different types, respectively. Unfortunately, the most of previous studies just measured apparent digestibility of nutrients. The nutrients ruminal degradability coefficients of different types of olive cake

Table I. Chemical composition of different types of olive cake

Chemical compositions	Types of olive cake: Crude olive cake	Exhausted olive cake	Partly destoned olive cake	Partly destoned exhausted
Dry matter	87.6	86.7	88.1	87.5
Crude protein	7.6c	7.2c	8.8b	9.7a
Crude fiber	38.7a	39.6a	22.1b	21.4b
Ether extract	5.7b	3.4c	6.4a	3.3c
Crude ash	7.4	8.1	7.6	8.4
NFE	40.6b	41.7b	55.1a	57.2a
Neutral detergent fiber	68.9a	71.3a	50.3b	54.3b
Non fibrous carbohydrate	10.4b	10.0b	26.9a	24.3a
Acid detergent fiber	51.2b	56.5a	30.5d	36.3c
Lignin	31.3a	32.3a	22.5c	27.1b

Table II. Ruminal dry matter degradability and effective degradability of different olive cake by-products after incubation in the rumen of Zel ewes with ruminal canulaes using nylon bag technique

Digestion parameters[1]	Crude olive cake	Exhausted olive cake	Partly destoned olive cake	Partly destoned exhausted olive cake	SEM	P-values
			Dry matter			
a[2]	7.83[d]	8.85[c]	11.05[b]	14.40[a]	0.035	<0.0001
b[3]	24.33[d]	25.45[c]	29.80[b]	31.45[a]	0.055	<0.0001
(a+b)[4]	32.16[d]	34.30[c]	40.85[b]	45.85[a]	0.049	<0.0001
c[5]	67.85[a]	65.70[b]	59.15[c]	54.15[d]	0.050	<0.0001
Kd[6]	0.028[b]	0.030[b]	0.041[a]	0.042[a]	0.001	0.0004
Lag time (h)	17.9[a]	14.30[b]	11.10[c]	10.10[d]	0.074	<0.0001
		Effective degradability on different Kp[7]				
0.02	16.52[d]	30.10[c]	35.65[b]	40.55[a]	0.037	<0.0001
0.04	15.49[d]	18.65[c]	24.80[b]	28.90[a]	0.068	<0.0001
0.06	13.20[d]	17.55[c]	23.35[b]	27.45[a]	0.025	<0.0001

[1]- For each sample, the model: $D = a + b\,(1\text{-exp}^{(-kd\,(T\text{-}d)}))$ was fitted to the %age of disappearance of DM. Where, a, soluble fraction (%age); b, slowly digestible fraction (%age); Kd, fractional rate of disappearance (per hour); L, lag time (hours), and t, = time of incubation (hours; Ørskov and McDonald, 1979).
[2]- Soluble fraction (%)
[3]- Slowly digestible fraction (%)
[4]-Potential extent of DM degradation (a + b)
[5]-Indigestible fraction (%)
[6]-Fractional rate of disappearance (%/h)
[7]- The equation $ED = [a + b \times Kd/(Kd + kp)]$ was used to calculate effective degradability (ED). In this equation, *kp* represents the flow rate of particles out of the rumen that was considered equal to 0.02, 0.05 and 0.06

had not tested. As the apparent digestibility coefficients are not sufficient to evaluate the nutritive value of a feed in ruminants, therefore it is necessary to determine the ruminal kinetic of digestion, specially a, b, potential degradable, c and kd of feed nutrients. According to Nefzaoui (1983), since olive cake is rich in ADF, it has a low degradability in the rumen that the maximum value obtained for exhausted screened olive cake was 32% of DM after 72 h ruminal incubation. In addition, protein degradability was also very low in olive by-products, which may be because 75 to 90% of protein is linked to the ADF fraction. The DM and nutrients digestibility of different types of olive cake that were measured *in vivo* had a high variation. Using sheep, digestibility of OM, CP, EE and CF were 32.9 (Boza & Varela, 1960), 30.8 to 45.7, 10 to 24.5, 65.5 to 89.2 and 28.4 to 29.6% in crude olive cake, respectively (Theriez & Boule, 1970). Using sheep, digestibility of OM and CP were 69.4 and 28.0% in exhausted olive cake, respectively (Theriez & Boule, 1970). In addition, Nefzaoui (1978) found that protein degradability was very low, because 75 to 90% of the nitrogen in olive by-products is linked to the ADF fraction. Olive cake is particularly rich in lignin that protected carbohydrates that related to ADF and lignin.

Theriez and Boule (1970) explained that many factors such a high ADF, poor digestive degradation and utilization may result in decreasing ruminal microbial activity that may decrease by 40% after ingestion of crude olive cake. The ammonia production of sheep rumen liquor receiving olive cake also confirmed decreased activity of the rumen micro flora (Nefzaoui *et al*., 1982). The influence of different olive cakes on ruminal microbial activity may result in quantity, nature and condition of conversation. High concentrations of free fatty acids in the rumen can alter digestion and appetite. In addition, olives by-products have some phenolic compounds that would inhibit fermentation, or tannin, which would insolubilize the proteins in the diet or in the olive by-products (Theriez & Boule, 1970). However, exhaustion eliminates large quantities of polyphenols and tannins from by-products. Therefore, higher degradable fraction of DM, crude protein and NDF in partly destoned exhausted olive cake, may result in the removal of polyphenols and tannins from by-products. Unfortunately, in the current experiment, we did not measure the phenolic components and tannins in by-products. However, analyses of olive cake by Nefzaoui (1978) showed that tannin rates below 1% were not sufficient to act as a depressant on rumen microflora, and digestibility of protein and polyphenols levels between 0.15 and 0.75% of DM are not sufficient to inhibit fermentation. In addition, *in situ* ruminal DM and CP degradability of olive leaves was low that could be due to high olive leaves fat content and incomplete removal of ruminal microbes from un-degraded residues in the nylon bags by washing and stomaching procedures (Hvelplund & Weisbjerg, 2000). Martin Garcia *et al*. (2003) reported that *in vitro* digestibility either of olive cake and leaves showed low values, especially for CP (9.62 & 1.24% of DM, respectively). Potential degradability was lower in olive cake than in olive leaves (44.95 vs. 74.5% for DM & 55.45 vs. 70.15% of crude protein respectively). Effective degradability was also lower in olive cake than in olive leaves (41.05 vs. 53.55% of DM & 43.4 vs. 45.85% of crude protein respectively).

CONCLUSIONS

The chemical composition of olive by-products had high variability. The pulp or mesocarp had the highest oil content and stone or endocarp had the highest crude fiber, NDF and ADF content of ripe olive. Therefore, chemical

Table III. Ruminal crude protein degradability and effective degradability of different olive cake by-products after incubation in the rumen of Zel ewes with ruminal canulaes using nylon bag technique

Digestion parameters[1]	Crude olive cake	Exhausted olive cake	Partly destoned olive cake	Partly destoned exhausted olive cake	SEM	P-values
			Crude protein			
a[2]	6.52[b]	5.55[c]	6.45[b]	7.45[a]	0.026	<0.0001
b[3]	6.53[a]	5.45[c]	6.45[a]	5.75[b]	0.027	0.0002
(a+b)[4]	13.04[b]	11.00[d]	12.90[c]	13.20[a]	0.013	<0.0001
c[5]	86.96[c]	89.00[a]	87.10[b]	86.80[d]	0.014	<0.0001
Kd[6]	0.020[b]	0.021[ab]	0.024[ab]	0.025[a]	0.003	0.1069
Lag time (h)	9.20a	8.35[b]	7.35[c]	6.65[d]	0.054	0.0003
		Effective degradability on different Kp[7]				
0.02	6.35[d]	8.20[c]	12.25[a]	10.65[b]	0.021	<0.0001
0.04	6.10[c]	6.15[c]	9.80[a]	8.45[b]	0.031	<0.0001
0.06	9.90a	5.85[d]	9.55[b]	8.25[c]	0.033	<0.0001

[1-] For each sample, the model: $D = a + b\ (1\text{-exp}^{(-kd\ (T\text{-}d)}))$ was fitted to the %age of disappearance of crude protein. Where, a, soluble fraction (%age); b, slowly digestible fraction (%age); Kd, fractional rate of disappearance (per hour); L, lag time (hours), and t, = time of incubation (hours; Ørskov and McDonald, 1979).
[2-] Soluble fraction (%); [3-] Slowly digestible fraction (%)
[4]-Potential extent of DM degradation (a + b); [5]-Indigestible fraction (%)
[6]-Fractional rate of disappearance (%/h)
[7-] The equation $ED = [a + b \times Kd/(Kd + kp)]$ used to calculate effective degradability (ED). In this equation, *kp* represents the flow rate of particles out of the rumen that was considered equal to 0.02, 0.05 and 0.06

Table IV. Ruminal neutral detergent fiber degradability and effective degradability of different olive cake by-products after incubation in the rumen of Zel ewes with ruminal canulaes using nylon bag technique

Digestion parameters[1]	Crude olive cake	Exhausted olive cake	Partly destoned olive cake	Partly destoned exhausted olive cake	SEM	P-values
			Neutral detergent fiber			
a[2]	6.50[b]	5.65[c]	6.45[b]	7.55[a]	0.021	<0.0001
b[3]	14.35[d]	16.05[c]	18.55[a]	17.15[b]	0.025	<0.0001
(a+b)[4]	20.85[d]	21.70[c]	25.00[a]	24.70[b]	0.028	<0.0001
c[5]	79.15[a]	78.30[b]	75.30[c]	75.00[d]	0.028	<0.0001
Kd[6]	0.021[b]	0.022[b]	0.021[b]	0.032[a]	0.001	0.0009
Lag time (h)	17.38[a]	10.30[b]	10.55[b]	7.45[c]	0.078	<0.0001
		Effective degradability on different Kp[7]				
0.02	10.65c	17.65b	20.55a	20.65a	0.026	<0.0001
0.04	10.20c	10.25c	11.75b	14.35a	0.041	<0.0001
0.06	10.95[b]	9.80[c]	11.05[b]	13.50[a]	0.059	<0.0001

[1-] For each sample, the model: $D = a + b\ (1\text{-exp}^{(-kd(T\text{-}d)}))$ was fitted to the %age of disappearance of NDF. Where, a, soluble fraction (%age); b, slowly digestible fraction (%age); Kd, fractional rate of disappearance (per hour); L, lag time (hours), and t, = time of incubation (hours; Ørskov and McDonald, 1979).
[2-] Soluble fraction (%); [3-] Slowly digestible fraction (%)
[4]-Potential extent of DM degradation (a + b); [5]-Indigestible fraction (%)
[6]-Fractional rate of disappearance (%/h)
[7-] The equation $ED = [a + b \times Kd/(Kd + kp)]$ used to calculate effective degradability (ED). In this equation, *kp* represents the flow rate of particles out of the rumen that was considered equal to 0.02 0.05 and 0.06.

compositions, especially cell wall components of different types of olive cake are functions of proportion of that mesocarp to stone or endocarp. Olive by-products had low ruminal degradability for DM, NDF and crude protein. However, the protein in different olive by-products had the lowest degradable fraction and rate. A high amount of N attached to the cell wall in olive by-products may be due either to heating of the material during the production that can give Maillard's products or to the formation of tannin–protein complexes. Moreover, low degradable fraction of DM, crude protein and NDF of olive by-products are the most important limiting factors in ruminant's nutrition. However, the use of those by-products, with adequate supplements, could be of a great importance in semi-arid Mediterranean countries with shortage of natural pastures.

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