

# The chemical composition, rumen degradability, *in vitro* gas production, energy content and digestibility of olive cake ensiled with additives

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## Summary

The chemical composition, *in situ* rumen degradability coefficients of dry matter (DM) and crude protein (CP), *in vitro* gas production and *in vivo* digestibility were evaluated for olive cake (OC) silage treated (DM basis) with different additives as follows: (1) untreated OC; (2) OC, 8% molasses and 0.4% formic acid and (3) OC, 8% molasses, 0.4% formic acid and 0.5% urea. After addition of the additives, materials were ensiled for 60 days in plastic bags. The addition of molasses, formic acid and urea resulted in higher DM, CP, pH and NH<sub>3</sub>-N content. There were some reductions in neutral detergent fiber, acid detergent lignin and acid detergent fiber contents of silages especially with treatment 3. The additives increased the nutritive value and preserved well the ensiled OC. Ruminal maximum potential degradability (a+b), and effective degradability (ED) of DM and CP were higher for treatment 3. Total gas production was higher (P<0.05) for treatments 2 and 3 and was associated with increased (P<0.05) *in vitro* organic matter digestibility and a non-significant increase in metabolizable energy content. Only CP digestibility was affected by treatments and was highest for treatment 3. *In vitro* dry matter and organic matter digestibilities improved with treatments 2 and 3. The results indicated that, treating OC (DM basis) before ensiling with molasses (8%), formic acid (0.4%) and urea (0.5%) resulted in a satisfactory and economical source of a non-conventional feed for ruminants.

**Key words:** Olive cake, Degradability, Gas production, Digestibility, Additives

## Introduction

The provision of adequate quantities of high-quality cheap forage is a major challenge in the development of livestock production systems throughout the Middle-east. The main advantages of feeding by-products to livestock have been less dependency on grains that can be consumed by humans and the reduction in costs related to waste management (Grasser *et al.*, 1995; Orskov, 1998; Aregheore, 2000). Conventional feedstuffs for small ruminants in Iran are often in short supply and expensive, so there is a need to search for non-conventional feedstuffs.

One such feedstuff available in Iran is crude olive cake (OC), the by-product of oil extraction. Under the government planning, the annual production of olive fruit is expected to increase with the expansion of

land allocated to olive tree plantation (up to 700,000 ha).

The crude olive cake contains the seeds or pits and the pulp, and due to variable processing methods it varies widely in composition (Morgan and Trinder, 1980; Ohlde and Becker, 1982; Harb, 1986; Yanez *et al.*, 2004). This by-product is highly fibrous (Ohlde and Becker, 1982; Alibas and Berge, 1983) and low in crude protein (CP) content (Nefzaoui, 1983; Hadjipanayiotou, 1994). Besides, a large proportion of the protein (80 to 90%) is linked to the ligno-cellulose fraction (Nefzaoui, 1983). The low content of CP suggests that the nutritional value of the product would benefit from supplementation with urea.

The availability of olive by-products is seasonal and their use in animal feeding throughout the year requires preservation and storage. The main constraints to

preservation of OC are its high water and oil contents. Therefore, long-term storage of this by-product near small local factories may result in a considerable deterioration (mould formation) of the material and wastage of potential nutrients. Ensiling has been reported to be a simple, cheap and efficient procedure for preserving OC (Hadjipanayiotou, 1999) and several chemical, physical and biological methods have been used to improve the nutritive value of OC (Karapinar, 1977; Worgan, 1978; Hadjipanayiotou, 1994; Al-Jassim *et al.*, 1997; Molina and Aguilera, 1998; Rowghani and Zamiri, 2007; Moumen *et al.*, 2008).

The objective of this work was to study the effect of additives such as urea, formic acid and molasses on chemical composition, rumen degradability, *in vitro* gas production, energy content and *in vivo* digestibility of ensiled OC. The additives were chosen on the basis of the results reported by Rowghani and Zamiri (2007).

## Materials and Methods

### Silage preparation

Fresh, de-stoned and screened OC with dry matter content of 65.3% was obtained from an olive mill near Shiraz, Iran. The chemical composition of the raw material was determined and OC was ensiled for 60 days in 30-kg dark plastic bags after addition of the additives (DM basis) as follows: (1) untreated OC; (2) OC, 8% molasses and 0.4% formic acid and (3) OC, 8% molasses, 0.4% formic acid and 0.5% urea. After 2 months, representative samples were taken for chemical analysis and determination of rumen degradability, *in vitro* gas production and *in vitro* DM digestibility (Tilley and Terry, 1963).

### Chemical analysis

Ether extract (EE), DM, ash and CP contents of silage samples, feces and urinary nitrogen were determined (AOAC, 2000). Neutral detergent fiber (NDF), acid detergent fiber (ADF) and lignin (ADL) were measured according to the method of Goering and Van Soest (1970). Hemicellulose and cellulose were calculated

by subtraction (NDF-ADF and ADF-ADL, respectively).

The pH of each sample was determined in triplicates by adding 25 g wet material to 100 ml of distilled water. After hydration for 10 min using a blender, the pH was determined using a digital pH meter (Polan *et al.*, 1998). The filtrate was filtered through two layers of cheesecloth, centrifuged and stored for organic acid analysis, which was performed by using gas chromatography (Crompak, Model CP9002, The Netherlands) as described by Playne (1985). Ten ml of filtered liquid was acidified by adding 10 ml of 0.1 N HCl and kept frozen at -20°C for determining NH<sub>3</sub>-N concentrations using the distillation of Kjeldahl procedure. Gross energy (GE) content of untreated olive cake was measured by bomb calorimeter (Parr Instrument Co, Moline, IL).

### *In situ* rumen degradability of DM and CP

Rumen degradability was estimated *in sacco* (Orskov and McDonald, 1979). The dry samples were ground (2-mm sieve), and approximately 5 g of each sample (DM) was transferred into polyester bags (12 × 19 cm) with 50-µm pore size. Four bags per treatment and incubation time were incubated in the rumen of two fistulated male Sistani cattle (450 kg BW) for 2, 4, 8, 12, 24, 48 and 72 h. The cattle were fed a diet consisting of 90% of a mixture of wheat bran and alfalfa hay (50:50) and 10% pistachio hulls. A maintenance ration was fed in equal portions every 12 h to maintain a relatively stable ruminal environment.

Four bags were also washed with cold tap water to estimate zero time washout. After each incubation time (including the zero time), the bags were removed and hand-washed with cold water until the water remained clear. Samples were then dried in an oven at 55°C until a constant weight was achieved before determination of DM disappearance. Loss of DM and N at various incubation intervals was fitted to the non-linear equation  $p = a + b(1 - e^{-ct})$ , in which “p” is the amount degraded at time, “a” is the fraction that is soluble or immediately degraded, “b” is the fraction that is

potentially degradable but insoluble, and “c” is the fractional rate constant at which the fraction “b” will degrade per h. These data (a, b, c and p) were analyzed by one-way analysis of variance.

#### *In vitro gas production, in vitro organic matter digestibility (IVOMD) and metabolizable energy estimation*

*In vitro* incubation was performed using 30 ml of buffered rumen fluid according to the method of Menke and Steingass (1988). Approximately 200 mg of sample were weighed and placed in 100 ml graduated glass syringes. Buffer mineral solution was prepared and placed in a water bath at 39°C under continuous flushing with CO<sub>2</sub>. Rumen fluid was collected after the morning feeding from two ruminally fistulated male Sistani cattle which were used in the rumen degradability experiment. Rumen fluid was pumped with a manually operated vacuum pump from the rumen into pre-warmed thermos flasks. The rumen fluid from the two cows was mixed and filtered through four layers of cheesecloth and flushed with CO<sub>2</sub>. The well-mixed and CO<sub>2</sub> flushed rumen fluid was added to the buffered mineral solution (1:2 v/v), which was maintained in a water bath at 39°C, and mixed. Buffered rumen fluid (30 ml) was pipetted into each syringe. The syringes were immediately placed in a water bath and maintained at 39°C. Gas production (GP) was recorded at 2, 4, 6, 8, 12, 24, 48 and 72 h to estimate the *in vitro* organic matter digestibility (IVOMD) and metabolizable energy (ME). Triplicates of each sample were used, with correction of the volume of gas according to a standard. Estimated ME concentration and IVOMD of samples were calculated as described by Close and Menke (1986):

$$\text{ME (MJ/kg DM)} = 2.20 + 0.136 \text{ GP} + 0.0057 \text{ CP} + 0.00029 \text{ EE}^2$$

$$\text{IVOMD} = (14.88 + 0.889 \text{ GP} + 0.045 \text{ CP} + 0.065 \text{ CA})/100$$

where GP is in ml/72 h, CP in g/kg DM, EE in g/kg DM and CA (crude ash) in g/kg DM.

#### *In vitro DM digestibility determination*

The IVDMD was determined according to the method described by Tilley and Terry (1963).

#### *Digestibility experiment*

Fifteen Ghezel male lambs (mean BW, 30.00 ± 0.52 kg and one year of age) were used in a completely randomized design digestibility experiment. They were divided into three equal groups with similar mean body weights and similar variations between lambs within group. The lambs were housed individually in crates and allowed 14 days of adaptation to the experimental diets. After adaptation, separate collections of total feces and urine were made in a period of 7 days. They had free access to fresh water and were fed experimental silages with alfalfa hay (90% DM and 23.23% CP content) in 50:50 ratio, since silages alone would not supply their nutrient requirements. They were fed silage DM at 4% BW in two equal meals (8:00 and 16:00 h). A 5-liter plastic bucket containing 100 ml of 10% (vol/vol) sulfuric acid, to keep the final pH below 3, was placed under crates for urine collection. Urine was collected for 7 d, the volume was recorded and a sub-sample of 100 ml was kept frozen (at -20°C) until N analysis. Each day, 10% of daily fecal samples were collected and kept frozen (at -20°C) until chemical analysis. The chemical composition of the silages are shown in Table 1.

#### **Statistical analysis**

Data were subjected to analysis of variance using general linear model procedure of SAS (1996). Mean separation was performed by Duncan's multiple range tests, and the level of significance was set at 5%.

#### **Results**

The dry matter content of untreated OC was 653 g/kg. The DM in OC contained 51.45 g/kg CP, 682 g/kg NDF, 506 g/kg ADF, 218 g/kg ADL, 142.5 g/kg EE, 54.30 g/kg ash, 945.70 g/kg OM, 5106.35 cal/g GE, and the pH was 6.08.

The chemical composition of OC silages treated with different additives is shown in Table 1. Table 2 shows pH, organic acids and ammonia-N concentrations of the silages. Addition of molasses, formic acid and urea (treatment 3) resulted in significant

increases ( $P < 0.05$ ) in DM, CP, ammonia-N concentrations and pH of the ensiled OC. Lactic acid levels were significantly higher in treatment 1 (Table 2). Other chemical parameters were not significantly affected by the additives (Tables 1 and 2). There was some reduction in NDF content of silages between days 0 and 60 of ensiling and the highest reduction was found in treatment 1 (154 g/kg DM).

**Table 1: Chemical composition (g/kg DM) of olive cake (OC) silages after 60 days of ensiling**

Parameters	Silages			
	1	2	3	SEM
DM	713.4 <sup>b</sup>	696.6 <sup>b</sup>	738.5 <sup>a</sup>	5.9
CP	77.07 <sup>b</sup>	78.24 <sup>b</sup>	105.55 <sup>a</sup>	5.84
NDF	528.15 <sup>b</sup>	590.20 <sup>a</sup>	578.07 <sup>a</sup>	11.29
ADF	296.49 <sup>a</sup>	443.26 <sup>a</sup>	387.69 <sup>a</sup>	41.63
ADL	229.25 <sup>a</sup>	220.65 <sup>a</sup>	155.51 <sup>a</sup>	22.43
Ash	51.67 <sup>a</sup>	60.67 <sup>a</sup>	53.00 <sup>a</sup>	3.67
Hemicellulose	231.66 <sup>a</sup>	146.94 <sup>a</sup>	190.37 <sup>a</sup>	49.32
Cellulose	112.39 <sup>a</sup>	222.61 <sup>a</sup>	232.18 <sup>a</sup>	38.00
Organic matter	948.33 <sup>a</sup>	939.33 <sup>a</sup>	947.00 <sup>a</sup>	3.67
EE	162.50 <sup>a</sup>	160.00 <sup>a</sup>	164.74 <sup>a</sup>	2.88

(1) Untreated olive cake; (2) OC, 8% molasses, 0.4% formic acid and (3) OC, 8% molasses, 0.4% formic acid, 0.5% urea. Means within each row with similar superscript are not significantly different (Duncan's test;  $P > 0.05$ ). SEM = standard error of the mean

**Table 2: pH values, organic acid and NH<sub>3</sub>-N content (% of DM) of olive cake (OC) silages**

	Silages			
	1	2	3	SEM
pH	6.12 <sup>b</sup>	6.06 <sup>c</sup>	6.31 <sup>a</sup>	0.01
Lactic acid	3.51 <sup>a</sup>	3.16 <sup>b</sup>	3.03 <sup>b</sup>	0.05
Acetic acid	0.38 <sup>a</sup>	1.02 <sup>a</sup>	1.10 <sup>a</sup>	0.39
Propionic acid	0.00 <sup>a</sup>	0.43 <sup>a</sup>	0.32 <sup>a</sup>	0.22
Butyric acid	0.09 <sup>a</sup>	0.44 <sup>a</sup>	0.27 <sup>a</sup>	0.16
NH <sub>3</sub> -N	0.88 <sup>b</sup>	0.60 <sup>b</sup>	2.59 <sup>a</sup>	0.26

(1) Untreated olive cake; (2) OC, 8% molasses, 0.4% formic acid and (3) OC, 8% molasses, 0.4% formic acid, 0.5% urea. Means within each row with similar superscript are not significantly different (Duncan's test;  $P > 0.05$ ). SEM = standard error of the mean

The DM degradation kinetics is presented in Table 3. Fraction "a" was higher ( $P < 0.05$ ) for treatments 2 and 3 than treatment 1. The degradation rate (c), was highest ( $P < 0.05$ ) for treatment 3. The maximum potential degradability (a+b) was higher for treatments 2 and 3 compared with treatment 1.

**Table 3: Dry matter degradation kinetics (%) of olive cake (OC) silages**

Constants	Silages			
	1	2	3	SEM
a	20.28 <sup>b</sup>	22.40 <sup>a</sup>	23.18 <sup>a</sup>	0.56
b	26.09 <sup>a</sup>	27.62 <sup>a</sup>	28.10 <sup>a</sup>	0.93
a+b	46.37 <sup>b</sup>	50.02 <sup>a</sup>	51.27 <sup>a</sup>	0.82
c (/h)	0.112 <sup>b</sup>	0.106 <sup>b</sup>	0.171 <sup>a</sup>	0.01
ED	38.31	41.16	44.91	--

(1) Untreated olive cake; (2) OC, 8% molasses, 0.4% formic acid and (3) OC, 8% molasses, 0.4% formic acid, 0.5% urea. Means within each row with similar superscript are not significantly different (Duncan's test;  $P > 0.05$ ). "a" Fraction that is soluble or immediately degraded. "b" Potentially degradable but insoluble fraction. "a+b": Maximum potential degradability. "c": Rate of degradation of the sample "b" fraction (per h). ED: Effective degradability values at 0.05 per h outflow rate. SEM = standard error of the mean

The CP degradation kinetics is presented in Table 4. Fraction "a" was higher ( $P < 0.05$ ) but fraction "b" was lower ( $P < 0.05$ ) for treatment 3, which reflected the inverse relationship between these fractions. There were not any significant differences for constants (c) and (a+b) between treatments. Effective degradability (ED) of CP was higher for treatment 3 (80.91%), showing the same trend as for DM degradability.

Table 5 presents *in vitro* gas production, estimated ME and IVOMD values of OC silages. Up to 4 h of incubation, the gas production was not different between

**Table 4: Crude protein degradation kinetics (%) of olive cake (OC) silages**

Constants	Silages			
	1	2	3	SEM
a	26.29 <sup>b</sup>	25.94 <sup>b</sup>	53.80 <sup>a</sup>	4.16
b	59.45 <sup>a</sup>	60.83 <sup>a</sup>	36.06 <sup>b</sup>	3.53
a+b	85.73 <sup>a</sup>	86.82 <sup>a</sup>	89.86 <sup>a</sup>	1.23
c (/h)	0.114 <sup>a</sup>	0.132 <sup>a</sup>	0.152 <sup>a</sup>	0.04
ED	67.61	70.15	80.91	

(1) Untreated olive cake; (2) OC, 8% molasses, 0.4% formic acid and (3) OC, 8% molasses, 0.4% formic acid, 0.5% urea. Means within each row with similar superscript are not significantly different (Duncan's test;  $P > 0.05$ ). "a" Fraction that is soluble or immediately degraded. "b" Potentially degradable but insoluble fraction. "a+b": Maximum potential degradability. "c": Rate of degradation of the sample "b" fraction (per h). ED: Effective degradability values at 0.05 per h outflow rate. SEM = standard error of the mean

treatments, but between 8 to 72 h, gas production level and IVOMD were significantly ( $P < 0.05$ ) higher for treatments 2 and 3. The digestibility, IVOMD and IVDMD are presented in Table 6. Only CP

**Table 5: Gas production (ml/g DM) at different hours of incubation and estimated metabolizable energy (ME, MJ/kg DM) and *in vitro* organic matter digestibility (IVOMD) of olive cake (OC) silages**

	Silages			SEM
	1	2	3	
Gas production after (h)				
2	8.99 <sup>a</sup>	12.04 <sup>a</sup>	10.56 <sup>a</sup>	1.43
4	10.44 <sup>a</sup>	15.92 <sup>a</sup>	14.48 <sup>a</sup>	1.90
6	12.21 <sup>b</sup>	19.90 <sup>a</sup>	18.11 <sup>ab</sup>	2.03
8	14.17 <sup>b</sup>	23.39 <sup>a</sup>	23.20 <sup>a</sup>	2.21
12	18.86 <sup>b</sup>	31.93 <sup>a</sup>	32.20 <sup>a</sup>	2.24
24	27.47 <sup>b</sup>	47.07 <sup>a</sup>	43.94 <sup>a</sup>	2.41
48	34.61 <sup>b</sup>	56.48 <sup>a</sup>	51.48 <sup>a</sup>	2.40
72	40.67 <sup>b</sup>	61.73 <sup>a</sup>	56.76 <sup>a</sup>	2.38
ME <sup>†</sup>	11.40 <sup>a</sup>	11.75 <sup>a</sup>	12.22 <sup>a</sup>	0.24
IVOMD <sup>††</sup>	0.289 <sup>b</sup>	0.333 <sup>a</sup>	0.332 <sup>a</sup>	0.005

(1) Untreated olive cake; (2) OC, 8% molasses, 0.4% formic acid and (3) OC, 8% molasses, 0.4% formic acid, 0.5% urea. Means within each row with similar superscript are not significantly different (Duncan's test;  $P > 0.05$ ). † Estimated from gas production measurement using equation: ME (MJ/kg DM) = 2.20 + 0.136 GP (ml gas from 200 mg OC DM for 72 h incubation) + 0.0057 CP + 0.00029 EE<sup>2</sup> and †† estimated from equation: IVOMD = 14.88 + 0.889 GP (ml gas from 200 mg OC DM for 72 h incubation) + 0.045 CP + 0.065 CA/100 (Close and Menke, 1986). SEM = standard error of the mean

**Table 6: Digestibility (%) of DM, OM, CP, NDF, ADF, EE and *in vitro* digestibility of OM (IVOMD) of olive cake (OC) silages**

Parameters	Silages			SEM
	1	2	3	
DM	47.94 <sup>a</sup>	50.39 <sup>a</sup>	49.51 <sup>a</sup>	1.19
OM	47.55 <sup>a</sup>	50.64 <sup>a</sup>	49.80 <sup>a</sup>	1.17
CP	45.79 <sup>ab</sup>	42.29 <sup>b</sup>	49.00 <sup>a</sup>	1.76
NDF	35.31 <sup>a</sup>	35.31 <sup>a</sup>	37.61 <sup>a</sup>	2.75
ADF	36.68 <sup>a</sup>	37.66 <sup>a</sup>	38.1 <sup>a</sup>	0.57
EE	57.80 <sup>a</sup>	57.41 <sup>a</sup>	57.33 <sup>a</sup>	0.35
Nitrogen retention (g per day)	1.34 <sup>a</sup>	1.14 <sup>a</sup>	1.14 <sup>a</sup>	0.12
IVOMD <sup>†</sup>	16.55	21.46	21.32	
IVDMD <sup>†</sup>	21.01	24.93	25.21	

(1) Untreated olive cake; (2) OC, 8% molasses, 0.4% formic acid and (3) OC, 8% molasses, 0.4% formic acid, 0.5% urea. Means within each row with similar superscript are not significantly different (Duncan's test;  $P > 0.05$ ). SEM = standard error of the mean. † From the methodology of Tilley and Terry (1963)

digestibility was affected by treatments and was higher ( $P < 0.05$ ) for treatment 3 than treatment 2, with no difference between treatment 1 and 3. IVOMD values for treatments 2 and 3 were much higher than treatment 1 and the same trend was seen for IVDMD.

## Discussion

In semi-arid areas which have a scarcity of natural pastures and conventional animal foods, using OC for ruminant feeding could contribute to the development of livestock production systems, thus replacing to some extent with more expensive feeds (mainly cereal grains). Higher DM content of silages treated with molasses may have resulted from the high DM content of molasses used (Hinds *et al.*, 1985) and less seepage from the silages with molasses (McDonald *et al.*, 1991). Also, the increased CP concentration in treatment 3 was a consequence of addition of urea, but molasses did not affect CP concentration. There are conflicting data about the effects of molasses on CP content of silages. Addition of molasses to silages increased (Lattermae *et al.*, 1985; Kennedy, 1990), did not affect (O'kiely, 1992), or even decreased (Moore and Kennedy, 1994) CP content of silages.

The lower NDF content in treatment 1, might be due to the acid hydrolysis of fiber (McDonald, 1981). The NDF content of silages decreased between days zero to 60 of ensiling and was 154, 92 and 104 g/kg DM for treatments 1, 2 and 3, respectively. The greater NDF reduction in treatment 3 compared with treatment 2, can be due to the effect of ammonia on the cell wall components (Deschard, 1983). There was 8.6 and 32.2% reduction in ADL content of treatments 2 and 3, respectively, compared with treatment 1, which shows greater effect of urea on cell wall content than formic acid alone. Nefzaoui and Vanbell (1986) reported 13 and 15% reductions in ADL content of OC treated with NH<sub>4</sub>OH and NaOH, respectively.

The addition of formic acid decreased pH below 6 immediately at ensiling (data not shown) (Mayne, 1993). The pH value in treatment 3 was highest ( $P < 0.05$ ) due to addition of urea, which can be explained by

the conversion of urea to ammonia which also resulted in higher ammonia-N concentration. The pH of treatment 2 was significantly ( $P < 0.05$ ) lower than other treatments due to formic acid; this silage had the lowest ammonia-N concentration as the result of restricted fermentation and proteolysis (Chamberlain *et al.*, 1990).

Treatments only affected the concentration of lactic acid among the studied organic acids. Treatment 1 had the highest ( $P < 0.05$ ) lactic acid concentration and was less stable after opening based on visual observation. Silages with high lactic acid level are less aerobically stable after opening (Moon *et al.*, 1980; Rust *et al.*, 1989). Lactic acid serves as a substrate for lactate- assimilating yeasts upon exposure to air (Wohlt, 1989). Treatment 1 also contained numerically less acetic and propionic acid concentrations. These short-chain acids inhibit the growth of yeast and moulds in the silage (Moon, 1983). On the other hand, when lactic acid is used as an energy source by the rumen microbes, it enters into the cell by active transport, which requires two-fold energy, thus it is not a good source of energy for rumen microbes (Baytok *et al.*, 2005). Thereby, silages high in lactic acid content (like treatment 1 in the present study) may result in low microbial protein synthesis in the rumen (Bosch *et al.*, 1988). This shows the beneficial effect of additives like formic acid or urea on quality and stability of fresh OC. The total acid content was higher for treatments 2 and 3 (5.05 and 4.72% DM, respectively) compared with treatment 1 (3.98% DM) which might be due to more soluble carbohydrates available for greater fermentation in these treatments.

Fraction "a" of DM degradation kinetics was higher ( $P < 0.05$ ) for treatments 2 and 3 which could be due to the synergistic effect of urea and formic acid in degrading ligno-cellulose fraction of the cell wall and also high water soluble carbohydrates of molasses. The potential (a+b) DM degradability was higher ( $P < 0.05$ ) in treatments 2 and 3, which reflected the higher effective DM degradability in both treated silages and were higher than 34% value reported for exhausted OC by Nefzaoui (1983). Effective degradability of

DM was higher for treatment 3 (44.91%), indicating the ability of the additives (molasses, formic acid and urea) in enhancing rumen degradability of OC. Effective degradability of DM increased by 7.4 and 17.2% in treatment 2 and 3, respectively compared to the control. The rate of degradation (/h) was highest ( $P < 0.05$ ) in treatment 3, which is another benefit of adding urea to OC. The higher "c" fraction in treatment 3 can result in higher DM intake through higher passage rate in the rumen.

The CP degradation kinetics ("a" and "b") and effective degradability were affected by treatments. Treatment 3 had a significantly higher "a" fraction and effective degradability, which can be attributed to the synergistic effect of additives on degradation of cell-wall components and provision of soluble carbohydrates for rumen microbes and also high urea solubility. The "a" and "c" fractions and effective degradability of DM and "a", "b" and "c" fractions and potential and effective degradabilities of CP were higher than values reported by Martin Garcia *et al.* (2003) for OC (0.38-0.44). Effective degradability of CP increased by 3.75 and 19.7% in treatments 2 and 3, respectively.

Up to 4 h of incubation, the gas production was not different between treatments (Table 5). Gas production was positively correlated to IVOMD, in agreement with the data of Al-Masri (2003). The gas production values following OC degradation ranged from 18.8 to 36.4 ml gas per g OC for 48 h incubation in the experiment of Al-Masri (2003). Gas production values in the present study were much higher than values reported by Al-Masri (2003). Van Soest and Robertson (1985) showed a highly significant and positive relationship between gas production and the *in vitro* apparent and true degradabilities.

The ME values estimated from gas production (Table 5) were much higher (11.4 to 12.2 MJ/kg DM) than values (4.2-6.4 MJ/kg DM) reported by Aguilera and Molina (1986) for OC. A higher gas production was associated with an increase in the values of ME in the study of Al-Masri

(2003); the same trend was observed in the present study. Supplementation of OC with additives, increased IVOMD (based on gas production) by about 15% (Table 5).

The IVOMD values (Table 6) obtained from gas production were higher than values obtained from the *in vitro* digestibility determined by the methodology of Tilley and Terry (1963) and were within the ranges (except for treatment 1) reported by Al-Masri (2003). The IVOMD and IVDMD (except for treatment 1) values were within the ranges obtained by Martin Garcia *et al.* (2003) and Molina Alcaide *et al.* (2003), 0.21 and 0.27, respectively. The variations in IVOMD and IVDMD values could be attributed to the differences in the chemical composition and method of oil extraction in different experiments. The IVOMD increased by 29.7 and 28.6% for treatments 2 and 3, respectively, which again showed the beneficial effects of additives. Only, the apparent digestibility of CP was significantly affected by the treatments, which was higher for treatment 3, possibly due to urea addition (McDonald *et al.*, 1991; Henderson, 1993). The NDF and ADF digestibility values tended to be higher for treatment 3. The digestibility values of CP and OM were higher than the value (0.2) reported by Theriez and Boule (1970). The digestibilities of NDF and ADF (especially for treatment 3) were also higher than the values (0.15 and 0.09, respectively) reported by Molina and Aguilera (1988) using untreated OC.

The results of this study confirm the findings of other researches that OC can be preserved well by ensiling. Incorporating molasses, urea and formic acid at ensiling, can improve its chemical composition and nutritive value. This by-product provides a cheap energy source and fiber for the ruminant animal which reduces the costs related to waste management of this potential pollutant.

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