Journal of Animal and Veterinary Advances 7 (12): 1589-1593, 2008
ISSN: 1680-5593

Study of the Effect of *Saccharomyces cerevisiae* on Nutritional Value of Exhausted Dry Olive Cake Using *In vitro Gas* Production Technique

Omid Khosravifar, Naser Maheri-Sis, Habib Aghdam-Shahriar,
Ramin Salamat-Doost and Ali-Reza Baradaran-Hasanzadeh
Department of Animal Sciences, Islamic Azad University, Shabestar Branch,
East Azarbayjan Shabestar, Iran

Abstract: The current study, was conducted to determines the chemical composition and estimation of nutritive value of Exhausted Dry Olive Cake (EDOC) and evaluate the effect of baker's yeast (*Saccharomyces cerevisiae*, SC) on the Metabolizable Energy (ME) and Organic Matter Digestibility (OMD) of exhausted dry olive cake using *in vitro* gas production technique in sheep. The feed samples (200 mg) were incubated with rumen liquor taken from 3 fistulated Ghezel rams at 2, 4, 6, 8, 12, 16, 24, 36 and 48 h. The results showed that, exhausted dry olive cake was highly fibrous material and CP content of this by-product was low. Cumulative gas production volume in 2, 4 and 6 h increased when exhausted dry olive cake supplemented with *Saccharomyces cerevisiae*. Addition of live yeast did not result in significant positive effect on Metabolizable Energy (ME), Organic Matter Digestibility (OMD) and gas production parameters on exhausted dry olive cake.

Key words: Exhausted dry olive cake, *Saccharomyces cerevisiae*, nutritive value, gas production, sheep

INTRODUCTION

In arid and semi-arid parts of the world like Iran, the major nutritional limitation for animal production is low availability of vegetation which limits intake of energy and protein (Rowghani and Zamari, 2007). Therefore, we need to find new source of feedstuffs to production systems.

Non-conventional feedstuffs such as olive by-products can play an important role as alternative source of nutrients for livestock. Crude olive cake is one of the by products of oil extraction from olive fruits that contains the olive kernel shell crushed into fragments, the skin and the crushed pulp. The main problem in preserving crude olive cake is its relatively high water content and the still large quantity of oil it retains (Sansoucy *et al*., 1985), consequently when this type of olive cake exposed to air, rancid and unfit for animal consumption (Yansary *et al*., 2007).

One of the ways to solve this problem is extraction and dehydration of crude olive cake that produced a new by product called "Exhausted Dry Olive Cake" (EDOC), therefore exhausted dry olive cake could be stored for over a year (Sansoucy *et al*., 1985).

The consumption of EDOC in ruminant is limited, because of their low nutritive value (Sansoucy *et al*., 1985) and condensed tannins (Garcia *et al*., 2003). This by-product is highly fibrous (Ohled and Becker, 1982) and is low in crude protein content (Hadjipanayiotou, 1994) as well as a large proportion of the protein (80-90%) is linked to the lingo-cellulose fraction (Sansoucy *et al*., 1985).

However, some researcher reported beneficial effect of utilization olive cake in ruminant nutrition (Jassim *et al*., 1997; Chiofalo *et al*., 2004) and others purposed some method to improving nutritive value of exhausted olive cake such as soda or ammonia treatment (Sansoucy *et al*., 1985; Jassim *et al*., 1997).

Nowadays, manipulating rumen digestion system through the addition of direct feed microbial (such as *Saccharomyces cerevisiae*) and a fibrolytic enzyme to ruminant rations so as to enhance cellulose digestion and improves the performance of the animal is the most interest in recent years (Salama *et al*., 2002; Nocek *et al*., 2003; Giger-Reverdin *et al*., 2004; Haddad and Goussous, 2005).

Unfortunately, the main limitation in olive by-products in animal feeding is ascribed to their variable composition, on the other hand, the variability of these by-products composition, depending on factors such as year, geographical origin, procedure of production or treatment (Alcaide *et al*., 2003). Thereby, we need a method to rapid evaluate the nutritive value of exhausted dry olive cake. In this regard, the *in vitro* gas production

Corresponding Author: Omid Khosravifar, Department of Animal Science, Islamic Azad University, Shabestar Branch, East Azarbayjan Shabestar, Iran

technique is beneficial assay to feed evaluation especially in developing countries because this method is capable of measuring rate and extend of nutrition degradation with less expensive (Menke and Stengass, 1988; Getachew *et al*., 2004; Chumpawadee *et al*., 2005). On the other hand, the Hohenheim Gas test is one of the *in vitro* methods used for the estimation of organic matter digestibility and the energy content of feedstuffs for ruminants (Menk *et al*., 1979) in addition, gas measurement provides a useful data on digestion kinetics of both soluble and insoluble fraction of feedstuffs (Makkar, 2003). This method relies on the measurement of total amount of gas production during fermentation of feed with buffered rumen fluid in calibrated glass syringes (Menk *et al*., 1979; Menke and Stengass, 1988). The gas produced in the gas technique is the direct gas produced as a result of fermentation and the indirect gas produced from the buffering of short chain fatty acids (Makkar, 2003).

Lee *et al.* (2000) showed a close relationship between the energy value of forage calculated on *in vivo* digestion trail and *in vitro* gas production parameters. Aiple *et al.* (1996) suggested that the cellulose technique and the gas test are suitable *in vitro* techniques in compound feeds for dairy cows. Seker (2002) also showed significant correlation between the energy levels obtained by *in vitro* gas test and *in vivo* trail.

The aim of this study, was to determine chemical composition and nutritive value of exhausted dry olive cake as well as the effects of inclusion *Saccharomyces cerevisiae* (EDOC+SA) on gas production parameters.

MATERIALS AND METHODS

Animal and feeds: Three fistulated Gezel rams were used for rumen liquor collection in order to application in gas production technique. Exhausted dry olive cake collected from an olive by-products processor manufacture near the city of Karaj.

Saccharomyces cerevisiae contained 5×10^9 live yeast cells per gram provided from Iran Mayeh Company.

Exhausted dry olive cake milled through a 1 mm sieve in animal nutrition laboratory. One kilogram of milled dry olive cake mixed with 28.66 g of *Saccharomyces cerevisiae* to evaluate of the effects of inclusion yeast on gas production parameters.

Chemical analysis: Exhausted dry olive cake milled through a 1 mm sieve for chemical analysis and gas production procedure. Dry Matter (DM) was determined by drying the samples at 105°C overnight and ash by igniting the sample in muffle furnace at 525°C for 8 h. Crude Fiber (CF) and Ether Extract (EE) were determined by the method of AOAC (1990). Nitrogen (N) content was measured by the Kjeldal method. Crude Protein (CP) was calculated as N×6.25 (AOAC, 1990).

***In vitro* gas production:** Rumen fluid was obtained from 3 fistulated Gezel rams fed twice daily at the maintenance level with a diet containing alfalfa hay (60%) and concentrate (40%). The samples incubated *in vitro* rumen fluid in calibrated glass syringes following the procedures of Menk *et al.* (1979). The 200 mg samples were weighed in triplicate into calibrated glass syringes of 100 mL. The syringes h syringe followed by incubation in a water bath at 39°C. Reading of gas production were recorded before incubation (0) and 2, 4, 6, 8, 12, 16, 24, 36 and 48 h after incubation. Total gas values were corrected for blank incubation and gas production from syringes contain rumen fluid and *Saccharomyces cerevisiae* without exhausted dry olive cake.

Cumulative gas production data were fitted to the model of Orskov and McDonald (1979).

$$Y = a+b\ (1-e^{-ct})$$

Where:

- a = The gas production from the immediately soluble fraction (mL).
- b = The gas production from the insoluble fraction (mL).
- c = The gas production rate constant for the insoluble fraction (b).
- a+b = Potential gas production (mL).
- t = Incubation time (h).
- Y = Gas production at time t.

The metabolizable energy (MJ Kg^{-1} DM) content of exhausted dry olive cake with and without of adding *Saccharomyces cerevisiae* were calculated using equations of Menk and Steingass (1988) as follows:

$$ME\ (MJ\ Kg^{-1}\ DM) = 2.20 + 0.136\ GP + 0.057\ CP + 0.0029\ EE^2$$

Where:

- GP = The 24 h net gas production (mL 200 mg^{-1}).
- CP = Crude Protein (%).
- EE = Crude fat (%).

Organic Mater Digestibility (OMD%) of exhausted dry olive cake with and without of adding *Saccharomyces cerevisiae* were calculated using equations of Menk *et al.* (1979) as follows:

$$OMD\% = 14.88+0.889GP+0.45CP+0.0651XA$$

Where:
GP = About 24 h net gas production (mL 200 mg^{-1}).
CP = Crude protein (%).
XA = Ash content (%).

Statistical analysis: All of the data were analyzed by using MSTATC software and means of 2 samples groups were separated by independent-samples t-test (Steel and Torrie, 1980).

RESULTS AND DISCUSSION

Chemical composition of the Exhausted Dry Olive Cake (EDOC) is presented in Table 1.

Exhausted dry olive was rich in cell wall content and low in CP content relatively.

Generally, wide variation existed in the chemical composition of the olive cake between investigations. Because chemical composition of this feedstuff influenced of many factor such as year, geographical origin, procedure of production or treatment (Alcaide *et al*., 2003).

Also chemical composition of olive cake influenced by method of extraction oil in olive mill and further processing on crude olive cake (Alcaide *et al*., 2003). Sansoucy *et al*. (1985) reported that crude olive cake has a low crude protein, high crude fiber and a relatively high fat content. Extraction by solvent decreased fat content and relatively increased the other contents in exhausted dry olive cake (Yansary *et al*., 2007).

The DM, CP, CF and EE content of EDOC were higher than reported by Yansari *et al*. (2007). The CP of EDOC was Higher than reported by Filya *et al*. (2006) and Garcia *et al*. (2003). Sansoucy *et al*. (1985) divided olive cake base on chemical composition, in this classification EDOC has 8-10, 35-40 and 4-6%, CP, CF and EE, respectively.

Gas production parameters (a, b, c) and gas production volume (mL 200 mg^{-1} DM) in different incubation times and calculated amounts of OMD and ME of EDOC and EDOC+SA are presented in Table 2 and 3.

Menk *et al*. (1979) suggested that gas volume at 24 h after incubation has been relationship with metabolizable energy in feedstuffs. Additionally, *in vitro* dry matter and organic mater digestibility were shown to have high correlation with gas volume (Sommart *et al*., 2000).

The gas volume for EDOC and EDOC+SA was significantly different in 2, 4 and 6 h. With increase in incubation times, differences gas volume between EDOC and EDOC+SA were not significant. Probably, yeast population or metabolic activity of *Saccharomyces cerevisiae* have decreased after 6 h incubation. El Hassan *et al*. (1993) reported that yeast cultures need to be both viable and metabolically active to have a full stimulatory effect on ruminal fermentation as well as they reported that after feeding, the number of viable yeast cells declined in the rumen of sheep at a rate of 8.6% h^{-1}. Likely, *Saccharomyces cerevisiae* in liveliness period could provide suitable condition for activation of rumen microorganisms. Stimulate utilization of hydrogen by ruminal acetogenic bacteria (Chaucheyras *et al*., 1995), scavenging excess oxygen (Newbold *et al*., 1996), help to buffer excess lactic acid production in the rumen and improvement in fiber digestion (Arambel *et al*., 1987) were noticeable effects of *Saccharomyces cerevisiae* in ruminal manipulation. Therefore, in 2, 4 and 6 h of incubation, cumulative gas production volume in EDOC+SA samples were more than EDOC.

The gas production parameters (a, b and c) were not affected by *Saccharomyces cerevisiae*. Although, ME and OMD increased with *Saccharomyces cerevisiae*, but these improvement were not significant.

The gross energy in olive Cake is high, presumably due to its high structural carbohydrate content (Molina-Alcaid and Yanez-Ruiz, 2007), but ME of this by-product is low. Garsia *et al*. (2003), Molin-Alcaide and Yanez-Ruiz (2007) reported high level of gross energy in

Table 1: Chemical composition of Exhausted Dry Olive Cake (EDOC)

		DM (%)				
	DM%	CP	EE	CF	Ash	NFE
EDOC	95	8.75	6.00	48.20	7.00	25.15
SE	1	0.19	0.19	1.65	0.26	2.89

Table 2: Cumulative gas production volume (mL per 200 mg) at different incubation times for EDOC and EDOC+SA

Incubation time (h)	EDOC	EDOC+SA	Significance
2	2.74	6.45	**
4	10.62	13.78	**
6	13.86	17.12	*
8	15.91	18.57	ns
12	17.26	20.17	ns
16	18.47	21.54	ns
24	20.37	23.24	ns
36	22.49	25.51	ns
48	24.23	27.58	ns

ns: Non significant, *: $p<0.05$, ** $p<0.01$

Table 3: The gas production parameters, Organic Mater Digestibility (OMD) and Metabolizable Energy (ME) contents of EDOC and EDOC+SA

Items	EDOC	EDOS+SA	Significance
a(mL)	-2.670	2.17	ns
b(mL)	24.860	23.32	ns
c(mL h^{-1})	0.158	0.14	ns
OMD (%)	37.380	39.93	ns
ME(Mj Kg^{-1} DM)	5.570	5.95	ns

a: The gas production from the immediately soluble fraction (mL), b: The gas production from the insoluble fraction (mL), c: The gas production rate constant for the insoluble fraction (b), ns: Non significant

olive cake that equal with 18.7 and 19.7 (MJ Kg^{-1} dry matter), respectively. The ME content of EDOC and EDOC+SC in this experiment were 5.57 and 5.95 Mj Kg^{-1} DM (Table 3), respectively that were almost in agreement with Filya *et al*. (2006) that they reported ME content of different type of olive cake between 4.97-6.53 Mj Kg^{-1} DM with *in situ* assay.

CONCLUSION

On the base of chemical composition, exhausted dry olive cake is a highly fibrous by product. The metabolizable energy and organic matter digestibility of this by-product is low, therefore we need assays to improvement of nutrient value of this food. *Saccharomyces cerevisiae* could not improve the organic matter digestibility and metabolizable energy content. But we think several investigation is need to verify of this hypothesis.In this trail, the gas production rate constant for the insoluble fraction was low, therefore we do not recommend to inclusion exhausted dry olive cake in transition dairy cows ration.

ACKNOWLEDGMENT

This research was carried out as M.Sc. thesis in animal sciences in Islamic Azad University-Shabestar Branch. We would like to acknowledge Mr. J. Doolgari-Sharaf, Mr. A. Noshadi, Mr. S. Khosravifar and Mr. H. Sadigi. Authors thanks from Animal Research Station of Islamic Azad University-Shabestar Branch and Tabriz University.

REFERENCES

Aiple, K.I., H. Steingass and W. Drochner, 1996. Prediction of the net energy content of raw materials and compound feeds for ruminants by different laboratory methods. Arch. Anim. Nutr., 49: 213-220.

Alcaide, M.E., D.Y. Ruiz, A. Moumen and I.M. Garcia, 2003. Chemical composition and nitrogen availability for goat and sheep of some olive by-products. Small Rumin. Res., 49: 329-336. DOI: 10.1016/S0921-4488(03)00148-2.

AOAC, 1990. Official Method of Analysis. 15th Edn. Washington DC, USA: Association of Official Analytical Chemists, pp: 66-88.

Arambel, M.J., R.D. Weidmeier and J.L. Walters, 1987. Influence of donor animal adaptation to added yeast culture and/or *Aspergillus oryzae* fermentation extract on *in vitro* rumen fermentation. Nutr. Rep. Int., 35: 433-437.

Chaucheyras, F., G. Fonty, G. Bertin and P. Gouet, 1995. *In vitro* H_2 utilization by a ruminal acetogenic bacterium cultivated alone or in association with an archea methanogen is stimulated by a probiotic strain of *Saccharomyces cerevisiae*. Applied Environ. Microbiol., 61: 3466.

Chiofalo, B., L. Liotta, A. Zumbo and A. Chiofalo, 2004. Administration of olive cake for ewe feeding: Effect on milk yield and composition. Small Rumin. Res., 55: 169-175.

Chumpawadee, S., K. Sommart, T. Vongpralub and V. Pattarajinda, 2005. Nutritional evaluation of non forage high fibrous tropical feeds for ruminant using *in vitro* gas production technique. Pak. J. Nutr., 4: 298-303.

El Hassan, S.M., C.J. Newbold and R.J. Wallace, 1993. The effect of yeast culture on rumen fermentation: Growth of the yeast in the rumen and requirement for viable yeast cells. Anim. Prod., 56: 463 (Abstr).

Filya, I., H. Hanoglu and Ö. Canbolat, 2006. Research feed value and possibilities in lamb fatting of dried olive-cake-determination of feed value by *in situ* method. Agric. J. Uludag Univ., 20 (1): 1-12.

Garcia, M.A.I., A. Moumen, D.R. Yanez Ruiz and E.M. Alcaide, 2003. Chemical composition and nutrient availability for goat and sheep of 2-stage olive cake and olive leaves. Anim. Feed Sci. Technol., 107: 61-74. DOI: 10.1016/S0377-8401(03)00066-X.

Getachew, G., P.H. Robinson, E.J. DePeters and S.J. Taylor, 2004. Relationships between chemical composition, dry matter degradation and *in vitro* gas production of several ruminant feeds. Feed Sci. Technol., 111: 57-71. DOI: 10.1016/S0377-8401(03)00217-7.

Giger-Reverdin, S., D. Sauvant, J. Tessier, G. Bertin and P. Morand-Fehr, 2004. Effect of live yeast culture supplementation on rumen fermentation in lactating dairy goats. S. Afr. J. Anim. Sci., 34: 89-91.

Haddad, S.G. and S.N. Goussous, 2005. Effect of yeast culture supplementation on nutrient intake, digestibility and growth performance of awassi lambs. Anim. Feed Sci. Technol., 118: 343-348. DOI: 10.1016/j.anifeedsci.2004.10.003.

Hadjipanayiotou, M., 1994. Laboratory evaluation of ensiled olive cake, tomato pulp and poultry litter. Livest. Res. Rural Dev., 6: 1-8.

Jassim, R.A. M.A., F.T. Awadeh and A. Abodabos, 1997. Supplementary feeding value of urea-treated olive cake when fed to growing awassi lambs. Anim. Feed Sci. Technol., 64: 287-292. DOI: 10.1016/SO377-8401(96)01044-9.

Lee, M.J., S. Hwang and P. Wen-Shyg Chiou, 2000. Metabolizable energy of roughage in taiwan. Small Rum. Res., 36: 251-259.

Makkar, H.P.S., 2003. Recnt advances in the *in vitro* gas method for evaluation of nutritional quality of feed resources. Animal Production and Health Section, Joint FAO/IAEA Division, International Atomic Energy Agency, Vienna, Austria. ISBN: 9251050465.

Menk, K.H. and H. Steingass, 1988. Estimation of energetic feed value obtained from chemical analysis and *in vitro* gas production using rumen fluid. Anim. Res. Dev., 28: 7-55.

Menk, K.H., L. Raab, A. Salewski, H. Steingass, D. Fritz and W. Schneider, 1979. The estimation of digestibility and metabolisable energy content of ruminant feedstuffs from the gas production when they incubated with rumen liquor *in vitro*. J. Agric. Sci., 93: 217-222.

Molina-Alcaid, E. and D.R. Yanez-Ruiz, 2007. Potential use of olive by-products in ruminant feeding: A review. Animal Feed Sciences Technology (in Press). DOI: 10.1016/j.anifeedsci.2007.09.021.

Newbold, C.J., R.J. Wallace and F.M. McIntosh, 1996. Mode of action of the yeast *Saccharomyces cerevisiae* as a feed additive for Ruminants. Br. J. Nutr., 76: 249-261.

Nocek, J.E., W.P. Kautz, J.A.Z. Leedle and E. Block, 2003. Direct-fed microbial supplementation on the performance of dairy cattle during the transition period. J. Dairy Sci., 86: 331-335.

Ohled, G. and K. Becker, 1982. Suitability of cell-wall constituents as predicators of organic matter digestibility in some tropical and subtropical by-products. Anim. Feed Sci. Technol., 7: 191-199. DOI: 10.1016/0377-8401(82)90053-0.

Orskov, E.R. and P. McDonald, 1979. The estimation of protein degradability in the Rumen from incubation measurements weighed according to rate of passage. J. Agric. Sci., 92: 499-503.

Rowghani, E. and M.J. Zamiri, 2007. Effect of additives on chemical composition, degradability coefficients and ruminal-intestinal disappearance of dry matter and crude protein of laboratory ensiled Olive cake. Iranian Vet. Res., 8 (1): 32-39.

Salama, A.A.K., G. Caja, D. Garin, E. Albanell, X. Such and R. Casals, 2002. Effects of adding a mixture of malate and yeast culture (*Saccharomyces cerevisiae*) on milk production of murciano granadina dairy goats. Anim. Res., 51: 295-303.

Sansoucy, R.R., X. Alibes, P. Berge, F. Martilotti, A. DbNefzaoui and P. Zoiopoulos, 1985. Olive by-products for animal feed. Food and Agriculture Organization of the United Nation, Rome, FAO. ISBN: 92-5-101488- 4.

Seker, E., 2002. The determination of the energy value of some ruminant feeds by using digestibility trail and gas test. Evue Méd. Vét., 153: 323-328.

Sommart, K., D.S. Parker, P. Rowlinson and M. Wanapat, 2000. Fermentation characteristics and microbial protein synthesis in an *in vitro* system using cassava, rice straw and dried ruzi grass as substrates. Asian-Aus. J. Anim. Sci., 13: 1084-1093.

Steel, R.G. and J.H. Torrie, 1980. Principles and Procedures of Statistics. 2nd Edn. McGrow-Hill., New York. ISBN: 0070609268 9780070609266.

Yansary, T.A., H. Sadeghi, Z. Ansari-Pirsarai and H. Mohammad-Zadeh, 2007. Ruminal dry matter and nutrient degradability of different olive cake by-products after incubation in the rumen using nylon bag technique. Int. J. Agric. Biol., 9 (3): 439-442.