

IMPROVING UTILIZATION OF OLIVE CAKE SILAGE BY TREATING WITH FIBROLYTIC ENZYMES ON DIGESTIBILITY AND GAS PRODUCTION IN THE RUMEN

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SUMMARY

In vitro study was carried out to determine the effect of replacing clover hay with olive cake silage with or without fibrolytic enzymes on rumen fermentation and gas production and dry matter disappearance. The experimental diets consist of 50: 50 concentrate: roughage ratio (on DM basis); the experiments divided into four groups, the first group diet was 50% concentrate feed mixture (CFM): 50% clover hay (control). The second group diets were replaced clover hay by olive cake by 25, 50 and 75% for T1, T2 and T3, respectively. The third group diets were replaced clover hay by olive cake silage by 25, 50 and 75% for T4, T5 and T6, respectively. The fourth group diets were replaced clover hay by treated olive cake silage with fibrolytic enzymes by 25, 50 and 75% for T7, T8 and T9, respectively. The results of ensiling olive cake showed that treated olive cake silage was recorded the lower values in pH and NH₃-N compared with untreated silage. Results showed a significant decrease in gas production and relative gas production to DM and fiber fractions in all treatments compared with control. DM disappearance showed that T1, T4, T7 and T8 recorded a significant increase compared with control. The current findings reflects a potential possibility of replacing clover hay with olive cake silage especially treated with fibrolytic enzymes.

Keywords: *In vitro*, ruminants, fibrolytic enzyme, olive cake, clover hay and digestibility.

INTRODUCTION

Olive cake is one of agro-industrial by-products which potentially used as feedstuff for ruminant animal. Olive cake incorporation into animal's diets lowering the ration cost (Molina-Alcaide and Yañez-Ruiz, 2008). Olive cake shows a different range in chemical composition according to different factors (such as olive species and method of extraction) (Abbeddou *et al.*, 2011). Low digestibility of olive cake might be due to high contents of fiber and presence of anti-nutritional factors (tannins) (Molina-Alcaide and Nefzaoui, 1996; Al-Masri and Guenther, 1995 and Abd El Tawab *et al.*, 2015 and 2016). Many studies were investigated inclusion of olive cake in ruminant diets either crude, chemically or biologically treated. Common investigated chemical treatments were ammonia and sodium hydroxide (Molina Alcaide and Nefzaoui, 1996 and Awawdeh and Obeidat, 2013) while, the most common biological method for improving olive cake characteristics was ensiling either with additives or without (Al Jassim *et al.*, 1997; Hadjipanayiotou, 1994, 1999). Different studies were suggested that fibrolytic enzymes have a positive effect on nutrients digestibility and animal performance (Feng *et al.*, 1996 and Dong *et al.*, 1999). Moreover, fibrolytic enzymes can be effective additive with agro-industrial by-product to improve nutritive value of fiber rich diets (Awawdeh and Obeidat, 2011; Salihu *et al.*, 2015 and Abd El Tawab *et al.*, 2016). Giraldo *et al.* (2004) determined that a pre-ingestive enzyme-feed interaction is substantial for enhancing effects on ruminal digestion. The aim of this study was to investigate the effect of ensiling olive cake treated with or without fibrolytic enzymes on rumen fermentation and gas production, dry matter, organic matter and fiber degradation.

MATERIALS AND METHODS

Enzyme sources:

Fibrolytic enzymes produced from anaerobic bacteria (*Clostridium butyricum*) at Dairy Science Department, National Research Centre, Dokki, Giza, Egypt. Each gram of enzymes contains 5000 IU/g of cellulase (Khattab *et al.*, 2017).

Collection and ensiling of crude olive cake:

Fresh olive cake (*Olea europaea*) was collected from Al-Salhiya Agricultural Company, Al-Sharqia, Egypt. Olive cake moisture content was adjusted to 65-70% before ensiling, treating silage was prepared by spraying fresh olive cake with four liter of enzyme solution per ton DM of olive cake (treated silage), or without crude enzyme, which sprayed with four liter of water (untreated silage). The material was ensiled in plastic bags for two months before feeding to the ewes. Chemical compositions of olive cake silage were determined as shown in Table (2).

Diets and in-vitro procedures:

In-vitro incubation was carried out as described by Khattab *et al.* (2016). The experimental diets consist of 50:50 concentrate: roughage ratio (on DM basis); the experiment diets divided into four main groups, the first group diet was 50% CFM, 50% clover hay (control). The second group diet was 50% CFM, replaced clover hay by olive cake by 25, 50, and 75% for T1, T2 and T3, respectively. The third group diet was 50% CFM, replaced clover hay by olive cake silage by 25, 50 and 75% for T4, T5 and T6, respectively. The fourth group diet was 50% CFM, replaced clover hay by treated olive cake silage with fibrolytic enzymes by 25, 50 and 75% for T7, T8 and T9, respectively (Table 1).

Table (1): Formula of the experimental rations (%).

Item		Concentrate feed mixture (CFM)	Clover hay	Crude olive cake	Untreated olive cake silage	Treated olive cake silage
Control		50	50	---	---	---
Crude olive cake	T1	50	37.5	12.5	---	---
	T2	50	25.0	25.0	---	---
	T3	50	12.5	37.5	---	---
Untreated olive cake silage	T4	50	37.5	---	12.5	---
	T5	50	25.0	---	25.0	---
	T6	50	12.5	---	37.5	---
Treated olive cake silage	T7	50	37.5	---	---	12.5
	T8	50	25.0	---	---	25.0
	T9	50	12.5	---	---	37.5

CFM: concentrate feed mixer; CFM consisted of 20% yellow corn, 20% wheat bran, 32% sugar beet pulp, 5% soybean meal, 20% cottonseed meal, 0.1% sodium bicarbonate, 1.5% limestone, 1% salt, 0.1% vitamins and 0.3% minerals.

The chemical composition of feedstuff ingredients and experimental rations are showed in Table (2).

Rumen fluid was collected from 3 ruminal cannulated Holstein dairy cows (mean weight 680±30 kg). The collected rumen fluid (before morning feeding) was mixed and squeezed through a 4-layers cheesecloth under continuous flushing with CO₂ and immediately transported to laboratory at 39° C, where it was used as a source of inoculum. Each treatment was tested in eight replicates (the experiment was repeated twice) accompanied by blank vessels (no substrate). 400 mg of milled substrate was added to the incubation vessels of 100 ml capacity. Each vessel was filled with 40 ml of the incubation medium (292 mg K₂HPO₄, 240 mg KH₂PO₄, 480 mg (NH₄)₂SO₄, 480 mg NaCl, 100 mg MgSO₄·7H₂O, 64 mg

CaCl₂.2H₂O, 4 mg Na₂CO₃ and 600 mg cysteine hydrochloride) per 1 liter of double distilled water (ddH₂O) and dispensed anaerobically in the 1:4 (v/v) ratio. Then the treatments were incubated at 39°C for 24h. The average of the volume of gas produced from the blanks was deducted from the volume of gas produced per sample. After 24 h digestion, the samples were transferred into test tubes and centrifuge for 1 h in order to obtain the residues which were then filtered and the residues dried at 65° C for 24 h. The dry residues were weighed and digestibility calculated using the equation as follows:

$$\text{IVDMD (\%)} = [(\text{initial DM input} - \text{DM residue} - \text{blank}) / \text{initial DM input}] * 100$$

Samples analysis:

Samples of rumen fluid samples were analyzed for pH and NH₃-N. Substrates and substrate residues after 48 h of incubation were dried at 70°C and analyzed for the amount of DM (DM digestibility) according to AOAC (1995). The NH₃-N concentration was determined as described by Khattab *et al.* (2016). Neutral detergent fiber (NDF) and acid detergent fiber (ADF) were analyzed by Ankom 200 Fiber Analyzer (Ankom Technology Corporation, Fairport, NY) according to Van Soest *et al.* (1991).

Statistical analysis:

Data were statistically analyzed using GLM procedure of SAS software (Version 9.2). Significant differences between means of treatments were carried out by the Duncan’s test, and the significance threshold was set at P<0.05.

RESULTS AND DISCUSSION

Silage quality:

Data in Table (2) showed the chemical composition of different ration ingredients. Results showed that treating olive cake silage with fibrolytic enzymes reduced NDF and hemicellulose and NH₃-N concentration as compared with untreated silage. Adding fibrolytic enzymes enhance degradation of plant cell wall and yielding soluble sugars which could be fermented by lactic acid bacteria to consequently lowering the silage pH (Kung, 2010). Reduction in NH₃-N concentration related to decrease deamination and degradation of silage proteins content by lower pH value (Kung, 2010).

Table (2): Chemical analysis of feed ingredients (on DM basis %).

Item	DM	OM	CP	EE	NDF	ADF	Hemicellulose	Ash	pH	NH ₃ -N (mM)
CFM	93.09	93.98	12.73	6.28	49.98	18.09	31.9	6.02	---	---
Clover hay	93.86	88.31	11.97	6.10	43.49	33.06	10.4	11.69	---	---
Crude olive cake	55.23	94.71	6.17	12.76	64.84	55.37	9.5	5.29	---	---
Untreated olive cake silage	45.06	97.37	5.16	14.79	71.04	54.87	16.2	2.63	4.10	3.50
Treated olive cake silage	46.34	96.78	5.38	12.36	66.84	58.53	8.3	3.22	4.07	2.61
Ration chemical composition, % (DM)										
Control	93.47	91.15	12.35	6.19	46.74	25.58	21.15	8.85	---	---
T1	88.65	91.94	11.63	7.02	49.40	28.36	21.04	8.06	---	---
T2	83.82	92.74	10.90	7.85	52.07	31.15	20.93	7.26	---	---
T3	78.99	93.54	10.17	8.69	54.74	33.94	20.81	6.46	---	---
T4	87.37	92.28	11.50	7.27	50.18	28.30	21.88	7.72	---	---
T5	81.27	93.41	10.65	8.36	53.62	31.03	22.60	6.59	---	---
T6	75.17	94.54	9.80	9.45	57.07	33.75	23.33	5.46	---	---
T7	87.53	92.20	11.53	6.97	49.65	28.76	20.89	7.80	---	---
T8	81.59	93.26	10.70	7.75	52.57	31.94	20.63	6.74	---	---
T9	75.65	94.32	9.88	8.53	55.49	35.13	20.36	5.68	---	---

CFM: Concentrate feed mixer.

Gas production:

The effects of experimental diets on total gas production (TGP) are illustrated in Table (3). Control recorded the highest (P<0.05) value compared with other treatments, but T1 showed the lowest TGP

($P < 0.05$). Whilst, the data showed no difference ($P > 0.05$) between other treatments T2 to T9. Reduction of TGP reflect the effect of ensiling, anti-nutritional substances like tannins and lignin contents in diets contain olive cake which influence microbial proliferation in rumen (Al-Masri and Guenther, 1995, Al Jassim *et al.*, 1997 and Hadjipanayiotou, 1994 and 1999). Also, the data were cleared the reduction ($P < 0.05$) in relative gas production either per DM, NDF, ADF or hemicellulose from all treatments compared with control. It well knows that there is a negative relationship between gas production and cell wall content (NDF and ADF), which influence on microbial activity (De Boever *et al.*, 2005 and Karabulut *et al.*, 2007).

Ruminal pH and ammonia concentrations:

The effects of experimental diets on pH and ammonia concentration are listed in Table (4). Generally, all experimental treatments slightly lowered pH compared with control, the decrease of pH values might due to increase olive cake oil in rations which known to lack of fiber digestibility and decrease ruminal pH (Ørskov and Ryle, 1990). Also, tannins and lignin contents in diets contain olive cake influence microbial proliferation in rumen (Al-Masri and Guenther, 1995). The results showed that ammonia concentration recorded higher value for silage groups (T4:T9) compared with control. Minimal ammonia concentration may depend on the substrate availability (Erdman *et al.*, 1986), or on the microbial species taking part in its degradation. These results were agreed with Sujani and Seresinhe (2015), who found that ruminal ammonia concentration, was higher in fibrolytic enzyme supplemented.

Nutrients digestibility:

The effect of experimental diets on *in vitro* nutrients digestibility is presented in Table (5). Replacements of clover hay with olive cake increased DM digestibility in all levels expect level 75% (T3, T6 and T9) was decreased in all groups than control. Also, fourth group (T7 and T8) was showed higher value of DM digestibility than the second groups (T1 and T2) and the third groups (T4 and T5). However, the NDF digestibility was recorded the highest values ($P < 0.05$) in T9 compared with other treatments. The results showed that ensiled olive cake with fibrolytic enzymes may be enhance the digestion and the utilization of dry matter and fiber and reduce DM losses (Muwalla *et al.*, 2007, Kung, 2010 and Salihu *et al.*, 2015). Bendaou (2003) found that the pre-degradation in cell walls by ensiling material by-product fermentation simplify microbial access in the rumen contents cell, which enhances the digestibility. Also, ADF digestibility was recorded higher values ($P < 0.05$) in all levels 75% replacement clover hay with olive cake (T3, T6 and T9) compared with other treatments.

CONCLUSION

The present *in vitro* study showed that incorporation treated olive cake silage with fibrolytic enzymes in ruminants' diet had positive effect on dry matter digestibility and ammonia concentration at replacement levels 25 and 50% of clover hay. Generally, replacement olive cake instead of clover hay had negative effect on gas production in ruminant's diets which enhance diet utilization.

Table (3): Effect of experimental diets on gas production (GP).

Item	Control	Crude olive cake			Untreated olive cake silage			Treated olive cake silage			±SEM
		T1	T2	T3	T4	T5	T6	T7	T8	T9	
Total GP	144.25 ^a	134.5 ^c	140.3 ^b	138.3 ^b	139.5 ^b	139 ^b	140.5 ^b	139.3 ^b	140.3 ^b	139.75 ^b	0.456
GP/ gm DM	356.8 ^a	331.5 ^c	342.3 ^b	343.5 ^b	343 ^b	343.8 ^b	348 ^b	343.8 ^b	346.7 ^b	346.3 ^b	1.217
GP/ gm NDF	763 ^a	671 ^{cd}	657.5 ^d	627.3 ^{ef}	683.5 ^{bc}	641.3 ^e	610.3 ^g	692.3 ^b	659.3 ^d	624.3 ^{fg}	7.012
GP/ gm ADF	1686.3 ^{ab}	1575.8 ^e	1635 ^d	1649.8 ^{abcd}	1567.3 ^e	1522.3 ^f	1492.3 ^f	1644.8 ^{cd}	1680.7 ^{abc}	1701.3 ^a	11.651
GP/ gm hemicellulose	1394 ^a	1169 ^c	1098.5 ^d	1011.8 ^{ef}	1212 ^b	1108.5 ^d	1031.5 ^e	1194.8 ^{bc}	1085.3 ^d	986.3 ^f	18.866

a, b, c,d,e,f and g means with different superscriptes in the same row are significant (P<0.05).

Table (4): Effect of experimental diets on ruminal pH and ammonia concentrations.

Item	Control	Crude olive cake			Untreated olive cake silage			Treated olive cake silage			±SEM
		T1	T2	T3	T4	T5	T6	T7	T8	T9	
pH	7.04 ^a	6.98 ^{ab}	6.98 ^{ab}	6.98 ^{ab}	6.97 ^{ab}	6.93 ^b	6.95 ^b	6.96 ^b	6.96 ^b	6.99 ^{ab}	0.007
NH ₃	32.31 ^{cd}	31.08 ^d	39.28 ^{ab}	32.49 ^{cd}	38.81 ^{abc}	34.40 ^{abcd}	33.66 ^{bcd}	40.59 ^a	37.73 ^{abcd}	38.10 ^{abc}	0.766

a, b, c and d means with different superscriptes in the same row are significant (P<0.05).

Table (5): Effect of experimental diets on nutrients digestibility.

Item	Control	Crude olive cake			Untreated olive cake silage			Treated olive cake silage			±SEM
		T1	T2	T3	T4	T5	T6	T7	T8	T9	
DM digestibility	51.28 ^{cd}	59.33 ^{ab}	52.95 ^{cd}	49.63 ^{cd}	58.75 ^{ab}	54.53 ^{bc}	49.33 ^{cd}	60.42 ^a	55.8 ^b	48.13 ^d	0.837
NDF digestibility	33.28 ^c	29.62 ^{de}	34.02 ^c	38.80 ^b	28.93 ^e	34.02 ^c	39.17 ^b	28.25 ^e	32.08 ^{cd}	42.20 ^a	0.778
ADF digestibility	23.58 ^{cd}	21.44 ^{de}	25.97 ^{bc}	28.62 ^{ab}	20.51 ^e	24.80 ^c	29.32 ^a	21.40 ^{de}	23.67 ^{cd}	30.98 ^a	0.623

a, b, c,d and e means with different superscriptes in the same row are significant (P<0.05).

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تحسين الاستفادة من سيلاج تفل الزيتون المعامل بالانزيمات المحللة للالياف علي الهضم و انتاج الغاز بالكرش

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اجريت هذه التجربة بغرض دراسة تأثير استبدال دريس البرسيم بسيلاج تفل الزيتون المعامل او غير المعامل بالانزيمات المحللة للالياف علي تخمرات الكرش و انتاج الغاز ومعدل اختفاء المادة الجافة. اشتملت علائق التجربة علي 50% علف مركز : 50% علف خشن. قسمت التجربة المجموعة الاولى الكنترول 50% علف مركز : 50% دريس برسيم ، المجموعة الثانية 50% علف مركز و استبدال دريس البرسيم بتفل الزيتون الخام بنسب 25 و 50 و 75% (T1, T2 , T3) علي التوالي. المجموعة الثالثة 50% علف مركز و استبدال دريس البرسيم بسيلاج تفل الزيتون غير معامل بنسب 25 و 50 و 75% (T4, T5 , T6) علي التوالي. المجموعة الرابعة 50% علف مركز و استبدال دريس البرسيم بسيلاج بتفل الزيتون المعامل بالانزيمات المحللة للالياف بنسب 25 و 50 و 75% (T7, T9 , T8) علي التوالي. اظهرت النتائج ان سيلاج تفل الزيتون المعامل بالانزيمات المحللة للالياف اظهر انخفاضاً في قيمة الرقم الهيدروجيني و نيتروجين الامونيا مقارنة بغير المعامل. كما اظهرت النتائج انخفاضاً معنوياً في إنتاج الغاز ونسبة إنتاج الغاز إلى المادة الجافة ومكونات الألياف في كل المعاملات مقارنة الكنترول. سجلت المعاملات الاولى والرابعة والسابعة والثامنة زيادة معنوية في معدل اختفاء المادة الجافة مقارنة بالمجموعة الكنترول.

الخلاصة: تظهر النتائج الحالية ان هناك امكانية لاستبدال دريس البرسيم بسيلاج تفل الزيتون وخاصة المعامل بالانزيمات المحللة للالياف.