OLIVE CAKE SILAGE AS ALTERNATIVE ROUGHAGE FOR RUMINANT: EFFECT ON RUMEN DEGRADABILITY AND *IN-VITRO* GAS PRODUCTION

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SUMMARY

The current *In vitro* study was carried out to investigate the effect of replacing green maize (darawa) with crude olive cake or treated silage with or without fibrolytic enzymes; on nutrients digestibility, rumen fermentation, pH, ammonia nitrogen and gas production. Nine TMR with different replacement ratios were formulated and tested *In vitro* as follow: C: control group (50% concentrate: 50% darawa), G1: 50% concentrate: 25% of darawa replaced with crude olive cake ratio, G2: 50% concentrate: 25% of darawa replaced with untreated olive cake silage, G3: 50% concentrate: 25% of darawa replaced with treated olive cake silage, G4: 50% concentrate: 50% of darawa replaced with crude olive cake, G5: 50% concentrate: 50% of darawa replaced with untreated olive cake silage, G6: 50% concentrate: 50% of darawa replaced with treated olive cake silage, G7: 50% concentrate:75% of darawa replaced with crude olive cake, G8: 50% concentrate: 75% of darawa replaced with untreated olive cake silage, G9: 50% concentrate: 75% of darawa replaced with treated olive cake silage. Total gas production (TGP) and Dry matter digestibility were recorded the highest values when replaced 25% of darawa with crude olive cake, untreated or treated olive cake silage, whereas TGP and dry matter digestibility were decreased at level 75%. Moreover, the highest NH_3-N concentration (37.63 mM) was observed when replacing 25% of darawa with untreated olive cake silage (G2). Also, NDF and ADF digestibility were enhanced in treated groups compared with control group. From results it can be concluded that using fibrolytic enzymes and ensiling processing led to enhance fiber fractions digestion in olive seeds cell wall. Also, there is high positive effect when replacements darawa with olive cake at level 75% % regarding to reduce gas production and increase NDF and ADF digestibility.

***Keywords:** In-vitro,* olive cake, silage, fibrolytic enzymes, nutrients digestibility and gas production.

INTRODUCTION

Extracting Olive oil process produces large amount of by-product (olive cake) that are potentially have a negative environmental impact. Olive cake is one of the agro-industrial byproduct consisting of olive pulp, skin, stone and water (Alburquerque *et al*., 2004 and Abd El Tawab *et al*., 2018); It is poor in crude protein (8% of DM), but containing a high level of fiber fractions (NDF 58%, ADF 46% and ADL 24% of DM) and ether extract (9% of DM) (Abbeddou *et al.,* 2011). Also, 80 - 90% of its protein content is linked to lingo-cellulose fraction (Nefzaoui, 1983). So that, olive cake may consider as a low cost ingredient in total mixed rations due to its high content of residual oil, also, the administration method and the proportion of olive cakes in ruminant diets can promote different responses in rumen fermentation, (Molina-Alcaide and Ya˜nez-Ruiz, 2008).

Olive by-products is seasonally available and using it as an animal feed along the year require preservation and storage, the main problem to preserve olive cake is its contain a large percentage of water and oil, so that, long-term storage may results in mold formation and wastage of its nutrients (Rowghani *et al*., 2008). Silage preservation method is a simple, cheap, and efficient manner to preserve olive cake and improve its nutritive value, (Nefzaoui, 1991; Hadjipanayiotou, 1994; Al-Jassim *et al*., 1997; Hadjipanayiotou, 1999; Rowghani and Zamiri, 2007; Moumen *et al.*, 2008 and Abd El Tawab *et al*., 2018). Olive cake silage have been included as a part (100–780 g/kg) of multi-nutrient blocks (Hadjipanayiotou, 1996) and/or as partial replacement (300 g/kg) of barley hay, straw, or concentrates in diets for growing (Hadjipanayiotou and Koumas, 1996) and lactating (Hadjipanayiotou, 1999) livestock.

Many studies reported a positive effect of fibrolytic enzymes on the digestion of nutrients (Feng *et al.*, 1996; Dong *et al*., 1999; Abd El Tawab *et al*., 2016 and 2018 and Khattab *et al.*, 2019a). Giraldo *et al*. (2004) stated that a pre-ingestive enzyme-feed interaction induce a significant beneficial effect on ruminal digestion. The enzyme addition onto feeds may create a stable enzyme-feed complex that protects free enzymes from proteolysis in the rumen as reported by Kung *et al*. (2000) and Khattab *et al.* (2019b). So, our study aimed to investigate the effect of replacing green maize (darawa) with crude olive cake or treated silage with or without fibrolytic enzymes; on nutrients digestibility, rumen fermentation, pH, ammonia nitrogen and gas production.

MATERIALS AND METHODS

Olive cake preparation:

Fresh olive cake (*Olea europaea*) obtained from newly extracted olive seeds for oil, at Al Salhiya Agricultural Company, Al Sharqia, Egypt. Treated olive cake silage was prepared by mixing four liters of enzyme solution per each ton dry matter of fresh olive cake. Untreated and treated olive cake ensiled in plastic bags for two months before analysis.

Enzyme sources:

Production of fibrolytic enzymes carried out at dairy science department, National Research Centre, Dokki, Giza, from anaerobic bacteria (*Clostridium butyricum*) according to Khattab *et al.* (2017). Each gram of the used enzymes contains 5000 IU/g of cellulase.

Experimental diets and In-vitro incubation:

In vitro incubation was carried out according to Menke and Steingass (1988) as described by Khattab *et al.* (2016). The basal diet consisted of 50% Concentrate: 50% darawa as roughage; the experimental diets were as follow: C: control group (50% concentrate: 50% darawa), G1: replacing 25% of darawa with fresh olive cake, G2: replacing 25% of darawa with untreated olive cake silage, G3: replacing 25% of darawa with treated olive cake silage, G4: replacing 50% of darawa with fresh olive cake, G5: replacing 50% of darawa with untreated olive cake silage, G6: replacing 50% of darawa with treated olive cake silage, G7: replacing 75% of darawa with fresh olive cake, G8: replacing 75% of darawa with untreated olive cake silage, G9: replacing 75% of darawa with treated olive cake silage. The chemical composition of feed ingredients and experimental diets were presented in Table (1). Rumen fluid was collected before morning feeding from Ossemi sheep. The collected rumen fluid was mixed and squeezed through 4 layers cheesecloth under continuous flushing with CO_2 and immediately transported to laboratory at 39°C where it was used as a source of inoculum. Each treatment was tested in eight replicates accompanied by blank vessels (no substrate). 400 mg of milled substrate was added to the incubation vessels of 100mL capacity. Each vessel was filled with 40 mL of the incubation medium (292 mg K_2HPO_4, 240 mg KH_2PO_4, 480 mg $(NH_4)_2SO_4$, 480 mg NaCl, 100 mg $MgSO_4.7H_2O$, 64 mg $CaCl_2.2H_2O$, 4 mg Na_2CO_3 and 600 mg cysteine hydrochloride) per 1 liter of double distilled water (ddH_2O) and dispensed anaerobically in the 1:4 (v/v) ratio. Then the treatments were incubated at 39°C for 48h.

Table (1): Chemical composition of feed ingredients (% on DM basis).

Items	DM	OM	CP	EE	NDF	ADF	Hemicellulose	Ash	pH	NH3-N (mM)
CFM	93.09	93.98	12.73	6.28	49.98	18.09	31.9	6.02	---	---
Darawa	96.92	86.95	7.45	6.25	64.51	31.64	32.87	13.05	---	---
Fresh 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	16.20	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
*Chemical composition of experimental diets (on DM basis %).										
C	95.00	90.46	10.09	6.27	57.25	24.87	32.39	9.54	---	---
G1	89.79	91.43	9.93	7.08	57.29	27.83	29.46	8.57	---	---
G2	84.58	92.40	9.77	7.89	57.33	30.8	26.54	7.6	---	---
G3	79.37	93.37	9.61	8.71	57.37	33.76	23.62	6.63	---	---
G4	88.52	91.77	9.81	7.33	58.06	27.77	30.3	8.23	---	---
G5	82.04	93.07	9.52	8.40	58.88	30.67	28.22	6.93	---	---
G6	75.56	94.37	9.23	9.47	59.69	33.58	26.13	5.63	---	---
G7	88.68	91.69	9.83	7.03	57.54	28.23	29.31	8.31	---	---
G8	82.36	92.92	9.57	7.79	57.83	31.59	26.24	7.08	---	---
G9	76.04	94.15	9.32	8.55	58.12	34.95	23.17	5.85	---	---

**C: control group (50% concentrate: 50% darawa), G1: replacing 25% of darawa with fresh olive cake, G2: replacing 25% of darawa with untreated olive cake silage, G3: replacing 25% of darawa with treated olive cake silage, G4: replacing 50% of darawa with fresh olive cake, G5: replacing 50% of darawa with untreated olive cake silage, G6: replacing 50% of darawa with treated olive cake silage, G7: replacing 75% of darawa with fresh olive cake, G8: replacing 75% of darawa with untreated olive cake silage, G9: replacing 75% of darawa with treated olive cake silage.*

After 48 h digestion, gas production (GP) was recorded using the pressure reading technique, bottles were uncapped, pH was measured using a pH meter, and the contents of each bottle were filtered to obtain the non-fermented residue for determination of degraded substrate. The samples were transferred into test tubes and centrifuge for 1h in order to obtain the residues and placed for drying at 65^{o}C for 24 h. The dry residues were weighed and digestibility calculated using the equation as follows:

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

Samples analysis:

Samples of fermented fluid were analyzed for pH and NH_3-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 NH_3-N concentration was determined as described by Khattab and Abd El Tawab (2018). Neutral Detergent Fiber (NDF) and Acid Detergent Fiber (ADF) were analyzed by Ankom200 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 treatment:

Chemical compositions of fresh olive cake, untreated and treated olive cake silage are presented in Table (1). Treated olive cake silage showed lowest values of neutral detergent fiber, hemicellulose, pH and NH_3-N. The pH value decreased in olive cake silage due to the production of lactic acid, acetic acid and VFAs' during fermentation (McDonald *et al.*, 1991), this results were in agreement with those found by Rowghani *et al*., (2008) they noted that NDF content of olive cake silage was decreased after 60 days of preservation. Treating olive cake silage with fibrolytic enzymes enhance the digestion of cellulose and hemicelluloses in olive seeds cell wall resulting in improving the release of soluble sugars which could be fermented by lactic acid bacteria inducing reduction of pH, increasing lactic acid content and improving the lactic acid : acetic acid ratio which reduce DM losses (Yitbarek, and Tamir, 2014). It is worth mention that, NH_3-N in ensiling olive cake was less than 5% of the total N which indicating good fermentation quality of silage (Chamberlain and Wilkinson, 2000). Low pH level during the fermentation period decreased proteins deamination and degradation which reduced NH_3-N production (Yitbarek, and Tamir, 2014).

Gas production:

Data presented in Table (2) showed that total gas production (TGP) increased significantly (157.5, 156.0 and 154.5 ml) in G1, G2, and G3, respectively. On the other hand, it decreased in other groups; the lowest values (129.5 and 123.5 ml) were sighted in G8 followed by G9, which both contained treated olive cake silage as replacement of darawa by 75 %, using high level of olive cake tend to decrease gas production due to high content olive cake from EE especially unsaturated fatty acids which lead to increase biohydrogenation .Data also, showed that gas production per each gram of DM, NDF and ADF took the same trend, where, the highest values found in treatments that contained 25 % olive cake (fresh, untreated or treated silage), but, the lowest values obtained in treatments which contained 75% untreated or treated olive cake silage. These results may be related to the high levels of tannins and lignin (anti-nutritional factors) in olive cake which affect on microbial proliferation in rumen (Al-Masri and Guenther, 1995; Abd El Tawab *et al*., 2018 and Abd El Tawab & Khattab, 2018). Akinfemi *et al.* (2009) suggested that gas production from protein fermentation is relatively small as compared to carbohydrate fermentation. Data also showed that, the highest level of gas production per each gram of hemicelluloses stated in G4 and G7 which contain 50 and 75% fresh olive cake, respectively. But, the control (C) showed the lowest level (1112.0). Ruminal degradability is a good indicator to estimate nutritive quality of different grass species (Murillo *et al.,* 2003 and Jančíc, 2010). Also, the determination of gas production *In vitro* provides information on fermentation kinetics of forage consumed by ruminants.

Table (2): Effect of experimental diets on gas production.

Item	Treatment*									
	C	G1	G2	G3	G4	G5	G6	G7	G8	G9
TGP	144.5[c]	157.5[a]	156.0[a]	154.5[ab]	152.5[b]	153.5[ab]	131.0[d]	137.0[d]	129.5[d]	123.5[e]
GP/g DM	360.2[c]	388.0[ab]	390.2[a]	386.4[ab]	376.1[b]	379.0[ab]	326.2[d]	328.7[d]	326.6[d]	306.6[e]
GP/g NDF	629.1[d]	677.2[a]	672.1[ab]	671.5[ab]	656.0[bc]	643.7[cd]	564.0[ef]	573.0[e]	547.1[f]	527.6[g]
GP/g ADF	1448.2[a]	1394.1[b]	1405.1[b]	1368.7[b]	1221.0[c]	1235.9[c]	1032.5[d]	973.8[e]	972.5[e]	877.4[f]
GP/g Hemicellulose	1112.0[f]	1316.9[bc]	1287.8[cd]	1318.2[bc]	1417.0[a]	1343.2[b]	1243.0[e]	1391.8[a]	1249.8[de]	1323.5[bc]
GP/ gm dDM	690.8[bc]	632.5[c]	632.1[c]	642.9[c]	671.4[bc]	686.1b[c]	723.6[ab]	767.2[a]	743.7[ab]	797.6[a]
GP/ gm dNDF	1784.0[b]	2473.8[a]	2457.5[a]	2384.8[a]	2049.7[b]	1988.7[b]	1314.7[c]	1415.1[c]	1312.8[c]	1091.0[c]
GP/ gm dADF	7079.4[a]	7543.9[a]	7422.7[a]	7559.4[a]	5379.6[b]	5370.3[b]	3592.8[c]	3440.9[c]	3301.6[c]	2587.3[c]

* *Treatments: C: control group (50% concentrate: 50% darawa), G1: replacing 25% of darawa with fresh olive cake, G2: replacing 25% of darawa with untreated olive cake silage, G3: replacing 25% of darawa with treated olive cake silage, G4: replacing 50% of darawa with fresh olive cake, G5: replacing 50% of darawa with untreated olive cake silage, G6: replacing 50% of darawa with treated olive cake silage, G7: replacing 75% of darawa with fresh olive cake, G8: replacing 75% of darawa with untreated olive cake silage, G9: replacing 75% of darawa with treated olive cake silage.*

The *In vitro* gas production almost used to estimate the nutritive quality of different classes of forages (Njidda, 2010). Gas production per each gram of digested dry matter recorded the highest levels when replacing 75% of darawa with fresh olive cake or treated olive cake silage; the values were 767.2 and 797.6 ml GP/ gm dDM for G7 and G9, respectively. But, the lowest levels (632.5, 632.1 and 642.9 ml GP/ gm dDM) found when replacing 25% of darawa with fresh, untreated and treated olive cake silage. In regard to gas production per each gram of digested NDF / ADF, recorded the same trend for total gas production, the highest values were at level 25% and lowest values at level75%.

Rumen pH and NH_3-N concentration:

It can be seen from the data presented in Table (3) that treated groups were differed significantly in pH level; the highest levels (7.09 and 7.07) were recorded for G9 and G6, respectively. But G3, G2, and G1 had the lowest pH levels (6.85, 6.88 and 6.88). Regarding to NH_3-N concentration (table 3), there were significant differences among the groups, where, G1 recorded the highest NH_3-N concentration (37.63 mM), but G8 showed the lowest NH_3-N concentration (29.65 mM), this may be due to increasing the synthesis of microbial protein by increasing the level of olive cake in the diet (Yáñez et al., 2004 and Abd El Tawab *et al*., 2018). This result in agreement with Park *et al.* (1994), who found that, ruminal NH_3-N concentrations decreased when diet CP content decreases. The ruminal NH_3-N concentrations in all groups were greater than the 5 mg/dl concentration suggested for microbial growth and more than the 1 to 2 mg/dl concentration proposed by Petersen (1987) as necessary for optimal degradation of fiber.

Table (3): Effect of experimental diets on pH value and Ammonia concentration.

Items	Treatments*									
	C	G1	G2	G3	G4	G5	G6	G7	G8	G9
pH	6.93^{b}	6.88^{bc}	6.88^{bc}	6.85^{c}	6.95^{b}	6.91b^{c}	7.07^{a}	7.06^{a}	7.02^{a}	7.09^{a}
NH3-N (mM)	31.90^{ab}	37.63^{a}	32.60^{ab}	31.60^{ab}	37.24^{a}	35.00^{ab}	35.51^{ab}	31.03^{ab}	29.65^{b}	34.04^{ab}

**Treatments: C: control group (50% concentrate: 50% darawa), G1: replacing 25% of darawa with fresh olive cake, G2: replacing 25% of darawa with untreated olive cake silage, G3: replacing 25% of darawa with treated olive cake silage, G4: replacing 50% of darawa with fresh olive cake, G5: replacing 50% of darawa with untreated olive cake silage, G6: replacing 50% of darawa with treated olive cake silage, G7: replacing 75% of darawa with fresh olive cake, G8: replacing 75% of darawa with untreated olive cake silage, G9: replacing 75% of darawa with treated olive cake silage.*

Nutrients digestibility:

The effect of replacing darawa with fresh olive cake, untreated or treated olive cake silage on nutrients digestibility were presented in Table (4). Dry matter digestibility was decreased gradually by increasing level of olive cake and recorded the lowest values at level 75%. These results may be attribute to high content of olive cake of unsaturated fatty acids which had negative effect on fibrolytic bacteria and protozoa. however, NDF and ADF digestibility showed the highest levels (48.40 and 34.00 %) when replacing 75% darawa with treated olive cake silage, (G9) compared with control and other treatments, these results due to that the ensiling – enzyme feed interaction increase the ruminal digestion, this results agreed with those found by Feng *et al.* (1996), Dong *et al*. (1999), Giraldo *et al*. (2004), Abd El Tawab *et al.* (2019) and Khattab *et al.* (2019b).

Table (4): Effect of experimental diets on nutrients digestibility.

Item	Treatment*									
	C	G1	G2	G3	G4	G5	G6	G7	G8	G9
DM digestibility	52.14^{c}	61.36^{a}	61.43^{a}	60.16^{ab}	56.03^{bc}	55.26^{bc}	45.14^{d}	43.55^{d}	44.43^{d}	38.52^{e}
NDF digestibility	35.29^{c}	27.69^{d}	27.81^{d}	28.74^{d}	32.13^{cd}	32.42^{cd}	43.02^{b}	41.22^{b}	42.46^{b}	48.40^{a}
ADF digestibility	20.46^{c}	18.75^{c}	19.07^{c}	18.91^{c}	22.88^{c}	23.08^{c}	28.88^{b}	28.61^{b}	30.18^{ab}	34.00^{a}

**Treatments: C: control group (50% concentrate: 50% darawa), G1: replacing 25% of darawa with fresh olive cake, G2: replacing 25% of darawa with untreated olive cake silage, G3: replacing 25% of darawa with treated olive cake silage, G4: replacing 50% of darawa with fresh olive cake, G5: replacing 50% of darawa with untreated olive cake silage, G6: replacing 50% of darawa with treated olive cake silage, G7: replacing 75% of darawa with fresh olive cake, G8: replacing 75% of darawa with untreated olive cake silage, G9: replacing 75% of darawa with treated olive cake silage.*

CONCLUSION

The obtained results showed that the treatment of olive cake with fiber degrading enzymes and ensiling contributed to increasing the efficiency of digesting the fibers of olive cake and increasing degrading cellulose and hemicellulose molecules. Gas production reached the highest levels when replacing 25% of the studied with crude olive and reached to the lowest values at 75%. The higher content of the unsaturated fatty acids in olive cake is used to reduce the production of gas which lead to more utilization from energy while overcoming the high content of olive fiber via the process of silage and enzymatic treatments .The results obtained can be used in the diets of lactating animals at level up to 75% of this studies.

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سيلاج تفل الزيتون كمادة علف خشنة للمجترات: تأثيرها علي الهضم وانتاج الغاز بالكرش

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أجريت الدراسة الحالية بغرض دراسة تأثير استبدال الدراوة بتفل الزيتون (الخام أو كسيلاج غير معامل أو المعامل بالإنزيمات المحللة للألياف) على هضم العناصر الغذائية وتخمرات الكرش و الأس الهيدروجيني و الأمونيا وإنتاج الغاز ، تم تكوين ٩ توليفات علفية باستبدال نسب مختلفة من الدراوة بتفل الزيتون كما يلى : C : المقارنة (٥٠% علف مركز + ٥٠% دراوة) ،G1 : (٥٠% علف مركز + ٢٥ % استبدال الدراوة بتفل زيتون الخام) ، G2 : (٥٠% علف مركز + ٢٥ % استبدال الدراوة بتفل زيتون فى صورة سيلاج غير المعامل بالإنزيم) ، G3 : (٥٠% علف مركز + ٢٥ % استبدال الدراوة بتفل زيتون فى صورة سيلاج معامل بالإنزيم) ، G4 : (٥٠% علف مركز + ٥٠% استبدال الدراوة بتفل زيتون الخام) ، G5 : (٥٠% علف مركز + ٥٠% استبدال الدراوة بتفل زيتون فى صورة سيلاج غير المعامل بالإنزيم) ، G6 : (٥٠% علف مركز + ٥٠% استبدال الدراوة بتفل زيتون فى صورة سيلاج معامل بالإنزيم) ، G7 : (٥٠% علف مركز + ٧٥% استبدال الدراوة بتفل زيتون الخام) ، G8 : (٥٠% علف مركز + ٧٥% استبدال الدراوة بتفل زيتون فى صورة سيلاج غير المعامل بالإنزيم) ، G9 : (٥٠% علف مركز + ٧٥% استبدال الدراوة بتفل زيتون معامل بالإنزيم). أظهرت النتائج المتحصل عليها أن معاملة تفل الزيتون بالإنزيمات المحللة للألياف قبل إجراء عملية السيلجة ساهم في رفع كفاءة هضم جدر خلايا بذور الزيتون و زيادة تكسير جزيئات السليلوز والهيميسيلولوز، مما نتج عنه زيادة تحرر السكريات القابلة للذوبان. كما وضح جلياً أن إنتاج الغاز وصل إلى أعلى المستويات عند استبدال ٢٥٪ من الدراوة بتفل الزيتون (الخام أو السيلاج الغير معامل أو المعامل بالإنزيمات المحللة للألياف)، بينما كان اقل ما يمكن عند مستوى ٧٥%. علاوة على ذلك، لوحظ ان أعلى تركيز للأمونيا (37.63 mM) كان عند استبدال ٢٥٪ من الدراوة بسيلاج تفل الزيتون غير المعامل بالإنزيم (G2). كما كان هناك إنخفاض تدريجى فى معاملات هضم المادة الغذائية بزيادة مستويات تفل الزيتون. من النتائج المتحصل عليها يمكن استخدام تفل الزيتون كسيلاج معامل بالانزيمات المحللة للالياف فى علائق الحيوانات الحلابة بنسب إحلال حتى ٧٥% من الدراوة بحيث يتم الاستفادة من إرتفاع محتواه من الأحماض الدهنية غير المشبعه فى تقليل إنتاج الغاز بالكرش مع التغلب على إرتفاع محتوى تفل الزيتون من الألياف بعملية السيلجة والمعاملات الإنزيمية.