

# ***In situ* degradability of dry matter, crude protein, acid and neutral detergent fiber of olive cake and greenhouse wastes of tomato and cucumber**

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## **SUMMARY**

Two agricultural by-products (crude olive cake, OC and a mix of greenhouse wastes of tomato and cucumber, GHW) were evaluated for ruminal dry matter (DM), crude protein (CP), acid detergent fiber (ADF) and neutral detergent fiber (NDF) degradation kinetics. Duplicate bags containing 3 g ground raw material each were incubated in the rumen of two ruminally cannulated Assaf rams for 4, 8, 16, 24, 48 and 72 h. Rate and extent of ruminal degradation were estimated. Significant effects of by-product type were observed in rapidly soluble and potentially degradable fractions, and degradation rates of DM, CP, ADF and NDF. The rapidly soluble DM, CP, ADF and NDF fractions were 16.19, 15.21, 10.64 and 14.06% for GHW, and 7.21, 6.64, 6.00 and 5.62% for OC. The potentially degradable DM, CP, ADF and NDF fractions, respectively, were 55.82, 62.30, 49.40 and 55.09% for GHW, and 47.61, 44.26, 46.34 and 48.53% for OC. The DM, CP, ADF and NDF disappearance in GHW were higher ( $P < 0.05$ ) compared to those in OC. Similarly, the effective degradability (ED) of DM, CP, ADF and NDF in GHW were higher compared to OC ( $P < 0.05$ ).

**Keywords:** sheep, olive cake, tomato and cucumber wastes, *in situ* degradability

## **RÉSUMÉ**

**Dégradabilité *in situ* de la matière sèche, des protéines brutes et des fibres de grignons d'olive et déchets de tomates et concombres**

Deux sous-produits agricoles (le grignon d'olive et un mélange de déchets de tomate et de concombre) ont été évalués en ce qui concerne la cinétique de dégradation ruminale de la matière sèche (DM), les protéines brutes (CP) et les fibres solubles acides (ADF) et neutres (NDF). Des sacs contenant 3 g de matière première chacun ont été incubés en double exemplaire dans le rumen de deux béliers Assaf fistulisés pendant 4, 8, 16, 24, 48 et 72 h. Le taux et le niveau de dégradation dans le rumen ont été estimés. Des différences significatives ( $P < 0,05$ ) entre sous-produits ont été observées. Les fractions rapidement soluble de DM, CP, ADF et NDF étaient respectivement de 16, 15, 10 et 14 % pour le mélange de déchets de tomate et concombres et 7, 7, 6 et 6 % pour le grignon d'olives. Les fractions potentiellement dégradables représentaient pour le DM, CP, ADF et NDF respectivement 56, 62, 49 et 55 % pour le mélange de déchets de tomate et concombres et 48, 44, 46 et 49 % pour le grignon d'olives.

**Mots-clés :** ovins, grignon d'olive, tomate, concombre, dégradation ruminale

## **Introduction**

The population in Palestine is growing at a high rate (2.9%, [21]). This population growth is accompanied by harsh economic conditions and long dry periods and simultaneous increase in the demand for food ingredients (*i.e.* meat, milk). This has already resulted in increased demand on ruminants' feed and then deterioration of natural grazing areas, in addition to rising prices of raw materials used in the manufacture of feed. It is therefore important to study the utilization of agricultural by-products as alternative feed ingredients for farm animals especially ruminants. The use of agro-industrial by-products as feed ingredients is practiced in different arid and semi arid areas of the world [9, 7, 2].

Olive cake (OC), tomato waste (TW), and cucumber waste (CW) are the most locally abundant agricultural by-products with amounts of 50, 500, and 500 thousand tons/year, respectively [21]. Tomato waste (TW) has been evaluated in rations for poultry [11], small ruminants [10, 8, 6] and dairy cows [28]. Similarly, OC has been used in poultry [3, 25], fattening lambs [1, 2]. Information about degradability

of nutrients, effective degradability (ED) values and digestion kinetics of agricultural wastes (*i.e.*, OC and green house wastes) is important for feed manufacturing industry and farmers performing feed mixing practices who currently rely on nutritive values published by research institutions. Unfortunately, the accuracy of ration formulation depends on the assumption that all wastes are represented by these limited published values. This study was undertaken to evaluate the nutritive value of OC and a mixture of green house wastes of tomato and cucumber (GHW) by means of chemical composition and ruminal degradability, fractional rates of digestion and effective degradability of DM, CP, ADF and NDF.

## **Materials and methods**

Initially, three 2-yr old Assaf rams were used in this study. Rams were fitted with rumen cannulas with a 4 cm internal diameter. One of the rams was later removed from the study due to health problems. A mixture of good quality hay and concentrate (60% concentrate and 40% hay) was offered to meet  $1.25 \times$  maintenance requirements. Hay contained 910,

90, 330, 450, 70 and 20 g/kg of DM, CP, ADF, NDF, ash, and fat, respectively. Chemical composition of the concentrate was 880, 160, 120, 340, 60, 30, 18 and 6 g/kg of DM, CP, ADF, NDF, ash, fat, Ca, and P, respectively. Concentrate contained vitamins A, D3 and E at levels of 50000, 700, 30 IU/kg, respectively.

The TW and CW were obtained from nearby greenhouses (mixtures of leaves, stems and some fruits) during summer after finishing a green house cultivation period, while OC was collected in November during the olive fruit pressing season. Raw ingredients (*i.e.* OC, TW, and CW) were dried and ground to pass through a 2 mm screen. Three-gram samples were weighed into nylon bags (8 cm × 16 cm) with 40–45 µ pore size. Equal amounts of TW and CW were used to form each sample of GHW mixture (2.5g TW, 2.5g CW). Prior to *in situ* degradability procedure, proximate analyses were performed on double samples (3 g) according to the Association of Official Analytical Chemists, AOAC [5]. Two samples from each ingredient (*i.e.* OC and GHW) were incubated in the rumen of each of the two remaining rams for 4, 8, 16, 24, 48 and 72 hr. After incubation, the bags were removed from the rumen and rinsed with cold tap water, until the rinsing water ran clear, then dried at 65 C for 48 h in an oven and weighed as described by Ørskov [18] and Janicki and Stallings [12]. Ruminal disappearance (*D*) at each incubation time was calculated as the difference between the residues and original samples. The amounts of DM, CP, ADF and NDF in the residues, expressed as percentages of original samples, were determined for each bag. Two observations from each ram were obtained for each raw material and occupation time. Ruminal kinetics parameters were estimated using PROC NLIN procedure of SAS, [24] fitting the exponential model of Ørskov and McDonald [20]:

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

Where *D* is rumen disappearance at time *t*, *a* is the rapidly soluble fraction, *b* is the potentially degradable (fermentable) fraction, and *c* the constant rate of degradation of *b* (percentage per hour).

Effective degradability (ED) of nutrient components was calculated applying the equation of Ørskov and Mc Donald [20]:

$$ED = a + [(bc)/(c+k)] \quad (2)$$

Where *a*, *b*, *c*, *t* are the same as defined in (1) and *k* is the rumen outflow rate of 2, 5 and 8% h<sup>-1</sup>.

The data obtained (degradation characteristics, effective degradability and disappearance rates) were subjected to statistical analyses using PROC MIXED procedure of SAS [24]. Because of the inherent differences between rams, each ram was considered as a block (randomized complete block design with two replicates per treatment). Treatment (type of by-product) was fitted as a fixed factor while block (animal) and block\*treatment were fitted as random factors according to the following model [13]:

$$y_{ijl} = \mu + \tau_i + \beta_j + \tau\beta_{ij} + \varepsilon_{ijl} \quad (3)$$

Where *y<sub>ijl</sub>* is observation *l* in treatment *i* and block *j*, *μ* is the overall mean, *τ<sub>i</sub>* is the effect of treatment *i*, *β<sub>j</sub>* is the effect of block *j*, *τβ<sub>ij</sub>* is the interaction effect of treatment *i* and block *j*, and *ε<sub>ijl</sub>* is random error with mean 0 and variance *σ*<sup>2</sup>.

## Results

Proximate analyses of OC and GHW are presented in Table 1. The rapidly soluble fraction 'a' of DM was higher (P<0.05) in GHW (16.19%) compared to OC (7.21%), Table 2. The insoluble but rumen degradable fraction of DM, 'b' was lower (P<0.05) in OC (47.61%) compared to that in TCW (55.82%). GHW had higher (P<0.05) amount of potentially degradable DM (a+b) than OC (72.01% and 54.82%). The same trends were found for CP, ADF, and NDF where GHW had consistently higher values (P < 0.05) of a, b, and a+b (Tables 3-5).

The effective degradability of DM, CP, ADF and NDF in OC and GHW are given in Tables 2- 5. The data have been calculated using rumen outflow rates of 3, 5 and 8 h<sup>-1</sup>. There were significant differences (P<0.05) between OC and GHW in effective degradability of DM, CP, ADF and NDF (ED of GHW was higher than that of OC for all tested nutrients).

Disappearance rates (%) of DM, CP, ADF and NDF for OC and GHW at different rumen incubation times are presented in Tables 2- 5. GHW had higher (P<0.05) disappearance rates of DM, CP, ADF and NDF at all incubation times. At 72 h of incubation, disappearance rates of all tested nutrients were consistently above 50% for GHW and below 50% for OC.

	GHW	OC
Dry matter	88.2	89.0
Crude protein	17.5	9.5
NDF	43	49
ADF	27	33
Ash	3.1	1.4

<sup>1</sup>these values are the means of two samples  
NDF = neutral detergent fiber; ADF = acid detergent fiber

TABLE I: Chemical composition<sup>1</sup> of olive cake (OC) and a mixture of greenhouse wastes of tomato and cucumber (GHW), % of DM

	GHW	OC	SEM <sup>6</sup>
<b>Degradation characteristics</b>			
a <sup>1</sup> %	16.1 <sup>a</sup>	7.2 <sup>b</sup>	0.10
b <sup>2</sup> %	55.8 <sup>a</sup>	47.6 <sup>b</sup>	0.27
c <sup>3</sup>	0.02 <sup>b</sup>	0.03 <sup>a</sup>	0.006
a+b <sup>4</sup> %	72.0 <sup>a</sup>	54.8 <sup>b</sup>	0.35
<b>Effective degradability (ED)<sup>5</sup></b>			
k=0.02	48.2 <sup>a</sup>	36.6 <sup>b</sup>	0.45
k=0.05	35.7 <sup>a</sup>	25.9 <sup>b</sup>	0.41
k=0.08	30.2 <sup>a</sup>	20.9 <sup>b</sup>	0.36
<b>Disappearance (%)</b>			
24hrs	47.3 <sup>a</sup>	34.9 <sup>b</sup>	0.52
48hrs	53.5 <sup>a</sup>	46.4 <sup>b</sup>	0.66
72hrs	65.3 <sup>a</sup>	49.2 <sup>b</sup>	0.40

GHW= green house waste; OC= olive cake

<sup>1,2,3,4</sup> constants in the equation  $D = a + b(1 - e^{-ct})$ , where P is level of degradation at time "t"; "a", readily soluble fraction; "b", insoluble fraction but degradable in rumen; "c", rate of degradation of "b" per hour; "a+b", potentially degradable fraction.

<sup>5</sup> ED: effective degradability calculated with outflow rates of 2, 5 and 8%.

<sup>6</sup> Standard error of the mean.

<sup>a,b</sup> Values within a row with different superscripts differ significantly at  $P < 0.05$ .

TABLE II: Degradation characteristics and disappearance of dry matter (DM) in olive cake (OC) and a mixture of greenhouse wastes of tomato and cucumber (GHW)

	GHW	OC	SEM <sup>6</sup>
<b>Degradation characteristics</b>			
a <sup>1</sup> %	15.2 <sup>a</sup>	6.6 <sup>b</sup>	0.14
b <sup>2</sup> %	62.3 <sup>a</sup>	44.2 <sup>b</sup>	0.41
c <sup>3</sup>	0.03 <sup>b</sup>	0.01 <sup>a</sup>	0.006
a+b <sup>4</sup> %	77.5 <sup>a</sup>	50.8 <sup>b</sup>	0.47
<b>Effective degradability (ED)<sup>5</sup></b>			
k=0.02	55.7 <sup>a</sup>	28.7 <sup>b</sup>	0.40
k=0.05	41.8 <sup>a</sup>	19.2 <sup>b</sup>	0.33
k=0.08	35.0 <sup>a</sup>	15.4 <sup>b</sup>	0.26
<b>Disappearance (%)</b>			
24hrs	55.7 <sup>a</sup>	25.6 <sup>b</sup>	0.63
48hrs	67.4 <sup>a</sup>	33.8 <sup>b</sup>	0.79
72hrs	72.7 <sup>a</sup>	40.3 <sup>b</sup>	0.43

GHW= green house waste; OC= olive cake

<sup>1,2,3,4</sup> constants in the equation  $D = a + b(1 - e^{-ct})$ , where P is level of degradation at time "t"; "a", readily soluble fraction; "b", insoluble fraction but degradable in rumen; "c", rate of degradation of "b" per hour; "a+b", potentially degradable fraction.

<sup>5</sup> ED: effective degradability calculated with outflow rates of 2, 5 and 8%.

<sup>6</sup> Standard error of the mean.

<sup>a,b</sup> Values within a row with different superscripts differ significantly at  $P < 0.05$ .

TABLE III: Degradation characteristics and disappearance of crude protein (CP) in olive cake (OC) and a mixture of greenhouse wastes of tomato and cucumber (GHW)

## Discussion

No statistical analysis of nutrient composition was conducted but the following trends were clear. OC had lower CP and higher ADF and NDF values compared to

GHW. These values for OC are comparable to previous reports [1, 2]. Alibes and Berge [4] and Ohlde and Becker [17] found that olive cake has higher NDF, ADF and lignin contents compared to GHW. However, a large proportion of the protein (80 to 90%) is linked to the ADF [16] and solubility of nitrogen is only 1.5 to 3% which is particularly

	GHW	OC	SEM <sup>6</sup>
<b>Degradation characteristics</b>			
a <sup>1</sup> %	10.6 <sup>a</sup>	6.0 <sup>b</sup>	0.17
b <sup>2</sup> %	49.4 <sup>a</sup>	46.3 <sup>b</sup>	0.44
c <sup>3</sup>	0.03 <sup>b</sup>	0.03 <sup>a</sup>	0.009
a+b <sup>4</sup> %	60.0 <sup>a</sup>	52.3 <sup>b</sup>	0.60
<b>Effective degradability (ED)<sup>5</sup></b>			
k=0.02	40.6 <sup>a</sup>	34.5 <sup>b</sup>	0.32
k=0.05	29.5 <sup>a</sup>	24.0 <sup>b</sup>	0.20
k=0.08	24.4 <sup>a</sup>	19.2 <sup>b</sup>	0.17
<b>Disappearance (%)</b>			
24hrs	40.3 <sup>a</sup>	32.8 <sup>b</sup>	0.55
48hrs	47.8 <sup>a</sup>	42.4 <sup>b</sup>	0.36
72hrs	54.9 <sup>a</sup>	47.3 <sup>b</sup>	0.41

GHW= green house waste; OC= olive cake

<sup>1,2,3,4</sup> constants in the equation  $D = a + b(1 - e^{-ct})$ , where P is level of degradation at time "t"; "a", readily soluble fraction; "b", insoluble fraction but degradable in rumen; "c", rate of degradation of "b" per hour; "a+b", potentially degradable fraction.

<sup>5</sup> ED: effective degradability calculated with outflow rates of 2, 5 and 8%.

<sup>6</sup> Standard error of the mean.

<sup>a,b</sup> Values within a row with different superscripts differ significantly at  $P < 0.05$ .

TABLE IV: Degradation characteristics and disappearance of acid detergent fiber (ADF) in olive cake (OC) and a mixture of greenhouse wastes of tomato and cucumber (GHW)

	GHW	OC	SEM <sup>6</sup>
<b>Degradation characteristics</b>			
a <sup>1</sup> %	14.0 <sup>a</sup>	5.6 <sup>b</sup>	0.21
b <sup>2</sup> %	55.0 <sup>a</sup>	48.5 <sup>b</sup>	0.63
c <sup>3</sup>	0.02 <sup>b</sup>	0.02 <sup>a</sup>	0.001
a+b <sup>4</sup> %	69.1 <sup>a</sup>	54.3 <sup>b</sup>	0.50
<b>Effective degradability (ED)<sup>5</sup></b>			
k=0.02	41.8 <sup>a</sup>	34.5 <sup>b</sup>	0.29
k=0.05	30.0 <sup>a</sup>	23.8 <sup>b</sup>	0.48
k=0.08	25.2 <sup>a</sup>	18.6 <sup>b</sup>	0.23
<b>Disappearance (%)</b>			
24hrs	40.7 <sup>a</sup>	32.9 <sup>b</sup>	0.42
48hrs	45.2 <sup>a</sup>	41.9 <sup>b</sup>	0.74
72hrs	57.9 <sup>a</sup>	48.1 <sup>b</sup>	0.57

GHW= green house waste; OC= olive cake

<sup>1,2,3,4</sup> constants in the equation  $D = a + b(1 - e^{-ct})$ , where P is level of degradation at time "t"; "a", readily soluble fraction; "b", insoluble fraction but degradable in rumen; "c", rate of degradation of "b" per hour; "a+b", potentially degradable fraction.

<sup>5</sup> ED: effective degradability calculated with outflow rates of 2, 5 and 8%.

<sup>6</sup> Standard error of the mean.

<sup>a,b</sup> Values within a row with different superscripts differ significantly at  $P < 0.05$ .

TABLE V: Degradable characteristics and disappearance of neutral detergent fiber (NDF) in olive cake (OC) and a mixture of greenhouse wastes of tomato and cucumber (GHW)

low [23]. Ventura *et al.* [26] showed similar NDF values of TW as values in the present research; however, the reported values for CP, ADF and ash were different. Factors such as plant maturity, cultivation management and type of soil and fertility could affect feed ingredients chemical composition.

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 kinetics of digestion of feed nutrients. *In situ* coefficients were used to develop a system to predict feed nutritive value [19]. The high readily fermented fractions (a) of DM, CP, ADF and NDF in GHW

compared to those in OC could be due to the high OC levels of ADF [2] and the non fibrous carbohydrates (*i.e.* nitrogen free extract, NFE) in GHW [15].

According to Nefzaoui [16], since olive cake is rich in ADF, it has a low degradability. The potentially degradable fractions (b) of all tested nutrients (*i.e.* DM, CP, ADF, NDF) in GHW were higher than that of OC. The high OC level of NDF [2] and low non fibrous carbohydrates [15] could explain this result.

The GHW had higher degradation rates compared to OC. The nutrient composition of GHW of high CP, NDF and lower ADF levels may explain the higher degradation rates observed. It was shown by previous research that degradation rates were different among types of roughage [27]. Effective degradability (ED) of DM, CP, ADF and NDF calculated at 2, 5 and 8% h<sup>-1</sup> outflow rates from the rumen showed that GHW had consistently higher values compared to that in OC. Effective DM and CP degradability decreased with increase in outflow rates. Yan and Agnew [29] showed that ED of DM was negatively related to NDF and ADF concentrations.

The disappearance of the tested nutrients in OC and GHW by the end of 48 hrs of incubation indicated that 48h disappearance allows a comparison or a classification of feedstuffs in vivo digestibility without reflecting exact values of in vivo digestibility. This information provides an insight into the level of rumen undegradable DM post incubation for 72 h.

Many experiments have shown that non-structural carbohydrates from some feed ingredients (cassava, barley and corn) have a positive effect on fiber digestion as fiber digestibility is increased using these carbohydrate sources [27].

## Conclusions

In this study, we attempted to develop a comparative data set of *in situ* DM, CP, ADF and NDF degradation parameters for some agricultural by-products which are used at narrow scale but have the potential to be used for ruminant nutrition in Palestine. Extensive differences in ruminal degradation kinetics of DM, CP, ADF and NDF were determined between OC and GHW. The new data presented in this study could be useful for the purposes of ration formulation and ruminants' performance. Considering these findings, one can propose using both by-products, together or separately as part of ruminant rations. It can be introduced in these rations as part of roughage (wheat and barley straw). GHW can be incorporated in ruminant rations as part of better quality roughage (legume hay). However, lower incorporation levels to replace corn or barley can be practiced

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