



## Evaluation of the nutritional value of Iranian melon (*Cucumis melo* cv. Khatooni) wastes before and after ensiling in sheep feeding

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**Abstract** There is little information on the nutritive value of ensiled Iranian melon (*Cucumis melo* cv. Khatooni) wastes (IMW) for sheep feeding; therefore, the nutritional value of IMW was investigated before and after ensiling. Treatments were 1) Fresh IMW as control [FIMW], 2) Ensiled IMW [EIMW], 3) Ensiled IMW with 1% grape vinegar [EIMW+1% GV], and 4) Ensiled IMW with 2% grape vinegar [EIMW+2% GV]. The IMW samples (after harvesting the ripe fruits) were ensiled in the polyethylene tubes for 60 days. Ensiling IMW increased the contents of dry matter (DM) and crude protein (CP). The concentration of CP was also increased ( $P<0.05$ ) in the silages supplemented with GV. However, neutral and acid detergent fiber (NDF and ADF, respectively) and ash contents were unchanged among treatments. The addition of grape vinegar to the silage increased the concentrations of lactic and acetic acids and decreased  $\text{NH}_3\text{-N}$  compared to control silage ( $P<0.05$ ). However, total volatile fatty acids (TVFA) and pH of silages were not significantly affected by the treatments. The highest values of gas production parameters ( $\text{gas}_{12, 24, 48, 72 \text{ h}}$  and  $\text{b}_{\text{gas}}$ ), true dry matter (TDMD) and organic matter degradability (TOMD) were observed in FIMW ( $P<0.05$ ). Ensiling IMW without additive, decreased TDMD and TOMD, whereas treating silages with GV increased its digestibility ( $P<0.05$ ). The highest partitioning factor (PF) and efficiency of microbial mass yield (EMMY) were detected in EIMW+2% GV ( $P<0.05$ ). Generally, it can be concluded that IMW can be used as a potential forage source in sheep feeding either as fresh or ensiled by-product. However, because of the high moisture content of IMW, ensiling with 2% GV could improve the nutritional value and fermentation parameters.

**Keywords:** nutritional value, Iranian melon wastes, gas production, silage

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### Introduction

With increasing global population, the demand for livestock products is also increasing. On the other hand, the shortage of forage resources for feeding livestock is a major constraint in developing countries. Therefore, providing adequate good quality feed to meet the nutrient requirements of ruminants is a big challenge to agricultural scientists (Kim et al., 2019). Using unconventional resources as a feed for the animal will maintain

their productivity and encourage feeding the millions and food security (Makkar, 2004). Agricultural by-products are defined as the residues from the growing and processing of agricultural products (Foster, 2015) which can be used in the dairy industry. Melon wastes are the residues that may contain materials which can be beneficial and economical in ruminant nutrition. Melon (*Cucumis melo* L.) is an important horticultural crop,

commonly cultivated in arid and semi-arid regions with a global production of 31.9 million tons annually (FAO, 2017). Iran with 1.59 million tons of melon annually ranks third in the world. Melon peels are rich in nutritional ingredients such as carbohydrates (69.77%) and ash (3.67%), and contain significant amounts of total dietary fibers (41.69%) and antioxidants such as polyphenols and flavonoids (Mallek-Ayadi et al., 2017). Due to abundant production of melon (*Cucumis melo*), this fruit has been used as an alternative feed source for sheep (Lima et al., 2012), and for supplementing ruminants diets during feed shortage (Lima et al., 2012; Oliveira et al., 2015). Sheep that received feed consisting of 75% melon exhibited a sharp drop in rumen pH, an increase in lactic acid concentration, rumen flora disorders, and a temporary increase in rumen osmolarity (Oliveira et al., 2016). Agro-industrial by-products are usually poor in nutrients and low in digestibility, thus applying different approaches to increase digestibility may result in improving growth or milk yield of ruminants and also reduced the feed costs. Preservation by ensiling increases the shelf life of crops and agro by-products for use as animal feed. As little information is available on the nutritive value of IMW, the present study was conducted to determine the *in vitro* fermentation characteristics and nutritional value of IMW (*Cucumis melo* cv. Khatooni) before and after ensiling. Drying IMW for use in livestock feeding is also a choice, but the high cost involved makes this uneconomical. We also tested the effect of GV as an additive on some fermentation parameters in the silage.

## **Materials and methods**

### *Sample preparation and ensiling*

The Iranian melon wastes (IMW, *Cucumis melo* cv. Khatooni) were collected just after fruit harvesting in early autumn 2018 from the educational greenhouse, University of Torbat-e Jam, Iran. The IMW, consisting of the whole plant and some ripe or unripe fruits, were chopped (1.5 cm) with a laboratory chopper. Four representative samples of fresh-chopped IMW were frozen for analysis. Samples of IMW (3 kg on fresh basis) were ensiled in fixed-volume polyethylene tubes equipped with a drain valve and preserved for 60 days. Treatments were as follows: 1) Fresh IMW as control [FIMW], 2) Ensiled IMW without additive [EIMW], 3) Ensiled IMW with 1% grape vinegar [EIMW+1% GV], and 4) Ensiled IMW with 2% grape vinegar [EIMW+2% GV].

### *In vitro procedures*

The *in vitro* gas production was measured according to

Menke and Steingass (1988). Rumen fluid was collected from three Baluchi male sheep (30±3.5 kg) fed a diet containing alfalfa and concentrate (60:40). Rumen fluid was strained through four layers of cheesecloth and flushed with CO<sub>2</sub>. About 200 mg of each treatment (1 mm screen) were transferred into 100 mL calibrated glass syringes. The amount of 30 mL of rumen-buffer mixture (1:2, v/v) was added to the pre-warmed (39°C) syringes containing the sample. All syringes were put in a water bath maintained at 39°C. Incubation was carried out in eight replicates. Gas production volume was recorded at 3, 6, 9, 12, 24, 48, 72 and 96 h (Menke and Steingass, 1988). The pH of the culture medium was measured using a pH meter (Metrohm 691) immediately after 24 h incubation. Then, the contents of four syringes from each treatment were filtered through four layers of cheesecloth. After that, 5 mL of 0.2N HCl was equally mixed with 5 mL of filtered rumen fluid for the determination of NH<sub>3</sub>-N. A subsample (5 mL) of filtered rumen fluid was mixed with 1 mL of 25% meta-phosphoric acid and then preserved at -20°C for volatile fatty acid (VFA) analysis. Total VFA (TVFA) and NH<sub>3</sub>-N production were determined according to Barnett and Reid (1957) and Komolong et al. (2001), respectively. The partitioning factor (PF), microbial mass yield (MMY), true organic matter digestibility (TOMD) and true dry matter digestibility (TDMD) were determined by the procedures described by Makkar (2010) and Vercoe et al. (2010). The partitioning factor is defined as the ratio of organic matter degraded *in vitro* (mg) to the volume of gas (mL) produced (Makkar et al., 2010). Lactic and acetic acids in the silage were measured according to Koc and Coskuntuna (2003). The method of Dubois et al. (1956) was used to measure the water-soluble carbohydrates (WSC). The efficiency of microbial mass yield (EMMY) was calculated as MMY divided by TOMD. The pH of silage was measured with a pH meter according to Keles and Demirici's (2011) method. The dry matter (DM), acid detergent fiber (ADF), crude protein (CP), crude fat (CF) and ash contents were determined according to AOAC (2005). Neutral detergent fiber (NDF) was determined according to the procedure of Van Soest et al. (1991).

### *Statistical analysis*

The data for gas production were analyzed according to the model of Ørskov and McDonald (1979):

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

in which, Y= cumulative gas production at the time of "t" (mL), b was potential gas production (mL), c was constant rate of gas production (mL/h) and "t" was the

time of incubation (h). The data were finally analyzed using the GLM procedure of SAS (2002) in a completely randomized design. The differences between treatment means were compared using the Tukey's test ( $P < 0.05$ ).

## Results

Dry matter and crude protein contents were increased by ensiling IMW with grape vinegar ( $P < 0.05$ ) (Table 1). The ADF, NDF, ash, and CF contents did not differ between treatments. Total volatile fatty acids, pH and WSC of the silages were not affected by treatments, but the concentrations of organic acids (acetic and lactic acids) increased and  $\text{NH}_3\text{-N}$  levels decreased in grape vinegar supplemented silages (Table 2).

Although TVFA in the culture medium was significantly reduced in the presence of 1% grape vinegar; pH,  $\text{NH}_3\text{-N}$  and MMY values were not affected ( $P > 0.05$ ) by

the treatments (Table 3). True DM and OM digestibilities were significantly decreased by ensiling and grape vinegar addition to the silages; however, PF and efficiency of MMY were lower in fresh IMW compared to other treatments. The addition of grape vinegar increased TDMD and TOMD compared to IMW silage without vinegar.

Except for the constant rate of gas production ( $c_{\text{gas}}$ ), other gas production parameters ( $\text{gas}_{12, 24, 48, 72 \text{ h}}$ ) were decreased by ensiling or supplementing the silages with vinegar (Table 4).

## Discussion

Similar to our results, previous studies have shown a slight increase of 2 percent in DM content which could be attributed to existing of a drain valve in the silo (Der Bedrosian et al., 2012; Young et al., 2012). However, Herrmann et al. (2011) observed that the DM content of whole plant maize silage was reduced after ensiling in

**Table 1.** Dry matter and chemical composition (%) of fresh and ensiled Iranian melon wastes (IMW)

Treatment	DM	NDF	ADF	CP	CF	Ash
FIMW	14.04 <sup>b</sup>	22.58	17.67	7.41 <sup>b</sup>	1.90	26.48
EIMW	15.14 <sup>ab</sup>	21.17	17.33	7.79 <sup>b</sup>	1.79	26.70
EIMW+1% GV	15.91 <sup>a</sup>	21.54	16.98	8.44 <sup>a</sup>	1.87	27.41
EIMW+2% GV	15.57 <sup>a</sup>	21.00	17.03	8.45 <sup>a</sup>	1.81	27.73
SEM	0.28	0.61	0.40	0.11	0.06	0.32

Within columns, means with common superscript (s) do not differ significantly ( $P > 0.05$ ).

DM: Dry matter; NDF: Neutral detergent fiber; ADF: Acid detergent fiber; CP: Crude protein; CF: Crude fat; FIMW: Fresh IMW; EIMW: Ensiled IMW; EIMW+1% GV: Ensiled IMW supplemented with 1% grape vinegar; EIMW+2% GV: Ensiled IMW supplemented with 2% grape vinegar.

**Table 2.** Fermentation parameters of Iranian melon waste (IMW) silages

Treatment	WSC	AA	LA	pH	$\text{NH}_3\text{-N}$	TVFA
EIMW	1.63	0.13 <sup>b</sup>	1.23 <sup>b</sup>	6.01	5.26 <sup>a</sup>	16.33
EIMW+1% GV	2.22	0.24 <sup>a</sup>	2.33 <sup>a</sup>	5.88	3.74 <sup>b</sup>	16.00
EIMW+2% GV	1.96	0.28 <sup>a</sup>	2.88 <sup>a</sup>	5.78	3.73 <sup>b</sup>	15.25
SEM	0.20	0.03	0.14	0.10	0.19	0.42

Within columns, means with common superscript (s) do not differ significantly ( $P > 0.05$ ).

WSC (%): Water soluble carbohydrate; AA (%): Acetic acid; LA (%): Lactic acid;  $\text{NH}_3\text{-N}$  (mg/dL): Ammonia nitrogen; TVFA (mmol/L): Total volatile fatty acids; EIMW: Ensiled IMW; EIMW+1% GV: Ensiled IMW supplemented with 1% grape vinegar; EIMW+2% GV: Ensiled IMW supplemented with 2% grape vinegar.

**Table 3.** *In vitro* parameters measured from fresh and ensiled Iranian melon wastes (IMW) incubated in a batch culture medium

Treatment	pH	$\text{NH}_3\text{-N}$	TVFA	TDMD	TOMD	PF	MMY	EMMY
FIMW	7.05	23.17	19.45 <sup>a</sup>	83.78 <sup>a</sup>	87.42 <sup>a</sup>	7.52 <sup>b</sup>	107.74	70.55 <sup>b</sup>
EIMW	7.07	24.83	17.87 <sup>ab</sup>	72.78 <sup>c</sup>	74.25 <sup>d</sup>	8.89 <sup>ab</sup>	106.43	75.07 <sup>ab</sup>
EIMW+1% GV	7.04	24.67	17.67 <sup>b</sup>	76.42 <sup>b</sup>	78.15 <sup>c</sup>	8.49 <sup>b</sup>	103.60	74.10 <sup>ab</sup>
EIMW+2% GV	6.99	25.17	18.10 <sup>ab</sup>	77.85 <sup>b</sup>	81.80 <sup>b</sup>	10.44 <sup>a</sup>	111.31	78.86 <sup>a</sup>
SEM	0.03	0.60	0.38	0.61	0.63	0.40	1.82	1.22

Within columns, means with common superscript (s) do not differ significantly ( $P > 0.05$ ).

TVFA: Total volatile fatty acids (mmol/L);  $\text{NH}_3\text{-N}$  (mg/dL): Ammonia nitrogen; TDMD (%): True dry matter digestibility; TOMD (%): True organic matter digestibility; PF (mg OM truly degraded/mL gas produced in 24h): Partitioning factor; MMY (%): Microbial mass yield; EMMY: Efficiency of microbial mass yield; FIMW: Fresh IMW; EIMW: Ensiled IMW; EIMW+1% GV: Ensiled IMW supplemented with 1% grape vinegar; EIMW+2% GV: Ensiled IMW supplemented with 2% grape vinegar.

**Table 4.** Gas production parameters of fresh and ensiled Iranian melon wastes (IMW) following incubation

Treatment	Gas <sub>12 h</sub>	Gas <sub>24 h</sub>	Gas <sub>48 h</sub>	Gas <sub>72 h</sub>	b <sub>gas</sub>	c <sub>gas</sub>
FIMW	14.70 <sup>a</sup>	20.43 <sup>a</sup>	26.22 <sup>a</sup>	28.73 <sup>a</sup>	28.47 <sup>a</sup>	0.066
EIMW	11.67 <sup>b</sup>	16.07 <sup>b</sup>	20.38 <sup>b</sup>	23.33 <sup>b</sup>	23.23 <sup>b</sup>	0.067
EIMW+1% GV	11.29 <sup>b</sup>	16.46 <sup>b</sup>	19.68 <sup>b</sup>	24.26 <sup>b</sup>	23.34 <sup>b</sup>	0.065
EIMW+2% GV	10.42 <sup>b</sup>	13.56 <sup>b</sup>	18.41 <sup>b</sup>	21.49 <sup>b</sup>	21.35 <sup>b</sup>	0.061
SEM	0.55	0.81	1.08	1.01	1.06	0.006

Within columns, means with common superscript (s) do not differ significantly ( $P>0.05$ ).

Gas<sub>12, 24, 48, 72 h</sub> (mL): cumulative gas production after 12, 24, 48 and 72 h incubation; b<sub>gas</sub> (mL): potential gas production; c<sub>gas</sub> (mL/h): constant rate of gas production; FIMW: Fresh IMW; EIMW: Ensiled IMW; EIMW+1% GV: Ensiled IMW supplemented with 1% grape vinegar; EIMW+2% GV: Ensiled IMW supplemented with 2% grape vinegar.

laboratory glass silos. One of the critical features of a crop to be ensiled is the appropriate level of DM, however, in spite of relatively high moisture in IMW (i.e., 84.1-86.0%), the quality of the silage (color, odor and fermentation parameters) were acceptable. It has been reported that silage additives reduce DM losses and enhance fermentation (Henderson, 1993). About 50% of the DM degradability was due to NDF loss, 40% due to WSC loss, and the rest to CP (Henderson, 1993). However, as a proportion of the initial content before ensiling, WSC loss differed from 0.49 to 0.72, while losses of DM, NDF, ADF, and CP differed from 0.08 to 0.12. The WSC was initially degraded during ensiling, while fractions of NDF, ADF or CP were digested with a similar rate (Sun et al., 2009). Although not directly fermented by lactic acid bacteria, the fibrous fraction of silages decreases as a result of fiber solubilization (Der Bedrosian et al., 2012). Bakshi and Wadhwa, (2013) reported that melon peels contained 14.9% ash, 9.5% CP, 5.78% ether extract, 59.3% NDF, and 35.7% ADF. In another research, the amounts of DM, OM, CP, NDF and ADF in melon by-products were 7.9, 9.09, 9.9, 27.4, and 23.9%, respectively (Dolores Megias et al., 2002).

The efficiency of rumen microbial protein synthesis is an essential parameter in protein evaluation systems for livestock, and it may differ considerably among diets (Firkins, 1996; Dijkstra et al., 1998). Microbial efficiency mainly depends on feed intake, rumen fluid, pH, availability of preformed monomers and protozoal activity (Dijkstra et al., 2005). Partitioning of the degraded substrate between microbial biomass, VFA and gas reflects this efficiency. A low microbial efficiency per unit substrate degraded implies that a high amount of degraded substrate is directed towards the formation of VFA and gases; thus high b<sub>gas</sub> and TVFA in FIMW may be reflected in this effect.

Approaches that can reduce the lignin content or break down the linkages to liberate the potentially degradable carbohydrates for rumen microbes should be used to improve the product degradability. Chemical additives such as acids or alkali have been shown to in-

crease the digestibility of maize stover (Hendriks and Zeeman, 2009) which was also found in the present work. Increasing forage digestibility will generally decrease greenhouse gas emissions from rumen fermentation and stored manure as well (Hristov et al., 2013) which is very important to reduce environmental pollution. The effect of ensiling on the PF values of IMW has not been extensively investigated. This is largely due to the difficulty in quantifying the amount of substrate degraded *in vitro*. It is important to determine the PF values for IMW because most of the agro-wastes may contain secondary metabolites such as tannins, which may cause harmful effects on rumen microbial fermentation (Jones et al., 1994). According to Blummel and Becker (1997), plants containing high PF are in general highly digestible and the values correlate well with DM intake in ruminants.

In agreement with our result, an OM digestibility of 73.5% was reported by Dolores Megias et al., (2002) for melon by-products. Miyaji and Matsuyama (2016) reported that lactic acid content increased, but the non-fiber carbohydrate and starch contents decreased during 2-3 months after ensiling. The silages had no pungent or unpleasant odors and showed a relative acidification level (pH 5.78-6.01). Although it is generally accepted that the silage pH values should be <4.5, lactate >30 g/kg DM (McDonald et al., 1991) and NH<sub>3</sub>-N content should not exceed 100 g/kg total nitrogen (Umana et al., 1991), pH and lactic acid of the present study were not within these ranges. Results of Sun et al. (2009) showed a positive correlation between lactic acid and WSC loss, whereas there was a negative correlation between lactic acid and DM loss, NDF, ADF, and CP. An increase in NH<sub>3</sub>-N was detected as an indicator of the rate of proteolysis (McDonald et al., 1991). In this study, it seems that vinegar supplementation prevented photolytic activity in the silage. The proportion of NH<sub>3</sub>-N to total nitrogen also determines the degree of proteolysis during ensiling, which also affects the utilization efficiency of N in the rumen (Thomas et al., 1980). Ammonia-N is commonly produced by *Clostridium* spp. via decompo-

sition of protein in the raw materials. Inappropriate fermentation will result in more ammonia-N production. Also using cellulase and lactic acid-producing bacteria as two additives enhanced lactic acid in the silage, which was associated with increased WSC loss and reduced losses of DM, NDF, ADF and CP during ensiling (Sun et al., 2009). Production of lactic acid is commonly the most important reason for the low pH in silage (Sun et al., 2009). The shift in anaerobic to aerobic fermentation during ensiling enables lactobacillus bacteria to gradually become the predominant microflora (Sun et al., 2009). Lactic and acetic acid contents increased when 1 or 2% vinegar were added to the silage. Santos and Kung (2016) observed that pH, lactic and acetic acids, and ammonia-N content of whole plant maize silage did not change significantly after 45 days of ensiling.

The *in vitro* gas production technique is widely used to determine the fermentation kinetics of various feedstuffs (Makkar, 2004) and some supplemented essential oils (Yadeghari et al., 2013), or especially for estimating the energy value of rangeland plants (Kazemi and Valizadeh, 2019) and agro-wastes (Lawal et al., 2016). The volume of gas produced at 6, 12, 24, 36, 48, 72 h,  $b_{\text{gas}}$  and  $c_{\text{gas}}$  for melon wastes were 27.8, 51.6, 72.5, 77.4, 80.4, 80.4, 81.4 mL and 0.085 mL/h, respectively (Dolores Megias et al., 2002) which are higher than our observations.

In this paper, decreases in gas production can be due to reduced production of VFA in the culture medium, as 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 SCFA (Getachew et al., 1998). The VFA produced by ruminal microorganisms during *in vitro* fermentation should be stoichiometrically related to gas production (Blummel and Becker, 1997). The high gas production during the first 24 h in IMW before ensiling suggests a higher extent of fermentation in the first 24 h of fermentation versus the other treatments. The increased PF value with ensiling (present study) is likely due to silage fermentation acids which are not fermentable by rumen microbes (AFRC, 1993). However, they do contribute to the truly degraded substrate, as they are not recovered in the incubation residues. This confounds the interpretation of PF values for ensiled feedstuff.

### Conclusions

Ensiling IMW with grape vinegar increased the content of DM and CP; however, the contents of NDF, ADF, and ash were not affected by ensiling or vinegar treating. An improvement in silage fermentation quality in-

cluding acid increment concentration and lower protein degradation was observed as a result of vinegar supplementation. Although the volume of gas produced and the true digestibility of DM and OM were higher in fresh IMW compared to the ensiled materials, the PF and efficiency of MMY was higher in EIMW+2% GV. Overall, it seems that fresh or ensiled IMW can be utilized in ruminant nutrition as an alternative forage source; however, due to high moisture content and the risk of mildew in fresh IMW, ensiling with 2% GV could improve the nutritional value and fermentation parameters.

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