



# NUTRITIVE VALUE, QUALITATIVE CHARACTERISTICS, *IN SITU* RUMEN DRY MATTER DEGRADABILITY AND *IN VITRO* GAS PRODUCTION PARAMETERS OF CITRUS PULP SILAGE SUPPLEMENTED WITH BARLEY GRAIN

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## ABSTRACT

The effect of grounded barley grain (BG), as a silage additive, on chemical composition, qualitative properties, *in situ* dry matter (DM) degradability and *in vitro* gas production of citrus pulp silage (CPS) was investigated. The whole fresh citrus pulp was manually chopped and used as either untreated or treated with 6, 12, or 18 g BG.kg<sup>-1</sup> fresh citrus pulp for ensiling. The data were analyzed in a completely randomized design. The data showed that the silage with 18 g BG had highest DM and CP content among the treatments ( $P < 0.05$ ). Also, with increasing of BG, the NDF and ADF concentrations linearly increased, but pH linearly decreased ( $P < 0.05$ ). Aerobic stability of silages exhibited a negative relationship with adding of BG ( $P < 0.05$ ). Data on *in situ* degradability of DM indicated that the soluble degradable fraction ( $a$ ) was highest in control group ( $P < 0.05$ ). Besides, the non-soluble degradation fraction ( $b$ ) was highest in silages with BG ( $P < 0.05$ ). The fractional degradation rate ( $c$ ) linearly increased with increasing of BG ( $P < 0.05$ ). After that, the potential DM degradability ( $a + b$ ) was lowest in the treatment with 18 g of BG ( $P < 0.05$ ). But, the effective degradability (ED) of DM was highest in treatments with BG ( $P < 0.05$ ). The data on gas production test indicated that the potential ( $b$ ) and fractional rate of gas production ( $c$ ) was highest in silages with 12 and 18 g of BG ( $P < 0.05$ ). Furthermore, treatments with 12 and 18 g of BG had higher OMD, NE<sub>i</sub>, ME and SCFA contents than other treatments ( $P < 0.05$ ). This data suggest that the addition of 12 and 18 g of BG to CPS can improve the nutritional value of this by-product.

**Key words:** aerobic stability; agricultural by-product; silage additives

## INTRODUCTION

Agricultural residues have historically been used as animal feed. Enhanced disposal prices in many parts of the world have increased interest in utilization of citrus by-product feedstuff as alternative feeds for ruminants (Arbabi *et al.*, 2008). Therefore, the use of these feeds in the ruminant diet can help dispose of these by-products in an ecologically sound manner, as ruminants can convert these feeds to valuable animal products (Amaral-Phillips and Hemken, 2006; Wood *et al.*, 2012).

Other benefit for these alternative feedstuffs is, that their use as a feed source is cheaper than grains in providing energy and protein to a diet and, therefore, may decrease the feed costs depending on prices of by-products and grains. Also, the use of these feedstuffs in livestock nutrition will reduce the dependence on the farm animal industry on cereal grains, and it can improve food security in a country (Kordi and Naserian, 2020).

The main by-product feedstuff from citrus processing is fresh citrus pulp, which is the whole residue after extraction of juice, representing between

492 and 692 g.kg<sup>-1</sup> of fresh citrus fruit with 600–650 g of dry matter (DM).kg<sup>-1</sup> peel, 300–350 g.kg<sup>-1</sup> of pulp and 0–100 g.kg<sup>-1</sup> of seeds (Ensminger *et al.*, 1990; Martinez-Pascual and Fernandez-Carmona, 1980). Citrus pulp is an appropriate energy source with high potential rumen degradability, high apparent digestibility, besides having a very low indigestible fraction and it is considered as pectin-rich feeds (Paya *et al.*, 2015; Andrade *et al.*, 2020). When citrus pulp is used in high concentrated diets to substitute traditional energy feeds, such as maize and barley grain, the performance of the ruminant animals was the same or premier, both for milk producing and weight gain. The nutritional properties of citrus pulp generally allow for the maintenance of favorable ruminal characteristics that certify acceptable animal performance (Andrade *et al.*, 2020).

The main citrus by-product feedstuffs fed to ruminants are fresh citrus pulp, citrus silage, dried citrus pulp, citrus meal and fines, citrus molasses, citrus peel liquor and citrus activated sludge. In most cases, the citrus pulp is used in a dried form, however, the drying process is expensive and often inconvenient. In fact, the use of the ensiled citrus pulp is cheaper than the dry processing and can be easily done by the farmers (Andrade *et al.*, 2020). Thus, there are many studies that make use of the ensiled citrus pulp as a feedstuff to dairy cattle (Volanis *et al.*, 2006; Williams *et al.*, 2018) and fattening lamb (Gado *et al.*, 2011). However, there is some challenge with feeding citrus pulp silage to ruminants. Storage of fresh citrus pulp is difficult due to its high moisture content. Therefore, due to the perishability of fresh citrus pulp in tropical countries, ensiling of this by-product would be convenient to develop an economical and efficient method of preservation that would enable these plant materials to be utilized as animal feeds for longer periods of time (Kordi and Naserian, 2012).

Furthermore, fermentation in the silo can be a very uncontrollable process leading to less optimal preservation of nutrients. Silage additives have been used to improve the ensiling process (better energy and DM recovery) with subsequent improvements in animal performance (Yitbarek and Tamir, 2014). Citrus pulp contains high amount of pectin and soluble carbohydrates (Bampidis and Robinson, 2006), so that the fermentation of high soluble carbohydrates in this by-product can lead to low final pH in CPS (Paya *et al.*, 2015).

However, pectin is fermented more slowly than starch, so that fermentation of pectin produced less lactate than fermentation of starch (Marounek and Duskova, 1999). Therefore, addition a starch-rich additive to citrus pulp before ensiling may speed up and intensify fermentation, which results in faster accumulation of lactic acid and lower pH at earlier stages of ensiling and improvement of silage conservation (Paya *et al.*, 2015). Rapidly decreasing pH conserves water soluble carbohydrates and declines proteolysis and deamination by inhibiting prolonged fermentation (Muck, 1993), which can improve DM and CP recovery in the silo.

Silage additives have been classified into various categories. One group of silage additives is fermentation stimulants that contain fermentable carbohydrates. Another type of silage additives is adsorbents. These additives, such as grains, have water absorption properties and, thus, supplement to forage with low DM content in order to reduce nutrient wastage and eutrophication of surface waters by silage leachate (Kordi and Naserian, 2012; Yitbarek and Tamir, 2014).

Thus, in this study barley grain used as a starch-rich silage additive to citrus pulp silage to provide fermentable substrate for achievement to lower pH at earlier stages of ensiling and also to direct the course of fermentation by absorbing excessive moisture. Barley grain has more than 95 % of dry matter content and it has water absorption properties. So, addition of this additive to CPS at ensiling may improve the DM content of this silage.

The objective of the present study was to evaluate the effect of different levels of BG on chemical composition, fermentational properties, *in situ* DM degradability and *in vitro* gas production by CPS.

## MATERIALS AND METHODS

### Silage preparation and sampling

The experiment was arranged as a completely randomized design with 4 replications. Whole fresh citrus pulp was collected from a local juice factory; it was manually chopped (4–5 cm length) and used either as untreated or treated with barley grain (BG) at 6, 12, and 18 g.kg<sup>-1</sup> of fresh citrus pulp. Barley grains were ground to pass through a 1-mm pore-size screen before adding to citrus pulp. Barley grain

**Table 1. Chemical composition of utilized fresh citrus pulp and barley grain for ensiling**

Item (% DM)	DM	CP	EE	NDF	ADF	Ash	pH
Fresh citrus pulp	12.50	10.61	3.50	26.30	25.80	5.33	4.33
Barley grain	91.97	11.30	1.69	27.31	6.89	3.43	-

was mixed with citrus pulp for about one minute. Chemical composition of fresh citrus pulp and barley grain is shown in Table 1. Each micro-silo was filled with approximately 2.5 kg of untreated or treated fresh citrus pulp, then manually compressed and capped. Silos were stored in the dark at ambient temperatures (20 and 22 °C) and opened after 60 days of ensiling. The content of each opened silo was thoroughly mixed and samples from individual silos were collected for chemical analyses.

#### Determination of pH and chemical analyses

For measurement of pH, 50 g samples of silage from each treatment were diluted with 450 ml sterile deionized water, blended for 2 min, strained through four layers of cheesecloth and pH was determined immediately by a pH meter (Model 691, Metrohm). The dry matter (DM) content of silage samples (150 to 200 g) was determined by drying in a forced-air oven at 60 °C for 48 h. After drying, samples were ground to pass a 1 mm sieve. Acid detergent fiber (ADF) and neutral detergent fiber (NDF) were analyzed by the method of Clovis *et al.* (2008). Crude protein (CP), ether extract (EE), ash and ammonia nitrogen were determined according to AOAC (2005). The quality of different silages was determined by estimating the flieg point data.

Flieg point was calculated using the following formula (Denek *et al.*, 2004):

$$\text{Flieg point} = 220 + (2 \times \text{dry matter \%} - 15) - 40 \times \text{pH}$$

Kilic (1986) expressed a positive relationship between this index and silage quality:

Determining the quality of silage according to the value of the number obtained from this formula is as follows: zero to 20 (bad), 21 to 40 (average), 41 to 60 (relatively good), 61 to 80 (good) and 81 up to 100 (excellent).

#### Aerobic stability

After opening the silos, the contents of each silo were thoroughly mixed and 1 kg of silage samples

were transferred into separate 1-L containers (3 containers per treatment). Each container was embedded with two thermometers in the lower and mid layers of the silage mass to record the temperature every 15 min. The containers were covered with a double layer of cheesecloth and stored at ambient temperature (20 to 22 °C) for 7 days. Ambient temperature was also simultaneously measured at 15 minute intervals during this period (Baah *et al.*, 2011).

#### *In situ* DM degradability of silages

Measurements of *in situ* DM degradability of treatments were performed in 3 rumen-fistulated steers using the nylon bag technique (Ørskov and McDonald, 1979). The nylon bags (9 × 18 cm<sup>2</sup>, pore size 50 µm) were filled with 5g of samples and put into the rumen. Feed ingredients of the diet of steers are shown in Table 2. Steers were fed at maintenance level, as recommended by NRC (2001). The bags were removed at 2, 4, 8, 12, 24, 48 and 72 h after the start of incubation, and each bag was washed immediately with tap water until color disappeared. For the  $t_0$  incubation time, the bags were simply washed in the water. *In situ* disappearance of DM was measured relatively to original feed. The rate and extent of DM degradation were estimated according to the equation:  $p = a + b(1 - e^{-ct})$  (Ørskov and McDonald, 1979). Effective degradability (ED) was calculated as  $ED = a + (b \times c) / (c + Kp)$ , assuming an outflow rate ( $Kp$ ) of 0.05/h.

#### *In vitro* gas production

The *in vitro* gas production was carried out using the method described by Menke and Steingass (1988). Samples (200 mg) were weighed in 100 ml calibrated glass syringes (3 replicates per treatment sample). Buffered mineral solution was prepared and placed in a water bath at 39 °C under continuous flushing with CO<sub>2</sub>. Rumen fluid was collected after the morning feeding from two adult ruminally fistulated sheep (42 ± 2.5 kg body weight), strained through

**Table 2. Feed ingredients of the diet of steers and sheep**

Ingredients, g.kg DM <sup>-1</sup> of Diet	
Alfalfa	300
Corn silage	200
Concentrate	500
Composition of concentrate, (g.kg DM <sup>-1</sup> )	
Barley grain	550
Canola meal	150
Wheat Bran	280
Vitamin-mineral mix	10
Calcium carbonate	5
Salt	5
Chemical composition(g.kg DM <sup>-1</sup> )	
CP	133
NDF	372
ADF	223
ME(MJ.kg <sup>-1</sup> )	11
Ca	10
P	6

four layers of cheesecloth and flushed with CO<sub>2</sub>. Feed ingredients of the diet of sheep are shown in Table 2. Sheep were fed at the maintenance level, as recommended by NRC (2001). The syringe was then filled with 30 ml of medium consisting of 10 ml of rumen fluid and 20 ml of buffer solution. All handlings were done under continuous flushing with CO<sub>2</sub>. The syringes were placed into a water bath at 38.6 °C. Gas production was measured at 2, 4, 6, 8, 12, 24, 36, 48, 72 and 96 h. Syringes were gently shaken after each recording. Rate and extent of gas production were determined for each feed by fitting gas production data to the non-linear equation:

$Y = b(1 - e^{-ct})$  (Ørskov and McDonald, 1979), where  $Y$  – is the volume of gas produced at time  $t$ ;  $b$  – is the potential gas production (ml.g DM<sup>-1</sup>);  $c$  – is the fractional rate of gas production.

Parameters  $b$  and  $c$  were estimated by an iterative least squares method using a non-linear regression procedure of the statistical analysis systems (SAS, 2003). Metabolizable energy (ME, MJ.kg DM<sup>-1</sup>), net energy lactation (NE<sub>l</sub>, MJ.kg DM<sup>-1</sup>) and *in vitro* organic matter digestibility (OMD, g.kg OM<sup>-1</sup>) were estimated according to Menke and Steingass (1988) and Menke *et al.* (1979).

$ME = 2.20 + 0.136 GP$  (mL.0.5 g DM<sup>-1</sup>) + 0.057 CP (g.kg DM<sup>-1</sup>),

$NE_l$  (MJ.kg DM<sup>-1</sup>) = (0.101 × GP) + (0.051 × CP (g.kg DM<sup>-1</sup>)) + (0.11 × CF (g.kg DM<sup>-1</sup>))

$OMD = 148.8 + 8.89 GP + 4.5 CP$  (g.kg DM<sup>-1</sup>) + 0.651 ash (g.kg DM<sup>-1</sup>), where

GP is net GP in mL from 200 mg of dry sample after 24 h of incubation.

Short-chain fatty acids (SCFA) were predicted as 0.0239 GV- 0.00601 (Getachew *et al.*, 2002), where GV is total gas volume.

### Statistical analyses

The data were analyzed in a completely randomized design using the general linear model procedure of SAS 9. 2 (2003). The statistical model used was as following:

$Y_{ij} = \mu + T_i + e_{ij}$ , where

$Y_{ij}$  is the observation;  $\mu$  is the overall mean;  $T_i$  is the effect of treatments and  $e_{ij}$  is the random error. Significant differences between individual means were identified using Duncan's multiple range test at a 0.05 probability level.

## RESULTS AND DISCUSSION

### Chemical composition and fermentational properties

The chemical composition, fermentational properties and quality parameters of experimental silages are given in Table 3. Data showed that DM content and CP concentrations were significantly different among treatments; with adding of BG in CPS, DM % and CP % increased ( $P < 0.05$ ) as treatment with 18 g of BG had highest DM and CP content among treatments. Ammonia nitrogen, EE and ash content were similar among treatments ( $P > 0.05$ ). Furthermore, with increasing the level of BG, the NDF and ADF concentrations were linearly increased, but pH decreased ( $P < 0.05$ ) as untreated silage had higher pH than treated groups. Aerobic stability of the silage exhibited a negative linear relationship with increasing the level of BG ( $P < 0.05$ ). Data of flieg point (Table 3) displayed that all treatments had a good quality, although the numerical value of flieg point was highest for treatments with BG.

**Table 3. Chemical composition, fermentational properties and quality of different treatments**

Items	Treatments <sup>1</sup>				SEM	P-value
	C	6 g BG	12 g BG	18 g BG		
DM %	11.82 <sup>b</sup>	12.14 <sup>ab</sup>	12.24 <sup>ab</sup>	13.48 <sup>a</sup>	0.326	0.076
CP %	11.25 <sup>c</sup>	11.68 <sup>b</sup>	11.69 <sup>b</sup>	11.74 <sup>a</sup>	0.011	0.0001
EE %	3.60	3.60	3.50	3.50	0.476	0.997
NDF %	32.00 <sup>b</sup>	38.00 <sup>ab</sup>	41.00 <sup>a</sup>	42.00 <sup>a</sup>	1.581	0.051
ADF %	29.00 <sup>c</sup>	33.00 <sup>b</sup>	35.00 <sup>ab</sup>	38.00 <sup>a</sup>	1.006	0.013
Ash %	6.16	6.08	6.24	6.16	0.155	0.899
pH	4.11 <sup>a</sup>	3.98 <sup>b</sup>	3.91 <sup>b</sup>	3.93 <sup>b</sup>	0.022	0.011
NH <sub>3</sub> -N (mg.dl <sup>-1</sup> )	0.01	0.01	0.01	0.08	0.006	0.558
Aerobic stability (h)	39.75 <sup>a</sup>	22.30 <sup>b</sup>	21.40 <sup>bc</sup>	20.80 <sup>c</sup>	0.233	<0.0001
Flieg point	65.08	69.89	71.97	74.57	-	-
Quality	good	good	good	good	-	-

<sup>1</sup>C: control group (silage untreated by barley grain); BG: Barley Grain.

Means in the same rows with different superscripts *a*, *b*, *c* are significantly different with *P* < 0.05.

Yitbarek and Tamir (2014) recommended that adding 45–90 kg of cracked or rolled grain to 1 ton of wet forages will increase the dry matter content of silage by about 5 percentage units. After that, fermentable substrate provided from BG resulted in lower pH of silages treated with this additive. Muck (1993) reported rapid decrease of pH conserves water soluble carbohydrates and declining proteolysis and deamination by inhibiting prolonged fermentation. Due to these subjects DM and CP content of treatments with BG was linearly higher than in control group. Besides, BG has absorbent properties (Yitbarek and Tamir, 2014; Kordi *et al.*, 2015) and it can result in increase of the DM content of the citrus pulp silage.

Higher NDF and ADF concentrations in silages with BG can be related to the cumulative effect of adding BG fiber to citrus pulp fiber, because in all treatments the amount of citrus pulp was fixed and the amount of BG was changed from 0 to 18 g.kg<sup>-1</sup> of citrus pulp.

The linear decrease in aerobic stability of silages with increasing the level of BG can be due to raising soluble carbohydrate content in the silages. More carbohydrates in the silage might induce the growth of fungi or molds. Subsequently, the temperature of silage with this activity has been increased (Kordi and Naserian, 2012). Data showed that ammonia nitrogen levels were not affected by treatments because of enough soluble carbohydrate for silage fermentation in different treatments.

Kordi and Naserian (2012) demonstrated that with adding 6, 12, and 18 g wheat bran/kg citrus pulp, CP and NDF concentration of silage linearly increased. Furthermore, in their study aerobic stability of CPS decreased as increasing in the level of wheat bran. But, ammonia nitrogen, EE, and ash content were similar among treatments, which our study was in agreement with them.

In another study, Kordi *et al.* (2014) reported linear increase of DM and CP content in CPS after adding 6, 12 and 18 g of sugar beet pulp/kg of citrus pulp. They attributed the higher DM content in silage with sugar beet pulp to the water absorbent properties of this additive. Also, they showed that EE and ash content in CPS did not differ among treatments with increasing of sugar beet pulp. However, oppositely to our study, they reported that with adding sugar beet pulp to CPS, ammonia nitrogen concentration and pH linearly increased among treatments.

Lashkari *et al.* (2014) reported that supplementation of orange pulp silage with poultry by-product meal resulted in increase of the DM and CP contents of the silage and improved the nutritive value of orange pulp.

Arbabi *et al.* (2008) reported that the addition of sugar beet pulp to CPS elevated the DM content in silages. They found that CPS with 5 % DM of sugar beet pulp had lowest ADF but highest NDF and CP concentrations in contrast to control group.

Moreover, they showed that the addition of sugar beet pulp to CPS did not affect a pH of the silage.

However, in contrast with our results, Kordi *et al.* (2010) reported that adding of 6, 12 or 18 g of molasses/kg of CPS, decreased CP % and significantly increased ash % and pH. Moreover, they showed that DM % and aerobic stability were not affected by different levels of molasses.

#### ***In situ* Rumen DM degradability of silages**

Data on degradation parameters after different treatments are presented in Table 4. Results indicate that soluble degradable fraction (*a*) was highest in control group ( $P < 0.05$ ), while with the adding BG (*a*) this parameter in treated silages was significantly decreased ( $P < 0.05$ ). Moreover, non-soluble degradation fraction (*b*) showed a positive relationship with the addition of BG to CPS, as with adding BG the (*b*) fraction was highest in silages with BG ( $P < 0.05$ ), but there were no differences among treated silages for (*a*) and (*b*) fractions. The fractional degradation rate (*c*) exhibited a positive relationship with adding BG to CPS, as with increasing the level of BG the (*c*) fraction linearly increased ( $P < 0.05$ ). After that, the potential degradability (*a* + *b*) of silages was lowest after treatment with 18 g of BG and it was similar compared to other groups ( $P < 0.05$ ). However, the effective degradability (ED) of dry matter was highest after treatments with BG ( $P < 0.05$ ). Higher content of soluble degradable fractions and lower concentration of non-soluble degradable fractions in untreated silage can be related

to higher ratio of pectin in the control group, as fiber content of external shells in BG can increase the content of (*b*) fraction in treated silages. Furthermore, linear rising of the (*c*) fraction with adding BG can be due to more degradable substrates being provided in silo by treating them with BG.

The control group had highest potential degradability among the treatments, what can be related to structural differences among CPS and BG for degradation, as untreated CPS had higher ratio of pectin for degradation. The effective DM degradability of experimental silages was highest for CPS with BG due to structural differences between pectin in citrus pulp and starch in BG.

Our present results are in agreement with our previous reports (Kordi and Naserian, 2012; Kordi *et al.*, 2014). Kordi and Naserian (2012), which used wheat bran as a silage additive for ensiled citrus pulp, reported that the addition of wheat bran to CPS caused decline in the soluble fraction (*a*) in treated silages. After that, fractional degradation rate (*c*) in silages treated with wheat bran was higher than obtained with other treatments. Furthermore, potential degradability of CPS linearly decreased with increasing the level of wheat bran.

Also, Kordi *et al.* (2014), who used sugar beet pulp as a silage additive for citrus pulp ensiling, concluded that with adding sugar beet pulp to CPS the soluble (*a*) degradable fraction was decreased, while non-soluble (*b*) fraction was increased. Besides, fractional degradation rate (*c*) linearly increased with increasing the level of sugar beet pulp

**Table 4. Degradation parameters for different experimental treatments**

Degradation parameters <sup>2</sup>	Treatments <sup>1</sup>				SEM	P-value
	C	6 g BG	12 g BG	18 g BG		
<i>a</i> (mg.g <sup>-1</sup> )	51.52 <sup>a</sup>	43.48 <sup>b</sup>	40.81 <sup>b</sup>	40.30 <sup>b</sup>	1.116	0.002
<i>b</i> (mg.g <sup>-1</sup> )	48.22 <sup>b</sup>	55.58 <sup>a</sup>	57.94 <sup>a</sup>	54.46 <sup>a</sup>	1.423	0.033
<i>c</i> (% h <sup>-1</sup> )	0.05 <sup>d</sup>	0.08 <sup>c</sup>	0.10 <sup>b</sup>	0.16 <sup>a</sup>	0.002	<0.0001
Potential dry matter degradability ( <i>a</i> + <i>b</i> ) (mg.g <sup>-1</sup> )	999.75 <sup>a</sup>	990.65 <sup>a</sup>	982.55 <sup>a</sup>	952.10 <sup>b</sup>	0.395	0.002
Effective dry matter degradability (%) ( $K_p = 0.05$ )	77.67 <sup>b</sup>	83.31 <sup>a</sup>	82.08 <sup>a</sup>	82.03 <sup>a</sup>	1.011	0.061

<sup>1</sup>C: control group (silage untreated by barley grain); BG: Barley Grain.

<sup>2</sup>*a* and *b* represent soluble and non-soluble degradable fractions, respectively; *c* is the fractional degradation rate of the *b* fraction for DM,  $K_p$  is the passage rate.

Means in the same rows with different superscripts *a*, *b*, *c* are significantly different with  $P < 0.05$ .

in silages. Then, effective DM degradability significantly increased by adding sugar beet pulp to CPS.

Lashkari *et al.* (2014) evaluated the influence of poultry by-product meal and urea, as nitrogen sources, on the orange pulp ensiling. These investigators reported that poultry by-product meal and urea did not change degradation rates (*c*) of DM and CP. They also reported that the effective degradability of DM was highest for silages supplemented with poultry by-product meal.

In another study, Paya *et al.* (2015) investigated the effect of adding bacterial inoculants (I), enzyme (E) or a combination of them (I + E) on fermentation, degradability and nutrient value of orange pulp silage. They found that silages with (I) and (I + E) had lowest pH and highest lactic acid concentration compared to the control. They also observed lowest ammonia nitrogen concentration in silages with bacteria inoculants. Furthermore, in their study the highest ME, NEI, digestible organic matter in dry matter, SCFA and microbial protein values were observed after (I) and (I + E) treatments. Besides, the *in vitro* degradability of dry matter was highest in (I + E) and effective degradability of dry matter was highest after (E) and (I + E) treatments.

#### ***In vitro* gas production**

The data on gas production test indicated that with increasing the level of BG in silage the volume of gas produced by 96 h was increased ( $P < 0.05$ ) (Table 5). The potential gas production (*b*)

and fractional rate of gas production (*c*) were highest by silages with 12 and 18 g of BG ( $P < 0.05$ ). Also, treatments with 12 and 18 g of BG had higher estimated OMD, NEI, ME and SCFA content than other groups ( $P < 0.05$ ). It can be related to more fermentable carbohydrate substrate provided by the adding BG to ensiled citrus pulp. Also, with adding BG to silage, the DM and CP contents were increased and these components were highest after treatment with larger amount of BG. Therefore, this treatment resulted in higher energy content in silages with 12 and 18 BG (Kordi and Naserian, 2012). Yitbarek and Tamir (2014) reported that addition of grain to the hay crop silage led to increase in the energy content of the silage.

Wheat bran (Kordi and Naserian; 2012) and sugar beet pulp (Kordi *et al.*, 2014) were used as silage additives for ensiling citrus pulp. Above mentioned authors concluded that with adding wheat bran or sugar beet pulp to CPS, the volume of gas produced by 96 h incubation was highest after treatments with 18 g of wheat bran or sugar beet pulp. Similarly, potential gas production fraction (*b*) was highest in silages with 18 g of wheat bran or sugar beet pulp. Moreover, fractional rate of gas production (*c*) linearly increased among treatments with increasing the levels of wheat bran or sugar beet pulp. Furthermore, Kordi and Naserian (2012) and Kordi *et al.*, (2014) concluded that adding wheat bran or sugar beet pulp to ensiled citrus pulp could significantly increase the OMD and ME contents of CPS.

**Table 5. Gas production properties and the estimated OMD, NE<sub>i</sub>, ME and SCFA of different experimental silages**

Items	C	Treatments <sup>1</sup>			SEM	P-value
		6 g BG	12 g BG	18 g BG		
96 h GP (ml.g DM <sup>-1</sup> )	237.58 <sup>c</sup>	244.44 <sup>c</sup>	285.61 <sup>b</sup>	304.97 <sup>a</sup>	5.805	<0.0001
<i>b</i> (ml)	236.00 <sup>b</sup>	258.10 <sup>ab</sup>	284.45 <sup>a</sup>	294.40 <sup>a</sup>	3.475	0.042
<i>c</i> (ml.h <sup>-1</sup> )	0.070 <sup>b</sup>	0.074 <sup>b</sup>	0.095 <sup>a</sup>	0.097 <sup>a</sup>	0.0031	0.008
OMD (%)	56.51 <sup>c</sup>	60.01 <sup>bc</sup>	64.59 <sup>ab</sup>	65.03 <sup>a</sup>	1.021	0.021
NE <sub>i</sub> (MJ.kg DM <sup>-1</sup> )	4.78 <sup>b</sup>	5.08 <sup>b</sup>	5.99 <sup>a</sup>	6.04 <sup>a</sup>	0.127	0.005
ME (MJ.kg DM <sup>-1</sup> )	7.95 <sup>b</sup>	8.42 <sup>b</sup>	9.65 <sup>a</sup>	9.72 <sup>a</sup>	0.189	0.006
SCFA (mmol.200 mg DM <sup>-1</sup> )	1.03 <sup>c</sup>	1.11 <sup>bc</sup>	1.28 <sup>ab</sup>	1.42 <sup>a</sup>	0.054	0.022

<sup>1</sup>C: control group (silage untreated by barley grain); BG: Barley Grain.

*b*: Potential gas production (ml.g DM<sup>-1</sup>); *c*: Fractional rate of gas production (ml.h<sup>-1</sup>); OMD: Organic matter digestibility; NE<sub>i</sub>: Net energy lactating; ME: Metabolisable energy; SCFA: Short chain fatty acids.

Means in the same rows with different superscripts *a*, *b*, *c* are significantly different with  $P < 0.05$ .

Lashkari *et al.* (2014) observed that orange pulp silage, supplemented with poultry by-product meal, had highest potential gas production (a), fractional rate of gas production (c) and ME value than silages treated with urea or the control group.

## CONCLUSION

The present study demonstrates that the adding 18 g of barley grain/kg of CPS can improve the nutritional value of silage; especially it can increase DM, CP, OMD, NE<sub>i</sub>, ME and SCFA contents of CPS. According to our study, if CPS will be the main or only feed offered for feeding to ruminant animals, then the supplementation of some barley grain to this by-product at ensiling will make it a more complete feed. Thus, this will reduce the amount of supplemental grain necessary for animal feeding.

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