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QUALITATIVE CHARACTERISTICS, MICROBIAL POPULATIONS AND NUTRITIVE VALUES OF ORANGE PULP ENSILED WITH NITROGEN SUPPLEMENTATION

S. LASHKARI[1]*, A. TAGHIZADEH[2], J. SEIFDAVATI[3], A. Z. M. SALEM[4]

[1]Department of Animal Science, Faculty of Agriculture, University of Kurdistan, Sanandaj, Iran
[2]Department of Animal Science, Faculty of Agriculture, University of Tabriz, Tabriz, Iran
[3]Department of Animal Science, Faculty of Agriculture, University of Mohaghegh Ardabili, Ardabil, Iran
[4]Facultad de Medicina Veterinariay Zootecnia, Universidad Autónoma del Estado de México, Estado de México, México

ABSTRACT

The aim of this study was to evaluate the qualitative characteristics, microbial profile and nutritive value of high-moisture orange pulp co-ensiled with poultry by-product meal (PBM) or urea as additional nitrogen (N) source. The optimum dry matter (DM) content of each experimental silage was achieved by adding the appropriate proportion of wheat straw. A control silage was also prepared with orange pulp and wheat straw, but without any supplemental nitrogen. Each experimental silage was ensiled in three replicates of 12 kg mini-silos and left intact for 90 days. The evaluated traits were: silage pH, microbial population, lactate, acetate, butyrate, crude protein (CP), true protein and NH_3-N contents. In addition to these, *in situ* dry matter and CP degradability as well as ruminal and post-ruminal CP disappearance rates were also measured. Both N-supplemented silages showed higher pH values and CP contents compared to the control silage ($P<0.05$). With regard to NH_3-N, the lowest values were detected in silage supplemented with PBM ($P<0.05$). The highest acetate production and total bacteria (TB) count ($P<0.05$) and the lowest lactic acid bacteria count ($P<0.05$) were observed in silage supplemented with urea. Control silage and urea supplemented silage had the highest and lowest lactate contents, respectively. Addition of PBM and urea did not alter degradation rates (c) of DM and CP, however, the latter caused a significantly increased ($P<0.05$) potentially degradable CP fraction (b). The highest ($P<0.05$) post-ruminal CP disappearance rate, gas production rate and ME value were observed in silage supplemented with PBM ($P<0.05$). In conclusion, both nitrogen sources used in this study enhanced nutritive value of orange pulp silage. Ensiling may be applied as a practical approach for long-term preservation of fresh orange pulp.

Key words: orange pulp; silage quality; *in situ* degradability

INTRODUCTION

Growing up feeds cost values in many parts of the world have increased attending in utilization of citrus by-product feedstuffs as specific feeds for ruminants. One of the citrus by-products that produced exceedingly is orange pulp and its cost is partly low compared to its nutritive value. Citrus pulp is by-product derived from the citrus juice industry and includes mixture of citrus peel, pulp and seeds (Lashkari and Taghizadeh, 2011). Citrus pulp is a suitable energy supplement, but is low in CP and neutral detergent fiber (NDF). Citrus pulp also has high potential rumen degradability, high apparent digestibility and considered as pectin-rich foods (Lashkari and Taghizadeh, 2012). It contains little starch and an excellent high-fiber energy source. High moisture content is main problem in conserving of this feeds and utilizing it with high moisture and high sugar content because of spoiling, fungi and mold exposed at risk human and animal health. More than 30 % of fresh citrus pulp was wasted by feeding wet citrus pulp (Arthington and Pate, 2001).

***Correspondence:** E-mail: S.Lashkari@hotmail.com
Saman Lashkari, Department of Animal Science, Faculty of Agriculture, University of Kurdistan, P. O. Box: 416, Sanandaj, Iran
Tel.: +98 9183713716459

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Therefore, it is used in animal feeding after dehydration or ensiling processes and most of research related to citrus pulp focus on dried products (Fegeros *et al*., 1995). The process of drying is costly and often inconvenient, but using the ensiling citrus pulp is cheaper than dry processing and can be easily accomplished by the farmer. Ensiling losses due to the high moisture content of citrus pulp are high and sticky nature makes it difficult to storage in sheds, bunkers or silos (Bampidis and Robinson, 2005) and high moisture silages promote seepage losses from the silo. To avoid these problems and prepare the appropriate dry matter for ensiling, citrus pulp was ensiled with high dry matter feeds such as chopped wheat straw which limits ensiling losses and gives to the silage the characteristics of a suitable and cheap substitute for farm forages (Scerra *et al.*, 2001). Nutritive value of ensiled feed such as crop residues and low quality feeds can be improved with such additive as non-protein nitrogen and animal protein source (Schingoethe *et al.*, 1980). Also, in order to increase the protein content in control silage (citrus pulp plus straw) poultry by-product meal and urea was added.

The objectives of this research were to evaluate the orange pulp silage quality and microbial contents, *in situ* degradability of DM and CP, *in vitro* gas production and fermentation characteristics, with the addition of straw and different nitrogen supplementation.

MATERIAL AND METHODS

Silage preparation and treatment

Fresh orange pulp without further processing after juicing was coarsely chopped to 5 cm pieces using a machine meant for chopping whole plant maize. Due to the high moisture content and the physical property (after chopping) of orange pulp, wheat straw was added as an absorbent. The PBM samples were randomly collected from rendering unit of industrial poultry slaughter-houses in the east Azerbaijan Province, Iran. Poultry by-product meal included the following ground, cleaned, and rendered carcass parts of poultry including heads, feet, viscera and trace amounts of feathers and blood. PBM was processed at approximately 142 °C and at approximately 380 kPas. Then PBM were dried at 110 °C. Silage masses were mixed after addition with protein additives, and the compositions of silages were as follows: 1) 73 % orange pulp + 27 % straw (control), 2) 74 % orange pulp + 12 % straw + 14 % poultry by-product meal (OSP) and 3) 63 % orange pulp + 25 % straw + 12 % urea solution (3 %). Silages were ensiled for 90 days in 12-kg plastic buckets (triplicate per treatment) and their compositions are listed in Table 1.

Silage analyses

Silage samples were obtained from each silo after opening and dried in a forced air oven at 60 °C for 48 h. Dried samples were ground using a grinder with a 1-mm sieve and analyzed for ash, ether extract and crude protein as described by AOAC (2005). Neutral detergent fibre (NDF) and acid detergent fibre (ADF) were determined using an ANKOM$^{200/220}$ Fibre Analyser (Ankom Technology Corporation, Macedon, NY) according to the manufacturer's instructions without sodium sulphite. In addition, NDF was analyzed without amylase with ash included. For measurement of pH, silage (15 g) was blended with 135 ml of deionized water for 30 s. The homogenate was filtered through two layers of cheesecloth and pH was immediately measured (Zahiroddini *et al*., 2004). The filtrate was used for the determination of volatile fatty acids (VFA) and NH_3-N. Subsamples of filtrate were prepared for analysis of VFA by adding 1 ml of 25 % (wt/vol) meta-phosphoric acid to 5 ml of filtrate. Lactic acid in water extracts of the silages was determined by spectrophotometry according to Barker and Summerson (1941). Another 5 ml of filtrate were combined with 0.4 ml of 65 % trichloric acid for analysis of NH_3-N, as described by Markham (1942). In order to analyze VFA and NH_3-N, samples were stored on ice and then stored at - 40 °C. Before analysis, the samples were thawed overnight at 4 °C. Silage VFAs were quantified using gas chromatography (WCOT Fused Silica Capillary,

Table 1: Chemical composition of silage ingredient (g/kg)

Type of silage	DM[1]	CP[2]	OM[3]	NDF[4]	ADF[5]
Orange pulp	130	78	912	234	164
Wheat straw	870	40	940	884	590
Poultry-by product meal	900	560	900	15	0

1 - DM = Dry matter, 2 - OM= Organic matter (g.kg^{-1} DM), 3 - CP = Crude protein (g.kg^{-1} DM), 4 - EE = Ether extract (g.kg^{-1} DM), 5 - NDF = Neutral detergent fiber (g.kg^{-1} DM), 6 - ADF = Acid detergent fiber (g.kg^{-1} DM)

chrompack CP 9002), flame ionization detection and crotonic acid was used as the internal standard.

Crude protein fraction

The true protein, neutral detergent insoluble nitrogen (NDIN) and acid detergent insoluble nitrogen (ADIN) fractions were determined according to standardization and recommendations published by Licitra *et al.* (1996). True protein was calculated as nitrogen precipitated with 100 $g.l^{-1}$ (w/v) of trichloroacetic acid. The procedure used here is based on NDF and ADF prepared. After that, association nitrogen into NDF and ADF measured as NDIN and ADIN, respectively.

Enumeration of microorganisms

For isolation and enumeration of microorganisms, 11 g of fresh silage were added to 99 ml of sterile 70mM potassium phosphate buffer (pH 7) and agitated for 60 s (Zahiroddini *et al.* 2004). Extracts required for enumeration of microorganisms were prepared from fresh silage as described by Zahiroddini *et al.* (2004). A semi-selective lactobacilli medium (MRS) and the nutrient agar (NA) were used for the isolation of lactic acid bacteria (LAB) and total bacteria (TB), respectively. Sabouraud's dextrose agar (SDA; Difco) was used for the isolation of yeasts and moulds. Serial dilutions (10^{-2} to 10^{-7}) of the suspension were prepared and 100 µl aliquots of three consecutive dilutions were plated onto a medium in triplicates. Lactobacilli MRS agar and nutrient agar contained 200 µg/ml of cycloheximide (Sigma, Mississauga) and SDA contained 100 µg/ml each of tetracycline and chloramphenicol. Lactobacilli MRS agar and NA plates were placed in an incubator at 37 °C for 24-48 h and SDA plates were incubated at 25 °C for 48-72 h. Colonies were counted from the plates at appropriate dilutions containing a minimum of 30 and a maximum of 300 colonies per plate and the number of colony forming units (cfu) was expressed per gram of fresh silages.

***In situ* study**

Ruminal disappearance of DM and CP of silages were determined using a nylon bag technique (Ørskov and McDonald, 1979). Silage samples were dried at 60 °C in a forced air oven for 48 h and ground through a 2 mm screen and ground samples (5 g) were placed in Dacron bags (12 cm × 6 cm with 50 µm pore size). Each feed sample was incubated in 6 replicates (2 replicates) in the rumen of three wethers (mean weight of 43.9 ± 4 kg). The incubation times for samples were 0, 2, 4, 6, 8, 12, 16, 24, 48, 72 and 96 h. Nylon bags were suspended in the rumen in a polyester mesh bag (25 × 40 cm, 3 mm pore size) and were removed from the rumen at the same time, so that all bags could be washed simultaneously. The nylon bags were then removed from the mesh bag and washed with a washing machine until the rinse water remained clear. Bags were then dried in forced air oven at 60 °C until a constant weight was achieved before determination of DM disappearance and CP analysis. The kinetics of *in situ* DM and CP disappearance were estimated using a non-linear procedure of SAS (1991). The model of McDonald (1979) was fitted to the percentage of DM and CP disappearance as:

$Y = a + b\,(1 - e^{-ct})$

Where, "*a*" - is the soluble fraction, "*b*" - the slowly disappearing fraction, "*c*" - the fractional rate of disappearance (per h) and t - is the incubation time (h). Effective ruminal disappearance was estimated using the following model:

$Y = a + bc/(c + k)$,

where "*k*" is the fractional rate of particulate passage, assumed to be 0.03 and 0.05/h (Ørskov and McDonald, 1979).

Modified three-step procedure

This part of the experiment followed the procedure of Gargallo *et al.* (2006). Approximately, 5 g of a sample were weighed and placed into a 5 cm × 10 cm nylon bags, 50 µm pore size dacron polyester bag (four bags per sample) and suspended in the rumen of three wethers (mean weight of 43.9 ± 2.4 kg) fitted with permanent rumen cannulae for 12 h. Bags were then removed and washed with washing machine and dried in a forced-air oven at 55°C for 48 h and weighed. Samples from each bag were taken for N analysis using the Kjeldahl method (Kjeltec 2300 Autoanalyzer, Foss Tecator). After weighing, the residue (0.5 g) was put in *in situ* bag and placed into an ANKOM Daisy incubator for determination of post ruminal digestibility (Gargallo *et al*., 2006). Briefly, samples were incubated in a pepsin/HCl solution for 1 h in a Daisy incubator, followed by the incubation in a pancreatin/KH_2PO_4 solution for 24 h. After 24 h, the liquid was drained from the bottles, and bags were again rinsed until the runoff was clear. Bags were allowed to drain and were dried in a forced hot air oven at 55 °C for 48 h. The dry weights of the samples and bags were recorded, and bags were opened and pooled by the sample for CP analysis.

***In vitro* gas production**

Dried samples (300 mg) were placed into the vial with 100 ml of serum and each sample was incubated in 6 replicates with 20 ml of rumen liquor and buffer solution (1:2). McDougall (1948) buffer solution was prepared and placed into a water bath at 39 °C. Rumen liquor samples were obtained from three wethers

(mean weight of 43.9 ± 2.4 kg) fitted with permanent rumen cannulae and fed on a diet comprising (DM basis) of 550 g.kg^{-1} alfalfa hay, 400 g.kg^{-1} barely grain, 48 g.kg^{-1} wheat bran and 2 g.kg^{-1} lime stone at maintenance level. Rumen fluid was collected after the morning feeding and pumped with a manually operated vacuum pump and transferred into pre-warmed thermos flask, combined, filtered through four layers of cheesecloth and flushed with CO_2. Each feed sample was incubated in six replicates with 20 ml of rumen liquor and buffer solution (1:2). Six vials were used as blank samples. The vials were sealed immediately after loading and were affixed to a rotary shaker platform (lab-line instruments Inc. Melors dark, USA) set at 120 rpm and housed in an incubator. Amounts of cumulative gas production were recorded at 2, 4, 6, 8, 10, 12, 16, 24, 36, 48, 72 and 96 h of incubation using a water displacement apparatus (Fedorak and Hurdy, 1983).

Cumulative gas production data were fitted to the model of Ørskov and McDonald (1979):

$P = A\ (1\text{-}e^{-ct})$

where p is the gas production at time t, "*A*" is the gas production of soluble fraction and potentially degradable fraction, and "c" is the gas production rate.

The metabolizable energy, short chain fatty acids and digestible organic matter in dry matter of silages were calculated using equations of Menke and Steingass (1988) and Getachew *et al.* (2002) as follows:

DOM (% DM) = 9.00 + 0.9991 GP + 0.0595 CP + 0.0181 ash (n = 200, $r^2 = 0.92$)

ME (MJ/kg DM) = 0.016 DOMD

SCFA (mmol/200 mg DM) = 0.0222 GP - 0.00425

where: DOMD is digestible organic matter in a dry matter; GP is 24 h net gas production (ml /200mg DM); CP and ash (% DM).

Statistical analysis

Experimental data were performed using GLM procedure of SAS (1991) for completely randomized design. Data of each mini-silo of the three individual silos within each treatment were averaged. Mean values of each individual silo within each treatment (three silos of each) were used as the experimental unit, and the statistical model was:

$Y_{ij} = \mu + T + e_{ij}$

Dependent variable representing the response for i treatment; μ = mean; T = treatment and e_{ij} = residual. The means were compared using Duncan's test used for the multiple comparisons among mean values for the treatments.

RESULTS AND DISCUSSION

Silage characteristics

Chemical composition and microbial counts (log10.cfu.g^{-1} fresh silage) of silages after 90 days of ensiling are outlined in Table 2. The higher protein content of silages supplemented with urea and PBM in comparison with control silage was undoubtedly due to the addition of urea and PBM. The pH value of the control and PBM silages was lower ($P<0.05$) than urea silage. Fegeros *et al.* (1994) reported that citrus pulp contains high amount of pectin and soluble carbohydrates. As a result, the fermentation of high soluble carbohydrates in this by-product leads to low final pH in the both control silage and silage supplemented with PBM (McDonald *et al.*, 1991). Its high soluble carbohydrates lead to a rapid production of alcohol as well as of VFA's and lactic acid. In our study, control silage and silage supplemented with PBM had considerable amounts of lactic acid (Table 2). Low pH and high lactic acid in the citrus pulp silages has been reported by Gado *et al.* (2011). Nevertheless, pH values of control silage and silage supplemented with PBM were near to required value for acceptable preservation of silage containing such high DM content (McDonald *et al*., 1991). Increased pH of silage supplemented with urea may be due to the extensive conversion of urea to ammonia-N resulting in high ammonia-N concentration (McDonald *et al*., 1991). In addition, CP content of silage supplemented with PBM was higher than in the control silage but ammonia-N was not affected by the addition of PBM to silage. Reduced protein degradation in silage with PBM was demonstrated by lower NH_3-N in silage supplemented with PBM versus silage supplemented with urea. This result indicated that the silage microorganisms were not capable to break down and convert the PBM protein to ammonia-N.

Highest mean value of total bacteria (TB) population was observed in silage supplemented with urea ($P<0.05$), whereas lactic acid bacteria (LAB) population was the lowest ($P<0.05$) in the silage supplemented with urea. Yeast counts were not affected ($P = 0.89$) by different N-supplements. It is noticeable that no yeasts and moulds were observed in silage supplemented with urea supplementation cultures. Highest mean value of the TB population in silage supplemented with urea with lower lactic acid bacteria (LAB) population could increase the silage pH and preservation of silage from spoilage (Table 2). The sufficient amount of lactic acid indicates the proper silage quality in this experiment. Holzer *et al*. (2003) suggested that lactic acid fermentation is a suitable method to preserve silage from spoilage

and pathogenic organisms, such as yeasts, moulds, enterobacteria and clostridia. The preservative effect is chiefly due to acid production and pH decline but is also a result of diminishing the oxidation-reduction potential and competition for essential nutrients. It is also possibly caused by the production of inhibitory compounds (Bonestroo *et al.*, 1993). High pH observed in silage supplemented with urea in our study was similar to the results of Sinclair *et al.* (2004), who added urea (20 g/kg DM) to crop silage and found that the final pH increased up to 8. Lactic acid bacteria were effective bacteria on the final fermentation products of silage. These bacteria produce more lactic acid and less acetic acid and butyric acid, resulting in low final pH of silage (Keles and Demirci, 2010).

Lower LAB and TB counts in control silage and silage supplemented with PBM compared to silage supplemented with urea might be due to microbial cell death (Inglis *et al*., 1999), also LAB contains an inhibitory effect on various gram-negative and gram-positive bacteria by their production of hydrogen peroxide and bacteriocins (Chateau *et al*., 1993). Danner *et al.* (2003) suggested that lactate and acetate in the silage have antimicrobial effects and proposed that lactate and acetate have a lipophilic character; that results in agglutination of acid molecule which penetrates the bacterial plasma membrane.

Yeast counts were not affected (P = 0.89) by introducing different N-supplements. High numbers of yeasts and molds in control silage and silage supplemented with PBM compared to silage with urea supplementation may be due to higher level of

Table 2: Chemical and microbial composition of orange pulp[1]

	Control silage	Silage supplemented with PBM	Silage supplemented with Urea	SEM
Dry matter (g/kg)	276. 5^{b}	311. 2^{a}	270^{b}	5.45
pH	4.14^{c}	4.29^{b}	8.13^{a}	0.70
Analysis (g/kg DM)				
Organic matter	898.61^{a}	904.42^{a}	844.38^{b}	7.60
Crude protein	63.00^{c}	196.40^{a}	148.00^{b}	1.97
Ether extract	10.39^{c}	36.38^{a}	18.51^{b}	3.84
Neutral detergent fiber	558.46^{a}	357.85^{b}	589.89^{a}	38.10
Acid detergent fiber	311.74^{a}	217.46^{b}	336.15^{a}	20.10
Total protein (g/kg total N)	540.46^{b}	824.92^{a}	464.54^{c}	54.90
NDIN (g/kg total N)	34.80^{b}	9.40^{a}	19.59^{c}	11.54
ADIN (g/kg total N)	16.82^{a}	6.64^{c}	10.68^{b}	1.49
NH_3-N (g/kg total N)	48.20^{b}	16.41^{c}	256.75^{a}	37.08
Lactic acid	37.20^{a}	30.00^{b}	12.46^{c}	3.68
Acetic acid	5.71^{c}	7.53^{b}	10.20^{a}	0.68
Propionic acid	2.77^{b}	5.53^{a}	5.51^{a}	0.48
Butyric acid	2.25^{b}	3.81^{a}	3.53^{a}	0.31
Total Fatty acid	47.61^{a}	47.19^{a}	30.06^{b}	3.01
Lactic acid : Acetic acid ratio	6.53^{a}	3.99^{b}	1.22^{c}	0.78
Enumerations (log cfu/g fresh silage)				
Total bacteria	3.48^{b}	3.50^{b}	3.65^{a}	0.02
Lactic acid bacteria	3.45^{a}	3.47^{a}	3.38^{b}	0.01
Yeasts	2.33	2.39	no	0.21
Molds	4.00	2.30	no	0.51

Means within a row with different subscripts differ (P<0.05).
[1]Control silage, orange pulp plus straw; silage supplemented with PBM, orange pulp plus straw plus poultry by-product meal; silage supplemented with Urea, orange pulp plus straw plus urea.
NDIN = Neutral detergent insoluble nitrogen, ADIN = Acid detergent insoluble nitrogen; [4]Standard error of the mean; no: not observed.

lactic acid in these silages. Kung *et al.* (2003) suggested that lactic acid exhibited lower activity for inhibition of growth the yeasts and moulds. It is noticeable that no yeasts and moulds were observed in silage with urea (Table 2), and it is likely due to the higher concentration of acetic acid and lower concentration of lactic acid in the silage supplemented with urea. Acetic acid is a known fraction inhibitor of both yeasts and moulds (Woolford, 1975), as well as it has been reported that acetic acid is one of the most effective substances for inhibition of spoilage microorganisms. Acetic acid in silages is necessary to successfull preservation the silages after the silos when they opened (Danner *et al.*, 2003). Inoculants containing *Lactobacillus buchneri*, a heterofermentative LAB aim to promote in acetic acid are specifically designed to reduce aerobic deterioration of silage by yeasts and moulds (Kung and Ranjit, 2001).

***In situ* DM and CP disappearance**

Soluble (*a*) and insoluble (*b*) fractions of DM were affected by N-supplementation (P<0.05, Table 3). Dry matter degradation rate (*c*) of the b fraction for all silages showed similar values (P = 0.10). The effective degradability of DM (EDDM) was highest for silage supplemented with PBM at two rumen flow rates (P<0.05). The results of DM degradability show that true protein content of the silages increased the EDDM and positively affected by PBM, however, in silage supplemented with urea true protein content did not increase, therefore EDDM was not influenced by increased CP content. These results are conflicting with those of Ramírez *et al.* (2004), who reported that CP content of grasses positively influenced EDDM, because this investigator reported, that with increasing of CP the EDDM content was also increased. Likewise, the high EDDM in silage supplemented with PBM may be related to lower NDF and ADF content in comparison to other silages. This result was in agreement with Woods *et al.* (2003), who found that there was high negative correlation between EDDM and the ADF and NDF components of the diet.

At two outflow rates the effective degradability of CP (EDCP) silage supplemented with urea was higher (P<0.05) than the other silages. According to this result, the "*c*" values were not affected by N-supplementation (P = 0.12). Woods *et al*. (2003) suggested that the "*c*" value of the "*b*" fraction has

Table 3: Dry matter and crude protein disappearance (g/g incubated) and estimated parameters of silage[1]

	Control silage	Silage supplemented with PBM	Silage supplemented with Urea	SEM
DM degradation[2]				
a	0.146[b]	0.152[b]	0.172[a]	0.0410
b	0.472[a]	0.450[a]	0.386[b]	0.0178
c (per h)	0.038[a]	0.031[a]	0.037[a]	0.0050
a + *b*	0.618[b]	0.651[a]	0.558[c]	0.0143
EDDM (0.03/h)	0.417[b]	0.437[a]	0.382[c]	0.0820
EDDM (0.05/h)	0.360[b]	0.384[a]	0.326[c]	0.0871
CP degradation[2]				
a	0.196[b]	0.050[c]	0.474	0.062
b	0.431[a]	0.444[a]	0.296[b]	0.023
c (per h)	0.041	0.043	0.048	0.061
a + *b*	0.496[c]	0.496[b]	0.770[a]	0.039
EDCP (0.03/h)	0.446[b]	0.313[c]	0.684[a]	0.005
EDCP (0.05/h)	0.391[b]	0.256[c]	0.65[a]	0.059

Means within a row with different subscripts differ (P<0.05).
[1]Control, orange pulp plus straw; silage supplemented with PBM, orange pulp plus straw plus poultry by-product meal; silage supplemented with urea, orange pulp plus straw plus urea.
[2]a = rapidly degraded fraction, b = slowly degraded fraction, a + b = maximum potential of degradability in the rumen. c = rate of degradation (per h), EDDM (0.03/h) = effective degradability of DM with passage rate of 0.03/h, EDDM (0.05/h) = effective degradability of DM with passage rate of 0.05/h. EDCP (0.03/h) = effective degradability of CP with passage rate of 0.03/h, EDCP (0.05/h) = effective degradability of CP with passage rate of 0.05/h. [4]Standard error of the mean.

a dramatic role in the determination of effective degradability. In the present study degradation rates were not influenced by N-supplementation, but the "*b*" values have significant difference between the silages; so that the "*b*" values showed a major role in determination of EDCP. Silage supplemented with urea had a higher "*a*" fraction and lower "*b*" value than the other silages and the EDCP value was higher for silage supplemented with urea, resulting in similar "*c*" values; "*a*" fraction had significant effect on EDCP. Also this fact was observed for silage supplemented with PBM and control, where the "*b*" value was low and resulted in decreased EDCP at two outflow rates.

Ruminal, post-ruminal and total tract protein disappearance

The results indicated an effect (P<0.05) of N-supplementation on ruminal, post-ruminal and total tract protein disappearance of silages (Tables 4). Silage supplemented with urea had the highest value for ruminal CP disappearance (P<0.05), whereas the highest (P<0.05) post-ruminal protein disappearance of ruminal-undegraded protein was observed in silage supplemented with PBM. Highest post-ruminal protein disappearance in silage supplemented with PBM supplementation may be due to the low NDF and ADIN. Van Soest (1994) suggested that the differences in ruminal and post-ruminal protein disappearance of the tropical feed sources may be due to differences in NDF and ADIN between these feeds. Low ruminal CP disappearance was compensated by digestion in post ruminal; resulting in a higher total tract disappearance in silage supplemented with PBM. Lashkari and Taghizadeh (2012) reported that in the citrus by-product such as sweet lemon pulp with low ruminal CP disappearance, the post ruminal disappearance has been high and this result may be due to the compensatory digestion in small intestine. For silage supplemented with PBM, higher values for post ruminal (P<0.05) showed that this silage can escape the rumen fermentation and supply the rumen undegradable protein requirement. The highest total tract CP disappearance in silage supplemented with urea was consistent with the result of Danesh Mesgaran and Stern (2005), who found that in maize silage treated with 24 g urea kg^{-1} DM had higher total tract protein disappearance compared with the other treatment.

***In vitro* gas production**

Potential (*A*) and rates (*c*) of gas production differed (P<0.05) among the silage treatments (Table 5). The "*A*" and "*c*" values were lowest (P<0.05) for silage supplemented with urea. Short chain fatty acid (SCFA) and ME in control silage and silage supplemented with PBM was higher (P<0.05) than silage supplemented with urea (P<0.05). Silage supplemented with PBM provides more potential gas production which is fermentable energy source. As an expected CP contents had an effect on gas production after 24 h of incubation. It has been reported that protein degradation influences gas production (Cone and Van Gelder, 1999). The CP content may have effect on gas production after 12 or 24 h of incubation that was also reported by Chenost *et al.* (2001). Differences in total gas production between silages could be explained by the differences in SCFA production and molar proportion of SCFA (Beuvink and Spoelstra, 1992). Likewise, because of the low contents of NDF and ADF the silage supplemented with PBM has high "*A*" value. This result was supported by the result of Gurbuz (2007) that estimated parameters and ME were negatively correlated with NDF and ADF, which is slowly fermented by microorganisms. In addition, it can be clearly observed that NDF and ADF in control silage and silage supplemented with urea were higher than silage supplemented with PBM. An increase in NDF and ADF resulted in the low gas production rate (*c*) in control silage and silage supplemented with urea. This result

Table 4: Ruminal, post-ruminal and total tract protein disappearance (g/g incubated) of silage[1]

Item	Control silage	Silage supplemented with PBM	Silage supplemented with urea	SEM
Ruminal[2]	0.487[b]	0.208[c]	0.621[a]	0.0609
Post-ruminal[3]	0.389[b]	0.619[a]	0.277[c]	0.0502
Total tract protein disappearance[4]	0.827[b]	0.876[a]	0.899[a]	0.0110

Means within a row with different subscripts differ (P<0.05).
[1]Control silage, orange pulp plus straw; silage supplemented with PBM, orange pulp plus straw plus poultry by-product meal; silage supplemented with urea, orange pulp plus straw plus urea.
[2]CP disappearance after 12 h incubation in the rumen using a nylon bag technique.
[3]Rumen- undegradable protein digestibility determined by the three-step procedure.
[4]Summation of ruminal and post ruminal.

Table 5: Estimated parameters and fermentation characteristics using *in vitro* gas production of silage[1]

	Control silage	Silage supplemented with PBM	Silage supplemented with urea	SEM
A	300.93[b]	322.25[a]	300.06[b]	3.97
c (per h)	2.90[b]	3.00[a]	2.00[c]	0.16
GP	182.16[a]	181.71[a]	96.16[b]	9.89
DOMD	459.78[a]	467.88[a]	294.15[b]	19.59
ME	7.35[a]	7.48[a]	4.70[b]	0.31
SCFA	0.80[a]	0.80[a]	0.42[b]	0.04

Means within a row with different subscripts differ (P<0.05).
[1]Control silage, orange pulp plus straw; silage supplemented with PBM, orange pulp plus straw plus poultry by-product meal; silage supplemented with urea, orange pulp plus straw plus urea.
A = potential gas production (ml/g DM); *c* = fractional rate of gas production (per h); GP = 24 h cumulative gas production (ml/g DM); DOMD: digestible organic matter in dry matter (g/kg DM); ME = metabolizable energy (MJ/kg DM); SCFA short chain fatty acid (mmol /200 mg DM)

was consistent with the findings of Kamalak (2006) who found that "*c*" value was negatively correlated with NDF and ADF. This suppressing effect probably results in the attachment of ruminal microorganisms to feed particles (McAllister *et al.*, 1994). Control silage and silage supplemented with PBM possess high DOM amounts; the resulted silage with higher gas production contains higher DOM.

CONCLUSION

The nutritive value of orange pulp was improved by the addition of PBM and urea, whereas control silage and silage with PBM were preserved, what can be illustrated by the proper fermentation characteristics, such as low pH, acetic, butyric acids and high lactic acid. Result indicated that LAB and TB counts were not affected by adding the PBM. Various microorganisms present in silage may affect the nutritive value of silages. LAB was probably mainly responsible for lowering the pH during ensiling. Fermentative products and microbiological assessments of silage can help us to define the type of fermentation that occurred in the silo.

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