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Influence of wheat bran as a silage additive on chemical composition, in situ degradability and in vitro gas production of citrus pulp silage

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The effect of wheat bran (WB) as a silage additive on chemical composition, in situ degradability and in vitro gas production of citrus pulp silage (CPS) was investigated. The whole fresh citrus pulp was manually chopped and used as untreated or treated with 6, 12, or 18 g WB/kg fresh citrus pulp for ensiling. The data were analyzed in a completely randomized design that showed experimental treatments had no significant effect on DM% of silages but with increasing WB, crude protein (CP%) linearly and quadratically increased (P < 0.05). Ammonia nitrogen, ether extract (EE%), ash% and pH were similar among treatments (P > 0.05). Result showed that with increasing WB, NDF concentration linearly increased (P < 0.05) but acid detergent fiber (ADF) concentration linearly and quadratically decreased among treatments (P < 0.05). Aerobic stability of silage exhibited a negative linear and quadratic relationship (P < 0.05), with increasing the level of WB. Data of flige point displayed that all treatments had very good quality. Data of in situ degradability indicated that soluble degradable fraction (a) was significantly higher in control group (P < 0.05) and with the addition of WB to silage, (a) parameter significantly decreased (P < 0.05). Non-soluble degradation fraction (b) was not affected by different treatments (P > 0.05). The fractional degradation rate (c) with increasing of WB significantly increased (P < 0.05). Potential degradability (a + b) of silages with increasing of WB linearly decreased (P < 0.05). Effective degradability (ED) of silages was not affected by different treatments (P > 0.05). Potential gas production (b) and fractional rate of gas production (c) demonstrated a linear and quadratic relationship (P < 0.05) with increasing WB. The organic matter digestibility (OMD), net energy (NEI), metabolisable energy (ME) and short chain fatty acids (SCFA) were significantly different between control group and treatments with WB (P < 0.05). This data suggest that the addition of WB to CPS can improve the nutritional value of citrus pulp silage without any adverse effects on this byproduct.

Key words: Aerobic stability, citrus pulp silage, silage additive, degradability.

INTRODUCTION

Feeding by-products from the crop and food processing industries to livestock is a practice as old as the domestication of animals by humans. Increased disposal

costs in many parts of the world have increased interest in utilization of citrus by-product feedstuff as alternative feeds for ruminants. It has two important advantages these being to diminish dependence of livestock on grains that can be consumed by humans, and to eliminate the need for costly waste management programs. This second advantage has become very important in recent years, as the world human population and the amount of crop and food by-product has increased, particularly in

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Table 1. Chemical composition of fresh citrus pulp before ensiling.

Item	DM	СР	EE	NDF	ADF	ASH	рН
(DM%)	12.5	10.61	3.5	26.3	25.8	5.33	4.33

developed countries (Grasser et al., 1995). The main citrus 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 of fresh citrus fruit with 600 to 650 g dry matter (DM)/kg peel, 300 to 350 g/kg pulp and 0 to 100 g/kg seeds (Ensminger et al., 1990; Martinez-Pascual and Fernandez-Carmona, 1980a).

Citrus pulp is a valuable, high energy by-product that can partly replace cereal grains in sheep rations without adverse effect on milk yield or composition (Fegeros et al., 1995). Leiva et al. (2000) reported that citrus by-product feedstuff can be used as a high energy feed in ruminant rations to support growth and lactation, with fewer negative effects on rumen fermentation than starch rich feeds. A large amount of the citrus by-product feedstuff is suitable for inclusion in ruminant diets because of the ability of ruminants to ferment high fiber feeds in the rumen (Grasser et al., 1995).

Ruminant feeding systems based on locally available by-product feedstuffs (BPF) are often a practical alternative because, the rumen microbial ecosystem can utilize BPF, which often contain high levels of structural fiber to meet their nutrient requirements for maintenance, growth, reproduction, and production (Arthington et al., 2002). 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. 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 (Chaudhry and Naseer, 2006).

Fermentation in the silo can be a very uncontrollable process leading to less than 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. Silage additives have been classified into various categories. In order for a silage additive to be useful, it must increase DM (nutrient) recovery, improve animal performance [milk (quantity and/or composition), gain, body condition, and reproduction] or decrease heating and molding during storage and feedout. Changes in fermentation end products without quantifiable improvements in one or more of these categories are not acceptable. One group of silage additives is adsorbents. These additives have water absorption properties and thus supplement to

forage with low DM content, in order to reduce nutrients wastage and eutrophication of surface waters by silage leachate. In this study, WB was added to citrus pulp silage (CPS) for water absorbability of this pectin feedstuff, because DM content of CPS is very low and it is difficult to ensile this high moisture by-product without the addition of a dry source. The objective of the present study was to evaluate the effects of different levels of wheat bran (WB) on chemical composition, fermentational properties, *in situ* degradability, and *in vitro* gas production of CPS.

MATERIALS AND METHODS

Silage preparation and sampling

The experiment was arranged as a completely randomized design with 3 replications. The whole fresh citrus pulp was collected from a local juice factory and it was manually chopped (4 to 5 cm length) and used as untreated or treated with WB at 6, 12, and 18 g/kg fresh citrus pulp. Chemical composition of utilized fresh citrus pulp 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 contents of each opened silo were thoroughly mixed and samples were collected for chemical analysis.

Determination of pH and chemical analysis

For measurement of pH, 50 g samples of silage from each treatment were diluted with 450 ml sterile deionized water and blended for 2 min, strained through four layers of cheesecloth, and pH determined immediately by a pH meter (Model 691, Metrohm). The 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 2 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 (1990). 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):

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

Aerobic satiability

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 each covered with a double layer of cheesecloth and stored at ambient temperature (20 to 22°C, for 7 days). Ambient temperature

Table 2. Feed ingredients of the diet of steers.

Ingredient	(g/kg DM of diet)
Alfalfa	300
Corn silage	200
Concentrate	500
Composition of concentrat	e (g/kg DM)
Barley grain	550
Canola meal	150
Wheat Bran	280
Vitamin- mineral mix	10
Calcium carbonate	5
Salt	5
Chemical composition (g/k	g DM)
CP	133
NDF	372
ADF	223
ME(Mcal/kg)	2.62
Ca	10
Р	6

was also simultaneously measured at 15 min intervals during this period (Baah et al., 2011).

In situ 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 x 18 cm², pore size 50 μ m) were filled with 5 g 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. 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 relative 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⁻¹.

In vitro gas production

In vitro gas production was carried out using the method as described by Menke and Steingass (1988). Samples (200 mg) were weighed into 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_2 . Rumen fluid was collected after the morning feeding from two adult ruminally fistulated sheep (42 \pm 2.5 kg body weight), strained through four layers of cheesecloth, and flushed with CO_2 . The syringe was then filled with 30 ml of medium consisting of 10 ml rumen fluid and 20 ml buffer solution. All handling was under continuous flushing with CO_2 . The syringes were placed in 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 nonlinear 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), and 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, 2000). Organic matter digestibility (OMD) was estimated using 24 h gas production as well as the CP and ash contents of the feeds as described by Menke and Steingass (1988). Short chain fatty acids were predicted according to the method of Getachew et al. (2002).

Statistical analysis

The data were analyzed in a completely randomized design using the MIXED procedure of SAS (2000). Comparison of means was performed according to the least square differences (LSD) test. The model used, was:

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

Where, μ = The common mean, T_i = the effect of treatments and e_{ij} = the random error. Levels of significance of linear and quadratic contrasts are presented.

RESULTS

Chemical composition and fermentational properties

The chemical composition and fermentational properties of experimental silages are given in Table 3. Experimental treatments had no significant effect on DM% of experimental silages (P > 0.05). Data showed that CP concentrations were significantly different among treatments, that with increasing WB in silage, CP concentration linearly and quadratically increased too (P < 0.0.5). Ammonia nitrogen concentration, EE%, ash%, and pH were similar among treatments (P > 0.05). The NDF concentration indicated a positive linear relationship (P < 0.05) with increasing WB in CPS. The ADF concentration decreased linearly and quadratically (P < 0.05) with increasing WB in CPS.

Aerobic stability of silage exhibited a negative linear and quadratic relationship (P < 0.05) with increasing level of WB. Data of flige point (Table 3) indicated that silage quality was similar among treatments.

In situ degradability of silages

Data of degradation parameters DM of different treatments are presented in Table 4. Data indicated that soluble degradability fraction (a) was significantly higher in control group (P < 0.05), and with addition of WB to silage, (a) parameter significantly decreased (P < 0.05) but among silages with WB, with increasing of WB, this parameter linearly increased (P > 0.05). However, (a) parameter displayed a linear and quadratic relationship

Table 3. Chemical composition and fermentational properties of different treatments.

Itam			Treatment ¹	Significance (P-value)				
Item	С	6 g WB	12 g WB	18 g WB	SEM	Treat	Linear	Quadratic
DM%	11.82	12.40	13.01	13.40	1.107	0.767	0.358	0.933
CP%	11.25 ^b	11.55 ^b	11.58 ^{ab}	11.98 ^a	0.090	0.039	0.011	0.610
EE%	3.60	4.00	3.00	3.00	0.550	0.485	0.282	0.704
NDF%	26.00 ^d	27.70 ^c	28.10 ^b	30.10 ^a	1.083	< 0.0001	< 0.0001	0.072
ADF%	24.00 ^a	23.59 ^b	23.12 ^c	23.09 ^c	1.056	0.003	0.0009	0.037
Ash%	6.16	7.00	6.65	5.99	0.251	0.171	0.505	0.057
рН	3.91	3.84	3.95	3.89	0.045	0.520	0.837	0.959
NH ₃ -N (mg/dl)	0.00	0.01	0.01	0.01	0.011	0.799	0.546	0.525
Aerobic stability	39.75 ^a	26.75 ^b	22.90 ^c	21.80 ^c	0.479	0.0003	0.0001	0.001
Flieg point	87.24	92.40	85.27	90.32	-	-	-	-
Quality	Very good	Very good	Very good	Very good	-	-	-	-

¹Control = Silage untreated by wheat bran, WB: wheat bran. ^{a, b, c}Means in the same row with different superscripts are significantly different with P < 0.05.

Table 4. Degradation parameters for the different experimental treatments.

Down detien neverter ²	Treatment ¹					Significance (P-value)		
Degradation parameter ²	С	6 g WB	12 g WB	18 g WB	SEM	Treat	Linear	Quadratic
a (mg/g)	510.75 ^a	412.65 ^d	441.30 ^c	485.70 ^b	12.24	0.0003	0.026	<0.0001
<i>b</i> (mg/g)	518.75	571.19	519.05	491.00	14.18	0.093	0.121	0.064
$c (h^{-1}\%)$	0.05^{c}	0.08 ^a	0.085 ^{ab}	0.09 ^a	0.001	0.0008	0.0004	0.003
Potential degradability (a + b) (mg/g)	998.50 ^a	984.45 ^{ab}	960.40 ^b	976.70 ^b	11.50	0.077	0.038	0.076
Effective degradability (%) ($Kp = 0.05$)	76.94	77.10	76.13	80.22	0.957	0.064	0.056	0.057

 $^{^{1}}$ Control= Silage untreated by wheat bran, WB: wheat bran. ^{2}a and b represent soluble and non-soluble degradable fractions, respectively; c is the fractional degradation rate of the b fraction for DM, Kp is the passage rate. $^{a, b, c}$ Means along same row bearing different superscripts are significantly different (P < 0.05).

(P < 0.05) with increasing WB. The non-soluble degradable fraction (b) was not significantly different among treatments (P > 0.05). The fractional degradation rate (c) exhibited a positive linear and quadratic relationship (P < 0.05) with increasing level of WB. Potential degradability (a + b) of silages demonstrated a negative linearly relationship (P < 0.05) with increasing WB to CPS. Experimental treatment had no significant effect on effective degradability of experimental silages (P > 0.05).

In vitro gas production

Data of gas production at different times of incubation and gas production parameters, OMD, NE_I, ME and short chain fatty acids (SCFA) are presented in Table 5. Gas production of silages at different times of incubation showed a linear and quadratic relationship (P < 0.05) with increasing WB at all times of incubation except in 2 and 4 h of incubation. Similarly, potential gas production (b) and

fractional rate of gas production (c) demonstrated a linear and quadratic relationship (P < 0.05) with increasing WB. The OMD, NE_I, ME and SCFA were significantly different among control group and treatments with WB (P < 0.05), but these variables were not affected by different levels of WB in CPS. These parameters displayed a linear and quadratic relationship (P < 0.05) with increasing WB.

DISCUSSION

Chemical composition and fermentational properties

Data showed that DM was not affected by treatments. In this study, by increasing WB in silage, CP linearly increased, which might be due to higher CP concentration in WB to CPS (17.3% versus 6.9%) (NRC, 2001). An increase was observed in the NDF concentration of silages with increasing WB, but ADF concentration decreased. Perhaps, this resulted from more NDF and less ADF concentration in WB. The aerobic stability of experimental

Table 5. Gas production and the estimated OMD, NE_I, ME, and short chain fatty acids (SCFA) of different experimental silages.

It a ma			Treatment ¹	Significance (P-value)				
Item	С	6 g WB	12 g WB	18 g WB	SEM	Treat	Linear	Quadratic
2 h incubation	6.73 ^b	15.26 ^a	15.38 ^a	15.81 ^a	0.603	0.0001	<0.0001	0.0004
4 h incubation	35.89	40.86	41.86	48.32	5.025	0.442	0.719	0.230
6 h incubation	82.86 ^b	104.61 ^a	109.45 ^a	108.21 ^a	4.891	0.026	0.023	0.021
8 h incubation	120.39 ^b	167.60 ^a	176.30 ^a	172.33 ^a	3.943	0.0002	0.0002	0.0003
12 h incubation	151.22 ^b	207.88 ^a	215.09 ^a	203.41 ^a	4.907	0.0003	0.0003	0.0004
24 h incubation	216.70 ^b	248.26 ^a	259.45 ^a	248.89 ^a	3.741	0.0009	0.0007	0.001
36 h incubation	233.85 ^b	264.67 ^a	277.34 ^a	267.15 ^a	4.232	0.001	0.0006	0.001
48 h incubation	241.18 ^c	273.24 ^b	288.40 ^a	278.83 ^{ab}	4.374	0.0007	0.0004	0.001
72 h incubation	243.67 ^c	276.97 ^b	293.87 ^a	283.68 ^{ab}	4.741	0.0008	0.0004	0.002
96 h incubation	249.51 ^c	281.32 ^b	297.73 ^a	288.53 ^{ab}	5.521	0.001	0.0006	0.004
b (ml)	253.00 ^c	276.97 ^b	296.11 ^a	286.68 ^{ab}	3.590	0.010	0.004	0.017
c (ml/h)	0.07 ^c	0.09 ^a	0.08 ^b	0.08 ^b	0.000	0.001	0.002	0.0006
OMD (%)	61.89 ^b	68.82 ^a	70.28 ^a	68.53 ^a	0.991	0.028	0.017	0.022
NE _I (MJ/kg)	5.28 ^b	6.05 ^a	6.13 ^a	5.89 ^a	0.094	0.021	0.014	0.016
ME (MJ/kg)	8.69 ^b	9.66 ^a	9.92 ^a	9.71 ^a	0.146	0.023	0.012	0.022
SCFA (mmol)	1.11 ^b	1.27 ^a	1.37 ^a	1.31 ^a	0.039	0.021	0.010	0.026

 1 Control = Silage untreated by wheat bran, WB: wheat bran, b: Potential gas production (ml/g DM); c: Fractional rate of gas production ($^{-1}$); OMD: organic matter digestibility; NE_i: net energy; ME: metabolisable energy: SCFA: short chain fatty acids. $^{a, b, c}$ Means in the same row with different superscripts are significantly different with P < 0.05.

silages with increasing WB linearly decreased, as a result of raising soluble carbohydrate content in the silages. More carbohydrates in the silage might motivate the growth of fungi or molds. Subsequently, with this activity, the temperature of silage has been increased. Data showed that pH and ammonia nitrogen were not affected by treatments because of enough soluble carbohydrate for silage fermentation in different treatments.

Arbabi et al. (2008) reported that by adding sugar beet pulp to CPS, DM of silages increased. They found that CPS with 5% DM sugar beet pulp had lower ADF, and higher NDF and CP concentration, compared with control group. Moreover, they showed that the addition of sugar beet pulp to CPS had no effect on silage pH. Chaudhry and Naseer (2006) added a mixture of poultry litter and corn forage to fresh citrus pulp and ensiled them. They concluded that composition of initial and ensiled mixtures were similar except that DM and CF contents decreased after ensiling. They suggested that, the decrease in cell wall constituents after ensiling may be due to the action of bacterial enzymes in hydrolyzing cell wall components, especially for the more digestible constituent of plant cell wall. In their study, inclusion of citrus pulp caused linear decrease in DM, CP, CF (crude fiber) and ash contents of the mixtures, and they concluded that these decreases were mainly attributed to the difference in chemical composition of citrus pulp. Similar results have been reported by Migwi et al. (2001) who ensiled fresh citrus pulp with high dry matter agro-industrial waste. Kordi et al. (2010a) found that by adding of 6, 12 or 18 g barley grain/kg of CPS, DM of silages significantly increased but ash concentration and pH were not affected. Also, they indicated that aerobic stability of silages significantly decreased with increasing amounts of barley grain. In another study, Kordi et al. (2010b) reported that adding of 6, 12, or 18 g/kg molasses to 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 degradability

As shown in Table 4, control group had highest soluble degradable fraction (a) and the silage with 18 g WB had the highest (a) parameter among silages with WB. Nonsoluble degradable fraction (b) of experimental silages was highest for silage with 6 g WB. Fractional rate parameter (c) was significantly higher for the treatment with 18 g WB. The control group had highest potential degradability among the treatments, which can be related to structural differences among CPS and WB for degradation. Effective degradability of experimental silages was highest for CPS, with 18 g WB. Possible reason for these findings can be the structural differences between pectin in CPS and WB, and different content of different components of these by-products, for example NDF concentration in CPS is lower than WB, but nonfiber carbohydrate (NFC) content in CPS is higher than WB (NRC, 2001).

In vitro gas production

Data of in vitro gas production test (Table 5) indicated that potential gas production for the control group was lower than for the other treatments. In addition, fractional rate of gas production was lowest for untreated silage. this resulted from more Perhaps carbohydrate concentration in silages with WB. Silage without any WB had lowest OMD among the treatments. Data shows that NE_I and ME of silages with WB were higher than control group. Similarly, SCFA of experimental silages significantly increased with increased WB. Possibly, the reason for these findings is related to increasing of carbohydrate, EE, and CP content in silages by increasing WB, causing an increase of OMD and energy content. Furthermore, with increasing WB, the EE content in silage increased too, and this might be the reason for increase of SCFA in silages with WB.

Generally, this study showed that fresh citrus pulp can be ensiled as a feedstuff. In addition, the present study concluded that wheat bran is a useful additive for citrus pulp silage that can improve the nutritional value of this by-product.

Abbreviations: ADF, Acid detergent fiber; NDF, neutral detergent fiber; WB, wheat bran; CPS, citrus pulp silage; DM, dry matter; CP, crude protein; EE, ether extract; OMD, organic matter digestibility; ME, metabolisable energy; SCFA, short chain fatty acids.

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