Journal of Biodiversity and Environmental Sciences (JBES)
ISSN: 2220-6663 (Print) 2222-3045 (Online)
Vol. 3, No. 6, p. 8-16, 2013
http://www.innspub.net

RESEARCH PAPER OPEN ACCESS

Determine of nutritive value of dried citrus pulp various using in situ and gas production techniques

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Article published on June 21, 2013

Key words: Citrus pulp, in situ, gas production, ruminants and wethers.

Abstract

Citrus pulp is an important by-product for sub-tropical and tropical ruminant animal production. In this study two wethers (38 ± 1.5) were used insitu method. Wetheres equipped with ruminal cannulas. Ruminal DM and CP disappearances were measured 0, 4, 8, 12, 24, 36, 48, 72 and 96h. The gas production was measured at 2,4,6,8,12, 24,36 and 48 h. Dry matter degradabilities of Orange, Grape fruit, Lemon and Tangerine at 96h were 92.1, 94.3, 86.3 and 92.1 respectively. Crude protein degradabilities of mentioned citrus pulps were 40.0, 37.1, 31.0 and 35.9 respectively. DM and CP in situ degradability at 96h showed significant differences ($P < 0.05$). Orange pulp showed high level of metabolizable protein (73.97 %) there were significant differences ($P < 0.05$). the most rate of gas production is related to grapefruit pulp showed significant differences ($P < 0.05$). data showed that citrus pulp 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.

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Introduction

Developing food industrial factories consequently produced large amount of wastes and by-products. Damping or burning wastes or agro-industrial by-products causes potential air and water pollution problems. High-moisture wastes are also difficult to burn. Many by-products have a substantial potential value as animal feedstuffs. Feeding by-products of the crop and food processing industries to livestock is a practice as old as the domestication of animals by humans. It has two important advantages (Grasser *et al.*, 1995), these being to diminish dependence of livestock on grains that can be consumed by humans (which was almost certainly the primary original reason), and to eliminate the need for costly waste management programs (which has become very important in recent years as the world human population has increased and the amount of crop and food by-product has increased, particularly in developed countries). Ruminant feeding systems based on locally available by-product feedstuffs (BPF) are often a practical alternative because the rumen microbial ecosysten can utilize BPF, which often contain high levels of structural fibre, to meet their nutrient requirements for maintenance, growth, reproduction and production. The term 'citrus by-product' includes numerous BPF, which vary according to the originating crop and method of production, that are an important component of ruminant feeding systems in many areas of the world (Bampidis and Robinson, 2006).

Citrus pulp contains a variety of energy substrates for ruminal microbes, including both soluble carbohydrates and a readily digestible NDF fraction (Ammerman and Henry, 1991; Ben-Ghedalia *et al.*, 1989; Miron *et al.*, 2001). Therefore, citrus pulp has been previously used as a high energy feed in rations for supporting growth and lactation of cattle (Ammerman and Henry, 1991; Belibasakis and Tsirgogianni,1996; Chapman *et al.*, 1983; Solomon *et al.*, 2000). Information on carbohydrate digestibility of diets containing ingredients such as citrus pulp is needed to optimize use of byproducts by high producing dairy cattle (Miron *et al.*, 2002). The nutritive value of citrus pulp depends on factors such as physical form (wet, dry, ensiled, or pelleted), citrus species, maturity, variety, growing conditions, and processing method, which dictate the proportion of rag (pulp residue), rind, and seed in the mixture (Kale and Adsule, 1995). Dried citrus pulp is typified (Arthington *et al.*, 2002; Bampidis and Robinson, 2006) by high concentrations of pectin (22 to 40%) and a high Ca:P ratio (3.5 1.7% Ca to 0.34 0.07% P), and relatively low concentrations of CP (7.2 0.2%), fat (3 1.0%), NDF (19.3 1.3%), and ADF (16.9 2%; DM basis) (Kim *et al.*, 2007). Pectin is the predominant carbohydrate in DCP, and it is quickly and extensively degraded by ruminal bacteria (Kim *et al.*, 2007). Total world citrus production averaged 69/4 million tonnes/year from 2000 through 2003. Inclusive (USDA/FAS, 2003). The genus Citrus includes several important fruits (Kale and Adsule, 1995), with the most important on a worldwide basis being sweet orange (C. Sinensis : 67/8 % of world citrus production), tangerine (C. Reticulata: 17/9 %), lemon (C. Limon: 6/3 %) and grapefruit (C. Paradisi: 5/0 %) (Bampidis and Robinson, 2006). Dried citrus pulp contains relatively large amounts of pectins and soluble carbohydrates (Rihani *et al.*, 1986) and very limited amounts of available N (Verite and Sauvant, 1981; Lanza, 1982).Increasing the amount of citrus pulp in ruminant rations increased mean acetic acid and widened the acetic/propionic ratio (Wing, 1974).

The in situ technique has been widely used to study ruminal digestion kinetics of feeds for cattle. Although in this technique the incubated feed is not subject to mastication and passage, it is no better way to simulate the rumen environment to study ruminal digestion kinetics (Nocek, 1988). This technique has been reported to be well correlated with animal performance (Orskov, 1989; Khazaal *et al.*, 1993), with voluntary feed intake and in vivo dry matter digestibility (Khazaal *et al.*, 1995). In Brazil, researchers have used the in situ method to evaluate tropical forages, agricultural residues and industrial by-products for feeding cattle (Vilela *et al.*, 1994; Gomes *et al.*, 1994; Aroeira *et al.*, 1995). The in vitro gas production system helps to better quantity the nutrient utilization and its accuracy in describing digestibility in animal has been validated in numerous experiments. Although, gases produced during rumen fermentation are colossal waste products and of no nutritive value to the ruminant, but gas production test are used routinely in feed research as gas volumes are related to both the extent and rate of substrates degradation (Akinfemi *et al.*, 2009). This experiment was designed to determine nutritive value of some citrus by-products using in situ and gas production techniques.

Materials and methods

Animals and feeding

Two yearling (Gizil) wethers (35±1.8 kg) were used. At least 30 d before initiation of the experiment, each wether was surgically fitted with a ruminal cannula. The wethers were housed in tie stalls under controlled environmental conditions with continueus lighting and constant temperature (24 to 26°C). All whether were fed a diet containing of 60% hay and 40% concentrate (NRC, 1989).The feed was fed in equal portions every 8 h to maintain a relatively stable rumen environment.

Sample collection

Orange (C. Sinensis), tangerine (C. Reticulata), lemon (C. Limon) and grapefruit (C. Paradisi) by-products were collected from at least 7 different areas whiten each Juice Company. All 7 samples were thoroughly mixed, and a composite sample (100g) was taken. If the pulps becomes too dry, its protein becomes indigestible from heat damage, because of that, our samples are dried in controlled condition. All samples were dried in an oven at 100°C until a constant weight was achieved. Samples were then ground to pass thought a 2-mm screen in Wiley mill (model 4, Arthur H. Thomas Co., Philadelphia, PA) before incubation.

Chemical analysis

DM was determined by drying the samples at 105° C. Nitrogen (N) content was measured by the Kjeldahl method. Neutral detergent fiber and ADF were measured according to the method of Van Soest *et al.* (1991).

In situ degradation

In situ methods procedures was determined using Nocek (1988) and reviewed by Palangi *et al.* (2012), the ground samples (5g) were placed in Dacron bags (5.5×10 cm;47-μm pore size) and were sealed with waterproof glue. Each feed sample was incubated in 4 replicates (2 replicates for each whether) in the rumen. The incubation times for citrus pulp samples were 0, 2,4,8,12,24,36,48,72 and 96 h. Nylon bags were suspended in the rumen in a polyester mesh bag (25×40 cm;3mm 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 washing until the rinse water remained clear. Samples were then dried in an oven at 55˚C until a constant weight was achieved before determination of DM disappearance. The DM and CP degradation data was fitted to the exponential equation $P = a+b(1 - e^{-ct})$ (Ørskov and Mc Donald, 1979), where P: is the disappearance of nutrients during time t, a: the soluble nutrients fraction which is rapidly washed out of the bags and assumed to be completely degradable, b: the proportion of insoluble nutrients which is potentially degradable by microorganisms, c: is the degradation rate of fraction b per hour and t is time of incubation.

In vitro gas production

Rumen fluid was obtained from two fistulated wethers fed twice daily with a diet containing alfalfa hay (60%) and concentrate (40%). Equal volumes of ruminal fluid from each sheep collected 2 h after the morning feeding squeized through four layers and mixed with McDougall (1948) buffer prewarmed to 39° C. The inoculums was dispensed (20 mL) per vial into 100 mL serum vial (containing of 300 mg sample per vial) which had been warmed to 39° C and flushed with oxygen free CO_2. The vials were sealed immediately after loading and were affixed to a rotary shaker platform (lab-line instruments Inc, Iran) set at (120 rpm) housed in an incubator. Vials for each time point, as well as blanks (containing no substrate), were prepared in triplicate. Triplicate vials were removed after 2, 4, 6, 8, 12, 24, 36 and 48 h of incubation. Cumulative gas production data were fitted to the model of Orskov and McDonald (1979):

$$P = a+b(1 - e^{-ct})$$

Where a: is the gas production from the immediately soluble fraction (ml), b: the gas production from the insoluble fraction (ml), c: the gas production rate constant for the insoluble fraction (b), t: the incubation time (h) and P: the gas production at the time "t".

Calculations and statistical analysis

Data were analyzed as a completely randomized design using a general linear model (GLM) procedure of SAS (1999), with Duncan's multiple range test used for the comparison of means. Feeds were the only sources of variation considered.

Results and discussion

Chemical composition

Nutrient composition of citrus by- products were shown at table. The data show that lemon pomace have greater ash and DM composition and the grapefruit have the lowest DM and ash composition. The ADIN percentage of grapefruit (0/92) was higher and the lemon's ADIN (0/59) was lower than other by- products. The obtained results for CP, NDF, ADF and ASH in consistent with NRC (1994). The obtained DM values in this study were more than Afsharmirzai *et al* (2007), NRC (2001) and lower than Kim *et al* (2007), Kostas *et al* (1995) and Madrid *et al* (1998). The difference between chemical can be resulted from citrus species, maturity variety, growing conditions and processing method.

Table 1. The chemical composition of citrus by-products

pulps	%DM1	%CP2	%EE3	%NDF4	%ADF5	%Ash6	%OM7	%ADIN8
Orange	88.73^{ab}	8.68	0.9^{c}	21.4	18.3^{a}	5.1^{ab}	94.9	0.78^{b}
Grapefruit	87.34^{ab}	8.01	1.0^{bc}	20.9	17.6^{a}	4.0^{b}	96.0	0.92^{a}
Lemon	90.69^{a}	7.82	1.2^{b}	19.7	15.1^{b}	6.9^{a}	93.1	0.59^{c}
Tangerine	91.02^{a}	6.81	1.6^{a}	22.0	17.9^{a}	4.8^{ab}	95.2	0.62^{c}
SEM	0.9129	0.8574	0.0764	0.9899	0.7354	0.7588	0.8636	0.0176

1: Dry matter, 2: Crude protein, 3: Ether Extrant 4: Neutral detergent fiber, 5: Acid detergent fiber, 6: Ash 7: Organic Matter and 8: Acid detergent insoluble nitrogen

Table 2. In situ DM disappearance (% of DM)

pulps	Incubation time (h)									
	0	2	4	8	12	24	36	48	72	96
Orange	46.8b	49.2b	59.4b	72.6b	80.3b	84.9b	88.8b	90.2b	91.0b	92.1b
Grapefruit	50.2a	63.7a	72.8a	80.5a	83.7a	88.4a	91.8a	93.2a	94.0a	94.3a
Lemon	35.1c	39.1c	41.3d	57.0d	61.7d	76.2d	81.1c	83.2c	85.7c	86.3c
Tangerine	45.7b	49.0b	54.7c	69.5c	76.2c	83.0c	87.5b	88.5b	90.8b	92.1b
SEM	0.511	0.772	0.827	0.478	0.916	0.743	1.000	1.206	0.800	0.975

^{a,b,c}: Means within a column with different subscripts differ (p<0.05).

In situ ruminal degradability

The degradability parameters of DM and CP are shown in table 2 and 3. and DM and CP degradation characteristics are shown in table 4. Grapefruit showed high ruminal DM disappearance in all of the incubation times there were significant differences (P < 0.05). And lemon showed the lowest ruminal DM disappearance in all of the incubation times (P <

0.05). The ruminal CP disappearance of orange is higher and the lemon showed lower ruminal CP disappearance there were significant differences ($P < 0.05$). The difference values for ruminal CP disappearance can be resulted from the higher CP composition of the orange. And pH of the lemon was rejected microbial mass in the rumen. Grapefruit showed high value for soluble fraction of DM in compare with other ones. Whereas lemon indicated high value for insoluble fraction (b) compared to other citrus variety ($P < 0.05$). The CP soluble fraction for grapefruit was more than the others, but the CP insoluble fraction of orange was higher than the others there were significant differences ($P < 0.05$). Silva *et al* (1997) showed the values of soluble (a) and insoluble (b) fraction for DM of orange about 30.06 and 69.94 respectively that were differed with our results. Perria and Konzalez (2004) reported 39.5 and 55.5 respectively for CP soluble and insoluble fractions, there were higher than ours. The achieved differences can be depended on the differences in citrus variety, drying processing, climate conditions, maturity, sample size: square area in used nylon bag and microbial contamination.

Table 3. In situ CP disappearance (% of DM)

pulps	Incubation time (h)									
	0	2	4	8	12	24	36	48	72	96
Orange	8.3	13.3^{ab}	16.8^{ab}	21.3^{a}	24.9	28.2^{a}	31.8^{a}	35.7^{a}	36.6^{a}	40.1^{a}
Grapefruit	9.2	14.5^{a}	17.9^{a}	21.0^{a}	23.7	26.5^{ab}	30.1^{ab}	34.8^{a}	35.9^{a}	37.1^{ab}
Lemon	7.6	9.8^{c}	12.5^{c}	17.1^{b}	22.3	25.7^{b}	27.2^{b}	29.0^{b}	30.1^{b}	31.0^{c}
Tangerine	8.1	11.8^{bc}	14.8^{bc}	18.2^{b}	22.9	26.1^{ab}	29.5^{ab}	33.3^{ab}	35.0^{a}	35.9^{b}
SEM	0.511	0.772	0.827	0.478	0.916	0.743	1.000	1.206	0.800	0.976

^{a,b,c}: Means within a column with different subscripts differ (p<0.05).

Table 4. In situ DM and CP degradation characteristics

pulps	DM degradation characteristics				CP degradation characteristics			
	a	b	c	ED	a	b	c	ED
Orange	44.17^{b}	46.58^{b}	0.1070^{b}	46.54^{b}	10.49^{ab}	27.7^{a}	0.0516^{b}	11.18^{ab}
Grapefruit	51.76^{a}	40.71^{c}	0.1556^{a}	54.69^{a}	11.77^{a}	24.93^{ab}	0.0482^{b}	12.35^{a}
Lemon	33.06^{b}	53.22^{a}	0.0652^{d}	34.74^{c}	7.18^{c}	22.97^{b}	0.0738^{a}	7.99^{c}
Tangerine	43.6^{b}	46.84^{b}	0.0878^{c}	45.57^{b}	9.60^{b}	26.18^{a}	0.0468^{b}	10.20^{b}
SEM	0.5195	0.6255	0.0054	0.4605	0.6072	0.9052	0.0054	0.5597

^{a,b,c}: Means within a column with different subscripts differ (p<0.05)

Metabolizable protein

The metabolizable protein parameters are shown in table 5. The data show that the orange and tangerine have highest and lowest MP. At the result of high CP, a and b fraction of orange pulp, this data were achieved. The obtained data for ERDP in consistent with AFRC (1998).

There can be resulted from variety in ruminal microbial yield, feeding level, type, variety, Processing, soluble and insoluble protein.

The gas production study

The data shows that orange have higher gas production at 2h ($P < 0.05$). According to the facts, orange has higher soluble fraction than others. At the 48h of incubation time, grapefruit and orange have higher and lower gas production there were significant differences ($P < 0.05$). Datt and Singh (1995) showed more gas production in feedstuffs can be correlated with high metabolically energy, high fermentable nitrogen for microbial activity, resulting high growth rate and enhanced ruminal biomasses. The achieved data for C fraction was lower than Akinfemi *et al* (2009). However, the obtained results for an insoluble fraction in consistent with Rodrigus *et al* (2009) and Kim *et al* (2007). The high gas yield in grapefruit probably resulted from high soluble CP, supply of N for growth of microorganism and high ruminal fermentation capacity for structural and nonstructural carbohydrate.

Table 5. Metabolizable protein

pulps	Parameters						
	QDP	SDP	RDP	ERDP	UDP	DUP	MP
Orange	9.10[a]	0.60[ab]	9.71[a]	7.88[a]	77.08[a]	68.94[a]	73.97[a]
Grapefruit	9.41[a]	0.46b[c]	9.88[a]	8.00[a]	70.21[c]	62.67[c]	67.77[b]
Lemon	5.61[b]	0.63[a]	6.25[b]	5.13[b]	71.94[b]	64.42[b]	67.69[b]
Tangerine	5.61[b]	0.40[c]	6.94[b]	5.63[b]	61.15[d]	54.69[d]	58.28[c]
SEM	0.469	0.047	0.433	0.340	0.433	0.390	0.173

[a,b,c]: Means within a column with different subscripts differ ($p<0.05$).

Table 6. In vitro gas production (mL g–1 DM)

pulps	Incubation time (h)								parameters	
	2	4	6	8	12	24	36	48	a+b	c
Orange	55.39[a]	88.02[b]	111.88[c]	134.31[c]	181.37[c]	214.22[c]	233.1[c]	250.08[c]	245.73[c]	0.1066
Grapefruit	25.31[c]	94.35[a]	141.64[a]	194.03[a]	253.52[a]	295.26[a]	321.01[a]	343.99[a]	326.75[a]	0.0791
Lemon	45.95[b]	73.37[c]	89.68[d]	106.34[d]	150.29[d]	193.25[d]	212.45[d]	232.99[d]	235.82[d]	0.0939
Tangerine	19.98[d]	70.60[c]	122.1[b]	170.05[b]	227.55[b]	226.4[b]	290.15[b]	312.46[b]	296.82[b]	0.0701
SEM	0.3084	1.7969	1.6672	1.4086	1.4558	2.1806	2.2918	2.2658	1.2247	0.017

[a,b,c]: Means within a column with different subscripts differ ($p<0.05$)

Conclusion

Citrus pulp widely 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.

Acknowledgment

The researcher wishes to thank Yavar Sharafi, Ali Nobakht and Somayyeh Shabestani for their assistance.

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