Journal of Animal and Feed Sciences, 21, 2012, 458–467

Influence of different levels of dried citrus pulp on *in vitro* ruminal fermentation kinetics of total mixed ration in goat rumen inocula

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(Received 14 April 2012; revised version 17 June 2012; accepted 14 September 2012)

ABSTRACT

Inclusion of dried citrus pulp (DCP) at different levels: 0 (control), 10% (DCP10), 20 (DCP20), and 30% (DCP30) of the total mixed ration (TMR) was evaluated by *in vitro* gas production (GP), and ruminal fermentation patterns, in a completely randomized design. Rumen fluid was collected before the morning meal from 8 gestating goats (Boer×Saanen, body weight 3±2.3 kg). GP was recorded at 2, 4, 6, 8, 10, 12, 24, 48, 72, and 96 h of incubation. Ruminal fermentation parameters such as 96 h partitioning factor (PF_{96}), *in vitro* organic matter digestibility (IVOMD), metabolizable energy (ME), short-chain fatty acids (SCFA), and metabolizable energy (ME) were also estimated. Inclusion of DCP linearly increased ($P<0.01$) cumulative gas production at 24, 48, and 96 h after inoculation, as well as asymptotic gas production (*b*). Rations at 30% and 10% DCP had the highest values of gas production, whereas control and 20% DCP presented the same values (quadratic effect $P<0.01$). Lag phase (*L*) decreased linearly ($P<0.001$) with DCP addition. IVDMD and IVOMD

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increased linearly (P<0.01) with the inclusion of DCP in rations. The ration at 30% DCP (DCP30) had the greatest value of ME and SCFA, but no differences were observed in the PF_{96} among treatments. The data suggest that the DCP30 ration had the best impact on rumen gas production and IVDMD, IVOMD, ME, and SCFA.

KEY WORDS: dried citrus pulp, gas production, *in vitro* fermentation, goat

INTRODUCTION

Conventional alternative feeds have been developed to meet the nutritional requirements of livestock during dry seasons. Food processing produces large amounts of by-products, but only a few of them have been successfully integrated as alternatives in livestock feeding because most of them are low in the major nutrients, and/or not balanced for them (Gado et al., 2011).

In Mexico, a large quantity of by-products from the citrus juice industry is obtained in the form of dehydrated citrus pulp (Ku et al., 1993). Dried citrus pulp (DCP) is a widespread by-product used mainly in ruminant diets (Volanis et al., 2006). Ensiled and dried citrus pulp have been extensively studied on dairy cows (Belibasakis and Tsirgoyianni, 1996), fattening calves (Hadjipanayiotou and Louca, 1976), and lambs (Gado et al., 2011). Citrus pulp (DCP) modifies ruminal fermentation (Pinzón and Wing, 1976), improves fibre digestion (Gado et al., 2011), and increases microbial protein synthesis in dairy cows (Belibasakis and Tsirgoyianni, 1996; Gado et al., 2009), and sheep (Aregheore, 2000). Citrus pulp could be an alternative energy source replacing some grain in concentrate diets (Gado et al., 2011). It is noteworthy that the effects of including DCP in diets for goats on fermentation and nutrient metabolism have not yet been fully examined. Therefore, the objective of this study was to investigate the effect of different levels of DCP in diets on *in vitro* fermentation of gas production kinetics and energy utilization in goat rumen inocula.

MATERIAL AND METHODS

Experimental diets

Four total mixed rations (TMR) were formulated to include 4 levels of dried citrus pulp (0%, 10%, 20%, and 30% of the TMR). The rations met the nutritional requirements for non-lactating mature does in early gestation (40 kg body weight, feed consumption of 1.05 kg DM; NRC, 2007; Table 1).

Table 1. Ingredients, chemical composition and secondary compounds (g/kg DM) of the total mixed rations (TMR) with the different levels of dried citrus pulp (DCP)

Indices	TMR				Dried citrus pulp
	Control	DCP10	DCP20	DCP30	
Ingredients					
sorghum grain	250	150	100	100	
soyabean meal	90	90	90	90	
urea, 46% N	10	10	10	10	
molasses	20	20	20	20	
mineral premix [b]	30	30	30	30	
Chenchus ciliaris	600	600	550	450	
dried citrus pulp	0	100	200	300	
Chemical composition					
organic matter	904	904	905	910	936
crude protein	154	155	153	155	47
neutral detergent fibre (NDF)	468	456	438	414	193
acid detergent fibre (ADF)	243	239	236	226	126
Secondary compounds					
total phenolics	38.9	35.7	47.7	37.9	134.1
saponins	40.0	48.5	39.7	53.6	45.9
aqueous fraction[a]	88.0	108.5	122.4	139.2	187.5

[a] aqueous fraction (lectins, polypeptides, starch; Cowan, 1999)

[b] contained per kg of mineral premix: g/kg: Ca 19.60, S 22.10; mg/kg: Co 4, I 15.93, Se 15.49

Preparation of diet extract to determine secondary metabolites

Secondary metabolites were determined as outlined previously in Salem et al. (2011). Briefly, the experimental diets were ground in a Willey-mill to pass a 1 mm screen and immediately extracted at 1 g/8 ml of solvent mixture. The mixture of solvents contained 10 ml methanol (99.8/100, analytical grade, Fermont®, Monterrey, Mexico), 10 ethanol ml (99/100, analytical grade, Fermont®, Monterrey, Mexico), and 80 ml distilled water. Rations were individually soaked and incubated with the solvent mixture in the laboratory at 25-30°C for 48-72 h in closed flasks. After incubation, all flasks were incubated in a water bath at 39°C for one h and then immediately filtered; the filtrates were collected and stored at 4°C for further use.

Proximate analysis and secondary metabolites

Samples of each diet were analysed for DM, ash, CP (N×6.25), and ADF according to AOAC (1990). The NDF analyses were according to the Van Soest et al. (1991) procedure, using an ANKOM 200 Fibre Analyzer Unit (ANKOM Technology Corporation, Fairport, NY, USA). Total mixed rations were extracted using a mixture of ethanol, methanol and water (40:40:20) and secondary

metabolites were determined using 10 ml of different TMR. The extract was fractionated using ethyl acetate (99.7/100, analytical grade, Fermont®, Monterrey, Mexico) to determine total phenolics by drying and quantifying the layer in the funnel. After total phenolics separation, n-butanol (99.9/100, analytical grade, Fermont®, Monterrey, Mexico) was added to fractionate the saponins (Makkar et al., 1998). The remaining solution was considered to be the aqueous fraction (lectins, polypeptides, starch; Cowan, 1999).

Donor animal's inocula

Rumen fluid was collected by a stomach tube before the morning meal from 8 gestating goats (Boer×Saanen, body weight 39±2.3 kg). The animals had access to clean water and were fed twice daily at 07.00 and 16.00 h for 15 days before sampling. Ruminal fluid was obtained using a stomach tube from multiple sites in the rumen, strained through two layers of muslin and then kept for 30 min at 39°C under a continuous CO_2 stream.

Gas production assay

The gas production assay was carried out according to Theodorou et al. (1994). A sample of 1 g DM (each one of the TMR; Table 1) was weighed in triplicate into 160 ml serum bottles. Anaerobic buffer solution (90 ml, containing micro- and macroelements, a reducing agent, and a reduction indicator of resazurin) was added to the bottles containing 10 ml of ruminal fluid. Negative controls (blank) containing buffered rumen fluid but no substrate were also included in triplicate for correction of gas produced from small particles present in the ruminal fluid. Cumulative gas production (ml/g DM) was recorded at 2, 4, 6, 8, 10, 12, 15, 19, 24, 30, 36, 48, 72, and 96 h after incubation at 39°C. The volume of gas produced after 24 h of incubation (GP_{24}) was used as an index of energy feed value of tree fodder samples (Menke and Steingass, 1988).

In vitro degradability

At the end of incubation (96 h), the contents of each serum bottle were filtered through sintered glass crucibles (coarse porosity no. 1, 100- to 160 μm pore size, Pyrex, Stone, UK) under a vacuum. Fermentation residues were dried at 105ºC overnight and then incinerated in a muffle furnace at 550ºC for 12 h. Loss in weight after incineration was used as a measure of ash. The *in vitro* organic matter degradability (IVOMD) at 96 h of incubation was calculated as the difference between the OM content of the substrate and its undegradable OM.

Calculations

The pressure generated by the gas accumulated in the upper part of the incubation bottles was measured through a pressure transducer connected to a digital reader. The equation previously obtained using regression analysis was as follows:

$$Y = -0.807 + 6.86X + 0.083X^2$$

where: Y - volume (ml), X - pressure (psi); $R^2 = 0.99$.

The gas production data (ml/g DM) were then fitted using the NLIN option of SAS (2002) to the model of France et al. (2000) as follows:

$$A = b \times (1 - e^{-c(t-L)})$$

where: A - volume of gas production at time t; b - asymptotic gas production (ml/g DM); c - rate of gas production (h), and L (h) - lag time.

Metabolizable energy (ME, MJ/kg DM) was estimated according to the procedure by Menke and Steingass (1988), by the following equation:

$$ME\ (MJ / kg\ DM) = 2.20 + 0.136\ GP + 0.057\ CP$$

where: GP_{24} - 24 h gas volume and CP (% DM) of the experimental diets.

Short-chain fatty acids (SCFA) were calculated according to the equation from Getachew et al. (2002):

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

where: GP - 24 h net gas production (ml/200 mg DM).

Partitioning factors (PF, used as a measure of fermentation efficiency) were calculated as the ratio of OM degradation to total gas production at 96 h (i.e., OM disappearance (IVOMD)/total gas production (GP_{96}); Blümmel et al., 1997).

Statistical analysis

Data for *in vitro* gas production, gas production parameters (b, c and L), *in vitro* degradability (IVDMD and IVOMD), ME, SCFA, and PF_{96} were analysed using the general linear model procedure (SAS, 2002) for a completely randomized design with four treatments of three repetitions; means were tested using the Tukey test (Steel and Torrie, 1980). Linear and quadratic contrasts of DCP level were performed.

RESULTS

The effect of including different levels of DCP in TMR on *in vitro* ruminal fermentation parameters (*b, c* and *L*), IVDMD, IVOMD, ME, SCFA, and PF_{96} are shown in Table 2 and Figure 1. Inclusion of DCP linearly increased (P<0.01) cumulative gas production at 24, 48, and 96 h after inoculation.

Table 2. *In vitro* fermentation characteristics of total mixed rations (TMR) with the different levels of dried citrus pulp (DCP) in goat rumen inocula

Parameter	TMR				SEM	Effects, P	
	Control	DCP10	DCP20	DCP30		linear	quadratic
Gas							
GP_{24}	174.6^{b}	183.9^{b}	194.4^{b}	221.3^{a}	5.52	<0.001	0.15
GP_{48}	256.6^{b}	265.9^{b}	277.5^{ab}	307.3^{a}	7.90	<0.01	0.230
GP_{96}	291.6^{b}	303.9^{ab}	314.4^{ab}	341.3^{a}	9.72	<0.01	0.47
Gas production parameters							
b	295.9^{b}	309.2^{ab}	319.1^{ab}	344.7^{a}	10.11	<0.01	0.56
c	0.047^{ab}	0.044^{b}	0.046^{ab}	0.049^{a}	0.0009	0.07	<0.01
L	5.02^{a}	3.62^{b}	3.52^{b}	3.64^{b}	0.192	<0.001	0.01
IVOMD	722.0^{b}	747.0^{b}	761.0^{ab}	793.7^{a}	10.26	<0.01	0.72
IVDMD	639.3^{b}	664.0^{ab}	672.7^{ab}	708.7^{a}	12.16	<0.01	0.65
ME	7.83^{b}	8.09^{b}	8.41^{ab}	8.98^{a}	0.151	<0.001	0.33
SCFA	0.77^{b}	0.81^{b}	0.86^{b}	0.98^{a}	0.0245	<0.001	0.15
PF_{96}	2.48	2.46	2.43	2.33	0.082	0.25	0.65

means in the same row with different superscripts differ (P<0.05). SEM - standard error of the means (*n*=3). GP - gas production (ml/g DM at 24, 48 and 96 h); *b* - asymptotic gas production (ml/g DM); *c* - fractional rate of gas production (/h); *L* - lag time (h); IVDMD - *in vitro* dry matter degradability (g/kg DM); IVOMD - *in vitro* organic matter degradability (g/kg DM); SCFA - short-chain fatty acid concentration (mmol); ME - metabolizable energy content (MJ/kg DM); PF - partitioning factor; OM - disappearance/total gas production (GP_{96})

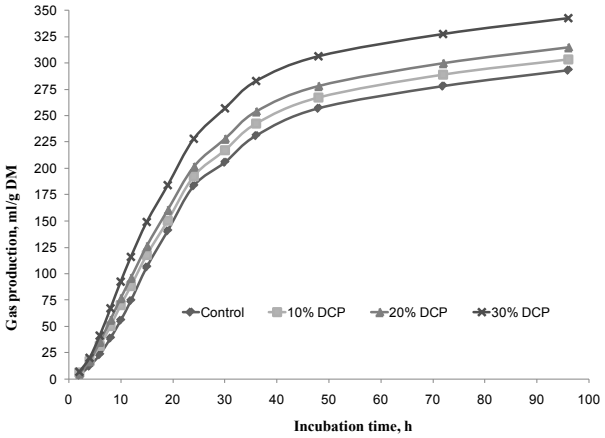

Figure 1. *In vitro* gas production profiles total mixed rations with the different levels of dried citrus pulp (DCP) in goat rumen inocula

Dried citrus pulp increased (P<0.01) asymptotic gas production (*b*) of TMR, while the fractional rate of gas production (*c*) was different among treatments. Rations at 30% and 10% DCP had the highest values, whereas control and 20% DCP presented the same values (quadratic effect P<0.01). The lag phase (*L*) decreased linearly (P<0.001) with DCP addition. The IVDMD and IVOMD increased linearly (P<0.01) with the inclusion of DCP in the diet. The ration at 30% DCP had the greatest ME and SCFA values, but no differences were observed among treatments in the PF_{96}.

DISCUSSION

Increase the DCP level in TMR (i.e., to DCP30) linearly increased gas production (Table 2; Figure 1), maybe because DCP contains a high amount of the neutral detergent soluble fibre fraction that includes pectin (250 g pectin/kg DM; Arthington et al., 2002)), soluble sugars (120 to 400 g/kg DM), and less than 10 g starch/kg DM (Volanis et al., 2006). Pectin is the main carbohydrate in dried citrus pulp, and is quickly and extensively degraded by ruminal bacteria (Sunvold et al., 1995). Moreover, higher concentrations of fermentable carbohydrates in DCP increase gas production, degradable N compounds, and decrease gas production because of the binding of CO_2 to ammonia (Krishnamoorthy et al., 1995). Substitution of feeds having high starch concentrations in TMR by others rich in rapidly fermentable carbohydrates, such as citrus pulp, avoids, at least in part, the negative effect on forage digestibility caused by high dietary starch levels (Barrios-Urdaneta et al., 2003).

The lower concentration of secondary compounds in DCP (Table 1) also improved gas production of the different TMR used, possibly due to the ability of rumen microorganisms to degrade them. These microorganisms can degrade alkaloids (Wachenheim et al., 1992), saponins (Hart et al., 2008) and phenolics (Varel et al., 1991) in plant extracts, however, and utilize them as an energy source. Administration of lower doses of leaf extracts rich in secondary compounds to ruminants as feed additives modified *in vitro* ruminal fermentation of high concentrate diets in lambs fed a daily dose of *S. babylonica* and *L. leucocephala* extract (Jiménez-Peralta et al., 2011), and improved *in vivo* digestibility and average daily gain of lambs (Salem et al., 2011).

Gas production reflects more on the digestible energy content of protein and fat (Aregheore, 2000) and it is generally a good indicator of digestibility, fermentability, and microbial protein production (Sommart et al., 2000). Macías-Cruz et al. (2010) indicated that the DM, OM, and CP apparent digestibility and intake increased with the inclusion of citrus pulp at 75% of the diet DM. The improvements observed in the apparent digestibility of the diet may be due to

the better nutrient profile and higher solubility of carbohydrates in the rumen, however, in the present study, IVDMD and IVOMD were improved with the increasing levels of DCP (linear effect $P<0.01$; Table 2).

CONCLUSIONS

Inclusion of dried citrus pulp (DCP) at the level of 30% in goat rations positively influenced ruminal microbial fermentation and digestion of the carbohydrates of rapid soluble DCP, suggesting the possibility of using this citrus by-product as a potential alternative feed in goat nutrition.

ACKNOWLEDGEMENTS

Authors would like to thank to Consejo Nacional de Ciencia y Tecnología (CONACYT) for the grant received by Javier Hernández Meléndez. Authors wish to thanks Dr. Nicolas DiLorenzo (NFREC, University of Florida, USA) for his assistance and advices during revision of the manuscript.

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