The Journal of Animal & Plant Sciences, 28(1): 2018, Page: 327-331
ISSN: 1018-7081

Short Communication

IN SITU RUMEN DEGRADATION KINETICS AND *IN VITRO* GAS PRODUCTION OF SEED, WHOLE PLANT AND STOVER OF *CHENOPODIUM QUINOA*

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ABSTRACT

The nutritional content, *in situ* rumen degradation of seeds, whole plant and stover of *Chenopodium quinoa*, as well as, their effect on *in vitro* gas production and rumen protozoa were evaluated. Six bulls (male castrated) with 412.3 ± 35.23 kg average body weight and fitted with rumen cannula were used. Treatments were: seeds (SEQ), whole plant (WPQ) and stover (SQ) of *C. quinoa*. The SEQ showed higher CP and gross energy ($P < 0.05$) content and lower fiber content than WPQ and SQ. Rumen degradation (97% DM, 89% CP and 80% NDF) and *in vitro* digestibility of DM and CP (80 and 88% respectively) were higher for SEQ ($P < 0.05$) than WPQ and SQ. *In vitro* gas production was 98 ml/0.5 mg fermented DM lower in SEQ and WPQ than SQ ($P = 0.0001$). However fermentation rate was higher for SEQ and WPQ. The protozoa count was lower in SEQ ($P = 0.0001$). Seeds of *C. quinoa* and whole quinoa plant can be incorporated into the diet of ruminants because their good chemical composition, reduced protozoa and high rumen digestion.

Keywords: quinoa, rumen degradation, *in vitro* gas production.

INTRODUCTION

Ruminant diet in dry inter-Andean valleys (above 3,000 m.a.s.l.) of Ecuador is based in low quality forage leading to severe nutritional constraints resulting in a low animal performance. In addition, low quality diets leads to a higher loss of dietary energy in form of greenhouse gases (Barros-Rodríguez *et al.,* 2014).

The use of products and sub-products of quinoa can be an option to improve ruminants diet. Quinoa has 10 - 16 % of CP, 14 - 15 MJ/kg DM of gross energy, 12 and 5 % NDF and ADF respectively, as well as, a high fatty acid composition (Peiretti *et al.,* 2013). It also contains saponins ranging from 1 to 4% in the seeds (Nowak *et al.,* 2016), which could help to reduce rumen methanogenesis and decrease the population of protozoa in the rumen (Jayanegara *et al.,* 2012). Thus and improvement in dietary energy utilization by the animal would be expected (Kurihara *et al.,* 1999).

Whole quinoa plant has been evaluated and low values of fiber and a high DM digestibility (80%) has been reported for ruminants (Peiretti *et al.,* 2013). However, information is lacking on rumen nutrient degradation kinetics of quinoa and its effect on rumen protozoa population. Based on the above, the objective of this research was evaluate the nutritional content, *in situ* rumen degradation of the seeds, whole plant and stover *Chenopodium quinoa*, *in vitro* gas production and rumen protozoa.

MATERIALS AND METHODS

Location, animals and treatments: The research was conducted at the Faculty of Agricultural Sciences, Technical University of Ambato, Ecuador, located at 2850 m.a.s.l.

Six bulls (male castrated) of 412.3 ± 35.23 kg average live weight, fitted with rumen cannula (4-inch internal diameter, Diamond Bar, Parma, Idaho, USA) were used. Animals were kept in individual pens with concrete floor. Bulls were fed with a diet based in *Medicago sativa* and *Lolium perenne* and water *ad libitum*.

The treatments were; seeds of *C. quinoa* (SEQ), whole plant of *C. quinoa* (WPQ) and stover of *C. quinoa* (SQ).

Chemical composition: The SEQ and WPQ (stems, leaves and seeds) were from harvested crops after six months growth. The SQ was collected after harvesting and threshing of seeds. The samples (30 kg of fresh material) of each part of the plant were collected from two provinces of Ecuador (two crops of Chimborazo and two of Tungurahua). Samples were oven dried at 60 °C and ground to a particle size of 1mm for chemical analysis. Then, samples were mixed to obtain a composite sample to perform both *in situ* and *in vitro* procedures.

Rumen degradation kinetics (DM, CP and NDF): The nylon bag technique (Ørskov *et al.,* 1980) was employed. On each bull, two bags containing 5 g DM of each part of the plant were incubated for 0, 4, 8, 12, 24, 36, 48, 72 and 96 h. At the end of incubation periods, bags were removed, washed under running water, and oven dried at 60 °C. Bags employed to measure washout loss (0 h) were only washed under running water. Residues were stored in polyethylene bags at −4 °C until chemical analysis. DM, CP and NDF disappearance were calculated as a proportion of incubated material. Data was fitted to the equation: $Y = a + b (1 – e^{–ct})$ (Ørskov and McDonald 1979).

Apparent digestibility, gas production and rumen protozoan: These tests were performed *in vitro*. Rumen contents (fluid and solid fractions) were obtained separately from four cannulated bulls. Rumen content was obtained before feeding and kept in a sealed plastic container and transported to the laboratory for further processing within 1 h of collection. Nitrogen-rich media was prepared as Menke and Steingass (1988). Gas production was measured as Theodorou *et al.* (1994). For each treatment, 0.5 g DM was placed in 100 ml serum bottles. Then, 60 ml rumen inoculum (70:30 media/rumen inoculum) was added under constant CO_2 flow. Bottles were sealed and incubated at 39–40 °C. At 3, 6, 9, 12, 18, 24, 36, and 48 h after incubation, gas pressure was measured with a transducer (DELTA OHM model DO 9704, Padova, Italia). At each time gas volume was measured using plastic syringes. For each treatment, six bottles were used at each time and three additional bottles were used as blank. At the end of incubation, DM and CP digestibility were estimated by filtering the residue and correcting with their respective blanks. Total gas production per 0.5 g fermented DM was estimated. Cumulative gas production data was fitted to the equation: $Y = a + b (1 – e^{–ct})$ (Ørskov and McDonald 1979).

Six additional bottles were used to determine the population of rumen protozoa. At 0, 12 and 24h incubation a 1 ml sample was removed from each bottle and preserved with phenol (one drop) and kept at 4 °C until protozoa were counted using an optical microscope (×40) and a Fucsh-Rosenthal chamber. Protozoa were stained using a methylene green formalin saline solution (Ogimoto and Imai, 1981).

Chemical analysis: DM (#7.007) and ash (#7.009) were determined according to AOAC (1990). Acid detergent fiber (ADF) and neutral detergent fiber (NDF) were determined by the methods 12 and 13 respectively, (Ankom^{2000} fiber analyzer, Ankom Technology, Macedon, NY, USA). Crude protein (CP) was determined by elemental analysis (N) using a LECO CHN 628 (LECO Corporation).

Experimental design and statistical analysis: All variables were analyzed as a completely randomized design with three treatments and six replications using PROC GLM. Protozoan counts were transformed (log_{10}) to normalize data and variance. All means were compared with the Tukey test (SAS Institute 2000), except for rumen degradation and gas production kinetics, which were analyzed with Graphpad Prism 6 Software, Inc. San Diego, CA, USA.

RESULTS

Chemical composition and rumen degradation: The SEQ showed higher (P<0.05) content of DM, CP, GE and lower fiber content that WPQ and SQ (Table 1). DM soluble fraction (A) of WPQ was higher (P <0.05) than SEQ and SQ, with 160 and 284 g/kg DM respectively. The insoluble but potentially degradable fraction (B) and degradation rate (%) per hour (c) of SEQ were higher (P <0.05) than 40% with respect to WPQ and SQ. Soluble fraction (A) of CP and NDF were higher in SEQ and WPQ with more than 15% compared to SQ (P <0.05). The insoluble fraction (B) but potentially degradable, as well as, degradation rate (%) per hour (c) of the CP and NDF in the SEQ were higher (P <0.05) at 12% versus WPQ and SQ (Table 1).

***In vitro* digestibility, gas production and rumen protozoan:** In relation with DM and CP *in vitro* digestibility, SEQ had the highest digestibility (80.6%) versus WPQ (58.3%) and SQ (30.9%) (P=0.0001). The *in vitro* gas production was lower (P=0.0001) for the SEQ and WPQ by approximately 98 ml/0.5 mg fermented DM respect to SQ. Fermentation rate (c) was higher for SEQ and WPQ than SQ (0.063 and 0.063 vs. 0.036, P<0.05) (Table 2). The protozoa population at 0 h were similar for both holotrich and entodinomorphid (P>0.05). After 12 and 24 h incubation, both holotrich and entodinomorphid counts were lower in SEQ and WPQ than SQ (P < 0.05) (Table 2).

Table 1. Chemical composition and rumen degradation kinetics of seeds (SEQ), whole plant (WPQ) and stover (SQ) of *Chenopodium quinoa* (g/kg DM).

	Treatments			*P* Value
	SEQ	WPQ	SQ	
DM	753.4±22.34a	612.7±19.76b	648.0±23.45b	0.0456
OM	936.9±7.98a	927.2±10.54a	927.9±7.01a	0.0865
CP (N*6.25)	183.0±2.32a	160.0±2.87b	72.0±1.98c	0.0001
NDF	81.9±2.98c	302.5±2.05b	548.7±3.12a	0.0001
ADF	25.2±1.87c	145.0±2.32b	244.8±2.50a	0.0001
GE (MJ/kg DM)	18.2±1.32a	13.7±1.76b	9.1±1.50c	0.0001
Degradation of DM				
T_0	324.7±19.86	478.7±9.49	175.7±13.22	
A	283.5±13.80b	452.0±21.37a	168.0±10.31c	<0.05
B	692.2±35.31a	356.6±20.11b	383.4±17.09b	<0.05
c	0.473±0.0486a	0.088±0.0091b	0.023±0.0031c	<0.05
A+B	977.7	808.6	551.4	
r^2	0.96	0.94	0.96	
Degradation of CP				
T_0	102.3±12.32	97.3±15.32	83.7±9.34	
A	365.7±11.23a	312.4±17.43a	134.2±12.32b	<0.05
B	527.2±16.43a	404.7±13.32b	286.8±14.43c	<0.05
c	0.386±0.0213a	0.062±0.0043b	0.028±0.0073c	<0.05
A+B	892.9	717.1	421.0	
r^2	0.98	0.96	0.98	
Degradation of NDF				
T_0	154.5±9.54	112±9.89	96.3±8.78	
A	302.8±14.32a	265.0±11.98a	101.6±12.76b	<0.05
B	498.2±12.54a	376.7±17.43b	304.0±10.87b	<0.05
c	0.213±0.020a	0.035±0.0072b	0.021±0.0012b	<0.05
A+B	801.0	641.7	405.6	
r^2	0.97	0.96	0.98	

[abc] Means with different letters in the same row differ at P < 0.05. T_0: washout loss. A, B, and c are equation parameters describing *in situ* rumen degradation, from: $y = a + b\ (1 - e^{-ct})$

	Treatments			SEM	*P* Value
	SEQ	WPQ	SQ		
IVDDM (g/kg DM)	806.3a	583.2b	309.6c	22.44	0.0001
IVDCP (g/kg DM)	883.4a	606.8b	330.1c	19.64	0.0001
IVGP ml/0.5g FDM	280.9b	281.0b	378.9a	4.59	0.0001
Gas production kinetics†					
a	-28.5[a]±5.29	-15.85[a]±5.80	3.3b±3.20		<0.05
b	325.8a±4.85	309.9[a]±5.34	452.0b±6.87		<0.05
c	0.064a±0.003	0.063[a]±0.003	0.036b±0.002		<0.05
r^2	0.990	0.987	0.997		
Protozoa					
h 0					
H	3.43a	3.25a	3.50a	0.019	0.3399
E	4.52a	4.54a	4.80a	0.094	0.1504
h 12					
H	0.00b	0.00b	1.01a	0.013	0.0056
E	3.10c	3.81b	4.21a	0.091	0.0005
h 24					
H	0.00	0.00	0.00	0.000	<0.05
E	1.20c	3.02b	3.81a	0.015	0.0001

[abc] Means with different letters in the same row differ at P < 0.05. SEM: standard error of the mean. † a, b, and c are equation parameters describing *in vitro* gas production, from: $y=a+b(1-e^{-ct})$, means±Standard error of the parameter SEQ: seeds of *C. quinoa*. WPQ: whole plant *C. quinoa*. SQ: stover of *C. quinoa*. IVDDM: *in vitro* digestibility of dry matter. IVDCP: *in vitro* digestibility of Crude Protein. FDM: fermented DM. H: Holotrichs. E: Entodinomorphs

DISCUSSION

The nutrient content of WPQ are consistent with those reported by Ramos and Cruz (2002). They assessed the crop of quinoa as fodder for animal feed in the Cuban dry season. The lower crude protein and higher fiber content of SQ, may be related directly to the characteristics of the plant, which upon reaching the physiological completion of cultivation tends to lignify the stem. According to Peiretti *et al.* (2013) older plant tends to decrease protein content and increase the fiber content.

The higher *in situ* rumen degradation and *in vitro* digestibility of nutrients in SEQ and WPQ, was possibly due to their lower fiber content (Table 1), and the lower values observed for SQ, was due to its increased cell wall lignification (Table 1). The high ADF content helps to explain the degradation and fermentation rates ("c" parameter for both *in situ* and *in vitro* techniques) for SQ which was approximately 50% lower than those of SEQ and WPQ (Tables 1 and 2).

In addition, a lower *in vitro* gas production of SEQ and WPQ could be associated; i) improved protein usage and higher microbial protein synthesis (Mao *et al.,* 2010). A lower IVGP together with higher IVDMD and IVOMD for SEQ and WPQ supports an improved fermentation efficiency (Blummel *et al.,* 1997). SEQ and WPQ would resemble beans and whole pods of tropical legumes as similar trends have been observed for example with *Mucuna pruriens* (Sandoval-Castro *et al.*, 2003) or the improved efficiency obtained when legume forage is mixed with an energy source (Estrada-Lievano *et al.*, 2009, Sandoval Castro *et al.*, 2002) and ii) reduction of methanogenic archaea and protozoan of rumen (Jayanegara *et al.,* 2012) possibly due to the saponin content of quinoa. Similar reduction of protozoa counts have been reported for foliage containing saponins (Monforte-Briceño *et al.,* 2005). Although it was not evaluated in the present experiment, a reduction of total gas yield and protozoa counts in diets containing quinoa might result in a lower greenhouse gas production due to the saponins in SQ and WPQ (Nowak *et al.,* 2016; Goel and Makkar, 2012), These results are consistent with findings by Barros-Rodriguez *et al.,* (2014) where saponins in the diet of ruminants reduced greenhouse gases without affecting the digestion of nutrients. Although further studies are needed to confirm its potential to reduce greenhouse gases *in vivo,* the current results show that due *C. quinoa* can be used as suitable ruminant feed and has potential to improve rumen fermentation and nutrient supply.

Conclusions: Seeds of *C. quinoa* and whole quinoa plant can be incorporated into the diet of ruminants because their good chemical composition and high rumen digestion.

Acknowledgements: The authors thank at the Technical University of Ambato, Ecuador for funding this research through the Dirección de Investigación y Desarrollo, project: CU-0135-P-2014.

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