EFFECT OF TWO LEVELS OF TEPOZAN BUSH (*Buddleia cordata*) HAY ON THE MICROORGANISMS IN THE RUMEN AND THE PRODUCTIVE BEHAVIOR IN GOATS

[EFECTO DE DOS NIVELES DE FOLLAJE DE TEPOZÁN HENIFICADO (*Buddleia cordata*) SOBRE LOS MICROORGANISMOS DEL RUMEN Y EL COMPORTAMIENTO PRODUCTIVO DE CABRAS]

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ABSTRACT

The effect of including (0, 15, and 30%) arboreal forage of *Buddleia cordata* (Tepozan) was evaluated on the productive behavior and microorganisms in the rumen of goats. Thirty native goats with an average live weight of 14 kg were randomly distributed into three treatments and kept in individual pens for 45 days. Daily weight gain (DWG), dry matter consumption (DMC), rumen pH, and total and cellulolytic bacteria, and protozoan concentrations were measured at 30 and 45 days into the experimental period. The experimental design was completely random with measurements done through time, analyzing DWG, DMC, and rumen pH data with PROC MIXED. The number of bacteria was analyzed statistically by trust intervals. There were no differences among treatments ($p>0.05$) with the addition of *B. cordata* in the diet on DWG, DMC, rumen pH, or total and cellulolytic bacteria, and protozoan concentration. No differences were seen among periods ($p>0.05$) in any variable. The conclusion is that *B. cordata* hay can be included in goat diets up to a maximum of 30% without any negative variations in the productive variables or rumen microorganisms.

Key words: *Buddleia cordata;* bacteria; rumen; protozoan; goats.

RESUMEN

Se evaluó el efecto de la inclusión (0, 15 y 30 %) de forraje arbóreo de *Buddleida cordata* (Tepozán) en el comportamiento productivo y los microorganismos del rumen en cabras. Se distribuyeron al azar 30 cabras criollas con un peso vivo promedio de 14 kg en tres tratamientos y fueron alojadas en corrales individuales durante 45 días. Se midió ganancia diaria de peso (GDP), consumo de materia seca (CMS), pH ruminal, concentración de bacterias totales, celulolíticas y protozoarios a los, 30 y 45 días del periodo experimental. El diseño experimental fue completamente al azar con mediciones repetidas a través del tiempo, analizando los datos de GDP, CMS y pH ruminal con PROC MIXED. El número de bacterias fue estadísticamente analizado por intervalos de confianza. No hubo diferencias entre tratamientos ($p>0.05$) por la inclusión de *B. cordata* en la dieta sobre la GDP, CMS, pH ruminal, concentración de bacterias totales, celulolíticas y protozoarios. No se presentaron diferencia entre periodos ($p>0.05$) en ninguna variable. Se concluye que el henificado de *B. cordata* muestra potencial para ser incluido en la dieta de cabras hasta un máximo de 30%, sin afectar negativamente las variables productivas ni a los microorganismos del rumen.

Palabras clave: *Buddleida cordata*; bacterias; rumen; protozoarios; cabras.

INTRODUCTION

B. cordata better known in Mexico as tepozan or zumpantle, is a tree that grows at altitudes of 1500 to 3600 masl. It is a fast growing tree commonly used as an ornamental plant (Martinez and Chacalo, 1994), and as a traditional medicinal plant (Ortiz, 1996). Moreover, some phytochemical studies of the leaves have shown its bactericidal and amebicidal properties (Ordaz, 1996). In forestry, it is considered as a fast growing species, resistant to contamination (Martinez and Chacalo, 1994). These data are an indicator of the potential that *B. cordata* represents; however, important aspects of its use in ruminant feeding are still unknown, such as the possible negative effects that can damage total and cellulolytic bacteria, and protozoan concentrations, which in turn could affect the productive variables of animals. This is mainly because of studies that show the presence of a phenylpropanoid glucoside mixed with two sugars, called verbascoside, which has a negative effect on the antitrembling action of DOPA, it is an analgesic, hypotensor, and cytotoxic. It is part of the plants' chemical defenses against pathogens (Andary *et al*., 1982; Lira-Rocha *et al*., 1987). To this regard, Diaz *et al*. (2000) mention that the phenylpropanoid extracted from *B. cordata* at a concentration of 50 mg L^{-1} eliminated 97.92% of *Costia necatrix*, which is an important protozoan in most fish. However, the parasitidal effects previously mentioned have not been reported in rumen bacteria. Thus, the objective of this research was to evaluate the effect of including 0, 15, and 30% of *B. cordata* hay on the productive behavior and rumen microorganisms in goats.

MATERIALS AND METHODS

General

Thirty 3-month old, native goats with an average live weight of 14.0 ± 2.0 kg were used. They were placed in 1.20 m x 1.10 individual pens, including a water and a food trough. The control diet was made up of alfalfa, corn hay, crushed corn, soy paste, molasses, and a mineral mixture (Table 1). The animals were randomly distributed into three treatments: T1 = control diet (without *B cordata*); T2 = control diet + 15% *B. cordata*; T3 = control diet + 30% *B. cordata*. The diets were formulated according to the requirements of the NRC (1985) for goats in keeping with an average weight of 14 to 16 kg. The used foliage of *B. cordata* was collected in the Pezmatlan Experimental Field, belonging to the Agronomics and Zootechnics Engineering Program of the Benemerita Universidad Autonoma de Puebla. The foliage was exposed in the sunlight for two days, thus obtaining the hay.

Alfalfa, corn, and *B. cordata* hay were ground using a 4 mm mesh. Table 1 shows the compositions of the experimental diets. Before taking the samples, a 10 day adaptation period was granted, to avoid digestive problems and rejection of the feed.

The research stage lasted **45 days**, animal weighing and the determination of the dry matter consumption (DMC) were done every **15 days**. The determination of rumen pH and the concentration of bacteria and protozoa were done at **30 and 45 days**, after beginning the experiment.

The *B. cordata* hay was determined for dry matter (DM), ash, and raw proteins (RP) through the procedure established by the AOAC, 1990; procedures: 934.01, 976.05, and 920.39, respectively. Acid detergent fiber (ADF), neutral detergent fiber (NDF), lignin, and ash were determined through the technique proposed by Van Soest *et al*., 1991.

Estimation of rumen bacteria, protozoa, and pH

The concentration of total, cellulolytic bacteria, and protozoa, were determined at **30 and 45 days**, according to the methodology described by Cobos *et al*. (2002, 2007), using the most likely number technique (Harrigan and McCance, 1979). The composition of the different anaerobic mediums are shown in Table 2. Possitive growth was confirmed after 24 hours for total bacteria, and 10 d for cellulolytic bacteria, after being incubated at 38 °C. Protozoa concentration was determined through direct counts in a Neubauer chamber, with a microscope at an amplification of 40X (Dehority, 1993). To measure rumen pH, 100 mL were taken from the ventral part of the rumen with a probe, four hours after the morning feeding. Immediately after obtaining it, the pH was measured with an Orion 710A portable potentiometer, calibrated at two points with a buffer solution set at pH 4.0 and 7.0, respectively.

The obtained data were analyzed in a totally random design with ten repetitions per treatment for DWG, DMC, and rumen pH. To evaluate the effect of time, the MIXED (SAS, 2001) procedure was used, as proposed by Littell *et al*. (1998), and Wang and Goonwardese (2004). The number of total and cellulolytic bacteria was statistically analyzed by trust intervals, as described by Harrigan and McCance (1979). For the analysis, the option AR (1) corresponding to the covariance structure was taken into account.

Table 1. Ingredients and chemical composition of the experimental diets (% BS)

Ingredient	Without *B. cordata*	With 15 % *B. cordata*	With 30 % *B. cordata*
Alfalfa	7	7	14
Corn hay	30	15	0
Crushed corn	39	43	42
Soy paste	21	17	11
B. cordata	0	15	30
Molasses	2	2	2
Mineral mixture[a]	1	1	1
Chemical composition		%	
DM	89.7	88.07	89.02
CP	14.9	14.84	14.89
ADF	17.71	16.5	17.78
NDF	29.6	26.5	26.01
Ash	3.04	2.8	3.3

[a]Calcium, 130 g; Phosphorus, 50 g; Sodium, 109 g; Chloride, 200 g; Iron, 4.30 g; Magnesium, 3.33 g; Manganese, 200 mg; Copper, 80 mg; Cobalt, 66.60 mg; Iodine, 4.0 mg; Zinc, 8.0 mg kg^{-1}

Table 2. Composition of the growth medium for total and cellulolytic bacteria count

Compound	Medium for bacteria (per 100 mL)	
	Total	Cellulolytic
Distilled water (mL)	52.6	52.6
Clarified rumen liquid (mL)(1)	30.0	30.0
Mineral solution I (mL)(2)	5.0	5.0
Mineral solution II (mL)(3)	5.0	5.0
Rezarsurine 0.1 % (mL)(4)	0.1	0.1
Soy peptone (g)	0.2	0.2
Yeast extract (g)	0.1	0.1
Sodium carbonate, sol. 8 % (mL)(5)	5.0	5.0
Sol. Sodium sulfite cistein (mL)(6)	2.0	2.0
Glucose (g)	0.06	0.0
Cellobiose (g)	0.06	0.0
Starch (g)	0.06	0.0
Watman 541 paper slip	-	One slip per tube

(1) Clarified rumen liquid, previously filtered in a triple gauze, and centrifuged at 12,000 rpm, for 15 minutes, three times, and sterilized for 15 min at 15 psi, 121 °C.
(2) Contains 6 g K_2HPO_4 per 1000 mL of H_2O.
(3) Contains 6 g KH_2PO_4; 6 g $(NH_4)_2SO_4$; 12 g NaCl; 2.45 g $MgSO_4$, and 1.6 g CaCl $^*H_2O$ per 1000 mL of H_2O (Bryant, 1972).
(4) Add 0.1 mL solution at 1 % in water and buffer in 100 mL distilled water.
(5) 8 g sodium carbonate in 100 mL distilled water.
(6) 2.5 g L- cistein (dissolved in 15 mL 2N NaOH); 2.5 g Na_2S-$9H_2O$, and 0.1 mL resarzurine in a final volume of 100 mL.

RESULTS

Proximal chemical analysis of *B. cordata*

Table 3 shows the results for the chemical analysis of *B. cordata*. It has a higher content of RP (8.23) than do other sources of fiber, like corn hay. This can be an advantage, given that in integral proportions, the addition of other protein sources, like soy paste, is decreased.

Daily weight gain (DWG)

Table 4 shows the DWG. The results obtained show that there were no differences among treatments in any period, nor in average DWG ($p>0.05$), thus indicating that up to 30% *B. cordata* can be added to goat fodder without negatively affecting this variable.

Table 3. Chemical analysis of *B. cordata* leaves

Compound	%
Protein	8.23 ± 2.1
Ash	4.63 ± 0.3
ADF	38.01 ± 5.6
NDF	51.34 ± 7.5
Lignin	10.3 ± 2.1
Phenols	1.65 ± 0.4

Dry matter consumption (DMC)

No digestive problems such as diarrhea, common during this stage (Owens *et al*., 1998), were observed during the 10 d adaptation period, which indicates that so much the goats as the population of rumen microorganisms adapted adequately to the rations. There were no differences in DMC among treatments ($p>0.05$) in any period, nor in the average of the whole experimental stage (Table 5). Moreover, time was not a factor that influenced the effect of the treatments ($p>0.05$). Therefore, the addition of up to 30% of *B. cordata* in goat diets had no negative effect on DWC in this experiment.

Rumen pH

Rumen pH is a determining factor in the fluctuations of bacterial populations. Most bacteria have a good development in an environment with a pH between 6.2 and 7.0; however, in a pH lower than 6.2, cellulolytic species, like *Fibrobacter succinogenes*, *Ruminococcus flavefaciens*, and *R. Albus*, reduce their growth, mainly since the concentration of bicarbonate in the medium is minimal if the pH nears 5.5 (Yokoyama and Johnson, 1988). In the present experiment, there were no differences in rumen pH among treatments ($p>0.05$), in any period, nor on average. The pH values are observed to be relatively low if we consider that pH is a determining factor in the fluctuations of bacterial populations (Table 6). Rumen pH is low 4 h post-feeding. It is probable that fiber fermentation is reduced, at least temporarily, because of this. Although the number of total and cellulolytic bacteria was not affected, it is possible that cellulolysis might have been negatively affected. The factors that determined the low rumen pH were the percentages of crushed corn (range = 39-43%) and soy paste (range = 11-21%) in the rations. It is possible that the osmotic pressure in the rumen might have also gone up (>350 mosmol L^{-1} H_2O), at least temporarily, for a time post-feeding.

Bacteria and protozoa concentration

The concentrations of total and cellulolytic bacteria, and protozoa, were similar ($p>0.05$) in all treatments after 30 and 45 days of evaluation. This indicates that there was no negative effect on rumen microorganisms from the addition of *B. cordata* in goat rations (Table 7).

Table 4. Average weight gain (g $animal^{-1}$ d^{-1}) in goats

Period[+]	**Without *B. cordata***	**With 15 % *B. cordata***	**With 30 % *B. cordata***	**SME**
1	59.08	-10.00	21.69	63.80
2	79.24	91.33	71.66	63.80
3	54.99	27.00	43.66	63.80
AVERAGE	64.44	36.10	45.67	42.71

There were no significant differences ($p>0.05$)
[+]Each period lasted 15 d
SME = Standard mean error

Table 5. Dry matter consumption (g $animal^{-1}$ d^{-1}) in goats

Period[+]	Without *B. cordata*	With 15 % *B. cordata*	With 30 % *B. cordata*	SME
1	553.50	637.30	581.60	63.19
2	643.34	625.92	564.01	63.19
3	669.00	607.92	537.84	63.19
AVERAGE	635.28	623.71	561.15	58.83

There were no significant differences ($p>0.05$)
[+]Each period lasted 15 d
SME = Standard mean error

Table 6. pH of the rumen liquid obtained four hours after feeding

Period	Without *B. cordata*	With 15 % *B. cordata*	With 30 % *B. cordata*	SME
1	5.81	5.56	5.60	0.13
2	5.92	5.89	5.82	0.13
AVERAGE	5.87	5.72	5.71	0.091

There were no significant differences ($p>0.05$)
SME = Standard mean error

Table 7. Concentration of total and cellulolytic bacteria and protozoa per mL of rumen liquid

Period	Without *B. cordata*	With 15 % *B. cordata*	With 30 % *B. cordata*
Total bacteria (10^{10} cells mL^{-1})			
1	11.2	12.3	15.3
2	35.1	18.4	15.8
Cellulolytic (10^{7} cells mL^{-1})			
1	3.27	2.3	2.3
2	6.2	6.2	6.3
Protozoa (10^{4} cells mL^{-1})			
1	3.27	2.3	2.3
2	6.2	6.2	2.3

There were no significant differences ($p>0.05$)

According to the pH obtained in this study, it would seem that the concentrations of cellulolytic bacteria would be affected, however, Cobos and Yokoyama (1995) mention that with a good period of adaptation of the animals and the rumen microorganisms to a substrate with a high content of easily fermented carbohydrates, normal concentrations of bacteria can be had, as was the case in this study, without drastically affecting the rumen conditions.

DISCUSSION

The contents of RP, NDF, and ADF in *B. cordata* are similar to those reported by Ayala *et al*. (2006) and are greater than those reported for corn hay when this is used as a source of fiber in ruminants (Yescas *et al*., 2004). If we consider that corn hay is of variable availability throughout the year, being rather scarce during the dry season (Cesin *et al*., 2007), while *B. cordata* has a constant forage production of good quality throughout the year (Nahed, 1998), it stands to reason that *B. cordata* can be used as an alternative forage in goat feeding. According to the results obtained with regard to DMC, there were no differences among treatments. These results agree with those reported by Nahed *et al*. (1998), who evaluated five foraging arboreal species in sheep feeding, and obtained a mean dry matter consumption of 317 g d^{-1} of *B. cordata* when offering it by itself. This indicates that it can be used as a feeding complement with economic benefits that could be quantified when evaluating the cost from adding another source of protein. There were no significant differences observed in the daily weight gain, thus indicating that *B. cordata* can be an alternative source of forage, mainly since the diets were designed for keeping, with the same nutrient input, and there was no negative effect on this variable.

Studies mention that the leaves of *B. cordata* have an average content of phenols of 1.8% (Ayala *et al*., 2006) that can affect the normal concentrations of rumen microorganisms, given their parasitidal effect on other species, such as fish (Diaz *et al*., 2000). This effect has not been reported in rumen microorganisms. The results obtained in this study show that the concentration of total and cellulolytic bacteria and protozoa are within the values reported by literature (Dehority, 2003b), which confirms the good adaptation of rumen microorganisms to this type of substrate.

CONCLUSIONS

The results obtained in this study indicate that rations for goats in keeping with up to 30% *B. cordata* added do no affect negatively the productive behavior nor the rumen microorganisms. Moreover, it can substitute other ingredients, such as corn hay.

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Submitted June 3, 2009– Accepted April 16, 2010
Revised received October 1, 2010