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Effect of Black Pepper (*Pepper Nigrum*) Essential Oil on In Vitro Gas Production Parameters of Alfalfa Hay, Barley Grain or Sugar Beet Pulp

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ABSTRACT

The objective of the present study was to evaluate the effect of black pepper (*Peppee nigrum*) essential oil (BPEO) on in vitro gas production parameters of alfalfa hay, barley grain or sugar beet pulp. The samples were left untreated or treated with BPEO (0.4 ml/g DM). The gas production data were fitted using an exponential equation of $P = b (1-e^{-ct})$, where b is the volume of gas produced, c is the fractional rate constant of gas production (/h), t is the incubation time (h) and P is the volume of gas produced at time t. A significant difference of gas production parameters ($p<0.05$) was observed among the feed samples evaluated in the present study. Present results indicate BPEO caused a significant increase ($p<0.05$) in both b and c fractions of alfalfa hay (49.63 vs. 52.24 and 0.06 vs. 0.11 for the treated and untreated samples, respectively). However, there was a negative significant effect ($p<0.05$) effect of BPEO on both b and c fractions when sugar beet pulp was considered (84.01 vs. 80.95 and 0.09 vs. 0.08 for the treated and untreated samples, respectively). The gas production parameters of barley grain did not alter when BPEO was applied. The most important finding of the present study is the different response of the feed samples evaluated when treated with BPEO.

INTRODUCTION

A goal of ruminant microbiologist and nutritionists is to manipulate the ruminal microbial ecosystems to improve the efficiency of converting feed to animal products consumable by humans. The use of feed additives such as antibiotics has proven to be a useful tool to reduce energy (in the form of methane) and nitrogen losses from the diet (Schelling, 1984). But, the use of antibiotics as growth promoters in animal feed is facing reduced social acceptance due to the appearance of residues and resistant strains of bacteria (Gustafson and Bowen, 1997). Antibiotic application in feed animal has been prohibited in the European Union since January 2006 (Regulation 1831/2003/EC). For this reason scientists have become interested in evaluating other alternatives to modulate rumen fermentation. Essential oils (EO) are plant secondary metabolites responsible for the odor and color of plants and spices and their antibacterial, antifungal and antioxidant properties make them useful as natural additives in animal feeds. The mechanism by which EO are thought to exert their antimicrobial activity is by disrupting the cell wall structure affecting electron transport ion gradients, protein translocation, phosphorylation steps and other enzyme dependent reactions (Ultee et al., 1999; Dorman and Deans, 2000). Early studies suggested that EO could alter rumen fermentation. Microbial activity measured by production of gas during in vitro incubation was either inhibited or promoted depending on the nature of the EO added (Oh et al., 1967, 1968). The objective of this study was to evaluate the effects of black pepper (*Pepper Nigrum*) essential oil (BPEO) on in vitro gas production parameters of alfalfa hay, barley grain, or sugar beet pulp.

MATERIALS AND METHODS

The effect BPEO was of evaluated in an in vitro gas production (Menke and Steingas, 1988). Samples were alfalfa hay (AH), barley grain (BG) and sugar beet pulp (BP). Approximately 0.3 g of each sample (four replicates) ground through a 2 mm screen and was placed in a 100 ml glass syringe. Treatment were control (no additive) and BPEO that were examined at 400 µl /g DM. Blank syringes were incubated with buffer alone and BPEO. Then, syringes were incubated in four replicate and blanks were incubated in two replicated. Two sheep (45±2kg, body weight) fitted with rumen cannulae were used as donors of rumen fluid. They were fed 1.5 kg DM alfalfa hay and 0.4 kg DM concentrates (165 g CP/kg DM) per head per day. Rumen content was collected before the morning feeding. The gas production method of Menke and Steingass (1988) was used. Rumen fluid was immediately strained through four layers of cheesecloth and mixed in a 2:1 with buffer, then, 40 ml of diluted fluid was added into the syringes. Each syringe was gassed with CO_2 then incubated at 38.6˚C. The volume of gas produced was determined at 2, 4, 8, 12, 24, 36, 72 and 96 h after the incubation. The gas production data were fitted using an exponential equation of $P = b (1 - e^{-ct})$, where b is the volume of gas produced, c is the fractional rate constant of gas production (/h), t is the incubation time (h) and P is the volume of gas produced at time t. The DM (dry matter) content of diets was determined by drying samples for 48 h in a 65˚C forced air oven (AOAC, 1990; method 950.01). Dry samples of diets were overnight at 550˚C in a furnace (AOAC, 1990; method 942.05) and OM (organic matter) was subsequently calculated as 100 minus the percentage ash (AOAC, 1990, method 942.05). Total nitrogen of diets was determined by the kjeldahl method (AOAC, 1990; method 976.05), crud protein was calculated as N×6.25. Ether extract was determined following the AOAC method (1990). The NDF (neutral detergent fiber) and ADF (acid detergent fiber) of diet were analyzed by the detergent system using the sequential procedure of Van Soest et al (1991). Statistical analysis was conducted using SAS (1999) software.

RESULTS AND DISCUSSION

The chemical composition of the diet is reported in Table1. Effect of BPEO on in vitro gas production parameters is shown in Table 2. BPEO caused to significant ($p<0.05$) increase in both b and c parameters of alfalfa hay. However, it had a negative ($p<0.05$) effect on b and c parameters of sugar beet pulp, while addition BPEO to barley grain had not significant different in both b and c parameters. Results of the present study demonstrated that the gas production parameters of the feed samples were significantly different ($p<0.05$). In addition, results indicated that the gas production parameters of the feed samples were significantly altered when BPEO was included in the medium. The most important finding of the present study is the different responses of the feed samples when incubated with BPEO. It was concluded that while BPEO caused an improvement in the gas production parameters of AH, there was a negative effect when BP was considered. Therefore, the effect of any plant extract and essential oil on rumen fermentation must be assessed using a wide range of feed samples.

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Table1 Composition of experimental diets

Item	Alfalfa hay	Barley grain	Sugar beet pulp
DM	90	92.3	85.25
CP	14.2	9.4	12.4
NDF	45%	25	43%
ADF	29.9	7.5	23.6
EE	2	2.1	0.5
ASH	9.5	3	9.5

DM = dry matter; CP = crude protein; NDF = neutral detergent fiber; ADF = acid detergent fiber; EE = ether extract

Table 2 Gas production parameters of alfalfa hay, barley grain and sugar beet pulp as untreated or treated with black pepper essential oil as 400 µl/g DM.

	Treatment					
Gas production parameters	AH	BG	BP	AH+BPEO	BG+BPEO	BP+BPEO
b (ml/ 0.3 g)	49.63±1.87 [a]	98.72±4.11 [c]	84.01±1.29 [d]	52.24±0.84 [b]	101.8±2.93 [c]	80.95±0.73
c (/ h)	0.063±0.007 [a]	0.039±0.003 [c]	0.088±0.004 [d]	0.11±0.006 [b]	0.041±0.003 [c]	0.079±0.002

[a, b, c, d] Means with difference letters in each row were significant ($p<0.05$). AH = alfalfa hay; BG = barley grain; BP = beet pulp; BPEO = black pepper essential oil. B = volume of gas produced; c = fractional rate constant of gas production