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"INFLUENCIA DEL ACEITE DE EUCALIPTO EN LOS COMPONENTES QUÍMICOS, PRODUCCIÓN DE GAS Y DEGRADABILIDAD DEL RASTROJO DE MAÍZ Y PAJA DE AVENA"

(Influence of eucalyptus oil on chemical constituents, gas production and degradability of corn stalk and oat straw)

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Influence of eucalyptus oil on chemical constituents, gas production and degradability of corn stalk and oat straw

Abstract

The effects of eucalyptus oils on ruminal fermentation of two agro-industry byproducts (corn stalk and oat straw), were studied using the *in vitro* gas production (GP) technique. Eucalyptus oil was added at 0, 30, 90, and 180 mg/l of incubation medium (equal to 0, 1.2, 3.6, and 7.2 mg/g DM substrate). Gas volumes were recorded at 2, 4, 6, 8, 10, 12, 24, 36, 48 and 72 h of incubation, and substrate DM, neutral detergent fiber (NDF) and acid detergent fiber (ADF) degradability were determined at 72 h of incubation. Eucalyptus oil increased (P<0.05) the asymptotic GP and GP of corn stalks and oat straw. Eucalyptu oil decreased fermentation pH (P<0.05) of corn stalks and oat straw. The inclusion of eucalyptu oil increased (P<0.05) DM degradability of corn stalks, oat straw. Eucalyptus oil decreased NDF degradability of corn stalks, with weak effects on NDF degradabilitie of oat straw. It can conclude that the application of eucalyptu oil positively affected rumen fermentation of the two agro-industry byproducts as roughage feeds. Increasing the dose of oils inclusion, enhanced the fermentation parameters; where the dose 180 mg oil/l increased GP, while the doses 30 and 90 mg oil/l increased nutrients digestibility.

Keywords: Degradability, eucalyptus oil, fibrous feed, garlic oil, gas production.

1. Introduction

Livestock production industry suffers from increasing cost of grains and quality forages. At the same time, there are large quantities of crop residues and by-products associated with the production of crops in the field. Such residues may have important economic and environmental impacts as feed in the diet of ruminants after upgrading their nutritive value (Kholif *et al*. 2014; Elghandour et al., 2016). Corn stalk, oat straw, roughages

have a poor nutritional value as animal feeds due to their low nitrogen and high fiber contents (Abdel-Aziz *et al*., 2015; Elghandour et al., 2016). Generally, the using of the raw fibrous residues as animal feeds within the farm is limited because their high fiber content, low crude protein (CP) content, poor palatability, and low nutrient digestibility (Khattab *et al*., 2013; Togtokhbayar *et al*., 2015), which invariably lowers the efficiency of digestive utilization (Khattab *et al.,* 2013; Rojo *et al.,* 2015). Thus and for better utilization as feeds for ruminant animals, improving the nutritive value of these feeds before feeding to animal, using different strategies is very important. One of the most effective and safe strategies is the using of feed additives, including essential and crude oils (Hernandez *et al*., 2017).

Experiments suggest that some crude and essential oils have appetite stimulating properties, anti-bacterial effects, and antioxidant functions (Bodas *et al*., 2012; Smeti et al., 2015). It has been reported that crude and essential oils improved feed utilization because of their ability to alter rumen fermentation and increase dietary digestibility (Bodas *et al*., 2012). Eucalyptus (*Eucalyptus robusta*) is a common plant grows in many parts of the world and used to produce oils for medicinal and pharmaceutical purposes (Liu *et al.,* 2014; Sartorelli e*t al*., 2007). α-pinene, 1,8-cineole, spathulenol, globulol, viridiflorol were the main component the essential oil of dried leaved of *E. robusta* from China (Liu *et al*., 2014), α-pinene from fresh leaves of *E. robusta* grown in Brazil (Sartorelli *et al*., 2007). Besides, Elaissi *et al.* (2012) reported that the main components of eucalyptus oil are: 1,8-cineole, cryptone, α-pinene, ρ-cymene, and α-terpineol. 1,8-cineole, linalool, spathulenol, α-pinene and α-terpineol were the main components in essential oils of different species of *Eucalyptus* (El-Baha *et al*., 2017; Hussein et al., 2017; Luís *et al.* 2016; Salem *et al*., 2016; Harkat-Madouri *et al*. 2015; Salem 2015a).

The eucalyptus oil has strong antibacterial properties due to its content of tannins, phenolics, flavonoids and volatile oils (Elansary *et al*., 2017; Elaissi et al., 2012). Moreover,

biological activities such as bacteriostatic, fungistatic, anti-inflammatory, modifying ruminal fermentation characteristics, anti-protozoal and CH_4 mitigation were reported by Thao *et al*. (2014). For example, The *E. robusta* oil presented good inhibition to the growth of *Staphylococcus aureus*, *Escherichia coli* and *Candida albicans* (Sartorelli *et al.*, 2007).

Our hypothesis was that eucalyptus essential oil, with their active compounds, would improve in vitro ruminal fiber degradation, and increase gas production during fermentation resulting higher nutritive value and feed utilization of the studied agro-industry byproducts. Therefore, the objective of the present study was to determine the effect of including eucalyptu oil on *in vitro* gas production and nutrient digestibility of two byproducts fibrous feeds.

2. Materials and Methods

2.1. Agro-industry byproducts as substrates

Two roughages byproducts, namely corn stalk and oat straw were used as incubation substrates. Two batches of each feed were selected randomly and manually harvested from different sites in the State of Mexico, Mexico. Samples of substrates were dried at 65°C for 72 h in a forced air oven, and then ground in a Wiley mill to pass a 1 mm sieve and stored in plastic bags for subsequent determination of chemical composition and *in vitro* incubation. Chemical composition of the fibrous feeds is shown in Table 1.

2.2. In vitro incubations

Rumen inoculum was collected before morning feeding from from two Brown Swiss cow (450 kg body weight) fitted with permanent rumen cannula and fed *ad libitum* a total mixed ration made up of 1:1 concentrate and alfalfa hay, formulated to cover their nutrient requirements (NRC 2001) with a full access to fresh water. Cows had free access to fresh

water at all times during the experiment. Straightway after collection, the rumen contents obtained from the donor sheep were flushed with CO_2, mixed and strained through four layers of cheesecloth into a flask with O_2 free headspace. Filtered rumen fluid was immediately transported to the laboratory where it was mixed in a 1:4 (v/v) proportion with the buffer solution described by Goering and Van Soest (1970), with no trypticase added. Diluted rumen fluid (50 ml containing 10 ml of rumen liquor) was added to each incubation bottle.

Feed samples (0.5 g DM) were weighed into 120 ml serum bottles with appropriate addition of oils. Eeucalyptus essential oil was included at (per liter of incubation medium): 30 mg, 60 mg, 90 mg and 180 mg (equal to 0, 1.2, 3.6, and 7.2 mg/g DM substrate). Three incubation runs were performed in three different weeks. Bottles were inoculated within each incubation run, three bottles as blanks (i.e., rumen fluid only, with no substrate). After filling all bottles, they were immediately closed with rubber stoppers, shaken and placed in a water bath at 39°C. The volume of gas produced was recorded at 2, 4, 6, 8, 10, 12, 24 and 48 h of incubation using a pressure transducer (Extech Instruments, Waltham, USA) following the technique of Theodorou et al. (1994).

After 48 h of incubation, bottles were opened, and the pH was measured using a pH meter (Conductronic pH15, Puebla, Mexico). The contents of each bottle were filtered under vacuum through sintered glass crucibles (coarse porosity no. 1, pore size 100 to 160 μm; Pyrex, Stone, UK). The incubation residues were dried then at 105°C overnight to estimate apparent DM degradability (DMD). Neutral (NDF) and acid detergent fiber (ADF) were determined in the dried residues for estimation of NDF degradability (NDFD) and ADF degradability (ADFD). Blanks were used to correct for substrate contamination from ruminal fluid.

2.3. Chemical analyses

Samples of the feeds were analyzed for DM (#934.01), ash (#942.05), nitrogen (#954.01) and ether extract (#920.39) using AOAC (1997) official methods. The NDF (Van Soest et al., 1991) and ADF (AOAC #973.18) contents in feeds and incubation residues were determined using an ANKOM200 Fiber Analyzer Unit (ANKOM Technology Corp., Macedon, NY, USA). The NDF analysis was done with sodium sulfite, but without α-amylase. Both NDF and ADF were expressed without residual ash.

2.4. Calculations and statistical analyses

To estimate kinetic parameters of GP, gas volumes recorded (ml/g DM) were fitted using the NLIN procedure of SAS (2004) according to France *et al.* (2000) model as:

$$y = A \times [1 - e^{-c\,(t-Lag)}]$$

where y is the volume of GP at time t (h); A is the asymptotic GP (ml/g DM); c is the fractional rate of fermentation (/h), and Lag (h) is the discrete lag time prior to any gas is released.

The experimental design for the in vitro ruminal GP and nutrients degradability parameter analyses was a completely random design considering, as fixed factors, type of feed and oil type, and oil dose in the linear model (Steel *et al*., 1997). Data of each of the three runs within the same sample were averaged prior to statistical analysis. Mean values of each individual sample within each feed (three samples of each) were used as the experimental unit (Udén *et al*., 2012). Linear and quadratic polynomial contrasts were used to examine responses of feeds to increasing addition levels of the enzyme preparation.

3. Results

3.1. Chemical composition of agro-industry byproducts and oils

The two byproducts roughages differed in their chemical composition. The NDF concentrations ranged between 459 g. The ADF concentration ranged between 281 g for corn stalk. On the other hand, CP concentrations were at 63 g for maize stubble.

Eighteen compounds were identified in the eucalyptus oil. The GC/MS analysis showed that the principal compounds of the oil were eucalyptol, α-pinene, spathulenol, terpinen-4-ol and 4-terpineol

3.2. Gas production and degradability

For corn stalks, oil type × oil dose interactions were observed for fermentation pH (P=0.002) and degradabilities (P≤0.009) of DM, NDF and ADF. The asymptotic GP (P=0.025) and the lag time of gas formation (P=0.002) differed between eucalyptus oil. Increasing the dose of eucalyptus oil increased (linear effect, P=0.019; quadratic effect, P<0.001) the asymptotic GP and GP (P<0.05), without affecting (P>0.05) the rate of GP or the lag time of GP were observed with the addition of eucalyptus oil at different hours of incubation. Fermentation pH (P<0.001), and degradabilities of DM, NDF and ADF (P≤0.004) differed between eucalyptus and garlic oils. Lower pH values were observed (linear effect, P=0.027; quadratic effect, P=0.003) with the addition of the oils, with more pronounced declines for garlic oil compared with eucalyptus oil. Eucalyptus oil decreased NDF (linear effect, P=0.004; quadratic effect, P<0.001) and ADF degradabilities (linear effect, P=0.048) compared with the control treatment (Table 2).

For oat straw, interactions between oil type and oil dose were observed (P<0.05) for the rat of GP, lag time of gas formation, GP at different incubation hours, fermentation pH and degradabilities of DM and ADF. Moreover, the rat of GP, lag time of gas formation, GP at different incubation hours, fermentation pH and degradabilities of DM, NDF and ADF differed (P<0.05) for eucalyptus. Greater asymptotic GP (linear effect, P=0.049), rate of GP

(linear effect, $P<0.001$), and GP at different incubation hours (linear and quadratic effects, $P<0.05$) were observed with the addition of eucalyptus and garlic oils (Table 3).

4. **Discussion**

The GP technique is a simple, sensitive and powerful screening method to evaluate fermentation of feeds and test efficacy of feed additives before in vivo evaluation (Getachew *et al*., 2005). The close relationship between rumen fermentation and GP has been recognized. Menke *et al*. (1979) reported a high correlation between in vitro GP and in vivo apparent digestibility. However, results and conclusions obtained in vitro are not always representative of what occurs in vivo due to different condition including the lack of nutrients absorption, differences in fluid and particle dilution rates, feed intake relative to rumen volume, homogeneity of the compartment, etc. (Hristov *et al*., 2012).

4.1. Agro-industry byproducts and oil type effects

In ruminant nutrition, the inclusion of crude and essential oils can alter microbial populations, digestion and fermentation of diets based on their chemical composition (Bodas *et al*., 2012). Wide types of essential oils produced by different plant species and vary in chemical structures, stereochemistry and bioactive activities (Burt, 2004). In the present experiment, one type of oil was evaluated to determine its efficacy to affect fermentation and GP of two fibrous substrates using GP technique in vitro.

Eucalyptus oil contained eucalyptol (423.2 mg/g), α-pinene (236 mg/g), spathulenol (87.7 mg/g), terpinen-4-ol (42.4 mg/g) and 4-terpineol (26.8 mg/g). α-pinene (23.9%), p-cymene (23.2%) and 1,8-cineole (14.5%) were the major components in the oil (Traoré et al., 2010). Additionally, α-pinene was the main constituent of *E. robusta* oil (Thiolali and Von Wandruzka, 1985). α-,β-pinene and limonene were found with high amount in *E. robusta* oil

and could be responsible for the highest activity against the microbial growth (Janssen, 1989). In addition, it was reported that the oil possessed strong repellency to *Blattella germanica* (Liu *et al.*, 2011), larvicidal activity against *Aedes aegypti* (Lucia *et al*., 2012), and antimicrobial activities (Sartorelli *et al*., 2007; Cimanga *et al*., 2002). Even that the major component is 1,8-cineole, but the main contributor for the oil bioactivity was resulted from α-terpineol and its isomers (4-terpineol and terpinen-4-ol) (Inouye *et al*., 2001).

The chemical composition of incubated roughages differed, and was quite consistent with that reported in other studies (Elghandour *et al*., 2016). Variation between plants in the chemical composition is mainly due to different cultivars and genotype of the plants, growing environments factors such as climate, the soil and agronomic practice, harvest conditions, post harvesting treatments, and morphological (stalk, stem, leaf, husk, chaff) fractions of the samples (Welch, 1995). Different chemical composition of substrates greatly affects the fermentation kinetics and parameters (Elghandour *et al*., 2016; Kholif *et al*., 2017). Generally, fermentation kinetics appeared to be related to the chemical composition, in particular to the fiber content of the substrates (Elghandour *et al*., 2015; Kholif *et al*., 2017). Fermentation is generally index with degradation yielding short chain fatty acids and various gases, principally CO_2, hydrogen (H_2), CH_4, and nitrous oxide. Increasing the level fiber (structural carbohydrates) is paralleled with decreasing fermentation efficiency and GP (Elghandour *et al.*, 2016; Kholif *et al*., 2017). Higher proportions non-structural carbohydrates in the substrate indicate a better nutrient availability for rumen microorganisms resulting in stimulated nutrients degradability (Elghandour *et al.*, 2015). In the contrary, higher fiber content will surprise and negatively affect the microbial growth and fermentation (Elghandour *et al*. 2015, 2016).

4.2. Gas production kinetics

Eucalyptus oil increased GP of corn stalks and oat straw. Different fermentation kinetics are mainly due to different chemical composition of the incubated substrates (Elghandour *et al.,* 2015; Kholif et al., 2017).

The greater GP with eucalyptus oil at all doses reveals that the investigated levels of eucalyptus oil were within range acceptable and tolerant for ruminal microbial activity and growth. The increased GP is a result of improved ruminal fermentation with the inclusion of eucalyptus oil, which contains some biologically active compounds including α-pinene, b-cymene, γ-terpinene, and 1.8-cineole (Pierre 2008). However, Manh *et al.* (2012) observed that the inclusion of eucalyptus leaf powder at increasing levels up to 6% of incubated substrate decreased GP *in vitro*. It is clear that the tested doses of eucalyptus oil in their experiments are very greater than those used in the present experiment, which explain the conflicted results. Eucalyptus oil contains volatile oils, which may exert an inhibitory effect on microbial activity at high levels of inclusion. However, at low and moderate levels of the phenolics, tannins and other secondary metabolites of plants, the microbial activity was improved (Cedillo *et al.,* 2014) due to the ability of rumen microorganisms to degrade and use them as energy source (Varel *et al*., 1991).

Greater rate of GP with the inclusion of the oil may be related to the ability of eucalyptus active compounds to the antioxidant activity and its ability to remove free radicals from the fermentation medium, making the fermentation condition more appropriate for microbial activity. The active compounds presented in the eucalyptus (1,8-cineole, cryptone, α-pinene, ρ-cymene, and α-terpineol) indicative different patterns of antioxidants as protective compounds against free radical (Chung, 2006; Elaissi *et al*., 2012).

4.3. Fermentation pH and nutrient degradability

Eucalyptus oil decreased fermentation pH of corn stalks and oat straw. The values for all roughages ranged between 6.41 to 6.85, which are within the acceptable range for fiber digestion (Ørskov and Ryle, 1990). The inclusion of oils in the diets of animals is known to decrease ruminal pH due to increased dietary energy density with the inclusion of oils15). Lower pH with oil versus control was expected in view of the probable altered total ruminal volatile fatty acids concentrations that occur with oil addition (Busquet *et al*., 2005). Lowering the pH fermentation may be responsible about the inconsistency between the present results of enhanced fermentation kinetics, and the other experiments showed negative effects or weak effects with the inclusion of essential oils in ruminant diets. Lowering the pH fermentation may be responsible about the inconsistency between the present results of enhanced fermentation kinetics, and the other experiments showed negative effects or weak effects with the inclusion of essential oils in ruminant diets. At low pH, ruminal microorganisms are more susceptible to the effects of essential oils (Skandamis and Nychas, 2000), with more positive effect due to the inclusion of essential oils in diet of ruminants (Mirzaei-Aghsaghali and Maheri-Sis, 2011).

Eucalyptus oil decreased NDF degradability of corn stalks and sugarcane bagasse, with weak effects on NDF degradabilities of sorghum straw and oat straw, indicating the effect of incubated substrates chemical composition. Both p corn stalks and sugarcane bagasse have lower NDF and greater NSC concentrations compared with sorghum straw and oat straw. Lower degradability could be due to a reduced number of cellulolytic bacteria (McSweeney *et al*., 2001), and/or impaired bacterial adhesion to substrate and fibrolytic activity of rumen microbes (Bento *et al*., 2005). Manh et al. (2012) reported a decreased in vitro degradability with the inclusion of eucalyptus leaf powder at increasing levels up to 6% of incubated substrate. The inconstant results may be related to the level and type of eucalyptus sources and incubated substrates. However, eucalyptus oil decreased NDF degradability, it increased

GP revealing that the increased GP was a result of the fermentation of other feed nutrient such as non-structural carbohydrates.

5. Conclusions

The inclusion of eucalyptus oil improved rumen fermentation (GP and nutrients degradability) of the four agro- industry byproducts (corn stalk and oat straw) in different manner depending on the chemical composition of each feed. Increasing the dose of eucalyptus oil enhanced the fermentation parameters, with greater effect withe the dose 180 mg oil/L of incubation medium. Further research should, however, be conducted to establish the efficacy of eucalyptus oil in *in vivo* trials.

Table 1.

Chemical composition of the fibrous feeds (g/kg DM).

	Corn stalk	Oat straw
Dry matter	960	896
Organic matter	956	924
Crude protein	63	39
Ether extract	13	15
Non-structural carbohydrates	403	332
Neutral detergent fiber	477	538
Acid detergent fiber	281	380
Lignin	48	66
Cellulose	233	314
Hemicellulose	196	158

Table 2.

In vitro rumen gas kinetics of corn stalk as affected by the addition of eucalyptus essential oils (mg/L incubation medium).

Oil	Gas production at:										Degradability[2]			
	2 h	4 h	6 h	8 h	10 h	12 h	24 h	36 h	48 h	72 h	pH	DM	NDF	ADF
Control	20	38	55	72	87	101	169	216	248	284	6.79	620	483	347
30	24	45	66	85	103	119	199	252	288	330	6.76	677	429	316
90	23	45	66	86	104	122	209	272	319	379	6.76	676	435	322
180	21	40	59	77	95	111	198	266	319	395	6.76	654	412	305
SEM	1.7	3.2	4.5	5.5	6.3	7.0	8.5	7.9	7.1	9.1	0.041	25.0	24.6	13.8
P value														
Oil	0.068	0.068	0.066	0.064	0.062	0.061	0.060	0.087	0.261	0.499	<0.001	0.004	<0.001	0.003
Oil dose														
Linear	0.016	0.013	0.011	0.008	0.006	0.005	0.005	<0.001	<0.001	<0.001	0.027	0.008	0.004	0.048
Quadratic	0.586	0.520	0.442	0.375	0.302	0.241	0.021	0.002	<0.001	<0.001	0.003	0.002	<0.001	0.060

[1]*A* is the asymptotic gas production (mL/g DM); *c* is the rate of gas production (/h); *Lag* is the initial delay before gas production begins (h).

[2]ADFD, acid detergent fiber; degradability DMD, dry matter degradability; NDFD, neutral detergent fiber degradability

Table 3.

In vitro rumen gas kinetics of oat straw as affected by the addition of eucalyptus essential oils (mg/L incubation medium).

Oil	Gas production at:										Degradability[2]			
	2 h	4 h	6 h	8 h	10 h	12 h	24 h	36 h	48 h	72 h	pH	DM	NDF	ADF
Control	8	16	23	31	38	45	84	118	148	198	6.81	503	597	393
30	11	21	31	40	49	58	106	146	179	229	6.71	490	627	418
90	11	21	31	41	51	60	111	155	192	250	6.76	522	612	439
180	15	29	43	57	69	82	146	196	236	292	6.78	527	593	435
SEM	1.0	1.9	2.8	3.6	4.4	5.2	8.6	11.0	12.5	3.9	0.012	24.1	18.9	19.6
P value														
Oil	0.003	0.003	0.004	0.004	0.005	0.006	0.002	0.004	0.009	0.034	<0.001	0.001	<0.001	0.018
Oil dose														
Linear	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	0.004	<0.001	0.015	0.004	0.908
Quadratic	0.011	0.011	0.009	0.008	0.007	0.006	0.003	0.001	0.007	0.002	0.734	0.306	0.067	0.869

[1]*A* is the asymptotic gas production (mL/g DM); *c* is the rate of gas production (/h); *Lag* is the initial delay before gas production begins (h).

[2]ADF, acid detergent fiber; degradability DM, dry matter degradability; NDF, neutral detergent fiber degradability.

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