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“SUSTAINABILITY INFLUENCE OF GARLIC OIL ON THE RUMINAL BIOGAS OF TWO AGRO-INDUSTRY BYPRODUCTS”

“INFLUENCIA DE LA SOSTENIBILIDAD DEL ACEITE DE AJO EN EL BIOGÁS RUMINAL DE DOS SUBPRODUCTOS AGROINDUSTRIALES”

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Sustainability influence of garlic oil on the ruminal biogas of two agro-industry byproducts

Short title: Garlic oils that affect ruminal fermentation.

Abstract

The effects of garlic oils on the environmental ruminal fermentation of two agroindustrial by - products (corn stalk, oat straw) were studied using the *in vitro* gas (GP) technique. Garlic oil at 0, 30, 90 and 180 mg / L of incubation medium (equal to 0, 1.2, 3.6 and 7.2 mg / g of DM substrate) was added to each. The gas volumes were recorded at 2, 4, 6, 8, 10, 12, 24, 36, 48 and 72 h of incubation. The kinetics of gas production differed in garlic oil. Garlic oil increased (P <0.05) the asymptotic GP and GP of corn stalks and oat straw. Garlic oil decreased the fermentation pH (P <0.05) of corn stalks and oat straw. It can be concluded that the application of garlic oil positively affected the ruminal fermentation of the two agroindustrial by-products as fodder feed. Garlic oil had a better environmental and sustainable effect on ruminal GP. By increasing the inclusion rate of oils, the fermentation parameters were improved; the dose of 180 mg of oil / L increased GP.

Key words: 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 and oat straw 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 (Bodas et al., 2012). Feeding garlic (*Allium sativum*) showed positive effects on feed utilization, antimicrobial activity, and ruminant performance (Yang and He, 2016). Garlic oil contains several active compounds, including organosulfur compounds, enzymes, sterols, steroids, triterpenoid glycosides, flavonoids, phenols and organoselenium compounds (Lawson, 1996). Feeding garlic oil to ruminants resulted in altered ruminal fermentation as reduced short chain fatty acids concentration and proportion of acetate, and increased the proportions of propionate and butyrate, with inhibition of methane (CH4) production (Busquet et al., 2005; Mirzaei-Aghsaghali and Maheri-Sis, 2011). The mode of action of garlic oil is not clear until now; however, its antimicrobial activity is related mainly to the active components in garlic oil (i.e., organosulfur compounds) which caused reduced CH4 proportions, revealing its role in rumen microbial modulation (Busquet et al., 2005).

Our hypothesis was that garlic oil, with their active compounds increases 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 garlic oil on in vitro gas production.

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. Four 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. 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 oil. Garlic 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, 48 and 72 h of incubation using a pressure transducer (Extech Instruments, Waltham, USA) following the technique of Theodorou et al. (1994).

After 72 h of incubation, bottles were opened, and the pH was measured using a pH meter (Conductronic pH15, Puebla, Mexico).

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. Statistical analyses

The experimental design for the in vitro ruminal GP analyses was a completely random design considering, as fixed factors, type of feed, 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 preparation. MODELO ESTADISTICO?

3. Results

3.1. Chemical composition of agro-industry byproducts and oils

The two byproducts roughages differed in their chemical composition. The ADF concentration ranged between 281 g for corn stalk, and for oat straw? On the other hand, the CP concentrations were between 27 to 63 g/kg for corn stalk, and for oat straw?

3.2. Gas production

For corn stalks, interactions DE QUE? were observed for fermentation pH (P=0.002). The asymptotic GP (P=0.025) and the lag time of gas formation (P=0.002) differed with garlic oil. Increasing the dose of garlic oils 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 garlic oil at different hours of incubation. Fermentation pH (P<0.001) differs with garlic oil. Lower pH values were observed with the addition of the garlic oil.

For oat straw, interactions between oil dose Y QUE OTRA COSA? were observed (P<0.05) for the rate of GP, lag time of gas formation, GP at different incubation hours, and fermentation pH. Moreover, the rate of GP, lag time of gas formation, GP at different incubation hours, fermentation pH differed (P<0.05) with garlic oil. 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 garlic oil. Lower fermentation pH (linear effect, P<0.001) were observed with the addition of garlic oil at different doses.

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). Menke et al. (1979) reported a high correlation between in vitro GP and in vivo apparent digestibility. The close relationship between rumen fermentation and GP has been recognized. 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, and homogeneity of the compartment (Hristov et al., 2012).

4.1. Agro-industry byproducts and oil type effects

Gas production kinetics differs with garlic oil as a result of differed chemical composition. 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 vary in chemical structures, stereochemistry and bioactive activities (Burt, 2004). In the present experiment, one oil typ with different chemical structures and stereochemistries were evaluated their efficacy to affect fermentation and GP of two fibrous substrates using the in vitro GP technique.

Garlic oil contained phenol,2-6-bis (1,1-dimethylethyl)–4-methyl; 177 mg/g), carbomethoxy carboethoxymethyl disulphide (31 mg/g), butylboronic acid (28 mg/g) and 2- propylheptanol (21 mg/g) as the most appendant compounds. Garlic oil had reported to have different chemical compounds such as dithin, allicin, diallyl trisulphide, and sallylcysteine; minerals like Mg, Zn, Se, germanium; amino acids, saponins and flavonoids (Block, 1992; Johnson et al., 2013). Garlic extract had various bioactivities such as bactericidal, fungicidal properties, antithrombotic, antitumor and anti-inflammatory properties (Harris et al., 2001; Curtis et al., 2004; Lanzotti, 2006).

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 yielding short chain fatty acids and various gases, principally CO2, hydrogen (H2), CH4, 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 of 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 negatively affect the microbial growth and fermentation (Elghandour et al., 2015, 2016).

4.2. Gas production kinetics

The garlic oil increased GP of corn stalks and oat straw. Different fermentation kinetics is mainly due to different chemical composition of the incubated substrates (Elghandour et al., 2015; Kholif et al., 2017). It was expected that increasing the doses of garlic oil would cause detrimental effects on ruminal fermentation due to the antimicrobial activity attributed to its sulfur compounds, particularly allicin (Feldberg et al., 1988) of garlic oil. This reveals that tested levels of garlic oils were within the acceptable range to modify the fermentation kinetics without negative effects. Busquet et al. (2005) observed a reduction on acetate production when added garlic oil at 312 mg/L of culture fluid. The improved fermentation and GP with increasing garlic oil doses can be explained based on its content of a highly active compound inulin which is considered as a prebiotic, which can act as a stimulator for the ruminal microorganisms leading to improved microbial eco-system (Kamra et al., 2012). Roland et al. (1995) showed that feeding of inulin resulted in a higher production of H2 without CH4 emission but accompanied with a four-fold increase in butyrate concentration.

Greater rate of GP with the inclusion of the oils may be related to the ability of garlic oil active compounds to have 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 garlic oil (e.g. allyl disulfide, alliin, allicin and allylcysteine) are indicative of different patterns of antioxidants as protective compounds against free radical (Chung, 2006; Elaissi et al., 2012).

4.3. Fermentation pH

Garlic 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. Lower pH with oil versus control was expected in view of the probable altered total ruminal volatile fatty acids concentrations that occurs with oil addition (Busquet et al., 2005). Hernandez et al. (2017) observed that garlic oil at 30, 120, 250 and 500 µL/g DM substrate decreased the fermentation pH.

Lowering the pH fermentation may be responsible about the inconsistency between the present results of enhanced fermentation kinetics, and the other experiments that 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).

Gas production is an indicator of DM digestibility. Increased DMD and NDFD with garlic supplementation suggests increased energy availability to ruminal microorganisms (Yang et al., 2007) when resulted in increased GP with garlic oil supplementation. Kongmun et al. (2010) observed that the addition of garlic essential oil increased ruminal DM and OM digestibility. Moreover, Patra et al. (2010) observed enhanced nutrient digestibility with feeding garlic to dairy cows at 1% of feed intake. Conversely, Busquet et al. (2005) observed that garlic oil had no effect on DM, OM, NDF and ADF digestibility suggesting that garlic oil cannot modify the overall diet fermentability. They included garlic oil at 31.2 mg/L incubation medium, which is very small amount compared with the used doses in the present experiment.

5. Conclusions

The inclusion of garlic oil improved the environment and sustainable effects on rumen fermentation of the two agro- industry byproducts (corn stalk and oat straw) in different manner depending on the chemical composition of each feed. Garlic oil had better effect. Increasing the dose of garlic oil enhanced the fermentation parameters, with greater effect with the dose 180 mg oil/L of incubation medium. Further research should, however, be conducted to establish the efficacy of garlic 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
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 garlic oil (mg/L incubation medium).

Oil	Gas production (ml/g DM) at:										
	2 h	4 h	6 h	8 h	10 h	12 h	24 h	36 h	48 h	72 h	pH
Control	20	38	55	72	87	101	169	216	248	284	6.79
Garlic oil											
30	25	49	71	92	111	129	213	269	306	347	6.61
90	27	51	75	96	117	136	227	288	328	374	6.62
180	25	49	71	92	111	130	217	276	317	364	6.41
SEM	1.7	3.2	4.5	5.5	6.3	7.0	8.5	7.9	7.1	9.1	0.041
P value											
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
Quadratic	0.586	0.520	0.442	0.375	0.302	0.241	0.021	0.002	<0.001	<0.001	0.003

Table 3.

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

	Gas production (ml/g DM) at:										
Trat	2 h	4 h	6 h	8 h	10 h	12 h	24 h	36 h	48 h	72 h	pH
Control	8	16	23	31	38	45	84	118	148	198	6.81
Garlic oil											
30	17	34	49	64	78	91	158	206	242	287	6.68
90	20	39	57	74	89	104	175	224	258	297	6.70
180	12	24	35	46	57	67	124	173	215	282	6.68
SEM	1.0	1.9	2.8	3.6	4.4	5.2	8.6	11.0	12.5	3.9	0.012
P value											
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
Quadratic	0.011	0.011	0.009	0.008	0.007	0.006	0.003	0.001	0.007	0.002	0.734

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