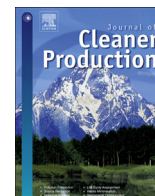


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The effect of garlic oil, xylanase enzyme and yeast on biomethane and carbon dioxide production from 60-d old Holstein dairy calves fed a high concentrate diet



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ABSTRACT

Ruminal fermentation is accompanied by production of methane (CH₄) and carbon dioxide (CO₂) which are greenhouse gases (GHG) that cause environmental pollution. The effect of natural feed additives on the *in vitro* fermentation and production of CH₄ and CO₂ in dairy calf has had less attention. Therefore, this study aimed to evaluate the effect of garlic oil, xylanase enzyme, and yeast on *in vitro* biogas production from dairy calves fed a high concentrate diet. Rumen contents from 60-d old Holstein calves fed a concentrate diet were used as inoculum source. Garlic oil was included at 30,120, 250 and 500 μL/g dry matter (DM), while xylanase was included at 3 and 6 μL/g DM and yeast at 2 and 4 mg/g DM. The substrate used was the same as the diet fed to calves. Garlic oil linearly decreased ($P < 0.05$) *in vitro* DM digestibility and there were no differences among levels of either xylanase or yeast. Garlic oil decreased ($P < 0.05$) DM degradability while xylanase and yeast had no effect. The lag phase was linearly increased ($P < 0.05$) with increasing level of garlic oil. Garlic oil quadratically decreased CH₄ and CO₂ production. The control treatment had the highest CH₄ and CO₂ production followed by xylanase, yeast and garlic oil. Increasing level of xylanase and yeast increased ($P < 0.05$) CO₂ production. It can be concluded that garlic oil followed by yeast and then xylanase can be used to mitigate *in vitro* CH₄ and CO₂ production from dairy calves fed a high concentrate diet. However, further research is warranted to establish the efficacy of such feed additives in *in vivo* trials.

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1. Introduction

Ruminant nutritionists aim to manipulate the ruminal microbial ecosystems and fermentation to improve feed utilization and feed conversion to animal products. Antibiotics have good effects on feed utilization and production, but have been banned due to the

Abbreviations: ADF, acid detergent fiber; *b*, the asymptotic gas production; *c*, the fractional rate of fermentation; CH₄, methane; CO₂, carbon dioxide; CP, crude protein; DM, dry matter; DMD, dry matter degradability; EE, ether extract; GHG, greenhouse gas; GP, gas production; *Lag*, the discrete lag time prior to any gas being released; N, nitrogen; NDF, neutral detergent fiber; O₂, oxygen.

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increasing public concern for their usage. Thus, looking for natural alternative has gained interest and importance. Phytochemical extracts (Cedillo et al., 2015), exogenous enzymes (Vallejo et al., 2016), and yeast (Hassan et al., 2016) are gaining increasing interest as feed additive for animal feeding. Such feed additives can reduce energy losses as methane (CH₄), and nitrogen (N) as ammonia, which reduce animal performance and contribute to the release of pollutants to the environment.

Currently, the livestock production sector is one of the largest contributors of anthropogenic sources of greenhouse gases (GHG), where livestock production accounts approximately for two-thirds of the direct emissions (Slade et al., 2016). Methane is the most important GHG since it accounts for about 50–60% of emitted GHG; it is also responsible for a loss of about 2–12% of gross energy of

feed in ruminant production systems (Mirzaei-Aghsaghali et al., 2012). Thus, it is important to reduce enteric CH₄ production in ruminants and contribute to reduce global GHG emissions without any negative effect on feed conversion efficiency.

Garlic (*Allium sativum*) has been shown to have positive effects on nutrient digestibility and antimicrobial activity in ruminant feeding. Garlic oil contains several active compounds, including organosulfur compounds, enzymes, sterols, steroids, triterpenoid glycosides, flavonoids, phenols, and organoselenium compounds (Lawson, 1996). The use of garlic oil as a feed additive in ruminant nutrition has not been researched until recently. In some experiments (Busquet et al., 2005; Mirzaei-Aghsaghali and Maheri-Sis, 2011), garlic oil reduced the concentration of short chain fatty acids and proportions of acetate, and increased the proportions of propionate and butyrate, with inhibition of CH₄ production. However, garlic oil has antimicrobial activity, which may be detrimental to ruminal fermentation at high levels (Busquet et al., 2005). The 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). Busquet et al. (2005) suggested that the anti-methanogenic effect of garlic is due to its active components which have direct inhibition on *Archaea* microorganisms in the rumen. However, the mechanism by which garlic decreases CH₄ production is not known; although the mechanism could be related to the capacity of organosulfur compounds to inhibit the enzyme 3-hydroxy-3-methylglutaryl coenzyme A reductase by a thiol-disulfide exchange reaction (Gebhardt and Beck, 1996).

Feeding ruminants on diets supplemented with fiber-degrading enzymes has been shown to improve feed utilization and animal performance (Rojo et al., 2015; Morsy et al., 2016), although the mode of action has not been fully elucidated. However, hydrolysis of dietary fiber and alteration of ruminal fermentation (Alseny et al., 2015), and enhanced ruminal microorganisms attachment and colonization to the plant cell wall (Wang et al., 2001) were observed. Synergistic enhancement of microbial enzyme activity in the rumen (Morgavi et al., 2000) have been suggested as possible modes of action. Morgavi et al. (2000) demonstrated a synergism between exogenous and endogenous rumen enzymes. The net result was a higher hydrolytic effect in the rumen with combined enzymes than that estimated from individual enzyme activities.

Saccharomyces cerevisiae is generally recognized as safe by the Food and Drug Administration organization of USA, and it can be legally used as an animal feed additive to stabilize rumen fermentation and prevents rumen microflora disorders (Elghandour et al., 2015; Hassan et al., 2016). Yeast has the ability to modify ruminal fermentation through provision of important nutrients and nutritional cofactors required for microbial growth and activity (Polyorach et al., 2014). Besides, yeast could enhance fungal colonization of plant cell walls resulting in increased dry matter (DM) and neutral detergent fiber (NDF) digestion (Patra, 2012) and mitigate CH₄ production due to stimulation of the acetogens to compete or co-metabolize hydrogen (H₂) with methanogens, thereby reducing CH₄ emissions (Hristov et al., 2013).

To the best of our knowledge, no study has been carried out to test the effect of natural feed additives on the *in vitro* fermentation of feeds using rumen inoculum from calves. Besides, the *in vitro* biogases produced (e.g., CH₄ and carbon dioxide (CO₂)) from dairy calves has not been investigated before. Therefore, the present experiment aimed to investigate the effect of natural feed additives i.e. garlic oil, xylanase enzyme and yeast at different levels on *in vitro* biogases production from 60-d old dairy calves fed a high concentrate diet.

2. Materials and methods

2.1. Treatments

Three feed additives were tested against a control treatment (no additives): garlic oil (Garlic oil, Chinese, Sigma-Aldrich, Saint Louis, MO, USA) at 30, 120, 250 and 500 µL/g DM, xylanase (Dyadic® Xylanase PLUS, Dyadic International, Inc., Jupiter, USA) at 3 and 6 µL/g DM and yeast (Procreatin 7, Safmix, Toluca, Mexico) at 2 and 4 mg/g DM. The substrate used was the same diet fed to calves.

2.2. *In vitro* incubations

Rumen inoculum was collected before morning feeding from 6 weaned calves at 60 days of age with 40–55 kg body weight using a stomach tube and hand pump. Calves were fed *ad libitum* a starter feed of a total mixed ration of a commercial concentrate (Ultra Malta Clayton®, Toluca, Mexico) containing (g/kg DM): 200 crude protein (CP), 230 NDF, 50.3 acid detergent fiber (ADF) and 35.6 ether extract (EE). The starter feed was formulated to meet the calves' nutrient requirements as per the National Research Council (NRC, 2001). Calves had free access to fresh water at all times during inoculum collection phase. Immediately after collection, the rumen contents from each calf were flushed with CO₂, mixed and strained through four layers of cheesecloth into a flask with an oxygen (O₂) free headspace. Filtered rumen fluid was immediately transported to the laboratory where it was mixed in a 1:4 (v/v) ratio with the buffer solution as described by Goering and Van Soest (1970), with no trypticase added. Diluted rumen fluid (40 mL containing 10 mL of rumen liquor) was added to each incubation bottle, where substrates (0.5 g DM) had been previously weighed out and additive solutions dispensed.

Three incubation runs were performed in three different weeks. In addition to bottles containing substrate, three bottles were incubated 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 production (GP) was recorded at 2, 4, 6, 8, 10, 12, 14, 16, 18, 24, 36, 48, 60 and 72 h of incubation using a pressure transducer (Extech Instruments, Waltham, USA) following the technique of Theodorou et al. (1994). At the same incubation period, CH₄ and CO₂ concentrations in the headspace of the bottles was measured using a diffusion based gas detector (Gas Analyzer CROWCON Model Tetra3, Abingdon, UK).

After sampling the supernatant and pH determination, the content of each bottle was filtered under vacuum through sintered glass crucibles (coarse porosity no. 1, pore size 100–160 µm; Pyrex, Stone, UK) and incubation residues dried at 105 °C overnight to estimate apparent DM degradability (DMD).

2.3. Chemical analyses

Samples of feeds were analyzed for DM (#934.01), ash (#942.05), N (#954.01) and EE (#920.39) using AOAC (1997) official methods. The NDF (Van Soest et al., 1991) and ADF (AOAC, 1997; #973.18) contents in both 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, and with 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 (2002)

according to France et al. (2000) model as:

$$y = b \times \left[1 - e^{-c(t-Lag)} \right]$$

where y is the volume (mL/g DM) of GP at time t (h); b 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 gas, CH₄ and CO₂ productions, and fermentation parameters analysis was a completely random design considering, as fixed factors, additive types (A) and additive doses (D) 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 extract within each species (three samples of each) were used as the experimental unit. The statistical model was:

$$Y_{jkl} = \mu + A_j + D_k + (A \times D)_{jk} + E_{jkl}$$

where: Y_{jkl} = is every observation of the j th feed additive (A_j) when incubated in the k th additive doses (D_k); μ is the general mean; and E_{jkl} is experimental error. Linear and quadratic polynomial contrasts were used to examine responses to increasing levels of additives.

3. Results and discussion

3.1. Gas production and fermentation

Extents 'b' and rate of GP were similar among different treatment at different levels; however, the rate of digestion was highest ($P < 0.05$) at 120 μ L garlic oil and intermediate in all other treatments (Table 1). The *in vitro* rumen GP (mL/g incubated DM) as affected by the different feed additives are shown in Fig. 1. Fermentation is generally index with degradation yielding short chain fatty acids and various gases, principally CO₂, H₂, CH₄, and nitrous oxide; some of which are affected by other substances including organic acids (Elghandour et al., 2016a), exogenous fibrolytic enzymes (Elghandour et al., 2016b; Kholif et al., 2016), yeast cell (Elghandour et al., 2016c,d; Velazquez et al., 2016).

Table 1
Effect of different feed additives on *in vitro* rumen gas kinetics of a high concentrate diet fed to young calves at 60 days of age.

Additive type	Dose (/g DM)	Gas production (mL/g DM) ^a			Fermentation kinetics	
		b	c	Lag	pH	DMD ^b
Control	No additive	382.9	0.058ab	2.69b	6.9	693.3a
Garlic oil	30 μ L	404.1	0.057b	2.72b	7.0	671.1a
	120 μ L	283.4	0.108a	6.33ab	6.8	684.9a
	250 μ L	372	0.085ab	8.94a	5.8	615.3ab
	500 μ L	213	0.067ab	6.45ab	7.0	554.1b
Xylanase	3 μ L	350.5	0.068ab	2.78b	6.9	701.1a
	6 μ L	354	0.077ab	3.14b	6.9	689.8a
	2 mg	345.2	0.069ab	2.92b	6.9	698.6a
Yeast	4 mg	347.5	0.063ab	2.44b	6.9	695.1a
	Pooled SEM ^c	74.61	0.0192	1.699	0.62	34.4
Additive effect		0.794	0.847	1.000	0.992	0.713
Dose effect						
Linear		0.718	0.073	<0.001	0.022	0.005
Quadratic		0.051	0.008	0.520	0.254	0.191
Additive \times Dose		0.5021	0.569	0.975	0.899	0.416

Means in the same column with different superscripts differ ($P < 0.05$).

^a b 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).

^b DMD is dry matter degradability.

^c SEM is standard error of the mean.

However, xylanase enzyme was expected to increase degradation and GP rate (Elghandour et al., 2016b; Kholif et al., 2016) but was not limiting fermentation in the rumen. Yeast cells consume O₂ molecules making the environment commensurate for optimum activity of various organisms (Newbold et al., 1996). The current study confirmed the lack of effect due to levels of yeast (Velazquez et al., 2016) but disagrees with others (Elghandour et al., 2016c,d) who report improved GP, suggesting that yeast can sometime but not always increase GP. These feed additives tended to exert a quadratic decrease in maximum GP. Garlic oil linearly delayed ($P < 0.001$) the onset of fermentation relative to xylanase, yeast and the control, suggesting that substances that directly affect the general population of rumen micro-organisms would generally delay the onset of fermentation.

The fermentation pH was affected linearly ($P = 0.022$) with different additives (Table 1). Garlic oil linearly decreased *in vitro* digestibility of DM ($P < 0.05$), while there was no difference between levels of either xylanase or level of yeast. Moreover, garlic oil ($P < 0.01$) decreased DMD, while xylanase and yeast had no effect. The linear decrease in the pH with increasing garlic oil level was unexpected since DM degradation decreased linearly with increasing additives, but these relationships are difficult to explain in fermentation terms. It was expected garlic oil would reduce the activity of all rumen micro-organism (bacteria, fungi and protozoa), therefore, a decrease in DM degradation in the current study agrees with the anti-microbial effects in various reports (Mirzaei-Aghsaghali et al., 2012).

3.2. Effect of additives on CH₄ production

Fig. 2 shows the *in vitro* rumen CH₄ production (mL/g incubated DM) as affected by the different feed additives. The effect of additive \times dose interaction was significant ($P < 0.05$) during all hours of incubation (Table 2). The control produced most ($P < 0.05$) CH₄ (expressed as: mL/g DM incubated, and mL/g degraded DM) followed in decreasing order by xylanase, yeast and garlic oil. These effects were rather amplified when CH₄ production was expressed as a proportion of total GP. It is accepted that the production of H₂ in the rumen limits the fermentation of fiber, and reduces digestibility; however, a copious quantity of H₂ is produced and together with CO₂ is used to synthesize CH₄ by methanogenic microorganisms. This process of CH₄ production uses reduced co-factors liberating oxidized ones, helping rumen microbes to recycle these co-factors. Although in agreement with Kholif et al. (2016), it is surprising that the control treatment yielded the highest amount of CH₄, followed by yeast and xylanase (Table 2). It is not clear why xylanase would depress CH₄ production when this supplementary enzyme is supposed to increase the availability of hemicellulose and CH₄ production (Elghandour et al., 2016b). Unless the digestion of hemicellulose liberates lignin compounds, increasing the chance of attachment to microorganisms or with microbial products forming antibiotic or bacteriostatic metabolite, as such having little or no effect on digestion as indicated in this study.

It is known that yeast products increase nutrient digestibility (Hassan et al., 2016) specifically by elevating populations of cellulolytic and amylolytic bacteria in the rumen accompanied by altered fatty acid production (Kumar et al., 1997). Some live *S. cerevisiae* added in very small amounts have been reported to reduce methanogenesis in the rumen (Hristov et al., 2013). In other studies, yeast supplementation had no impact on CH₄ production (McGinn et al., 2004). In addition, studies on yeasts appear somewhat conflicting, suggesting either an increase in CH₄ production (Elghandour et al., 2016c,d) or no effect (Opsit et al., 2011) in batch cultures with mixed rumen microflora; in agreement with the

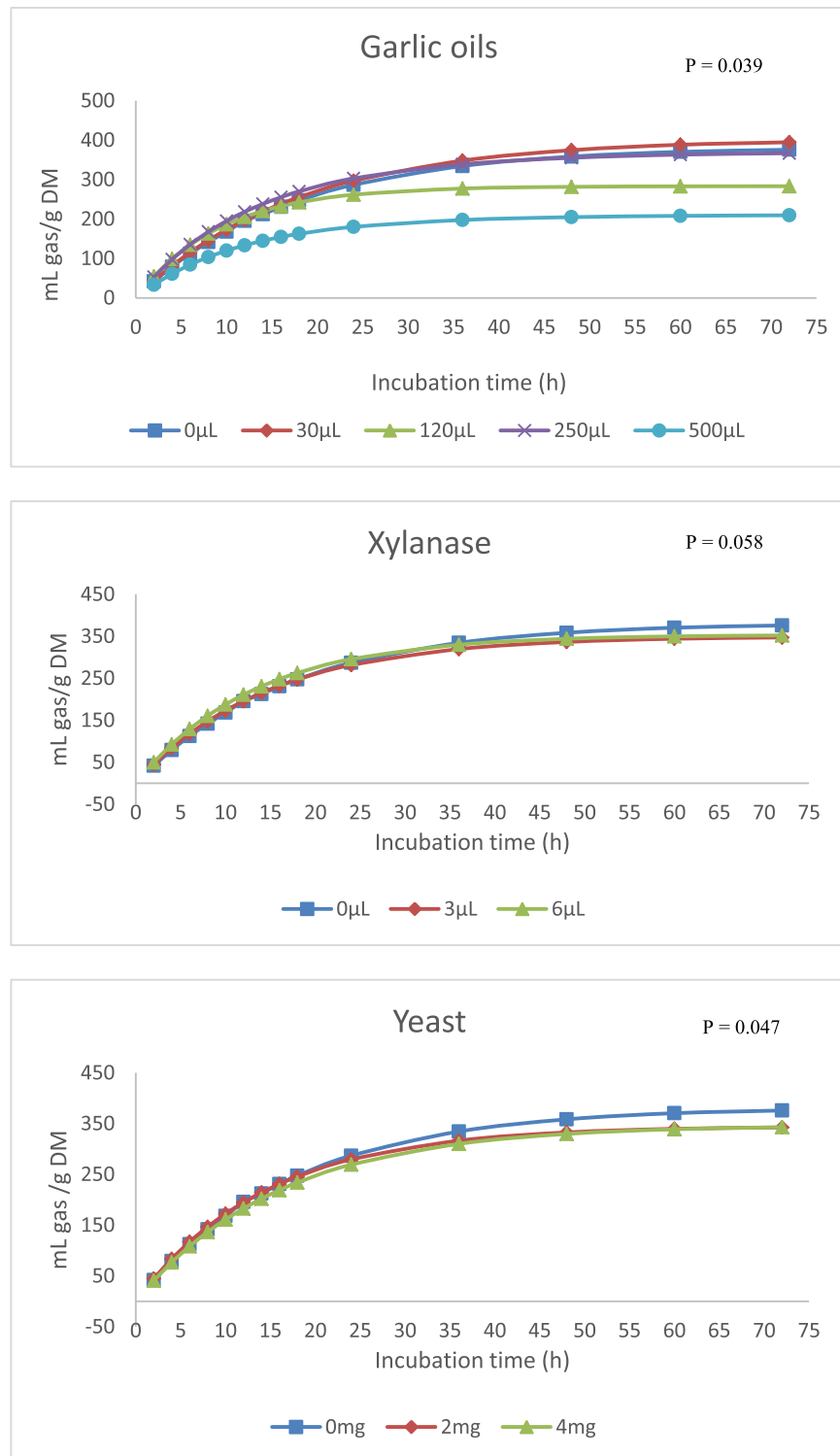


Fig. 1. *In vitro* rumen gas production (mL/g incubated DM) of a high concentrate diet fed to young calves at 60 days of age incubated with no additive, garlic oil, xylanase, and yeast.

observations in the current study. It was expected that yeast cultures would stimulate acetogens to compete or to co-metabolize H_2 with methanogens and reduce CH_4 emissions (Hristov et al., 2013) and/or scavenge O_2 and make the environment more conducive for fermentation and microbial growth. Implying a tendency of decreased CH_4 production with yeast was expected in the study. Thus, in a few studies, feeding live yeast products have consistently

reduced CH_4 production by 20–58% (Lynch and Martin, 2002; Newbold and Rode, 2006) but it remains uncertain whether a 60-day old rumen environment could be less responsive to yeast.

Garlic oil has been shown to have both Gram negative and Gram positive anti-bacteria properties largely due to allicin (allyl 2-propene thiosulfinate) (Cavallito and Bailey, 1944), anti-fungi (Adetumbi et al., 1986) and anti-viruses (Weber et al., 1992)

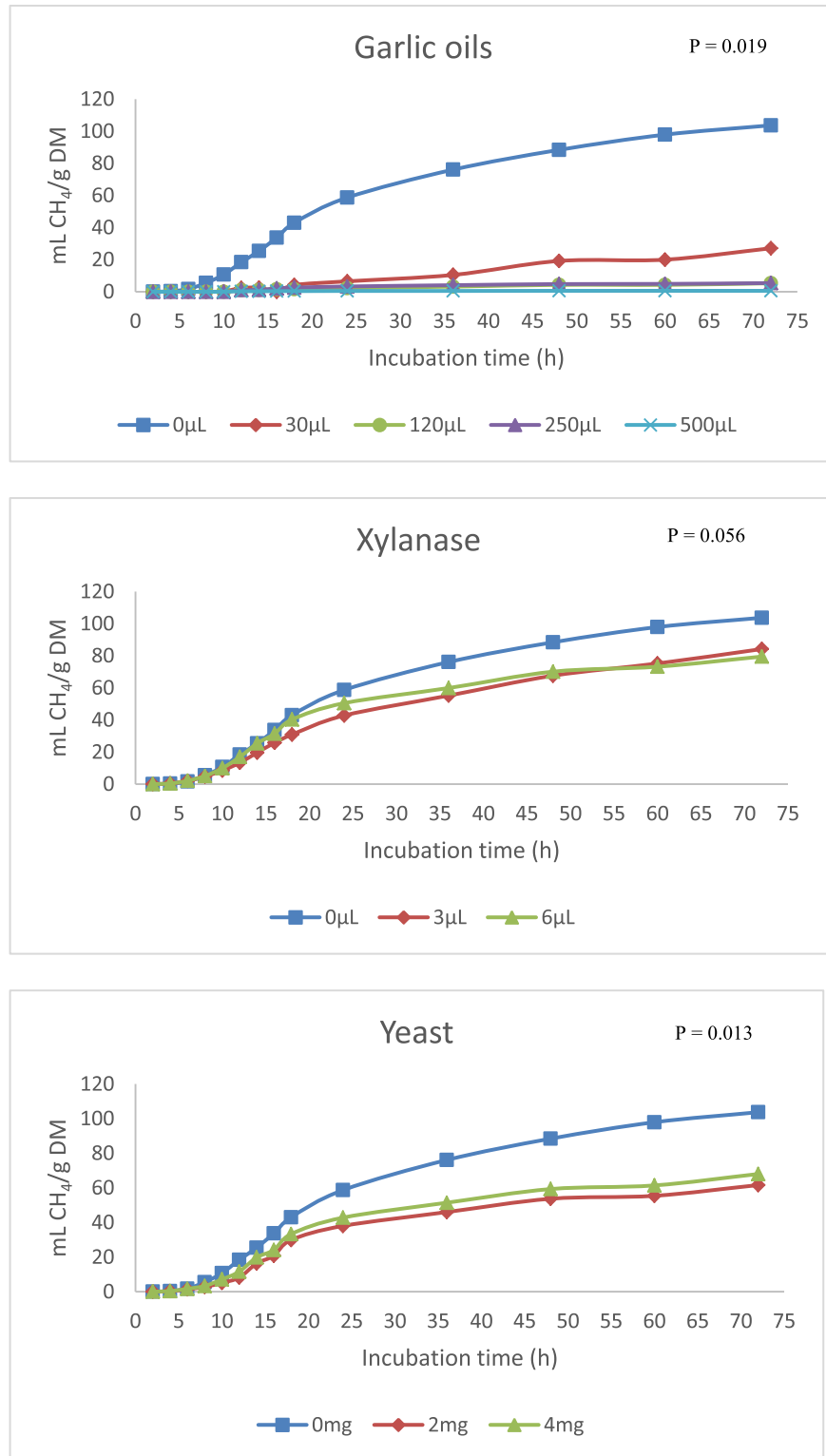


Fig. 2. *In vitro* rumen methane (CH_4) production (mL/g incubated DM) of a high concentrate diet fed to young calves at 60 days of age incubated with no additive, garlic oil, xylanase, and yeast.

properties. Based on these properties, others have either defined the minimum inhibitory concentration (Nejad et al., 2014) or pointed to the antiparasite role (Goncagul and Ayaz, 2010) of this food item. In rumen fluid, CH_4 producing organisms live in symbiotic relationship with cellulolytic organism, protozoa and fungi,

the latter two relationships being involved in extensive production of CH_4 ; methanogens also shift H_2 among themselves (McAllister et al., 1994). Both linear and quadratic decreases in CH_4 production due to increasing levels of garlic oil reported here may be related to any one of the following explanation. Garlic oil might

Table 2Effect of different feed additives on *in vitro* methane (CH₄) production of a high concentrate diet fed to young calves at 60 days of age.

Additive type	Dose (/g DM)	CH ₄ production											
		mL/g incubated DM				mL/g degraded DM				Proportional CH ₄ production			
		6 h	24 h	48 h	72 h	6 h	24 h	48 h	72 h	6 h	24 h	48 h	72 h
Control	No additive	1.71a	58.7a	88.4a	103.7a	2.47a	84.9a	127.7a	149.6a	1.53a	20.55a	24.83a	27.81a
Garlic oil	30 µL	0.00b	6.5c	19.2d	27.1bc	0.00b	9.6b	28.5c	40.3bc	0.00b	2.17b	5.00b	6.67bc
	120 µL	0.00b	2.2c	4.3d	5.3c	0.00b	3.3b	6.2c	7.0c	0.00b	0.83b	1.50b	1.83c
	250 µL	0.00b	3.3c	4.8d	5.4c	0.00b	5.4b	7.6c	8.6c	0.00b	1.17b	1.50b	1.67c
	500 µL	0.00b	0.5c	0.6d	0.6c	0.00b	0.9b	1.0c	1.1c	0.00b	0.50b	0.50b	0.50c
Xylanase	3 µL	2.04a	42.8ab	67.6ab	84.2a	2.92a	61.1a	96.5ab	120.2a	1.70a	15.08a	20.08a	24.28a
	6 µL	2.14a	50.5ab	70.1ab	79.5a	3.11a	73.4a	102.0ab	115.7a	1.67a	17.09a	20.22a	22.39a
Yeast	2 mg	1.39a	38.0b	53.7bc	61.6ab	2.00a	54.5b	76.9b	88.2ab	1.19a	13.53a	16.11a	17.93ab
	4 mg	1.66a	42.8ab	59.3ab	68.0ab	2.39a	61.7b	85.4ab	97.9ab	1.54a	15.92a	18.01a	19.86a
Pooled SEM ^a		0.440	7.79	11.69	16.76	0.669	12.39	18.01	24.87	0.371	2.886	3.444	4.693
Additive effect		0.001	0.001	0.003	0.015	0.002	0.003	0.006	0.020	0.001	0.001	0.002	0.006
Dose effect													
Linear		0.101	0.176	0.043	0.045	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
Quadratic		0.001	0.031	0.203	0.229	0.009	<0.001	<0.001	0.001	0.004	<0.001	<0.001	0.001
Additive × Dose		0.001	0.001	0.001	0.004	0.001	0.001	0.002	0.006	0.001	0.001	0.001	0.002

Means in the same column with different superscripts differ ($P < 0.05$).^a SEM standard error of the mean.

suppressed the activity of all organisms in the rumen with increasing concentration thus delaying colonization, decreasing digestion, and reducing the population of protozoa (Harris et al., 2001) and methanogens.

3.3. Effect of additives on CO₂ production

Fig. 3 shows the *in vitro* rumen CO₂ production (mL/g incubated DM) as affected by the different feed additives. The effect additive × dose interaction was significant (at least at $P < 0.04$) during all hours of incubation (Table 3). The control produced more ($P < 0.05$) CO₂ (expressed as: mL/g DM incubated, mL/g degraded DM, and as a proportion of total GP) than any other treatment. Consequently, the yield of CO₂ was highest ($P < 0.001$) in the control followed in decreasing order by xylanase, yeast and garlic oil. Within each additive, CO₂ production increased linearly with xylanase and yeast levels ($P < 0.001$) but decreased linearly ($P < 0.001$) and in a quadratic fashion ($P < 0.001$) with increasing level of garlic oil. Carbon dioxide in the most produced gas in rumen fermentation during metabolism of pyruvic acid in the production of acetic acid. Throughout the incubation period the control treatment yielded significantly more CO₂ than any of these feed additives in agreement with Kholif et al. (2016) for reasons advanced below. Xylanase is viewed simply as an enzyme that hydrolyses hemicellulose to xylose before further fermentation can take place. However, it is a xylan-degrading enzyme system which includes the main-chain-cleaving enzymes, endo-b-1,4-xylanases and b-xylosidases, and the side-chain enzyme activities, acetylxy-lan esterases, a-glucuronidases, ferulic acid esterases and a-arabino-fur-anosidases (Coughlan et al., 1993). Therefore, in the process of fermentation or digesting of hemicellulose, some of its metabolites and intermediates of lignin might react forming products that slows or reduce microbial activity. In some situations, addition of lignin compounds (vanillic acid, protocatechuic acid, acetovanillone, and vanillin) increased the rate of hydrolysis of xylan probably changing the structure of xylanase (Kaya et al., 2000); therefore, it is possible some other substances would have a negative effect. Since xylanase is sometimes none-limiting in the rumen fluid there is generally little to high changes in digestion following xylanase supplementation (Elghandour et al., 2016b). A tendency of decreased CO₂ production with increasing level of xylanase as demonstrated in this study was observed suggesting

that xylanase enzyme may help to increase sugars for the survival of propionate-producing microorganisms.

Live yeast cells naturally scavenge for O₂ and produce CO₂ in the rumen, thus increasing rumen anaerobiosis and microbial viability. That is why most groups of rumen microbes that do not compete for a common substrate (glucose) would tend to increase in the presence of live yeast cells. An increase in the population of fibrolytic organisms was expected to increase the rate of fiber digestion; this process majors in the production of acetate and CO₂ in the rumen. Naturally, it was expected that more CO₂ would be produced with this feed additive but the results showed an opposite trend from the control treatment. It is speculated that yeast in these feed additive produced anti-microbial metabolites that tended to affect digestion adversely. That is why the control treatment produced more CO₂ at all incubation times than yeast treatments. Yeast is known to shift H₂ utilization from methanogenesis to reductive acetogenesis through the homoacetogenic bacteria that produces acetate from CO₂ and H₂ (Mwenya et al., 2004), even in the presence of methanogens (Chaucheyras-Durand et al., 1997), thus helping to explain a tendency for yeast to decrease CO₂ emission.

Consequently, both CO₂ and CH₄ production would reduce as observed in this study, where CH₄ production was highest in the control treatment and lowest in the intermediate garlic treatment, suggesting that 250 µL of garlic oil might be the level at which the least CH₄ would be produced.

4. Future outlook

The research area of including natural feed additives in ruminant nutrition has gained the attention of researchers to improve feed utilization, animal productivity and reduce GHG emission. However, studies dealing with dairy calves are still relatively scarce, with almost no study on GHG emission. The tested feed additives showed promising results to reduce CH₄ and CO₂ production in the *in vitro* scale. However, further research is warranted to establish the efficacy of such feed additives in *in vivo* trials.

5. Conclusions

Garlic oil, yeast, and xylanase at different levels did not affect the extents of GP; however, garlic oil increased the rate of digestion and the lag phase. Besides, garlic oil decreased CH₄ and CO₂

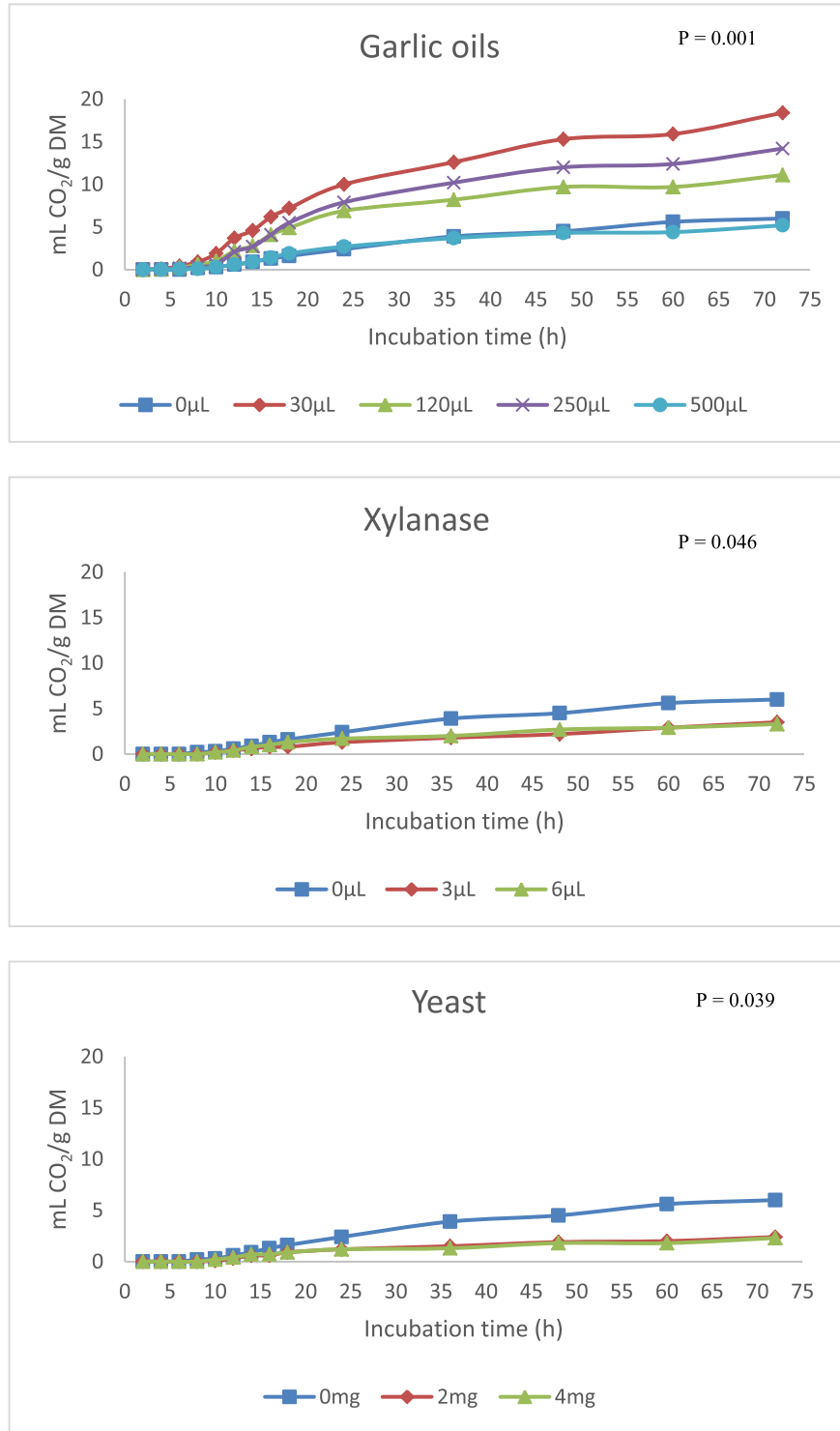


Fig. 3. *In vitro* rumen carbon dioxide (CO₂) production (mL/g incubated DM) of a high concentrate diet fed to young calves at 60 days of age incubated with no additive, garlic oil, xylanase, and yeast.

Table 3Effect of different feed additives on *in vitro* carbon dioxide (CO₂) production of a high concentrate diet fed to young calves at 60 days of age.

Additive type	Dose (/g DM)	CO ₂ production											
		mL/g incubated DM				mL/g degraded DM				Proportional CO ₂ production			
		6 h	24 h	48 h	72 h	6 h	24 h	48 h	72 h	6 h	24 h	48 h	72 h
Control	No additive	0.00d	2.4bc	4.5bc	6.0bc	0.00d	3.42cd	6.47bc	8.57bc	0.00c	0.83c	1.27bc	1.60abc
Garlic oil	30 µL	0.42a	10.0a	15.3a	18.4a	0.62a	14.77a	22.60a	27.40a	0.36a	3.33a	4.05a	4.64a
	120 µL	0.23abc	6.9ab	9.7abc	11.1abc	0.37b	10.10abc	14.16abc	16.28abc	0.19b	2.62ab	3.42ab	3.91ab
	250 µL	0.16bcd	7.9a	12.0ab	14.2ab	0.25bc	12.77ab	19.50ab	23.11ab	0.12bc	2.67ab	3.45ab	3.96a
	500 µL	0.07cd	2.7bc	4.3bc	5.2bc	0.13cd	4.96bcd	8.01bc	9.75abc	0.09bc	1.19bc	1.65abc	1.60abc
Xylanase	3 µL	0.00d	1.3c	2.2c	3.5bc	0.00d	3.09cd	6.25bc	9.07bc	0.00c	0.77c	1.27bc	1.76abc
	6 µL	0.00d	1.7c	2.7c	3.3bc	0.00d	2.02d	3.62c	4.80c	0.00c	0.47c	0.70c	0.90c
Yeast	2 mg	0.00d	1.2c	1.9c	2.4c	0.00d	1.89d	2.68c	3.58c	0.00c	0.47c	0.57c	0.73c
	4 mg	0.00d	1.2c	1.8c	2.3c	0.00d	1.81d	2.70c	3.32c	0.00c	0.47c	0.57c	0.67c
Pooled SEM ^a		0.075	1.97	3.26	4.33	0.085	3.024	5.264	6.861	0.044	0.655	0.931	1.158
Additive effect		<0.001	0.001	0.004	0.011	<0.001	0.005	0.024	0.058	<0.001	0.004	0.031	0.082
Dose effect													
Linear		0.011	0.033	0.052	0.085	0.001	0.001	0.003	0.009	0.002	0.001	0.004	0.012
Quadratic		0.494	0.046	0.031	0.041	0.001	0.312	0.731	0.920	<0.001	0.049	0.088	0.144
Additive × Dose		<0.001	0.001	0.001	0.003	<0.001	0.001	0.007	0.018	<0.001	0.001	0.009	0.027

Means in the same column with different superscripts differ ($P < 0.05$).^a SEM standard error of the mean.

productions, followed by yeast and xylanase. It can be concluded that garlic oil followed by yeast and then xylanase can be used to mitigate *in vitro* CH₄ and CO₂ production from dairy calves fed a high concentrate diet. However, further research is warranted to establish the efficacy of such feed additives in *in vivo* trials.

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