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Modulation of Murrah Buffalo (*Bubalus bubalis*) Rumen Functions for In Vitro Fatty Acid Bio-Hydrogenation, Methane Production and Fermentation Pattern of Total Mixed Ration Supplemented with *Allium sativum* (Garlic) Essential Oils

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Abstract: The potential for plant-origin essential oils to modulate rumen functions for reducing bio-hydrogenation of fatty acids and methane production has been a significant area of research in recent times. This study investigated the effects supplementation of garlic (*Allium sativum*) essential oils have on in vitro bio-hydrogenation of fatty acids, methanogenesis and fermentation characteristics of total mixed ration in buffalo with the aim of enhancing conjugated linoleic acid (CLA) content in animal products as well as reducing environmental pollution. *Allium sativum* (AS) essential oils were examined at four levels [0 (Control), 33.33 µL (AS-1), 83.33 µL (AS-2) and 166.66 µL (AS-3) per litre of buffered rumen fluid] in a radio-frequency based automatic gas production system (ANKOM-RF). Two bottles per treatment per run over two incubation runs were undertaken to gain representative results. Oats hay and concentrate mixture (1:1) was used as a substrate (500 ± 5 mg) and incubated with 60 mL of buffered rumen fluid in 250 mL ANKOM bottles fitted with automatic an gas recording system at 39 °C for 24 h, following standard in vitro gas production protocols. The results demonstrated a reduction ($p < 0.01$) in lipid bio-hydrogenation, measured by lowered saturated fatty acids and enhanced unsaturated fatty acids on the supplementation of AS essential oils, irrespective of the dose levels. Moreover, the increased ($p < 0.01$) production of *trans* vaccenic (*trans* C18:1) acid (TVA) following graded dose supplementations of the AS essential oils increased the production of conjugated linoleic acids (CLA) in animal products. Although, reduced methane production ($p < 0.01$) was evidenced, the decrease in total gas production and feed digestibility (TDDM) demonstrated the strong antimicrobial properties of AS at all dose levels. The study reveals that the *Allium sativam* (Garlic) essential oils have the potential to be an agent for the reduction of the rumen biohydrogenation of fatty acids and methanogenesis. However, in vivo examination is necessary to validate the findings and confirm its suitability for use as an additive to enhance nutraceutical and organoleptic properties in animal products.

Keywords: rumen bio-hydrogenation; *Allium sativum*; methane production; feed fermentation; in vitro

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1. Introduction

Climate safety and the generation of quality products through animal husbandry practices are burning issues in the modern era. Minimising greenhouse gas (GHGs) emissions from livestock production and reducing health-detrimental saturated fatty acids in livestock products are key research considerations, owing to deteriorating climate safety and social awareness towards health-influencing food items. India has the world's largest livestock population and generates huge quantities of GHGs through enteric fermentation, manure, dung cake etc. and such high emissions compromise climate safety [1]. Aside from climate safety, a parallel, crucial aspect is wastage of dietary energy in the form of unproductive

gaseous losses. Nutrition is a major input asset (60–70% of total rearing cost) when dealing with animal husbandry practices. Tropical climatic conditions and the country's existing feeding practices result in excessive losses of dietary energy in the form of wasteful GHGs. In feeds, lingo-cellulosic fodder content and a hot, humid climate contribute to around 6–10% of gross dietary energy losses and 8–14% of digestible energy losses [2]. Thus, animal husbandry practitioners suffer two-way losses in the form of climate safety and nutritional losses. India has a vast livestock population (536.76 million) with a growth rate of 4.82% [3]. However, feed resources and land availability are limited. Thus, the existing judicial and scientific use of existing feed resources is a seriously demanding scenario for livestock owners trying to harvest maximum profits through animal husbandry practices and compromising minimally on environmental safety.

Indian social structure reflects the maximum population in the rural part of the country. Such rural society primarily depends upon agriculture and animal husbandry practices for livelihood. The animal husbandry sector is presumed to be the driving force behind agriculture and sustainability in the rural population [4]. Vital products from livestock, viz. milk, meat and various other dairy products, nourish the country and exist as integral parts of daily meals throughout Indian society. In the vegetarian community of the country, milk is considered a complete food and the entire country consumes it either fresh or in value-added processed form. However, milk is deficient in vitamin C, iron, copper and dietary fibre. Moreover, meat is a pivotal source of animal protein, especially for the richer essential amino acid index (EAAI) that is required for various biochemical reactions and physiological processes inside the human body [5]. For two decades, India has held the top ranking in milk production and an appreciable scenario for meat production [3]. However, dairy production is not qualitatively optimal owing to various issues including a larger concentration (approx. 70%) of health-detrimental saturated fatty acids (SFA); low content of the health-influential PUFAs, ω-3 and CLA (<3.0%); and more trans-FAs (~4.0%) [6,7]. Thus, to improve the health and life expectancy in society, two-way strategies are needed, i.e., for climate safety (minimal GHG emissions) along with quality dairy production (suppression of saturation fatty acid profiles and enhancing CLA content).

To overcome the significant dietary loss, influence the persistence of the environment and enrich dairy products, several rumen modulators have recently been investigated for their impact on rumen functions. However, many of the questions raised on dietary inclusion of rumen modulators, viz. antibiotics premixing in livestock production, have been interrogated in recent years owing to the development of antibiotic-resistant bacteria and their zoonotic importance [8,9]. Moreover, several naturally active phytogenic substances have been examined for their pharmacological impact on methane mitigation and their desired biohydrogenation characteristics. Essential oils are active phytogenic substances, mainly terpenoids and phenylpropanoid compounds, present in many herbs and common spices with a varied array of anti-microbial properties [10]. Essential oils' extensive beneficial properties and rumen modifier actions has stimulated researchers to focus their use in animal feed additives to modulate rumen microbiome, enhancing feed efficiency. Garlic (*Allium sativum*) belongs to the *Amaryllidaceae* family, has a wide range of pharmacological actions and is an integral component of kitchens in Asia and Europe. It has a wide range of phytoactive, low molecular weight, aliphatic hydrocarbons, acids, alcohols, aldehydes, acyclic esters, and lactones and is exceptionally high in nitrogen and sulphur-containing compounds, coumarins and homologues of phenylpropanoid [11]. Out of the many active phytochemicals existing in garlic oils, allicin, a diallyl sulphite is a major part, having marked properties that suppress the activities of enteropathogenic bacteria and the archaeal population in rumen [12]. The rumen-stimulating abilities of AS oils could be an excellent substitute for modifying rumen functions, particularly altered biohydrogenation and reduced methanogenesis. Of the world's total buffalo population of 200 million, India alone harbours 57%, contributing to about 50% of the total milk production of the country [3,13]. The rumen microbiota and their functions, methanogenesis, ruminal passage rates of feed and uptake of nutrients from buffalo rumen, are better than other bovines [13–16], there-

fore, the modulation of rumen fermentation by the supplementation of phytogenic feed additives, including *Allium sativum* oils, could differ between buffaloes and other bovine species. Therefore, this experiment was conducted to investigate in vitro modulation of rumen functions for fatty acid bio-hydrogenation, methane production and fermentation patterns of total mixed rations with *Allium sativum* (garlic) oils supplementation in Murrah buffalo (*Bubalus bubalis*).

2. Materials and Methods

The experiment was conducted at the Division of Animal Nutrition and Feed Technology, ICAR-Central Institute for Research on Buffaloes (CIRB), Hisar, Haryana, India (29.1203_N, 75.8069_E). The guidelines of the Institute Animal Ethics Committee of CIRB were compiled (IAEC-CIRB/19-20/A/010 dated 5 August 2019) for the care of the rumen fistulated animals and rumen fluid collection.

2.1. Experimental Design and Substrate

A total mixed feed (500 mg) consisting of oats hay and concentrate mixture (1:1) was used as a substrate for the in vitro fermentation study [17]. Purified *Allium sativum* oil (W250309) from Sigma-Aldrich Ltd. Was examined at four concentrations (0, 2.0 µL, 5.0 µL and 10.0 µL per 60 mL of buffered rumen fluid) to get a concentration of AS, 0 (Control), 33.33 µL (AS-1), 83.33 µL (AS-2) and 166.66 µL (AS-3) per L of fermentation fluid. Two bottles per treatment per run with two incubation runs were undertaken to get representative results.

Oats fodder was cultivated at ICAR-Central Institute for Research on Buffaloes, Hisar, Haryana, India and harvested at the pre-bloom stage and oven dried at 65 °C for 48 h and ground to pass through 1.0 mm sieve for subsequent use. A concentrated mixture was prepared by mixing ingredients (% parts) wheat grains, 20; maize grains, 15; mustard cake, 20; groundnut cake, 10; wheat bran, 32; mineral mixture, 2.0; and common salt, 1.0.

2.2. Collection of Rumen Inoculum

Rumen liquor was collected from two rumen-fistulated Murrah buffalo steers (*Bubalus bubalis*) maintained on roughage-based diets at the animal farm of the ICAR-Central Institute for Research on Buffaloes, Hisar, India (29.1203° N, 75.8069° E). The mixed semisolid contents of rumen digesta were collected manually from different locations at different depths in the early morning before offering feed and water to the animals and hand squeezed to obtain a liquid portion of rumen fluid. Equal volumes of rumen fluid from both the fistulated animals were pooled to completely fill a 1.0 L, pre-warmed, oxygen-free thermos flask and brought to the laboratory. The rumen fluid was filtered through four layers of muslin cloth under a continuous flow of CO_2 for use as a source of inoculums for the in vitro investigations [18].

2.3. In Vitro Incubation

Investigations were carried out using a control (CON) of total mixed feed as substrate and various treatment groups with different graded dose regimens (2.0 µL, 5.0 µL and 10.0 µL per 60 mL of buffered rumen fluid) of AS essential oil. The in vitro incubations were carried out in 250 mL capacity ANKOM bottles fitted with radio frequency (RF) based pressure-temperature sensitive modules (ANKOM-RF Gas Production System). The day before the incubation, 500 mg mixed feed substrate was weighed into the bottles. On the day of in vitro incubation, various concentrations of *Allium sativum* essential oils were added to their respective bottles in duplicate. Incubation media (60 mL) consisting of rumen liquor and in vitro buffer media in 1: 2 ratios [19] were added to the bottles under continuous gassing with CO_2 before being capped and returned to the incubator rack set at 39 °C and 85 oscillations/minutes for 24 h. The in vitro buffer media (1 L) was prepared by mixing distilled water (534.0 mL), buffer solution (232.40 mL containing NH_4HCO_3, 4.0 g; $NaHCO_3$, 35.0 g; per L distilled water), macro-mineral solution (232.40 mL containing Na_2HPO_4

anhydrous, 5.70 g; KH_2PO_4 anhydrous, 6.20 g; $MgSO_4.7H_2O$, 0.60 g per L distilled water), micro-mineral solution (0.122 mL containing $CaCl_2.2H_2O$, 132.0 g; $MnCl_2.4H_2O$; 100.0 g; $COCl_2.6H_2O$, 10.0 g; $FeCl_3.6H_2O$, 80.0 g per L distilled water) and resazurin (1.0 mL per L distilled water) [20]. Three bottles containing only incubation media without substrate were used as blanks for the experiment and two replicates for each treatment were maintained.

2.4. Estimation of Fatty Acids Biohydrogenation

After 24 h fermentation, sample preparation for studying fatty acid biohydrogenation of the substrate in vitro was carried out in accordance with Mandal, et al. [21] with slight modifications and finally subjected to GC analysis. The incubated buffered rumen fluid (BRF) was initially prepared for esterification compounds i.e., FAME (fatty acid methyl esters). For this, 20 µL of fermented BRF was taken in pyrex culture tubes (dimension, 16 × 125) and mixed with 0.35 mL of 10 N KOH and 2.65 mL of methanol. The contents were subjected to 1.5 h incubations at 55 °C with 5 s of vigorous shaking at 20 min intervals. After incubation, the sample was cooled in running tap water and 0.29 mL of 24 N H_2SO_4 was added with inverse manner mixing. The content was again incubated and cooled in a similar manner. Finally, 1.5 mL n-hexane was mixed in the content and vortexed for 5 min and afterward subjected to centrifugation @ 2500 rpm for 5 min. The acquired glassy transparent supernatant n-hexane layer containing FAME was subjected to GC analysis (Agilent GC system-8890, Agilent Technologies, Palo Alto, CA, USA), maintained at 260 PSI inlet pressure, 175 °C column temperature and 260 °C detector temperature. An individual fatty acids composition was calculated as follows;

$$\text{Individual fatty acid composition (g/ 100 g of fatty acid methyl ester)} = \frac{Area\ of\ individual\ fatty\ acid}{Total\ area\ of\ fatty\ acid} \quad (1)$$

2.5. Estimation of Gas and Methane Production

After exactly 24 h of incubation, the recording was stopped and the total gas production (mol) in the bottles was recorded automatically through cumulative pressure and temperature in a ANKOM *RF* Gas Production System and the total gas was determined as follows [22];

$$\text{Total gas production (mol)} = \frac{Pressure\ in\ ANKOM\ RF\ Gas\ Production\ bottle\ (KPa) \times Head\ space\ volume\ of\ ANKOM\ RF\ Gas\ Production\ bottle\ (ml)}{Temperature\ of\ ANKOM\ RF\ Gas\ Production\ bottle\ (Kelvin) \times Avogadro's\ number\ (8.3144)} \quad (2)$$

The methane concentration (%) in the headspace gas was estimated after 24 h of incubation using a gas chromatograph (Nucon-5700, New Delhi, India) installed with a flame ionization detector (FID) and a column (Porapak 'Q'). The gas sample (200 µL) with the help of a 1000 µL graduated gas-tight micro-syringe (Hamilton, Bonatus, Switzerland) was received from bottles and injected into the injector port of GC. A mixed gas (CH_4:CO_2: 50:50) (Centurion Scientific, New Delhi, India) was used as the standard for comparison. The temperature of the injector, detector and oven of the GC were maintained at 140 °C, 200 °C and 70 °C, respectively. The proportion of methane (%) in the total gas concentration was calculated as follows;

$$\text{Methane in headspace (\%)} = \frac{[Area\ coverd\ by\ the\ sample] \times 50}{[Area\ coverd\ by\ standard\ of\ gas]} \quad (3)$$

The total methane production was calculated by multiplying total gas production with methane concentration.

2.6. In Vitro Dry Matter Degradability and Ammonia Production

The content of each ANKOM bottle was transferred into a spoutless beaker by repeated washings with a neutral detergent solution. After refluxing the contents for 1 h, the residue was recovered in pre-weighed filter crucibles (G1). After drying the crucibles to the constant

weight, ashing was done at 550 °C. The truly degradable dry matter (TDDM) was calculated as follows [17];

$$\text{TDDM } (\%) = \frac{(\text{Dry weight of substrate incubated} - \text{Dry weight of residue leftover}) \times 100}{\text{Dry weight of substrate incubated}} \quad (4)$$

The ammonia nitrogen (NH_3-N) concentration was estimated in accordance with the Conway microdiffusion disk method [23].

2.7. Chemical and Statistical Analysis

The mixed feed substrates (Oats hay and concentrate mixture) were chemically analysed in accordance with AOAC [24]. However, fibre constituents were analysed using the method of Van Soest, et al. [25]. Data obtained were subjected to analysis of variance (ANOVA) using SPSS 17.0 software [26] (SPSS, 2008, Armonk, NY, USA) and the treatment means were ranked using Duncan's multiple range tests according to Snedecor and Cochran [27].

3. Results and Discussion

The chemical composition of the oats hay and concentrated mixture (Table 1) used as the substrate for in vitro studies showed values within the range for Indian feeds and fodders [28]. The active components present in essential oils, their dose of inclusion, as well as the dietary composition of the animals actively affect the rumen biohydrogenation of fatty acids and microbial fermentation [29]. The essential oils of *Allium sativam* contain fat-soluble organo-sulphur compounds derived from allicin during the steam distillation process of oil extraction, which has the potential to shift rumen microbial populations to modulate biohydrogenation of fatty acids as well as methanogenesis [30,31]. In this study, the reduction in total saturated fatty acids (Figure 1), stearic acid (C18:0) and the increase in *t*-vaccenic acid (C18:1) in the fermented fluid (Table 2) demonstrated modulation of the microbial populations engaged in biohydrogenation. Numerous ruminal microbes have marked efficacy towards biohydrogenation of unsaturated fatty acids [32]. Ruminal lipid biohydrogenation is extensively associated with both the Gram-positive-Gram-negative microbes and *Butyrovibrio fibrisolvens* present in the rumen. An increased concentration of *t*-vaccenic acid in the rumen fluid in this study (Table 3) envisaged more production of *cis* 9 *trans* 11 conjugated linoleic acid (CLA) in animal products. In recent studies, many researchers [33,34] also reported a reduction in saturated fatty acids and enhanced t-vaccenic acid on supplementation of garlic oils under in vitro fermentation systems, which corroborates our findings. The reduction in the proportions of C15:0, C16:0 and C18:0 and the increase in the proportion of C18:1 in the garlic oil supplemented groups in this study, suggests reductions in *de novo* fatty acids synthesis in the rumen and partial inhibition of microbial biohydrogenation through the inhibition of *B. proteoclasticus* and other unknown bacteria involved in the final step of the biohydrogenation process [8,35].

Table 1. Chemical composition (%DM basis) of feeds used as the substrate for in vitro studies.

Attributes	Oats Hay	Concentrate Mixture *
Organic matter (OM)	93.30	87.42
Crude protein (CP)	7.80	19.50
Ether extract (EE)	2.07	4.12
Total ash (TA)	6.70	12.58
Neutral detergent fiber (NDF)	58.40	31.06
Acid detergent fiber (ADF)	43.70	19.64

* Composed of ingredients (% parts) wheat grains, 20; maize grains, 15; mustard cake, 20; groundnut cake, 10; wheat bran, 32; mineral mixture, 2.0 and common salt, 1.0.

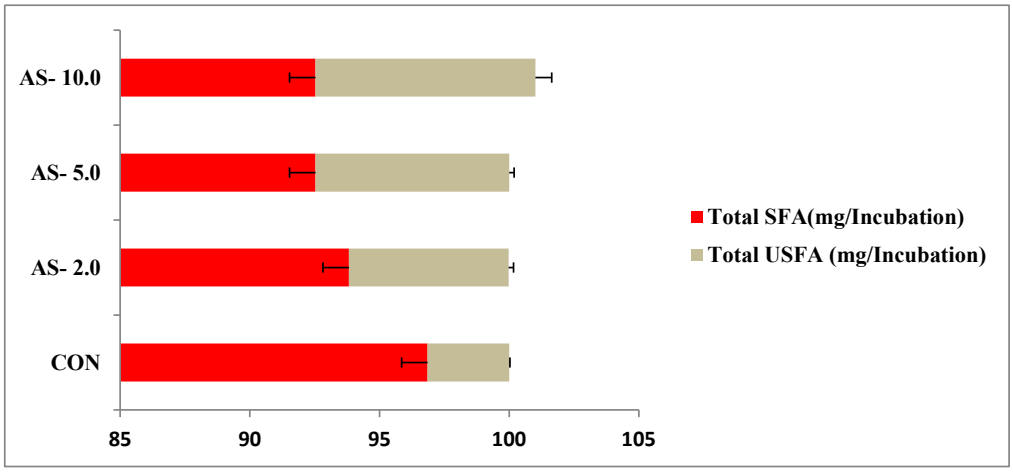

Figure 1. Effects of the graded levels of AS supplementation with mixed feed on rumen biohydrogenation of fatty acids (SFA, Saturated Fatty Acids; USFA, Un Saturated Fatty Acids).

Table 2. In vitro fatty acid biohydrogenation (mg/incubation) profiles upon incubation of mixed feed substrate with buffered rumen fluid supplemented with graded levels of *Allium sativum* (AS) essential oils.

Fatty Acids	Control	AS-2	AS-5	AS-10	SEM	*p* Value
	Individual saturated fatty acids (SFA)					
C8:0	36.23	35.99	35.76	34.69	0.26	0.21
C11:0	5.95 ^{b}	5.58 ^{ab}	5.20 ^{a}	5.33 ^{a}	0.11	0.02
C12:0	12.51 ^{ab}	13.0 ^{b}	12.17 ^{ab}	11.82 ^{a}	0.23	0.09
C13:0	3.81	3.66	3.59	3.55	0.07	0.62
C14:0	6.40 ^{b}	5.83 ^{a}	5.49 ^{a}	5.47 ^{a}	0.14	0.02
C15:0	3.27 ^{b}	3.15 ^{b}	0.0 ^{a}	0.0 ^{a}	0.55	<0.001
C16:0	11.41 ^{b}	11.08 ^{ab}	10.14 ^{a}	10.00 ^{a}	0.25	0.06
C18:0	20.51 ^{b}	18.55 ^{ab}	17.69 ^{a}	18.96 ^{ab}	0.64	0.08
Total SFA	100.09 ^{b}	98.1 ^{b}	90.04 ^{a}	89.82 ^{a}	0.76	<0.001
t-vaccenic acid (C18:1)	3.15 ^{a}	6.16 ^{b}	7.47 ^{c}	8.48 ^{c}	0.76	<0.001

Control, AS-2, AS-5 and AS-10 are the treatment groups @ 0.0, 2.0, 5.0 and 10.0 µL of pure AS (Sigma Aldrich Ltd., St. Louis, MO, USA) essential oil/60 mL of buffered rumen fluid (BRF), respectively. Mean values bearing a, b, c superscripts in a row vary significantly ($p < 0.05$).

Table 3. Modulation (%) of rumen bio-hydrogenation of fatty acids, total gas production, methane production and truly degradable dry matter on incubating graded doses of AS essential oils with mixed feed substrate.

Dose Incubated (µL/60 mL BRF)	SFA Inhibited	*t*-Vaccenic Acid (C18:1) Enhanced	Gas Production Inhibited	Methane Production Inhibited	TDDM Inhibited
2	1.99	95.56	24.09	64.02	7.55
5	10.04	137.14	30.74	83.64	14.80
10	10.26	169.21	36.78	100.0	26.26

The antimicrobial effects of garlic oils, especially on Gram-positive bacteria [36], are responsible for the reduction ($p < 0.01$) of total gas production (Figure 2) as well as feed digestibility (TDDM). Methane concentration (%) in the head space gas as well as the total methane production after 24 h fermentation was linearly reduced ($p < 0.001$) in response to graded doses of AS supplementation (Table 4). The antimicrobial efficacy of essential oils (EO) is due to various terpenoid and phenolic compounds, as well as the chemical constituents and functional groups contained in the EO [37]. Allicin, or

diallyl sulphite, is the major phyto-active constituents of AS, having marked depression abilities towards enteropathogenic bacteria and rumen archaeal populations [11]. Thus, direct dose-dependent microbial inhibitory activities or alterations in the phylogenetic distribution of methanogenic archaea owing to associated protozoa population destructions were responsible [38] for the present experimental graded dose depression in methane production (Figure 3). This study gets support from the findings of other researchers [39–42], where garlic oils (*Allium sativam*) alone or in combination with other essential oils were examined to reduce methanogenesis in ruminants. In contrast, Kamel, et al. [43] did not find any inhibitory effect on in vitro methane production on supplementation of diallyl disulfide at any of the investigated dose levels (0.5. 5 and 1.0 mg/L) to a mixed ration.

Moreover, ammonia-N concentrations were comparable ($p > 0.05$) in all AS supplementation groups (Table 3), except the highest dose (AS-10), where a reduction was evident ($p < 0.01$). Many researchers reported that essential oil supplementations resulted in a reduced population and diversity of hyper-ammonia-producing bacteria with a decrease in the rate of NH_3 production through compromising the deamination of dietary amino acids [44–46]. Cardozo, et al. [47], while investigating the effects of natural plant extracts in a concentrate-based diet on rumen fermentation, reported a reduction in ammonia-N concentration upon the supplementation of garlic oil at various dose levels (0.3, 3, 30, 300 mg/L). In contrast, an increased concentration of ammonia-N and unsaturated fatty acids without affecting the total VFA production was reported by Zhu, Mao and Zhu [34].

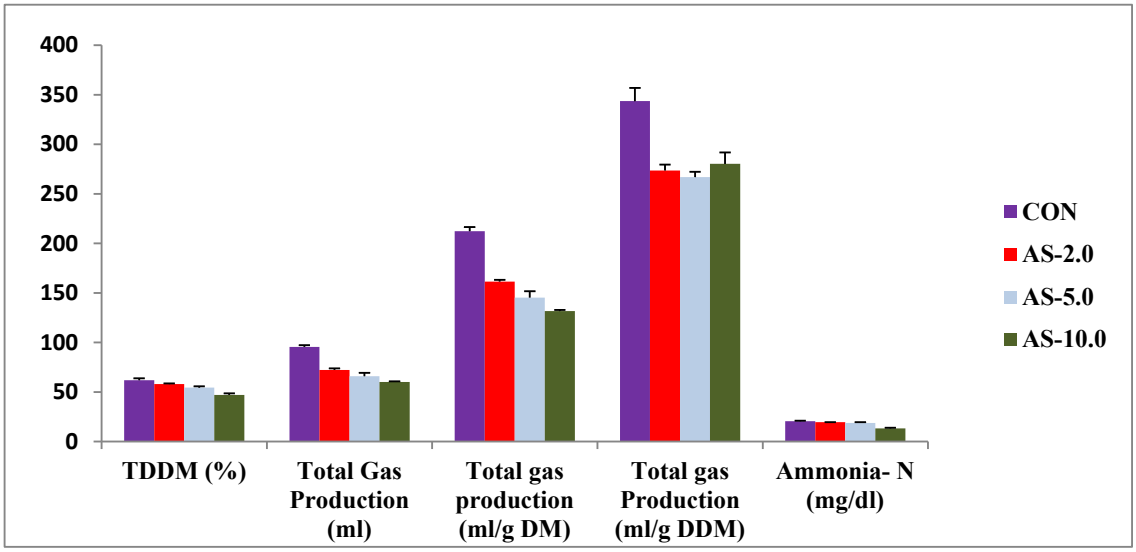

Figure 2. Effects of the AS supplementation with mixed feed on truly degradable dry matter, total gas production and ammonia-N.

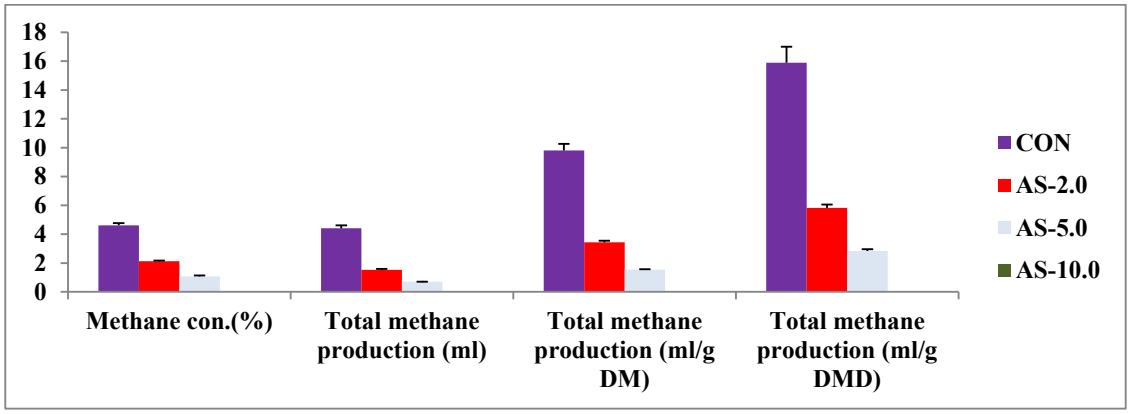

Figure 3. Effects of the graded levels of AS supplementation with mixed feed on methanogenesis.

Table 4. Effects of graded doses of *Allium sativum* (AS) essential oils on in vitro gas production, truly degradable dry matter, methanogenesis and the fermentation characteristics of mixed feed substrate.

Attributes	Control	AS-2	AS-5	AS-10	SEM	*p* Value
			Total gas production			
mL	95.15 ^{c}	72.23 ^{b}	65.90 ^{ab}	60.15 ^{a}	5.11	0.002
mL/g DM	211.69 ^{c}	161.45 ^{bc}	145.22 ^{b}	131.73 ^{a}	11.59	0.001
mL/g DMD	331.53 ^{c}	273.57 ^{b}	266.84 ^{a}	280.17 ^{bc}	10.20	0.020
			Total methane production			
Methane concentration, %	4.49 ^{d}	2.13 ^{c}	1.07 ^{b}	0.0 ^{a}	0.63	<0.001
Total methane, mL	4.28 ^{d}	1.54 ^{c}	0.70 ^{b}	0.0 ^{a}	0.62	<0.001
mL/g DM	9.52 ^{d}	3.44 ^{c}	1.54 ^{b}	0.0 ^{a}	1.37	<0.001
mL/g DMD	14.92 ^{d}	5.82 ^{c}	2.84 ^{b}	0.0 ^{a}	2.12	<0.001
TDDM, %	63.85 ^{d}	59.03 ^{c}	54.40 ^{b}	47.08 ^{a}	2.37	0.002
Ammonia N, mg/dl	21.00 ^{b}	19.60 ^{b}	18.90 ^{b}	13.30 ^{a}	1.12	0.001

Control, AS-2, AS-5 and AS-10 are the treatment groups @ 0.0, 2.0, 5.0 and 10.0 µL of pure AS (Sigma Aldrich Ltd., St. Louis, MO, USA) essential oil /60 mL of buffered rumen fluid (BRF), respectively. Mean values bearing a, b, c, d superscripts in a row vary significantly ($p < 0.05$).

4. Conclusions

Although part of the composition of a basal diet, the bioactive compounds present in garlic oils and their levels of inclusion play an important role in modulating rumen functions, *Allium sativam* (Garlic) essential oils and the potential they have to be an agent used to reduce the rumen biohydrogenation of fatty acids and methanogenesis without affecting feed utilization. However, in vivo examination is necessary to validate the findings before they can be used as feed additives to enhance the nutraceutical and organoleptic properties of animal products.

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Informed Consent Statement: Not applicable.

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