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Influence of dietary supplementation of garlic (*Allium sativum* L.) extract on cecal productions of total gas, carbon dioxide and fermentation profiles in rabbits

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Abstract The aim of this present study was to evaluate the effect of rabbit diets supplemented with garlic (*Allium sativum* L.) extract on in vitro cecal gas production (GP), carbon dioxide (CO_2) productions and some cecal fermentation parameters. Garlic extracts were prepared at 0.125 g/mL and administered at four different doses: 0 (control without garlic extract), 0.6, 1.2 and 1.8 mL extract/g dry matter (DM). The in vitro gas production was measured at 2, 4, 6, 8, 10, 12, 14, 16, 18, 24, 36, 48, 60 and 72 h incubation. The results showed quadratic increase in asymptotic GP ($P = 0.027$) and rate of GP ($P = 0.037$) but no linear or quadratic effects ($P > 0.05$) was observed for initial delay before GP. However, inclusion of garlic extracts at 0.6 and 1.2 mL extract doses/g DM increased asymptotic GP compared to control. In in vitro gas yield (GY), there were quadratic effects for GP_{24} ($P = 0.043$) and GP_{48} ($P = 0.029$) incubation times. There were no linear and quadratic effects ($P > 0.05$) in in vitro CO_2 production for all the incubation times. However, the dose of 1.2 mL DM had the highest in vitro CO_2 production at 12 h, 24 h and 48 h incubations. The results suggest that 1.2 mL/g DM garlic extract was the most effective to improve rabbit cecal fermentation compared with the other tested doses.

Keywords Carbon dioxide · Garlic extract · Gas production · Rabbits · Fermentation

Introduction

In agro-based industries chemical additives such as antibiotics, ionophore, gas inhibitors and defaunating agents use in supplementing feed diets to enhance the efficiency of nutrient use and also reduce methane (CH_4) production have been prohibited in the European Union due to their potential toxic effects to humans and its environment (Official Journal of the European Union 2003). These negative impacts especially from antibiotics have compelled the nutritional scientists to exploit the use of natural safe feed additives; such as, tree leaves, tree by-products and

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plant bioactives to improve nutrient utilization, digestibility of fibrous feeds, increase microbial protein production and also reduce both protein degradability and emission of greenhouse gases (Patra and Saxena 2011).

Many tree leaves and tree by-products contain reasonable amount of secondary metabolites also known as phytochemicals; for example, alkaloids, phenolics, saponins, tannins and terpenoids. These secondary metabolites are part of grazing animal diets and play essential roles as antibacterial, antioxidants, anticoccidial and anthelmintic with the capacity to modify rumen fermentation pattern (Salem et al. 2011). Rumen microbes have the capacity to metabolize and utilize lower concentration of alkaloids, saponins and phenolics as a source of energy without any residual effects on rumen productions (Salem et al. 2014a). These chemical components from tree leaves or tree by-products also inhibit enteric methanogenesis and reduce ammonia release in rumen. However, consumption of higher doses of tannins and saponins may have haemolytic effect and lead to animal death (Athanasiadou and Kyriazakis 2004).

Currently, the use of tree leaves and tree by-products as feed additives have gained substantial interest; hence, leading researchers into a new, safer and inexpensive way to improve ruminal production performance. Researchers have reported that the use of tree leaves, tree by-products and plant bioactives as natural feed additives improve rumen fermentation efficiency, enhance protein metabolism, decrease greenhouse gas production, reduce nutritional stress such as bloating or acidosis (thus contributing to animal welfare) and also improve animal health and productivity (Mclntosh et al. 2003; Patra et al. 2006; Benchaar et al. 2007).

Salem et al. (2014b) reported that the extracts of *B. crassifolia*, *C. pallida*, *E. cyclocarpum*, *F. excelsior*, *F. trigonata*, *P. brevifolium* and *P. domestica* at doses of 1.2–1.8 mL/g in diet positively modified rumen fermentation. Plant extracts with high flavonoid contents have potential to decreased methane production and yield more microbial biomass; as consequence, resulting in higher degradability and utilization of CP and cell wall constituents (Broudiscou et al. 2002). Salem (2012) found that ingestion of tree leaf extracts of *S. babylonica* and *L. leucocephala* reduced ruminal in vitro GP, fermentation and increased digestibility. Ishtiyak et al. (2010) observed that addition of *Trigonella foenumgraecum* improved the in vitro dry matter and organic matter digestibility. El-Adawy et al. (2008) reported positive effect of ZADO® addition on browse leaves degradation in rabbits indicating positive effects on the caecal microbial activity and nutrient digestion.

Rabbit diets present an endogenic complexity together with its digestive physiology (Cecotrophy) making it an extreme sensitive animal during diet compositions. Rabbit is considered as an ideal meat-producing animal. Rabbit meat is a highly digestible and tasty. It has great nutritional values and dietetic properties making it more preferable by nutritionists over other meats. Rabbit meat is healthy and characterized by high contents of polyunsaturated fatty acids, proteins, essential amino acids, high-energy values, and low fat, sodium and cholesterol levels (Dalle Zotte and Szendrő 2011). It has a short life cycle, short gestation period, very prolific, and high feed conversion capacity (Lebas et al. 1997). Due to its digestive physiology, rabbit exploits cellulose-rich feed (forages) and converts about 20% of its protein into edible meat (Dalle Zotte 2014), thus making it useful in the context of a sustainable livestock production.

Nowadays several researches are focused on the evaluation of the potential use of natural antimicrobials; such as, garlic and other plant extracts to improve rumen ecology. Garlic has been recognized not only as spice or herb plant but also as an antimicrobial agent. The antimicrobial properties of garlic are associated to the presence of organosulfur compounds such as allicin, diallyl sulphide, dialyl disulphide, S-allylcysteine and allyl mercaptan among others (Lawson 1996). These organosulfur compounds manipulation could change rumen fermentation patterns by decreasing acetate and increasing propionate and butyrate proportions. They can inhibit methanogenesis and decrease the methane (CH_4) and volatile fatty acid (VFA) ratio. (Busquet et al. 2005a, b, 2006). Mbiriri et al. (2016) have shown that garlic oil blending, fumarate and nitrate suppressed in vitro CH_4 production without affecting total volatile fatty acid concentration.

Kongmun et al. (2010) measured the in vitro effect of garlic powder and coconut oil fermentation in buffalo ruminal fluid and concluded that, this mixture has the potentials to improve ruminal fluid fermentation and could reduce CH_4 production and protozoal

population. Patra et al. (2010) reported that ethanol and methanol extract of fennel, cloves and garlic had positive inhibition effects on CH_4 production.

The in vitro gas production methodology has been used to evaluate the rumen degradation of ruminant diets (Vallejo et al. 2016), which allows the determination of short chain fatty acid, the energy value of the feed and the amount of fermented substrate used in microbial protein synthesis. This study was conducted to investigate the effects of supplementing Rabbit diets with garlic extract at different doses on the productions of cecal gas and carbon dioxide as well as some fermentation profiles.

Materials and methods

Aqueous extract preparation

Exactly 50 g of powdered garlic (*Allium sativum* L.) were dissolved in 400 mL distilled H_2O (0.125 g/mL) as a garlic extract main solution. This mixture was blended for 5 min (Oster 6630-13) and immediately filtrated twice with medical gauze pads. The filtrates (Garlic extract (GE) were collected and stored at 4 °C until they were used.

Substrates and treatments

Garlic extract main solution was dosed (treatments) into four different volumes; 0, 0.6, 1.2 and 1.8 mL DM of rabbit diet. A commercial diet (Union plus Rabbit Tepexpan) base was used as substrate and its nutritional composition (%) was 16.5 crude protein, 3 crude fat, 15 crude fibre, 9 ashes, 12 moisture and 44.5 free nitrogenous extract.

Cecal inoculum preparation

Three Chinchilla/New Zealand crossbreed rabbits (2.21 ± 0.13 kg of 11 weeks old) were used as cecal contents donor. Rabbit donors were fed with the Plus Union Tepexpan commercial diet, and samples of cecal contents were collected after rabbits were slaughtered.

In vitro incubation

Gas production (GP) was evaluated according to method of Theodorou et al. (1994) modified by Mauricio et al. (1999). Three aqueous garlic extract doses were used as treatments (0.6, 1.2 and 1.8 mL). Exactly 0.5 g of commercial feed were placed in 115 mL volume capacity amber coloured glass jars with appropriate addition of garlic extract dose mL/g DM and then 10 g of cecal inoculum were added, followed by addition of 40 mL of the buffer solution of Goering and Van Soest (1970). A fourth garlic extract dose (0.0 mL) served as a control dose without any garlic extract added. After all addition, the glass jars were flushed with CO_2 and closed immediately with stoppers, shaken and incubated at 39 °C for 72 h. Volume of gas production was measured at 0, 2, 4, 6, 8, 10, 12, 16, 18, 24, 36, 48, 60 and 72 h (Extech Instruments Waltham, EE. UU). Total and CO_2 gas productions were measured with a pressure gauge (Extech 407910) and a Gas meter (Crowcon Gas-Pro), respectively. All amber glass jars were uncapped at the final incubation time (72 h) and pH was measured with pH meter (Orion Star A215, Thermos Cientific, 2015). Content of each amber glass jars was filtered under vacuum through sintered filter with glass crucible. Fermentation residues were dried at 65 °C for 72 h to estimate the potential dry matter disappearance (DMD).

Calculations

Gas accumulation pressure at the top jars was measure with a pressure transducer connected to a digital reader. Psi unit conversion was determinate through a previously equation obtained by SAS program (SAS 2002):

$$Y = 0.024 + 5.34X + 0.031X^2 \quad (1)$$

where y = is the volume (8 mL); X is pressure (psi) with a $R^2 = 0.99$. Kinetic parameter of gas production (GP) results (mL/g DM) were fitted using NLIN option of SAS (2002) according to France et al. (2000) model as:

$$A = b\,x\left[1 - e^{-C\,(t-L)}\right] \quad (2)$$

where A is the volume of GP at time t, b is the asymptotic GP (mL/g DM), k is the rate of GP (/h) and L (h) are the discrete lag time prior to GP.

Metabolizable energy (ME, MJ/kg DM) was estimated according to the method of Menke et al. (1979) as:

$$\text{ME (MJ/kg DM)} = 2.20 + 0.136\ \text{GP} + 0.057\ \text{CP} \quad (3)$$

Gas yield at 4 and 6 h (GY_4, GY_6) was calculated as the volume of gas (mL gas/g DM) produced after and before incubation periods divided by the amount of DM (g) as:

$$\text{Gas yield (GY4/GY6)} = \text{mL gas/g DMD} \quad (4)$$

Short chain fatty acids (SCFA) were calculated (Getachew et al. 2002) as:

$$\text{SCFA (mmol/200 mg DM)} = 0.0222\ \text{GP} - 0.00425 \quad (5)$$

where GP is 24 h net gas production (mL/200 mg DM).

Microbial crude protein production was calculated (Blümmel et al. 1997) as:

$$\text{Microbial crude protein(mg = g DM)} = \text{mg DMD} - (\text{mL gas} \times 2.2\ \text{mg/mL}) \quad (6)$$

where the 2.2 mg/mL is a stoichiometric factor that expresses mg of C, H and O required for the SCFA gas associated to 1 mL gas production (Blümmel et al. 1997).

Statistical analyses

The experimental design for the in vitro test was a completely randomized design. Values of gas production within each extract doses were used as the experimental unit. Linear and quadratic polynomial contrasts were used to examine response to increasing addition of additives doses. Multiple comparisons of means were performed using the Tukey's test. Significance was declared at a level of $P < 0.05$.

Results

The asymptotic GP (*b*, mL/g DM) quadratically increased ($P = 0.027$) with increasing doses of garlic extract. Addition of 0.6 and 1.2 mL extract/g DM garlic extracts doses resulted in higher asymptotic GP compared to control (without garlic extract addition). In Fig. 1. dose effects (quadratic ($P = 0.037$)) on the rate of GP but the extract had no linear or quadratic effects ($P > 0.05$) on the initial delay before GP.

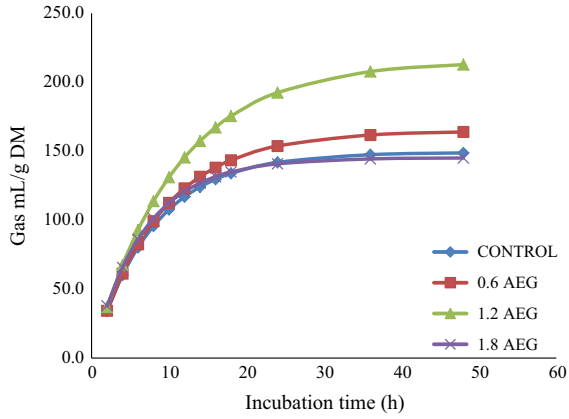

Fig. 1 In vitro gas production (mL/g DM) at different times of dietary incubation with different doses of aqueous garlic extract (AEG)

In in vitro GY, there was no linear and quadratic effects ($P > 0.05$) at GP_{12} h incubation but there were quadratic effects for GP_{24} ($P = 0.043$) and GP_{48} ($P = 0.029$) incubation, respectively. However, the dose of 1.8 mL/g DM had the lowest in vitro GY at 24 h and 48 h incubation times compared with the control and other doses. In in vitro CO_2, there were no linear and quadratic effects ($P > 0.05$) for all the incubation times. However, the dose of 1.2 mL/g DM had the highest in vitro CO_2 production at 12, 24 and 48 h incubations. Whereas the dose of 1.8 mL/g DM had the lowest CO_2 production at 48 h incubation time (Table 1, Fig. 2).

There were no linear and quadratic effects ($P > 0.05$) of the garlic extract on pH, DMD and GY24. However, garlic extract showed a quadratic effect for microbial crude protein ($P = 0.043$), and the highest garlic extract concentration at 1.8 mL/g DM had the lowest microbial crude protein when compared to the control or other extract doses. Metabolizable energy (ME) quadratically increased ($P = 0.042$) and had highest value in 1.2 mL dose than the control. Short chain fatty acids (SCFA) had no linear effect ($P > 0.05$) but quadratic effect was observed ($P = 0.044$). The dose of 1.8 mL garlic extract addition resulted in the lowest SCFA compared with the control (Table 2).

Table 1 In vitro gas production after 72 h incubation as influenced by three different doses of aqueous extract of garlic

AEG dose (mL/g DM)	GP *Kintetics*			GP (mL/g DM)			CO_2 (mL/g DM)		
	b (mL/g DM)	*c* (/h)	*L* (/h)	12 h	24 h	48 h	12 h	24 h	48 h
0	149.15	0.13	2.42	116.91	141.86	148.73	1.46	4.37	6.93
0.6	164.76	0.12	3.41	123.07	153.71	163.92	2.19	6.42	9.55
1.2	215.26	0.91	1.86	145.43	192.41	212.75	3.50	9.09	13.45
1.8	145.20	0.15	3.28	120.75	140.82	145.04	1.65	4.45	5.58
SEM	33.351	0.0253	1.390	18.591	28.144	32.821	1.444	3.353	4.632
Linear effect	0.900	0.373	0.519	0.826	0.969	0.905	0.885	0.978	0.760
Quadratic effect	0.027	0.037	0.366	0.093	0.043	0.029	0.110	0.100	0.073

AEG aqueous extract of garlic, *b* asymptotic gas production, *DM* dry matter, *c* rate of gas production, *L* initial delay before gas production begins, *h* hours, *GP* gas production and CO_2 carbon dioxide

SEM standard error of the mean

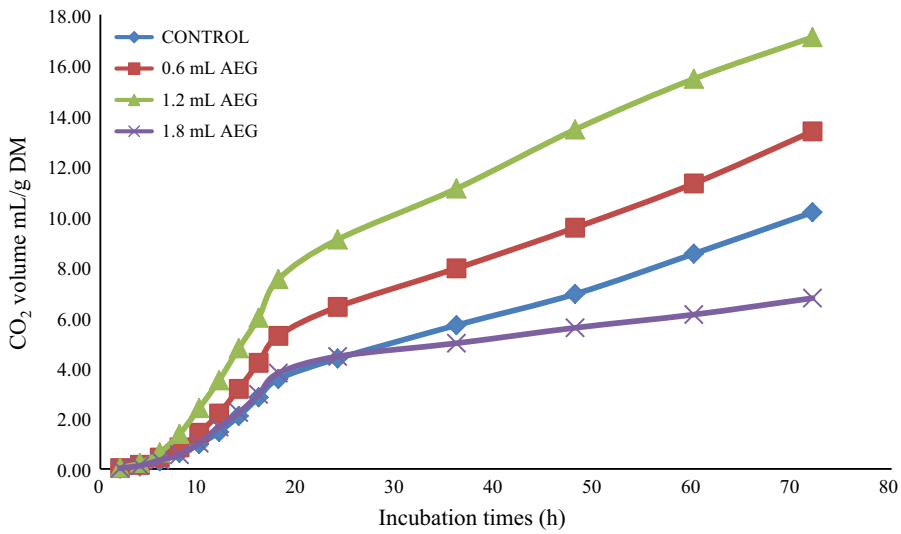

Fig. 2 In vitro CO_2 production (mL/g DM) at different times of dietary incubation with different doses of aqueous garlic extract (AEG)

Discussion

In vitro fermentation technique is a simple and sensitive screening method for investigating nutritive values of diets and for evaluating the efficacy of feed additives (Salem et al. 2014a). It is a powerful tool used to study forage utilization, feed digestibility, fermentability and microbial protein production. It is also used to measure the in vitro gas rumen kinetics such as methane (CH_4), Carbon dioxide (CO_2) and Hydrogen (H_2) (Elghandour et al. 2015, 2016). Recently, there has been an increased interest in using natural additives such as tree leaves and tree by-products to modify rumen microbial fermentation. Many studies have shown that tree leaves, tree by-products and their bioactives have the potential to improve rumen fermentation and GP at lower or moderate concentrations (Jiménez-Peralta et al. 2011; Abarghuei et al. 2013; Salem et al. 2014a). In this study, garlic extract is exploited by evaluating its effects on in vitro gas production and the possibility of improving fermentation profiles of rabbits. Garlic is a natural antimicrobial agent and it is used to prolong the shelf life of meat products because of its bactericidal effects.

In this study, garlic extract had quadratic effects on the asymptotic GP ($P = 0.027$) and rate of gas production ($P = 0.037$), so no effect on initial delay

Table 2 Effects of aqueous extract of garlic on fecal fermentation parameters

AEG dose (mL/g DM)	pH	DMD (mg/g DM)	MCP (mg/g DM)	GY24 (mL gas/g DMD)	ME (MJ/kg DM)	SCFA (mmol/g DM)
0	6.1	67.57	541.29	165.80	6.85	3.12
0.6	6.4	62.36	563.44	168.07	7.17	3.39
1.2	5.7	64.12	635.81	181.64	8.23	4.25
1.8	6.4	62.36	539.33	165.28	6.82	3.10
SEM	0.28	3.051	52.640	10.901	0.762	0.624
Linear effect	0.369	0.104	0.968	0.957	0.968	0.968
Quadratic effect	0.053	0.718	0.043	0.085	0.0426	0.044

AEG aqueous extract of garlic, *DM* dry matter, *DMD* DM degradability, *MCP* microbial crude protein production, *GY24* gas yield at 24 h of incubation, *ME* metabolizable energy, *SCFA* short chain fatty acids

SEM standard error of the mean

before gas production began. This result is in contrast with Salem et al. (2014b), who reported that an increased GP was parallel with decreased initial delay before GP begins in their study involving seven tree species extracts. Garlic extract at 0.6 and 1.2 mL/g DM doses increased GP, but GP was lower than the control with addition of 1.8 mL/g DM dose. Higher gas values are good indication of better nutrient availability for rumen microorganisms (Mahala and Fadel Elseed 2007). This finding is similar to the result of Salem et al. (2014b) which reported that ruminal GP increased with *Salix babylonica* extract at 0.6 and 1.2 mL/g DM, but not with the dose 1.8 mL/g DM. The authors were of the opinion that this improvement in GP could be attributed to the capacity of rumen microorganisms to degrade low or moderate level of plant secondary metabolites in plant extracts thus utilizing them as energy source without negatively effecting rumen fermentation. GP increase also agrees with the results of previous studies of Jiménez-Peralta et al. (2011) and Salem et al. (2011). However, high doses of plant secondary metabolites in plant extracts are well known antimicrobial agents that inhibit bacteria, protozoa and fungi activities (Bodas et al. 2012) as observed at dose of 1.8 mL/g DM in this study.

In in vitro GY, there was no linear and quadratic effects ($P > 0.05$) at GP_{12} h incubation for all the doses, but there were quadratic effects at GP_{24} and GP_{48} incubation times. However, the dose of 1.2 mL/g DM had the highest in vitro GY of 212.75 mL/g DM at GP_{48} compared with the control and other doses. No linear or quadratic effects ($P > 0.05$) were observed at in vitro CO_2 production. However, 1.2 mL/g DM dose garlic extract had the highest CO_2 production; whereas, the dose of 1.8 mL/g DM had lower CO_2 production compared to their respective control at 48 h incubation time. There are no published or documented reports on the effect supplementing rabbit's diets with garlic extract on in vitro GY and CO_2 productions; therefore, the results of this present study could not be compared from previous studies.

There were no significant effect ($P > 0.05$) of the garlic extract supplementation on pH and GY24. The pH values were within the range of 5.74 in the 1.2 mL/g DM dose to 6.11 in the control, showing no adverse effect of the garlic extracts on in vitro system. This result was similar to the findings of Cardozo et al. (2005). The authors reported that the ethanol extract of garlic dose of 30 mg/L in rumen liquor from beef cattle fed on high concentrate diet stimulated the required changes in in vitro batch culture fermentation at pH 5.5 with the better value of total VFA. In the present study, the pH of 5.74 at the dose 1.2 mL/g DM had the highest SCFA compared to the control and other doses. Positive correlations between pH and volatile fatty acids have been properly documented (Ramos et al. 2009) and this is in agreement with the findings of this study. The addition of different doses of garlic extract did not significantly influence DMD ($P > 0.05$) in this study. Busquet et al. (2005a, b) reported that the addition of garlic oil and its compounds did not have influence on true DM; while, Yang et al. (2007) observed that addition of garlic and

berry essential oil did not influence total digestibility's of DM. However, Sirohi et al. (2009) reported 15% increase in DMD due to addition of *Aloe barbadanis* extract.

In this study, the supplementation of garlic extract had a quadratic effect ($P < 0.05$) over the microbial crude protein, metabolize energy (ME) and short chain fatty acids (SCFA). Salem et al. (2014a) reported that addition of *Leucaena leucocephala* and *Salix babylonica* extracts at (0.6, 1.2, 1.8 mL extract/g DM increased gas volume, and microbial crude protein when compared to the control. This finding was similar to the results of our study except that addition of garlic extract at 1.8 mL extract/g DM decreased the gas volume, microbial crude protein, ME and SCFA. The observed decrease could be as a result of antimicrobial activities of the garlic extract. This correlates with the findings of Sreekanth et al. (2006), who reported that high levels of plant secondary metabolites might reduce the feed intake, and impair the nutrient digestibility or even be toxic to the rabbits. In contrast, Alexander et al. (2008) reported that extracts of *Moringa oleifera* and *Picrorhiza kurroa* decreased DM degradability and GP_{24} but had no effect on microbial crude protein.

There was significant increase ($P < 0.05$) in SCFA at the dose of 1.2 mL/g DM compared to the control and other doses. This result suggests that garlic extract at the dose of 1.2 mL/g DM produced large amounts of asymptotic gas which leads to large production of SCFA and decrease in the caecal pH. Kholif et al. (2015) indicated that increase in SCFA can be as result of direct improvement on digestion of *Moringa oleifera* diets leading to more efficient fermentation. Morever, at the higher dose of 1.8 mL/g DM garlic extract there was a decreased in SCFA and increased in pH. This result was similar to the work of Wanapat et al. (2008a, b), who observed that increasing garlic powder in diets rumen resulted in a reduced SCFA.

Conclusion

The moderate dose of 1.2 mL/g DM supplemented garlic extracts had more positive influence on the gas production and fermentation parameters than the lower doses of 0, 0.6 mL/g DM doses and higher dose of 1.8 mL/g DM. In this study, supplementation of garlic extract at the dose 1.2 mL/g DM resulted in more available energy for increasing SCFA production and ME density than other doses. It could be an alternative means of feed additive to improve rabbit's fermentation end products leading to reduced fermentation losses, improving ME, SCFA, and increasing microbial crude protein production. The results of our findings showed that in vitro gas production and some fermentation parameters such as the microbial crude protein, ME and SCFA demonstrate that garlic extract is rich in plant secondary metabolites and could be a promising potential feed additive in rabbit diets.

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Compliance with ethical standards

Conflict of interest The authors declare they have no conflict interest.

Human and animal rights The authors declare they followed the guide for the care and use of laboratory animals.

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