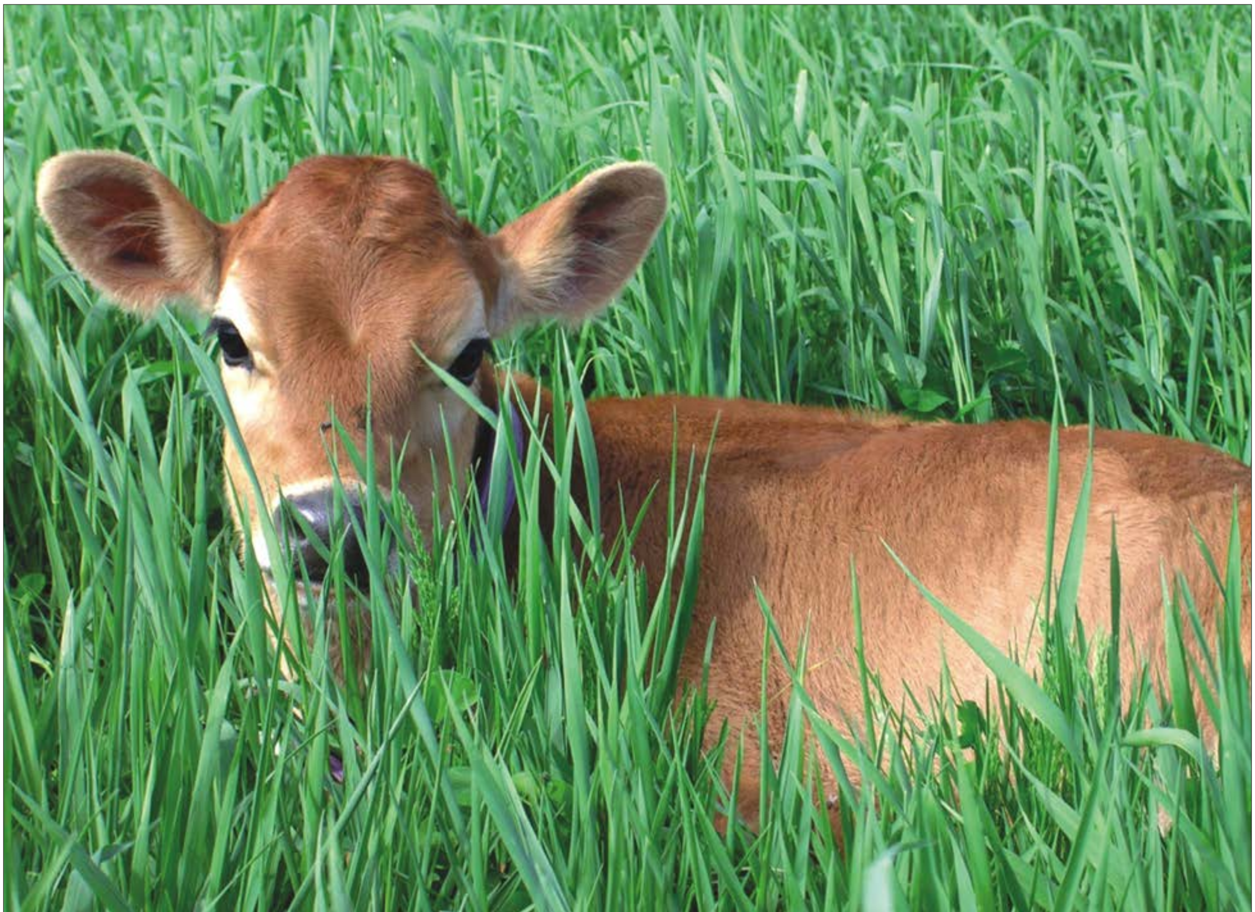

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Research Article
Utilizing of Celery and Thyme as Ruminal Fermentation and Digestibility Modifier and Reducing Gas Production

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Abstract
Background and Objectives: Improving ruminant environmental impacts and productivity get a great interest in last years, herbal plants were investigated as feed additive for decreasing gas production from rumen and enhancing nutrients digestibility. So, in the current study was carried out to investigate the effect of supplementing ruminant diets with different levels of thyme or celery on rumen fermentation, digestibility and gas production. **Materials and Methods:** Seven experimental treatments were done using rumen *in vitro* batch culture technique. Treatments were: 60% CFM, 40% clover hay (control), control diet+5 g thyme kg^{-1} DM (T1), control diet+10 g thyme kg^{-1} DM (T2), control diet+20 g thyme kg^{-1} DM (T3), control diet+5 g^{-1} celery kg^{-1} DM (T4), control diet+10 g^{-1} celery kg^{-1} DM (T5), control diet+20 g celery kg^{-1} DM (T6), control diet+0.4 g salinomycin kg^{-1} DM (T7). **Results:** The obtained results showed no significant ($p>0.05$) change in ruminal pH, neutral detergent fiber (NDF) and acid detergent fiber (ADF) degradation. Thyme addition to diet (T1 and T2) significantly lowered ($p<0.05$) total gas production (TGP) compared with control (152, 152 vs. 157 mL, respectively). Dry matter and organic matter disappearance (DMd, OMd) appeared showed no significant difference ($p<0.05$) between control and thyme treatments (T1, T2 and T3) and celery treatments (T4, T5 and T6). **Conclusion:** It could be concluded the adding thyme or celery at low levels to ruminant diets could improve ruminal fermentation and reducing gas production without adverse effect on nutrients digestibility.

Key words: Thyme, celery, rumen fermentation, gas production, nutrients digestibility, ionophores

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

The term "sustainability" has gained a strong attention in recent years from both scientific and productive point of view. Interestingly, agriculture contribute in all three pillars of sustainability in both positive way, by triggering the worldwide economy and social acceptability and negative way; being a source of environmental pollution and high concentration of greenhouse gasses (GHG)[1,2]. Hobson and Stewart[3] highlighted that methane is one of the rumen byproduct released due to the inability of the ruminants to benefit from hydrogen and carbon dioxide production during fermentation, which is later converted to CH_4 by the methanogenic population. It was estimated that dairy farms contribute in no less than 3% of the total GHG[4] that is in addition to a loss in feed energy by up to 12% in the form of emitted methane[5,6]. Developing strategies to mitigate methane from dairy cows represents an inevitable challenge in both environmental and economic perspectives. Antibiotics were widely used as a supplement in ruminant diet for their ability to modulate the rumen fermentation, mitigate methane emission and improve animal productivity has been proven for years[7,8]. However, the excess of using antibiotic would lead animals to develop a resistance against different drugs, additionally, antibiotics transfer to ruminant product (milk or meat) which could threaten human health[7,9]. Since European Union (EU) banned using antibiotics as feed additive, this radical change in laws resulted in an intensive development of research that relates to find effective natural compounds that could inhibit GHG and modulate the rumen fermentation and fatty acid composition in the produced milk[10]. Development of antibiotics resistant bacteria cannot be ignored; therefore, it is essential to find more desirable alternatives[10-12] from natural and safe sources.

Recently, many attempts were carried out to evaluate plant secondary metabolites (saponins, tannins and essential oils) as natural rumen modifier[10-13]. Generally, the antimicrobial activity of essential oils, is mainly a result of its content of terpenoid and phenolic compounds, these compounds have noted as antimicrobial activity by inactivation of some microbial enzymes. Many of herbs contains essential oils have been investigated (such as lemongrass, galangal, rosemary, cinnamomum, etc.)[11,12] on modifying rumen fermentation and positivity affected volatile fatty acids, methane production, starch, protein degradation and decrease ruminal bio-hydrogenation. So, the current study was aimed to evaluate the effect of two herbs (thyme and celery) at different supplementing levels on rumen fermentation and gas production, dry matter and fiber degradation and ammonia production *in vitro*.

MATERIALS AND METHODS

The experiment was carried out at the laboratory of dairy animal production, National Research Centre (Egypt) during spring of 2019 (March-May, 2019).

Experimental treatments: *In vitro* incubation procedures were carried out as described by Khattab *et al.*[11], rumen fluid was collected before morning feeding from 3 ruminally cannulated Holstein dairy cows (mean weight 680±30 kg), mixed and squeezed through 4 layers cheese cloth under continuous flushing with CO_2 and immediately transported to laboratory at 39°C (used as a source of inoculum)[11]. Treatments were: 60% CFM, 40% clover hay (control), control diet+5 g thyme kg^{-1} DM (T1), control diet+10 g thyme kg^{-1} DM (T2), control diet+20 g thyme kg^{-1} DM (T3), control diet+5 g celery kg^{-1} DM (T4), control diet+10 g celery kg^{-1} DM (T5), control diet+20 g celery kg^{-1} DM (T6) and control diet+0.4 g salinomycin kg^{-1} DM (T7) (Table 1). Each treatment was tested in 8 replicates accompanied by blank bottles (no substrate). The experiment run were replicated twice in different weeks. Substrate (400 mg) was added to the incubation bottles of 100 mL capacity. Each bottle was filled with 40 mL of the incubation medium (292 mg K_2HPO_4, 240 mg KH_2PO_4, 480 mg $(NH_4)_2SO_4$, 480 mg NaCl, 100 mg $MgSO_4.7H_2O$, 64 mg $CaCl_{2}.{}_2H_2O$, 4 mg Na_2CO_3 and 600 mg cysteine hydrochloride)/1 L of double distilled water (ddH_2O) and dispensed anaerobically in the 1:4 (v/v) ratio[13]. Then the bootles were incubated at 39°C for 48 h.

Table 1: Chemical composition of feed ingredients

Items	DM (%)	OM (%)	CP (%)	EE (%)	NDF (%)	ADF (%)	Ash (%)
CFM	93.09	93.98	12.73	6.23	49.98	18.09	6.02
Clover hay	93.86	88.31	11.97	6.10	43.49	33.06	11.69
Experimental diet composition	93.40	91.71	12.43	6.18	47.38	24.08	8.29

CFM: Concentrate feed mixture, consisted of 20% yellow corn, 20% wheat bran, 32% sugar beet pulp, 5% soybean meal, 20% cottonseed meal, 0.1% sodium bicarbonate, 1.5% limestone, 1% NaCl, 0.1% vitamins and 0.3% minerals, DM: Dry matter, OM: Organic matter, CP: Crude protein, EE: Ether extract, NDF: Neutral detergent fiber, ADF: Acid detergent fiber

Substrates sampling and gas production recording: After 48 h of incubation, gas production (GP) was recorded using the pressure reading technique according to Khattab and Abd El Tawab[13], bottles were uncapped, pH was measured using a pH meter and then contents were filtrated of each bottle to obtain the non-digested residue for determination of degradation percent.

Chemical analysis: The non-fermented residues were dried, weighed and digestibility calculated using the equations as follows[11]:

$$\textit{In vitro}\ \text{dry matter disappearance (\%)} = \frac{\text{Initial DM input-DM residue-Blank}}{\text{Initial DM input}} \times 100$$

$$\textit{In vitro}\ \text{organic matter disappearance (\%)} = \frac{\text{Initial OM input-OM residue-Blank}}{\text{Initial OM input}} \times 100$$

Neutral detergent fiber (NDF) and acid detergent fiber (ADF) were analyzed by Ankom200 Fiber Analyzer (Ankom Technology Corporation, Fairport, NY) according to Van Soest *et al*.[14]. Microbial protein production was calculated as 19.3 g microbial nitrogen per kg OMD according to Khattab *et al*.[11]. The NH_3-N concentration was determined as described by Khattab *et al*.[15].

Statistical analysis: Data were statistically analyzed using GLM procedure of SAS software (Version 9.2). Significant differences between means of treatments were carried out by the Duncan's test and the significance threshold was set at $p<0.05$.

RESULTS

Ruminal fermentation parameters: The effects of supplementing diet with different levels of thyme or celery or supplementing with salinomycin on some ruminal fermentation parameters are listed in Table 2. The results showed no significant differences ($p>0.05$) between treatments in pH. Also, microbial protein showed no significant changes between treatments except for salinomycin (T7) which significantly decreased ($p<0.05$) by recording 259.22 mg g^{-1} DM while supplementing diet with high dose of celery (T6) recorded the highest value 295.81 mg g^{-1} DM.

Ammonia-N concentrations showed a converged and similar values between different treatment except for T5 that recorded the lowest value (24 mg/100 mL) whereas control and T7 recorded the highest value (37.1 and 37 mg/100 mL, respectively).

Supplementing diets with different levels of celery improved ($p<0.05$) short chain fatty acids concentrations (SCFA) especially high level of addition (T6) by recording 7.06 mmol. Whilst, T2, T3 and T7 significantly ($p<0.05$) decreased SCFA concentration (6.73, 6.74 and 6.07 mmol, respectively).

Gas production: Table 3 illustrates the effect of experimental diets on total gas production (TGP). The values cleared that thyme addition to diet (T1 and T2) significantly lowered ($p<0.05$) TGP compared with control (152, 152 vs. 157 mL, respectively). T7 recorded the lowest ($p<0.05$) record (137 mL) for TGP compared with other treatments. In the other hand

Table 2: Effect of supplementing diet with different level of thyme or celery on some ruminal parameters

		Thyme			Celery				
Items	Control	T1	T2	T3	T4	T5	T6	T7	Pr>
pH	6.92	6.89	6.88	6.86	6.87	6.87	6.88	7.00	0.07
Ammonia-N (mg/100 mL)	37.10^{a}	35.70^{ab}	32.60^{ab}	30.50^{ab}	31.40^{ab}	24.00^{c}	34.00^{ab}	37.00^{a}	0.0016
SCFA (mmol)	6.97^{ab}	6.73^{c}	6.74^{c}	6.96^{ab}	6.78^{bc}	6.80^{bc}	7.06^{a}	6.07^{d}	0.0001
Microbial protein (mg g^{-1} DM)	281.06^{a}	280.12^{a}	280.90^{a}	290.35^{a}	281.75^{a}	283.65^{a}	295.81^{a}	259.22^{b}	0.0073

Control: 60% CFM, 40% clover hay, T1: Control diet+5 g thyme kg^{-1} DM, T2: Control diet+10 g thyme kg^{-1} DM, T3: Control diet+20 g thyme kg^{-1} DM, T4: Control diet +5 g celery kg^{-1} DM, T5: Control diet+10 g celery kg^{-1} DM, T6: Control diet+20 g celery kg^{-1} DM, T7: Control diet+0.4 g salinomycin kg^{-1} DM, SCFA: Short chain fatty acids, different superscript letters in a the same row differ significantly ($p<0.05$)

Table 3: Effect of supplementing diet with different level of thyme or celery on ruminal gas production

		Thyme			Celery				
Items	Control	T1	T2	T3	T4	T5	T6	T7	Pr>
Total GP (mL)	157^{ab}	152^{c}	152^{c}	157^{ab}	153^{bc}	153^{bc}	159^{a}	137^{d}	0.0001
GP/g DM (mL)	344^{ab}	333^{c}	333^{c}	345^{ab}	335^{bc}	336^{bc}	351^{a}	302^{d}	0.0001
GP/g NDF (mL)	721^{ab}	698^{c}	697^{c}	722^{ab}	701^{bc}	704^{bc}	735^{a}	632^{d}	0.0001
GP/g ADF (mL)	1485^{ab}	1438^{c}	1436^{c}	1487^{ab}	1444^{bc}	1451^{bc}	1515^{a}	1302^{d}	0.0001

Control: 60% CFM, 40% clover hay, T1: Control diet+5 g thyme kg^{-1} DM, T2: Control diet+10 g thyme kg^{-1} DM, T3: Control diet+20 g thyme kg^{-1} DM, T4: Control diet +5 g celery kg^{-1} DM, T5: Control diet+10 g celery kg^{-1} DM, T6: Control diet+20 g celery kg^{-1} DM, T7: Control diet+0.4 g salinomycin kg^{-1} DM, Total GP: Total gas production after 48 h, different superscript letters in a the same row differ significantly ($p<0.05$), GP: Gas production, NDF: Natural detergent fiber, ADF: Acid detergent fiber

Table 4: Effect of supplementing diet with different level of thyme or celery on ruminal nutrients disappearance (%)

		Thyme			Celery				
Items	Control	T1	T2	T3	T4	T5	T6	T7	Pr>
DMd	54.71[a]	53.73[ab]	53.50[ab]	55.05[a]	54.85[a]	53.50[ab]	54.54[a]	42.18[b]	0.032
OMd	55.90[ab]	54.19[c]	54.27[c]	55.83[ab]	54.58[bc]	54.66[bc]	56.45[a]	49.61[d]	0.0001
NDFd	30.39	30.59	30.48	30.31	29.32	31.14	30.55	26.96	0.09
ADFd	20.32	20.71	20.84	21.03	19.86	20.88	21.57	18.04	0.085

Control: 60% CFM, 40% clover hay, T1: Control diet+5 g thyme kg^{-1} DM, T2: Control diet+10 g thyme kg^{-1} DM, T3: Control diet+20 g thyme kg^{-1} DM, T4: Control diet +5 g celery kg^{-1} DM, T5: Control diet+10 g celery kg^{-1} DM, T6: Control diet+20 g celery kg^{-1} DM, T7: Control diet+0.4 g salinomycin kg^{-1} DM, DMd: Dry matter disappearance, OMd: Organic matter disappearance, NDFd: Natural detergent fiber disappearance, ADFd: Acid detergent fiber disappearance, different superscript letters in a the same row differ significantly (p<0.05)

celery addition (T4 and T5) did not change TGP vs control except T6 which significantly (p<0.05) increased TGP. The data showed that T6 recorded the highest value of TGP as 159 mL followed by T3 (157 mL) whilst T7 recorded the lowest value (137 mL). Other results of relative gas production per DM, NDF or ADF showed the same trend of TGP.

Nutrients degradability: Nutrients degradability are listed in Table 4. The data of dry matter disappearance (DMd) appeared that there were no significant variance (p<0.05) between control and thyme treatments (T1, T2 and T3) and celery treatments (T4, T5 and T6) but salinomycin (T7) actually depressed DMd (54.71, 53.73, 53.5, 55.05, 54.85, 53.5, 54.54 and 42.18%, respectively).

Organic matter disappearance (OMd) significantly (p<0.05) affected by supplementing diets with thyme (T1 and T2) compared with control (54.19, 54.27 vs. 55.9%, respectively), while, high level of thyme (T3) and celery supplementation (T4, T5 and T6) were not significantly differ with control. It well noted that salinomycin (T7) recorded the lowest (p<0.05) value of OMd by 49.61%.

Fiber fraction degradability (NDFd and ADFd) results showed non-significant (p>0.05) change between control and other treatments.

DISCUSSION

The main active compounds of thyme and celery are thymol, carvacrol, eugenol and ϒ-terpinene[16,17]. Overall decrease in rumen fermentation in supplementing diets with thyme and celery may be due to their effective antimicrobials components (thymol, eugenol and ϒ-terpinene) because of the presence of a hydroxyl group in the phenolic structure and results into loss of solidity of bacterial cell membrane which ultimately resulted in reduction in glucose uptake by bacteria[18-21].

Reduction in concentration of NH_3-N suggested the potentiality of celery for inhibiting deamination. Evans and Martin[22] in their study in pure culture reported that thymol affected the energy metabolism of two major rumen deaminating bacteria, *Streptococcus bovis* and *Selenomonas ruminantium*.

The current results by NH_3-N reduction was agreed with results of previous studies Wanapat *et al.*[23], Macheboeuf *et al.*[24] and Cobellis *et al.*[25], this reduction might be due to effect of the active essential oils especially cinnamaldehyde and cinnamon which noted as ammonia concentrations reducer in the rumen, It proposed that essential oils act on inhibiting attachment of bacteria on feed particles and deamination activity of amino acids[26]. Another study concluded that number and diversity of Hyper-NH_3 producing bacteria was reduced by supplementing diets with essential oils which reflect as a decrease in NH_3 production from amino acids[27]. It was proposed that essential oil inhibit proteolysis, peptidolysis and deamination of amino acids[28] different studies reported that the effective dose of essential oils which needed to decrease ammonia production was lower than that needed to decrease gas production[24,25,29].

Corroborating the study of Busquet *et al.*[30], present experiment also reported reduction of short chain fatty acids (SCFA) in low and medium dose level of thyme which could be a good indicator of simultaneous methane reduction in the rumen. Finally, under the conditions of the present study, using celery and/or thyme (as natural feed additive) in the diet of ruminants had potential positive impacts on ruminal fermentation and digestion.

CONCLUSION

The present study indicated that supplementing ruminant diet with herbal plants (thyme and celery) had potential positive effect on rumen fermentation and gas production and reduced NH_3-N concentration with no negative effect on DM, OM, NDF and ADF digestibility. Because of the high variability of the compounds and dosages used, the results found so far are inconsistent. Currently, further *in vivo* experiments are carrying out to elucidate topics such as the effect of those plants on voluntary intake, rumen fermentation and methane emission.

SIGNIFICANCE STATEMENT

The current study cleared that using natural herbal plants could play an effective role on improving ruminal activity and fermentation and digestion, in addition to reduce ruminal gas production which help in decreasing negative environmental impacts (gas emission from ruminant) and improve diet energy availability for animal performance. The current study could contribute in improving the knowledge of reducing the negative impacts of ruminant animal due its digestion performance and help in the new trend and theory of using natural products to improve ruminant production and decline its environmental negative effects.

REFERENCES

1. Von Keyserlingk, M.A.G., N.P. Martin, E. Kebreab, K.F. Knowlton and R.J. Grant *et al*., 2013. Invited review: Sustainability of the US dairy industry. J. Dairy Sci., 96: 5405-5425.
2. Lyons, T., A. Bielak, E. Doyle and B. Kuhla, 2018. Variations in methane yield and microbial community profiles in the rumen of dairy cows as they pass through stages of first lactation. J. Dairy Sci., 101: 5102-5114.
3. Hobson, P.N. and C.S. Stewart, 1997. The Rumen Microbial Ecosystem. 2nd Edn., Blackie Academic and Professional, London, UK., Pages: 744.
4. Knapp, J.R., G.L. Laur, P.A. Vadas, W.P. Weiss and J.M. Tricarico, 2014. Enteric methane in dairy cattle production: Quantifying the opportunities and impact of reducing emissions. J. Dairy Sci., 97: 3231-3261.
5. Arndt, C., J.M. Powell, M.J. Aguerre, P.M. Crump and M.A. Wattiaux, 2015. Feed conversion efficiency in dairy cows: Repeatability, variation in digestion and metabolism of energy and nitrogen and ruminal methanogens. J. Dairy Sci., 98: 3938-3950.
6. Li, Z.J., H. Ren, S.M. Liu, C.J. Cai, J.T. Han, F. Li and J.H. Yao, 2018. Dynamics of methanogenesis, ruminal fermentation and alfalfa degradation during adaptation to monensin supplementation in goats. J. Dairy Sci., 101: 1048-1059.
7. Zhan, J., M. Liu, C. Wu, X. Su, K. Zhan and G. Qi Zhao, 2017. Effects of alfalfa flavonoids extract on the microbial flora of dairy cow rumen. Asian-Aust. J. Anim. Sci., 30: 1261-1269.
8. Bayat, A.R., I. Tapio, J. Vilkki, K.J. Shingfield and H. Leskinen, 2018. Plant oil supplements reduce methane emissions and improve milk fatty acid composition in dairy cows fed grass silage-based diets without affecting milk yield. J. Dairy Sci., 101: 1136-1151.
9. Byarugaba, K.D., 2004. Antimicrobial resistance in developing countries and responsible risk factors. Int. J. Antimicrobial Agents, 24: 105-110.
10. Matloup, O.H., A.M. Abd El Tawab, A.A. Hassan, F.I. Hadhoud and M.S.A. Khattab *et al*., 2017. Performance of lactating Friesian cows fed a diet supplemented with coriander oil: Feed intake, nutrient digestibility, ruminal fermentation, blood chemistry and milk production. Anim. Feed Sci. Technol., 226: 88-97.
11. Khattab, M.S.A., H.M. Ebeid, A.M. Abd El Tawab, S.A.H. Abo El-Nor and A.A. Aboamer, 2016. Effect of supplementing diet with herbal plants on ruminal fiber digestibility and gas production. Res. J. Pharmaceut. Biol. Chem. Sci., 7: 1093-1097.
12. Khattab, M.S.A., H.M. El-Zaiat, A.M. Abd El Tawab, O.H. Matloup and A.S. Morsy *et al*., 2017. Impact of lemongrass and galangal as feed additives on performance of lactating barki goats. Int. J. Dairy Sci., 12: 184-189.
13. Khattab, M.S.A. and A.M. Abd El Tawab, 2018. *In vitro* evaluation of palm fronds as feedstuff on ruminal digestibility and gas production. Acta Scientiarum. Anim. Sci., Vol. 40. 10.4025/actascianimsci.v40i1.39586.
14. Van Soest, P.J., J.B. Robertson and B.A. Lewis, 1991. Methods for dietary fiber, neutral detergent fiber and nonstarch polysaccharides in relation to animal nutrition. J. Dairy Sci., 74: 3583-3597.
15. Khattab, M.S.A., A.E. Abd-El-Gawad, S.H.A. Abo El-Nor and M. El-Sherbiny, 2015. The effect of diet supplemented with vegetable oils and/or monensin on the vaccenic acid production in continuous culture fermenters. Anim. Nutr., 1: 320-323.
16. Hristov, A.N., C. Lee, T. Cassidy, K. Heyler and J.A. Tekippe *et al*., 2013. Effect of *Origanum vulgare* L. leaves on rumen fermentation, production and milk fatty acid composition in lactating dairy cows. J. Dairy Sci., 96: 1189-1202.
17. Roy, D., S.K. Tomar and V. Kumar, 2015. Rumen modulatory effect of thyme, clove and peppermint oils *in vitro* using buffalo rumen liquor. Vet. World, 8: 203-207.
18. Devi, K.P., S.A. Nisha, R. Sakthivel and S.K. Pandian, 2010. Eugenol (an essential oil of clove) acts as an antibacterial agent against *Salmonella typhi* by disrupting the cellular membrane. J. Ethnopharmacol., 130: 107-115.
19. Pei, R.S., F. Zhou, B.P. Ji and J. Xu, 2009. Evaluation of combined antibacterial effects of eugenol, cinnamaldehyde, thymol and carvacrol against *E. coli* with an improved method. J. Food Sci., 74: M379-M383.
20. Bassole, I.H.N. and H.R. Juliani, 2012. Essential oils in combination and their antimicrobial properties. Molecules, 17: 3989-4006.
21. Ultee, A., M.H.J. Bennik and R. Moezelaar, 2002. The phenolic hydroxyl group of Carvacrol is essential for action against the food-borne pathogen *Bacillus cereus*. Applied Environ. Microbiol., 68: 1561-1568.

22. Evans, J.D. and S.A. Martin, 2000. Effects of thymol on ruminal microorganisms. Curr. Microbiol., 41: 336-340.
23. Wanapat, M., A. Cherdthong, P. Pakdee and S. Wanapat, 2008. Manipulation of rumen ecology by dietary lemongrass (*Cymbopogon citratus* Stapf.) powder supplementation. J. Anim. Sci., 86: 3497-3503.
24. Macheboeuf, D., D.P. Morgavi, Y. Papon, J.L. Mousset and M. Arturo-Schaan, 2008. Dose-response effects of essential oils on *In vitro* fermentation activity of the rumen microbial population. Anim. Feed Sci. Technol., 145: 335-350.
25. Cobellis, G., M. Trabalza-Marinucci, M.C. Marcotullio and Z. Yu, 2016. Evaluation of different essential oils in modulating methane and ammonia production, rumen fermentation and rumen bacteria *in vitro*. Anim. Feed Sci. Technol., 215: 25-36.
26. Wallace, R.J., N.R. McEwan, F.M. McInotoch, B. Teferedegne and C.J. Newbold, 2002. Natural products as manipulators of rumen fermentation. Asian-Australasian J. Anim. Sci., 10: 1458-1468.
27. McEwan, N.R., R.C. Graham, R.J. Wallace, R. Losa, P. Williams and C.J. Newbold, 2002. Effect of essential oils on ammonia production by rumen microbes. Reprod. Nutr. Dev., 42: S65-S65.
28. Patra, A.K., 2011. Effects of essential oils on rumen fermentation, microbial ecology and ruminant production. Asian J. Anim. Vet. Adv., 6: 416-428.
29. Lin, B., J.H. Wang, Y. Lu, Q. Liang and J.X. Liu, 2013. *In vitro* rumen fermentation and methane production are influenced by active components of essential oils combined with fumarate. J. Anim. Physiol. Anim. Nutr., 97: 1-9.
30. Busquet, M., S. Calsamiglia, A. Ferret and C. Kamel, 2006. Plant extracts affect *in vitro* rumen microbial fermentation. J. Dairy Sci., 89: 761-771.