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Research Article

Impact of Lemongrass and Galangal as Feed Additives on Performance of Lactating Barki Goats

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Abstract

Background: The current study was carried out to investigate addition of lemongrass or galangal to diet and its effect of productive performance of lactating Barki goats. **Materials and Methods:** Thirty lactating Barki goats were divided into three groups (10 animals per each treatment), first group was fed control diet without additives, consist of Egyptian clover hay, corn silage and concentrate feed mixture (10:30:60% on DM basis, respectively) (Control); second group was fed control diet plus 4 g of lemongrass kg⁻¹ DM and the third group was fed control diet plus 4 g galangal kg⁻¹ DM. **Results:** The results showed that adding galangal increased propionate concentrations ($p < 0.05$) compared with control (41.13, 37.75 and 39.46 mM for galangal, control and lemongrass, respectively); while, there were no differences ($p > 0.05$) between treatments in acetate and butyrate concentrations. Ammonia concentration was higher ($p < 0.05$) in lemongrass compared with other treatments (21.49, 15.92 and 15.91 mM for lemongrass, control and galangal, respectively). Milk yield were significantly increased ($p < 0.05$) by adding lemongrass or galangal to the diets (825 and 771 g day⁻¹ for lemongrass and galangal) compared with control (652 g day⁻¹). Also, milk lactose content was significantly increased ($p < 0.05$) in lemongrass compared with control (44, 40 and 39 g kg⁻¹ for lemongrass, control and galangal, respectively). **Conclusion:** It could be concluded that adding lemongrass or galangal the diet could enhance the performance of lactating Barki goat.

Key words: lemongrass, galangal, digestibility, milk, Barki goats

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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

Herbs have been used as helpful materials to cure various diseases in animals and human¹. Recently, there are more attention to utilize herb on ruminant nutrition. Herbs are used in animal feeding as feed additives to solve many problems in livestock production and animal nutrition². Increasing of its use in animal nutrition in the European Union, regard to the risk of the presence of antibiotic residues in meat and milk which have harmful effects on human health³. Herb extracts have gained interest in animal nutrition strategies after prohibition of the most of antimicrobial growth promoter⁴. Herbal extracts have been evaluated for its ability to improve nutrients utilization in ruminants as a result of altering ruminal fermentation⁵. In addition, herb feeding to ruminants are possibly beneficial for the animal and decreases stress⁶. Many herbs like galangal and lemongrass have antioxidant properties and antimicrobial activities which make it more useful as natural animal feed additives. Recent studies on galangal (*Alpinia galanga*) and lemongrass (*Cymbopogon citratus*) showed that its anti-plasmodium⁷, antibacterial⁸, antioxidant and anti-microbial properties⁹. Recently, herbal plants and essential oil were recorded as feed additive for decreasing gas production from rumen and enhancing nutrients digestibility^{8,10}. Essential oil and other compounds are responsible for such activities^{7,11}. Moreover, essential oils have attracted observation for their ability as an alternative to feed antibiotics and growth promoters in livestock¹². The aim of the study was to determine the effect of adding lemongrass or galangal to lactating Barki goat's diet on feed intake, nutrient digestibility and milk yield and composition.

MATERIALS AND METHODS

Animals and experimental design: Thirty lactating Barki goat of 35 ± 1.5 kg b.wt., were randomly assigned to one of three groups (10 animals per each treatment): First group was fed control diet composed of Egyptian clover hay, corn silage and

concentrate feed mixture (Table 1) (10:30:60% on DM basis, respectively) (Control), the second group was fed control diet plus powdered lemongrass (4 g kg⁻¹ DM) and the third group was fed control diet plus powdered galangal (4 g kg⁻¹ DM). The experiment extended for 90 days started 1 week after parturition. Goats were fed according to Nutrient Requirements of Domestic Animals No. 15 Nutrient Requirements of Goats. Animals were fed individually twice per day at 07:00 and 15:00 h. The animals had free access to water. Feed refusals were measured daily and feed intake was determined by difference. Animal care and procedures were conducted under established approved standards of the Animal Production Department, Faculty of Agriculture, Alexandria University, Egypt.

Sampling and chemical analysis: Samples analysis were carried out at the Laboratory of Dairy Science Department, National Research Center and Laboratory of Animal Nutrition, Department of Animal and Fish Production, Faculty of Agriculture, Alexandria University. Feed and feces samples were collected at the last 3 days of each 30 day of the experiment; samples chemical analysis were performed according to AOAC¹³. Apparent nutrients digestibility coefficients were determined using acid detergent insoluble ash (AIA) content of feed and feces as an internal marker according to Khattab *et al.*⁸. The ruminal fluid was collected after 21 days of the begin of the experiment and each 28 day after that. Samples were collected via the stomach tube at 1.0, 3.0 and 6.0 h after feeding for consecutive 2 days. Rumen pH was measured directly within 2-3 min of sampling using a portable pH meter (GLP 21 model; CRISON, Barcelona, Spain). The rumen fluid was separated from the feed particles through four layers of gauze and stored at -20°C for later analysis. A volume of 10 mL of the filtrated ruminal fluid was mixed with 10 mL of 0.2 N HCl or 2 mL of 25% (w/v) metaphosphoric acid for NH₃-N and VFA's analysis, respectively. The concentrations of ruminal VFA's were determined according to Sallam¹⁴.

Table 1: Chemical composition of experimental ration

Parameters	Concentrate	Clover hay	Corn silage	Diet
Chemical composition of diet (g kg⁻¹ DM)				
OM	821.0	895.3	895.0	850.63
CP	133.7	145.4	106.2	126.62
EE	28.3	25.2	19.0	25.20
NDF	413.9	496.5	578.3	471.48
ADF	192.1	395.1	432.4	284.49
Hemicellulose	221.8	101.4	145.9	186.99
Cellulose	172.0	318.6	370.6	246.24
Lignin	20.2	106.5	61.8	41.31

Ruminal NH₃-N concentration was measured colorimetrically by spectrophotometer (Alpha-1101 model; Labnics Equipment, California, USA) using commercial lab test described by Konitzer and Voigt¹⁵.

Total protozoal count were counted by using Neubauer improved bright-line counting chamber according to the procedure described by Dehority *et al.*¹⁶.

Blood samples were collected from the jugular vein at the last 3 days of the trial (3 h after the morning feeding) by heparinized syringe. Samples were centrifuged at 3,500×g for 15 min at 4°C and collected plasma were immediately transported to the laboratory and frozen at -20°C until analyzed. Plasma concentrations of total protein, glucose, triglyceride, aspartate aminotransferase (AST) and alanine aminotransferase (ALT) were spectrophotometrically measured (T80 UV/VIS Spectrometer, PG Instruments Ltd., UK) according to the standard protocols of the suppliers.

Milk samples and yield was recorded twice weekly, animals were milked at 07:00 and 16:00 h as described by Sallam¹⁴. Milk samples were analyzed as described by Abo El-Nor and Khattab¹⁷ for total solids, fat, protein, lactose, solids not fat and somatic cells count by using infrared method (EKOMILK-M ultrasonic milk analyzer, EON Trading INC, Bulgaria, 2000).

Statistical analysis: Data were statistically analyzed according to a completely randomized design 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

Rumen fermentation: The effect of lemongrass and galangal treatments on rumen fermentation are presented in Table 2. The results showed that rumen pH values were not changed ($p > 0.05$) between control and other treatments (6.01, 6.07 and

6.12 respectively). Also, acetic, butyric, valeric acids concentrations were not affected by different treatments ($p > 0.05$). On the other hand, propionic acid concentrations were significantly increased ($p < 0.05$) in galangal which recorded the highest value (41.13 mM) followed by lemongrass (39.46 mM) then control (37.75 mM). Lemongrass and galangal addition to diet were decreased acetate: Propionate ratio compared with control. But adding galangal significantly decreased ($p < 0.05$) iso-valeric acid (1.8 mM) compared with control and lemongrass (2.71 and 2.70 mM, respectively).

Addition of lemongrass encouraged proteolytic activity in rumen and significantly ($p < 0.05$) increased ammonia concentration (21.49 mM) compared with control and galangal (15.92 and 159.91 mM, respectively).

Results showed that lemongrass slightly ($p > 0.05$) increased protozoa count ($3.78 \times 10^5 \text{ mL}^{-1}$) while galangal decreased its count ($3.34 \times 10^5 \text{ mL}^{-1}$) compared with control ($3.46 \times 10^5 \text{ mL}^{-1}$).

Apparent nutrients digestibility: The effect of experimental treatments on nutrients digestibility are presented in Table 3. Results of lemongrass and galangal showed that there were no effect ($p > 0.05$) of adding lemongrass or galangal to diets on apparent digestibility of OM, CP, cellulose and hemicellulose compared with control. While, lemongrass significantly ($p < 0.05$) increased DM digestibility (74.14/100 g) compared with galangal (69.30/100 g) but there was no differences ($p > 0.05$) between control and other treatments (72.08/100 g).

Lemongrass and galangal significantly ($p < 0.05$) decreased NDF and EE digestibility compared with control. While, galangal decreased ADF digestibility compared with control.

Blood metabolites: The results of current study revealed that supplementing diets with lemongrass or galangal had no negative effect on blood plasma constituents. Plasma total

Table 2: Effect of experimental treatments on rumen fermentation kinetics

Parameters	Control	Lemongrass	Galangal	p-value
pH	6.01±0.07	6.07±0.07	6.12±0.04	0.79
Acetic acid (mM)	74.11±1.26	74.83±1.35	73.16±1.61	0.77
Propionic acid (mM)	37.75±1.92 ^b	39.46±1.48 ^{ab}	41.13±1.72 ^a	0.0001
Butyric acid (mM)	32.26±2.02	32.09±1.62	32.19±1.82	0.28
Valeric acid (mM)	3.61±0.44	3.61±0.16	3.48±0.22	0.23
Iso-butyric acid (mM)	2.49±0.71	1.62±0.47	2.02±0.70	0.54
Iso-valeric acid (mM)	2.71±0.15 ^a	2.70±0.32 ^a	1.80±0.21 ^b	0.06
Acetate: Propionate ratio	1.97±0.09	1.92±0.08	1.81±0.11	0.53
Ammonia (mM)	15.92±0.25 ^b	21.49±1.19 ^a	15.91±0.56 ^b	0.0008
Protozoa count ($\times 10^5 \text{ mL}^{-1}$)	3.46±0.22	3.78±0.33	3.34±0.21	0.13
*Methane (mM)	41.30±0.28	41.97±0.23	40.40±1.15	0.43
*Gas production (mM)	73.07±0.23	73.52±0.58	73.27±0.32	0.74

Values within rows with different superscript letters are significantly different ($p < 0.05$). *Calculated as described by Blummel *et al.*²¹

Table 3: Effect of experimental treatments on nutrients digestibility (g/100 g)

	Control	Lemongrass	Galangal	p-value
Dry matter intake (g h ⁻¹ day ⁻¹)	1037.00±0.81 ^b	1050.00±1.86 ^a	1031.00±1.54 ^c	0.0004
Dry matter	72.08±0.91 ^{ab}	74.14±1.62 ^a	69.30±1.27 ^b	0.035
Organic matter	74.70±1.23	74.40±1.39	72.50±1.26	0.17
Crude protein	69.16±1.54	67.50±1.28	65.80±1.47	0.60
Ether extract	75.10±0.66 ^a	70.80±1.03 ^b	69.04±1.39 ^b	0.023
Neutral detergent fiber	61.35±1.16 ^a	55.18±1.43 ^b	53.87±1.43 ^b	0.048
Acid detergent fiber	54.77±1.77 ^a	52.84±0.59 ^a	48.65±1.34 ^b	0.006
Hemicellulose	65.63±2.55	59.97±1.89	59.54±1.76	0.25
Cellulose	62.40±1.49	60.10±0.93	58.30±1.68	0.62

Values within rows with different superscript letters are significantly different (p<0.05)

Table 4: Effect of experimental treatments on blood metabolites

Parameters	Control	Lemongrass	Galangal	p-value
Total protein (g dL ⁻¹)	6.58±0.18	6.15±0.15	6.06±0.42	0.17
Glucose (mg dL ⁻¹)	84.40±3.83	84.40±2.73	84.90±4.52	0.44
Tri-glycerides (mg dL ⁻¹)	355.00±23.1	356.00±43.7	322.00±40.6	0.62
AST	25.00±2.47	26.00±2.99	28.00±3.35	0.63
ALT	34.00±2.09	32.00±1.63	35.00±2.96	0.66

AST: Aspartate aminotransferase, ALT: Alanine aminotransferase

Table 5: Effect of experimental treatments on milk yield and constituents

Parameters	Control	Lemongrass	Galangal	p-value
Milk yield (g day ⁻¹)	652.00±71.92 ^b	825.00±100.23 ^a	771.00±55.78 ^{ab}	0.046
FCM (4%) (g day ⁻¹)	723.00±92.68 ^b	818.00±68.95 ^a	845.00±45.53 ^a	0.0628
Fat (g kg ⁻¹)	36.00±0.32	39.00±0.21	39.00±0.36	0.84
Protein (g kg ⁻¹)	26.00±0.11 ^b	29.00±0.064 ^a	26.00±0.14 ^b	0.034
Lactose (g kg ⁻¹)	40.00±0.15 ^b	44.00±0.096 ^a	39.00±0.21 ^b	0.039
SNF (g kg ⁻¹)	65.00±0.25 ^b	72.00±0.18 ^a	64.00±0.35 ^b	0.057
Ash (g kg ⁻¹)	5.60±0.019 ^b	6.30±0.013 ^a	5.40±0.031 ^b	0.045
Somatic cell count	972.00±46.61 ^a	669.00±82.66 ^b	683.00±82.77 ^b	0.022

Values within rows with different superscript letters are significantly different (p<0.05)

protein was insignificantly decreased (p>0.05) in supplemented diets compared with control as shown in Table 4 (6.58, 6.15 and 6.06 mg dL⁻¹ for control, lemongrass and galangal respectively). Tri-glycerides results showed a non significant (p>0.05) differences between treatment as shown in Table 4.

Also, insignificant increase had noticed (p>0.05) in plasma glucose concentration for galangal compared with control and lemongrass (Table 4) (84.9, 84.4 and 84.4 mg dL⁻¹, respectively).

Table 4 shows the effect of treatments on liver function enzymes (AST and ALT) and the results cleared that supplementation had no significant effect on AST and ALT enzymes values.

Milk yield and composition: Results of milk yield and milk constituents are presented in Table 5. Data showed an increase (p<0.05) in milk yield and FCM by adding lemongrass or galangal to diet compared with control. Lemongrass increased milk SNF, protein, lactose and ash comparing with galangal or control. While, milk fat content was not affected (p>0.05) by different treatments.

Addition of lemongrass or galangal to diet significantly decreased (p<0.05) somatic cell count compared with control.

DISCUSSION

Results reflects that adding lemongrass and galangal to diet had no negative effect on fibre degradation which showed as acetic and butyric acids concentrations in rumen liquor samples. These results were in agreement with previous studies^{6,18}, which reported that adding lemongrass leaf at 5% to diet was not changed VFA's concentrations in the rumen in dairy steers. Other study¹⁹ cleared that adding essential oil to ruminant diet could change microbial populations and rumen fermentation in the rumen. It seems to be there are positive effect of galangal addition in propionic acid bacteria activity. Generally, Gram positive bacteria appeared to be more sensitive to be inhibited by plant essential oil compounds than Gram-negative bacteria²⁰. This effect could related to presence of the outer membrane of Gram negative bacteria in the rumen, which protect them with a hydrophilic surface, it gives a great impermeability barrier²¹. The activity of lemongrass affects electron transport, ion gradients, protein translocation, phosphorylation steps and other enzyme-dependent reactions, causing the affected bacteria to lose chemiosmotic control²².

The current study revealed that lemongrass significant increase in rumen ammonia concentration which means that

this additive enhance bacterial proteolytic activity in the rumen which could be noted by the increase of DM digestibility and protozoa count.

Our results concluded that adding lemongrass increased protozoa count in the rumen which disagreed with previous study¹⁸ which reported that protozoal populations tended to be decreased with increasing concentration of lemongrass leaf in the diets.

Insignificant increase of protozoal count in lemongrass treatment could be clear the slight increase of methane produced which explained by the role of protozoa on methanogenesis by consuming oxygen which provides more anaerobic environment allows the anaerobic bacteria and archae to carry out methanogenesis^{23,24}.

Dry matter intake was not affected by lemongrass or galangal supplementation. These results were agreed with other studies^{6,18} which reported that adding lemongrass leaf to dairy steers had no effect on dry matter intake when dairy cows fed a mixture of essential oil compounds²⁵ at 750 mg day⁻¹.

In our study we observed that supplementing diet with lemongrass or galangal could decrease digestion coefficients of ether extract, NDF and ADF. These findings could explained by sensitivity of ruminal Gram positive bacteria to be inhibited by plant essential oil compounds than did Gram-negative bacteria which be reflect the decrease in cellulytic activity and digestion in the rumen²⁰.

Significant increase of milk yield, FCM, SNF, protein and lactose were reported when diet supplemented with lemongrass. This enhancement of yield and components might be due to the increase of dry matter digestibility which also reflected as increase in propionic acid concentration which had been cleared as an increase in milk lactose. Our results agreed with others^{26,27} who concluded that adding essential oil may increase milk yield and composition. While, others^{28,29} recorded a non-significant increase in milk yield, milk fat and protein when diet supplemented with essential oil.

Somatic cell count which used as indirect indication for mastitis resistance³⁰ were decreased when diet supplemented with lemongrass or galangal.

CONCLUSION

In general, supplementing lactating goat diets with lemongrass or galangal did not have any negative effect on performance and productivity but might improve nutrients digestibility, rumen fermentation and milk yield and composition and animal health status. Lemongrass have a

positive effect which appeared in significant increase in propionic acid concentration in the rumen and dry matter digestibility, milk yield, FCM, milk fat, lactose and protein, in other side reduced somatic cell count in milk which reflect more mastitis resistance.

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REFERENCES

1. Tipu, M.A., M.S. Akhtar, M.I. Anjumi and M.L. Raja, 2006. New dimension of medicinal plants as animal feed. Pak. Vet. J., 26: 144-148.
2. Wallace, R.J., N.R. McEwan, F.M. McInoch, B. Teferedegne and C.J. Newbold, 2002. Natural products as manipulators of rumen fermentation. Asian-Australasian J. Anim. Sci., 10: 1458-1468.
3. Ishlak, A., M. Gunal and A.A. AbuGhazaleh, 2015. The effects of cinnamaldehyde, monensin and quebracho condensed tannin on rumen fermentation, biohydrogenation and bacteria in continuous culture system. Anim. Feed Sci. Technol., 207: 31-40.
4. Kamel, C., 2000. A novel look at a classic approach of plant extracts. Feed Mix, 8: 16-18.
5. Nanon, A., W. Suksombat, K.A. Beauchemin and W.Z. Yang, 2014. Short Communication: Assessment of lemongrass oil supplementation in a dairy diet on *in vitro* ruminal fermentation characteristics using the rumen simulation technique. Can. J. Anim. Sci., 94: 731-736.
6. Hosoda, K., K. Kuramoto, B. Eruden, T. Nishida and S. Shioya, 2006. The effects of three herbs as feed supplements on blood metabolites, hormones, antioxidant activity, IgG concentration and ruminal fermentation in Holstein steers. Asian-Australasian J. Anim. Sci., 19: 35-41.
7. Latha, C., V.D. Shriram, S.S. Jahagirdar, P.K. Dhakephalkar and S.R. Rojatkar, 2009. Antiplasmid activity of 1-acetoxychavicol acetate from *Alpinia galanga* against multi-drug resistant bacteria. J. Ethnopharmacol., 123: 522-525.
8. 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.
9. Wong, L.F., Y.Y. Lim and M. Omar, 2009. Antioxidant and antimicrobial activities of some *Alpina* species. J. Food Biochem., 33: 835-851.

10. Cobellis, G., M. Tralbalza-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.
11. Zhu, X.L., M.H. Yang, J.G. Luo, X.F. Huang and L.Y. Kong, 2009. A new phenylpropanoid from *Alpinia galanga*. Chin. J. Nat. Med., 7: 19-20.
12. Wallace, R.J., 2004. Antimicrobial properties of plant secondary metabolites. Proc. Nutr. Soc., 63: 621-629.
13. AOAC., 1995. Official Methods of Analysis of AOAC International, Volume 1: Agricultural Chemicals, Contaminants, Drugs. 16th Edn., Association of Official Analytical Chemists (AOAC), Washington, DC., USA.
14. Sallam, S.M.A., 2005. Nutritive value assessment of the alternative feed resources by gas production and rumen fermentation *in vitro*. Res. J. Agric. Biol. Sci., 1: 200-209.
15. Konitzer, K. and S. Voigt, 1963. [Direct determination of ammonium in blood and tissue extracts by means of the phenol by chlorite reaction]. Clinica Chimica Acta, 8: 5-11, (In German).
16. Dehority, B.A., W.S. Damron and J.B. McLaren, 1983. Occurrence of the rumen ciliate *Oligoisotricha bubali* in domestic cattle (*Bos taurus*). Applied Environ. Microbiol., 45: 1394-1397.
17. Abo El-Nor, S.A.H. and M.S.A. Khattab, 2012. Enrichment of milk with conjugated linoleic acid by supplementing diets with fish and sunflower oil. Pak. J. Biol. Sci., 15: 690-693.
18. 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.
19. Calsamiglia, S., M. Busquet, P.W. Cardozo, L. Castillejos and A. Ferret, 2007. Essential oils as modifiers of rumen microbial fermentation. J. Dairy Sci., 90: 2580-2595.
20. Davidson, P.M. and A.S. Naidu, 2000. Phyto-Phenols. In: Natural Food Antimicrobial System, Naidu, A.S. (Ed.). CRC Press, Boca Raton, FL., pp: 265-294.
21. Nikaido, H., 1994. Prevention of drug access to bacterial targets: Permeability barriers and active efflux. Science, 264: 382-388.
22. Ultee, A., E.P.W. Kets and E.J. Smid, 1999. Mechanisms of action of carvacrol on the food-borne pathogen *Bacillus cereus*. Applied Environ. Microbiol., 65: 4606-4610.
23. Williams, A.G., 1986. Rumen holotrich ciliate protozoa. Microbiol. Rev., 50: 25-49.
24. Scott, R.I., N. Yarlett, K. Hillman, A.G. Williams, D. Lloyd and T.N. Williams, 1983. The presence of oxygen in rumen liquor and its effects on methanogenesis. J. Applied Bacteriol., 55: 143-149.
25. Benchaar, C., H.V. Petit, R. Berthiaume, D.R. Ouellet, J. Chiquette and P.V. Chouinard, 2007. Effects of essential oils on digestion, ruminal fermentation, rumen microbial populations, milk production and milk composition in dairy cows fed alfalfa silage or corn silage. J. Dairy Sci., 90: 886-897.
26. Shaver, R.D. and M.D. Tassoul, 2008. Essential oils as dietary supplements for dairy cows. Proceedings of the 6th Mid-Atlantic Nutrition Conference, March 26-27, 2008, Baltimore, MD., USA.
27. Kung, Jr. L., P. Williams, R.J. Schmidt and W. Hu, 2008. A blend of essential plant oils used as an additive to alter silage fermentation or used as a feed additive for lactating dairy cows. J. Dairy Sci., 91: 4793-4800.
28. Tager, L.R. and K.M. Krause, 2011. Effects of essential oils on rumen fermentation, milk production and feeding behavior in lactating dairy cows. J. Dairy Sci., 94: 2455-2464.
29. Morsy, T.A., S.M. Kholif, O.H. Matloup, M.M. Abdo and M.H. El-Shafie, 2012. Impact of anise, clove and juniper oils as feed additives on the productive performance of lactating goats. Int. J. Dairy Sci., 7: 20-28.
30. Mavrogenis, A.P., A. Koumas, C.K. Kakoyiannis and C.H. Taliotis, 1995. Use of somatic cell counts for the detection of subclinical mastitis in sheep. Small Ruminant Res., 17: 79-84.
31. Blummel, M., H.P.S. Makkar and K. Becker, 1997. *In vitro* gas production: A technique revisited. J. Anim. Physiol. Anim. Nutr., 77: 24-34.