

Effect of cinnamaldehyde thymol mixture on growth performance and some ruminal and blood constituents in growing lambs fed high concentrate diet

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Abstract: The objective of this study was to evaluate the effect of thymol and cinnamaldehyde mixture (TCM) supplementation on growth performance and some ruminal and blood parameters of lambs fed high concentrate diets. Twenty-Four Rahmani lambs with initial live weight of 21.98 ± 1.43 kg were randomly divided into three groups (8 animals each). The first group fed diet without supplementation (control) while 2nd and 3rd group were supplemented with 100 or 200 mg TCM/kg of diet respectively *ad libitum* over 18-week period. Addition of TCM tended to improve nutritive values (TDN, DCP, SE), DMI ($P=0.97$), ADG ($P=0.25$) and feed efficiency ($P=45$). Ruminal pH, total VFA concentrations and ammonia concentration were similar between lambs fed TCM and those fed the control diet. Mean number of protozoa and microbial protein concentration were not altered in response to any of TCM levels. Serum concentrations of glucose, cholesterol, triglyceride, urea-N, alanine aminotransferase and aspartate aminotransferase were not changed by feeding TCM. Overall, results from this study suggest that supplementing growing lambs fed high concentrate diet with TCM may have limited potential to improve their growth rate and feed efficiency without any detrimental effect on ruminal and blood constituents.

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1. Introduction

Over the last decades, antibiotic as growth promoters have been included successfully in ruminant animal diet additives to improve animal performance besides reducing diseases probabilities (Nagaraja, 1995, Tedeschi *et al.*, 2003 and Chaves *et al.*, 2008a;b). Nevertheless, the World Health Organization (WHO) and the Food and Agriculture Organization (FAO) have drawn attention to the threat of the transfer of antibiotic resistant pathogens to humans beside the hazard of their residues in meat and milk on human health (FAO/OIE/WHO, 2004). Therefore, the European Union has prohibited their use (EC, 2003).

In recent years, plant bioactive compounds and their major elements, essential oils (EO), have been considered a unique feed additives alternative to growth promoters in livestock nutrition as most of them are categorized under GRAS (Generally Recognized as Safe) for human consumption (FDA, 2004 and Geraci *et al.*, 2012). The EO known as volatile or ethereal oils, are naturally occurring blend of volatile secondary metabolites that can be obtained from plants through steam distillation or by extraction using organic solvents (Benchaar *et al.*, 2007).

Recent literatures have shown the potential of some EO components to favorably modify rumen metabolism and increase animal production (McIntosh *et al.*, 2003, Fraser *et al.*, 2007, Calsamiglia *et al.*, 2007 and Benchaar *et al.*, 2008).

Moreover, previous report of Burt, (2004) has suggested that combinations of EO secondary metabolites, may result in additive and/or synergetic effects which may enhance efficiency of rumen microbial fermentation.

Among EO compounds, thymol (THY) and cinnamaldehyde (CIN) are two potential components of interest. Thymol is a phenolic monoterpene and one of the main active compounds of thyme oil (*Thymus vulgaris*), while CIN, a phenylpropanoid with antimicrobial activity, is the main active component of cinnamon (*C. cassia*) oil (Calsamiglia *et al.*, 2007).

Data from *in vitro* (Castillejos *et al.*, 2006, Martinez *et al.*, 2006 and Fraser *et al.*, 2007) and *in vivo* (Cardozo *et al.*, 2006, Benchaar *et al.*, 2006a;b and Yang *et al.*, 2010a;b) studies on effects of both THY and CIN are contradictory. Additionally, information on their mixture effect on rumen fermentation elements and growth performance in ruminant animals is ambiguous.

Therefore the objective of this trail was to evaluate the effects of THY and CIN mixture at two levels (100 and 200 mg/kg of diet) on feed intake, growth performance, and some ruminal and blood parameters in lambs fed high concentrate diet.

2. Material and methods

2.1. Animals and experimental rations

This study was conducted at private farm located at Belbies, Sharkia Governorate, Egypt and the laboratories measurements conducted in the

Department of Animal Production, Faculty of Agriculture, Zagazig University, Zagazig, Egypt.

Twenty-Four Rahmani lambs of 4-5 months old and of 21.98 ± 1.43 kg average body weight were used over 18 week period in this study. Animals were randomly divided into 3 similar groups (8 animals each) with respect to their initial live body weight and fed high concentrate diet (Table 1) without or with (100 or 200 mg/kg ration) THY and CIN mixture (TCM).

Lambs of each group were kept in a separate shaded pen and adapted for the control diet for 10 days before using the tested diets. The tested animals were fed pelleted complete diet (Table 1) *ad-libitum* and water was available at all times. Thymol (purity of 99%) and CIN (purity $\geq 98\%$), synthetically manufactured, were provided by Oxford laboratory Company Mumbai, India (THY) and Flaka chemical, Switzerland (CIN). The chemical compositions of ingredients and the experimental ration were shown in Table (1).

2.2. Feed intake and gain:

Quantities of feed offered and refusals were recorded daily for daily feed intake determination. Animals were individually weighed at 14 d-intervals in the morning before feeding and the average daily gain (ADG) was determined by dividing weight gain (initial LW–final LW) by the number of days in the study. Feed efficiency was calculated as the ratio between ADG and DMI (kg of LW gain/kg of DMI).

2.3. Digestibility trials:

Three digestibility trials were carried out using 12 lambs (4 lambs/each) to evaluate the tested diets at the end of the experiment. Throughout the digestibility trial period, the experimental diets were weighed and offered to the individual animals *ad-libitum* and the refusal (if any) were recorded daily once at 8:00 a.m. Feces were daily collected from each lamb once daily using fecal collecting bags. Representative samples of offered and refused feeds and feces were collected for chemical analysis. The chemical composition of different ingredients and feces were analyzed according to the A.O.A.C. (1990).

Item	Amount
Ingredients (g/kg of diet)	
Yellow corn	230
Soybean meal	200
Wheat bran	140
Barley	200
Wheat straw	200
Limestone	15
Salt	7
Sunflower oil	5
Mineral-vitamin premix *	3
Chemical composition	
Dry matter (g/kg)	892.9
Organic matter (g/kg of DM)	942.1
Crude protein (g/kg of DM)	159.3
Ether extract (g/kg of DM)	38.3
Crude fiber (g/kg of DM)	113.6
Nitrogen free extract (g/kg of DM)	630.9
Ash (g/kg of DM)	57.9
* each 1 kg contains: vitamin A 580000 IU, vitamin D ₃ 8600 IU, vitamin E 720 mg, vitamin K ₃ 142 mg, vitamin B ₁ 58 mg, vitamin B ₁ and B ₆ 34 mg, vitamin B ₁₂ 58 mg, vitamin C 0.1 mg, folic acid 86 mg, pantothenic acid 8 mg, copper 3400 mg, iodine 25 mg, selenium 25 mg, iron 2000 mg, manganese 65 mg, zinc 3000 mg, cobalt 572 mg and calcium Carbonate as carrier up to 1 kg	

2.4 Ruminant fermentation characteristics

Ruminal fluid samples were collected 3 hrs after morning feeding from four lambs randomly selected from each treatment group for 3 consecutive days every two weeks up to 12th week of the experimental period. Ruminal fluid samples were obtained by using rubber stomach tube. Samples were strained through four layers of cheese cloth. Each sample was divided into four portions. The first

and 2nd were used immediately for the estimation of rumen pH (using the Consort pH meter model P 107 with combined electrode) and protozoa count (was determined according to **Ogimoto and Imai, 1981**), while 3rd and 4th portions were kept in deep freeze at -20 until further analysis of both ammonia-N and microbial protein.

Ammonia nitrogen concentration (NH₃-N) of rumen fluid was estimated according to **Conway (1957)**. The total VFA's concentration was determined by steam distillation as mentioned by **Warner (1964)**. Ruminal microbial protein was measured according to the method of **Shultz and Shultz (1970)**.

2.5. Blood metabolites

From the same animals used for ruminal sampling, blood samples were withdrawn once before morning feeding (fasting) from the jugular vein every two weeks till 12th week. Blood serum was collected after centrifugation at 3000 rpm for 20 minutes and stored at - 20 C until analysis. Spectro-photometric determination of serum glucose, ALT, AST, Urea, cholesterol and triglycerides were done according to the methods described by **Trinder (1969)**, **Harold (1975)**, **Patton and Crouch (1977)**, **Finley et al. (1978)** and **Scheletter and Nussel (1975)**, respectively.

2.6. Statistical analysis:

The data related to ruminal and blood constituents were analyzed using the MIXED procedure by SAS User's Guide (**SAS, Institute, 2002**). The mathematical model was $Y_{ijk} = \mu + T_i + P_j + TP_{ij} + e_{ijk}$, where μ = the overall mean, T_i = the fixed effect of treatment, P_j = the fixed effect of period (weeks), TP_{ij} = the fixed effect of interaction between treatment and period and e_{ijk} = residual error. While the data of other measured variables were analyzed by simple one way classification as the model: $Y_{ij} = \mu + T_i + e_{ij}$ Where μ = the overall mean, T_i = the fixed effect of treatment and e_{ij} = residual error. Significant

differences were determined by Duncan's Multiple Range test (**Duncan, 1995**).

3. Results and discussion

1. Feed intake and growth performance

The obtained results showed that the addition of 100 and 200 mg of TCM / kg diet insignificantly improved DMI (1.297 Kg/d, $P = 0.97$) and average daily gain (ADG) (233.16 g /d, $P = 0.25$) (Table 2). Also, feed efficiency relatively improved (185g gain/g feed, $P = 0.45$) by TCM addition to the tested diet. This findings are consistent with **Yang et al., 2010b** who confirmed that low and moderate doses of CIN (400 and 800 mg/d) improved feed intake. In the same line, **Chaves et al. (2011)** confirmed that no detrimental effect on DMI by addition of CIN in ruminant diets within the range of the dosage level (43 to 400 mg/kg of dietary DM). Similarly, **Chaves et al. (2008b)** found that addition of CIN (200 mg/kg of dietary DM) had no effect on DMI in growing lambs. Also more recently, **Vakili et al. (2013)** reported that supplementation of feedlot calves fed high-concentrate diets with (5 g/d/calf) thyme and (2 g/d/calf) cinnamon EO did not affect DMI, ADG and feed efficiency. However, various literatures confirmed reduction DMI response to THY or CIN addition. **Busquet et al. (2003)** observed a 12% reduction in concentrate DMI in dairy cattle fed 600 mg of CIN/kg of DM. furthermore, reduction in DMI was recorded by **Vakili et al. (2013)** with the high dose addition of CIN (1600 mg/d) but this was not the matter with the low or moderate doses. Taken together, the reduction in DMI might be related to palatability problems as their odor could be detected through olfaction.

Item	Control	100 mg	200 mg	S.E.M	P-value
Number of lambs	8	8	8		
Initial BW (kg)	22.97	21.34	21.62	1.43	0.69
Final BW (kg)	51.51	51.89	52.78	1.62	0.80
Total gain (kg)	27.76	29.71	30.66	1.15	0.25
Average daily gain (g/d)	220.29	235.83	243.37	9.12	0.25
DMI (kg/d)	1.279	1.299	1.313	0.100	0.97
Feed efficiency *	0.172	0.182	0.185	0.007	0.45

* = Gain: feed, calculated as total BW gain divided by total feed intake (DM basis).

2. Nutrient digestibility

In the current experiment, Digestibilities of DM, OM, CP, EE, CF and NFE were not affected by addition of TCM (100 and 200 mg/kg diet). Mean values of TDN, DCP and SE were 77.88, 13.11 and 70.06 %, respectively and did not significantly differ among the experimental treatments (Table 3). Our findings are in parallel with **Yang et al., 2010a** who recorded non significant change in digestibility of OM in the rumen of growing beef heifers fed high-concentrate diets supplemented with low dose of CIN

(400 mg/d). On the contrary, at a high dose (1,600 mg/d), OMD and NDF were decreased by 8% and 35% respectively compared with the control value. Similarly, **Castillejos et al. (2006b)** found that addition of THY at high dose (500 mg/L) significantly reduced the DMD, OMD, NDFD and ADFD digestibility. **Kamalak et al. (2011)** suggested that the decrease in the DMD and OMD with THY addition in high doses could be possibly owed to decrease in fiber digestion as a result to the higher sensitivity of cellulotic bacteria to THY.

Table 3. Nutrients digestibility and nutritive values as affected by supplementation of thymol and cinnamaldehyde mixture (TCM).

Item	Control	100 mg	200 mg	S.E.M	P-value
Nutrients digestibility (%)					
DM	73.98	74.67	75.21	1.39	0.83
OM	77.60	78.51	78.40	1.28	0.87
CP	81.93	82.27	82.62	1.10	0.91
EE	88.86	88.72	88.08	1.21	0.90
CF	50.59	50.24	53.61	1.79	0.40
NFE	80.69	82.03	81.21	1.33	0.78
Nutritive values (%)					
TDN	77.36	78.20	78.07	1.24	0.88
DCP	13.05	13.11	13.16	0.17	0.91
SE	69.54	70.39	70.25	1.22	0.87

DM= dry matter, OM= organic matter, CP= crude protein, EE= ether extract, CF= crude fiber, NFE= nitrogen free extract, TDN= total digestible nutrients, DCP= digestible crude protein, SE= starch equivalent.

3. Rumen characteristics

Mean ruminal pH values were not significantly differ among treatments and ranged from 5.57 to 5.48 (Table 4). The absence of effect of these additives on ruminal pH values were in line with previous researches in sheep (Chaves *et al.*, 2008a;b), dairy cows (Yang *et al.*, 2007, Kung *et al.*, 2008 and Tager and Krause., 2011), and beef cattle (Cardozo *et al.*, 2006, Fandino *et al.*, 2008, Meyer *et al.*, 2009 and Yang *et al.*, 2010a;c). On the contrary, various *in vitro* studies showed a notable increase (Evans and Martin 2000 and Castillejos *et al.*, 2006a) or decrease in pH values Castillejos *et al.* (2008) when incubated with THY or CIN. The opposite results of pH values were correlated with the high doses and could be partially described by the type of diets used and the short term of these studies (Vakili *et al.*, 2013).

Supplementation of TCM had no effect on either total VFA or ammonia concentrations at tested levels and averaged 22.15 to 22.5 mg/dL and 9.90 to 9.99 ml eq. /dL, respectively (Table 4). The lack effects on total VFA concentration when CIN or THY was added separately to the diet was also recorded in other *in vivo* studies with sheep (Newbold *et al.*, 2004 and Chaves *et al.*, 2008a), dairy cows (Benchaar *et al.*, 2008) and beef cattle (Yang *et al.*, 2010b). Furthermore, no difference in ruminal total VFA concentration was recorded with combination of CIN with other EO component like eugenol (Cardozo *et al.*, 2006). In the same line, most of *in vivo* studies have been shown EO often do not influence concentration of ruminal NH₃ -N (Benchaar *et al.*, 2006b;2007, Devant *et al.*, 2007, Yang *et al.*, 2007 and Yang *et al.*, 2010a;c) that are compatible with our finding in this research. In sharp

contrast, some *in vitro* studies in batch fermentation and continuous culture recorded a reduction in both total VFA and ammonia concentrations with addition of CIN or THY (Castillejos *et al.*, 2006a, b and Busquet *et al.*, 2006). This contradictory effect between *in vitro* and *in vivo* results may be related to concentration of EO or their mixtures used, pH of incubation medium, the *in vitro* technique which used (Benchaar *et al.*, 2008) and the capacity of rumen microbes to adapt and/or degrade these components (Benchaar and Greathead, 2011).

It is well known that rumen ciliate protozoa play diverse roles in ruminal metabolism and in their absence the numbers of bacteria and starch degradation increase, and ammonia N concentration decreases (Van Nevel and De-meyer, 1988). In the current research, the average mean of protozoa number and microbial protein concentration in experimental treatments were 3.33x10⁵ and 1.01 g/mL, respectively and was similar in all treatments. The lack of treatment effect on protozoal numbers in the rumen is consistent with the literature that indicates only very large doses of EO reduce protozoal numbers. Hart *et al.* (2008) concluded in a review that the effect of EO on rumen protozoa varies with the type of EO tested and that relatively large concentrations of EO are required to decrease protozoal numbers. Benchaar *et al.* (2006b) reported that supplementation of dairy cow diets with 1,000 mg/d of CIN had no effect on the numbers or generic composition of ciliate protozoa. However, feeding 2,000 mg/d of anise extract containing 100 g/kg of anethol to beef heifer decreased counts of holotrichs and entodiniomorphs (Cardozo *et al.*, 2006).

The absence effect of TCM on microbial protein flow in our study is compatible with the

previous *in vitro* study of by **Busquet *et al.* (2005)** who confirmed the absence of change in bacterial N flow in a dual-flow continuous culture supplied with 31.2 or 312 mg/L of CIN. Also, in an *in vivo* study

with another EO named juniper berry oil in dairy cow diets, microbial protein synthesis was not differ than the control one (**Yang *et al.*, 2007**).

Table 4. Some ruminal parameters of growing lambs as affected by supplementation of thymol and cinnamaldehyde mixture

Item	Control	100 mg	200 mg	S.E.M	P-value		
					T	Week	T × Week
pH							
2 nd week	5.68	5.50	5.71	0.10	0.17	0.01	0.60
4 th week	5.74	5.64	5.58	0.10			
6 th week	5.50	5.40	5.57	0.08			
8 th week	5.47	5.37	5.48	0.06			
10 th week	5.34	5.40	5.57	0.03			
12 th week	5.54	5.58	5.53	0.08			
Overall	5.55	5.48	5.57	0.04			
Ammonia-N (mg/dL)							
2 nd week	20.83	21.00	22.75	0.86	0.05	0.11	0.33
4 th week	20.20	21.44	22.05	1.07			
6 th week	22.23	23.40	22.40	0.74			
8 th week	22.23	22.77	22.05	0.67			
10 th week	19.83	22.05	22.44	0.78			
12 th week	23.14	22.23	23.63	0.68			
Overall	21.41	22.15	22.55	0.34			
TVFA (ml eq./dL)							
2 nd week	7.89	7.84	7.98	0.37	0.92	0.00	0.25
4 th week	8.20	8.15	8.90	0.49			
6 th week	9.94	10.29	11.00	0.33			
8 th week	10.98	10.71	10.84	0.31			
10 th week	11.28	11.38	10.38	0.30			
12 th week	11.50	11.05	10.85	0.38			
Overall	9.96	9.90	9.99	0.24			
Protozoa count (×10⁵/mL)							
2 nd week	3.05	3.14	3.14	0.15	0.56	0.03	0.99
4 th week	3.10	3.24	3.13	0.16			
6 th week	3.44	3.49	3.24	0.18			
8 th week	3.39	3.54	3.34	0.45			
10 th week	3.40	3.45	3.40	0.13			
12 th week	3.43	3.49	3.55	0.10			
Overall	3.30	3.39	3.30	0.07			
Microbial protein (gm/dL)							
2 nd week	1.17	1.31	1.23	0.12	0.18	0.000	0.34
4 th week	1.36	1.28	1.55	0.08			
6 th week	1.23	1.11	1.06	0.08			
8 th week	1.38	1.47	1.54	0.12			
10 th week	1.27	1.61	1.66	0.10			
12 th week	1.31	1.28	1.38	0.09			
Overall	1.28	1.34	1.40	0.05			

4. Blood metabolites

In spite of the well established pharmacological potential of both THY and CIN, serum hepatic enzymes ALT and AST concentrations were not significantly altered among treatments

ranging 24.29 and 53.80 μ L, respectively (Table 5). These results were in accordance with **Vakili *et al.* (2013)** who confirmed that addition of these compounds had no effect on hepatic enzymes.

Table 5. Blood metabolites of growing lambs as affected by addition of thymol and cinnamaldehyde mixture							
Item	Control	100 mg	200 mg	S.E.M	P-value		
					T	Week	T × Week
ALT (μ/L)							
2 nd week	18.90	21.53	22.56	1.46	0.64	0.000	0.92
4 th week	21.25	22.00	20.97	2.23			
6 th week	25.71	27.59	25.66	1.20			
8 th week	22.84	23.22	22.99	1.36			
10 th week	29.09	28.57	26.79	1.36			
12 th week	26.18	25.85	25.52	1.05			
Overall	23.99	24.79	24.08	0.80			
AST (μ/L)							
2 nd week	48.64	52.08	47.87	2.85	0.90	0.000	0.96
4 th week	44.18	46.22	43.67	2.54			
6 th week	50.55	51.95	54.12	2.64			
8 th week	60.11	62.78	62.15	2.73			
10 th week	58.33	56.79	59.47	4.06			
12 th week	58.71	55.52	55.27	2.78			
Overall	53.42	54.22	53.76	1.63			
Glucose (mg/dL)							
2 nd week	76.00	66.75	74.00	2.89	0.08	0.001	0.20
4 th week	77.25	82.75	84.50	2.72			
6 th week	77.00	80.25	79.75	2.97			
8 th week	78.25	80.50	81.00	2.88			
10 th week	70.00	79.00	77.75	2.99			
12 th week	75.25	79.75	78.25	1.50			
Overall	75.63	78.17	79.21	1.34			
Urea (mg/dL)							
2 nd week	41.76	42.79	46.73	1.37	0.39	0.000	0.05
4 th week	35.91	35.77	39.94	0.86			
6 th week	45.84	45.27	46.21	1.08			
8 th week	49.11	47.90	46.54	1.51			
10 th week	49.02	50.28	48.60	0.91			
12 th week	48.55	49.67	47.80	1.09			
Overall	45.03	45.28	45.97	0.98			
Cholesterol (mg/dL)							
2 nd week	40.88	41.18	44.71	1.77	0.051	0.362	0.99
4 th week	37.65	39.12	40.30	1.70			
6 th week	37.35	39.12	39.12	2.99			
8 th week	38.82	43.24	43.82	1.94			
10 th week	38.53	41.77	43.24	2.13			
12 th week	39.12	42.06	43.24	2.22			
Overall	38.73	41.08	42.40	0.98			
Triglycerides (mg/dL)							
2 nd week	34.46	34.26	32.27	0.93	0.19	0.000	0.40
4 th week	28.69	27.09	29.88	1.74			
6 th week	26.10	24.30	27.29	1.17			
8 th week	28.29	26.49	25.70	0.99			
10 th week	26.30	26.70	28.29	0.89			
12 th week	27.29	25.90	27.89	0.71			
Overall	28.52	27.46	28.55	0.71			

It is well known that serum cholesterol and triglyceride level is an indicator of fat mobilization **Chaves et al. (2008a)**. Along twelve weeks of the present experiment, serum concentrations of cholesterol and triglyceride were not affected by any of TCM levels (Table 5). These results suggested no change in fat mobilization that may be contributed to lack of DMI alternation by tested levels of TCM. Similar findings were observed by **Chaves et al. (2011)** who recorded that cholesterol and triglycerides concentration of lambs were not affected by CIN supplementation at 200 mg/kg diet compared to those of the control one. A comparable results were given by **Vakili et al. (2013)** who observed no change in cholesterol and triglyceride levels in feedlot calves fed high-concentrate diets supplemented with thyme (5 g/d/calf) or cinnamon (5 g/d/calf) oil. On the contrary, **Chaves et al. (2008b)** detected an 18-fold higher total triglycerides serum concentration in lambs supplemented with 200 mg CIN/kg of dietary DM in comparison with those fed control diet. These authors suggested that CIN might have a potential antidiabetic activity.

Previously, **Petit and Flipot, 1992** and **Davidson et al., 2003** illuminated that blood urea nitrogen is the end product of the excess ruminal ammonia-N that passed from ruminal wall to liver via portal vein. Thus serum urea concentration in this experiment was not varied among different treatment as shown in table 5 because of the absence of change of ruminal ammonia-N as previously mentioned in table 4. These results are in accordance with a previous research used THY and CIN supplementation separately (**Vakili et al., 2013**) and also with some studies that used other EO (**Tassoul and Shaver, 2009** and **Özdoğan et al., 2011**). Nevertheless, this result contrasts with the findings of **Yang et al. (2010a)** and **Chaves et al. (2011)** in beef cattle and lambs respectively but this could be owed the high doses of additives used in this study.

Compared to control, 100 and 200 mg / kg TCM treated group showed no significant change in blood glucose concentration (Table 5). Similar findings were reported by (**Devant et al., 2007**, **Chaves et al., 2008a**, **Tassoul and Shaver, 2009** and **Yang et al., 2010b**) for THY and CIN at different doses.

Conclusion

Our results suggest that a mixture of THY and CIN had a moderate improvement DMI, ADG and feed efficiency without counteractive effect on nutrient digestibility and ruminal and blood constituents. So this study could suggest these additive as promising feed additive as an

alternative for antibiotic use in ruminant nutrition when used in moderate doses with suitable ration.

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