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Kurniawati A, Widodo, Artama WT, Yusiati LM

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Amomum compactum Soland ex Maton ADDITION AS ESSENTIAL OIL SOURCE AND ITS EFFECT ON RUMINAL FEED FERMENTATION BY IN VITRO ANALYSIS**

Asih Kurniawati[1], Widodo[2], Wayan Tunas Artama[3] and Lies Mira Yusiati[1]*

[1]Department of Animal Nutrition and Feed Science, Faculty of Animal Science, Universitas Gadjah Mada, Yogyakarta 55281, Indonesia

[2]Department of Animal Products Technology, Faculty of Animal Science, Universitas Gadjah Mada, Yogyakarta 55281, Indonesia

[3]Department of Biochemistry, Faculty of Veterinary, Universitas Gadjah Mada, Yogyakarta 55281, Indonesia

*Corresponding author, e-mail: liesmira@ugm.ac.id

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Running title: *Amomum compactum* Soland ex Maton addition as essential oil source

ABSTRACT

Essential Oil (EO), as feed additive, has positive effect on ruminal feed fermentation by increasing feed efficiency and reducing methane production. This research was done to studied java cardamom (*Amomum compactum* Soland ex Maton) effect, be essential oil source as feed additive, on ruminal feed fermentation. In vitro gas production technique was used in this research to elaborate the effect of cardamom on nutrient digestibility of substrate and parameters of fermentation in rumen. Cardamom meal was added into feed sample equal to end concentration of EO in fermentation medium as much as 0, 25, 50, 75 and 100 mg/l. Substrate of fermentation consist of *Pennisetum purpureum*, rice bran and wheat pollard. Addition of cardamom have no effect on substrate dry matter digestibility except at 100 mg/l was decreased. Protein digestibility in all added cardamom fermentation were lower than control, whereas organic matter and crude fiber digestibility increased up to 13.5 and 24% at level of EO 100mg/l respectively. Fermentation parameters including volatile fatty acid (acetate, propionate, butyrate) production, pH and microbial protein synthesis did not affected by cardamom addition except ammonia concentration. Methane production and protozoa population also did not change by the treatment. In conclusions of this study that, utilization of java cardamom as feed additive have positive effect on ruminal feed fermentation by increase organic matter and crude fiber digestibility and reduce protein digestibility.

Keywords: *Amomum compactum* Soland ex Maton, essential oil, ruminal fermentation, methane

INTRODUCTION

Digestion system of ruminant is unlike of monogastric animal. Rumen as a unique organ in ruminansia is a part of its system which is a large natural fermenter. Bacteria, fungi, and protozoa habitated the rumen (Nagaraja, 2016) and plays the important and main role in feed ruminal fermentation particularly plant materials including fibrous plants cell wall (Wang and McAllister 2002). From fermentation process energy supply for the host animal was served in form of volatile fatty acid (Puniya et al. 2015), and from those process the other useful product also produced for

instance high quality of protein from non-protein nitrogen in the form of bacteria cell, and vitamins particularly vitamins B beside several "waste gaseous CO_2 and CH_4. Methane emission from enteric rumen fermentation represent ineffectiveness of feed energy utilization. About 6 to 10% (Eckard et al. 2010) and 2-15% (Kumar et al., 2009) of total consumed feed gross energy release and loss through the breath as methane. Methane is potent greenhouse gas with global warming potency 25 folds of CO_2 (Eckard et al., 2010). Some research has been done to increase efficiency of rumen fermentation and reduce methane production through rumen microbes and rumen fermentation manipulation.

Antibiotic utilization, ionophore, for modification of rumen fermentation briefly illustrated by (Russell & Strobel, 1989). Monensin, one of ionophore antibiotic increase efficiency of feed utilization by decreasing 30% of ruminal methanogenesis, reduce ammonia concentration in the rumen by interfere proteolytic bacteria and mostly deamination bacteria. Reduction of ammonia in the rumen lead to decreasing of protein loss in urine. In growing cattle monensin increasing rumen VFA concentration, digestibility and protein retention, thus improving food use and weight gain (Salles, Zanetti, Titto, & Conti, 2008). Ionophore in feedlot cattle increase of daily gain 1.6% and feed efficiency 7.5% respectively (Jouany & Morgavi, 2007). However, utilization of antibiotic recently was banned in several country regarding of antibiotic residue in animal product and emerging of resistance bacteria. Hence safely feed additive as antibiotic alternative are needed. EOs have antimicrobial activities and are currently considered safe for human and animal consumption and are categorized as Generally Recognized as Safe (U.S. Food & Drug Administration, 2017) (U.S. Food & Drug Administration, 2017).

Essential oil is plant secondary metabolite, a natural product which exhibit activity against a wide range of microorganisms, including bacteria, protozoa and, fungi (Chao et al. 2012; Cosentino et al. 1999; Deans and Ritchie 1987; Sivropoulou et al. 1996). Naturally, essential oil is part of plant defending mechanism again predator as antibacterial, antifungals, insecticide, antivirals (Bakkali, Averbeck, Averbeck, & Idaomar, 2008). Therefore, based on the activity, essential oil has change to be used as antibiotic an alternative to modified rumen microbes and ruminal fermentation.

Several researches in essential oil utilization as feed additive perform positive effect i.e. increasing of productivity and decreasing of methane production by in vitro and or in vivo studies (Bodas et al., 2012; Geraci, Garciarena, Gagliostro, Beauchemin, & Colombatto, 2012). Mix oil of cinnamaldehyde, eugenol, and capsicum in feedlot cattle have the same effect as monensin (Geraci et al., 2012) increase growth and health performance, optimized of feed fermentation in the rumen and increased immune system (Compiani, Sgoifo Rossi, Pizzi, & Dell'Orto, 2013) hence essential

oils could be used as alternative antibiotic for feed additives (Khorrami, Vakili, Mesgaran, & Klevenhusen, 2015).

Essential oil from rosemary, oregano, ceylon cinnamon, dill seeds, cinnamon leaves, cinnamon bark, and eucalyptus, at level 1,125 ml/L of fermentation media, reduce methane production and ammonia concentration in rumen medium with no detrimental effect in NDF degradability except eucalyptus. Oregano individually and combinations of several those essential oil reduce abundance of archaea (Cobellis et al. 2016).

Essential oil of clove, eucalyptus, garlic, oregano and peppermint at concentration of 0.25, 0.5, and 1 g/L of medium reduced methane production in line with the increasing of essential oil level, reduced population of protozoa and archaea, but NDF digestibility also decreased except in garlic oil due to the decreasing of cellulolytic bacteria. Those essential oils have no effect on volatile fatty acid production except in addition of clove and oregano oil (Benchaar & Greathead, 2011; Calsamiglia, Busquet, Cardozo, Castillejos, & Ferret, 2007).

Biological activity of essential oils in rumen fermentation are varies. Effects of essential oils depend on their chemical composition. Same essential oil which obtained from different plant in same genus may have opposite effect, stimulatory or inhibitor (Ferme et al. 2004; Patra A. K. 2011). Purity and dose also influenced activity of essential oil (Macheboeuf, Morgavi, Papon, Mousset, & Arturo-Schaan, 2008).

Amomum compactum Soland ex Maton (Java cardamom), commonly called as Java cardamom, or false cardamom, ismember of Zingiberaceae family. In Indonesia java cardamom used as spices in several dishes and part of traditional medicine called jamu. Identified active component in java cardamom essential oil according to (Chempakam & Sindhu, 2008) 98% of the total oil consist of 1,8-cineole (38.7%), β- pinene (13.6%), α-terpineol (12.6%), spathulenol (8.3%), 4-terpineol (4.5%), germacrene D (3.0%), α-pinene (2.8%) and β- selinene (2.7%). Whereas acoording to (Sardar, Tarade, & Singhal, 2013) the major active components of cardamom essential oil is 1,8-cineole (30.2%) and a-terpinyl acetate (46.6%) (Sardar et al., 2013). This study present in vitro effect of addition java cardamom in the diet on nutrient digestibility, methane production and other parameter of ruminal fermentation.

MATERIALS AND METHODS

Feed, Treatments and In Vitro Fermentation

The effect of java cardamom on nutrients digestibility, ruminal fermentation and methane production were studied in this research using batch culture of in vitro gas production technique. Feed sample for in vitro fermentation consist of Pennisetum purpureum, which cut before flowering stage, rice bran and wheat pollard, obtained from feed shop, with ratio 60:20:29 based on dry

matter. Java cardamom meal was prepared by drying its seed in dryer incubator at 55°C and ground to pass through a 1 mm pore size sieves. Addition of java cardamom were based on final concentration of essential oil in fermentation media i.e. 0, 25, 50, 75, and 100 mg/L.

Inoculum for the in vitro gas production was obtained from two ruminally canulated Ongole grade cattle which fed diet consisting of Pennisetum purpureum and beef cattle concentrate 60:40 DM bases. Rumen fluid was collected before morning feeding, and squeezed through polyester cloth into a vacuum flask thermos, and immediately sent to the laboratory.

Serum bottles have volume 125 ml, were used for in vitro incubations. Bottles were set into three triplicate bottles, one set for dry matter digestibility (DMD) and organic matter digestibility (OMD) determination, gas and methane production, one set for crude protein digestibility (CPD), and one set for rumen fermentation parameter. Sufficient anaerobic media was prepared the day before the incubation according to (Theodorou, Williams, Dhanoa, McAllan, & France, 1994). Sixty-three milliliters of media were added in to serum bottles which previously filled with 700 mg of substrate and java cardamom powder according to the treatments and continuous flushed by oxygen free carbon dioxide. Bottles were sealed immediately with butyl rubber stopper plus aluminum crimp cap and pre-warmed overnight at 39°C. In the next morning rumen fluid was collected, and 7 ml was added into each bottle using 10 ml plastic syringe. Bottles then incubated for 24 h at 39°C. Bottle head space gas pressure were zeroing before incubation by inserting 0.6 mm needle attached to a pressure transducer.

At the end of incubation gas were collected using calibrated syringe and 5 ml of gas were transferred into 5 ml plain vacuum tube (Becton Dickinson Vacutainer System) for methane analysis. DMD, OMD and CPD were determined by filtered the bottle content, and residual feed were collected for residual nutrients analysis, including DM, OM and CP. Procedure for nutrient analysis according to AOAC (2005). Sample for protozoa calculation were prepared by pipetting 1 ml of bottle content and be added to 0.8ml of formaldehyde saline (1ml of 37%formaldehyde + 9 ml 0.9% NaCl). One microliter sample then transferred to haemocytometer for direct calculation under microscope according to method explained by Abreu et al. (2004).

For ammonia measurement, 1 ml of bottle content were preserved with 1 ml NaCl 20% and be frozen until later analysis of ammonia base on phenol hypochlorite reaction as explained by Chaney and Marbach (1962). Media, as much as 1 ml for volatile fatty acid (VFA) analysis were added into tube containing 1 ml of 20% metha-phosphoric acid and stored in freezer for further analysis using gas chromatography. Prior to sampling for ammonia, VFA, microbial protein and protozoa, pH media were measured. Rumen microbial protein was determined by Lowry method (Alexander and Griffiths, 1993). Microbial cell was separated from residual feed by centrifugation 1.5 ml of bottle content at 500g. Cell were precipitated from supernatant by spin down at 15.000 g.

Pellets were re-suspension in physiology solution and re centrifuge. Re-suspension was repeated for twice. The last suspension was subjected for protein determination.

Calculation and statistical analysis

Variables observed of this studied were nutrients digestibility including dry matter digestibility (DMD), organic matter digestibility (OMD), crude protein digestibility (CPD) (expressed in %), total VFA, acetate, propionate and butyrate concentration as mmol/100mL, rumen microbial protein, ammonia concentration as mg/100L, methane production as ml/g DM digested, and protozoa number. Data were subjected to one-way analysis of variance with level of Java cardamom as the treatment factor. Comparisons between means were analysis using Duncan Multiple Range test due to the treatment effects.

RESULTS AND DISCUSSION

Nutrient Digestibility

Nutrient digestion in the rumen by rumen microbes is importance point for ruminant production. Rumen microbes help ruminant, the host animal, to extract energy and serve protein by digestion and ferment the feeds. Feeds for ruminant commonly are fibrous material that cannot be used by monogastric animal. Modification of rumen fermentation were done to achieve the higher of nutrient utilization by: improving fiber digestion, reduced feed protein degradation to increase the availability of amino acid to be absorb in small intestine, reduce the degradation rate of rapidly fermentable carbohydrate, and shift methane production to propionate (Jouany & Morgavi, 2007). Essential oils is compounds which have change to be exploited to modify the feed fermentation in rumen due to their activity in exhibit microorganism growth including bacteria, fungi, and protozoa.

As shown in Table 1 addition of java cardamom significantly reduced dry matter digestibility ($P<0.05$) at levels 100 mg/l. The reduction as much as 12.29% compared to control. Slightly increase of dry matter digestibility (10.42%) occurred at addition level of 25%, while addition at level 50 and 75mg/l d (Castillejos etal. 2006) did not change digestibility of dry matter.

Organic matter digestibility also affected by several level of java cardamom addition ($P<0.05$) except 25mg/l of addition Table 1). Addition at level 50 mg/l and up increased organic matter digestibility. The increasing were 7.46, 7.27 and 13.05% for java cardamom addition at level 50, 75, and 100 mg/l respectively. Increasing of dry matter and organic matter digestibility also reported by Wang and Wang, (2016) by Chinese herbal addition. This result was not parallel with finding reported by Cobellis et al. (2016), they said that addition of essential oils in in vitro ruminal fermentation i.e. essential oil from dill seed oil, cinnamon leave, cinnamon bark, Ceylon cinnamon bark, eucalyptus leaves oregano leave and rosemary leave at level 1125 mg/l or their combination in

lower concentration (800 mg/l) reduced dry matter digestibility, but have no effect neutral detergent fiber digestibility. Major component of those essential oil was carvone in dill seed oil, trans-cinnamaldehyde, in cinnamon leave, cinnamon bark, Ceylon cinnamon bark, 1,8-cineole, in eucalyptus leaves and rosemary leave, and carvacrol, in oregano leaves. Even though main component of java cardamom is 1,8 cineole the same main component of essential oil from eucalyptus and rosemary leave but the effect on nutrient digestibility are different. It might be caused by different concentration of essential oil administered. Addition 1,8-cineole in in vitro rumen fermentation at level 50 mg/75 ml medium which correspond to 666 mg/l have no effect on organic matter digestibility (Araujo et al., 2011). Mixture of thymol, limonene and guaiacol at lower level, 1.5 mg/l, have no effect on dry matter, organic matter neutral detergent fiber, acid detergent fiber and crude protein digestion (Castillejos, Calsamiglia, Ferret & Losa, 2005). Castillejos et al. (2006) reported addition of thymol at level 5 and 50 mg/l have no effect on dry matter, organic matter, neutral detergent fiber and acid detergent fiber digestibility, but at high level, 500 mg/l, those nutrient degradability reduced. In the same research addition of eugenol up to 500 mg/l did not interfere the digestion of dry matter, organic matter, neutral detergent fiber and acid detergent fiber. Effect of essential oils in rumen fermentation were determined by their chemical composition (Ferme et al., 2004; Patra & Saxena, 2009). Purity and doses also influenced activity of essential oil (Macheboeuf, Morgavi, Papon, Mousset, & Arturo-Schaan, 2008).

Table 1 Effect of java cardamom addition as source of essential oil on ruminal in vitro nutrient digestibility.

Parameters	Level of Essential Oil (mg/l)				
	0	25	50	75	100
True Nutrient Digestibility (5%)					
Dry matter*	49.31^{ab}	54.45^{b}	47.24^{ab}	47.03^{ab}	43.25^{a}
Organic matter*	47.45^{b}	45.59^{b}	50.99^{ab}	50.90^{ab}	53.64^{a}
Crude protein**	56.02^{c}	44.21^{ab}	32.80^{a}	48.17^{bc}	36.42^{ab}
Crude fiber**	31.30^{a}	39.69^{b}	43.88^{c}	46.82^{c}	51.79^{d}

^{a,b,c,d} different superscript in the same row significantly different; * (P<0.05); ** (P<0.01)

Crude protein digestibility decreased by java cardamom addition (P<0.01) (Table 1). Crude protein digested by rumen microbes were lower in all level of java cardamom addition. In contrary to crude protein digestibility, Crude fiber digestibility rise up in line with rising of java cardamom level (P<0.01). The raise were 26.81, 40.19, 49.58, and 65.46% compared to control in order of java cardamom addition at 25, 50, 75, and 100 mg/l. Essensial oil may inhibit colonization of proteases bacteria in rumen that indicated by lower activity of protease (Wallace et al. 2002). Lowering crude protein digestibility in the rumen is an advantage since escaped feed protein from microbial degradation flushed abomasum and small intestine to be digested by indigenous animal proteases

and absorbed for animal metabolism. In rumen Feed protein are digested and broken down into small peptides, further into amino acid then ammonia. Ammonia which do not incorporated to microbial protein were absorbed across rumen wall into bloodstream. Ammonia converted to urea in liver and excreted through urine (Moran, 2005).

Previously researches shown essential oil did not affect fiber digestion in rumen et al. 2006; Cobellis, et al. 2016; Wallace et al. 2002). Addition of java cardamom in this research gave positive effect on fiber digestion (Table 1) since one of the main goals of rumen modification is improving fiber digestion (Jouany & Morgavi, 2007).

Parameter of Rumen Fermentation

Volatile fatty acid production and its composition as well as acetate: propionate ratio did not shift by treatment even though crude fiber digestibility increased (TABLE 2.). Volatile fatty acids in the rumen are come from digestion and fermentation of carbohydrate by rumen microbe (Moran, 2005). Effects of essential oils on VFA production in the rumen VFA are inconsistent. With the use of blend essential oil (oregano, cinnamon, thyme, orange peel) (Spanghero, Zanfi, Fabbro, Scicutella, & Camellini, 2008) reported decreases in VFA concentrations. VFA production also decreased as effect of single essential oils (dill seeds; cinnamon leaves; cinnamon bark; Ceylon cinnamon bark; eucalyptus leaves; oregano leaves; rosemary leaves) except eucalyptus (Cobellis et al. 2016). Moreover, this research showed changes in VFA component and acetate: propionate ratio. Reduction VFA also reported by (Castillejos et al. 2006), when thymol and eugenol were added at high level 500 mg/l in contrary thymol and eugenol at low level 5 and 50 mg/l did not change neither VFA concentration nor VFA composition.

Addition of several individual essential oil in several doses (Busquet, Calsamiglia, Ferret, & Kamel, 2006) showed addition at 0 to 30 mg/l did not changed VFA production, at level 300 mg/l some essential oil reduced or increased slightly and addition at 3000 mg/l almost all essential oil changed VFA production and VFA composition depend on essential oil source. Addition spices as source of essential oil at low level reported by (Chaudhry et al. 2012) cinnamon have no effect on VFA, clove and coriander lower VFA, whereas cumin and turmeric increased VFA and all treatment reduced acetate: propionate ratio except cumin and turmeric.

Eucalyptus and rosemary with main component 1,8-cineole the same main component with essential oil from java cardamom exhibit different effect. In the same level eucalyptus have effect VFA while rosemary reduced VFA (Cobellis, Massimo, Marcotullioc, & Yu, 2016). Essential oils may also alter the VFA profile, even when essential oils are added at doses below their capacity to depress VFA production (Spanghero et al., 2008). Observed effects of essential oils on VFA molar proportions are reduced acetate and increased butyrate proportions (Castillejos et al., 2006),

increased acetate proportion (Castillejos et al., 2005, 2007; Spanghero et al., 2008) or increased propionate proportion (Busquet et al., 2005; Cardozo et al., 2005).

Table 2 Effect of java cardamom addition as source of essential oil on parameters of ruminal in vitro fermentation

Parameters	Level of Essential Oil (mg/l)				
	0	25	50	75	100
Total VFA (mmol/100 ml)	18.28	17.19	18.80	18.41	19.77
Asetat	13.77	12.79	13.69	13.78	13.97
Propional	2.64	2.84	3.22	2.83	3.80
Butirat	1.87	1.55	1.89	1.80	2.00
Acetate:Propionate	5.22	4.57	4.52	5.00	3.97
Protozoa (x 104)	9.42	12.15	10.67	9.61	10.38
Microbial protein (mg/100 ml)	241.73	247.84	278.95	299.16	255.29
NH3 concentration (mg/100ml)**	25.79[a]	26.60[a]	25.77[a]	30.11[b]	26.13[a]
Methane/DM Digested (ml/g)	42.15	50.06	44.52	43.89	42.74
pH	6.78	6.77	6.78	6.79	6.78

[a,b,c,d] different superscript in the same row significantly different; * (P<0.05); ** (P<0.01)

Effects essential oils on rumen microbe protozoal and bacteria population vary. Data on Table showed that addition of java cardamom did not affect protozoa number and microbial protein synthesis. Some studies report different effect of herb and extract and essential oil on protozoal numbers. Anise extract reduced protozoa number while capsicum and blend of cinnamon and eugenol did not affect (Cardozo et al. 2006). (Patra & Yu, 2012) reported that effect of essential oil on protozoa number depend on source and doses. Eucalyptus and clove oil have stimulatory effect at level 250 mg/l but at 500 and 1000 mg/l inhibit protozoa, garlic, oregano and peppermint inhibit protozoa at level 250 up to 1000 mg/l. Individual essential oil have varies effect while combination of those essential oil reduce protozoa number (Cobellis et al. 2016). Similar tendency as in protozoa number effect of essential oil on microbial synthesis in consistent. Some research shows stimulation (Fraser et al., 2007), have no effect (Castillejos et al. 2005; Cobellis et al. 2016) and decreased (Metha Wanapat et al., 2012). Even essential oils were reported have ability to inhibit rumen microbes (Chao et al. 2012; Cosentino et al. 1999; Deans and Ritchie 1987; Sivropoulou et al. 1996) but the activities depend on doses and functional group of compounds. Kalemba dan Kunicka (2003) explain antimicrobial activity of functional group from the strongest are phenol follow by cinamic aldehyde, alcohol, aldehid, ketone, eter and hydrocarbon respectively. Phenol is component of essential which frequently found in essential oil and broadest spectrum activity (Kalemba et al, 2012). In general, the mechanism of action is considered to be the disturbance of the cytoplasmic membrane, disrupting the proton motive force, electron flow, active transport, and coagulation of cell contents (Kotzekidou et al. 2008).

As Shown in Table 2 ammonia in treatment level 75 mg/l is found to be different ($p<0.01$) from other treatments. The higher ammonia in this treatment might be caused by the higher or protein digestibi lity. Mostly in several research, addition of herb and essent ial oil reduced ammonia concentration (Chaudhry et al. 2012; Cobellis et al. 2016; Fraser et al. 2007; Wanapat et al. 2013) due to reduce the peptidolytic activity of ruminal bacteria (Busquet et al., 2005), and inhibit growth of hyper ammonia producing bacteria, a ruminal bacteria involved in ammonia production by the active compound of herb and essential oil (McIntosh et al., 2003; Newbold et al., 2004; Wallace, 2004). Otherwise it seems that java cardamom does not have effect on ammonia producing bacteria, but only on peptidolitic.

As shown in Table 2 Methane production were not affected by the treatment. The existence of protozoa which were not influenced by treatment might be one of the possible reasons. Most of methanogens archaea in rumen are associated with protozoa by endosymbiosis (Belanche et al. 2014). Defaunation (elimination of protozoa number) decrease methane production (Morgavi et al. 2010).

pH of medium in vitro with addition Java cardamom range from 6.77 to 6.79 (TABLE 2). Those range is in the physiology pH for activity rumen microbes that support for feed metabolism in rumen. The physiological pH range is between 5.5 and 6.9, and it is one of the most variable factors in the rumen environment (Puniya et al. 2015).

CONCLUSION

Java cardamom is potential as a rumen modifier feed additive. Utilization of java cardamom in in-vitro rumen fermentation increase feed efficiency by increasing organic matter and crude fiber digestibility and reduce ruminal feed protein digestion.

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REFERENCES

Abreu, A., J. E. Carulla, C. E. Lascano, T. E. Díaz, M. Kreuzer & H. D. Hess. 200). Effects of Sapindus saponaria fruits on ruminal fermentation and duodenal nitrogen flow of sheep fed

a tropical grass diet with and without legume. Journal of Animal Science, 82: 1392–1400. https://doi.org/10.2527/2004.8251392x

Administration Departement of Health And Human Services Subchapter B Food for Human Consumption, revised April 1 2017. Retrieved from ttp://www.accessdata.fda.gov/scripts/cdrh/cfdocs/cfcfr/CFRSearch.cfm?CFRPart=50&showFR=1&subp artNode=21:1.0.1.1.20.2

Araujo, R. C., A. V. Pires, G. B. Mourão, A. L. Abdalla, & S. M. A. Sallam. 2011. Use of blanks to determine in vitro net gas and methane production when using rumen fermentation modifiers. Animal Feed Science and Technology, 166–167: 155–162. https://doi.org/10.1016/j.anifeedsci.2011.04.009

Bakkali, F., S. Averbeck, D. Averbeck & M. Idaomar. 200). Biological effects of essential oils - A review. Food and Chemical Toxicology, 46: 446–475. https://doi.org/10.1016/j.fct.2007.09.106

Belanche, A., G. De. Fuente & C. J. Newbold. 2014. Study of methanogen communities associated with different rumen protozoal populations. FEMS Microbiology Ecology. 90: 663-677. https://doi.org/10.1111/1574-6941.12423

Benchaar, C., & H. Greathead. 2011. Essential oils and opportunities to mitigate enteric methane emissions from ruminants. Animal Feed Science and Technology 166–167: 338–355. https://doi.org/10.1016/j.anifeedsci.2011.04.024

Bodas, R., N. Prieto, R. García-González, S. Andrés, , F. J. Giráldez & S. López. 2012. Manipulation of rumen fermentation and methane production with plant secondary metabolites. Animal Feed Science and Technology. 176: 78–93. https://doi.org/10.1016/j.anifeedsci.2012.07.010

Busquet, M., S. Calsamiglia, A. Ferret & C. Kamel. 2006. Plant Extracts Affect In Vitro Rumen Microbial Fermentation. Journal of Dairy Science. 89: 761–771. https://doi.org/10.3168/jds.S0022-0302(06)72137-3

Calsamiglia, S., M. Busquet, P. W. Cardozo, L. Castillejos, & A. Ferret. 2007. Invited Review: Essential Oils as Modifiers of Rumen Microbial Fermentation. Journal of Dairy Science, 90: 2580–2595. https://doi.org/10.3168/jds.2006-644

Cardozo, P. W., S. Calsamiglia, A. Ferret & C. Kamel. 2006. Effects of alfalfa extract, anise, capsicum, and a mixture of cinnamaldehyde and eugenol on ruminal fermentation and protein degradation in beef heifers fed a high-concentrate diet. Journal of Animal Science. 84: 2801–2808. https://doi.org/10.2527/jas.2005-593

Castillejos, L., S. Calsamiglia & A. Ferret. 2006. Effect of Essential Oil Active Compounds on Rumen Microbial Fermentation and Nutrient Flow in In Vitro Systems. Journal of Dairy Science. 89: 2649–2658. https://doi.org/10.3168/jds.S0022-0302(06)72341-4

Castillejos, L., S. Calsamiglia, A. Ferret & R. Losa. 2005. Effects of a specific blend of essential oil compounds and the type of diet on rumen microbial fermentation and nutrient flow from a continuous culture system. Animal Feed Science and Technology. 119: 29–41. https://doi.org/10.1016/j.anifeedsci.2004.12.008

Chao, S. C., D. G. Young & C. J. Oberg. 2012. Screening for Inhibitory Activity of Essential Oils on Selected Bacteria , Fungi and Viruses Screening for Inhibitory Activity of Essential Oils on Selected Bacteria , Fungi and Viruses. Journal of Essential Oil Research 12: 37–41.

Chaudhry, A. S., M. Mehedi & H. Khan. 2012. Impacts of different spices on in vitro rumen dry matter disappearance , fermentation and methane of wheat or ryegrass hay based substrates. Livestock Science, 146: 84–90. https://doi.org/10.1016/j.livsci.2012.01.007

Chempakam, B., & S. Sindhu. 2008. Large cardamon in: Chemistry of Spices. V. A. Parthasarathy, B. Chempakam, & T. Zachariah, Eds. Cab International, London, pp: 59-69.

Chiba, L. I. 2014. Rumen microbiology and fermentation. in : Animal Nutrition Handbook. L. I. Chiba (Ed.), pp: 57–81.

Cobellis, G., M. Trabalza-Marinucci, & Z. Yu, Z. 2016. Critical evaluation of essential oils as rumen modifiers in ruminant nutrition: A review. Science of the Total Environment. 545-546: 556-568https://doi.org/10.1016/j.scitotenv.2015.12.103

Cobellis, G., T. Massimo, M. C. Marcotullioc, & Z. Yu. 2016. Evaluation of different essential oils in modulating methane and ammonia production, rumen fermentation, and rumen bacteria in vitro. Animal Feed Science and Technology. 215: 25-36. https://doi.org/10.1016/j.anifeedsci.2016.02.008

Compiani, R., C. A. Sgoifo Rossi, A. Pizzi, & V. Dell'Orto. 2013. Administration of essential oils cinnamaldehyde, eugenol, and capsicum to beef cattle: Effects on health status and growth performance. in : Trends in Veterinary Sciences: Current Aspects in Veterinary Morphophysiology, Biochemistry, Animal Production, Food Hygiene and Clinical Sciences. pp: 117-180. https://doi.org/10.1007/978-3-642-36488-4_32

Cosentino, S., C. Tuberoso, B. Pisano, M. Satta, V. Mascia, E. Arzedi, & F. Palmas. 1999. In vitro antimicrobial activity and chemical composition of Sardinian Thymus essential oils. Letters in Applied Microbiology, 29: 130–135.

Deans, S.G. and G. Ritchie. 1987. Antibacterial properties of plant essential oils. International Journal of Food Microbiology: 165–180.

Eckard, R. J., C. Grainger, & C. A. M. de Klein .2010.. Options for the abatement of methane and nitrous oxide from ruminant production: A review. Livestock Science. 130: 47–56. https://doi.org/10.1016/j.livsci.2010.02.010

Extracts on Microbial Community Structure in a Rumen-Simulating Continuous-Culture System as Revealed by Molecular Profiling. Folia Microbiol., 49: 151–155.

Ferme, D., M. Banjac, S. Calsamaglia, M. Busquet, C. Kamel, & G. Augustin. 2004. The Effects of Plants

Fraser, G. R., A. V. Chaves, Y. Wang, T. A. Mcallister, K. A. Beauchemin, & C. Benchaar. 2007. Assessment of the Effects of Cinnamon Leaf Oil on Rumen Microbial Fermentation Using Two Continuous Culture Systems 1. Journal of Dairy Science, 90: 2315–2328. https://doi.org/10.3168/jds.2006-688

Geraci, J. I., A. D. Garciarena, G. A. Gagliostro, K. A. Beauchemin & D. Colombatto. 2012. Plant extracts containing cinnamaldehyde, eugenol and capsicum oleoresin added to feedlot cattle diets: Ruminal environment, short term intake pattern and animal performance. Animal Feed Science and Technology, 176: 123–130. https://doi.org/10.1016/j.anifeedsci.2012.07.015

Jouany, J.-P., & D. P. Morgavi. 2007. Use of "natural" products as alternatives to antibiotic feed additives in ruminant production. Animal, 1: 1443–1466. https://doi.org/10.1017/S1751731107000742

Khorrami, B., A. R. Vakili, M. D. Mesgaran, & F. Klevenhusen. 2015. Thyme and cinnamon essential oils: Potential alternatives for monensin as a rumen modifier in beef production systems. Animal Feed Science and Technology, 200: 8–16. https://doi.org/10.1016/j.anifeedsci.2014.11.009

Kotzekidou, P., P. Giannakidis, & A. Boulamatsis. 2008. Antimicrobial activity of some plant extracts and essential oils against foodborne pathogens in vitro and on the fate of inoculated

pathogens in chocolate. LWT - Food Science and Technology, 41: 119–127. https://doi.org/10.1016/j.lwt.2007.01.016

Kumar, S., A. K. Puniya, M. Puniya, S. S. Dagar, S. K. Sirohi, K. Singh, & G. W. Griffith. 2009. Factors affecting rumen methanogens and methane mitigation strategies. World Journal of Microbiology and Biotechnology, 25: 1557–1566. https://doi.org/10.1007/s11274-009-0041-3

Macheboeuf, D., D. P. Morgavi, , Y. Papon, , J. L. Mousset, & M. Arturo-Schaan. 2008. Dose-response effects of essential oils on in vitro fermentation activity of the rumen microbial population. Animal Feed Science and Technology, 145: 335–350. https://doi.org/10.1016/j.anifeedsci.2007.05.044

Mahmud. L. E. 1994. Antifungial action and antiflatoxenic properties of some essential oil constituents.pdf. Letter in Applied Microbiologi. 19: 110–113.

Moran, J. 2005. How the rumen works. in :Tropical Dairy Farming: Feeding Management for Small Holder Dairy Farmers in the Humid Tropics. Landlinks Press. pp: 41–49.

Morgavi, D. P., E. Forano, C. Martin, & C. J. Newbold. 2010.. Microbial ecosystem and methanogenesis in ruminants. Animal, 4: 1024–1036. https://doi.org/10.1017/S1751731110000546

Nagaraja, T. G. 2016. Microbiology of the Rumen. In Rumenology. D. Millen, Millen, D. D., M. De Beni Arrig, & R. D. Lauritano Pacheco (Eds.). Switzerlan: Springer International Publishing. pp: 39–61 https://doi.org/10.1007/978-3-319-30533-2

Newbold, C. J., F. M. Mcintosh, P. Williams, R. Losa, & R. J. Wallace. 2004. Effects of a specific blend of essential oil compounds on rumen fermentation. Animal Feed Science and Technology. 114: 105–112. https://doi.org/10.1016/j.anifeedsci.2003.12.006

Patra A. K. 2011. Effects ofessential oils on rumen fermentationT microbial ecology and ruminant production.pdf. Asian Journal of Animal and Veterinary Advanceds. 6: 416–428.

Patra, A. K., & Z. Yu. 2012. Effects of essential oils on methane production and fermentation by, and abundance and diversity of, rumen microbial populations. Applied and Environmental Microbiology, 78: 4271–4280. https://doi.org/10.1128/AEM.00309-12

Patra, A. K., & J. Saxena. 2009. Dietary phytochemicals as rumen modifiers: A review of the effects on microbial populations. Antonie van Leeuwenhoek, International Journal of General and Molecular Microbiology, 96: 363–375. https://doi.org/10.1007/s10482-009-9364-1

Puniya (Eds.), pp. 3–16. Springer India. https://doi.org/10.1007/978-81-322-2401-3

Puniya, A. K., R. Singh, & D. N. Kamra. 2015. Rumen microbiology: An overview. in: Rumen Microbiology: From Evolution to Revolution. P. K. Choudhury, A. Z. M. Salem, R. Jena, S. Kumar, R. Singh, A. K.

Russell, J. B., & H. J. Strobel. 1989. Minireview. Effect of lonophores Ruminal Fermentation, applied and environmental microbiology. 55: 1–6.

Salles, M. S. V., M. A. Zanetti, E. A. L. Titto, & R. M. C. Conti. 2008. Effect of monensin on performance in growing ruminants reared under different environmental temperatures. Animal Feed Science and Technology, 147: 279–291. https://doi.org/10.1016/j.anifeedsci.2008.01.008

Sardar, B. R., K. M. Tarade, & R. S. Singhal. 2013. Stability of active components of cardamom oleoresin in co-crystallized sugar cube during storage. Journal of Food Engineering, 117: 530–537. https://doi.org/10.1016/j.jfoodeng.2013.03.035

Sivropoulou, A., E. Papanikolaou, C. Nikolaou, S. Kokkini, T. Lanaras, & M. Arsenakis. 1996. Antimicrobial and Cytotoxic Activities of Origanum Essential Oils, J.Agric.Food Chem. 44: 1202–1205.

Spanghero, M., C. Zanfi, E. Fabbro, N. Scicutella, & C. Camellini. 2008. Effects of a blend of essential oils on some end products of in vitro rumen fermentation . Animal Feed Science and Technology 145: 364–374. https://doi.org/10.1016/j.anifeedsci.2007.05.048

Theodorou, M. K., B. A. Williams, M. S. Dhanoa, A. B. McAllan, & J. France. 1994. A simple gas production method using a pressure transducer to determine the fermentation kinetics of ruminant feeds. Animal Feed Science and Technology. 48: 185–197. https://doi.org/10.1016/0377-8401(94)90171-6

U.S. Food & Drug Administration. 2017. Substances Generally Recognized as Safe. In: Food and Drug

Wallace, R. J., N. R. McEwan, F. M. McIntosh, B. Teferedegne, & Newbold, C. J. 2002. Natural manipulators for rumen fermentation. Asian-Aust. J. Anim. Sci, 15: 1458–1468.

Wanapat, M., P. Kongmun, O. Poungchompu, A. Cherdthong, P. Khejornsart, R. Pilajun, & S. Kaenpakdee. 201). Effects of plants containing secondary compounds and plant oils on rumen fermentation and ecology. Tropical Animal Health and Production, 44: 399–405. https://doi.org/10.1007/s11250-011-9949-3

Wanapat, M., S. Kang, P. Khejornsart, & S. Wanapat. 2013. Effects of plant herb combination supplementation on rumen fermentation and nutrient digestibility in beef cattle. Asian-Australasian Journal of Animal Sciences. 26: 1127–1136. https://doi.org/10.5713/ajas.2013.13013

Wang, S. P., & W. J. Wang. 2016. Effects of dietary supplementation of Chinese herb medicine mixture on rumen fermentation , nutrient digestion and blood profile in goats. South African Journal of Animal Science, 46: 247–260.

Wang, Y., & T. A. McAllister. 2002. Rumen microbes, enzymes and feed digestion-A review. Asian- Australasian Journal of Animal Sciences, 15: 1659–1676. https://doi.org/10.5713/ajas.2002.1659