Research Article

Effect of Galangal (*Alpinia galanga*) Essential Oil Supplementation on Milk Production, Composition, and Characteristics of Fatty Acids in Dairy Cows

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Abstract | Galangal essential oil (EO) has a promising impact as a rumen modifier because it contains 24% of cineole and known to have antimicrobial activity on Gram positive and Gram negative bacteria. Improving the quality of milk composition can be achieved by modification of microbial abundance in the rumen. The study was conducted to measure milk production and composition of Holstein Friesian cow ration, which was supplemented with galangal (EO). The experiment was designed a completely randomized design, 60% of mott elephant grass and 40% of concentrate (dry matter basis) were supplemented by galangal EO as much as 0, 1.25 and 2.50 g/head/day with four replications. The data on milk production were collected every day of milking. Milk composition (lactose, protein, and fat) were analyzed each week for 30 days, and milk fatty acids were analyzed at the end of the research. The results showed no significant difference in milk production, milk composition among rations. Furthermore, milk urea nitrogen (MUN) and blood urea nitrogen (BUN) concentration was also absorbed and galangal EO did not effect on both treatments. Galangal EO at dose of 1.25 g/head/day decreased significantly on blood of volatile fatty acids. The concentration of unsaturated fatty acids (ɣ-linolenic acids) ratio 1.25 was higher than 0 and 250 g/head/day. In conclusion, Galangal EO supplementation increased the polyunsaturated fatty acids ratio at level 1.25 g/head/day of galangal EO, but did not affect milk production, lactose, protein, and fat content.

Keywords | Galangal EO, Cineole, Milk composition, Linolenic acids, Fatty acids

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INTRODUCTION

The biohydrogenation process in the rumen causes polyunsaturated fatty acids (PUFA) to turn into saturated fatty acids (SFA) as much as 80-90% (Bauman and Lock, 2006). Because of this result, ruminant products are always high in saturated fat. Efforts to optimize the quality of milk fatty acids have been made by manipulating the byohydrogenation to protect unsaturated fatty acids in the rumen (Lanier and Corl, 2015). Strategies to reduce biohydrogenation in the rumen have been widely reported, one of which is using secondary metabolites such as phenolic compounds (Gadeyne et al., 2017). According to the research of Jayanegara et al. (2012), Tannins, which are also phenolic compounds, can reduce biohydrogenation in the rumen because they can inhibit the activity of lipolytic bacteria. Phenolic is also found in many essential oil compounds and has function as antibacterial agents.

Essential oils consist of various bioactive compounds, and more than 200 types have been identified (Young, 2019). The mechanism of action of essential oils depends on the components of the main compound, but it is possible that minor compounds also have an influence (Faleiro, 2011).The mechanism of action of essential oils varies greatly depending on the chemical composition (both the components present and their proportions), the structure of the chemical components, and the interactions between the components and are very different when compared to pure compounds (Macheboeuf et al., 2008; Cobellis et al., 2016; Castañeda-Correa et al., 2019). The complexity of the rumen microbial ecosystem and EO composition lead to mixed results on rumen fermentation parameters (Schären et al., 2017; Lee et al., 2020). Furthermore, the mechanism of action of EO as antimicrobial can replace antibiotics as growth promoters of livestock. However, the type of essential oil and the species of livestock used will also have a different effect on production performance (Torres et al., 2020; Irawan et al., 2021; Torres et al., 2021). The selection of EO as a feed additive needs to be considered for its type and availability.

Indonesia, a country with a high biodiversity of aromatic plants, is a home for hundreds EOs-producing plant that can be used as sources of EO to improve the milk quality of dairy cows. Indonesia is among the top three countries with highest galangal production is easily found in various regions (Ministry of Trade, 2011). Galangal is known to have bioactive compounds that can be used as antibacterial in food storage (Verma et al., 2011). Determination of novel in the animal feeding requirement balancing of production animals is key to development of animal industry in future trends (Adli, 2021; Sjofjan et al., 2021a, b). One of EO that can be used as antibiotic growth promoters (AGPs) replacement were Galangal. Galangal EO consist of cineole, phenol, alkaloids (Rangan, 2013). According to Tang et al. (2018) hydroxyl groups (-OH), ketone groups (=O) and ether groups (-O-) in galangal essential oil compounds are thought to play a role in interactions with amino acids on the active side of bacteria. This study aimed to evaluate the dose of galangal essential oil on production, milk composition, milk fatty acids, BUN and MUN, and blood VFA levels in dairy cows.

MATERIALS AND METHODS

Galangal EO production

The galangal rhizome harvested from a local farmer in Boyolali district, Central Java. Plant taxonomy has been identified in Purwodadi Botanical Gardens, National Research and Innovation Agency (BRIN), Indonesia, with letter number No. B-324/IPH.6/KS.02/IX/2020. The identification results stated that the type of rhizome used was white galangal (*Alpinia galangal*). The rhizome has been sliced thin as much as 1000 Kg and dried at room temperature for three days until the water content decreases by 65%. 150 kg of the dried galangal put into a steam distillation device equipped with a condenser, then heated. The water flowed into the condenser and kept flowing. Condenser temperature kept cool so that all the evaporated oil is condensed and does not escape into the air. The water and oil components separated via Clevenger-type apparatus. The distillation process lasts for 5 hours (Raina and Abraham, 2017). The essential oils was yielded as much as 500 ml from 150 kg of dried of galangal rhizome.

Bioactive compound of galangal EO

The essential oil obtained was analyzed using gas chromatography-mass spectrometry (GC-MS). Quantitative data obtained from the % area of the essential oil component spectra to the total area of the essential oil components (Rana et al., 2010). The cineol content in galangal essential oil used to determine the dosage of adding galangal essential oil to dairy cattle feed *in vitro*. The reported percentage (Table 1) is a percentage by weight in the extracted.

Table 1: Bioactive compound of galangal oil by GC-MS.

Bioactive compounds	%
1,8-Sineol	24.38[2]
cis-ß-Farnesene	12.19
ß-Pinene	8.48
Phenol, 4-(2-propenyl)-, acetate	6.01
(S)-4-(1-Acetoxyallyl)phenyl acetate	5.66
Eugenol	3.00
Geranyl acetate	2.97
Caryophyllene	2.16
Farnesol, acetate	2.21
ß-Bisabolene	1.79
Cyclohexene	2.36
3-Octen-5-yne	1.21
Terpineol	1.27
(E)-Hexadec-2-enal	0.93
Terpinene	0.83
trans-Carveyl acetate	0.73
Bornyl acetate	0.50

[1]Essential oil represented 9.22% of galangal DM; [2]n=3; SD: 0.52

Animal grouping

This study used Friesian Holstein (FH) mid-lactation dairy cows totalling 12 heads with an average production of 6.97± 0.77 kg/head/day and a bodyweight (BW) of 407±11.83 kg. Dairy cows were divided into three groups of treatments, namely 0, 1.25 and 2.50 g/head/day, which consisted of four replications. Dosis of galangal EO 2.50 g/head/day equal to 24% of pure cineole. All animal procedures approved by Ethics Committee of Faculty of Veterinary Medicine, Universitas Gadjah Mada Number of Letter: 0055/EC-FKH/Eks./2020.

Feeding treatments

Galangal EO is given directly on top of the concentrate according to the feeding time. Adaptation in feeding was carried out at seven days, then continued with feeding treatment using galangal EO for 21 days. The ratio of basal feeding consisted of 60% of mott elephant grass: 40% of the concentrate. The basal ratio was calculated based on NRC (National Research Council, Nutrient requirement for Dairy Cows) (NRC, 2001). Forage was distributed after concentrate, namely two times a day at 07.00 am and 15.00 pm. Concentrate produced from Cooperative Agro Niaga Jabung, (KAN Agro) Malang. Nutrient and fatty acids composition of basal feed are presented on Table 2. Drinking water was provided ad libitum. The amount of feed and the residual for each animal was weighed and recorded daily. The residual of the feed was collected every day.

Table 2: The composition and nutrient content of feed ingredients in treatment.

Nutrient content % (DM basis)	Mott elephant grass	Concentrate
Nutrient compositions		
Dry matter	18.79	91.65
Organic matter	76.21	88.42
Crude protein	13.57	14.38
Ether extract	2.77	2.60
Crude fiber	30.40	29.30
Nitrogen free extract[a]	29.82	42.03
Total digestible nutrient[b]	70.46	70.48
Fatty acids profile (% from total of fat)		
Caproic (C6:0)	Nd	0.25
Caprylic (C8:0)	Nd	3.01
Capric (C10:0)	0.12	3.10
Lauric (C12:0)	0.23	32.38
Trideciclyc (C13:0)	0.10	0.12
Myristic (C14:0)	1.46	13.15
Palmitate (C16:0)	Nd	17.59
Stearate (C18:0)	0.73	0.10
Saturated Fatty Acids (SFA)	2.77	69.93
Myristoleic (C14:1)	0.13	0.10
Palmitoleate (C16:1)	85.32	0.17
Margaric (C17:1)	0.13	0.19
Oleic (C18:1)	5.54	4.21
Linoleic (C18:2)	1.85	23.81
Unsaturated Fatty Acids (UFA)	92.84	30.07

Milk production and compositions

Milk production collected and measured morning and evening during the study simultaneously as the intake of feed consumption data. Furthermore, milk composition and milk fatty acids were analysed at the end of treatments on day 30. Milk composition (fat, lactose and protein) were analysed using Lactoscan MCCW - V1, Milkotronic Ltd, Bulgaria. Milk fatty acid was carried out using gas chromatography with the following procedure: Milk samples were prepared using the AOAC (2005) method, which consisted of two stages: hydrolysis and methylation. FAME analysis was carried out with GC following the procedure by (Jenkins et al., 2008). Milk Urea Nitrogen (MUN) were analysed using a photometer Microlab 300 with a spectrophotometer (Berthelot method).

Blood sample analysis

Collection and analysis of blood samples were collected from each dairy cow at the end of the study. Blood is taken from the jugular vein and stored in a tube (vacuum tube) containing ethylene diamine tetraacetic acid (EDTA) 0.5% to obtain plasma blood and tubes without EDTA for the serum. Blood urea nitrogen (BUN) from serum were analyzed using a photometer Microlab 300 with a spectrophotometer. Blood plasma was used to analyze in VFA by using gas chromatography (Sigma, 1998).

Data analysis

Milk production data were recorded every morning and evening, but milk quality was analyzed every week in the morning and evening during the adaptation and collection period. Milk fatty acid, MUN, BUN, and blood VFA data were analyzed at the end of the study. The data were examined by a completely randomized design (CRD) with One-way analysis of variance (ANOVA). Furthermore, significant differences between the means have been identified with Duncan multiple range tests (DMRT) (Steel et al., 1997). SPSS software (Windows version of SPSS, release 16) was used for running of data.

RESULTS AND DISCUSSION

Effect of galangal EO on milk production and composition

The addition of galangal essential oil did not affect ($P>0.05$) on milk production and milk composition (fat, protein, and lactose, which are presented on Table 3. According to Daning et al. (2020, 2021); the use of essential oils at a dose of 1-2 g/head/day did not affect dairy cows' milk production and quality. However, each type and dose of EO will affect the nutrient metabolism of dairy cows. The addition of 1.74 g/head/day of eucalyptus essential oil in dairy feed equivalent to cineol 91.5% increased milk production as much as 6.69% However, it decreased as much as 12.90% at a dose of eucalyptus oil of 6.96 g/head/day (Shwerab et al., 2014). Compared with the dose of Galangal EO, 2.50 g/head/day, equivalent to 24% cineol, was insufficient compared to eucalyptus essential oil. In Shwerab et al. (2014) study, it was also explained that the increase in milk production was due to increased VFA levels and decreased methane in dairy cows. Furthermore, Kahvand and Malecky (2018) explained that Sage EO as much as 37.5 g/300 mg of feed (DM basis) equivalent to cineol by 24% in rumen fermentation *in vitro* increased VFA levels, digestibility of dry matter and organic matter, and decreased methane.

Table 3: Effect of galangal essential oils on milk production and composition.

Measurements	Dosage of galangal EO (g/head/day)		
	0	1.25	2.50
Milk yield	7.48 ± 0.25	7.51 ± 0.24	7.73± 0.79
Fat			
Kg	0.32 ± 0.02	0.31 ± 0.02	0.32 ± 0.03
%	4.35 ± 0.36	4.17 ± 0.45	4.22± 0.61
Protein			
Kg	0.21 ± 0.01	0.21 ± 0.01	0.21 ± 0.01
%	2.90± 0.09	2.80 ± 0.04	2.81 ± 0.10
Lactose			
Kg	0.32 ± 0.01	0.31 ± 0.01	0.33 ± 0.02
%	4.39 ± 0.14	4.23 ± 0.06	4.29 ± 0.11

To prove whether the main component of cineol has the same role as galangal EO. This study also compared the galangal dose of EO and pure cineol in rumen fermentation *in vitro*. The addition of galangal EO of 60 mg/300 mg of feed (DM basis) and cineol 5 mg/300 mg of feed (DM basis) with the calculation of the two treatments are equivalent to 24% cineol. There are differences in rumen fermentation results. Galangal EO decreased VFA, methane, and dry matter (DM) organic matter (OM) digestibility, while cineol increased VFA, DM and OM digestibility but also reduced methane levels (Supplementary Table 2). Furthermore, it was also explained that in a study Colombini et al. (2021) which compared cineol with the essential oil of *Achillea moschata*, there were also differences in the yield pattern of *in vitro* rumen fermentation. The difference in fermentation results between the dose of cineol and galangal EO could be possible that the mechanism of action in the rumen by galangal EO not only depends on the main compound but also minor compounds have a role. According to (Young, 2019) the mechanism of action of essential oils as antimicrobials is synergistic in both major and minor compounds.

The addition of galangal essential oil up to a dose of 2.5 g. head per day did not affect the production and composition of dairy cows. It could be possible that the bioactive components that work in modifying the fermentation product in the rumen are not sufficient if it depends on the type of cineol. Another possibility is that cineol, a type of monoterpene, is degraded in the rumen. According to Malecky and Broudiscou (2009), monoterpene compounds in lower dosage of EO were degraded after 3-6 hours of *in vitro* rumen fermentation. In Cobellis et al. (2015, 2016); study, essential oil from rosemary at a dose of 7 g/head/day equivalent to 24% cineol content did not affect the total abundance of rumen bacteria. Several studies Cardozo et al. (2018a, b) show that rumen microbes can adapt to essential oils, especially at low levels. One of the mechanisms is the reduction of the active components of essential oils into inert alcohol by some microbes (Chizzola, 2004) In *in vivo* nutrient digestibility data, there is no difference between control and galangal essential oil (Supplementary Table 4). The nutrient digestibility *in vivo* experiments also proves that the dose of galangal EO as much as 2.50 g/head/day has not been optimal for modifying the rumen microbes of dairy cows. Milk production and composition are closely related to the nutrient composition used in dairy cow feed (Drackley et al., 2006). Recently, several trials evaluated phytochemicals as feed additives in ruminant nutrition and reported improvements in feed utilization and animal performance (Stevanović et al., 2018; Jimenez, 2018; Kholif and Olafadehan, 2021). Results varied between no effect, positive affect, or negative effect on rumen fermentation, feed utilization and performance, revealing the importance of optimizing phytochemical dosages and types (Cobellis et al., 2016).

Effect of galangal EO on milk fatty acids

The addition of galangal essential oil at a dose of 1.25 g/head/day increased significantly Linoleic (C18:2) as much as 7.98% compared to the control. In comparison, at a dose of 2.50 g/head/day, it decreased by 5.87% compared to an amount of 1.25 g/head/day (Table 4). The decrease in ɣ-Linoleic at high doses of galangal EO was in line with the results of our study on *in vitro* rumen fermentation. The higher the amount of galangal EO, the linoleic content decreased compared to the control. According to Sterk et al. (2011) dairy cows that have C18:3 levels are higher than SFA levels because the proportion of forage is higher than concentrate. This study used basal feed with a ration 60% forage and 40% concentrate. Beside the feed ration, differences in the abundance of bacterial hydrogenase also play a role in the fatty acid composition of milk (Lourenço et al., 2010a). In a Sterk et al. (2011) study, it also has been reported that a diet rich in starch increased trans-10-C18:1. (Tina et al., 2006; Nielsen et al., 2006) explained that feeds high in starch content stimulated the growth of the *Megasphaera elsdenii YJ-4* bacterial strain. Different spices of bacteria can convert C18:2n-6 and C18:3n-3 through different biohydrogenation pathways. *Megasphaera elsdenii YJ-4* can convert C18:2n-6 to trans-10, cis-12-C18:2 and trans-10-C18:1 (Bauman and Griinari, 2001) while the bacterial strain *Butyrivibrio fibrisolvens* converts C18:2n- 6 to cis-9, trans-11-C18:2 and trans-11-C18:1 (Lourenço et al., 2010). Shingfield et al. (2008) suggested that starch content and starch to Neutral Detergent Fibre (NDF) ratio in feed are essential determinants of the trans-C18:1 isomer profile in milk due to their effect on specific populations' relative abundance and activity bacteria in the rumen.

Table 4: Effect of galangal essential oils on milk fatty acids.

Fatty acids (% relative on total of fat)	Dosage of galangal EO (g/head/day)		
	0	1.25	2.50
Caproic (C6:0)	1.15±0.09	0.99±0.16	1.06±0.01
Caprylic (C8:0)	0.91[b]±0.11	0.72[a]±0.11	0.84[b]±0.01
Capric (C10:0)	2.08[b]±0.27	1.50[a]±0.21	1.93[b]±0.10
Laurate (C12:0)	7.56±0.12	7.45±0.21	7.41±0.18
Pentadecyclic (C15:0)	1.71±0.07	1.78±0.09	1.92±0.39
Palmitic (C16:0)	1.35±0.13	1.52±0.10	1.34±0.29
Margaric (C17:0)	33.75±1.52	32.64±1.47	33.89±1.05
Myristoleic (C14:1)	15.03[b]±0.30	14.04[a]±0.60	14.61[ab]±0.60
Linoleic (C18:2)	35.61[a]±0.40	39.32[b]±1.57	37.01[ab]±1.25
Saturated fatty acids (SFA)	49.07±1.62	46.61±1.64	48.42±1.89
Unsaturated fatty acids (UFA)	50.82±1.59	53.36±1.66	51.63±1.62

[ab]Means in the same row without common letter are different at P<0.05

The addition of galangal essential oil did not affect the total concentrations of both SFA and UFA, although the concentrations of myristoleic and linoleic significantly increased compared to control. According to Chilliard et al. (2007), biohydrogenation in the rumen involves complex biochemical reactions, so it cannot be determined which process is mono unsaturated fatty acids (MUFA) or polyunsaturated fatty acids (PUFA) first. Almost all unsaturated fatty acids going into Saturated Fatty Acids (SFA). Similar to our study that several studies using EO to evaluate milk fatty acids' results reported no significant difference with the control (Silva et al., 2020; Kalaitsidis et al., 2021). Milk fatty acids are related on abundance of lypolytic bacteria and their enzyme activities. Our study showed that the abundance of lipolytic microbes increased for the genus *Butirivibrio in vitro* (Supplementary Table 2). Nevertheless, the cineole compounds in galangal EO also can bind to protein enzymes. Therefore, biohydrogenation is still inhibited on the MUFA level (Supplementary Table 3). *Butirivibrio,* known as Gram negative resistant to EO, so that EO did not affect growth of bacteria. Based on the data from the analysis of fatty acids in the basal feed, there were 85% of palmitoleate (MUFA) in forage and 23.81% of linoleic (PUFA) in concentrate. Furthermore, the analysis of fatty acid characteristics *in vitro* rumen fermentation showed that a dose of galangal essential oil of 60 µL/300 mg of feed (DM basis) increased MUFA levels in the rumen. However, with the same dose, linoleic (PUFA) decreased significantly compared to control and dose of galangal EO (Supplementary Table 3). The characteristic pattern of fatty acids occurring in the rumen may also affect the yield of fatty acids in milk.

The dose of galangal EO in dairy cattle feed caused linoleic acid levels to increase by 3.84% at a dose of 1.25 g/head/day and by 3.78% at a dose of 2.50 g/head/day. In a study Güney et al. (2021), rosemary EO at a dose of 0.5 g/head/day equivalent to 32% cineol caused a decrease in conjugated linoleic acid (CLA) levels in goat meat. Cineol compounds may have limited the activity of bacteria involved in the biohydrogenation process. Furthermore, linoleic acid was not significant compared to control at a dose of 0.25 g/head/day but increased at a dose of 0.5 g/head/day. However, in several studies, linolenic acid in lamb was significantly increased by adding high doses of rosemary EO. Researchers explain this situation because the cineol compounds in rosemary EO protect unsaturated fatty acids by inhibiting rumen bacteria involved in biohydrogenation (Nieto et al., 2010). In this study, it can be concluded that galangal EO increased linoleic levels at a dose of 1.25 g/head/day.

Effect of galangal EO on BUN and MUN

The presence of galangal EO did not effect of Milk Urea Nitrogen (MUN) and Blood Urea Nitrogen (BUN). All MUN data are presented on Table 4. In contrast with Hristov et al. (2013) observed lower MUN in cows fed with supplementation of oregano leaf as much as 0.25,0.5, and 0.75 g/head/day compared to cattle fed a diet without supplements. Benchaar (2021) reported that the addition of thyme oil did not affect MUN levels. High MUN levels are often associated with specific causes, including too much rumen degraded protein (RDP), too little energy, imbalanced carbohydrate to protein ratio, and too much rumen un-degraded protein (RUP). Under normal production conditions, most dairy farms should have MUN concentrations range of 10 to 14 mg/dl (Jonker et al., 2002). In dairy cattle, blood urea reflects protein degradation by ruminant tissues and protein catabolism in the rumen by bacteria. Digestion of protein in the rumen releases ammonia, which can be utilized by rumen bacteria or absorbed into the bloodstream. When milk is secreted, urea diffuses in and out of the mammary glands, balancing with blood urea. Due to this process, milk urea nitrogen

(MUN) is proportional to blood urea N, and total urinary N excretion is linearly related to MUN (Hristov et al., 2019).

In vivo experiments reported that nutrient digestibility, milk production, and quality were not significant by the dosage of galangal EO. So that, in the MUN and BUN results, that was also not significant because it also related to nutrient digestibility in rumen especially protein degradation in rumen. Furthermore, BUN concentration is a result of the NH3–N concentration produced in the rumen. BUN concentration showed a similar trend with NH3–N concentration in this study, and did not change in fattening groups with rosemary EO supplementationin (Güney et al., 2021). Several studies using a mixture of essential oils at a dose of 1-2 g/head/day also showed the same results on BUN and MUN values (Blanch et al., 2016; Silva et al., 2020; Benchaar, 2021). In addition, the dose of 1.25-2.50 g/head/day for dairy cows has not been obtained optimally for use as a rumen modifier. Another possibility is that EO compounds need a long adaptation in their use as dairy cattle feed. According to Geiger et al. (2020), the nutrient efficiency of dairy cows has increased by EO if given in a rotational system during long term treatment.

Effect of galangal EO on VFA blood

Volatile Fatty Acids are absorbed through the rumen wall and then transported in the blood to the liver. In the liver, they are converted into other sources of energy (Nagaraja, 2012). In this study, it is necessary to do blood VFA testing to determine the level of VFA absorption in the blood. Based on Storm et al. (2012), VFA production in the rumen is correlated with the concentration of VFA in the blood. Recently, research related to the effect of essential oils on VFA in the blood of ruminants is limited. This discussion which is a related effect of galangal EO on VFA will focus on *in vitro* results. Levels of Volatile fatty Acids (VFA) in the blood decreased significantly at a galangal EO dose of 1.25 g/head/day compared to controls (Table 6). At a galangal dose of EO 2.50 g/head/day, VFA levels increased compared to a galangal dose of EO 1.25 g/head/day, but not significantly different when compared to controls. According to Dijkstra et al. (1993) absorption of VFA levels in the blood of ruminants correlated with VFA levels in the rumen. Based on the results of an *in vitro* evaluation, a dose of galangal EO 60µL/300 mg DM of feed equivalent to 1.25 g/kg DM of feed also caused a decrease in the levels of acetate, propionate, and butyrate (Supplementary Table 2). At a dose of 2.50 g/kg DM of feed, the levels of VFA (acetate, propionate, and butyrate) increased compared to levels of 1.25 g/kg DM of feed but were not significantly different from the control. In the *in vitro* evaluation, the increase in galangal essential oil by 120 µL/300 mg DM of feed also increased the levels of propionate and butyrate compared to lower doses of 30 and 60 µL/300 mg DM of feed.

Table 5: Effect of galangal EO on MUN and BUN.

Urea nitrogen content (mg/dL)	Dosage of galangal EO (g/head/day)		
	0	1.25	2.50
Milk	15.23±2.48	12.72±1.36	14.48±1.72
Blood	12.67±1.24	12.41±0.61	11.42±0.73

Table 6: Effect of galangal EO on blood VFA.

VFA proportion (mM)	Dosage of galangal EO (g/head/day)		
	0	1.25	2.50
Acetate	2.89^{ab}±0.26	2.67^{a}±0.20	3.71^{b}±0.69
Propionate	5.01^{b}±0.05	4.69^{a}±0.04	4.95^{b}±0.21
Butyrate	3.59^{b}±0.04	3.27^{a}±0.14	3.70^{b}±0.17
VFA total	11.49^{ab}±0.23	10.64^{a}±0.21	12.37^{b}±0.98

[ab]Means in the same row without common letter are different at P<0.05

According to Castillejos et al. (2008) essential oils to decrease methane levels and increase propionate requires high doses. However, many researchers do not recommend high doses *in vivo* use in dairy cows with various considerations, namely the high cost of essential oil production and palatability. Several studies on EO have been carried out using *in vitro* methods such as batch culture and continuous culture (Castillejos et al., 2006; Benchaar et al., 2008; Tager and Krause, 2011). Data from *in vitro* studies are often inconclusive and lead to conflicting results due to variations in dosage, the chemical structure of EO compounds, diet, mixtures, and EO providers (Calsamiglia et al., 2007). Often, *in vitro* studies require very high doses (>100 mg/L per day) to obtain an observable response, which is equivalent to feeding >10 g EO/cow per day (assuming a 100-L rumen volume) in life. For most EOs, this is >5 times the recommended food dose of EO products that have been commercialized as feed additives. However, to the best of our knowledge, no studies using such high doses have been performed *in vivo*, making it difficult to extrapolate *in vitro* studies to real-life applications (Tager and Krause, 2011).

CONCLUSIONS AND RECOMMENDATIONS

This study concludes that galangal EO did not affect milk production and composition of Holstein Friesian cow ration. Nevertheless, the dosage of galangal EO 1.25 g/head/day increased linoleic acids in milk fatty acids.

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NOVELTY STATEMENT

This study is the first to evaluate an EO originating from Indonesia, namely Galangal. Galangal is known to have bioactive components such as cineol and phenol. The use of essential oils is expected to be able to increase the function of bioactive compounds in modifying rumen microbes and optimizing the production and quality of dairy cows'.

AUTHOR'S CONTRIBUTION

DRAD, LMY, CH & BPW: Idea and research design. DRAD: *In Vivo* collection and lab analysis. DRAD and CH: Write the manuscript. LMY and BPW: Revision.

Conflict of interest

The authors have declared no conflict of interest.

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Supplementary Table 1: The effect of galangal essential oil and cineol on rumen fermentation product.

Parameters	Dose µL/300 mg Dry Matter of feed				
	0	30	60	120	5 (cineole)
Total gas production (ml)	51.58[a]±0.65	51.16[a]±1.09	41.52[b]±0.86	29.00[c]±0.28	53.83[a]±0.79
DMD (%)	47.54[a]±0.60	39.34[b]±0.56	36.89[b]±0.94	26.59[c]±1.71	46.14[a]±0.33
CH_4 (mL)	5.84[a]±0.44	6.04[a]±0.50	3.53[c]±0.86	2.73[d]±0.28	4.85[b]±0.75
CH_4 per DMD (mL/g)	0.125[a]±0.03	0.128[a]±0.03	0.18[b]±0.05	0.124[a]±0.05	0.117[b]±0.04
pH	6.90 ±0.07	6.93±0.091	7.07±0.25	7.07±0.07	7.00±0.13
NH_3 mg/100 ml	40.11[b]±0.48	39.24[b]±1.15	37.66[c]±1.31	39.01[b]±0.64	46.73[a]±1.18

pH	6.90^{ns} ±0.07	6.93±0.091	7.07±0.25	7.07±0.07	7.00±0.13
Protozoa (x 10^3/ml)	8.94^{a}±0.05	6.07^{b}±0.33	2.62^{c}±0.23	3.60^{d}±0.22	7.08^{e}±0.11
Total VFA mM	88.78^{b}±1.96	89.31^{b}±1.46	85.14^{c}±2.09	78.50^{d}±2.36	101.98^{a}±1.10
Acetate mol/100 mol	66.26^{b}±1.74	61.15^{c}±0.97	62.07^{c}±1.05	57.07^{d}±1.05	69.29^{a}±1.32
Propionate mol/100 mol	23.46^{b}±1.56	22.63^{b}±0.71	30.11^{a}±1.72	29.94^{a}±1.55	23.48^{b}±1.09
Butirate mol/100 mol	10.27^{c}±1.45	16.20^{a}±0.91	7.81^{d}±0.79	12.98^{b}±1.08	7.22^{d}±1.28
Acetate:propionate	2.83^{a}±0.24	2.70^{a}±0.11	1.91^{b}±0.19	2.08^{b}±0.29	2.95^{a}±0.16
Microbial protein (mg/mL)	0.55^{a}±0.07	0.60^{a}±0.06	0.53^{a}±0.05	0.41^{b}±0.06	0.54^{a}±0.06

Supplementary Table 2: Genus-level composition of the rumen samples from dosage of galangal oils and cineole (% total observation).

Phylum	**Genus**	**Dose µL/300 mg Dry Matter of feed**				
		0	**30**	**60**	**120**	**5 (cineole)**
Bacteriodetes		52.03	55.47	43.95	34.66	58.17
	Rikenellaceae_RC9_gut_group	12.39	11.70	12.17	12.19	11.69
	F082-uncultured_rumen_bacterium	4.75	5.21	2.33	1.82	5.93
	Bacteroidales_BS11_gut_group	3.28	3.94	2.44	2.12	3.58
	SP3-e08	2.85	2.70	3.76	3.02	2.76
	Prevotellaceae_UCG-003	0.61	0.36	0.35	0.26	0.86
Proteobacteria		7.85	8.60	12.08	16.59	3.67
	Sutterella	0.77	1.42	2.78	7.81	0.44
	Succinivibrio	0.86	1.79	2.93	1.85	0.63
	Ruminobacter	0.40	0.99	0.86	0.57	0.34
Firmicutes		14.71	15.89	20.73	26.53	15.49
	Ruminococcaceae_UCG-011	0.86	0.98	1.30	2.37	0.83
	Clostridium_sensu_stricto_1	0.003	0.002	0.008	0.006	0.224
	Streptococcus	0.07	0.05	0.10	0.44	0.06
	Lachnospiraceae_XPB1014_group	0.70	0.69	0.89	0.94	0.54
Euryarchaeota	*Methanobrevibacter*	0.03	0.02	0.06	0.06	0.04

Supplementary Table 3: Characteristics of fatty acids from rumen fermentation *in vitro*.

% Fatty acids	**Dosage of galangal essential oils/300 mg dry matter of feed**				**Dosage of cineole**
	0 µL	**30 µL**	**60 µL**	**120 µL**	**5 µL**
Caproic (C6:0)	15.03^{c} ± 1.70	8.02^{b} ± 0.48	3.89^{a} ± 0.73	6.02^{b} ± 0.89	16.86^{c} ± 1.16
Caprilic (C8:0)	2.69^{a} ± 0.75	2.83^{a} ± 0.39	3.08^{a} ± 0.32	3.68^{a} ± 0.32	4.84^{b} ± 0.90
Capric (C10:0)	2.72^{b} ± 0.57	3.15^{b} ± 0.41	2.68^{b} ± 0.30	0.73^{a} ± 0.16	2.66^{b} ± 0.32
Lauric (C12:0)	12.79^{b} ± 2.00	9.71^{a} ± 1.01	10.23^{a} ± 0.40	10.87^{ab} ± 0.18	9.03^{a} ± 0.59
Trydecylic (C13:0)	1.56^{c} ± 0.37	1.19^{bc} ± 0.20	0.68^{a} ± 0.08	1.18^{bc} ± 0.23	1.01^{ab} ± 0.19
Myristic (C14:0)	7.11^{ab} ± 0.45	6.85^{ab} ± 0.48	6.94^{ab} ± 0.43	7.49^{b} ± 0.22	6.38^{a} ± 0.52
Pentadecylic (C15:0)	1.47^{ab} ± 0.15	1.57^{ab} ± 0.17	1.18^{a} ± 0.47	1.91^{b} ± 0.42	1.36^{ab} ± 0.04
Palmitate (C16:0)	25.78^{a} ± 1.15	31.63^{c} ± 1.27	31.81^{c} ± 0.65	30.58^{c} ± 1.12	27.78^{b} ± 0.72
Myristoleate (C14:1)	2.77^{c} ± 0.19	2.02^{b} ± 0.05	1.25^{a} ± 0.12	1.00^{a} ± 0.09	2.42^{bc} ± 0.43
Palmitaloate (C16:1)	1.27^{a} ± 0.22	3.03^{b} ± 0.27	4.12^{c} ± 0.76	3.37^{bc} ± 0.81	1.10^{a} ± 0.06
Oleic (C18:1)	14.22^{a} ± 1.05	18.27^{b} ± 1.16	24.99^{c} ± 1.85	24.60^{c} ± 0.63	16.28^{ab} ±2.68
Linoleat (C18:2)	3.09^{b} ± 0.27	3.15^{b} ± 0.15	1.57^{a} ± 0.31	2.01^{a} ± 0.41	3.27^{b} ± 0.38
Docosadienoic (C22:2)	8.46 ± 1.02	8.01 ± 0.08	7.95 ± 0.78	7.49 ± 0.78	6.96 ± 1.09
Saturated fatty acids	70.17^{c} ± 1.13	64.41^{b} ± 1.52	60.10^{a} ± 0.59	61.50^{a} ± 1.51	69.95^{c} ± 0.90
Mono unsaturated fatty acids	18.26^{a} ± 0.39	23.32^{b} ± 0.26	30.36^{c} ± 0.88	28.97^{c} ± 0.87	19.82^{a} ± 0.48
Poly unsaturated fatty acids	11.55^{b} ± 0 .76	11.26^{ab}± 1.64	9.52^{a}± 1.47	9.52^{a} ± 0.80	10.23^{ab} ± 1.38
Unsaturated fatty acids	29.82^{a}±1.15	34.59^{b}±0.71	38.49^{c}±0.59	39.89^{c}±1.51	30.05^{a}±0.90

Supplementary Tabel 4: Effect of galangal essential oil on nutrient digestibility.

Nutrient digestibility (%)	Dosage of galangal essential oils (g/head/day)		
	0	**1.25**	**2.50**
Dry matter	76.62±3.41	77.32±3.77	75.00±1.69
Organic matter	76.82±2.46	74.81±2.79	74.82±2.48
Ether extract	84.00±1.16	84.01±1.76	84.22±1.14
Crude fiber	81.85±3.21	79.87±2.69	78.81±3.03
Crude protein	77.39±2.18	76.85±1.85	77.07±1.46
Nitrogen free extract	74.51±1.97	72.86±1.76	70.44±1.14