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Effects of Four Essential Oils on Nutrients Digestibility of *In Vitro* Ruminal Fermentation

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ABSTRACT

This research was done to study the effect of four essential oils (EOs), as feed additives, on ruminal nutrient digestibility in order to find out candidate of rumen modifier. Those four EOs were destilated from *Foeniculum vulgare* (Mill), *Pinus merkusii* (Jungh. & de Vriese), *Cymbopogon nardus* (L.) Rendle and *Melaleuca leucadendra* (L.). Their effect on dry matter (DM), organic matter (OM), crude protein (CP) and crude fiber (CF) digestibility were studied using *in vitro* rumen fermentation technique according to Theodorou method. Data were statistically analysed using analysis of variance factorial 4x5 design. Feed for fermentation substrate consist of *Pennisetum purpureum*, rice bran and wheat pollard. Essential oil individually was added and mix with substrate to meet the final concentration in fermentation media of 0, 100, 200, 400 and 800 mg/l. *P. merkusii*, *C. nardus* and *M. leucadendra* EOs reduced DM, OM, and CF digestibilities. The decreasing were in line with the increasing of EOs doses. Therefore *F. vulgare* only reduced CF digestibility. The value of DM, OM and CP digestibilities in fermentation with *F. vulgare* did not differ from control. CP digestibility differed among EO treatments. In *F. vulgare*, and *P. merkusii*, treatments, CP digestibility did not differ from control, whereas overall CP digestibility in *C. nardus* tended to be higher than control and CP digestibility in *M. leucadendra* was significantly higher. All EOs addition reduced CF digestibility at all level. CF digestibility in fermentation added 800 mg/l of *M. leucadendra* EO was slumped to only 14.29% of control. Among four EOs *M. leucadendra* is the most potent on interrupt rumen feed fermentation whereas *P. merkusii* and *C. nardus* in moderate ways and *F. vulgare* in delicate manner.

Key words: Doses, Essential oils, Nutrient digestibility, Rumen fermentation

Introduction

Essential oils (EOs) are plants secondary metabolites in the form of complex compounds. They are characterized by volatile, natural, strong odor and are composed of aromatic compounds. They can be obtained by steam or hydrodistillation. In nature, essential oils is important part in plants defending mechanism from predators attack, as antibacterial, antivirals, antifungals, insecticide (Bakkali *et al*., 2008). EOs have demonstrated activity against a wide range of microorganisms, including bacteria, protozoa and, fungi (Deans and Ritchie, 1987; Sivropoulou *et al*., 1996; Chao *et al*., 2012).

Bacteria, fungi and protozoa are microorganisms which lives in rumen habitat. They play a key role of feed digestion and fermentation in the rumen. Since essential oils have antibacterial, antifungal, and insecticide activity, therefore addition of essential oils in rumen may modify the activity of the microorganisms either positively or negatively effect. Several studies indicated that selected essential oils and their component have potency as alternative compounds to substitute antibiotic in animal production. Monensin, an antibiotic, have been used to manipulate rumen fermentation and successfully increase feed efficiency and animal production. Effect of monensin in the rumen fermentation was briefly explained by Russell and Strobel (1989). Several essential oils demonstrated actively in inhibition of ruminal methane emission, increase ruminal propionate proportion (Roy *et al*., 2014) and increase by-pass protein to the intestine (Macheboeuf *et al*., 2008; Cobellis *et al*., 2015).

Anti-microbial activities of essentials oil are various depend on their chemical structure from its plant origin (Calsamiglia *et al*., 2007; Bakkali *et al*., 2008). Different essential oil component has different mechanism in manipulating ruminal fermentation (Calsamiglia *et al*., 2007). The activities are determined by the bioactive compound and the doses (Tajkarimi *et al*., 2010).

Several essential oils from *Foeniculum vulgare* (Mill)*, Pinus merkusii* (Jungh. & de Vriese), *Cymbopogon nardus* (Linn.) Rendle and *Melaleuca leucadendra* (Linn.) are locally available in Yogyakarta, Indonesia, with relatively cheap in price. They are produced by home industries. Twenty eight components are identified in hydrodistilate of *F. vulgare* seeds, major components among them are Trans-anethole and estragole (Diao *et al*., 2014; Pavela *et al*., 2016). In *C. nardus* 28 compounds are identified by β-citronellal, nerol, β-citronellol, elemol and limonene as major component (Kpoviessi *et al*., 2014). The essential oils of *M. leucadendra* are composed of mainly oxygenated sesquiterpenes (81.23% to 93.50%), followed by sesquiterpene hydrocarbons (1.84% to 11.41%), whereas the main constituent is (E)-nerolidol (76.58% to 90.85%) (Padalia *et al*., 2015). Furthermore, main component of essential oil from *P. merkusii* are α-pinene, followed by γ-cymene and β-pinene (Wiyono *et al*., 2006). Indonesia has a lot of essential oils, but nowadays research using local essential oils as feed additive for ruminant have not intensively conducted. This research was aimed to study the effect of four thus essential oil on nutrient digestibility as preliminary study in selection of suitable essential oil to increase feed efficiency and animal production.

Materials and Methods

Diet and treatments

The effect of four distillate of essentials oil were evaluated in an *in vitro* batch fermentation trial with a 60:40 forage: concentrate diet according to Theodorou *et al*., (1994) with modification. Smaller serum bottles were used in this research with same ratio of liquid and headspace volume. The diet consists of *Pennisetum* purpureum (Schumach), which cut before flowering stage, rice bran and wheat pollard, obtained from feed shop, with ratio 60:20:20 based on dry matter. Four distillates of EOs were added to the diet as additives. Treatments were control group (no additive) and other three group with addition of essential oil of *F. vulgare, P. merkusii*, *C. nardus* and *M. leucadendra*. All EOs are commercial product obtained from local shop. Four doses of each additive were used: (100, 200, 400, and 800) mg/l of the fermentation fluid based on final concentration. Incubations were conducted using rumen fluid from ruminal canulated Ongole grade cattle fed diet consisting of *P. purpureum* and beef cattle commercial concentrate 60:40 DM bases TDN 88.57% and CP 9.34%. Rumen fluid was collected before morning feeding and squeezed through polyester cloth into a vacuum flask, and immediately sent to the laboratory. The incubation was conducted in a 125 mL serum bottle containing 70 mL of diluted rumen fluid and 700 mg of the diet per bottle.

The bottles were set into three triplicate bottles, the first, second, and third set bottles were used for dry matter digestibility (DMD) and organic matter digestibility (OMD) determination, crude protein digestibility (CPD), and crude fiber digestibility (CFD) respectifully. EOs were dissolved in ethanol before added into the bottle according to treatments. The control treatment was also supplied with ethanol in the same volume with diluted EOs which added. Each tube was gassed with CO_2 gas before sealing with butyl rubber stopper plus aluminium crimp cap and pre-warmed overnight at 39˚C. In the next morning, 7 ml collected rumen fluid was added into each bottle using 10 ml plastic syringe. Bottles then incubated for 24 h at 39˚C. The gas pressure in bottle head space was zeroing before incubation by inserting 0.6 mm needle attached to a pressure transducer.

Sample collection and chemical analyses

After 24 h, the pH was measured immediately after opening the bottles and then residual feed were collected by filtration using filter paper for further residual nutrients analysis, including DM, OM and CP. Procedure for nutrient analysis according to AOAC (2005). The data of residual nutrients were used to calculate DMD, OMD, CPD and CFD respectively.

Statistical analyses

Results of the calculation of nutrients digestibility were analyzed by analysis of variance using 4x5 Factorial design. Comparisons between means were analyzed by using t-test of Duncan multiple range test.

Result and Discussion

pH medium did not affect by all treatment Table 1, its range from pH 6.80 to pH 7.01, in normal pH for optimum rumen fermentation. Nutrient digestibility significantly affected by essential oils and it doses ($p<0.01$), except crude protein digestibility. Among essential oil, there were variations in type of response as well as in the concentration of product needed to produce this response.

As shown in Table 1, All essential oils affected DMD, but when the effect of each essential oils was analyzed there were varies in response. *F. vulgare* essential oils were not changed the dry matter digestibility at all doses treatment, in contrary *P. merkusii*, *C. nardus* and *M. leucadendra*, reduced dry matter digestibility. The higher dose of essential oil the greater dry matter digestibility reduced. Reduction of DMD were start at dose of 400 mg/l for *P.merkusii* , 200 mg/l for *C. nardus* and 100 mg/l for *M.*

leucadendra with reduction of DMD were 26%, 38.80% and 17.83% respectively . The greatest reduction occurred at addition of 800 mg/l *M. leucadendra* the DMD decline 50.80%. Other previous researches resulted varies result as well. The increasing suplementation level of orange oil as essential oil source resulted in lower DMD (Kamalak *et al*., 2011). The reduction of DMD regarding the reduction of another nutrient including OM, CP and CF. The higher concentration of EO in the diet affect fiber, starch and protein degradability, thus, decrease the ruminal dry matter digestibility (Beauchemin and McGinn, 2006; Yang *et al*., 2007; Yang *et al*., 2010). However, several studies with essential oils monoterpenoids group did not change nutrient digestibility (Malecky *et al*., 2009; Meyer and Erickson, 2009; Santos *et al*., 2010). Inconsistency effect of different essential oils in nutrient digestibility may affected by several factors, for instant; the plant origin; the bioactive compounds; level of oils, which provided various results (Castillejos *et al*., 2006; Castillejos *et al*., 2007).

Table 1 showed essential oils and increasing of their dosages reduced OMD. The effect pattern of essential oils addition in feed and also the doses effect were similar to their effect on DMD. The lowest CPD was happened at addition *M. leucadendra* 800 $mg \cdot L^{-1}$. OMD at this level was 28.17% almost a half of the control. There is considerable evidence that EO affected rumen microbial fermentation and nutrient degradation (Mcintosh *et al*., 2003; Castillejos *et al*., 2006; Kamalak *et al*., 2011; Cobellis *et al*., 2016). Suggested that high supplementation of EO showed anti-microbial activity which inhibited the ruminal feed degradation and fermentation process, thus, decreased organic matter degradability.

Effect of essential oils on CPD was differed among them but the doses did not affect CPD. CPD in fermentation with addition of *F. vulgare* and *P. merkusii* relatively similar with control. Therefore CPD in fermentation treated with *C. nardus* tended to be higher than control, and CPD in *M. leucadendra* was higher than control. This result was in accordance with several researches, addition of CRINA a mixture of essential oils for ruminants was not changed the CPD (Benchaar *et al*., 2007; Hart *et al*., 2008; Klevenhusen *et al*., 2011). Other study showed increasing in CPD by addition of 5 $mg \cdot L^{-1}$ garlic oil and contrary most studies reported that reduction of CPD by essential oil addition which resulted decreased of ammonia concentration (Hart *et al*., 2008; Cobellis *et al*., 2016).

All treatment reduced CFD. The reduction of CFD started at doses 100 $mg \cdot L^{-1}$ in all essential oil and consistent decreased with the increasing of essential oils concentration. At the highest dose, 800 $mg \cdot L^{-1}$, CFD dropped sharply except *F. vulgare*. CFD at dose 800 $mg \cdot L^{-1}$ of *F. vulgare* was 58.98% of control CFD therefore at

Table 1. Effect of essential oils and doses on ruminal nutrients digestibility of diet *in vitro*

Nutrients digestibility	Essential oils doses (mg.L^{-1})	Essential oils doses (mg.L^{-1}) 0	100	200	400	800	Means **
pH	*F. vulgare*	6.80	6.81	6.82	6.81	6.85	6.82
	P. merkusii	6.80	6.86	6.92	6.90	6.92	6.88
	C. nardus	6.80	6.88	6.92	6.96	6.97	6.90
	M. leucadendra	6.80	6.89	6.91	6.89	7.01	6.90
	Means	6.80	6.86	6.89	6.89	6.90	
Dry matter	*F. vulgare*	44.88^{m}	41.44^{m}	42.75^{m}	43.19^{m}	49.34^{n}	44.31^{a}
	*P. merkusii***	44.88^{m}	41.48^{m}	30.73^{n}	33.21^{n}	26.78^{o}	35.41^{b}
	*C. nardus***	44.88^{m}	45.97^{m}	27.47^{n}	28.46^{n}	28.89^{n}	35.13^{b}
	*M. leucadendra***	44.88^{m}	36.88^{n}	29.01^{o}	26.09^{op}	22.08^{p}	31.78^{c}
	Means**	44.88^{x}	41.44^{y}	32.49^{z}	32.74^{z}	31.77^{z}	
Organic matter	*F. vulgare*	48.26	49.67	46.62	48.21	53.07	49.08^{a}
	P. merkusii	48.26	49.63	39.73	40.25	34.16	42.41^{b}
	*C. nardus**	48.26^{m}	53.30^{m}	36.73^{n}	38.43^{n}	37.08^{n}	42.76^{b}
	*M. leucadendra***	48.26^{m}	46.50^{mn}	39.32^{no}	35.88^{o}	28.17	39.61^{b}
	Means**	48.26^{x}	49.78^{x}	40.60^{y}	40.69^{y}	38.02^{y}	
Crude protein	*F. vulgare*	45.54	45.84	45.18	45.03	46.32	45.21^{b}
	P. merkusii	45.54	45.09	46.65	43.05	43.93	44.85^{b}
	C. nardus	45.54	44.67	47.14	48.84	47.01	46.64^{ab}
	M. leucadendra	45.54	49.10	48.58	48.97	50.01	48.44^{a}
	Means^{ns}	45.54	46.18	46.89	46.47	46.35	
Crude fibre	*F. vulgare*	41.42	34.07	32.07	32.61	32.31	30.88^{a}
	*P. merkusii***	41.42^{m}	27.24^{n}	17.29^{o}	13.03^{0}	13.54^{o}	22.50^{b}
	*C. nardus***	41.42^{m}	21.47^{n}	19.08^{n}	13.98^{o}	13.42^{o}	21.87^{b}
	*M. leucadendra***	41.42^{m}	27.59^{n}	22.69^{no}	15.63^{o}	5.92^{p}	22.65^{b}
	Means**	41.42^{x}	25.10^{y}	22.78^{y}	18.81^{z}	14.28^{z}	

* ($P<0.05$), ** ($P<0.01$).
a,b,c different superscript in same column indicate significantly different
x,y,z different superscript in same row indicate significantly different
mnop different superscript in the same row indicate significantly different.

the same level of *P. merkusii*, *C. nardus* and *M. leucadendra* the CFD only 32.69%, 32.40% and 14.29% of control CFD value respectively. This result is different with another previous study. No inhibition effect of essential oil addition in several fibrolytic bacteria (Castillejos *et al*., 2007). This finding is suggesting the reason in several researches which reported CFD was not affected by the essential oils supplementation [33, 10, 22, 30].

Conclusions

Essential oils of *Pinus merkusii*, *Cymbopogon nardus* and *Melaleuca leucadendra* reduces DMD, OMD and CFD except for *F. vulgare* only reduces CFD. All essential oils do not change the CPD. The pattern effects differ among essential oils regarding the doses. Essential oil of *M. leucadendra* is the most potent on interrupt rumen feed fermentation whereas *P. merkusii* and *C. nardus* in moderate ways and *F. vulgare* in delicate manner.

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