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Effect of Increasing Doses of Essential Oil Extracted from Berastagi Orange (*Citrus sinensis* L.) Peels on Performance, Rumen Fermentation and Blood Metabolites in Fattening Bali Cattle

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Abstract: The aim of the current study was to evaluate the increasing doses of OEO extracted from Berastagi orange peels on performance, rumen fermentation and blood metabolites in fattening Bali cattle. Fifteen male Bali beef cattle of similar age 1.5-2 years and initial body weight (194.4±22.31 kg) were used in a Completely Randomized Block Design (RCBD) for 90 d. The doses of OEO supplementation ware applied by oral infusion. Dose of the OEO supplementation treatments were: 0 ppm (basal diet + 0 ppm OEO/kg DMI), 200 ppm (basal diet + 200 ppm OEO/kg DMI), 400 ppm (basal diet + 400 ppm OEO/kg DMI), 800 ppm (basal diet + 800 ppm OEO/kg DMI) and 1200 ppm (basal diet + 1200 ppm OEO/kg DMI). The basal diets were formulated based on protein and TDN content of 13 and 65% with swamp grass: concentrate ratio of 60: 40%. Swamp grass and concentrate were given separately. The animal were fed twice a day, morning and afternoon. Oral infusion of OEO treatments were given after the animal fed a morning of concentrate using disposable syringe. The obtained result showed that the increasing doses of OEO did not influence on final BW, DMI, ADG and FCR. OEO supplementation was significantly ($p<0.05$) decreased on ruminal NH_3-N, molar proportion of acetate and ratio acetate to propionate, whereas total VFA and molar proportion of propionate tended to increase. Supplementation of OEO at the dose 800 ppm and 1200 ppm was tended to decrease of total VFA and molar proportion of propionate. There were no effect of increasing dose of OEO on pH, molar proportion of butyrate, valerate, iso-valerate. Serum concentration of glucose, total triglycerides, albumin were no changed by increasing dose of OEO, but serum total protein tend to increased ($p<0.15$) by OEO supplementation. Moreover, supplementation of OEO decreasing serum concentration of total cholesterol, LDL, urea and BUN and increasing serum concentration of HDL. Our results suggest that supplementation of berastagi orange oil at dose 400 ppm could be considered as suitable feed additives to manipulate rumen microbial fermentation and to improve blood metabolites in fattening Bali cattle.

Key words: Orange essential oil, performance, rumen fermentation, blood metabolites, Bali cattle

INTRODUCTION

In beef cattle production, antibiotics have been used as feed additives at non-therapeutic level to increasing performance, feed efficiency and as fermentation modifiers in the rumen besides reducing diseases probabilities (Wallace, 2004; Benchaar *et al*., 2007; Calsamiglia *et al*., 2007; Soltan, 2009). The use of antibiotics as feed additives for above reason have been successfully. Nevertheless, the World Health Organization (WHO) and the Food and Agruculture Organization (FAO) have drawn attention to the threat of the transfer of antibiotic resistant pathogens to human besides the hazard of their residues in meat and milk on human health (FAO/OIE/WHO, 2004). Therefore, the European Union was prohibited their use (EC, 2003). The prohibition of the use of antibiotics in animal feed has effort to explore in natural bioactive compounds as alternative means to increasing performance, feed efficiency, modifier of ruminal fermentation and metabolic products of the ruminant (Soltan, 2009; Gandra *et al*., 2012; Vakili *et al*., 2013). Last few years, there has been increasing attention in potential of plant extracts such as essential oils (EO) as alternatives for feed antibiotics, growth promoters and manipulate the rumen metabolism in ruminant nutrition (Cowan, 1999; McIntosh *et al*., 2003; Wallace *et al*., 2003; Benchaar *et al*., 2006a; Kamalak *et al*., 2011). However, the effect of essential oils and their derivatives on rumen bacteria and ruminal fermentation are not consistent and are variable (Busquet *et al*., 2006; Castillejos *et al*., 2006; Benchaar *et al*., 2007). The difference among some research were caused by difference in type, doses and difference in technique used in these research (Busquet *et al*., 2006; Fraser *et al*., 2007). Recently several research have been done to studies the effect of orange essential oil (OEO) extracted from some type of citrus peels on their anti microbial activity and rumen microbial fermentation (Nam *et al*., 2006; Kirbaslar *et al*., 2009;

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Kamalak *et al*., 2011). The result of the studies showed OEO have different ability related to a lot of factors such as chemical contains, soil composition, growing region, climate and extraction process (Kirbaslar *et al*., 2009). Berastagi is one of the most important district producer sweet orange named "*jeruk Berastagi*" that important to supply the requirement of orange fruits in Indonesia. It was considered that EO extracted from Berastagi orange peels could be used as alternative feed additives for ruminant. Therefore, the present study was carried out to assess the effect of increasing doses of essential oil extracted from berastagi orange peels on performance, rumen fermentation and blood metabolite in fattening Bali cattle.

MATERIALS AND METHODS

The experiment was conducted in June-September 2014, taken place at the Desa Tangkit as village farm of Animal Science Faculty, University of Jambi, Indonesia. Chemical analyses of the experimental diets were done in Animal Nutrition Laboratory, Faculty of Animal Science, Jambi University. Blood samples were analysis in Health Service Laboratory of Jambi Province.

Animal, diets and management: Fifteen male bali beef cattle of similar age 1.5-2 years and initial body weight (194.4±22.31 kg) were placed in individual cage. The animals and treatments were randomly according to Randomized Complete Block Design (RCBD) with three blocks as replication and five treatments of the increasing dose of OEO supplementation. The dose OEO supplementation ware applied by oral infusion. Dose of the OEO supplementation treatments were as follows: 0 ppm (basal diet + 0 ppm OEO/kg DMI), 200 ppm (basal diet + 200 ppm OEO/kg DMI), 400 ppm (basal diet + 400 ppm OEO/kg DMI), 800 ppm (basal diet + 800 ppm OEO/kg DMI) and 1200 ppm (basal diet + 1200 ppm OEO/kg DMI). The basal diets were formulated based on protein and TDN content of 13% and 65% with swamp grass: concentrate ratio of 60: 40%. Swamp grass (*Hyampeacne amplexicaulis* Rudge Ness) and concentrate were given separately, The swamp grass were given after the animals consume of concentrate. The ingredients and chemical composition of experimental diets are show in Table 1 and 2. Before the experiment started, animals were given basal diets to know daily voluntary dry matter intake in order to maintain oral infusion dose of OEO supplied. The animal were fed twice a day, morning and afternoon. Oral infusion of OEO treatments were given after the animal fed a morning of concentrate using disposable syringe. The cattle were scaled for daily gain (ADG) parameter with interval 30 days.

Preparation of orange essential oils: Berastagi orange peels were collected from by product of the orange juice shops at District of Jambi. Essential oil in orange peels were extracted with hydro distillation method using clavenger type apparatus (Kumar, 2010). The recovered oil was transferred into dark bottle and stored at 4°C in a lab refrigerator.

Ruminal liquor collection and analysis: Ruminal liquor was collected from animals at the end the experiment (3 h after the morning feeding) using stomach tube. The rumen liquor was filtered trough a double layer of cheesecloth into bottle glass (50 ml). Rumen pH was measured immediately after sampling using a pH meter (Mettler Toledo FE20) The ruminal liquor concentration of $N\text{-}NH_3$ was determined using micro-diffusion Conway technique (General Laboratory Procedures, 1969). The concentration of ruminal VFA were measured by gas chromatography Hewlet Packard model 5890 with Flame Ionization Detector (FID), column steel with 0.20 cm and 0.40 cm internal and external diameter. Nitrogen was used as carrier gas with flow rate of 0.5 ml/sec and the burning gas using oxygen and hydrogen with flow rate 5 and 0.5 ml/sec, respectively. Column temperature was set in 125°C, while injector and detector temperature was set in 160 and 200°C.

Blood sample and analysis: Blood sample were withdrawn once before morning feeding (fasting) at a week before final research. Blood sampling was collected by jugular vein puncture using 10 ml plastic syringe and placed in vacutainer. Blood serum was separated by centrifugation at 3000 rpm for 10 minutes and analyzed for concentration of blood serum glucose, total protein, albumin, triglyceride, total cholesterol, HDL and LDL. Blood metabolites were analyzed using Auto analyzer (HumaStar 80®).

Statistical analysis: Statistical analysis was carry out using SPSS program, version 17.0. Differences between treatments in dry matter intake, performance, ruminal fermentation products and blood metabolites were evaluated by univariate ANOVA. Differences among means were tested using Duncan's multiple range tests. Data were presented in means±SEM. Level significance was set at $p<0.05$.

RESULTS AND DISCUSSION

Feed intake and growth performance: There are little information on effect of OEO on DMI and growth performance in fattening cattle. Dry matter intake and growth performance data are presented in Table 3. Initial and final BW averaged 194.40 and 231.53 kg, respectively and did not different among doses of OEO and as result ADG was not effected by doses of OEO treatments. The obtained result showed that the increasing doses of OEO did not influence on final BW, DMI, ADG and FCR. Benchaar *et al*. (2006a) who reported no change in DMI of beef cattle fed a silage based diet supplemented with 2 or 4 g/d of commercial

Table 1: Ingredients composition (%) concentrate mixture of experimental diets

Item	Dry matter (%)
Rice bran	58
Corn grain, ground	25
Soybean cake	6
Coconut cake	9
Mineral mix[1]	1
Salt	1

[1]Mineral mix per kg Contain: Ca 165.000 mg, P 52.000 mg, Na 157.000 mg, Fe 2.500 mg, Cu 2.500 mg, Mg 2.000 mg, I 125 mg, Co 50 mg, Zn 5.000 mg, Se 10 mg

Table 2: Chemical composition of swamp grass, concentrate and basal diets (% DM basis)

Nutrients composition	Swamp grass	Concentrate	Basal diets
DM	22.25	91.48	91.62
CP	12.94	12.47	13.18
CF	27.67	8.81	18.99
EE	1.37	3.64	1.51
NFE	50.13	68.74	59.54
NDF	71.98	50.85	57.94
ADF	34.65	14.78	29.24
Ash	7.89	6.34	7.49
TDN	61.92	70.48	65.34

mixture of EO compound consisting thymol, eugenol, vanillin and limonene. Similar results have been reported by Rofiq (2013) that oral infusion 300 ppm of PO (orange peel oil) did not increase DMI and ADG in dairy goats.

Gandra *et al.* (2012) reported that increasing doses of ricinoleic acid from castor oil (*Ricinus communis* L.) did not effect on DMI, ADG and FCR of nellore steers in feedlots. In the others studies, Hassan and Abdel-Raheem (2013) reported that no significant effect on DMI, final weight, weight gain and feed conversion in growing buffalo calves fed dietary supplementation of caraway and garlic as natural additives. Our results confirm these reports which showed that oral infusion of OEO in bali cattle had no effect on DMI and performance. Some data from the literature show that EO have attractive and palatable properties that influence intake by animal (Wallace, 2004), Benchaar *et al.* (2008). Supplementing beef cattle with 2 and 4 g/d of EO observed an increase in DMI when compared with non-supplemented animal, but also others studies showed that EO can decrease DMI (Cardozo *et al.*, 2006; Fandino *et al.*, 2008). In some of studies, depression of DMI in cattle supplemented with EO might be related to palatability problems (Calsamiglia *et al.*, 2007). Meanwhile, no difference in ADG might be related due to similarity in DMI of the cattle in different treatments. NRC (2001), DMI accounts for 60-90% of the variations in animal performance. Furthermore, it has been reported that DMI can be effected by a number of dietary or management factors, such as body weight, animal growth stage, specific physical and chemical characteristics of diets (i.e., fiber content, particle size, amount and ruminal degradation of protein, etc.), digestion, or rumen fermentation metabolites (Allen, 2000; Yang *et al.*, 2007).

Rumen fermentation characteristics: Rumen ecology parameters for pH, NH_3 and VFAs are shown in Table 4. Rumen pH was similar in all treatments with the range from 7.13 to 7.15. The rumen pH was found in the optimal pH (6.6±0.5) to maintain normal cellulolytic organisms activity (Van Soest, 1994). OEO supplementation significantly decreased on ruminal ammonia nitrogen (NH_3-N) concentration ($p<0.05$). This result is similar with finding of Kamalak (2011) who showed that increasing of OEO supplementation significantly reduced the ammonia concentration. Manh *et al.* (2012) reported that eucalyptus supplementation significantly decreased ruminal NH_3-N and tended to decreases with enhancing levels of eucalyptus. The concentration of NH_3-N from this study was variously from 4.47 to 7.55 mg/dl. This result was lower than those reported by Benchaar *et al.* (2007) (18.53 mg/dl) and Kamalak *et al.* (2011) (19.40 to 47.58 mg/dl). Ammonia-N production can differ between *in vitro* and *in vivo* studies because of the contribution of rumen recycled nitrogen (Vallimont *et al.*, 2004). However, Preston and Leng (1987) reported that the crucial level for ammonia has been variously reported as 5 to 25 mg NH_3-N/dl. Meanwhile, Slyter *et al.* (1979) reported that concentration of 2 to 5 mg NH_3-N/100 ml of rumen fluid is sufficient to allow maximum growth of rumen microbes. Decreasing in NH_3-N production was associated with a reduction number of group of bacteria called hyper-ammonia producing (HAP) bacteria. Collectively, results of studies by Newbold *et al.* (2004) suggest that effects of EOs on ruminal protein metabolism are on amino acid (AA) degradation and these effects are likely due to inhibition of HAP bacteria. Supplementation of OEO also had significant ($p<0.05$) effect on the total VFA, molar proportion of acetate, propionate and no significant ($p>0.05$) effect on molar proportion of butyrate, valerate, iso-butyrate and iso-valerate.

Supplementation of OEO at 400 ppm were higher on total VFA production than all treatments. Increasing dose of OEO supplementation tended to decreases of total VFA production. Meanwhile, supplementation of OEO at 0 ppm (control) had significantly higher acetate proportion than other treatments. Supplementation of OEO tended to increasing proportion of propionate, while at the dose 800 ppm and 1200 ppm tended to decreases. Nam *et al.* (2006) reported that supplementation at dose 0.112 g/kg citrus essential oil (CEO) consistently produce higher VFA than control. Moreover, Busquet *et al.* (2006) noted that effect EOs on ruminal fermentation at 24 h batch culture increased total VFA concentration, while the highest concentration

Table 3: Effect increasing doses of OEO on performance in fattening bali cattle

	Doses of OEO (ppm)						
Item	0	200	400	800	1200	S.E.M	p-value
Initial BW (kg)	193.00	192.33	197.67	197.67	191.33	5.13	0.84
Final BW (kg)	229.67	230.00	235.00	235.67	227.33	5.22	0.75
DMI (kg/d)	7.63	7.25	7.70	7.46	7.54	0.37	0.92
ADG (kg/d)	0.61	0.63	0.62	0.64	0.60	0.04	0.97
FCR	13.43	14.58	14.19	11.63	14.18	1.80	0.78

Table 4: Effect increasing doses of OEO on rumen fermentation characteristics in fattening bali cattle

	Doses of OEO (ppm)						
Item	0	200	400	800	1200	S.E.M	p-value
pH	7.14	7.15	7.13	7.14	7.15	0.01	0.76
NH_3-N (mg/dl)	7.55^{a}	6.08^{b}	5.49^{c}	5.13^{c}	4.47^{d}	0.15	0.00
Total VFA (mM)	100.62^{b}	100.46^{b}	103.89^{a}	97.82^{b}	81.41^{c}	0.89	0.00
Acetate (mM)	62.84^{a}	59.10^{b}	57.08^{b}	58.02^{b}	43.82^{c}	0.62	0.00
Propionate (mM)	28.57^{c}	30.83^{b}	35.56^{a}	29.86^{bc}	26.78^{d}	0.45	0.00
Butyrate (mM)	4.65	4.75	5.36	4.77	4.82	0.18	0.12
Valerate	1.34	1.35	1.34	1.35	1.36	0.01	0.42
Isobutyrate	1.63	2.68	2.84	2.47	2.62	0.11	0.00
Isovalerate	1.59	1.74	1.71	1.35	2.10	0.14	0.10
A: P[1]	2.20^{c}	1.92^{b}	1.60^{a}	1.94^{b}	1.64^{a}	0.03	0.00

Means in the same row with different letters (a, b, c and d) are significantly ($p<0.05$). [1]Acetate:Propionate ratio

Table 5: Effect increasing doses of OEO on serum blood metabolites in fattening bali cattle

	Doses of OEO (ppm)						
Item	0	200	400	800	1200	S.E.M	p-value
Glucose (mg/dl)	40.00	38.67	42.00	41.33	40.00	1.72	0.69
Triglycerides (mg/dl)	10.00	11.00	10.00	10.67	10.67	0.97	0.92
Cholesterol (mg/dl)	137.67^{a}	117.67^{b}	118.33^{b}	113.67^{b}	92.33^{c}	1.77	0.00
HDL (mg/dl)	52.60^{b}	52.07^{b}	51.97^{b}	61.91^{a}	61.63^{a}	0.96	0.00
LDL (mg/dl)	74.23^{a}	67.23^{b}	68.10^{b}	68.63^{b}	61.77^{c}	0.94	0.00
Urea (mg/dl)	38.33^{a}	27.00^{b}	26.67^{b}	20.33^{c}	20.33^{c}	1.42	0.00
BUN (mg/dl)	18^{a}	12.70^{b}	12.57^{b}	9.53^{c}	9.57^{c}	0.30	0.00
Protein total (mg/dl)	4.06	4.08	4.16	4.17	4.61	0.15	0.15
Albumin (mg/dl)	2.37	2.34	2.32	2.32	2.33	0.08	0.98

Means in the same row with different letters (a, b and c) are significantly ($p<0.05$)

in most treatments decreased total VFA concentration. The result under this study was noted that, the C_2:C_3 ratios were significantly different ($p<0.05$). These results were in agreement with Castillejos *et al.* (2006) who stated that Rosemary oil (*Rosmarinus officinale*) had effect on rumen fermentation at 500 mg of EOs/l, leading increasing propionate proportion but reducing acetate proportion. The pattern of VFA observed in this study were related with the ability of OEO inhibition acetate and propionate bacteria. In the rumen, Gram-positive bacteria are generally acetate and butyrate-producing bacteria, while Gram-negative bacteria are generally propionate-producing bacteria (Stewart, 1991). In this study, the supplementation of OEO at 400 and 800 ppm have been effective inhibition *K. pnemoniae* (gram+) than *E. coli* (gram-) bacteria. Burt (2004) suggested that Gram-positive bacteria appear more susceptible to the antibacterial properties of plant EO than Gram-negative bacteria. This may be expected as Gram-negative bacteria have an outer layer surrounding their cell wall that acts as a permeability barrier, limiting the access of hydrophobic compound.

Blood metabolites: There is limited information about the effect of OEO supplementation on serum blood metabolites in fattening cattle. There was no significant effect of OEO supplementation until dose 1200 ppm on serum glucose and triglycerides concentration (Table 5). Similar finding were reported by Devant *et al.* (2007), Chaves *et al.* (2008), Yang *et al.* (2010) and Alsaht *et al.* (2014) that supplementation of EOs at different doses did not effect on serum blood glucose. Vakili *et al.* (2013) reported that supplementation of thymol and cinnamon in the diet of feedlot calves resulted in no change in the value of plasma triglycerides. There are no change in serum triglycerides in this study my be related to lack of DMI alteration by OEO. It has been reported that concentration of some blood metabolites such as triglycerides can be influenced by EO supplementation via changing of feed intake (Yang *et al.*, 2010). In this study, supplementation of OEO had significant effect on total serum cholesterol, HDL and LDL. Total serum cholesterol and LDL were tended to decrease and HDL were increase with enhancing levels of OEO. Improvement of blood lipid profile was also important

in animal studies, because it has been used as indicator for good health of animal products for human consume. Kurowska *et al.* (2000) reported that limonoid and limonene had effect on reduction apolipoprotein B and apolipoprotein A as an important structural protein of LDL and HDL. In this studies, limonene is major constituent (65%) in the OEO content. Other studies showed that OEO had ability as antimicrobial agent mainly more effective to gram-positive bacteria than gram-negative bacteria (Patra and Yu, 2012). Several of the gram-positive bacteria are involved in rumen biohydrogenation of unsaturated fatty acid (Harfoot and Hazelwood, 1988). Benchaar *et al.* (2006b) reported that supplementation of bland EO at higher concentration (2 g/d) were increase in the concentration of conjugated linoleic acid in the milk. Blood urea nitrogen is the end product of the excess ruminal ammonia-N that passed from ruminal wall to liver via portal vein. Serum urea and BUN concentration in this study was significantly ($p<0.05$) decrease in parallel with increasing doses of OEO supplementation. Average value of urea and BUN serum in this research are in normal ranges. Swenson *et al.* (1996) reported that the BUN value considered normal for cattle and ranged from 10 to 30 mg/dl or 21.74 to 65.21 mg/dl of urea.

Increasing urea or BUN in blood in ruminant that may to imply an increase of protein catabolism or protein digestion and had negative effect in energy efficiency, nitrogen utilization or environment. Putrino *et al.* (2006) reported that BUN is used as indicator of protein balance and the limits indicating adequate protein intake are 9-12 mg/dl or 19.56-26.09 mg/dl of urea. OEO Supplementation until 1200 ppm tend to increase serum total protein ($p<0.15$), since no effect was observed on serum albumin. Higher serum total protein with OEO supplementation may be indicated that OEO had positive effect to decrease ruminal proteolysis by inhibiting the conversion of amino acid (AA) to NH_3. Patra and Yu (2012) showed that clove oil and origanum oil in cultures resulted inhibition of deamination of AA. McIntosh *et al.* (2003) reported that bacteria such as *Clostridium sticklandii*, *Peptostreptococcus anaerobius* and *C. aminophilum* are known hyper-ammonia-producing bacteria very sensitive to EOs.

Conclusion: The result of this study show that Berastagi orange oil have a potential as natural feed additive to improve rumen fermentation and blood metabolites without counteractive effect on DMI and performance of fattening Bali cattle. Our results suggest that supplementation of Berastagi orange oil at doses 400 ppm could be considered as suitable feed additives to manipulate rumen microbial fermentation and to improve blood metabolites in fattening bali cattle.

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