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Study of Local Herb Potency as Rumen Modifier: Red Ginger (*Zingiber officinale Var. Rubrum*) Addition Effect on In Vitro Ruminal Nutrien Digestibility

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Abstract. Addition of red ginger in ruminant diet was studied using in vitro gas production technique to evaluate its effect on nutrient digestibility. Red ginger meal was added to meet essential oil level in fermentation medium of 0 mg/l (control), 25, 50, 75 and 100 mg/l. The diet consisted of *Penisetum hybride*, rice bran, and wheat pollard with 60:20:20 ratio DM basis. Feed fermentation was incubated for 24 h at 39°C. At the end of incubation, residual feed was collected for further nutrient analysis to calculate the digestibility of dry matter (DM), organic matter (OM), crude protein (CP) and crude fiber (CF). Data were subjected to variance analysis, followed by DMRT analysis. Addition of red ginger increased the total volume of gas production at $\geq$ 50 mg/L but significantly decreased CP digestibility at 50 mg/l, whereas DM and OM digestibility was not affected. In contrast, CF digestibility of 50 mg/l treatment was significantly higher than that of control. In conclusion, red ginger could modify rumen and supplementing red ginger linear to essential oil 50 to 100 mg/l could improve ruminal nutrient fermentation.

Keywords: essential oil, red ginger, rumen fermentation, in vitro

Abstrak. Penambahan jahe merah di dalam ransum terhadap kecernaan nutrien dipelajari dalam penelitian ini dengan menggunakan teknik in vitro produksi gas. Jahe merah yang ditambahkan setara dengan konsentrasi essential oil di dalam medium in vitro: 0, 25, 50, 75 dan 100 mg/l. Ransum tersusun dari rumput raja, dedak dan pollard dengan perbandingan 60:20:20 berdasar berat kering. Fermentasi pakan dilakukan selama 24 jam pada suhu 39°C. Pada akhir inkubasi, sisa pakan dikoleksi untuk penentuan sisa nutrien untuk menghitung kecernaan bahan kering, bahan organik, protein kasar dan serat kasar. Data hasil penelitian dianalisis dengan analisis variansi pola searah dilanjutkan dengan DMRT. Penambahan jahe merah meningkatkan total volume gas pada level 50mg/l dan di atasnya. Kecernaan bahan organik dan bahan kering tidak dipengaruhi oleh penambahan jahe merah. Namun kecernaan serat kasar mengalami penurunan mulai pada level 50 mg/l. Sebaliknya kecernaan serta kasar meningkat dengan penambahan jahe merah. Dapat disimpulkan bahwa jahe merah dapat digunakan sebagai rumen modifier dan penambahan setara dengan level essential oil 50 mg/l sampai 100 mg/l memberikan efek yang menguntungkan

Kata kunci: essential oil, jahe merah, fermentasi rumen, in vitro

Introduction

Feed digestion in the rumen is the key point of ruminants feed utilization particularly fibrous feed material, and it determines feed efficiency. In this way, feed digestions are conducted by rumen microbes (Wang and McAllister, 2002) namely bacteria, fungi, and protozoa (Nagaraja, 2016). Interference to microbial population and rumen condition may affect feed fermentation proses, positively or negatively.

Efforts to increase feed efficiency include the modification of rumen fermentation to increase of nutrient utilization. Some measures to achieve this goal include reducing feed protein degradation due to an increased amino acid for absorption in small intestine; improving digestion of feed fiber; minimizing the degradation rate of rapidly fermentable

carbohydrate; and shifting methane production to propionate (Jouany and Morgavi, 2007). Utilization of antibiotic, such as monensin, has successfully modified rumen fermentation and increased feed efficiency (Russell and Strobel, 1989). However, antibiotic use has been banned due to residual antibiotic in animal product and increasing bacteria resistance bacteria.

Like antibiotic, essential oils have a wide range of antimicrobial activities against bacteria, fungi and protozoa (Deans, S.G. and Ritchie, 1987; Sivropoulou et al., 1996; Cosentino et al., 1999; Bassolé et al., 2011; Chao et al., 2012). Moreover, essential oils are considered safe for human and animal consumption, as evident from the 'Generally Recognized as Safe' label (U.S. Food & Drug Administration, 2017). Essential oil is a natural product and a plant secondary metabolite which exhibits antibacterial, antifungals, insecticide, and antiviral properties as a defense mechanism against predator (Bakkali et al., 2008). Accordingly, essential oil is a potential antibiotic alternative (Khorrami et al., 2015).

The addition of essential oil has varied effects on rumen fermentation. Meanwhile, garlic oil did not negatively affect nutrient fermentation (Bodas et al., 2012). Kouazounde et al, (2014) reported that essential oil from five plants did not significantly affect dry matter digestibility and ammonia concentration at 100 to 300 mg/l, and some oils could reduce dry matter digestibility at 300 to 400 mg/l. Garlic oil, cinnamaldehyde (the main active component of cinnamon oil), capsaicin (the active component of the hot pepper), eugenol (the active component of the clove bud), and anethol (the active component of anise oil) are reported to improve the fermentation profile in a continuous culture of rumen microorganisms (Calsamiglia et al., 2007).

Essential oil from several plants parts at low level can reduce ammonia concentration and methane production in rumen with no detrimental effect on NDF degradability except eucalyptus (Cobellis et al. 2016). Essential oil of clove, eucalyptus, garlic, oregano and peppermint at the concentration of 0.25, 0.5 and 1 g/l of medium reduced methane production (linear to the concentration level) and the population of protozoa and archaea; however, NDF digestibility also decreased except in garlic oil due to the decreased cellulolytic bacteria. (Calsamiglia et al., 2007; Benchaar and Greathead, 2011). Further studies reported that garlic oil could improve fiber digestibility (Klevenhusen et al., 2011). Reviewing previous studies, Hashemi (2014) concluded that carvacrol prevents protein degradation and stimulation of lipid breakdown.

The varied effects of essential oils depend on the chemical composition. Identical essential oils obtained from different plants of one genus may have opposite effect, stimulatory or inhibitor (Ferme et al., 2004; Patra A. K., 2011). Purity and dose also influenced the activity of essential oil (Macheboeuf et al., 2008).

Indonesia has three kinds of ginger based on color, shape, and size, i.e. the large white ginger or *gajah* (elephant) ginger (Z. officinale var. Roscoe), small white or yellow ginger called *emprit* ginger (Z. officinale var. Amarum) and red ginger or *sunti* ginger (Z. officinale var. Rubrum) (Wiedhayati, 2016). Red ginger contains the highest essential oil. As red ginger is extremely hot, it is more common for raw materials for *jamu* (herbal medicines) and pharmaceutical industries than for spices in cooking. Essential oil of ginger could be found in leaf or rhizomes. Essential oil component in rhizome is dominated by monoterpenoid including camphene (14.5%), geranial (14.3%), and geranyl acetate (13.7%). These components are active against the

Gram-positive bacteria (*Bacillus licheniformis* and *Bacillus spizizenii*) and the Gram-negative bacteria (*Escherichia coli*, *Klebsiella pneumoniae* and *Pseudomonas stutzeri)* (Sivasothy et al., 2011). Similarly, Jayanudin et al., (2015) reported that red ginger was rich in monoterpenoids (81.9%), mainly containing camphene (14.5%), geranyl acetate (13.7%), geranial (14.3%), neral (7.7%), geraniol (7.3%) and 1.8-cineole (5.0%).

Materials and Methods

Diet and Treatments

Red ginger as the source of essential oil was evaluated using an *in vitro* batch fermentation trial to study its effect on ruminal nutrient digestibility in rumen. In vitro rumen fermentation was run according to in vitro gas production technique of Theodorou et al., (1994). Some modification was made to the volume of bottle serum as fermenter. Smaller volume was used in this research with identical ratio of liquid and head space. Rumen microbe was obtained from rumen fluid of two cannulated Ongole grade cattle which collected before morning feeding. Cattle were fed *P. purpureum* and beef cattle concentrate 60:40 DM bases. Substrate for in vitro fermentation consisted of forage (*Pennisetum purpureum* (Schumach), rice bran and wheat pollard in ratio 60:20:20 based on dry matter. Forage was cut before flowering stage and dried in driying oven 50°C, grinded to pass 1 mm sieves, whereas rice bran and wheat pollard were obtained from local feed shop. Dried red ginger was obtained from local traditional marker then grounded to pass 1 mm sieves. As sources of essential oil, red ginger meals were added into the diet to obtain final concentration of essential oil in medium of 0 (control), 25, 50, 75, and 100 mg/l.

Fermentation was carried out in a 125 ml serum bottle containing 70 ml fermentation medium and 700 mg of the diet per bottle. The bottles were set into three triplicates (one bottle each) to determine dry matter (DMD) and organic matter digestibility (OMD), crude protein digestibility (CPD), and crude fiber digestibility (CFD). Anaerobic fermentation was prepared by continuous flushing with CO_2 gas. The filled serum bottles were sealed with butyl rubber stopper plus aluminium crimp cap and pre-warmed overnight at 39˚C. On day 1 fermentation, 7 ml of collected rumen fluid was added into each bottle using a 10-ml plastic syringe. Bottles then incubated for 24 h at 39˚C. The gas pressure in bottle headspace was zeroing before incubation and every 2 hours by inserting 0.6 mm needle attached to a pressure transducer.

Sample Collection and Chemical Analyses

After 24 h, residual feed were collected by filtration using filter paper for further residual nutrients analysis of DM, OM, CF, and CP. The procedure for nutrient analysis was according to AOAC, (2005). The data of residual nutrients were used to calculated DMD, OMD, CPD and CFD respectively.

Statistical Analyses

The data from nutrients digestibility were subjected to one-way analysis of variance. The comparisons between means were analysis using Duncan Multiple Range Test.

Result and Discussion

Red ginger added to in-vitro rumen fermentation reduced crude protein digestibility at level 75 mg/l and above ($P<0.01$) (Figure 1.). Digestibility value in the 75 and 100 mg/l treatment was more than half of the control. The decreased protein digestion was followed by the reduced ammonia concentration across red ginger treatments.

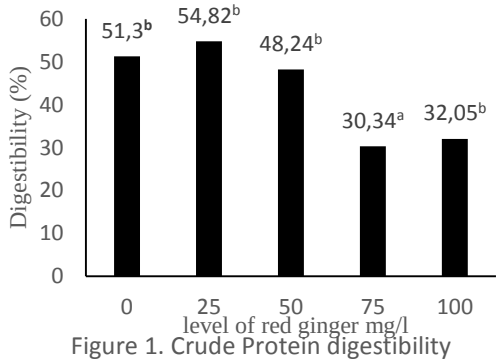

Figure 1. Crude Protein digestibility of in vitro rumen fermentation with different levesl of red ginger addition

Figure 2. Crude fiber digestibility of in vitro rumen fermentation with different levels of red ginger addition

The higher doses of red ginger in the diet resulted in lower protein digestibility. Addition of raw materials containing essential oil either terpene group or phenyl propane had a negative effect on protein degradation and other parameters of rumen fermentation in dose-dependant fashion (Macheboeuf et al., 2008). Colonization and degradation of readily degraded substrate, like starch and protein, could be reduced by essential oil (Cobellis et al., 2016b). The effect of essential oil on the compositions of microbial community depend on chemical of essential oil (Patra and Yu, 2015).

The attachments of proteolytic bacteria initiated feed protein breakdown. Protein then hydrolyzed into peptides and further amino acids. Some peptides and amino acids can be assimilated by rumen bacteria into microbial protein or fermented to produced VFA and ammonia (Cobellis et al., 2016b). Wallace et al., (2002) stated that colonization of protease bacteria in the rumen might be inhibited by essential oil indicated by the decreased protease activity. The mix of several essential oils did not affect protein degradation but reduced ammonia concentration (Newbold et al., 2004). Rumen bacteria assimilated some of the released peptides and amino acids into microbial protein or ferment amino acids to produce VFA and ammonia (Cobellis et al., 2016b).

Commercial essential oil showed an inhibition effect on the hyper-ammonia production bacteria with no effect on proteolytic (Mcintosh et al., 2003). In this research, the reduced crude protein digestion from 75 mg/l essential oil was higher than the level to start reducing ammonia (25 mg/l). It suggested that deamination microbes were more susceptible to red ginger addition compared to proteolytic. Cardozo et al., (2006) reported that eugenol tended to increase the ammonia concentration at low level, 0.3 and 3 mg/l, did not have effect at intermediate level (30 mg/L), and significantly reduced ammonia at the higher levels (300, 3000 and 5000 mg/l) in an in vitro batch culture system.

A decreased crude protein digestibility in rumen is an advantage since the escaped feed protein from microbial degradation passed to abomasum and small intestine for digestion by proteases and absorbed for metabolism. In rumen Feed protein are digested and broken down into small peptides, further into amino acid then ammonia. Ammonia is not incorporated to microbial protein is absorbed across rumen wall into bloodstream. Ammonia is converted to urea in liver and excreted through urine (Moran, 2005), and potentially contributes to greenhouse gas by nitrous oxide emission and ground water pollution (Cobellis et al., 2015).

TABLE 1. Effect of red ginger 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					
Dry matter	50.20	55.47	50.79	47.03	47.05
Organic matter	48.16	44.51	48.24	45.57	51.61

Fiber digestibility were higher in all addition of red ginger groups compared to control (P<0.05) (Figure 2.). Digestibility values in 25, 50, 75, and 100 mg/l treatment were 9.87; 18.75; 33.27 and 22.05%, respectively, whereas in control group was 31.20%. Meanwhile, red ginger did treatment did not affect organic matter and dry matter digestibility (Table 1.)

Ruminants digestive system is characterized by functional and anatomical adaptations that allow food energy in fibrous plant material to be extracted, mainly energy from cellulose and others stubborn carbohydrates. Ruminant animals derive about 70% of their metabolic energy from microbial fermentation (Niwińska, 2012). An extensive fiber digestion in rumen leads to a higher supply of energy for animal and increases feed efficiency.

Furthermore, the effect of essential oil on dry matter, organic matter and crude fiber varied. 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 et al., 2005). Addition of 5 and 50 mg/l thymol did not affect dry matter, organic matter, neutral detergent fiber and acid detergent fiber digestibility, but the high level (500 mg/l) could reduce nutrient degradability. Also, 500 mg/l eugenol did not affect the digestibility of dry matter, organic matter, neutral detergent fiber and acid detergent fiber (Castillejos et al., 2006). Essential oil from rosemary, ceylon cinnamon, oregano, dill seeds, cinnamon bark, cinnamon leaves, and eucalyptus, at the level of 1,125 ml/l of fermentation media had no detrimental effect on NDF degradability except eucalyptus (Cobellis et al. 2016). Essential oil of clove, eucalyptus, garlic, oregano and peppermint at 0.25, 0.5, and 1 g/l of medium decreased NDF digestibility except in garlic oil due to the decreasing cellulolytic bacteria (Calsamiglia et al., 2007; Benchaar and Greathead, 2011). Further studies showed that garlic oil could improve fiber digestibility (Klevenhusen et al., 2011). The effect of essential oils in rumen fermentation was determined by the chemical composition (Ferme et al., 2004; Patra and Saxena, 2009). Purity and doses also influenced activity of essential oil (Macheboeuf et al., 2008). The addition of 30 mg/l cinnamon oil increased dry matter and organic matter digestibility, but 300 and 600 mg/l concentration tend to reduce both nutrient digestibility. Similar pattern was observed in garlic oil. However, oregano oil only reduced dry matter and organic matter digestibility at the highest level (600 ml/l). On the contrary, feed dry matter and organic matter digestibility were not affected by rosemary oil addition (Roy et al., 2014). *Fructus agni-casti* containing essential oil (1-5% DM) did not change organic matter (Soycan-Önenç, 2016) In the present research, the declining protein degradation was compensated by the increased fiber digestion, hence the total organic and dry matter digested did not change significantly by the addition of red ginger.

Conclusion

Addition of red ginger in ruminal diet improved the efficiency of feed fermentation

through increasing fiber digestion and minimizing protein digestion in the rumen.

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