Nutrient digestibility on ruminal fermentation in vitro with addition of rumen modifier based on Clove (*Syzygium aromaticum*. L.) and Fennel (*Foeniculum vulgare*. Mill.)

**Essential Oil**

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**Abstract.** Effect of rumen modifier (RM) with clove or fennel essential oil on nutrient digestibility was studied in in vitro batch fermentation. RMs were mix with feed material to meet concentration of 0, 25, 50, 75 and 100 µl/l of medium. Feed consist of forage, wheat pollard and rice bran (60:20:20DM based). In vitro gas production technique was used with Ongole grade cattle as rumen microbe donor. Fermentation was done at 39ºC. Residuals feed were collected after 24 hours of incubation for nutrient content analysis as data for calculation of dry matter (DMD), organic matter (OMD), crude protein (CPD) and crude fibre (CFD) digestibility. RMs reduced DMD and OMD (P<0.01) start at clove RM of 25 µl/l whereas fennel RM at 50 µl/l. Range of DMD and OMD value were 30.17 to 43.55% and 35.53 to 47.98% respectively. CPD reduced by clove RM (P<0.05) otherwise did not affected by fennel RM. CPD decreased at clove RM 50 µl/l and gradually decline with increasing of RM. RM increased CFD (P<0.01), but at 100 µl/l CFD was not significant different from control. In conclusion, fennel essential oil have lesser negative effect on nutrient digestibility as RM at doses 25 to 50 µl/l.

1. **Introduction**

Feed efficiency is one of the determining factors in success of ruminant livestock production. An efficient production system lead to fewer resources utilization which support to global food security.

Feed utilization in ruminant is characterized by fermentation processes in the rumen, a part of the digestive tract. Rumen contain a large number of microbes including bacteria, protozoa and fungi [1]. Rumen microbes play an important role in digestion and extraction of feed energy and nutrient, and convert it into more valuable metabolites for the host animal [2]. End product of nutrient fermentation in the rumen are short chain fatty acid majority volatile fatty acid (VFA), microbial cell, ammonia (NH₃), CO₂, and methane (CH₄) [3]. VFA and cell microbial protein are the main energy and protein sources for ruminant. Almost 50 to 70% energy for ruminant come from VFA [4]. However, end product of ruminal fermentation in the form of methane indicate that there was an energy loss and generate environmental problem since methane is potent greenhouse gas[5].
Some efforts have been done to increase the ruminal feed efficiency thru rumen ecosystem modification, for instance utilization of antibiotic as feed additive in term of antibiotic growth promotors (AGP). AGP has proven increased feed efficiency in the rumen, and reduce the methane production [6]. But, antibiotics application as feed additive in animal production has been banned regarding disadvantage to consumer and environment.

Essential oils (EO), one of the plants secondary metabolite, recently attract several research to exploit its modifier of rumen fermentation due to their antimicrobial activity [7,8]. Fennel EO with main active compounds trans-anethole and estragole have activity again gram negative and gram positive strains of bacteria [9]. Furthermore clove EO with eugenol, a phenolic compound is one of the most valuable antimicrobial oil [10]. Due to the antimicrobial properties, EO has a chance to become an alternate of AGP. However application EOs or their compounds in several research reduced methane production was associated by decreasing in feed digestion [11]. Therefore this research aimed to find out doses of clove and fennel EOs as RM with no negative effect on nutrient digestibility.

2. Material and Methods

2.1 Material

Feed material as substrate for fermentation consist of Pennisetum purpureum, rice bran and wheat pollard, in ratio on dry matter was 60:20:20 (DM 88.94, OM 84.53, CP 13.40 and CF 21.44 and NNE 46.5%). Clove and fennel EOsas component of RM were bought from local EO store Lansida, in Yogyakarta. Rumen liquor as rumen microbe source was obtained from two ruminal cannulated Ongole grade cattle which fed diet consisting of Pennisetum purpureum and beef cattle concentrate 60:40 DM bases.

2.2 Methods

Serum bottle batch culture of in vitro gas production technique was used to study the effect of clove based and fennel based RMs on ruminal nutrients digestibility. Five of triplicate bottles (125 ml) respectively were set for determination of DMD and OMD, CPD, and CFD. Essential oils were added to 600 mg of feed ration to get EO final concentration in medium of 0, 25, 50, 75 and 100µl/ l and filled into the bottle. At the day before incubation, medium for anaerobic fermentation was prepared according to [12].Sixty millilitres of media was added in to substrate filled serum bottles and continuous flushed by oxygen free carbon dioxide. Immediately bottles were sealed with butyl rubber stopper and crimped then pre-warmed overnight at 39°C. Rumen liquor was collected before morning feed in the day of incubation and strained using polyester cloth then as much 30 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 residual feed were filtered and collected for analysis of residual nutrient included dry matter, organic matter, crude protein, and crude fibre for calculation of dry matter, organic matter and crude protein digestibility. Procedure for nutrient analysis according to [13]. Obtained data were subjected to one-way analysis of variance. Duncan Multiple Range Test was done to indentify an significantly different among treatments.

3. Result and Discussion

As shown in Table 1 dry matter digestibility was decreased by addition of clove based RM at all level (P<0.01) and relatively lower than control at addition of fennel based RM 75 and 100 µl/l. Reduction of dry matter digestibility at clove based RM treatment range from 17.89 to 33.02% compare to control. Based on component analysis main component of clove essential oil were eugenol (71.40%) and followed by Caryophyllene (22.48%). Antibacterial activity of eugenol relatively high due to its contain of phenol group. A high activity against bacteria are displayed by essential oil component that contain phenol group. Phenol group in essential oil have a high reactivity an tend to bind toward enzyme and protein by hydrogen bond [14], and cause cell lysis [15]. Feeds digestion and metabolism in the rumen is performed by a complex microbiota which inhabited in it[16]. A disruption of rumen
microbial due to the addition of antimicrobials agent will be followed by alteration of feed fermentation including nutrient digestibility.

Similar to dry matter digestibility, as shown in Table 1, value of organic matter digestibility also decreased with addition of clove and fennel based RM (P<0.01). Both clove and fennel based RM reduced organic matter at doses 50 µl/L and above. Dry matter digestibility and organic matter digestibility at fennel based RM treatments reduced more gradual compare to clove based RM. Based on GCMS analysis, dominant active compound of fennel essential oil in this research, was trans-anethole (68.76%). Compare to eugenol, trans-anethole is less active. Higher concentration is needed to perform the toxic effect [17]. Activity of essential oils are depend on chemical structure and composition which affected by essential oil sources [18] and doses administered [19].

Value of dry matter digestibility was reduced by administered of geraniol at the higher doses 300, 600, and 900 µl/l whereas addition of Eucalyptus essential oil with active compound 1,8-cineole reduce dry matter digestibility at level 1000 µl/l however addition at lower doses 250 and 500 µl/l did not change the dry matter digestibility and crude fibre digestibility [20].

Effect of clove and fennel base RM on crude protein digestibility was in different way. Addition of both RM up to doses 75 µl/L did not affect crude protein digestibility but at doses 100 µl/l clove based RM decreased crude protein digestibility and fennel based RM give an opposite result, crude protein digestibility was higher at doses of 100 µl/L fennel based RM. Addition of RMs had tendency to increase crude protein digestibility particularly on high protein feedstuffs [21].

Both clove and fennel based RM up to level 75 µl/l increase crude fibre digestibility (Table 1) then followed a decreasing at level 100 µl/L (P<0.01). Several previous research showed addition of essential oil did not have an effect on crude fibre digestibility ([11,22]. Increasing of crude fibre digestibility is an beneficial since improvement of fibre digestion is one of rumen modification main goal [23].

Table 1. Nutrient digestibility (%) of ruminal feed fermentation in vitro with addition of different source and doses of essential oil.

| Essential oil source | Doses (µl/l) | Parameters |
|----------------------|-------------|------------|
|                      |             | DMD        | OMD        | CPD        | CFD        |
| Clove                |             |            |            |            |            |
| 0                    |             | 45.05<sup>de</sup> | 43.15<sup>c</sup> | 49.14<sup>ab</sup> | 19.47<sup>bc</sup> |
| 25                   |             | 36.90<sup>ab</sup> | 42.12<sup>bc</sup> | 46.68<sup>d</sup> | 25.27<sup>d</sup> |
| 50                   |             | 33.78<sup>ab</sup> | 38.97<sup>ab</sup> | 42.88<sup>ab</sup> | 28.32<sup>d</sup> |
| 75                   |             | 33.17<sup>ab</sup> | 38.15<sup>ab</sup> | 43.03<sup>ab</sup> | 28.63<sup>d</sup> |
| 100                  |             | 30.17<sup>a</sup> | 35.52<sup>a</sup> | 38.85<sup>a</sup> | 16.46<sup>ab</sup> |
| Fennel               |             |            |            |            |            |
| 0                    |             | 46.58<sup>d</sup> | 41.98<sup>bc</sup> | 49.14<sup>ab</sup> | 17.80<sup>b</sup> |
| 25                   |             | 46.63<sup>d</sup> | 41.97<sup>bc</sup> | 44.44<sup>b</sup> | 18.66<sup>bc</sup> |
| 50                   |             | 47.37<sup>d</sup> | 35.44<sup>a</sup> | 49.09<sup>ab</sup> | 23.86<sup>cd</sup> |
| 75                   |             | 39.77<sup>cd</sup> | 42.88<sup>c</sup> | 46.16<sup>ab</sup> | 22.15<sup>c</sup> |
| 100                  |             | 40.22<sup>cd</sup> | 36.07<sup>a</sup> | 52.01<sup>b</sup> | 12.10<sup>a</sup> |

** Essential oil x Doses

** Parameters

ns = non significant

* Different superscript in the same column is significantly different

** (P<0.01); *(P<0.05); ns non significant;

DMD: dry matter digestibility; OMD: organic matter digestibility; CPD: crude protein digestibility; CFD: crude fiber digestibility
4. Conclusion

Fennel essential oil have lesser negative effect when used as rumen modifier at doses 25 to 50 µl/l. Dry matter, organic matter and crude protein digestibility did not change and resulting better crude fiber digestibility. Further research needs to be done with different parameters including parameter of fermentation, methane production, enzymes activity and microbial diversity.

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