Meta-analysis of the effect of essential oil usage towards the production and milk composition of dairy cow

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Abstract. One of the strategies to increase dairy cow production is by using antibiotics to manipulate the rumen fermentation process; nevertheless, there has been a ban on antibiotics usage in Europe and Indonesia. Essential oils consist of many secondary metabolites that own anti-microbes characteristics as antibiotics. This study aims at evaluating the effect of essential oil dosage on feed efficiency and dairy cow production by the meta-analysis method. There are two data types, namely rumen fermentation and in vivo production performance of milking cows based on the independent variable. The variable is in the form of essential oils dosage taken from thirteen journals from previous studies conducted from the year 2013 to 2020. The result of the meta-analysis analysis shows that the dosage of essential oil only has any impact on the population of protozoa. On the other hand, it does not have any effect on the fermentation result such as pH, methane, volatile fatty acids, and ammonia. The usage of a specific dosage of essential oils has significant impacts on milk production, fat corrected milk (FCM), and feed efficiency. Still, it does not impact the milk composition parameters such as lactose, fat, protein and milk urea nitrogen (MUN). The result of the study concluded that the usage of a specific dosage of essential oils has significant impacts on milk production, protozoa, and fat corrected milk.

1. Introduction
The manipulation of rumen fermentation is an attempt to achieve feed efficiency and to improve the ruminants’ productivity. Therefore, there have been many studies focusing on the modification of rumen fermentation. The main goal of rumen fermentation is to increase beneficial processes and at the same time is to minimize, change, or eliminate the inefficient process for the cattle [1]. One of the strategies to achieve nutrient efficiency as the focus of the research is by manipulating the nutrient fermentation as the carbohydrate and protein sources [2]. Another purpose of the fermentation process is to optimize several functions of rumen for the benefit of ruminants in the form of 1) decreasing methane production that increases the propionate to achieve the balance of energy for the cattle; 2) decreasing the protein degradation of cattle feed to improve the bio-availability of amino acid in the small intestine; 3) reducing the fast rate of carbohydrate degradation during the fermentation process (amylum, sucrose) and controlling the lactacid acid; and 4) improving the digestibility level for fibres [3].
During the last couple of years, the strategy of rumen fermentation uses secondary metabolites compounds due to its bioactive content after being extracted and thickened which has anti-microbes capacity towards the rumen microbes [4]. Secondary metabolite compounds that have a mechanism of anti-microbial mostly derive from phenol due to its aromatic core and significantly reactive OH cluster and the ability to create a bond between hydrogen and targeted protein and enzyme. Phenol components exist in essential oils such as thymol, carvacrol, and eugenol [5].

The use of cajeput oil as a form of essential oil that has the main component of cineol during the *in vitro* rumen fermentation can reduce the proteolytic bacteria population, namely protozoa [6] and inhibit the methanogen [7]. Apart from its significant impact on the bacteria population, essential oils also affect the rumen fermentation result in the form of the reduction of methane and NH3 and the increase of propionate contents [6]. According to Kurniawati *et al.* [8] the use of essential oils that derive from Indonesian herbal plant namely red ginger by *in vitro* procedure did not affect the rumen fermentation (pH, VFA, and microbial protein) but had a potential effect on the efficiency of nitrogen usage and the reduction of protozoa population. The *in vitro* result of the study by reducing the population of protozoa, proteolytic bacteria, and archaea resulted in the open possibility to transform them into rumen modifiers to fulfill the need for energy efficiency and feed protein for the dairy cow. The use of bioactive compounds derived from the essential oil by *in vitro* process can become a basis of the feed’s additive potential to improve the efficiency of nutrient. This review aims at evaluating the result of the study by applying various dosages of essential oils towards the parameters of fermentation, production, and composition of milk by *in vivo* procedure published between the year 2013 to 2020 using the meta-analysis approach.

2. Materials and Methods

2.1. The database

Reference searches are conducted through the ScienceDirect (http://www.sciencedirect.com/) and Google Scholar literature databases ranging from 2013 to 2020, with keywords used in the search: essential oil, rumen fermentation, dairy production, milk composition, and rumen manipulation. Also, the search can be done by finding the name of the author that corresponds to the research field use essential oil. The search results of research journals obtained about 100 journals, then grouped by year, type of EO, and the dose of EO between 0.2–3.5 g/tail/day. The database includes pH rumen, ammonia (mg/100 ml), total VFA mmol, VFA composition (acetate, propionate, and butyric, molar % of total VFA), number of protozoa (x 105/ml), and DMI (kg/d). In addition, the production of methane (mmol/100 mol VFA) is obtained from the calculation of the formula $(0.45 \times \text{Acetate})-(0.275 \times \text{propionate})+(0.4 \times \text{butyrate})$ [27]. Milk production and milk composition are obtained from the publication date, while the composition of the milk production is calculated in kg/d. Then, 3.5% fat-corrected milk $(0.0432 \times \text{Milk yield} + 16.23 \times \text{milk fat yield})$ is calculated in the determination of feed efficiency unit DMI.

2.2. Statistical analysis

The results of the database from the journal extracted were analyzed using linear regression to decide the correlation between the dosage of essential oil on rumen fermentation and dairy cows performance. The random effect of experiment represents the variance between experiments accounted for by the other variables such as the physiological status of the animals, experimental design, and measurement methods. The initial model used was

$$Y_{ij} = B_0 + B_1X_{ij} + B_2X_{ij}^2 + s_i + b_iX_{ij} + e_{ij}.$$  \hspace{1cm} \text{(1)}$$

where $Y_{ij}$ is the dependent variable, $B_0$ is the overall intercept across all experiments (fixed effect), $B_1$ and $B_2$ are the overall linear and quadratic regression coefficients, respectively, across all experiments (fixed effect), $X_{ij}$ is the value of the continuous predictor variable (EOBC doses, fixed effect), $s_i$ is the random effect of experiments, $b_i$ is the random effect of experiment on the regression
coefficient of $Y$ on $X$, and $eij$ is the unexplained residual error. Descriptive statistics of the dependent and independent variables shown in Tables 1 and 2 were computed using SPSS 16.

3. Results and Discussions

3.1 Rumen fermentation

The meta-analysis result can be seen in Table 1 that the application of a certain amount of essential oils on cattle feed did not significantly affect the fermentation parameters such as pH, VFA total, ammonium, and methane. The average pH reached around 6.20, the total amount of VFA 125.65 mmol, which consisted of acetate 64.34%, propionate 22.35%, and butyrate 14.67%. The observation on the usage of essential oils for cattle feed must also consider the methane production concerning its energy efficiency during the rumen fermentation. The meta-analysis result showed that the adding of essential oils in the cattle feed did not give any significant impact on methane production by its average value of 21.54 mmol/100 mol VFA. Methane production during the fermentation in rumen also depends on the population of protozoa; the production occurs due to the endo-symbiosis between protozoa and metanogenic bacteria. Based on the meta-analysis, the addition of essential oils has a significant impact on the reduction of the population of protozoa with its average value of 8.59 x 105/ml. The result of ammonium production shows the efficiency of rumen fermentation in using protein sources. Based on the result of the meta-analysis study, the use of a particular amount of essential oil does not show any significant difference; with the average ammonium content of 28.67 mg/dl.

Table 1. Meta-analysis of essential oils effects on rumen fermentation data.

| Parameter            | Intercept | SEintercept | P_intercept | Slope | SEslope | P_slope | R  | R²   |
|----------------------|-----------|-------------|-------------|--------|---------|---------|-----|------|
| pH                   | 5.60      | 0.530       | <0.05       | 0.287  | 0.469   | 0.55    | 0.16| 0.026|
| Methane mmol/100 mol | 27.61     | 1.47        | <0.05       | 1.22   | 1.17    | 0.39    | 0.30| 0.09 |
| VFA, mmol            | 128.76    | 6.034       | <0.05       | -4.287 | 5.33    | 0.43    | 0.21| 0.040|
| Acetate %            | 61.64     | 3.518       | <0.05       | 3.106  | 2.81    | 0.29    | 0.31| 0.100|
| Propionate %         | 23.51     | 1.304       | <0.05       | -1.334 | 1.04    | 0.22    | 0.36| 0.131|
| Butyrate %           | 15.58     | 1.35        | <0.05       | -1.354 | 1.08    | 0.23    | 0.35| 0.124|
| Acetate:propionate   | 2.68      | 0.22        | <0.05       | 0.342  | 0.17    | 0.08    | 0.52| 0.275|
| Ammonia, mg/dL       | 24.65     | 5.919       | <0.05       | -6.16  | 5.63    | 0.29    | 0.30| 0.091|
| Protozoa, 10³/ml     | 3.31      | 1.39        | <0.05       | 7.07   | 1.07    | 0.00    | 0.94| 0.895|

According to Friggens et al.[9], monosaccharide fermentation mainly produces VFA, especially acetate (A), propionate (P), butyrate (B), and valerate (V). Apart from the n-butyrare and n-valerate, the monosaccharide also has isobutyrate and isovalerate contents. The composition of VFA within the rumen ranges from 65%A, 20% P, and 10% B, and 5% V in general [10]. The meta-analysis result shows that the use of essential oils with the dosage of 0.2 to 3.5g/cow/day for cattle feed by in vivo procedure does not affect the VFA total amount negatively; the finding is in line with several previous studies that proved the inconsistency result between the in vitro and in vivo procedures on the use of essential oils towards the VFA [11] [12]. The use of essential oils with single eugenol and carvacrol components did not have any significant impact on the total VFA and the microbial variety of rumen, both in the high and low fibre concentration of the feed [13] [14]

According to several studies on the usage of essential oils, there is inconsistent diversity of rumen fermentation due to the action mechanism of essential oils as an anti-microbe. The action mechanism occurs due to the hydroxyl cluster on phenol which acts as a transmembrane carrier, namely monovalent cation and proton, which have a similar mechanism as ionosphere in antibiotics. Another mechanism on the function of essential oils acting as an anti-microbe is the ability of phenol compound to coagulate cell components, such as protein denaturation mechanism [15]. The phenol compound has the coagulation ability because it can interact with active molecular group such as protein and enzyme [16].
Based on the previous findings, there has been a significant need to conduct a more in-depth review of the effect of essential oils towards the diversity of rumen microbes affected by the adding of the mixture. The diversity of microbes that occurred in the rumen can explain the microbial work mechanism in degrading the cattle feed. According to Benchaar et al. [12], the use of essential oil has affected bacteria that is reactive towards ammonium production, provotela bacteria that has proteolytic activity, and methanogenic.

3.2. Milk production and compositions

The quality of milk production and composition from milking cattle depends significantly on the rumen fermentation result. According to meta-analysis result on the milk production parameter can be shown on Table 3, the adding of essential oils has an impact on the milk production by an average of 29.06 kg and 3.5% fat corrected milk with an average value of 30.1 kg; while on the other hand, the feed consumption does not have a significant difference by an average value of 22.76 kg. An evaluation on the usage of essential oils using the meta-analysis approach towards the composition of milk lactose, protein, and fat shows that there is no significant effect with the average value of the three parameters of 4.83%, 3.32%, and 3.58% consecutively.

| Parameter                           | Intercept | SE_intercept | P_intercept | Slope | SE_slope | P_slope | R     | R²    |
|-------------------------------------|-----------|--------------|-------------|-------|----------|---------|-------|-------|
| DMI, kg                             | 20.41     | 1.902        | <0.05       | 2.472 | 1.478    | 0.12    | 0.435 | 0.189 |
| Milk yield, kg                      | 24.10     | 2.479        | <0.05       | 5.984 | 2.14     | 0.01    | 0.539 | 0.291 |
| 3.5% fat corrected milk, kg        | 24.52     | 2.592        | <0.05       | 6.795 | 2.24     | 0.007   | 0.571 | 0.326 |
| Milk fat %                          | 3.58      | 0.124        | <0.05       | 0.137 | 0.10     | 0.21    | 0.281 | 0.072 |
| Milk protein %                      | 3.39      | 0.066        | <0.05       | -0.084| 0.057    | 0.15    | 0.321 | 0.103 |
| Milk lactose %                      | 4.91      | 0.089        | <0.05       | -0.104| 0.077    | 0.19    | 0.313 | 0.098 |
| Milk fat yield, kg                  | 0.87      | 0.096        | <0.05       | 0.259 | 0.08     | 0.06    | 0.581 | 0.337 |
| Milk protein yield, kg              | 3.39      | 0.066        | <0.05       | -0.084| 0.057    | 0.15    | 0.32  | 0.103 |
| Milk lactose yield, kg              | 4.91      | 0.08         | <0.05       | -0.104| 0.07     | 0.19    | 0.098 | 0.313 |
| Milk Urea Nitrogen (MUN), mg/100 ml | 13.92     | 1.621        | <0.05       | -1.202| 1.29     | 0.37    | 0.258 | 0.067 |
| Feed efficiency                     | 1.52      | 0.162        | <0.05       | 0.019 | 0.12     | 0.88    | 0.045 | 0.02  |

Researches related to in vivo process of essential oil adding to cattle feed showed different results because the rumen fermentation condition for in vivo differs from the one of in vitro (Khiiosa-ard and Zebeli 2013). Several current studies also mention that the adding of essential oil, both single and blend essential oils with a dosage of 0.5 to 1 g/cow/day did not affect the milk production; yet it tends to increase the milk fat and protein [18] [19] [20]. Research by Braun et al.[21] mentioned that the mixture of essential oils consisting of eugenol, menthol, and anethol with a dosage of 1.2 g/cattle/day could increase the production of milk, milk fat, and protein in a dry feed consumption. According to Drong et al. [22] the cattle feeding in purpose to formulate ketogenic inhibition, the blend essential oils (BEO) can significantly increase milk fat, corrected milk energy, and feed efficiency; nevertheless, it also increases the β-hydroxybutyrate (BHB) and non-esterified fatty acid NEFA. The result shows a negative impact on the balance of milking cow energy. Nevertheless, other researchers also found that the adding of essential oils mixture on the milking cow feed did not affect the BHB and NEFA [23].

Kung, L. et al. [24] reported that there was an increase in milk production and had no effect on milk fat at the beginning until the middle of lactation with the addition of 1 g/cow/day (CRINA). The mechanism of the EO's influence on milk production is still different depending on the stages of lactation (early and mid-action), but this may be attributed to the alteration of the microbe profile and nutritional needs as cattle-stage transition cows. Some studies have been published, but until now, in general, the conclusion is that EO does not affect milk production. The research of [25] is very interesting because
the plasma urea significantly dropped, it is explained that the low urea milk during the addition of EO, milk urea nitrogen (MUN) can be used as a prediction of total excretion of nitrogen in the urine, other research about the effect of EO on MUN is also explained by Giannenas et al. [26]. The decline in the MUN level of cow feed added commercial EO. Furthermore, the results of this research are known to produce milk proteins increased, it is possible that nitrogen is not directly related to efficiency into proteins and amino acids such as glutamine compared to no nitrogen proteins such as urea.

4. Conclusions

Effect of the dose of the type of bioactive composition both single and blend have no effect on the fermentation parameters except the number of protozoa, while the production parameters only affect the parameters of milk production and FCM. The results of the meta-analysis recommendations for further research on the influence of essential oil on the diversity of microbial rumen affected by the dose of essential oil in vivo, and blood profile, so it will give profound effectiveness on the influence of essential doses of protozoa, milk production, and FCM by in vivo.

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