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Effect of *Curcuma zedoaria*, *Curcuma mangga* and *Cuminum cyminum* on Rumen Ecology and Pb Profile in the Rumen of Mastitis Dairy Cows (*in vitro*)

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ABSTRACT
The aim of this study was to evaluate the effect of *Curcuma zedoaria*, *Curcuma mangga* and *Cuminum cyminum* on rumen ecology and Pb profile in the rumen of mastitis dairy cows (*in vitro*). The research design was complete randomized design with 4 treatments (A Control (without herbs), B *Curcuma zedoaria* 0.02% of b.wt., C *Curcuma mangga* 0.06% b.wt. And D *Cuminum cyminum* 0.03% b.wt.) and 8 replication. This study was carried out *in vitro* in the laboratory by a pressure transducer technique (using rumen fluid of FH dairy cows. Sample was conditioned by adding 3 ppm Pb sulfate in rumen fluid. The variables measured were rumen pH, rumen bacteria counts (CFU mL^{-1}), Volatile Fatty Acids (VFA) partial concentration (mg/100 mL) by using Gas Liquid Chromatograph (Chrompack® CP90022) and heavy metal Pb profile in rumen fluid by using Absorption Atomic Spectrophotometer (AAS) 6300 A with minimum reading limit 0.01 ppm. The result of study group of *Curcuma zedoaria* and *Cuminum cyminum* showed the highest number of rumen bacterial acetic acid, propionic acid and butyric acid and a lower profile of heavy metals in rumen fluid. Lead (Pb) was found only in the control without herb use.

Key words: Mastitis, *Curcuma zedoaria*, *Curcuma mangga*, *Cuminum cyminum*, Pb, *in vitro*

INTRODUCTION

Milk was reported to be contaminated by toxic substances such as heavy metals and pesticides (Corrigan and Seneviratna, 1990). This condition occurred in Indonesia (Indraningsih *et al*., 2003; Nurdin *et al*., 2013) and some of them exceeded the allowed threshold. This is one of the causes of children's intelligence deficiency. The materials of this contamination (heavy metals and pesticides) came from the air, water, soil and residue material in agricultural product and consumed by cattle through feed and water that will be accumulated in the body and excreted in milk.

Heavy metal residues in milk will cause many problems to human health and intelligence. Feed additives that containing ingredients that can neutralize pesticide residues and heavy metals can be used to solve that problem, as well as reducing the use of antibiotics for treatment of mastitis by using natural antioxidant ingredients and organic minerals. Nurdin (2006, 2007a) has reported that natural antioxidants such as sunflower, *Cuminum cyminum*, *Curcuma zedoaria* and *Curcuma mangga* can reduce the incidence of mastitis, improve milk production and increase the number of rumen bacterial (Nurdin, 2007b; Nurdin and Arief, 2009; Nurdin *et al*., 2011a, 2013). As presented by Sunaryadi (2006), lowercase number of rumen bacterial can increase the amount of heavy metals (Pb) excreted through faeces.

The aim of this study was getting the best herb that can eliminate Pb residues contaminated in dairy cattle rumen and eliminate metal residue accumulated in milk. Finally, It will increase milk yield and quality, antioxidant milk production and eliminate the heavy metal residues.

MATERIALS AND METHODS

This study was carried out *in vitro* in the laboratory by a pressure transducer technique using rumen fluid of FH mastitis dairy cows. Sample was conditioned by adding 3 ppm Pb sulfate in rumen fluid.

A complete randomized design with 4 treatments and 8 replications was used this experiment; A as Control, B *Curcuma zedoaria* (0.02% of b.wt.), C *Curcuma mangga* (0.06% b.wt.) and D *Cuminum cyminum* (0.03% b.wt.).

The variables measured were rumen pH, rumen bacteria counts (CFU mL^{-1}), by Ogimoto and Imai (1981) method, Volatile Fatty Acids (VFA) partial concentration (mg/100 mL) by using Gas Liquid Chromatograph (Chrompack® CP90022) and heavy metal Pb profile in rumen fluid by using Absorption Atomic Spectrophotometer (AAS) 6300 A with minimum reading limit 0.01 ppm.

Statistical analysis: Subsequently, the data was analyzed by analysis of variance. After a significant F-test ($p<0.05$), Duncan's multiple range test was use to inspect differences among treatment (Gaspersz, 1995).

RESULTS AND DISCUSSION

The number of rumen bacterial and rumen pH recorded from each group was shown in Table 1.

Total rumen bacteria ranged between 2.4033×10^9-3.850×10^9 CFU mL^{-1}, with highest total rumen bacteria was achieved by adding *Curcuma zedoaria* 0.02% and *Cuminum cyminum* 0.03%. Conversely control (A) have lowest total rumen bacteria 2.4033×10^9 CFU mL^{-1}. It is indicated that adding herbs in dairy cattle feed can increase total rumen bacteria from 45.63-60.20%. The results of this study are still within the normal range of total rumen bacteria, ranged from 10^9-10^9 CFU mL^{-1} in accordance with that proposed by Ogimoto and Imai (1981), Czerkawski (1986) and Donald *et al*. (1988).

Levels of VFA *partial* (acetic acid, propionate acid, butyric acid and valeric acid) ranged from 82.470-143.020, 23,560-43, 160, 8.440-14 and 1,200-3,330 mg/100 mL, respectively. The highest acetic acid obtained in treatment B and D, were 143.020 and 138.030 mg/100 mL. The average of the highest propionic acid is in treatment D and C were 43, 160 and 36,560 mg/100 mL. The highest butyric acid is in treatment B and D which is 14,100 and 12,180 mg/100 mL. The highest valerate acid was on treatment B and D were 3.330 and 3.050 mg/100 mL (Table 2).

The increase of rumen bacteria number was followed by increasing concentrations of VFA in every treatment. It is caused by the doses and the condition of the rumen ecosystem to support microbes growth development that will produce higher VFA. Because microbes have an important role as protein sources in body, it can produce energy as the result of fermentation (Theodorou and France, 1993).

Dairy cattle with subclinical mastitis condition will change the balance of microbes in the rumen and increase the amount of microbial pathogens. By adding herbs as antioxidants and anti-inflammatory compounds can enhance the ecological balance of the rumen. Based on the results of the phytochemical screening of *Curcuma zedoaria*, *Curcuma mangga* and *Cuminum cyminum* saponin contained can improved the balance of microbes in the rumen and reduce the number of pathogenic microbes. Based on Nurdin and Susanti (2009) and Nurdin and Arief (2009), adding *Curcuma zedoaria* (0.02% b.wt.) and *Curcuma mangga* (0.06% of b.wt.), *Cuminum cyminum* (0.03% b.wt.) *in vitro* will improve ecological conditions better. Consequently the amount of rumen bacterial, total VFA and propionic acid will increase. *Curcuma zedoaria*, *Curcuma mangga* and *Cuminum cyminum* were containing flavonoids, phenolic and saponin as an antioxidant and anti-inflammatory that was able to increase the cell permeability so that the body resistance will increase as well (Nurdin and Susanti, 2009).

The heavy metal (Pb) was found in the group control only. The entire treatment by adding *Curcuma zedoaria*, *Curcuma mangga* and *Cuminum cyminum* showed no Pb contents dissolved in rumen fluid, with Pb concentrations below the 0.01 ppm. It may be due to the ability of the instruments used can only detect above 0.01 ppm. Lead (Pb) content had not detected in treatment B, C and D, because Pb easily bound together by fatty acids bonds which generally become constituent of *Curcuma zedoaria*, *Curcuma mangga* and *Cuminum cyminum* (Nurdin *et al*., 2011b). Subsequently Pb-phenolic bond has a large molecular weight and toughly absorbed by the body, it will be excreted from the body through the faeces (Nurdin *et al*., 2013).

Table 1: Average pH and rumen bacteria

Treatments	pH	TCB ($\times10^9$ CFU mL^{-1})
A	6.26±0.1131	2.4033 ± 1.6599^{ns}
B	6.33±0.1200	3.5000±3.5000*
C	6.28±0.0058	2.5400 ± 2.5400^{ns}
D	6.23±0.0231	3.8500±0.1825*

Same superscript means no significant difference, A: Control, B: *Curcuma zedoaria* (0.02%), C: *Curcuma mangga* (0.06%) and D: *Cuminum cyminum* (0.03%)

Table 2: Volatile fatty acids partial concentration and dissolved Pb

Treatments	Acetate acid (mg/100 mL)	Propionate acid (mg/100 mL)	Butyric acid (mg/100 mL)	Valeric acid (mg/100 mL)	Dissolved Pb (ppm)
A	82.470 ± 0.1617^{ns}	23.560 ± 0.0404^{ns}	8.440 ± 0.4792^{ns}	0.0520 ± 1.850^{ns}	0.0289
B	143.020±1.2759**	36.560±0.2771*	14.140±0.0529**	3.330±0.0346*	Not detected
C	94.520 ± 0.0115^{ns}	27.540 ± 0.0635^{ns}	0.0635 ± 7.070^{ns}	0.0058 ± 1.200^{ns}	Not detected
D	138.030±0.0289**	43.160±0.9185*	12.190±0.0100*	3.050±0.0462*	Not detected

Same superscript means no significant difference A: Control, B: *Curcuma zedoaria* (0.02%), C *Curcuma mangga* (0.06%) and D: *Cuminum cyminum* 0.03

Based on the research results, the best treatment is a treatment that provides results of the highest number of rumen bacteria, optimum pH, acetic acid, propionic acid and butyric acid as well as heavy metal profiles in the rumen fluid.

CONCLUSION

Cuminum zedoaria, *Curcuma mangga* and *Cuminum cyminum* provided favorable effect on rumen ecology.

The best treatment is giving white turmeric (*Cuminum zedoaria*) about of 0.02% and cumin (*Cuminum cyminum*) about of 0.03%

Giving *Cuminum zedoaria*, *Curcuma mangga* and *Cuminum cyminum* showed a decrease dissolved Pb contained in the rumen fluid.

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