Milk production and milk quality of sub-clinical mastitis cows feed with different supplementation of herbal in the diet

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D W Harjanti[1], F Wahyono[1] and D N Afifah[2]

[1]Department of Animal Science, Faculty of Animal and Agricultural Sciences, Diponegoro University Indonesia
[2]Centre of Nutrition Research, Medical Faculty, Diponegoro University Indonesia

E-mail : dianharjanti@undip.ac.id

Abstract. This study was aimed to evaluate effect of five Indonesian herbals on milk production and bacterial count in sub-clinical mastitis cows. A completely randomized design split plot in time with 4 replications and 4 treatments was used. The main plot was basal diet supplemented with 12.5 g/100kgBW of mixed-herbal. Dietary treatments were basal diet supplemented with various composition, T0 (without herbal additive); T1 (70% *Sauropus androgynus*, 25% *Curcuma xanthorrhiza*, and 5% *Alpinia galanga*), T2 (70% *Piper betle*, 25% *Curcuma domestica*, 5% *Curcuma xanthorrhiza*), and T3 (35% Sauropus androgynus, 35% *Piper betle*, 10% *Curcuma xanthorrhiza*, 10% *Curcuma domestica* and 10% *Alpinia galanga*). Sub plot was treatment duration (0, 5 and 10 days). Data showed that bacterial count in T1, T2 and T3 were lower ($P<0.05$) than T0. Among herbal groups, cows in T3 had lowest bacterial count and reached Indonesian standard for milk quality (< 10^6 cfu/ml) after 5 days treatments. Cows fed with T1 and T3 produced more milk ($P<0.05$). Milk total solid and lactose contents in T1 were the highest ($P<0.05$), whereas highest milk fat in T3 ($P<0.05$). In conclusion, herbal feed additive could be used as an alternative for mastitis treatment to ensure milk productivity and milk safety.

1. Introduction

Herbs that grows in tropical area might have positive impact for milk production and mastitis treatment. Mastitis is an infection of mammary gland in dairy cows. It causes huge economic losses due to low production and quality of milk. Clinical and sub-clinical mastitis in lactating cows is most commonly treated by intramammary infusion of antibiotic. However, both antibiotic injection and antibiotic feed additives have caused public concern due to potential of antibiotic residue in milk. Thus, the exploration and evaluation of herbal remedies that have potential to increase milk production and reduce the severity of mastitis infection in dairy cow must be conducted. Indonesia is rich in natural resources with many benefits. Plants herbs such as *Piper betle* leaves and *Curcuma domestica* are potential to be use as anti-bacterial and anti-inflammatory agent. Our previous data suggested that *Piper betle* leaves is effective as antibacterial agents against mastitis causing bacteria [1]. Curcumin, the active compound of *Curcuma domestica* could induce the immune-stimulatory action by activating CD4 cells expansion in dairy cows [2]. *Curcuma xanthorrhiza* and *Alpina galanga also* use as herbal remedies in Indonesia since many years ago to cure stomach illness, increase eating appetite and cure liver disease (hepatoprotector) [3]. Moreover, natural agents such as *Sauropus androgynous* serves to initiate, maintain and increase milk production in human, as well as dairy animal due to its

galactagogic substances. Based on the efficacy of each herbal, if those herbals are being combined, it is expected to get better efficacy for bovine mastitis treatment. Moreover, the information about the appropriate duration of treatments is needed for the safety and efficiency of herbal utilization. Therefore, present study was conducted to evaluate the effect of mixed-herbal on milk production, milk components (total solid, fat, lactose and protein), and microbiological quality of milk in sub-clinical mastitis cows. The duration of herbal additive treatment for appropriate medication also observed.

2. Material and method

Sixteen lactating dairy cows with sub-clinical mastitis infection were used in this study. The cows were tested by California Mastitis Test and showed positive 2-3 for each teat, indicating similar mammary inflammation degree. The cows assigned in completely randomized design split plot in time with 4 treatments and 4 replications. The main plot was dietary treatments that is herbal feed additive given at 12.5 g/100kgBW in the basal diet. The basal diets were Elephant grass (22% dry matter, 11.5% crude protein, 1.89% crude fat, 33.50% crude fiber, 53.72% TDN) and commercial concentrate (88.76% dry matter, 15.28% crude protein, 4.51% crude fat, 6.72% of crude fiber, 80.60% TDN), at a ratio of 60:40. Dietary treatments were basal diet added with various composition of mixed-herbal additives: T0 (without herbal additive); T1 (70% *Sauropus androgynus*, 25% *Curcuma xanthorrhiza*, and 5% *Alpinia galanga*), T2 (70% *Piper betle*, 25% *Curcuma domestica*, 5% *Curcuma xanthorrhiza*), and T3 (35% Sauropus androgynus, 35% *Piper betle*, 10% *Curcuma xanthorrhiza*, 10% *Curcuma domestica* and 10% *Alpinia galanga*). Sub plot was the duration of treatment (0, 5 and 10 days). Milk production were recorded twice daily at morning and evening milking. Milk sample for bacteria and milk components (total solid, fat, protein) analysis were taken aseptically on day 0, 6 and 11 at morning milking to evaluate the effect of 5 and 10 days of mixed-herbal additive intake. Milk sample was dried in oven at 105°C for 1 h to determine total solid. Milk fat content was analyzed using Gerber Method, milk protein content using Kjeldahl Method and lactose content using Luff Schrool Method. Milk samples were incubated in Nutrient Agar at 37°C for 24 h to evaluate the bacterial count. Data were analysis by ANOVA.

3. Result and discussion

Milk production in mastitis cows fed herbal additives were presented in Table 1. There was no interaction between dietary treatments and the duration. Milk production in T3 was the highest ($P<0.05$) among treatments. Comparison between the duration of treatments showed that the length of herbal additive intake did not significantly affect milk production. The milk production remained constant during the study, indicating that adding mixed-herbal probably not change cow's appetite and feed intake. After 10 days of treatments, milk total solid and lactose in T1 were higher than T0, T2 and T3. Milk fat content in the T3 was the highest among treatment groups, whereas there were no differences on milk protein contents among treatments (Figure 1).

Table 1. Milk production (l/day) of sub-clinical mastitis cows feed with different supplementation of herbal in the diet

Treatment	Duration of Treatment (days)			
	0	5	10	Mean
T0	11.67	11.50	10.83	11.33[b]
T1	12.67	12.33	13.37	12.79[ab]
T2	11.83	12.33	12.33	12.17[b]
T3	12.83	13.37	14.03	13.41[a]
Mean	12.25	12.38	12.64	

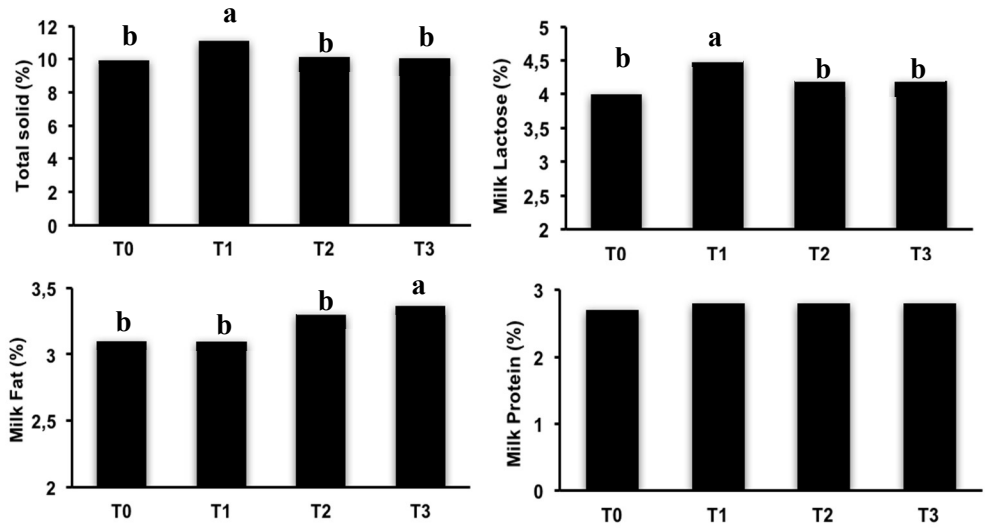
[ab] Different superscript in the same column were significantly different ($P<0.05$)

Although T1 contained with 70% of *S. androgynous* leaves powder, milk production did not significantly different with T0. Interestingly, milk production in T3 which contained with 35% of *S. androgynous* was the highest among treatments. Previous study by Suprayogi et al. [4] revealed that adding *S. androgynous* leaves powder in the diet of Holstein cows at 100 g, 150 g and 200 g/head increase milk production up to 30%, 40% and 34% respectively. Phytochemical investigation of *S. androgynus* leaves reported that they contained sterol, resin, tannin, saponin, alkaloid, flavonoid, terpenoid, glycoside, phenol, catechol, cardiac glycosides, acidic compounds, anthocyanins, carotenoid, ascorbic acid [5]. *Sauropus androgynous* leaves known to have galactagogic effects which is believed to increase lactation in women [6,7], Friesian Holstein cattle [4] and rat [8]. *Sauropus androgynous* intake may increase prolactin secretion which could induce milk secretion [4]. Feed additive T1 had the highest proportion (70%) of *S. androgynous*, however, milk production in cows fed T1 did not significantly different with those fed T0. The possibility of the different result between present study and Suprayogi et al. [4] might be the dosage of *S. androgynous* given. The amount of mixed-herbal added in the diet in present study was 2.5 g/ kg BW/day. Feed additive T1 and T3 containing 70% and 35% of *S. androgynous* leaves powder and given to the cows at 12.5 g/100 kg BW/day, hence the amount of *S. androgynous* added in the diets were equal to 35 g/day and 17.5 g/day for T1 and T3 (BW 400 kg). These amounts were lower than those reported previously by Suprayogi et al. [4]. Nonetheless, milk production in cows fed T3 additive was the highest among treatments although the amount of *S. androgynous* added in the diet of T3 group was lower than those in T1 group. In this case, it seems that other herbal components might contribute to the increase of milk production. *Curcuma xanthorizza* is having two important bioactive compounds, named curcuminoid (3.16%) and atriric oil (15.5%). These compounds have several functions such as anti-inflammation, anthelminthic, hypocholoretic, feed intake stimulant and substances hormone-like prolactin (maintaining milk production) and oxytocin (milk let down) [2]. *Alpinia galangal* rhizome contains flavonoids, some of which have been identified as kaemperol, kaempferide, galangin, alpinin [3]. Unique aroma components of galangal rhizome such as hydroxyl-1,8-cineole glucopyranosides, trans-2-hydroxy-1,8-cineole β-D glucopyranosides and trans-3-hydroxy-1,8-cineole β-D glucopyranoside are known to improve appetite and taste [9]. The amount of milk produced is influenced by nutrient supply from feed consumed. Additive T1 and T3 contain 5% and 10% *Alpinia galangal*, which causes an increase in milk production 5.2% and 8.6% for T1 and T3 respectively, whereas the additive T2 did not contain *Alpinia galangal rhizome.*

Milk total solid and lactose in T1 were higher ($P<0.05$) than T0, T2 and T3. Milk lactose is synthesized from blood glucose. Previous study reported that active compound of *S. androgynous* leaves increases glucose metabolism [4]. Since the proportion and intake of *S. androgynous* leaves powder in T1 group was the highest among treatments, the increase milk lactose concentration in T1 might be the result from the increase of glucose metabolism. Present study showed that milk fat contents in T3 was the highest among treatments. Milk fat is mainly affected by acetate production in rumen [10]. Although another study stated that adding *Curcuma xanthorizza* in the diet of lactating goat decrease milk fat, however milk fat contents in T1 and T2 did not change significantly and milk fat in T3 was increase in present study. Milk protein is synthesized from blood amino acid as the end product of rumen protein degradation and the ungradable protein digestion [10,11]. Furthermore, the similar milk protein contents within treatments groups indicated similar protein metabolism among groups. Present result indicating that mixed-herbal additives did not influence proteolytic microbes in the rumen.

Mixed-herbal additives in present study could decrease bacterial count in milk. Total bacterial count in T1, T2 and T3 were lower ($P<0.05$) than those in T0 (Table 2). The bacterial count decreased ($P<0.05$) with the duration of herbal feed additives intake. Among the herbal groups, cows fed T3 additive had the lowest ($P<0.05$) bacterial count in milk and has reached the Indonesian standard for milk quality (less than 10^6 cfu/ml) after 5 days of treatments. This result indicating that cows fed T3 additive had the highest cure rate and it could be the reason why the cows in T3 groups produce more milk. The high number of bacteria in milk reflects the high bacteria infection and inflammation degree

inside the mammary gland [12]. Lactating cows with mammary inflammation usually produce less milk [13]. The decrease of bacteria in milk of cows in the control group (T0) caused by routine antiseptic teat dipping after milking only. Based on this study, it can be seen that combined treatment between antiseptic teat dipping and herbal additives resulted in faster curative for sub-clinical mastitis cows. The T3 additive contains 35% Sauropus androgynus, 35% *Piper betle*, 10% *Curcuma xanthorrhiza*, 10% *Curcuma domestica* and 10% *Alpinia galangal*, which have active ingredients that act as antibacterial and anti-inflammatory agents, such as alkaloid, flavonoid, saponin, tannin, glycoside, triterpenoid, phenolic and essential oils. Our previous study revealed that *Piper betle* leaves could inhibit the growth of bacteria in mastitis milk and it had similar antibacterial effects with penicillin-dihydrostreptomycin [1]. Piper betle leaves contain eugenol rich essential oil (1-3%), hydroxychavicol, saponin, alkaloid [14]. The bioactive compound curcumin in *Curcuma xanthorrhiza* and *Curcuma domestica* known as anti-inflammatory agent. Curcumin is able to reduce the activity of myeloperoxidase in mammary gland, an enzyme used as indicator of neutrophil infiltration [15]. It is interesting to note that in the T3 herbal additive which contained with five herbals, the cure rate was the fastest among treatment. Bioactive compounds from each herbal might act to inhibit the growth of bacteria and reduce mammary inflammation. Flavonoid and saponin in herbal plants are characterized for their antimicrobial, antifungal, antioxidant and anti-inflammation activities [1,13,14].



[ab] (P<0.05)

Figure 1. Milk total solid, lactose, fat and protein contents of sub-clinical mastitis cows feed with different supplementation of herbal in the diet

Table 2. Bacterial count in milk (CFU/ml) of sub-clinical mastitis cows feed with different supplementation of herbal in the diet

Treatment	Duration of Treatment (days)			
	0	5	10	Mean
T0	14,000,000	10,333,300	2,233,300	8,855,533[a]
T1	16,300,000	1,633,000	403,000	6,112,000[b]
T2	15,667,000	1,400,000	178,300	5,748,433[b]
T3	15,600,000	240,000	37,000	5,292,333[b]
Mean	15,391,750[a]	3,401,575[b]	712,900[b]	

[ab] Different superscript in the same column and row were significantly different (P<0.05)

4. Conclusion

In conclusion, herbal feed additive could be used as an alternative for mastitis treatment to ensure milk productivity and milk safety. The recommendation of herbal feed additive for mastitis treatment is formula T3 and the duration of treatment is minimum 5 days.

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