p-ISSN 1410-5659 e-ISSN 2621-5144
Published by Fakultas Peternakan UNPAD
Unpad Press

Jurnal Ilmu Ternak Universitas Padjadjaran, Desember 2022, 22(2):88-92
DOI: 10.24198/jit.v22i2.40525
Available online at http://jurnal.unpad.ac.id/jurnalilmuternak

Effect of Betel Leaf Meal (*Piper Betle* L.) as Feed Additive on Milk Composition and Somatic Cell Count of Subclinical Mastitis

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Abstract
The aim of this experiment was to study the effect of betel leaf meal (BLM) as feed additive on milk composition and somatic cell count of subclinical mastitis infected cow. The experiment design was a completely randomized design with five dietary treatments of BLM level and four replications in concentrate feed. The treatments were T0 (without betel leaf meal, as control), T1 (2% of betel leaf meal every day), T2 (4% of betel leaf meal every day), T3 (2% of betel leaf meal skip two days), and T4 (4% of betel leaf meal skip two days). Milk samples from infected quarter would be analysed at first, second, and third week of experiment. Parameters measured were milk composition and somatic cell count. Data were analysed by using analysis of variance (ANOVA) and the differences among treatment were examined by Duncan multiple range test. Results showed that in T1 decreased somatic cell count by 83%, but addition of BLM did not significantly affect milk composition. It could be concluded that addition of 2% of BLM as feed additive has potency to prevent mastitis in lactating cow.
Keywords: Betel leaf meal, dairy cow, milk composition.

Pengaruh Tepung Daun Sirih (Piper Betle L.) sebagai Feed Additive terhadap Komposisi Susu dan Jumlah Sel Somatik Mastitis Subklinis

Abstrak
Penelitian ini bertujuan untuk mempelajari pengaruh tepung daun sirih / betel leaf meal (BLM) sebagai feed additive terhadap komposisi susu dan jumlah sel somatik sapi yang terinfeksi mastitis subklinis. Rancangan percobaan adalah rancangan acak lengkap dengan lima perlakuan ransum tingkat BLM dan empat ulangan pada pakan konsentrat. Perlakuan tersebut adalah T0 (tanpa tepung daun sirih, sebagai kontrol), T1 (2% pemberian tepung daun sirih setiap hari), T2 (4% pemberian tepung daun sirih setiap hari), T3 (2% pemberian tepung daun sirih melewatkan dua hari). , dan T4 (4% dari makan daun sirih melewatkan dua hari). Sampel susu dari kuartal yang terinfeksi akan dianalisis pada minggu pertama, kedua, dan ketiga percobaan. Parameter yang diukur adalah komposisi susu dan jumlah sel somatik. Analisis data menggunakan analisis varians (ANOVA) dan perbedaan antar perlakuan diuji dengan uji jarak berganda Duncan. Hasil penelitian menunjukkan bahwa pada T1 terjadi penurunan jumlah sel somatik sebesar 83%, namun penambahan BLM tidak berpengaruh nyata terhadap komposisi susu. Dapat disimpulkan bahwa penambahan 2% BLM sebagai feed additive berpotensi mencegah mastitis pada sapi laktasi.
Kata kunci: Tepung daun sirih / betel leaf meal, sapi perah, komposisi susu.

Introduction

Dairy industry is play important role to supply protein from animal sources for human consumption. Milk is the main product obtained from dairy industry then processed to be other product including yogurt, butter, cheese etc. Milk is one of the animal products that has a great demand. Thus, it should be produced healthy for human consumption. The main case of dairy farm usually occur is mastitis, especially subclinical mastitis which is costly disease in dairy animals (Salum and Abdul, 2022). Mastitis also reduced milk production, lactose and protein (Hiitiö *et al.*, 2017; Khasanah *et al.*, 2021).

Mastitis is one of the factors affecting milk quality such a hygienic quality of the milk. Milk composition including fat, protein, total solid and solid non-fat usually become variables measurement to determine milk quality.

Somatic cell count is also measured in milk. The highest quality milk most likely resulted from the lowest somatic cell count in the milk (Zigo et al., 2021). The way to overcome this case is prevention through the feed by using herb as feed additive in animal diet. Herbs are usually used as traditional medicine for a long time.

Betel leaf is one of the herbs which has antibacterial compounds to inhibit bacterial growth. Thus, the goal of this research was to observe the effect of betel leaf meal (BLM) as feed additive on milk composition and somatic cell count of subclinical mastitis cows. Yamin et al. (2013) reported that 2% of BLM could inhibit *Staphylococcus sp.* after in vitro fermentation with rumen liquor. Essential oil of betel leaf by 50%, 25%, and 12.5% could also inhibit *Streptococcus agalactiae* and *Staphylococcus epidermis*, but 50% and 25% only inhibit *Staphylococcus* aureus (Poeloengan *et al.*, 2006). Based on previous research, BLM has large potential as feed additive in concentrate feed for healing and protecting cow from subclinical mastitis (Lubis *et al.*, 2020).

Material dan Method

Cows used in this research were 12 Fries Holland dairy cows with normal lactation. All quarters were identified using IPB-1 reagent. Twenty quarters were positively infected by subclinical mastitis (+++) and treated for 3 weeks. Milk samples from all quarters were collected every week for analysis. Complete randomized design was used with 5 treatment and 4 replications. Treatments were applied below;

1. T0: Control
2. T1: Addition 2 % BLM in concentrate diet daily (2d)
3. T2: Addition 4 % BLM in concentrate diet daily (4d)
4. T3: Addition 2 % BLM in concentrate diet skip two days (2s)
5. T4: Addition 4 % BLM in concentrate diet skip two days (4s)

Parameters measured were SCC and milk composition such as protein, fat, total solid, and solid non-fat. All samples of quarters were taken for Somatic cell count, and then analyzed based on Breed method (Sanjaya et al. 2009). Fat determination was done by Gerber Method. There is correlation between fat and protein, it could be measured when the fat is already known. Total solid was measured using Fleischmann equation, but it is needed fat and milk specific gravity at 27.5 ^{o}C. Solid non-fat was archived by reducing of total solid value with milk fat value (Sanjaya *et al*. 2009). Data obtained were analysed statistically (analysis of variance), and then difference between the treatment effects was tested by using Duncan's Multiple Range Test.

Result and Discussion

Effect of betel leaf addition somatic cell count

Cell somatic count is the important variable to evaluate the mammary gland health. Somatic cell count is also used as indicator to determine pathogenicity of mastitis. Subclinical mastitis (+++) was detected and assessed using reagent IPB 1 and treated with betel piper powder in the diet. Result obtained that BLM has beneficial effect on decreasing SCC. Two percent of BLM every day (2d) in concentrate diet could decrease SCC in milk compared among the treatments (Table 1).

The treatment 2d in first week had 1800 x 10^3 cell/ml and decreasing in the third week 300 x 10^3. While 0, 4d, 2s, and 4s also decreased, but those were not stable. Addition 2d of BLM could decrease 494.05% SCC until the end of experiment. Some treatments were decreasing and increasing along the experiment. Subronto (2003), stated that normal milk contains 0 – 200.000 cell/ml and consisted of 65-70% mononuclear, 0-8% neutrophils, <5% lymphocyte, and monocyte. Antibacterial compounds of BLM could kill mastitis bacteria, so infected cell of mammary gland would be improved and decrease somatic cell. SCC is greater than 2 × 105 cells/mL milk according to severity of intramammary infection; with severe intramammary infection, the SCC may reach 1 × 106 cells/mL milk or more within a few hours (Alnakip *et al.*, 2014).

Table 1. Effect of betel leaf meal on cell somatic count (x1000)

Observation	Treatment				
	0	2 d	4 d	2 s	4 s
Week I	4185	1800	5230	3310	1750
Week II	1450	390	4260	7750	5570
Week III	1020	303	8000	2360	1520
Average	2218^{ab}	831^{a}	5830^{b}	4473^{ab}	2946^{ab}

0 = 0% (Control), 2d= 2% everyday, 4d = 4% everyday, 2s = 2% skip two days, 4s = 4% skip two days

Cell somatic is leukocyte degradation in milk involving macrophage, lymphocyte, neutrophil, and epithelial cell (Hossain *et al.*, 2021). Leucocyte mobilization was natural defence mechanism to inhibit bacteria colony which has penetrated in cistern of mammary gland (Rainard, 2017). Therefore. bacterial infection is correlated for increasing of SCC in milk (Sudarwanto, 2008). Leitner et al. (2004) reported that sheep infected *Streptococcus* in mammary gland caused an increasing SCC in the milk. Cell somatic assessment is should be done frequently for early detection of mastitis case in the farm. Even though it is needed additional cost and extra time. SCC is greater than 2 × 105 cells/mL milk according to severity of IMI; with severe IMIs, the SCC may reach 1 × 106 cells/mL milk or more within a few hours.

Effect of betel leaf addition on milk composition

Milk was obtained from BLM treated cows showed the changes on milk composition such as fat, protein, total solid, and solid non-fat (Table 2). Sonea et al. (2009) reported that decreasing 11% of milk fat at subclinical mastitis infecting due to alveolus damage in mammary gland. Level and application of BLM on subclinical mastitis infecting cow showed the change of milk fat. Milk Composition was different significantly ($P<0.05$) among the treatment. Untreated cow (2.63%) was not far different with 2d and 4d (2.56% and 2.58%). 2s and 4s (2.56% and 2.58%) were different ($P<0.05$) among the treatments. These were tending to decrease average of milk fat. Milk fat synthesis depend on number of forages intake. Forage contains cellulose and hemicellulose which is fermented in rumen and producing volatile fatty acid (VFA) such as acetate acid. Acetate acid is precursor for fat milk synthesis (Tyler and Ensminger, 2006). Yamin *et al*. (2013) reported 2 % of BLM in concentrate diet could increase VFA production compared to control (untreated), but 4, 6, and 8% of BLM decreased VFA production. Castillegos *et al*. (2006) reported that essential oil containing eugenol decreased concentration of total VFA and changed VFA profile but it increased propionate concentration.

Diet containing BLM did not significantly influence milk protein than control. However average for each treatment 4d was higher (2.73%) and followed by 2d (2.68%) than control (2.66%). Diet containing 2% skip two day and 4% skip two day (1.85% and 2.04%) of BLM were lower than control. National Standardization Agency for Indonesia (SNI) 01-3141-1998 had been determined that fresh milk should contain 2.70% of protein. Susanty and Nurdin (2011), reported that milk of infected by subclinical mastitis did not change milk protein, decreased casein, and increase whey. Whey is synthesized *de novo* and resistant to proteolytic activity (Raluca and Gavan, 2010). Other result was reported by Kifaro *et al.* (2009) that mastitis decreased protein, milk fat, and chloride but did not affect lactose.

The different results were due to the mastitis pathogenicity of each case. Somatic cell count had a bad effect on milk protein by decreasing total protein (Forsback *et al.*, 2011). Protein also plays important role on lactose synthesis, α-lactalbumin and β-lactoglobulin. It is also a part of *lactose synthase* enzyme which has main function in combining glucose and galactose molecule to be lactose as well as lactose influences milk production.

Table 2. Effect of Betel Leaf Meal Addition on average milk composition from First Week to Third Week.

Parameter	Treatment				
	0	2d	4d	2s	4s
Fat	2.63[a]	2.56[a]	2.58[a]	1.38 [ab]	1.87[b]
Protein	2.66[a]	2.68[a]	2.73[a]	1.85[b]	2.04[b]
Total Solid	9.88[ab]	10.39[a]	9.73[ab]	9.61[ab]	9.39[b]
Solid Non-Fat	8.30[a]	8.08[ab]	7.28[c]	8.00[ab]	7.72[b]

0 = 0% (Control), 2d= 2% everyday, 4d = 4% everyday, 2s = 2% skip two days, 4s = 4% skip two days

Previous experiment of in vitro fermentation on diet containing of BLM did not affect NH_3, but there was tendency that 2% of BLM addition was higher NH_3 concentration compared those control and the other level of BLM addition. Ammonia is the main nitrogen sources of microbes to synthesize amino acid (Sitoresmi *et al.*, 2009) for their growth. Thus, the higher level of BLM addition could inhibit rumen microbe to growth. On the other hand, a decrease in feed protein degradation by essential oil would increase the by-pass protein and thus increase the protein supply to ruminant (Lin et al. 2012) as single-cell protein sources.

Fat, lactose and protein affect total solid of milk. Addition of BLM significantly ($P<0.05$) affect total solid. Total solid tends to be increased by 2d and 4d (10.39% and 9.73%). Fat is the main factor that increases total solid of milk, the higher fat in milk, the higher total solid. The other components of milk are solid non-fat involved protein and lactose. Diet containing BLM did not affect solid non-fat. Control, 2d (8.08%) and 2s (8.00%) were higher than those 4d (7.28%) and 4s (7.28%). Solid non-fat was depend on SCC, the higher SCC could decrease solid non-fat in milk (Raluca and Gavan, 2010). Antanaitis, *et al.* (2021) reported the change in milk lactose is the indicator of mastitis presence because carbohydrates represent approximately 40% of the total solid and 50% of the solid non-fat in milk and milk production is affected by lactose content in milk. The higher lactose in milk, the higher the milk production.

Conclusion

Addition 2% of BLM daily in concentrate diet has beneficial effect on total solid, solid non-fat, protein, fat and tend to decrease SCC in milk. Therefore, it can be used as feed additive to prevent subclinical mastitis in dairy cow.

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