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Original Article

Effect of dietary supplementation of binahong leaf meal, betel nut meal or their combination on serum albumin and globulin, fecal endoparasites and bacterial counts in milk of Saanen goats suffering from subclinical mastitis

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ABSTRACT

The effect was investigated of dietary supplementation of binahong (*Anredera cordifolia* (Ten.) Steenis) leaf meal, betel nut (*Arecha catechu* L.) meal or their combination on serum albumin and globulin, fecal endoparasites and bacterial counts in the milk of Saanen goats suffering from subclinical mastitis. The goats were randomly allotted to one of four experimental groups: control diet (diet without binahong leaf meal or betel nut meal; CON), diet supplemented with binahong leaf meal (1 g/kg body weight; BNH), diet supplemented with betel nut meal (1 g/kg body weight; BTN) and diet supplemented with a combination of binahong leaf meal and betel nut meal (both 0.5 g/kg body weight; BNH + BTN). After 14 d treatment, the pH was higher ($p < 0.05$) in the milk of the CON group compared to the treated groups. The numbers of udders positive for subclinical mastitis were reduced ($p < 0.05$) following the treatments with BNH, BTN or BNH + BTN. Total viable bacteria were higher ($p < 0.05$) in the milk of the CON group than in the treated groups. The numbers of oocytes and coccidial oocysts were higher ($p < 0.05$) in the feces of the CON group than those in the treated groups. There were no differences ($p > 0.05$) in levels of serum albumin and globulin and milk production across the treatment diets. In conclusion, dietary supplementation of binahong leaf meal, betel nut meal and a combination of both showed potential to reduce the load of endoparasites in the gastrointestinal tract and to reduce subclinical mastitis in lactating Saanen goats.

Introduction

The number of commercial dairy goat farms in Indonesia is now increasing due to an increased demand of goat milk especially for medicinal purposes; however, the milk production per goat is still low (Astuti and Sudarman, 2012). Several factors may contribute to low goat milk production, one of which is subclinical mastitis (Gelasakis et al., 2016). The intramammary administration of antibiotics has commonly been practiced to control subclinical mastitis in goats (Poutrel et al., 1997). Although it has proved to be effective for treating subclinical mastitis, such treatment may cause antibiotic resistance which has become a global concern both in human and animals (Virdis et al., 2010). With this background, any efforts to find alternatives to antibiotics would be valuable for the treatment of subclinical mastitis in lactating goats.

A variety of plants have been proposed to substitute the use of antibiotics in animal husbandry, including binahong (*Anredera cordifolia* (Ten.) Steenis) normally known as Malabar spinach, which is a leafy vegetable that is widely found in tropical and sub-tropical area including Indonesia (Miladiyah and Prabowo, 2012). This plant, particularly its leaf, has been reported to exhibit antimicrobial activity against *Escherichia coli*, *Aspergillus niger*, *Vibrio cholerae*, *Staphylococcus aureus* and *Salmonella typhi* (Sen et al., 2010). Accordingly, Priya et al. (2015) confirmed that the presence of secondary metabolites such as steroids and triterpenoids in the leaves of binahong may be responsible for the inhibitory activity of binahong against *E. coli*, *Pseudomonas aeruginosa*, *Bacillus subtilis* and *Aspergillus flavus*. This may suggest that binahong could be exploited for new potential antibiotics. Binahong leaf has also been reported to have potent antihelmintic activity (Deshmukh and Gaikwad, 2014). In such cases, the presence of tannins in binahong seems to be responsible for its antihelmintic potential (Rubesh et al., 2011). In addition to antimicrobial and antihelmintic activities, binahong has

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been reported as a potential source of natural antioxidants (Anusuya et al., 2012), and may thus be beneficial in reducing the incidence of mastitis (Yang and Li, 2015). Indeed, some active components present in binahong leaf, such as flavonoids, betacyanins, phenols, vitamin C and tannins, may be responsible for the antioxidant potential of binahong leaf meal (Anusuya et al., 2012; Deshmukh and Gaikwad, 2014).

Betel nut (*Areca catechu* L.) is another plant known as a good source of antimicrobial compounds such as fatty acids, procyanidins and phenol (Panutat and Vatanyoopaisarn, 2005; Jaiswal et al., 2011; Amudhan et al., 2012). It is grown widely in South and Southeast Asia, and has been popular as a traditional herbal medicine (Peng et al., 2015). Rahman et al. (2014) reported that betel nut may act as an antibacterial agent against Gram-positive bacteria (*S. aureus* and *B. Bacillus*). Panutat and Vatanyoopaisarn (2005) showed the inhibitory effect of betel nut to Gram-positive *Bacillus cereus* and *S. aureus* and Gram-negative *Proteus mirabilis*. Betel nut has also been reported to have anti-nematodal and helmintic effects due to its high content of arecoline (Jaiswal et al., 2011; Peng et al., 2015). Apart from the antimicrobial and antihelmintic effects, betel nut has been known to be a strong antioxidant due to its high content of monomeric, dimeric and trimeric procyanidins (Amudhan et al., 2012; Sazwi et al., 2013). Taken together, the antimicrobial, antihelmintic and antioxidant activities of binahong and betel nut may be beneficial for the treatment of subclinical mastitis in lactating goats. Therefore, the aim of the present study was to investigate the effect of dietary supplementation of binahong leaf meal, betel nut meal or their combination on serum albumin and globulin, fecal endoparasites and bacterial counts in the milk of Saanen goat suffering from subclinical mastitis.

Materials and methods

Preparation of binahong leaf meal and betel nut meal

Binahong leaves and betel nut were purchased from the local market. The binahong leaves were sundried for 2 d, ground and sifted. The betel nut was peeled, sliced and then sundried for 3 d. The dried betel nut was then ground and sifted. The binahong leaf meal and betel nut meal were stored in a dry place until use.

In vivo experiment on lactating Saanen goats

Goats were initially selected that were not pregnant and had 3–4 lactation number, an average milk yield (1.6 ± 0.44 L/d; mean ± SD) and body weight (44.03 ± 3.06 kg; mean ± SD). Goats that did not meet these criteria were excluded. The selected goats were then tested for subclinical mastitis (based on pH and a mastitis test). In brief, the samples of fresh milk (from the 10 udders of 5 goats for each treatment group) were aseptically collected and analyzed for pH using pH indicator strips (pH 0–14, Macherey-Nagel GmbH & Co.KG.; Düren, Germany). Subclinical mastitis was defined as milk having a pH in the range 6.9–7.2 (Andrews et al., 2004). Mastitis indicator paper (Bovivet indicator paper; Kruuse, Denmark) was also used to define the prevalence of subclinical mastitis in the goats. The mastitis test was performed by adding a couple of drops of milk to the spot indicated on the indicator paper. A possible color change was then observed. A change of color from yellow to green or bluish green on the indicator paper was regarded as positive for subclinical mastitis (Megersa et al., 2010). Upon the selection and determination of subclinical mastitis (using mastitis indicator paper on 40 udders from 20 goats with 5 goats per treatment), 20 Saanen goats were eventually selected for the present *in vivo* experiment. These goats were randomly allotted to one of four experimental groups with five goats in each. These experimental groups were: the control diet (diet without binahong leaf meal or betel nut meal; CON), diet supplemented with binahong leaf meal (1 g/kg body weight; BNH), diet supplemented with betel nut meal (1 g/kg body weight; BTN) and diet supplemented with a combination of binahong leaf meal and betel nut meal (both 0.5 g/kg body weight; BNH + BTN). The basal diets provided for the entire experiment consisted of elephant grass (*Pennisetum purpureum*), *Gliricidia sepium*, legumes and concentrate (wheat pollard [63%], cornmeal [20%], coconut cake [10%], soybean meal [5%] and mineral mix [2%]).

Samples of blood, feces and milk were collected on the day before treatment to obtain the baseline values of serum albumin and globulin, fecal endoparasites and bacterial counts, respectively, in the milk of Saanen goats. After 14 d of treatment, samples of blood, feces and milk were again collected. The samples of milk were collected from the two udders of each goat. Blood was obtained by puncturing the jugular vein of each goat and was collected in vacutainers containing no anticoagulant. The blood was then allowed to clot for 2 h at room temperature. The serum was obtained after centrifugation at 2000 rpm for 15 min, and stored at −20 °C until analysis. Total protein in serum was measured using a photometric test according to the biuret method using a kit (total protein kit, DiaSys Diagnostic System GmbH; Holzheim, Germany). Albumin was determined using a photometric test with bromocresol green (DiaSys Diagnostic System GmbH; Holzheim, Germany). Globulin was obtained by subtracting albumin values from the total serum protein. The albumin-to-globulin ratio (A/G ratio) was then calculated by dividing the albumin values by the globulin values.

Fecal samples were obtained aseptically from each goat and immediately analyzed. The numbers of oocytes in the feces were enumerated according to a simple flotation method (Dryden et al., 2005), while coccidial oocysts were enumerated based on a sedimentation technique (Ryley et al., 1976). The samples of fresh milk from each goat were aseptically collected and analyzed for pH, prevalence of subclinical mastitis and total viable bacteria in the milk. The pH and mastitis test (on milk) were determined as described above using the pH indicator strips and mastitis indicator paper, respectively. Total viable bacteria in the milk were enumerated in natrium agar according to the total plate count method (Raikwar and Shukla, 2015).

Data analysis

The data before and after the treatments were analyzed separately according to a completely randomized design using the analysis of variance component in the General Linear Models Procedure in SAS (SAS Inst. Inc.; Cary, NC, USA). To evaluate the time effect, the data within each experimental group (before versus after treatment) were compared using the Student's t-test. The data on number of udders with subclinical mastitis were analyzed with the GLIMMIX procedure in SAS. The data were tested for normality prior to the analysis. Animal was treated as the experimental unit and the results were presented as means and standard error of the means (SEM). Significant differences among treatment groups were further analyzed using Duncan's multiple-range test at a significance level of $p < 0.05$.

Results

pH and Bovivet score of milk

The pH levels of Saanen goat milk before and after the treatments are presented in Table 1. There was no difference ($p > 0.05$) in the pH of milk within each treatment group (before versus after treatment), nor was the pH of milk different ($p > 0.05$) among the groups before the treatments. However, the treatments, the pH was