Total bacteria, total fungus, and water activity content of multinutrient block with green betle leaf levels added as feed supplements for goat

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Abstract. Multinutrient blocks (MNB) have high water content (24.46%) which during storage can damage and reduce its quality. High water content can trigger the growth of bacteria and fungus, so it’s necessary to add natural ingredients such as green betle leaf which functions as anti-bacterial and anti-fungus. This study aims to evaluate the effect of green betle leaf juice addition on total bacteria, total fungus and water activity in MNB during storage. The research used completely randomized design with level addition of green betle leaf juice specifically at 0% (S0), 3% (S1), and 6% (S2). The results showed that the addition of green betle leaf juice with different levels to the multinutrient blocks during storage had a significant effect (p <0.05) on total bacteria and fungus. The addition of 6% green betle leaf juice to the MNB resulted in the lowest average total bacteria and fungus totalling 4.32 x 10^4 CFU / g and 4.66 x 10^4 log cfu / g. Water activity is below 0.6, so that fungus and bacteria in the multinutrient blocks cannot grow. So, it can be stated that multinutrient blocks with the addition of 6% green betle leaf juice can be given safely as feed supplements.

1. Introduction
Multinutrient block is a feed supplement that contains good enough nutrients so that it becomes a solution in meeting the nutritional needs of goats [1]. The purpose of giving multinutrient blocks to livestock is as a supplementary feed that can catalyze poor quality feed into a good ones [2]. Multinutrient blocks are generally given to ruminants in the form of blocks and contain energy, urea, minerals and important proteins for livestock as well as a solution to malnutrition in livestock during the dry season [3].

The nutritional content of MNB is 24.46% water content, 22.25% ash content, 9.34% crude fiber, 1.77% crude fat and 4.69% crude protein [1]. The water content of the multinutrient block (24.46%) can be classified as an Intermediate Moisture Food (IMF) product which has a moisture content in the range of 10 - 40% and a water activity value ranges from 0.6 to 0.9 [4]. The value of water activity can trigger the growth of microorganisms. Each microorganism has a minimum water activity limit so that it can grow in feed, such as bacteria growing on water activity of 0.90 and fungi from 0.60 to 0.70 [5]. The factors that cause the growth of pathogenic bacteria are contamination with air, high water content and nutrient content in materials that are sufficient for the growth of bacteria [6]. The growth of pathogenic...
bacteria in feed during storage can reduce the quality of feed [7]. Humid tropical conditions in Indonesia can facilitate the growth of fungus, especially pathogenic fungus. Feed that has been contaminated by pathogenic fungi can decrease its nutrient content, does not last long and can lead to death in livestock [8]. The decrease in feed quality during storage can be prevented by adding ingredients that are antibacterial and anti-fungus. The addition of natural ingredients to feed can also be used as a substitute for artificial preservatives so that the shelf life of feed is longer and does not leave a residue that is harmful to livestock. Betle leaf is an alternative herbal medicine that has antimicrobial and anti-inflammatory functions.

The part of the green betle plant that is commonly used is the leaf part as a natural herbal medicine. The humid area of Indonesia makes it easy for green betle plants to grow so that green betle leaves are easy to find and obtain [9]. Green betle leaves contain essential oils that are antibacterial with the main components being phenolic compounds and phenol derivatives (eugenol and chavicol) [10]. The betle leaf used is green betle leaf because it has a higher essential oil content (4.2%) than red betle leaf which is only 0.727%. The higher the betle leaf concentration, the more it will reduce bacterial activity. The phenol content found in green betle leaves has a higher bacterial exterminating capability [11]. Thirty percent levels of phenol compounds and some of their derivatives in green betle leaves can damage and penetrate the bacterial walls, function as toxins in the protoplasm and inactivate essential enzymes in the bacterial cells so that bacteria cannot grow [12].

Betle leaf can be used as a natural preservative because it contains phenolic compounds which can prevent the growth of putrefactive bacteria [13]. The essential oil content in green betle leaves can inhibit the growth of bacteria such as Salmonella sp., Streptococcus mutans, Pneumococcus sp, Streptococcus agalactiae and Staphylococcus epidermidis [14]. Betle leaf can also inhibit fungus growth because phenolic compounds are anti-fungus, especially Aspergillus sp. [8]. The addition of green betle leaf to feed with safe levels for consumption by ruminants has not been found much information. Microbiological feed quality testing is necessary to determine the presence of bacteria and fungus. Therefore, in this study, the addition of green betle leaf juice to the multinutrient block is expected to suppress the presence of bacteria and fungus during storage. Apart from being used as a natural preservative, betle leaf is also expected to have a positive effect on livestock because it can inhibit the activity of several gram-positive and negative bacteria, one of which is Escherichia coli [12].

This study aims to examine the effect of different levels of betle leaf juice added to multinutrient blocks on total bacteria, total fungus, and water activity during storage. Water activity is the amount of free water contained in feed and used by microorganisms for activity [15]. Each microorganism has a minimum water activity limit in order to thrive in feed, for example, bacteria can thrive at 0.90 water activity; yeast 0.80 - 0.90; yeast 0.88; halophilic bacteria 0.75; xerophilic bacteria 0.65 and fungi 0.60 - 0.70 [5]. The high water activity value in feed can reduce feed resistance caused by microorganism activity [16]. The level of water activity in the feed is influenced by the moisture content of the feed and the ambient temperature [15]. Multinutrient blocks have a high water activity so that additional preservatives are needed to stop the activity of bacterial and fungus growth. The research hypothesis is that the additional concentration of green betle leaf juice will suppress the growth of bacteria and funguss in multinutrient blocks, which are intermediate moisture food during storage.

2. Materials and methods

Green betle leaf juice and multinutrient block consisting of molasses, fermented rice straw, urea, bentonite, clamshell flour and salt were used as materials. The equipment used includes a blender, a pipe-shaped fungus (8 cm in diameter with a thickness of 4 cm), analytical scales, measuring glass, trays, diskmill type grinders (80 mesh), mixer, plastic (0.4 mm of thickness), paper wrap (thickness of 0.3 mm), incubator, petridishes, test tubes, 1 ml and 10 ml pipettes, colony counters, microscopes, and water activity meters. Multinutrient blocks formulation is as follows:
2.1 Design study
This study was used completely randomized design. The addition of green betle leaf juice was managed as treatment for S0 (0%), S1 (3%), and S2 (6%) with 5 replications in each treatment.
S0 : MNB + green betle leaf juice 0%
S1 : MNB + green betle leaf juice 3%
S2 : MNB + green betle leaf juice 6%

2.2 Method
Protocol research was based on preparation stage, providing multinutrient block, data collection and analysis. The preparation stage was started by making rice straw fermentation for 14 days with 50 ml EM4 as the starter per 25 kg of chopped rice straw, 5 kg of rice bran and 20 litres of water. Fermentation processing was providing in a barrel added with plastic tarpaulin and tightly closed to perform anaerob condition. After 14 days, fermented rice straw was dried under the sun and grinded into a particle size of 80 mesh.
MNB processing was begin with heating molasses for 10 minutes at 40-50°C. This process will reduce the moisture content of molasses and make its texture thicker so that bonding process becomes easier. Furthermore, all the multinutrient blocks ingredients were mixed until they are homogeneous. And then, fresh green betle leaves that have been mashed using a blender and added with 50 ml of distilled water were added according to the treatment level (0%, 3% and 6%) into the multinutrient block mixture and stirred until homogeneous. The homogeneous multinutrient block dough was then weighed in the amount of 100 grams and pressed using a pipe with 8 cm diameter and 4 cm thickness. Pressed multinutrient blocks were then dried in the shade for 3-4 days before packaged using paper packaging. The packed multinutrient blocks were then stored for 40 days at room temperature. Analysis and data collection were carried out after that.

2.3 Parameters observed
The parameters observed in this study were total bacteria, total fungus and water activity. All analyzes were performed after the multinutrient blocks were stored for 40 days. Analysis of total bacteria using the Total Plate Count (TPC) test with the pouring method [7]. The number of colonies growing on the plate is calculated using the following formula:
Number of bacteria = Number of colonies × volume planted × dilution
Analysis of total fungus using the Total Plate Count (TPC) test with the pouring method [17]. The number of fungus colonies growing on a plate is calculated using the following formula:
Number of fungus = Number of colonies × volume planted × dilution
Analysis of water activity is measured using a water activity meter [18].

3. Results and discussion
3.1 Total bacteria on the multinutrient block
The results of the analysis of total bacteria of the multinutrient blocks added with green betle leaf juice with concentrations of 0, 3 and 6% can be observed in Table 2.
Table 2. Total number of flavonoids, fungus, and bacteria in multinutrient blocks given additional betle leaves with different levels.

| Parameter observed    | S0                | S1                | S2                |
|-----------------------|-------------------|-------------------|-------------------|
| Total Flavonoid (ppm) | 15.8304±0.615a    | 29.463±1.0189b    | 31.7855±0.924c    |
| Total Fungi (CFU/g)   | 4.90×10⁴±1.3×10⁴  | 4.71×10⁴±1.7×10⁴  | 4.66×10⁴±1.1×10⁴  |
| Total Bacteria (CFU/g)| 7.82×10⁴±2.2×10⁴  | 5.06×10⁴±5×10³    | 4.32×10⁴±2.7×10⁴  |

a,b,c different superscripts on the same line presented significant differences (P < 0.05), ns (non significant) : not significantly different (P > 0.05). S0 = 0 % betle leaf level; S1 = betle leaf level 3 %; S2 = 6 % betle leaf level.

Analysis of variance showed that multinutrient blocks with the addition of different levels of green betle leaf juice had a significant effect (p <0.05) on total bacteria. The average calculation of total bacteria showed that multinutrient blocks without the addition of green betle leaf juice had the highest total bacteria (7.82 × 10⁴ cfu / g) while multinutrient blocks with the addition of 6% green betle leaf juice had the lowest total bacteria (4.32 × 10⁴ cfu / g). This shows that increasing the level of green betle leaf juice can reduce the total bacteria in the multinutrient block. Duncan's multiple region test showed that the mean total bacteria in the S1 and S2 treatments was significantly (p <0.05) lower than that of S0. The differences in the concentration of the green betle leaf juice may effect to the difference of total number of bacteria [19]. Green bettle leaf extract is effective in infecting both gram-positive and gram-negative bacteria as a natural antibiotic additional to replace artificial antibiotics which are now gradually resistant to several microbes [20].

The addition of green betle leaf juice showed that the higher the concentration of betle leaf juice, the lower the bacterial contamination in the multinutrient block. The higher the concentration of betle leaf, the more antibacterial content (flavonoids and phenol derivatives) so that the ability to inhibit bacterial growth in multinutrient blocks is greater [21]. From the analysis (table 2), it was found that the flavonoid compounds in the multinutrient block with the addition of green betle leaf juice were 3% lower (29.463 ppm) than the multinutrient block with the addition of 6% green betle leaf juice (31.7855 ppm). One of the mechanisms of flavonoid and phenol derivative compounds found in betle leaves in inhibiting bacterial growth is removing wall permeability in bacteria so that the survival of the bacteria is disrupted. The ability of flavonoid compounds in betle leaf is being able to damage bacterial cell walls so that bacterial cells cannot develop properly [22]. The addition of green betle leaf juice with concentration of 5 ml in feed causes the number of worm eggs containing Escherichia coli and Salmonella typhi bacteria to be lower than in feed without betle leaf juice [14].

3.2 Total fungus on the multinutrient block

Data regarding total fungus of the multinutrient blocks added with green betle leaf juice with concentrations of 0%, 3%, and 6% can be seen in Table 2. Based on Table 2, flavonoid content of 31.7855 ppm is mostly found in multinutrient blocks with the addition of green betle leaf juice level of 6%. This flavonoid content belongs to moderate levels of flavonoids [23]. Flavonoid in the range of 10 - 50 ppm belongs to the moderate category, below 10 ppm fits low levels and above 50 ppm indicates high levels. The higher level of flavonoids becomes available for a better impact as an antimicrobial. Flavonoids in green betle leaf juice have a function as antimicrobial compounds [24], as shield against pathogenic microbes and are recognized to be antioxidants, anti-inflammatory, anti-mutagenic and anti-carcinogenic [25].

Analysis of variance showed that the multinutrient block with the addition of different levels of betle leaf juice had a significant effect (p <0.05) on the total number of fungus. The average total fungus yield showed that S0 had the highest total fungus, namely 4.90 log cfu / g compared to S2 which had the lowest total fungus, that is 4.66 log cfu / g. This shows that increasing the level of green betle leaf juice on the multinutrient blocks can reduce the total fungus during the storage period. The total value of
fungus in each treatment is still below the limit. The limit for the amount of fungus growing in feed is $10^5$ cfu [8]. Based on the results of the Duncan multiple area test, the average total fungus of S2 and S1 treatments was significantly (p <0.05) lower than that of S0. The average total fungus in S1 treatment was significantly (p <0.05) lower than S0.

The addition of 3% green betle leaf juice to the multinutrient block was able to reduce the total fungus after the multinutrient block was stored for 40 days. This is referring to the information that a stuff which has a wetness content ranging from 15% to 50% can be understood to be Intermediate Moisture Food (IMF) or semi-wet material. It was able to storage for up to 1 month [26]. Fungus that grow in feed will produce mycotoxins which, if it’s being consumed, may cause mycotoxicosis [27], furthermore once toxins provide in the body of livestock, it will trigger pathological abnormalities to death.

The decrease of total fungus in the multinutrient blocks may increase the durability time of the multinutrient blocks. The addition of green betle leaf flour with concentration of 1%, 5% and 10% in cow feed may reduce Aspergillus flavus contamination, so that the longevity of feed is extended [8]. The decrease of total fungus in the addition of green betle leaf juice is thought to be caused by the presence of anti-inflammatory compounds in green betle leaf juice, that is flavonoids. The flavonoid compounds in green betle leaf can damage the fungal cell membrane so that the cell activity will be disrupted. Essential oil in green betle leaf contains flavonoids and derivative compounds which was able to damage cell membranes in fungus that resulting in cell leakage marked by the release of components in the cell so that cell life is messed up [28]. The reduction in total fungus along with the increase in the concentration of green betle leaf juice is thought to be due to the higher content of flavonoids in green betle leaf juice, so that more fungus cannot grow. An increasing in the concentration of green betle leaf extract is followed by an increasing in the phenol content and its derivative compounds so that the reaction to damage the cell membrane in the fungus is getting stronger[29]. The decrease in total fungus in each treatment can also be influenced by the value of water activity.

### 3.3 Water activity.

Water activity in each treatment is shown in Table 3. This value is safe enough to prevent feed from growing fungus. The minimum value of water activity for fungus growth is 0.60 - 0.70 [5]

| Treatment | Replications | Total | Average |
|-----------|--------------|-------|---------|
| S0 (0%)   | 0.439        | 2,089 | 0.4178  |
| S1 (3%)   | 0.440        | 2,177 | 0.4354  |
| S2 (6%)   | 0.404        | 2,157 | 0.4314  |

$S0 = 0 \% \text{ green betle leaf level}; S1 = \text{ green betle leaf level 3 \%}; S2 = 6 \% \text{ green betle leaf level}.$

The analysis of variance showed that multinutrient blocks with addition of different levels of green betle leaf juice had no significant effect (P> 0.05) on the water activity of the multinutrient blocks. The value of water activity in multinutrient blocks is the amount of free water contained in the multinutrient blocks used by the fungus to grow. The concentration of green betle leaf juice addition is believed not affect the value of water activity and water content in multinutrient blocks so that the growth of bacteria and fungi is inhibited. This condition can be caused by the nature of one of the multinutrient block feed ingredients, specifically salt which has the property of being able to draw water from the feed material so that the resulting water activity value is low. Salt in multinutrient blocks can reduce the activity of water because it draws water out of the material [16]. The mean value of water activity at each level of betle leaf juice addition was 0% (0.4178); 3% (0.4354) and 6% (0.4314) are relatively the same. The low water activity value (less than 0.60) causes microorganisms such as bacteria and fungus to not develop in feed products [15]. The lower the value of water activity in feed ingredients during storage, the lower the growth of microorganisms [5].
4. Conclusion
Based on the research results, it can be informed that the addition of 6% green betel leaf juice was the best level to reduce total bacteria and total fungus in the multinutrient blocks that were stored for 40 days. The low value of water activity notifies that multinutrient block which was added by green betel leaf juice can be given safely as feed supplement for goat.

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