Potential local microorganisms of local plants as a source of probiotics for livestock

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Abstract. The use of antibiotics as a growth promotor in livestock in Indonesia has been banned since January 2018 due to the negative effects of antibiotic resistance. Therefore, this study was conducted aiming to obtain the potential of natural probiotics derived from local plants as an alternative of antibiotics. This study used materials in the form of roots and leaves that exist on the coast, in plantation areas, and in mountainous areas. The coastal area is the crown flower (Calotropis gigantean Dryand), in the plantation area is the fern plant (Nephrolepis biserrata), and in the mountainous area is the citronella plant (Cymbopogon nardus). Plant parts taken are roots and leaves. Each part of the roots and leaves was chopped by 1-2 cm, then as much as 1 kg of material soaked in 4 l of water containing 100 grams of sugar and then stored at room temperature for 21 days in a closed container. This research was conducted in vitro. The parameters measured were physical, namely odor, chemical in the form of pH and total acid, and microbiology, namely total lactic acid microbes. The data obtained were analyzed descriptively and displayed in the form of figures and tables. The results showed that all materials were seen from the physical odor that smelled rather acidic, chemically pH of 3.09-3.58 and had a total acid of 1.07-2.09\% microbiologically there was total lactic acid bacteria (LAB) of 1.5-223 cfu/ml. Potential the best amount of LAB is in crown flower and ferns is in the roots, while the citronella is in the leaves. In conclusion, the three plants studied have the potential of local microorganisms to be used as natural probiotics for livestock, so that alternative antibiotics are cheap, safe and easy to obtain.

1. Introduction
Animal products such as milk, meat and eggs that are exposed to antibiotic residues cause negative effects on human health. Therefore, the Government of the Republic of Indonesia has banned the use of antibiotics as a growth booster of cattle since January 2018. The use of local herbs or plants with...
fermentation technology is expected to be one of the inexpensive, safe and easy antibiotic alternatives. The fermentation process is informed to be able to produce substances that can inhibit pathogenic bacteria namely *Salmonella*, *E. coli*, *Campylobacter* and improve the surface area of the small intestine of ducks [1] and laying hens [2], so that in addition to provide health effects for livestock, it also increases the ability of feed absorption. Ammonia content in manure is also positively influenced by the fermentation process [3]. It was further reported that fermented feed given to broilers could increase body weight and reduce drinking water consumption [4] [5]. In one study, the fermentation feed process will reduce the body weight gain of cattle [6]. However, feeding with fermentation technology needs to be preceded by an adaptation period [2].

Probiotics that are produced through the fermentation process on ingredients found in various regions are expected to be an alternative antibiotic. Probiotics are supplements that are still alive and can thrive in the digestive tract and are usually known as Lactic Acid Bacteria (LAB) which has a positive impact on its host. The existence of probiotics in feed will improve the digestive tract of livestock by inhibiting the growth of pathogens [7]. However, regarding the herbal ingredients that will be used as a source of probiotics from various regions, the potential is not yet known as a source of local microorganisms (LMO). Therefore, this is an opportunity to explore local wealth, as well as complete information from herbal ingredients that represent coastal areas, plantations and mountainous areas or plateaus to be a source of natural probiotics for livestock.

### 2. Research Methods

This research was carried out for 45 days. Herbal ingredients were taken at three locations in Aceh. The research material consisted of roots and leaves collected from the coastal area which is the crown flower (*Calotropis gigantean Dryand*), in the plantation area which is the fern plant (*Nephrolepis biserrata*), and in the mountainous area which is the citronella plant (*Cymbopogon nardus*). As much as 1 kg of roots after being removed from the soil and leaves, was chopped 1-2 cm each, then 1 kg of material was soaked in 4 liters of distilled water containing 100 grams of sugar which was then incubated for fermentation in rooms temperature in silo without LAB addition. The fermentation process was carried out at the place of taking materials (crown flower on the Coast in Banda Aceh city, fern plant in the Aceh Jaya plantation area and citronella plant in the Gayo Lues plateau area).

After the fermentation period, the fermented herbal analyzed at the Laboratory of Milk Processing Science and Technology, Faculty of Agriculture, Syiah Kuala University.

The solution taken as much as 100 ml to be taken physically was odor, chemically that were pH and total acid, and microbiologically was total LAB. Physical test was done by using the sense of smell by giving a score; 1 = bad odor, 2 = slightly acidic, 3 = discarded acid. Furthermore the pH chemical test was carried out using a pH meter that is put into the solution, the number printed on the monitor shows the pH content of the sample. The digital pH meter used was calibrated by comparing standard specifications first. Total acid use the titration method. Microbiology test used the Total Plate Count method on a 10^-4 dilution. Fermentation solution added with distilled water by adding 1: 2 taken as much as 1 ml put into 5 ml of distilled water, then diluted by taking 0.5 ml put into 5 ml of distilled water until dilution 4 times. Then as much as 1 ml of the fourth dilution was planted in the medium containing MRS agar media. Media agar that has been planted with fermented feed samples was incubated for 3 days in an incubator. Colonies that grew roundish rather yellowish are LAB colonies calculated using a colony counter. LAB population (cfu / ml) = number of colonies x dilution. The data analyzed descriptively.

### 3. Results and Discussion

#### 3.1. Physical Test

Physical test results consisting of odors on probiotics fermented on several herbs from various differentials can be seen in Table 1.
Table 1. Physical test results in the form of odor on the fermented herbs for 21 days

| Herb               | Roots | Leaves |
|--------------------|-------|--------|
| Crown flower       | 3     | 3      |
| Fern plant         | 3     | 3      |
| Citronella plant   | 2     | 2      |

Note: Value 1 = bad odor, 2 = mildly sour odor and 3 = sour odor

The smell of acid indicates that the fermentation process was happening well. This was indicated by numbers 2-3 which means the smell was slightly acidic. This figure was based on a simplification of a similar study [8]. The better the anaerobic silo condition, the better the fermentation process so that it will produce a product that smells sour. On the contrary, if the silo is not in good condition, then the fermentation process will be disrupted so that it will be contaminated by spoilage bacteria that degrade protein into ammonia so that the fermentation product does not smell acidic but has a bad odor. The fermentation process can be seen in Figure 1.

Figure 1. Roots and leaves cut and put into silos with water and sugar added

Additives that were given in probiotics process also accelerate the fermentation process and improve physical quality so that the acid odor was achieved [9]. Dissolved carbohydrate [10] added in this research in the form of sugar was an additive so that it showed the success of the fermentation process so that the odor ranges in value 2-3. High water content used in the fermentation process also increases the chance of local microbes in the form of LAB in all three herbs that can work optimally [11, 12]. The smell of acid indicates that the three herbs have the potential to be a source of probiotic ingredients that contain local microorganisms.

3.2. Chemical tests are pH and total acid
Chemical test results in the form of pH and total acid in fermented probiotics on several herbs from various different plains can be seen in Table 2.

Table 2. pH and total acid values in fermented herbs for 21 days

| Ingredient               | pH   | Acid Total (%) |
|--------------------------|------|----------------|
| Roots of crown flower    | 3.38 | 1.75           |
| Leaves of crown flower   | 3.22 | 1.83           |
| Roots of fern plant      | 3.60 | 1.60           |
| Leaves of fern plant     | 3.57 | 1.52           |
| Roots of citronella plant| 3.09 | 1.07           |
| Leaves of citronella plant| 3.58| 2.09           |

Note: Results of the analysis of the Milk Processing Science and Technology Laboratory, Faculty of Agriculture, Universitas Syiah Kuala (2019)
The value of pH ranging from 3.09 to 3.60 indicates the state of acidic probiotic solutions. LAB as a gram-positive bacterium is able to produce acid, high pH or acidity indicates that LAB content is also high [13]. The highest LAB content when viewed based on the lowest pH value is in the herbal ingredients of citronella plant root, while the highest pH is in the roots and fern leaves, and citronella plant leaves. However, in general the pH of the probiotic solution shown in Table 2, showed that the three herbal ingredients have the potential to be a source of local probiotics. The maximum growth of LAB can reduce the pH value [14], so that the acid atmosphere will suppress the growth of pathogens in the digestive tract when given to livestock.

The highest percentage of total acid at the time of observation after 21 days of fermentation [14]. The three herbs were fermented using 21 days, allegedly the fermentation process was optimal. The total amount of acid corresponds to the total LAB presents in the probiotic solution. The total acid contained in fermented herbs is around 1.07-2.09%. According to a study a post-harvest feed material before fermentation had $10^6$ LFB per gram of material [15], so the amount of LAB after fermentation would increase linearly with the total acid contained. If the material does not contain LAB, then acidic and total acid pH will not occur. The three herbs both leaves and roots originating from various plains were able to reach pH 3, this was not only determined by the fermentation process but also determined by the state of LAB available in the chemical ingredient.

3.3. Microbiology test that is total LAB

Microbiological test results, namely the total LAB on probiotics fermented on several herbs from various different plains can be seen in Table 3.

| Herb            | Total LAB cfu/ml |
|-----------------|------------------|
|                 | Roots | Leaves |
| Crown flower    | 223   | 130,5  |
| Fern plant      | 130,5 | 1,5    |
| Citronella plant| 14    | 143,5  |

Table 3. Total LAB on fermented herbs for 21 days

Note: The results of the analysis of the Laboratory of Milk Processing Science and Technology, Faculty of Agriculture, Universitas Syiah Kuala (2019), on dilution 4 times.

The total LAB of the three herbs both in the roots and in the leaves varies. In crown flower and ferns, the roots had a higher total LAB compared to the leaves, but on the other hand, citronella plant leaves had higher BAL than the roots. Although the total LAB are vary greatly, the pH can reach 3 and the total acid formed above 1%. This showed that the condition of probiotics derived from the three herbs has undergone a perfect fermentation process, however probiotic treatment highly depend on the total amount of LAB and its ability to live in the host digestive tract. The amount of dissolved carbohydrate and water content greatly influences the total LAB in the fermented product [12, 14], so that the fermented product did not undergo decay by pathogenic bacteria [16].

4. Conclusions

Sources of probiotics derived from local microorganisms for livestock can be used as crown flower plants and fern roots in coastal areas and plantations, while Citronella plant originating from the highlands can use leaves.

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