Effect of incubation time of sago (*metroxylon sago*) waste by local microorganism "*ginta"* on pH, crude protein, and crude fiber content

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Abstract. This study aims to examine the effect of incubation times of sago waste by local microorganism (MOL) “*Ginta*” to the crude protein and crude fiber content in relation to finding a cheap and good quality ruminants feed alternative. Incubation times were 0 hours to 144 hours. The data obtained were analyzed using Completely Randomized Design consisting of seven treatments and three replications. The result showed that the duration of incubation of sago waste by local microorganism (MOL) “*Ginta*” caused pH reduction, improved crude protein and crude fiber content. pH reduction was from 7.03 at 0 hour to 4.05 at 144 hours incubation. The highest increased in crude protein was H6U3 (5.58%) : 144 hours incubation and the lowest was H0U2 (3.22%) : 0 hour incubation while the highest crude fiber was H0U1 (19.99%) : 0 hour incubation and the lowest was H6U3 (18.23%) : 144 hours incubation. It can be concluded that incubation of sago waste triggered lower pH, higher crude protein and lower crude fiber than unoinoculated. A recommendation could be given on using MOL “*Ginta*” in order to produce a cheap and good quality ruminants feed alternative.

Keywords: sago waste, local microorganism (MOL) “*Ginta*”, crude protein, crude fiber

1. Introduction
Sago waste which is a by product of sago industry, has the opportunity and potential to be used as one of the alternative source of fibrous feed ingredients, because it has very high organic contents that potential as a source of energy. However, sago waste has low protein and high fiber content as for a feed [1].

According to Rumalatu [2] the processing of sago flour was obtained on flour and sago waste with a ratio of 1: 6. Sago waste was used to discharge in the location of sago flour processing which caused environmental pollution. Sago planting area in Indonesia has a land area of 1.6 million hectare BPS 2015 [3], where sago could be produced as much as 15 million tons because each sago stalk can produce 200 kg sago [4]. In Indonesia sago planting spread from Irian Jaya 1,406,469 Ha, Maluku 41,949 Ha, Sulawesi 45,540 Ha, Borneo 2,795 Ha, West Java 292 Ha to Sumatra 31,872 Ha [3].

Sago processing waste is classified as wet by-products because it still contains 75-80% moisture content, which can be degraded quickly if not processed immediately. Treatment by drying is commonly done before it is given to livestock. The nutrients contained in sago waste such as; states That the sago pulp contains 65.7 % starch, 14.8 % crude fiber, 1 % crude protein, and 4.1 % ashAdb-
Aziz[5]; The content of nutrients contained in sago waste are: crude protein of 3.36%, NDF 67.40%, ADF 42.11 and crude energy of 3,738 Kcal / kg, relatively comparable to grass [5]. With the nutrient content, sago waste is estimated to be only able to meet the basic needs of life, so for growth, pregnancy and lactation required additional feed to meet protein and energy needs of ruminants.

An alternative technique to improve nutrition quality of sago waste is by doing fermentation. In doing so, it is not only nutrition quality improved but also digestion and elimination of aflatoxin or toxic compounds. The fermentation process is done by adding for example a starter of local microorganisms (MOL) "Ginta" which corresponds to the substrate and the purpose of the fermentation process. The use of mold of local microorganism (MOL) "Ginta" as a starter in the fermentation process is deemed most suitable and in accordance with the purpose of fermentation, namely to reduce the levels of crude fiber and at the same time to increase levels of crude protein. "Ginta" was created in the purpose that people in rural area could do fermentation without depend on commercial fermentor. This study aims to investigate effectiveness "Ginta" in fermenting sago waste on improving low protein content and high fiber.

2. Methods
This research had been conducted at Research and Technology Laboratory Faculty of Agriculture University of Sumatera Utara, Medan. Research started from December 2016 until February 2017. The materials used in this research were: sago waste, Local Microorganism (MOL) "Ginta" as source of isolate, aquadest as addition of water in MOL of "Ginta", alcohol as aseptic material, spiritus as bunson fuel while working with media, media agar as new culture media for bacterial isolates. The tool were used autoclave, Erlenmeyer flask, petri dish, and ose loop.

The study was prepared based on Completely Randomized Design (RAL) pattern with 7 (seven) treatments with 3 (three) replications so that 21 units of experiments were obtained. The treatment arrangement based on the duration of incubation as follows [7]:

H0 : incubation time 0 hour, H1 : incubation time 24 hour, H2 : incubation time 48 hour, H3 : incubation time 72 hour, H4 : incubation time 96 hour, H5 : incubation time 120 hour, H6 : incubation time 144 hour.

Fermentation of sago waste with local microorganism inoculum MOL "Ginta" was done with initial pH 6 and temperature 35 °C with long treatment incubation 0, 24, 48, 72, 96, 120 and 144 hours. The fermentation path can be seen in Figure 1.

The data obtained were analyzed using orthogonal polynomial test to see the influence trend of incubation duration on crude protein content and crude fiber of sago waste [7]. Furthermore, the data were analyzed statistically with the help of SPSS software Ver.13.0 to see the response curve. Parameters observed in this research were crude protein content [8] and crude fiber content [8].

![Figure 1. Flow chart of Sago Waste Fermentation with Inoculum “Ginta”](image-url)

*Nutrient mix consists of: Sucrose 25-50 g/l, Ammonium nitrate (NH4NO3) 2.25 g/l, Potassium hydrogen phosphate (KH2PO4) 0.3 g/l, Magnesium (MgSO4) 0.25 g/l.*
3. Results and Discussion
The mean data of crude protein and crude fiber content of sago waste (*Metroxylon sagu*) inoculated with Local Microorganism (MOL) "Ginta" can be seen in Table 1. Inoculation with variation of time influenced pH, crude protein and crude fiber of sago waste.

3.1. pH
"Ginta" was created by adding kefir while it contained a lot lactic acid bacteria [9]. As the result bacteria metabolized carbohydrate became lactic acid which caused declined of pH. In this research, it was found that at 0 hour pH was 7.03 and at 144 hour pH was 4.05. Lower pH such as 4 was not a problem for sago to be ruminant feed as in the early stage of feed chewing, ruminant produced a lot of saliva which has an alkaline pH. As the result of chewing process was finely feed with normal pH Campbell et.al [10]. Research by Araujo [11] on youghurt while it also contained of BAL, there was a decrease in pH between 3.84 and 3.98. Sunarlim [12] found that there was a dominated bacteria amongs BAL which was *Lactobacillus plantarum* and that the benefits of BALs for a lot of health were included interfering with pathogenic bacteria, lowering cholesterol, anti-mutagenic, anti-carcinogenic and improving immune system.

3.2. Crude Protein
The influence of the incubation duration of sago waste (*Metroxylon sagu*) by using Local Microorganism (MOL) "Ginta" on crude protein content based on response curve analysis can be seen in figure 2. Based on the results of the response curve analysis, it is known that crude protein gives polynomial response (quadratic) to the duration of incubation of sago pulp. The amount of correlation incubation duration of sago to crude protein content is 68.1%. The increment of crude protein content in the sago pulp of H6U3 is incubation for 144 hours due to the addition of microbial protein from local microorganism (MOL) "Ginta" which is called single cell protein. This is supported by the opinion of Tampoebolon [13], which states that the local microorganism (MOL) was a single cell protein (mold) containing high protein, so that like in this research the addition of starter with Local Microorganism (MOL) "Ginta" on sago waste added raw protein content of fermented sago waste. Najafpour [14] mentioned that microbial protein known as Single Cell Protein (SCP) or Single Cell Protein. Single Cell Protein is a term used for rough or pure protein derived from microorganisms, one of which is mold.

| No. | Treatment | CP content (%) | CF content (%) |
|-----|-----------|----------------|---------------|
| 1.  | H0U1 (0 h) | 3.25           | 19.52         |
| 2.  | H0U2 (0 h) | 3.22           | 19.96         |
| 3.  | H0U3 (0 h) | 3.23           | 19.94         |
| 4.  | H1U1 (24 h) | 4.11           | 19.99         |
| 5.  | H1U2 (24 h) | 4.15           | 18.99         |
| 6.  | H1U3 (24 h) | 4.18           | 18.99         |
| 7.  | H2U1 (48 h) | 4.28           | 18.80         |
| 8.  | H2U2 (48 h) | 4.30           | 18.72         |
| 9.  | H2U3 (48 h) | 4.32           | 18.68         |
| 10. | H3U1 (72 h) | 4.50           | 18.57         |
| 11. | H3U2 (72 h) | 4.52           | 18.55         |
| 12. | H3U3 (72 h) | 4.55           | 18.50         |
| 13. | H4U1 (96 h) | 5.01           | 18.49         |
| 14. | H4U2 (96 h) | 5.11           | 18.48         |
| 15. | H4U3 (96 h) | 5.18           | 18.46         |
16. H5U1 (120 h)  5.20  18.43
17. H5U2 (120 h)  5.23  18.40
18. H5U3 (120 h)  5.25  18.38
19. H6U1 (144 h)  5.36  18.28
20. H6U2 (144 h)  5.46  18.26
21. H6U3 (144 h)  5.58  18.23

Figure 2. Respond Curve of The Influence of Incubation Duration of Sago (Metroxylon sago) Waste Using Local Microorganism (MOL) ‘Ginta’ against Crude Protein

| Time | Crude Protein |
|------|---------------|
| H0   | 1            |
| H1   | 2            |
| H2   | 3            |
| H3   | 4            |
| H4   | 5            |
| H5   | 6            |
| H6   | 7            |

The content of crude protein in the sago waste begins to decrease in the treatment H0U2 (0 hours). This is because within 0 hours Local Microorganism (MOL) "Ginta" has not developed yet. The first and second day of the Local Microorganism (MOL) "Ginta" is in the lag phase, this phase is a microbial adjustment phase with the new environment. During this phase, cell division was slow. Day 2 (24 hours) to 3 (72 hours) growth of Local Microorganism (MOL) "Ginta" experiencing logarithmic phase, in this phase microbe is actively doing metabolism. Day 4 (96 hours) to 6th (144 hours) Local Microorganism (MOL) "Ginta" enters stationary phase, in this phase no longer develops. In this phase the cells become small because the cells keep splitting although the availability of nutrients in the medium has been greatly reduced. After undergoing the stationary phase the microbes begin to enter the phase of death. According to Vikineswary et.al [1], prior to incubulation, sago waste could be supplemented with source of nitrogen and that the result of microbial utilization was a good quality of animal feed.

3.3. Crude Fiber
The influence of the incubation duration of sago pulp (Metroxylon sago) by using Local Microorganism (MOL) "Ginta" on crude fiber content based on response curve analysis can be seen in Figure 3.
Based on the results of the response curve analysis, it was known that the crude fiber gives polynomial response (linear) to the duration of incubation of the sago pulp. The amount of correlation duration of sago dregs incubation by using Local Microorganism (MOL) "Ginta" to crude fiber content is 92.7%. The decrease of crude fiber content of fermented sago waste with Local Microorganism (MOL) "Ginta" from day 0 to day 6 that is in treatment H0 until the treatment of H6 indicates that Local Microorganism (MOL) "Ginta" play an active role to produce enzyme Cellulase that serves to degrade the sago pulp. This is supported by the opinion of Tampoebolon [13] that the increase of incubation time causes the increase of local microorganism (MOL) "Ginta" to grow and fermentation, so that the incubation time will increase the chance of local microorganism (MOL) "Ginta" to degrade the higher. Cellulase enzyme is one of the enzymes produced by microorganisms that function to degrade cellulose into glucose. The decline in crude fiber in the sago fermentation results was due to the work of the cellulase enzyme produced by the local microorganism (MOL) "Ginta" which works to overhaul the crude fiber. This is supported by the opinion of Nurhayati[15], that the good growth of molds of local microorganisms (MOL) "Ginta" is expected to produce enzyme cellulase in large quantities so that it can be used to remodel and lower crude fiber.

4. Conclusion

The inoculation sago by local microorganisms (MOL) "Ginta" caused less pH, higher crude protein and lower crude fiber than non-inoculation. Increased duration of incubation from 0-144 hours in protein content gave a quadratic response where the highest peak of protein content was more in the duration of incubation of 120 hours. The increase in incubation duration from 0-144 hours on crude fiber content gave a linear response wherein the lowest crude fiber content was seen at the time of incubation of 144 hours.

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