

# Innovative Bio-processing: Turning Water Hyacinth and Vegetable Waste into High-Quality Animal Feed

Apriansyah Susanda Nurdin\*, Anggriawan Naidilah Tetra Pratama\*, Fitri Nova Liya Lubis\*, Dyah Wahyuni\*, Sofia Sandi\*

\* Department of Technology and Livestock Industry, Faculty of Agricultural, Sriwijaya University, Indralaya, South Sumatera, Indonesia

**Abstract-** The study was aimed at developing innovation in the utilization of water hyacinth as animal feed through bio-processing or fermentation process derived from vegetable waste in the form of cabbage and mustard greens. The research findings were water hyacinth and bacterial starter obtained from the extraction process of vegetable waste. The design used was completely randomized consisting of four treatments and five replicates with the following composition P0 as control, P1: Starter 20%; P2: Starter 40%; and P3: Starter 60%. The observation variables consisted of 2 stages, which were physical and chemical post-fermentation quality, and in vitro material digestibility. The results of descriptive analysis showed that the treatment of 20 and 40% starter produced the best physical quality when compared with other treatments, however, in the observation of chemical quality, the 60% treatment showed a decrease in crude fiber content and an increase in crude protein which was then followed by an increase in dry matter digestibility, organic matter digestibility and in vitro feed fermentation quality which included an increase in VFA and  $\text{NH}_3$  values and a decrease in pH ( $p < 0.05$ ). Based on this study, it can be concluded that the use of starter bacteria obtained from vegetable waste such as cabbage and mustard by 60% is able to have a major impact on the physiological and chemical quality and digestibility of water hyacinth.

**Index Terms-** Bio-Processing, Water Hyacinth, bacterial starter, vegetable waste.

## I. INTRODUCTION

The realization of the uses of grasses or forages as animal feed has been a common occurrence, including the development of ephipitic microorganisms from these forage sources ([1]–[4]). Indonesia is part of a developing country with a tropical climate that has great potential in increasing the potential of animal feed biodiversity potential, because the environmental status is suitable for the development of various forage varieties and microorganisms, especially since these varieties have a tendency to need solar energy to carry out the photosynthesis process ([5], [6])

Among the types of weeds that are widely developed in Indonesia is water hyacinth or better known by the local name as eceng gondok, however, the area of life that requires water makes this type of plant grow rapidly on several islands in Indonesia such as

Kalimantan and Sumatra the large area of swamp areas on both islands is a factor indicating the rapid development of water hyacinth, besides that the rapid growth of water hyacinth is often considered a threat to agricultural land because it can inhibit the growth of food crops in the region ([7]). Nevertheless, a rapid development can be an interesting factor as a reference for use as animal feed because of the availability that was able to be fulfilled in a certain time period ([8])

Proximate analysis results that have been conducted in many studies show that water hyacinth has a fairly high content of organic matter, vitamins, and fat. The crude protein content can reach 21% in the leaves, while the stems and roots range from 6-9%; with a fairly complete amino acid composition compared to other types of weeds ([9]). Interestingly, despite its many advantages, water hyacinth cannot be used as an independent feed (basal); because water hyacinth has a limiting factor, the high fiber content which can reach above 30%. Therefore, to solve this problem, advanced feed processing techniques are needed to optimize the use of water hyacinth as animal feed ([10]–[12]).

Fermentation is a feed processing technique that is currently being used as a method to answer problems in the limited utilization of fiber in various types of forage. Several benefits are obtained including increasing digestibility and aroma which in turn can have an effect on the high absorption of nutrients and consumption rates. The group of microbes that have an important role in the fermentation process are yeast (yeast), mold (mold), bacteria and several species of actinomycetes. The principle of fermentation is to separate lignin and cellulose. The research by Susanti et al [12] showed that the fermentation process was able to increase the content of water hyacinth nutrient content, which was Protein by 10-14%, and reduce crude fiber up to 14%.

Basically, the starter used as a source of decomposers during the fermentation process can be found commercially or sourced in the inoculation process independently obtained from various types of vegetable or animal products available in nature ([13], [14]). Based on this, an experiment was conducted on Bioprocessing Technology or water hyacinth fermentation using starters derived from cabbage and mustard waste as animal feed

## II. MATERIALS AND METHODS

### A. Preparing samples and experimental design

This study embarked on a journey to explore the potential of locally available resources, specifically water hyacinth, cabbage, and mustard waste, as primary substances. The water hyacinth

samples were not just randomly picked; instead, they were carefully collected from a diverse range of swamplands scattered across the area Indralaya, ogan ilir-south Sumatera, Indonesia. This ensured a broad representation of the local ecosystem in the study. In parallel, vegetable waste, comprising cabbage and mustard remnants, was gathered from the closest traditional market. This step not only promoted recycling of waste but also added another layer of local relevance to the research. Once the collection phase was completed, the study moved into its next critical phase: the fermentation process. The water hyacinth waste, along with the cabbage and mustard waste, underwent fermentation. This process was not merely about breaking down the waste; it was primarily aimed at cultivating microorganisms. These microorganisms served as the primary starter in this study, playing a pivotal role in the subsequent stages of the research. The research design employed was a completely randomized design, a robust statistical method that ensures the validity of the results. It focused on four distinct treatments, each replicated five times to account for any variability and to strengthen the reliability of the findings. The treatments used in this study were carefully chosen to cover a broad spectrum: P0 served as the control, providing a baseline for comparison; P1 incorporated a 20% starter, offering a moderate level of intervention; P2 escalated the starter to 40%, exploring the impact of a higher level of intervention; and P3 pushed the boundaries with a 60% starter, investigating the effects of a substantial level of intervention. This comprehensive and meticulous approach ensured that the study was not only grounded in the local context but also held up to rigorous scientific standards. It is a testament to the potential of integrating local resources, scientific methods, and sustainable practices in conducting meaningful and impactful research.

#### B. Preparation of vegetable waste fermentation starter

The method of making starter in this experiment was based on the method of Wikanastri et al [15] which was then modified at different quantities. The process was as follows: Mustard cabbage waste is washed and sliced with a size of approximately 1-2 cm; then drained for approximately 10 minutes. After that, cabbage and mustard waste were weighed as much as 1 kilogram and salt as much as 30 grams or as much as 3%. Furthermore, the ingredients were mixed evenly into a closed jar, then closed tightly

### III. RESULTS AND DISCUSSION

#### A. Physical and chemical quality

Based on the results of descriptive analysis, it shows that the pH value, and temperature in each treatment are still within the range of reasonableness or acceptable. The pH value ranging from 4.64 - 5.44 can be categorized in acidic conditions, which seems to illustrate that the derivate compounds during fermentation are dominated by acids. These acidic compounds are generally produced in the form of highly volatile acids such as acetate and butyrate ([21]), while lactic acid compounds that are usually produced by lactic acid bacteria in the silage making process have the possibility to appear because the results of various types of research report that cabbage and mustard seeds have the potential to produce lactic acid bacteria which are quite abundant ([22], [23]). The temperature can describe the type of fermentation that occurs, where the higher the energy of the raw material used as the

and incubated for 9 days. The fermentation results were then filtered to take the supernatant.

#### C. Water hyacinth fermentation processing

Water hyacinth was washed and cut into 2-3 cm pieces. then dried for 6 hours. After that, prepare twenty 5 kg plastic containers. Each plastic was filled with 1 kg of dried water hyacinth and added fermentation extract/supernatant according to the treatment, namely: five plastic inoculated with 0% starter (first treatment as control with five replications), five plastic inoculated by 20% starter (second treatment with five replications), five plastic inoculated by 40% starter (third treatment with five replications), and five plastic inoculated by 60% starter (fourth treatment with five replications). After completion, the samples were opened and analyzed for physical and chemical quality. as for the digestibility test, the samples were dried and grinded until fine..

#### D. Observed variables

The methods employed for these observations were not arbitrary; they were based on established and recognized techniques in the field. The physical quality test was conducted in accordance with the method proposed by Rajan Katoch [16] and Tingshuang et al. [17]. These methods are known for their robustness and reliability in assessing the physical quality of materials. The nutritional quality test, on the other hand, was carried out based on the Tiley and Terry method [18]. This method is renowned for its accuracy in measuring the digestibility value, a key indicator of the nutritional value of feed. In addition, the Association of Official Analytical Chemists (AOAC) method was used to measure the nutrient value of the treatment [19]. The AOAC method is a globally recognized standard for such analyses, further enhancing the credibility of the findings.

#### E. Statistical analysis

Analysis consisted of 2 forms: descriptive physical quality data and quantitative digestibility and nutrient composition data. Quantitative data obtained were analyzed using Statistical Package for the Social Sciences (SPSS) version 20 if there were differences between treatments then continued with the Duncan multiple range test (DMRT) [20].

main ingredient of fermentation, the higher the resulting temperature [24]). However, in reality there are still many studies that report that there is no relationship between the source of fermentation material and the temperature produced ([25]).

In line with these results, the physical quality of each treatment also shows interesting results, where the color, aroma, and texture indicate the presence of lactic acid bacteria activity which can be seen from the color of the treatment sample which is still greenish; has a strong sour aroma; and has a medium to hard texture. However, this was only found in the P0 and P1 treatments, while P3 and P4 had changes in color and texture, which were yellowish and brown and had a soft texture. This change can occur allegedly due to many factors such as the activity of cellulolytic and fibrolytic bacteria that can change the texture of the fibers in the forage so that the structural treatment samples become soft or due

to the influence of acid during the fermentation process which can loosen fiber bonds which further affect the color pigments possessed by the sample ([26]–[28]).

Table 1. Physical quality and chemical composition of water hyacinth fermented with vegetable waste starter bacteria

Parameters	Treatments			
	P0	P1	P2	P3
PH value	5,34	5,44	5,42	4,64
Temperature	28	27,6	27,6	28
Color	Greenery	Greenery	Yellowish	Chocolate
Aroma	Acid	Very Acidic	Very Acidic	Very Acidic
Texture	Hard	Medium	Slightly Soft	Flabby
Water content	6,54 ± 0,19	6,48 ± 0,05	6,38 ± 0,45	6,67 ± 0,25
Crude protein	11.33 <sup>a</sup> ± 0.64	10.53 <sup>a</sup> ± 0.75	11.35 <sup>a</sup> ± 0.43	12.53 <sup>b</sup> ± 0.73
Crude Fiber	20.90 <sup>d</sup> ± 1.62	17.83 <sup>c</sup> ± 0.74	15.96 <sup>b</sup> ± 1.43	13.98 <sup>a</sup> ± 0.82
Extract ether	1.36 <sup>b</sup> ± 0.31	1.22 <sup>ab</sup> ± 0.45	0.81 <sup>a</sup> ± 0.06	0.97 <sup>ab</sup> ± 0.33

<sup>abc</sup>Different superscript letters in the same column showed a significant difference ( $p < 0.05$ ); P0 (Control); P1(20% Starter); P2(40% Starter); and P3 (60% Starter).

Table 2. In vitro digestibility and fermentation quality of water hyacinth fermented with vegetable waste starter bacteria

Parameters	Treatments			
	P0	P1	P2	P3
DMD	46,29 <sup>a</sup> ±1,51	53,09 <sup>b</sup> ±1,17	57,31 <sup>c</sup> ±1,03	63,59 <sup>d</sup> ±0,81
OMD	34,43 <sup>a</sup> ±2,07	37,10 <sup>b</sup> ±1,65	37,69 <sup>b</sup> ±1,54	42,99 <sup>c</sup> ±1,99
pH	6,98 <sup>d</sup> ±0,02	6,86 <sup>c</sup> ±0,01	6,81 <sup>b</sup> ±0,01	6,78 <sup>a</sup> ±0,01
VFA	73,04 <sup>a</sup> ±10,48	104,13 <sup>b</sup> ±5,76	124,42 <sup>c</sup> ±9,50	143,07 <sup>d</sup> ±13,10
NH <sub>3</sub>	11,26 <sup>a</sup> ±0,45	12,63 <sup>b</sup> ±0,39	14,16 <sup>c</sup> ±0,84	16,30 <sup>d</sup> ±0,55

<sup>abc</sup>Different superscript letters in the same column showed a significant difference ( $p < 0.05$ ); P0 (Control); P1(20% Starter); P2(40% Starter); and P3 (60% Starter); Dry Mater Digestibility (DMD); Organic Matter Digestibility (OMD); Volatile Fatty Acid (VFA).

This can be seen from the observation of aroma where although changes in color and texture occur, but in the P3 and P4 treatments, the aroma produced is very sour. The strong sour aroma during the fermentation process can be strongly suspected due to the presence of acidic compounds, although in detail it cannot be classified as entirely lactic acid because the fermentation process of organic materials such as forage can produce other acidic compounds such as acetate and butyrate ([29], [30]).

Nutrient composition based on statistical analysis shows that there is a significant change in the composition of crude fiber, protein, and fat in the P3 treatment ( $p < 0.05$ ). these changes are thought to be due to the activity of bacterial starters that were able to breakdown the complex fiber bonds present in water hyacinth, which is known that water hyacinth contains high fiber. Crude fiber is a limiting factor in feed digestibility because crude fiber is a cell wall that protects the cell nucleus consisting of protein ([31][32]). Thus, the breakdown of crude fiber can certainly cause the protein content contained in water hyacinth to be released optimally. Although this process is also suspected to be the cause of fat reduction, especially in the 40% starter treatment. In line with this statement, shown in the P3 treatment, the decrease in crude fiber followed by an increase in crude protein is strongly suspected to occur due to the use of 60% starter which is assumed to be dominated by cellulolytic bacterial activity that reduced the fiber composition in water hyacinth. The results of the study revealed that the results of extracts or inoculants of microorganisms obtained from vegetable waste besides having consisted of lactic acid bacteria activity also consisted of cellulolytic bacteria ([33], [34]).

### B. In vitro digestibility

Statistical analysis showed that the use of 60% starter was able to increase the digestibility of dry matter and organic matter in the P3 treatment ( $p < 0.05$ ). This result was confirmed by the high value of VFA and NH<sub>3</sub> in the treatment ( $p < 0.05$ ). As explained in the changes in nutrient composition, the pattern might be occurred due to the changed fiber composition and thus the digestibility of feedstuffs might be increased. Furthermore, during the in vitro process, microbial penetration such as cellulolytic, and proteolytic can more quickly decompose related components due to the loosening of cell walls due to the fermentation process or in terms of the pre-digest process by microorganisms ([35], [36]). Previous research studies have revealed that fiber is a limiting factor in the digestibility of other nutrient components so that if crude fiber can be treated to reduce or break down the components, certainly will affect the digestibility ([32]).

Based on the results of statistical analysis, the pH value is significantly different in the P3 treatment when compared to other treatments ( $p < 0.05$ ). The decrease in pH value was caused by the high value of VFA (volatile fatty acid) produced, the VFA value in this study can be seen in table 2. The increase in VFA value in line with the increased bacterial starter ratio indicated that the massive activity of cellulolytic bacteria during the pre-digest process was very influential in breaking down fiber bonds without removing the fiber components themselves such as cellulose and hemicellulose. This statement is assumed because the VFA value is still quite high even though the crude fiber value has decreased, so it can be assumed that the fiber component does not disappear but only undergoes a simple reformation of the cell wall / crude

fiber component. The results of research on various types of fermented feed reported that the decreased fiber during the fermentation or pre-digest process did not affect the VFA value ([37], [38]). Volatile Fatty acid is a compound dominated by acetate and butyrate produced by the fiber fermentation process during in vitro, so the composition of fiber will affect the high and low VFA. As an illustration, some studies show that the value of VFA is strongly influenced by fiber content or composition in various types of forage with different fiber compositions ([39]). Furthermore, the NH<sub>3</sub> value, which is the result of the protein fermentation process, has increased because of the sequential pattern that occurs, where protein access is easier due to crude fiber. Not much can be described from the NH<sub>3</sub> value, but the resulting value is quite good considering the NH<sub>3</sub> value produced can reach 16%, which in many similar studies the value of NH<sub>3</sub> produced is around 18-20% ([8], [40], [41]).

#### IV. CONCLUSIONS

Based on this study, it can be concluded that the use of starter bacteria obtained from vegetable waste such as cabbage and mustard by 60% is able to have a major impact on the physiological and chemical quality and digestibility of Water hyacinth.

#### ACKNOWLEDGMENT

We would like to thank the Public Service Agency of Sriwijaya University for the assistance of the DIPA Budget for Fiscal Year 2021, No. SP DIPA-023.17.2.677515/2021 which consistently provides support so that this research project was able to proceed successfully.

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## AUTHORS

**First Author** – Apriansyah Susanda Nurdin, Animal Feed Science and Technology, Department of Technology and Livestock Industry, Faculty of Agricultural, Sriwijaya University, Indralaya, South Sumatera, Indonesia,

**Second Author** – Anggriawan Naidilah Tetra Pratama, Animal Feed Science and Technology, Department of Technology and Livestock Industry, Faculty of Agricultural, Sriwijaya University, Indralaya, South Sumatera, Indonesia,.

**Third Author** – Fitri Nova Liya Lubis, Poultry Nutrition, Department of Technology and Livestock Industry, Faculty of Agricultural, Sriwijaya University, Indralaya, South Sumatera, Indonesia,

**Fourth Author** – Dyah Wahyuni, Quality and Safety of Animal Foods, Department of Technology and Livestock Industry, Faculty of Agricultural, Sriwijaya University, Indralaya, South Sumatera, Indonesia,

**Fifth Author** – Sofia Sandi, Animal Feed Science and Technology, Department of Technology and Livestock Industry, Faculty of Agricultural, Sriwijaya University, Indralaya, South Sumatera, Indonesia,

**Correspondence Author** – Anggriawan Naidilah Tetra Pratama,