Dry matter, crude fiber and nitrogen free extract contents of Seligi leaf (*Phyllanthus buxifolius*) powder fermented with different duration and probiotic’s dose as a fish feed ingredient

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Abstract. Seligi (*Phyllanthus buxifolius*) is a herbaceous plant which still rarely used as feed additives. Seligi has a high crude fibre and low protein so it needs to be improved by fermentation using probiotics. The fermentation process is influenced by duration and dose. This study aims to determine whether there are effects and interactions of duration and dose of probiotics on the content of dry matter, crude fibre and nitrogen free extract (NFE) in Seligi leaves. This study is experimental research with factorial completely randomized design with 2 factors, namely the duration of fermentation, consisting of 2 treatments, 5 and 7 days, while the other factors are the dose, consisting of 4 treatments namely 0%, 2%, 4% and 6%. The results showed that there was an effect of fermentation duration on dry matter, which on day 5 produced the highest dry matter, but there was no dose-effect and no interaction. In crude fibre, there is an effect of probiotic dose where at a dose of 2% it produces the lowest crude fibre, but there is no effect on the duration and there is no interaction. In the NFE, there was no influence and interaction between time and dose.

1. Introduction

Feed is one of the most important factors in aquaculture production due to 60-70% total production cost comes from the feed [1]. To reduce this production cost, fish farmers usually use alternative materials that are easily available and cheap. Seligi contains high crude fiber and low crude protein [2]. One way to improve feed quality was through fermentation with probiotics. The fermentation of feed ingredients is influenced by the the probiotic dose and fermentation time [15].

Based on these problems, it is necessary to research the use of probiotics that contain *Actinomyces* spp., *Bacillus* sp., *Cellulomonas* spp., and *Enterobacter* sp. [3, 4]. In the fermentation process of Seligi leaves in improving the nutritional quality of Seligi leaves, specifically increasing dry matter and nitrogen-free extract (NFE) and also reducing crude fiber, which then can be used as an alternative ingredient in fish feed. This study aims to determine the effect of fermentation duration and probiotic dose on the nutrient content of dry matter, crude fibre and NFE on Seligi leaves as well as to find out the interaction between the two variables.
2. Material and methods

2.1. Duration and place
This study was conducted at the Laboratory of Anatomy and Aquaculture Faculty of Fisheries and Marine Airlangga University. Proximate analysis of this study was conducted at the Faculty of Veterinary Medicine, Airlangga University. The study was held from December 2019 to April 2020.

2.2. Material
The materials used in this study were the Seligi leaves, probiotics, molasses and aquades. The Seligi leaves used in this study were obtained from the garden belong to the Faculty of Veterinary Medicine, Airlangga University. Probiotics used were Bio MC-4. This probiotic contains bacteria Actinomyces spp., Bacillus sp., Cellulomonas spp., and Enterobacter sp., with the number of bacteria $1.2 \times 10^{-9}$ CFU/ml.

2.3. Experimental design
This research method was an experimental method using a completely randomized design (CRD) factorial pattern with 2 factors. The first factor was the duration of fermentation, consisting of 2 treatments (5 and 7 days) and the probiotic dose, consisting of 4 treatments (0%, 2%, 4%, 6%). There are 8 combinations of treatments (2 treatments of fermentation duration x 4 probiotic doses treatments). The treatments in this study were carried out in 3 replications, so that as a whole produced 24 combinations. The following table is a combination of treatment factors.

| Duration of Fermentation (H) | Probiotic Dose (P) |
|-----------------------------|--------------------|
|                             | P0 (Control)       |
| H1 (5 days)                 | H1P0               |
| H2 (7 days)                 | H2P0               |
|                             | P1 (2%)            |
| H1P1                        | H2P1               |
|                             | P2 (4%)            |
| H1P2                        | H2P2               |
|                             | P3 (6%)            |
| H1P3                        | H2P3               |

The dose used was based on the results of the best dose analysis study conducted on the proximate analysis of papaya leaves fermentation using BioMC4 probiotics with a bacterial count of $1.2 \times 10^{-9}$ CFU/ml [5]. The dose of molasses used was 2%, while the water used was 30% of the total leaves flour.

2.4. Experimental procedure
Seligi leaves dried using the sun heat for 2-3 days. The dried leaves were finely ground using a grinding machine to turn it into flour. Seligi leaves flour was weighed 100 grams each sample. The flour was put into a basin to be homogeneously stirred with fermentor solution. Making fermenter solution was done by mixing 2% molasses and 30% water with probiotics in accordance with the treatment. The fermentor solution was then left for 24 hours in a closed container (sample pot). It aims to rejuvenate bacterias contained in probiotics. After 24 hours, the fermentor solution was put into a spray bottle and then sprayed on the Seligi powder evenly. Every material that has been mixed evenly is put into a plastic bag and tied in an anaerobic state. The fermentation process was carried out within 5 and 7 days according to the treatment.

2.5. Observed parameters
The parameters observed in this study were the content of dry matter, crude fiber and nitrogen-free extract (NFE) of Seligi leaves which were fermented with different duration and probiotic dose. The value of the parameter content is known by doing a proximate analysis.

2.6. Data analysis
Data analysis was performed using Analysis of Variance continued with the Duncan Multiple Range Test for any significant difference among treatments with a 95% confidence interval [6].
3. Results and discussion

3.1. Dry matter

The results of statistical tests on dry matter content of fermented Seligi leaves powder with different duration and doses of probiotics can be seen in Table 2.

| Probiotic Dosage | Duration of Fermentation | Average     |
|------------------|--------------------------|-------------|
|                  | H0 (5 days)              | H1 (7 days) |
| P0 (0%)          | 75.78 ± 0.10b            | 73.66 ± 0.52a | 74.72 ± 1.21a |
| P1 (2%)          | 75.22 ± 0.19b            | 73.86 ± 0.63a | 74.54 ± 0.86a |
| P2 (4%)          | 75.54 ± 0.51b            | 74.03 ± 0.64a | 74.76 ± 0.97a |
| P3 (6%)          | 75.64 ± 0.14b            | 73.94 ± 0.41a | 74.79 ± 0.95a |
| Average          | 75.55 ± 0.33b            | 73.87 ± 0.50a |

Note: Different superscripts in the same column and row show significantly different effects (p <0.05)

Based on the average proximate analysis of the Seligi leaves which are fermented at different duration, there is a significant difference (p <0.05) between H0 treatment (5-day fermentation) and H1 (7-day fermentation), while based on the average treatment dose, not significantly different (p >0.05) between treatments P0 (0%), P1 (2%), P2 (4%) and P3 (6%). The results of the Analyze of Variance (ANOVA) test show that there is an effect on fermentation duration of the dry matter content on Seligi leaves (P. buxifolius). The best result of dry matter was in the treatment H0, while the results of the probiotic dose test showed that there was no effect of probiotic doses on the content of dry matter on the Seligi leaves. Furthermore, the interaction between the duration and probiotic dose showed that there was no interaction between duration and probiotic dose on the dry matter content of the leaves.

The decrease in dry matter is caused by the usage of nutrients from the substrate by microbes as a source of carbon, nitrogen, and minerals, as well as the release of CO\textsubscript{2} and energy in the form of heat that evaporates with water particles. The water molecule is formed from the process of catabolism which breaks down complex compounds into simpler materials. The longer the fermentation duration, resulting in decreasing levels of dry matter [7].

The decrease in the dry matter also occurs due to bacterial activity. Bacteria will use the materials on Seligi leaves for the anaerobic fermentation process which will then produce carbon dioxide and water. In 5 days fermentation results in high dry matter content because at that duration the bacteria contained in probiotics have not to use the feed ingredients optimally, whereas in fermentation with 7 days duration produces low dry matter content because this duration is an effective duration for bacterial growth where the bacteria will convert existing materials into energy and by-products in the form of carbon dioxide and water [8].

3.2. Crude fiber

Based on the average proximate analysis of Seligi leaves that are fermented at different durations there is no significant difference (p <0.05) between H0 (5-day fermentation) and H1 (7-day fermentation), while based on the average treatment the dose did not differ between treatments P0 (0%) and P3 (6%), but differed from treatment P1 (2%) and P2 (4%).

Based on ANOVA shows that the lowest average crude fiber content is at doses of 2% and 4% because it has different notation with P0 and P1. In Table 3, the average results show that probiotic’s dose of 2% produces the lowest crude fibre yield. Thus it can be concluded that the best probiotic dose to produce the lowest crude fibre content is 2%. In the duration, treatment shows no effect and there is no interaction between fermentation duration and the probiotic dose of crude fibre content in Seligi leaves. The results of statistical tests on crude fibre content can be seen in Table 3.

There is a decrease in crude fibre content in fermentation with a duration of 5 days with a dose of 0%, 2% and 4%, but an increase in the dose of 6%. This also occurs in fermentation with 7 days where there is a decrease in crude fiber in the treatment P1 (2%) and then an increase in treatments P2 (4%).
and P3 (6%). The treatment P0 has the highest crude fiber content because there is no additional probiotics are given where there are no fermenting bacteria that can reduce crude fiber. In the treatment of P1 (2%) at 7 days is the result with the lowest crude fiber content. This shows that the bacteria have reached optimum conditions in growth where the bacteria will produce compounds to break down complex crude fibers into simple compounds [9]. In treatments P2 (4%) and P3 (6%), increasing content of crude fiber content is suspected because this nutrient source has been depleted, the formation of growth-inhibiting metabolites and unfavourable factors causing bacteria to die. Microbial growth is characterized by the amount of duration used so that the metabolic concentration increases until it becomes limited then it can cause death and decrease of growth rate [10]. Decrease crude fiber content in the fermentation process due to the presence of cellulytic bacteria in probiotics (Bacillus sp., Enterobacter sp., Cellulomonas sp., and Actinomyces sp.). Bacteria in probiotics will produce cellulase enzymes that can break the complex lignocellulose bonds into glucose. Some crude fiber fraction is used as an energy source for microbial growth. With the process of degradation of crude fiber there is a decrease in the content of crude fiber in the substrate used as a fermentation medium [11]. The decrease in crude fiber shows an increase in feed ingredient’s quality. This is because the low-value crude fiber will increase the digestibility of the feed ingredients. One of the advantages of the fermentation process using cellulytic bacteria is the faster growth rate compared to other microbial groups so that the duration needed for enzyme production is faster [12].

### Table 3. The average crude fibre content of the leaves during the treatment

| Probiotic Dosage | Fermentation time | Average |
|------------------|------------------|---------|
|                  | H0 (5 days)      | H1 (7 days) |         |
| P0 (0%)          | 15 ± 0.82<sup>b</sup> | 15.73 ± 0.35<sup>b</sup> | 15.65 ± 0.57<sup>b</sup> |
| P1 (2%)          | 14.12 ± 0.90<sup>ab</sup> | 13.63 ± 1.57<sup>a</sup> | 13.87 ± 1.18<sup>a</sup> |
| P2 (4%)          | 14.03 ± 1.10<sup>ab</sup> | 14.81 ± 0.26<sup>ab</sup> | 14.42 ± 0.83<sup>a</sup> |
| P3 (6%)          | 14.60 ± 0.84<sup>ab</sup> | 14.57 ± 0.52<sup>ab</sup> | 14.59 ± 0.63<sup>ab</sup> |
| Average          | 14.58 ± 1.01<sup>a</sup> | 14.69 ± 1.07<sup>a</sup> |         |

Note: Different superscripts in the same column and row show significantly different effects (p < 0.05)

### 3.3 Nitrogen free extract (NFE)

Based on the average proximate analysis of the Seligi leaves that are fermented with different duration showed there is no difference between treatments H0 (5 days fermentation) and H1 (7 days fermentation), whereas based on the average treatment dose, P0 (0%) was not significantly different from P2 (4%) and P3 (6%), but P0 was significantly different from P1 (2%). Based on the results of the study showed that the highest content of the nitrogen-free extract was found in the treatment of P1 (2%). It can be concluded that the treatment of P1 (2%) was the best treatment that produced the highest nitrogen-free extract content. Based on the results shows that there is no effect of the fermentation duration on NFE content on Seligi leaves (P. buxifolius), while based on the average dose treatment, P0 (0%) was not significantly different from P2 (4%) and P3 (6%), but P0 was significantly different from P1 (2%). Overall, there is no difference between treatments, this shows that there is no effect of probiotic dose on NFE content in Seligi leaves and there was no interaction between fermentation duration and probiotic dose on nitrogen-free extract (NFE) content in Seligi leaves (P. buxifolius) (Table 4).

In this study, the nitrogen-free extract material has decreased. This is caused by bacteria use organic material such as carbohydrates, where carbohydrate is the main component contained in the nitrogen-free extract. There is a tendency for bacteria to consume easily-digested carbohydrates first as source energy [13]. The decrease in NFE levels is seen in the aspect of nutrition being less profitable because the less NFE means the less organic component can be digested so that the less energy can be produced [14].
Table 4. The average of nitrogen-free extract content during treatment

| Probiotic Dosage | Fermentation time | Average      |
|------------------|-------------------|--------------|
|                  | H0 (5 days)       | H1 (7 days)  |               |
| P0 (0%)          | 54.39 ± 1.20a     | 53.50 ± 0.43a| 53.94 ± 0.95a |
| P1 (2%)          | 56.48 ± 1.64a     | 56.26 ± 2.97a| 56.37 ± 2.16b |
| P2 (4%)          | 56.72 ± 1.44a     | 54.86 ± 1.11a| 55.79 ± 1.53ab|
| P3 (6%)          | 55.74 ± 2.47a     | 55.42 ± 0.28a| 55.58 ± 1.58ab|
| Average          | 55.83 ± 1.77a     | 55.01 ± 1.73a|               |

Note: Different superscripts in the same column and row show significantly different effects (p < 0.05)

4. Conclusion
Probiotic dosage and duration of fermentation significantly affected dry matter, crude fibre and nitrogen-free extract contents in the Seligi leaf flour. The result also indicated that there was no interaction between the fermentation time and the probiotic dose of the ingredients dry, crude fibre and nitrogen-free extract on the Seligi leaves.

5. References
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