The Utilization of Vegetable Waste Silage as Feed Ingredient in Diets for Tilapia *Oreochromis niloticus*

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**Abstract.** The vegetable waste silage is potential as a feed ingredient for tilapia culture. Silage is a product of the processing of materials by the addition of acid or by anaerobic fermentation. This study aimed to evaluate the effect of vegetable waste silages on the growth of tilapia (*Oreochromis niloticus*). Formic acid is used in this study as organic acid for the preservative. Four diets concentration of formic acid in making silage (0%, 3%, 4%, and 5%) was formulated containing 27% crude protein. The result showed that total digestibility, protein digestibility, and fat digestibility were significantly higher (P<0.05) in 4% formic acid and 5% formic acid than Control. The value of growth was significantly highest (P<0.05) in 5% formic acid (2.96±0.08 %/day), followed by 4% formic acid (2.66±0.14 %/day), 3% formic acid (2.56±0.05 %/day) and control (2.54±0.21 %/day). The utilization of 5% formic acid in making vegetable waste silage showed the best result on growth and feed efficiency than the other treatments.

**Keywords:** formic acid, silage, tilapia.

1. Introduction

Tilapia (*Oreochromis niloticus*) is a type of fish that has high economic value as a consumption fish and is an important commodity in the freshwater fish business in the world [1]. On the other hand, the intensive tilapia culture faces several problems, including the relatively expensive price of artificial feed that is not followed by the selling price of the product. This certainly has an impact on the income of fish farmers because fish farmers have to incur other production costs such as seeds and medicines. This problem also has an impact on the decline in tilapia production in Indonesia. The annual report of the Directorate of Production, the ministry of marine and fisheries in 2016 states that national tilapia fish production is 1.02 million tons and this number is below the production target targeted by the ministry in 2016, which is 1.65 million tons. The high price of feed is due to high prices of feed raw materials such as fish meal and soybeans meal. Therefore, it's needed local alternative raw materials in the tilapia feed.

Vegetable waste is an alternative material used as raw material for fish feed [2, 3], because it has a high protein content of 15-24% [4]. In addition, the availability of vegetable waste is abundant, inexpensive and does not compete with human needs [5]. Utilization of vegetable waste as feed raw material can also reduce environmental pollution because vegetable waste is waste that pollutes the environment [6]. However, the use of vegetables as feed raw materials is limited by the high durability.
of vegetable waste and crude fiber. Vegetable waste is easy to rot and voluminous (large) with a crude fiber content of 20.76-29.18%. Therefore, it is necessary to increase shelf life and decrease the value of crude fiber of vegetable waste.

Silage is a technology that is expected to increase shelf life and reduce crude fiber from vegetables. According to [7], the mechanism of action of silage is that the addition of acid will cause the pH to decrease (<4.2), thereby inhibiting the growth of spoilage bacteria and only lactic acid bacteria in the silage material. The acid commonly used in making silage is formic acid [8, 9, 10, 11]. However, there is no research related to the use of silage derived from vegetable waste as feed raw material in tilapia. Therefore, research is needed related to the use of silage from vegetable ingredients as raw material for tilapia fish feed. This study aimed to evaluate the effect of vegetable waste silages on the growth of tilapia.

2. Material and Methods
2.1 Silage preparation

Vegetable waste consisting of cabbage waste, water spinach, spinach, mustard is cut into small pieces to facilitate the fermentation process. Furthermore, vegetable waste is weighed and added formic acid according to treatment. The dosage of formic acid used consisted of 0%, 3%, 4% and 5% (w/w). The mixture of vegetable waste and formic acid is then put into a bucket and sealed tightly to create anaerobic conditions. The fermentation process lasts for 10 days, pH silage is measured every day to ensure the fermentation process occurs in vegetable waste. The silage is then dried in the sun to dry and ground into silage meal.

2.2 Preparation of experimental diets

The feed used is commercial feed with a protein content of 32%. Feed was pollinated into the meal and then chromium oxide (Cr2O3) of 0.5% as an indicator of digestibility and 3% tapioca as a feed binder were added to the meal before the pelleting process. Feed formulation refers to [12], i.e. feed formulation consisting of 70% commercial feed and 30% test material, while for control (formic acid 0%) using a vegetable waste meal. The feed is made in the form of a wet pellet using 2 mm size feed molding machine. The formulations of feed are presented in Table 1.

2.3 Experimental design

Tilapia used were male monosex tilapia with an average weight of 10 g with a density of 15 fish/aquarium with a recirculation system. Tilapia is obtained from Ary Farm, Serang, Banten Province, Indonesia. This study is conducted at the Laboratory of Aquaculture, Department of Fisheries, University of Sultan Ageng Tirtayasa, Indonesia. fish were reared in 12 aquariums with a size of 50 x 40 x 30 cm, for 40 days. After the acclimation period is completed (7 days), fish fasted for 24 hours with the aim of eliminating the remaining food in the body. Feeding is done three times a day with satiation. Feces collection was conducted in the day-5 after stocking by siphoning out the faecal material from the bottom of the aquarium. The faecal material was dried at 60°C for 24 h.

2.4 Growth parameters

At the end experiment, the growth parameters of the tilapia were analysed for the specific growth rate (SGR), feed efficiency (FE), survival rate (SR) were calculated using equations described by [13]. Nutrient digestibility (ND) including protein digestibility, fat digestibility, and energy digestibility, and total digestibility (TD) were calculated according to [14].

\[
\text{SGR} \% = \frac{100 \times (\ln W_2 - \ln W_1)}{\text{time (days)}} \quad (1)
\]

\[
\text{SR} \% = \frac{\text{NT} / N_0 \times 100}{(2)}
\]

\[
\text{FE} \% = \frac{100 \times [\text{wet weight gain (g)} / \text{dry feed intake (g)}]}{3}
\]

Where, \( W_1 \) and \( W_2 \) indicate the initial and final weight (g), respectively. \( N_T \) is the number of fish at the end (individuals), \( N_0 \) is the number at the beginning (individuals).
\[ ND = 100 - [1 - a/a' \times b'/b] \]  
(4)

\[ TD = 100 - [1 - a/a'] \]  
(5)

Where \( a \) is \( \text{Cr}_2\text{O}_3 \) percentage in the feed, \( a' \) is \( \text{Cr}_2\text{O}_3 \) percentage in the faeces, \( b \) is a nutrient percentage in the feed, \( b' \) is the nutrient percentage in the faeces.

2.5 Chemical Analysis

The proximate composition of experimental diets and faecal samples was analysed according to the standard method of [16]. The proximate composition including crude protein, crude lipid, moisture, nitrogen-free extract, and the chromium content of diets and faecal.

2.6 Statistical analyses

Statistical analyses of growth parameters were carried out using the Statistical Package for the Social Sciences (SPSS) program for Windows (v. 16.0). Where significant differences were found, the means within each treatment and among treatments were compared using Duncan of multiple comparisons with a 95% significant level.

Table 1. Formulation of the diet in this study

| Ingredients* | Treatments |
|--------------|------------|
|              | 0% formic acid | 3% formic acid | 4% formic acid | 5% formic acid |
| Commercial feed (%) | 67.55 | 67.55 | 67.55 | 67.55 |
| Vegetable waste (A) | 28.95 | - | - | - |
| Silage meal (B) | - | 28.95 | - | - |
| Silage meal (C) | - | - | 28.95 | - |
| Silage meal (D) | - | - | - | 28.95 |
| Tapioca | 3 | 3 | 3 | 3 |
| \( \text{Cr}_2\text{O}_3 \) | 0.5 | 0.5 | 0.5 | 0.5 |
| Total | 100 | 100 | 100 | 100 |

Proximate analyses (% dry matter)

|                | 0% formic acid | 3% formic acid | 4% formic acid | 5% formic acid |
|----------------|----------------|----------------|----------------|----------------|
| Crude protein (%) | 27.91          | 27.97          | 27.00          | 27.80          |
| Crude lipid (%)   | 4.20           | 4.26           | 4.22           | 4.24           |
| Fiber (%)         | 7.93           | 6.15           | 6.14           | 6.11           |
| NFE (%)**         | 39.38          | 39.40          | 43.31          | 41.00          |
| Energy (Kcal/kg feed)*** | 230.155 | 230.901 | 236.957 | 234.144 |

Note: * A = commercial feed + vegetable waste, B = commercial feed + vegetable waste silage (3% formic acid), C = commercial feed + vegetable waste silage (4% formic acid), D = commercial feed + vegetable waste silage (5% formic acid).

** Nitrogen free extracts

*** DE: Digestible Energy = carbohydrate: 2.5 kcal DE; protein: 3.5 kcal DE, fat: 8.1 kcal DE [13]

3. Results and Discussions

3.1 Growth parameters of tilapia

Formic acid is routinely used in the manufacture of silage and formic acid salts have been shown to increase disease resistance in tilapia [9]. Application of formic acid in the manufacture of silage has been observed in fingerlings of common carp \textit{Cyprinus carpio} [8, 21], Mozambique Tilapia (\textit{Oreochromis mosaambicus}) [9], discard fish (\textit{Equulites klunzingeri} and \textit{Carassius gibelio}) [10], abalone [11]. In the present study, 5% formic acid treatment can promote best growth compared to control for all parameters. Growth parameters of tilapia fed experimental diet is shown in Table 2.
Table 2. Effect of dietary vegetable waste silage as feed ingredients on growth parameters of tilapia*.

| Parameters** | 0% formic acid | 3% formic acid | 4% formic acid | 5% formic acid |
|--------------|----------------|----------------|----------------|----------------|
| FI (%)       | 337.5±9.04     | 337±7.22       | 335±7.07       | 324±10.32      |
| TD (%)       | 44.30±4.12     | 49.98±4.16     | 55.61±5.92     | 55.66±4.15     |
| PD (%)       | 78.56±2.63     | 81.78±2.30     | 82.13±3.58     | 84.59±1.26     |
| FD (%)       | 74.35±1.60     | 80.38±1.54     | 81.53±4.52     | 84.24±2.80     |
| ED (%)       | 59.09±3.04     | 65.42±3.01     | 69.22±4.08     | 69.48±4.06     |
| SGR (day⁻¹)  | 2.54±0.21      | 2.56±0.05      | 2.66±0.14      | 2.96±0.08      |
| FE (%)       | 47.04±2.53     | 47.78±3.62     | 45.80±6.46     | 57.93±2.67     |
| SR (%)       | 92.18±3.12     | 89.06±5.98     | 87.50±8.83     | 82.81±7.86     |

Note: *The value in the same row followed by similar superscript are not significantly different (p>0.05).
** (FW), feed intake (FI), total digestibility (TD), protein digestibility (PD), fat digestibility (FD), energy digestibility (ED), specific growth rate (SGR), feed efficiency (FE), survival rate (SR) of tilapia.

No significant difference (P<0.05) was obtained in feed intake. The value of feed intake in 0% formic acid treatment of 337.5±9.04%, 3% formic acid treatment of 337±7.22%, 4% formic acid of 335±7.07%, and 5% formic acid of 324±10.32%. No significant difference (P<0.05) was obtained in feed intake. This is indicating that the utilization of vegetable waste silage did not affect to feed palatability. Palatability is the level of preference for fish to consume the feed given at a certain time period [17]. The similar result has been reported by [18] in the feed based the other vegetable protein. He noted that the utilization of Leucaena leucocephala leaf meal did not affect to the appetite of rohu, Labeo rohita (Hamilton). Digestibility describes the fraction of the nutrient or energy in the ingested feedstuff that is not excreted in the feces [19]. Total digestibility is the number of nutrients in the feed that can be digested by fish [13]. The value of total digestibility was significantly higher (P<0.05) in 5% formic acid treatment (55.66±4.15%) and 4% formic acid treatment (55.61±5.92%) compared to control (44.30±4.12%), but there was no significant (P>0.05) difference between 3% formic acid treatment (49.98±4.16%), and 4% formic acid treatment and 5% formic acid treatment. Protein is most feedstuffs that have been properly processed are highly digestible of fish [13]. Protein digestibility showed higher (P<0.05) in 5% formic acid (84.59±1.26%) than control (78.56±2.63%). There are no significant (P>0.05) difference between 4% formic acid treatment, 3% formic acid treatment (82.13±3.58%, 81.78±2.30%, respectively) than control. In the present study, the protein digestibility value (78-84%) of tilapia were lower than the previous study by [20] in tilapia (89-93%).

Fat when administered either alone or in a mixed diet routinely gives digestibility values of 85% to 95% for fish [13]. Fat digestibility showed higher (P<0.05) in 5% formic acid treatment and 4% formic acid treatment (84.24±2.80%, 81.53±4.52%, respectively) than control (74.35±1.60%) and there no difference (P>0.05), between control and 3% formic acid treatment (80.38±1.54%). The similar result was obtained in energy digestibility, the result showed that energy digestibility was significant higher (P<0.05) in 5% formic acid treatment (69.48±4.06%) and 4% formic acid treatment (69.22±4.08%) compared to control (59.09±3.04%) and no significant difference (P>0.05) between control and 3% formic acid treatment (59.09±3.04%). The high energy digestibility value in 4-5% formic acid treatment might be related to the high value of protein digestibility and fat digestibility in this treatment. In this study, nutrient digestibility was significantly higher observed in the treatment of 5% formic acid compared to other treatments. This shows that the dose of formic acid 5% is effective in breaking down the nutrients contained in vegetable waste so that the digestibility of nutrients in the treatment of 5% formic acid is better than other treatments.

The value of specific growth rate was significantly highest (P<0.05) in 5% formic acid (2.96±0.08 %/day), followed by 4% formic acid (2.66±0.14 %/day), 3% formic acid (2.56±0.05 %/day) and control (2.54±0.21 %/day). Feed efficiency in this study showed highest (P<0.05) in 5% formic acid treatment (57.93±2.67%) compared the other treatments, followed by 3% formic acid treatment, control, and 4% formic acid treatment (47.78±3.62%, 47.04±2.53%, 45.80±4.66, respectively). In the present study, the high growth and feed efficiency may be due to the higher nutrient digestibility value.
in 5% formic acid treatment than the other treatments. The similar result has been reported by [21] in major carp, he found that a higher growth response on major carp feed a 26.4 to 53.1 % silage-based diet than fish fed a fishmeal-based diet. The same results were founded for salmon. [22] noted that salmon fed a 25% silage-based diet grew faster than fish fed a commercial dry pellet. The survival rate of feeding trial in control, 3% formic acid treatment, 4% formic acid treatment, and 5% formic acid treatment (92.18±3.12%, 89.06±5.98, 87.50±8.83, 82.81±7.86, respectively). The result is indicating that the addition of formic acid in making vegetable waste silage as feed did not affect to fish health of tilapia. Similarly, [9] has reported that the utilization of formic acid in making rainbow trout silage not effect on survival rate and immune stimulant of tilapia.

4. Conclusions
Vegetable waste silage can be used as a feed ingredient of tilapia feed. Total digestibility, protein digestibility, and fat digestibility were significantly higher (P<0.05) in 4% formic acid and 5% formic acid than Control. The value of growth was significantly highest (P<0.05) in 5% formic acid (2,96±0.08 %/day), followed by 4% formic acid (2,66±0,14 %/day), 3% formic acid (2,56±0,05 %/day) and control (2,54±0,21 %/day). The utilization of 5% formic acid in making vegetable waste silage showed the best result on growth and feed efficiency than the other treatments.

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