The use of mangrove leaves flour *Avicenia rumphiana* as antioxidant feed additive in commercial feed towards growth and survival rate of Nile tilapia fry *Oreochromis niloticus*

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Abstract. Nile tilapia has high economic value, in its cultivation, to optimize the growth and survival rate of Nile tilapia could be done by adding vitamin C in its feed as antioxidant. However, vitamin C could not be synthesized in the fish body and expensive. Mangrove leaves *Avicenia rumphiana* contains antioxidant that suitable as the alternative of antioxidant likes vitamin C. This study aimed to observe the using of feed additive of mangrove leaves flour as antioxidant towards the growth and survival rate of Nile tilapia. This research applied a completely randomized design method which consist both of four treatments and replications that involve 0.02gr/kg vitamin C (K), amount of 0.2gr/kg, 0.4gr/kg, 0.6gr/kg mangrove leaves flour (P1, P2, P3). The result showed that the using of mangrove leaves flour as feed additive in commercial feed in 30 days at 0.02gr/kg has influenced the development of growth and survival rate of Nile tilapia. Therefore, it has proved that mangrove *Avicenia rumphiana* leaves flour is qualified to be the alternative of feed additive antioxidant in fish feed.

1. Introduction
Nile tilapia is high economical cultivated fish. Growth and survival rate of nile tilapia can be optimized with the supplementation of vitamin C in feed as an antioxidant. Vitamin C is antioxidant feed additive that play the role to increase growth, immune system, and survival rate of fish. However, vitamin C has a weakness as unable to be synthesized by fish body with expensive price, therefore it needs another alternative [1].

Mangrove leaves *A. rumphiana* has antioxidant activities, thus can be utilized to replace vitamin C. [2] explained that the mangrove leaves *A. rumphiana* have bioactive compounds that potentially utilized as natural antioxidant, namely phenols, flavonoids, and tannins. Natural antioxidants found in plants are usually flavonoid compounds, phenols, polyphenols, tannins, cinnamic acid derivatives, tocopherols, and organic acids [3].

This study was conducted to observe the growth and survival rate of nile tilapia *O. niloticus* fed with the supplementation of mangrove leaves flour *A. rumphiana* in feed.

2. Methodology
This study was conducted in Anatomy and Aquaculture laboratory, Faculty of Fisheries and Marine and animal feed laboratory, Faculty of Animal Medicine, Universitas Airlangga.
2.1. Materials
Materials used in this study were 160 nile tilapia fry sized 3-5 cm in a 20 L of volume aquarium at a density of 10 fish/12 L obtained from Gunungsari fish market, freshwater, commercial feed PF500, mangrove leaves flour, vitamin C, and egg whites. Equipments used in this study were sixteen aquariums sized 30×30×35 cm, aeration hose, aeration stone, aerator, water stock tank, net, analytic scale, thermometer, pH meter (0009-I), ammonia test kit (Tetra test NO2), DO meter (YSI), grinder, and ruler.

2.2. Treatment
This study used with completely randomized design experimental method containing four treatments, i.e. 0.02 gr/kg vitamin C (K) and 0.2 g/kg, 0.4 g/kg, 0.6 g/kg mangrove leaves flour. The study began with fish acclimatization against the new rearing environment. Treatment feed was made by mixing 1 kg PF500 feed sized 1 mm and mangrove leaves flour based on the treatment given, then binded with 5 ml egg whites. Mixed feed was given 3% of the fish body mass weight on three times a day. Aquariums were syphonized every 3-4 days in the morning before feeding. The parameter measurements were done once in 10 days.

The main parameters of this study were specific growth rate by measuring fish weight and length [4] and survival rate gained from the ratio of living fish at the end and beginning of rearing [5]. Supported parameters observed included feed conversion ratio obtained from the ratio of feed consumed against increased fish weight and dead fish weight during the study [6], the calculation of energy, protein, and lipid retention using the formula [7], feed antioxidant activity by DPPH assay continued with the determination of IC50 value, water quality. The formula of growth rate, survival rate, feed conversion ratio, energy retention, protein retention, fat retention can be seen as follows:

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SGR (L) = \frac{\ln(L_t) - \ln(L_0)}{t}
\]
\[
SGR (W) = \frac{\ln(W_t) - \ln(W_0)}{t}
\]
\[
SR = \frac{N_1}{N_0} \times 100\%
\]
\[
FCR = \frac{F}{(W_t + D) - W_0}
\]
\[
RE = \frac{\text{Final energy weight - Initial energy weight}}{\text{Total of consumed feed}} \times 100\%
\]
\[
RP = \frac{\text{Final protein weight - Initial protein weight}}{\text{Total of consumed feed}} \times 100\%
\]
\[
RL = \frac{\text{Final lipid weight - Initial lipid weight}}{\text{Total of consumed feed}} \times 100\%
\]

2.3. Data analysis
Data was statistically analyzed using ANOVA to determine the treatment influence given, whereas significant result of the data was continued to be analyzed using Duncan’s multiple range test.

3. Results and discussion

3.1. Antioxidant activity test
Antioxidant activity test after the supplementation of mangrove leaves flour on feed indicated that high mangrove leaves flour supplementation reduced antioxidant antivity characterized as elevated concentration of IC50. Result can be seen in Table 1. IC50 is a concentration inhibiting 50% free radical
activity of DPPH. The antioxidant activity in commercial feed supplemented with mangrove leaves flour, A. rumphiana was lower compared to vitamin C as a positive control. It corresponds to [8] that smaller value of IC\textsubscript{50} indicates stronger antioxidant activity of sample tested.

| Sample code                  | IC\textsubscript{50} |
|------------------------------|-----------------------|
| K (0.02g/kg Vitamin C)       | 71.74                 |
| P1 (0.2g/kg mangrove leaves flour) | 74.79               |
| P2 (0.4g/kg mangrove leaves flour) | 88.40               |
| P3 (0.6g/kg mangrove leaves flour) | 96.61               |

3.2. Specific growth rate
Based on the observations result on the length and weight specific growth rate of tilapia, it showed that the supplementation of mangrove leaves flour on 0.2 g concentration accelerated the growth process of nile tilapia characterized 1.04 g% for SGR (weight) and 1.29% for SGR (length). The graphic of specific growth rate on tilapia can be seen in Figure 1.

3.3. Survival rate
The observation result of survival rate in tilapia indicated that the supplementation of mangrove leaves flour on 0.2 g concentration affected the survival rate of nile tilapia with 85% survival rate. The graphic of observations of tilapia livelihoods can be seen in Figure 2.

3.4. Feed conversion ratio
Based on the observation result of feed conversion ration on tilapia, it indicated that the supplementation of mangrove leaves flour on 0.2 g concentration influenced the feed conversion ratio of nile tilapia with 1.37. Feed conversion ratio result is presented on Figure 3.

![Feed conversion ratio of Nile tilapia](image)

**Figure 3.** Feed conversion ratio of Nile tilapia.

### 3.5. Nutrient retention

Based on energy, lipid, and protein retention result, it indicated that the supplementation of mangrove leaves flour on 0.2 g concentration increased the nutrient retention as 17.26% energy, 17.51% lipid, and 35.67% protein retention. The result of nutrient retention can be seen on Figure 4.

![Energy retention, lipid retention, and protein retention of Nile tilapia](image)

**Figure 4.** Energy retention, lipid retention, and protein retention of Nile tilapia

### 3.6. Feed proximate analysis

Proximate analysis is chemical test to determine the nutrient content of food or feed ingredient [9]. Feed proximate analysis result is presented on Table 2.

| Treatment | Analysis result |
|-----------|-----------------|
|           | Moisture (%)    | Ash (%)     | Protein (%) | Lipid (%) | Fiber (%) | NFE (%) | GE (Kcal/kg) |
| K         | 88.1081         | 10.6615     | 32.9000     | 8.3436     | 8.0056    | 28.1974 | 2753.4768    |
| P1        | 81.9057         | 9.6080      | 33.8542     | 5.9282     | 4.5133    | 28.0020 | 2595.8978    |
| P2        | 80.1710         | 9.4650      | 31.9740     | 6.4314     | 5.2419    | 27.0587 | 2536.4884    |
| P3        | 79.4481         | 9.4721      | 30.3809     | 6.8315     | 4.5750    | 28.1886 | 2552.1457    |

Table 2. Feed proximate analysis on each treatment.

The supplementation of mangrove leaves flour with 0.2 g concentration in feed affected the growth rate, survival rate, feed conversion ratio, energy retention, lipid retention, and protein retention. Mangrove leaves flour supplementation in feed gave the same influence as vitamin C supplementation,
however higher concentration of mangrove leaf decreased the value of growth rate, survival rate, energy retention, lipid retention, protein retention, thus increasing feed conversion rate of nile tilapia. Factors affecting this condition is the content of bioactive substances, namely flavonoids and tannins in mangrove leaf that have bitter taste [2], fish type, and proximate analysis result. The fish used was nile tilapia, which is omnivorous fish with protein requirement of 25-35%, lipid requirement of 6-10%, and carbohydrate requirement of 30-40% [7]. 0.2 g concentration of mangrove leaves flour supplementation in feed indicated that the feed is still can be consumed well in fish body by means of maximized nutrient absorption to support fish growth. This condition is in accordance with the statement of [9], who stated that feed is used for survival, while the rest is for growth. Growth is related to feed conversion ratio. Important factors affecting feed conversion ratio are feed quality, density, stress levels and diseases [10]. Lower value of feed conversion ratio causes better feed efficiency [11]. Feeding using mangrove leaves flour with 0.2 g concentration in feed affected energy, lipid, and protein retention. Energy retention indicates as the magnitude of feed energy consumed contributed to increased energy in fish body [12]. The availability level of feed energy can be measured by feed caloric content in calorie unit [6]. Lipid retention illustrates the fish ability to store and utilize lipid in feed [13]. Feed lipid has an important role as energy source, besides maintaining membrane or tissue and retaining the body buoyancy [14]. Protein retention indirectly describes the amount of feed proteins consumed to build the protein tissue utilized for growth [13]. Fish feed contains components, such as proteins, fats, carbohydrates, and others that are useful for growth and energy source.

Survival rate is the percentage of fish that live at the end of rearing compared to the number of fish stocked at the beginning of rearing [15]. The higher value of survival rate shows better cultivation, because nile tilapia (O. niloticus) can adapt well to the environment. Factors affecting survival rate comprise internal factors (gender, tenacity, age, reproduction, and disease resistance) and external factors (water quality, stocking density, individual number, and feed) [16]. Feed additive is often given in feed. Feed additive serves to improve appetite, digestibility, endurance, besides reducing stress levels and enhancing growth [17]. Mangrove leaf A. rumphiana feed additive has phenolic compounds in the form of flavonoid and tannins that are natural antioxidant to increase the survival rate level in nile tilapia. The survival rate level of nile tilapia during 30 days rearing showed good result. This was in accordance with [18], who reported that survival rate with ≥ 50% indicates good rearing quality, 30-50% indicates medium rearing quality, and < 30% indicates ungood rearing quality. Water is fish rearing medium that must be considered to support the rearing success. Based on the temperature measurement, it showed that the temperature ranged 26-28.90°C, as in accordance with [19], who suggested that the optimum temperature for nile tilapia growth is 25-30°C. pH ranged 6.5-8.3. The pH value that can be tolerated by nile tilapia is 6.5-8.5, but for optimum growth and development should be 7-8 [19]. The dissolved oxygen measurements ranged from 3.7-6 mg/L, which was in accordance with [19], as optimum dissolved oxygen for nile tilapia should be > 3mg/L. The ammonia concentration during the study was 0-0.05 mg/L. [20] Ammonia compounds that are harmful to aquatic organisms ranges at 0.6 mg/L. Water quality during study reating was still in the optimum for nile tilapia growth.

4. Conclusion
Based on this study result, it can be concluded that the supplementation of mangrove leaves flour Avicennia rumphiana in feed can be utilized as anti oxidant feed additive alternative by influencing the growth and survival rate of nile tilapia fry.

5. References
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