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The influence of heat stress and powder of jaloh leaves supplementation into commercial fish feed on body weight gain, hematokrit level and malondialdehid content in the nila's liver

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Abstract. The aims of this experiment are to find out the effect of temperature stress and jaloh leaves supplementation on body weight gain, hematocrit level, and malondialdehid (MDA) content in the liver tissues of nila fish (*Oreochromis niloticus*). A total of 80 fishes with the weight of 40-50 gr were randomly allocated into 8 treatments. The treatments consisted of P1 (no heat stress and no jaloh leaves supplementation); P2 (no heat stress and 5% of jaloh leaves supplementation) ; P3 (no heat stress and 10% of leaves supplementation); P4 (no heat stress and 15 % of leaves supplementation); P5 (heat stress and no jaloh leaves supplementation); P6 (heat stress and 5% of jaloh leaves supplementation); P7 (heat stress and 10% of jaloh leaves supplementation); P8 (heat stress about 35 ± 1°C for 4 h per day in 30 days and 15% of jaloh leaves supplementation). The body weight was measured from 1d to 31d. Blood samples, lever tissues and statistical analysis were conducted on 31d. The results of the experiments indicated that supplementation of fish feed with jaloh leaves 5-15% had negative effects on body weight gain. On the other hand, supplementation of jaloh leaves 5-10% on commercial fish feed had positive effects on performances and immune system of experiment fishes.

Keywords: *heat stress, jaloh leaves and* Oreochromis *niloticus*

Introduction

Water condition is one of the important factors influencing growth and physiology aquatic animals (Inoue *et al*., 2008). Increase of water temperature results in decreasing of soluble oxygen in water. In addition, environmental manipulation such as feed density can also cause reducing of water quality. The effect of this condition can cause fish stress. Stress on fish can decrease productivity and immune system of fish and also fish mortality (Davis *et al*., 2008). Nila is a kind of fish that is more tolerable on environmental changes. Nila fish can be adaptable on the temperature of 14-36°C (El-Sherif dan El-Feky, 2009). However, based on the reported by Joseph dan Sujatha (2010) increase of water temperature at 34°C for 2 hours resulted in stress on nila fishes.

To protect fish from diseases can be conducted by increasing immune system of fishes. By this way, fishes are able to overcome from attracted pathogen micro-organisms. So far, the main methods that have been applied to protect fishes from diseases are by using chemical compounds or antibiotics. Researches relating to protect fish diseases based on environmental approach, for example by using plant sources (phytogenic), have increased recently. This method has positive effect by improving system immune of fish (imunostimulant). Several plants has been known and identified containing immune effects and antibacterial on fishes (Galina *et al*., 2009). Jaloh (*Jalŏh* atau *Sijalŏh*) in Aceh language is a kind of fodder plants with Latin name of *Salix tetrasperma* Roxb. Sugito *et al.* (2009) reported that Jaloh has effect as imunostimulant at broiler chickens. According to Kemp *et al*. (2001) several species of salix containing high nutritive values, with the protein content of 142 g/kg, and dry matter digestibility of 650 g/kg and metabolic energy of 9,8 MJ/Kg. So far there were no any reports the effeft of Jaloh or salix species on fish. The purpose of this experiment is to find out the effect of heat stress and jaloh leaves supplementation on body wight gain, level of hematocrit, and level of malondidaldehid (MDA) at liver tissues of nila fish (*Oreochromis niloticus*). The results of experiment can be used as sources of information how to increase productivity of fish in the condition of heat stress.

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Materials and Methods

Jaloh Leaves Samples

The characteristics of jaloh leaves that used as samples are fresh, dark green (about 2 months after falling old leaves) with diameter of trees 10-15 cm (about 2 years). Before being used as pellet, jaloh leaves were dried at the oven with the temperature of 60°C, then ground to be powder for pelleting with commercial feed.

Feed Formulation

Feed used in this experiment was commercial feed in the form pellet. Commercial feed was mixed with jaloh powder based on treatments. The percentage of commercial feed with jaloh leaves were 0, 5, 10, dan 15% from commercial feed. Process of jaloh supplementation was as follow; first all material was mixed and add 1% of binder. All mixed material was pelleted by using fish pelleting machine.

Animal and Experimental Procedures

Experimental fish that used in this experiment was nila gesit with the average wight og 40-50 g. These fishes were obtained from Fish Culture Institution (BBAP) Ujung Batee. Experiments were conducted at aquatic laboratory, Veterinary Faculty, Syiah Kuala University, Banda Aceh. Fishes were placed on chamber for acclimation process. Fishes were fed 3% of body weight per day for 7 days during acclimation process. After acclimation process, fishes were placed into aquarium (10 fishes per aquarium) with the size of aquarium (80cm x 60cm x 30cm). A number of aquariums used in this experiment were 8 aquariums and filled with clean water.

Procedures

Total of 80 nila fishes were randomly allocated into 8 treatments. Experimental treatments were as follow P1 (no heat stress and no jaloh leaves supplementation); P2 (no heat stress and 5% of jaloh leaves supplementation) ; P3 (no heat stress and 10% of leaves supplementation); P4 (no heat stress and 15 % of leaves supplementation); P5 (heat stress and no jaloh leaves supplementation); P6 (heat stress and 5% of jaloh leaves supplementation); P7 (heat stress and 10% of jaloh leaves supplementation); P8 (heat stress about 35 ± 1^{o}C for 4 h per day in 30 days and 15% of jaloh leaves supplementation). Body weight measurement, blood samples and tissue liver data were collected on 31d.

Fishes were fed three times a day (morning, noon and after noon) with the amount of 3% from body weight per day. Temperature in the aquarium was gradually increased from 09.00 and fishes was placed into the water temperature of 29 ± 1ºC and 35 ± 1ºC for 4 hours. Temperature into aquariums was increased by using heater and kept stable by automatic thermoregulatory. Heat treatment was started at 09.00 and finished at 17.00. Water in the aquarium was changed every 3 days with the amount of 80% from aquarium volume. Aquarium was cleaned from feces and refused feed every day.

Measurement Parameters

Parameters measured in this experiment were body weight gain, hematokrit value and value of malondialdehid (MDA) in the tissue livers. Heamatokrit measurement was conducted by mikrohematokrit method. Blood samples of 3 fishes from each treatment were collected at 31d after experiment. Before blood sampling, fishes were anesthetized, then bled by using spuit. Body weight gain were measured by putting fishes in the chamber and weighted (electronical balance 0,5 gr). Fish body weight was measured at the end of experiment (31d). Measurement of liver tissue was based on the ability to form pink color between MDA and TBA. Supernatant was measured and absorbed by using spectrometer with the wave of 532 nm. The unit of MDA was stated as µg per gr sample.

Statistical Analysis

All data were tabulated and statistically analyzed by using completely randomized factorial design. Differences between treatment was stated when $P<0.05$.

Results and Discussion

Water Quality

Water quality at the experimental period is shown in Table1. According to Indonesian National Standard (SN) 2009, water requirements for raising of nila fishes was with the pH of 6,5-8,6, temperature of 25 – 32° C and ammoniac (NH3) content of 0,02 mg/l.

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Table 1. Water quality during experiment

Parameter	P1	P2	P3	P4	P5	P6	P7	P8
NH3	3,49	3,57	3,68	3,36	2,91	3,45	3,48	3,79
pH	7,89	7,78	7,80	7,83	7,83	7,88	7,82	7,82
Organic	51,69	51,57	40,04	43,34	45,74	39,29	38,80	43,47

The range of pH during experiment was 7,78-7,89 and this range was still under SNI recommeded for growth of nila fishes. However, the ammoniac content and organic matter were relatively high based on optimal growth of nila fishes. The content of ammoniac and organic matter was 2,91-3,79 mg/l dan 38,80-51,69 mg/l, respectively.

Djokosetiyanto *et al*. (2006) stated that accumulation of organic matter caused poisonous compounds to the fishes, mineralization of organic compounds and high absorption of nitrogen. It can result in water quality reduction. Mineralization of nitrogen compounds consisting of protein and amino acids produced an-organic nitrogen such ammoniac (NH3), nitrite (NO2) and nitrate (NO3).

Body weight of Nila fishes

Body weight of nila fish during experiment was shown in Figure 1. As indicated in Figure 1, body weight of nila fish was higher in treatment P1 and P2 compared to other treatments. Body weight of P5 (with the aquarium temperature of 35°C ± 1°C) was 651 g. Meanwhile, body weght of treatment P1 was 435 g.

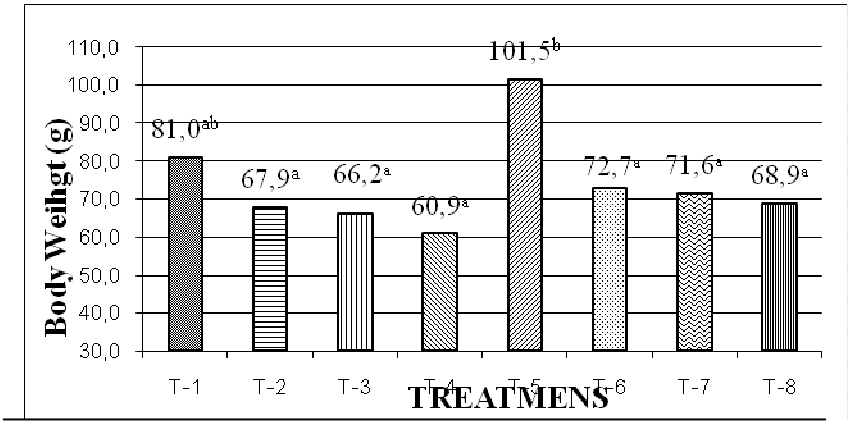

Figure 1. The average of body weight (g) nila fish after feeding for 30d experiment.

There was a big difference between nila fish applied heat stress and without heat stress. The difference between fish applied high temperature and without high temperature was 216 g. The same trend was also indicated at the treatment of supplementation jaloh leaves. It was more than body weight of fish applied without heat stress, compared to heat stress with 23 g and 53 respectively. Body weight of fish without heat stress and was supplemented with jaloh leaves 5% (P2), 10% (P3), and 15% (P4) between 243 to 312 g, meanwhile nila fish applied heat stress and were supplemented with jaloh leaves 5% (P6), 10% (P7), and 15% (P8) were 335 and 365 g respectively. It was indicated that application of heat stress at the temperature of 35°C ± 1°C was better body weight growth compared to nila fishes without application of heat stress. This finding was in accordance with El-Sherif *et al*. (2009) stated that raising of nila fish at the temperature of 32° C improved performance and livelybility of nila fishes. Furthermore, Turker *et al*. (2003) explained that nila fish was thermophilic animals and the ability of feeding was depending on increasing of water temperature in which the ability of feeding was relating to increase of metabolic rate. Morgan (1977) stated that increase of water temperature will stimulate metabolic rate at the body fish. El-Sherif *et al*. (2009) reported that increase of water temperature at aquarium increased the value of RPK.

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Treatment of nila fish supplemented with jaloh leaves in the commercial feed influenced body weight. It was indicated that body weight of control group (P1) dan P5. Fishes were fed jaloh leaves was lower compared to control both with heat and not heat stress. This is probably due to nutritive value of feed supplementation with jaloh leaves. Based on proximate analysis, amongst 4 feed formulation, feed supplemented jaloh leaves had lower of ash and protein content, on the other hand the content of crude fiber and extract-N was higher. The results of feed proximate analyses were shown in Table 2.

Table 2. Chemical analysis of experimental feed.

Feed	DM	Ash	CP	CF	CF	N-Extract	Energy
PK+TDJ 5%	90,54	9,59	28,37	7,35	2,58	42,67	4.183
PK+TDJ 10%	91,43	9,58	28,29	7,46	2,54	43,56	4.444
PK + TDJ 15 %	92,04	9,45	27,31	7,65	2,5	45,13	4.562
PK	89,28	10,82	31,29	6,15	2,46	38,56	4.445

Hematokrit Value of Nila Fishes

Physiological responses of nila fishes applied heat and without heat stress and supplementation of jaloh leaves on level of hematocrit was shown in Figure 2. The lowest value of hematocrit was found in fish supplementation of 5% jaloh leaves (T2) and the highest value of hematocrit was found in the treatment of fish applied heat stress and supplemented jaloh leaves 5% at commercial feed (T6). The result of experiment indicated that hematocrit value was not statistically difference (P>0.05) at the treatment of heat stress and jaloh leaves supplementation at commercial feed. There was linier correlation between value of hematocrit and body weight, as indicated at treatment of P4 and P8. At the treatment of P4 (supplementation of 15% jaloh leaves), the value of Hb was close to high value of 8,3 g/dl. In this observation, there was possibility that administration of fish feed with jaloh leaves at high temperature water increased fish health.

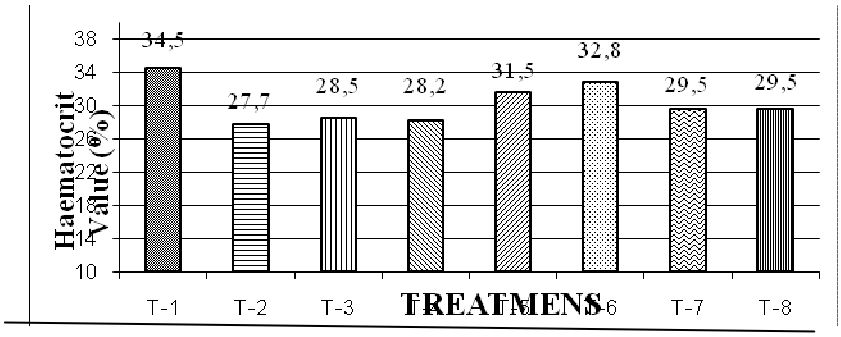

Figure 2. The avarage value of hematocrit nila fishes after treatment of 30d experiments.

Malondialdehid Values at Liver Fish

The results of malondialdehid (MDA) level at liver tissues of nila fish with and without stress application presented at Figure 3. As shown in the Figure 3 that the MDA level of fishes with heat stress and supplementation of jaloh leaves were not significantly differences (P>0,05). However, the level of MDA tented to increase, mainly heat stress treatment combined with supplementation of jaloh leaves (P5). Fish treated in heat stress and fed jaloh leaves were able to reduce the level of MDA in the liver mainly treatment 5% (P6). It was indicated also that fishes were supplemented with jaloh leaves increased MDA content both fish with and without heat stress treatments. In this experiment, fishes supplemented with jaloh leaves without heat stress treatment had bad effect which indicated by increasing of MDA level in the liver. It was shown in the treatment of P2, P3 and P4 in which the level of MDA was higher compared that of treatment P1.

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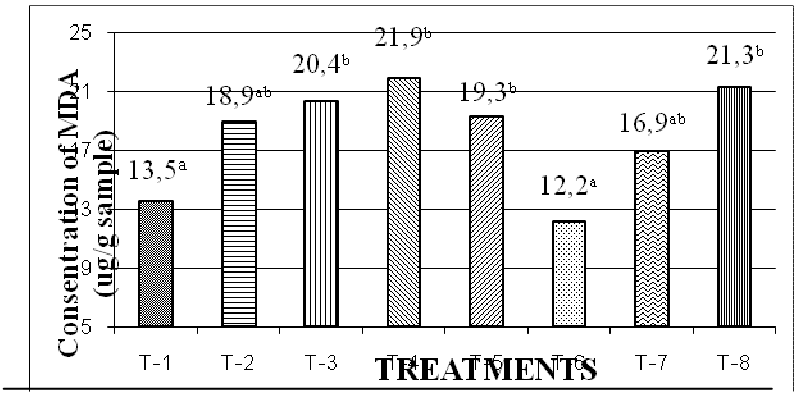

Figure 3. The avarage of malondialdehid (MDA) content in nila fish after treatment of 31d experiment.

Increase of MDA content at nila fishes supplemented by jaloh leaves was a result of lipid oxidative metabolic. It was the same reason for treatment of heat stress. According to Sreejai and Jaya (2010) one of secunder product from lipid acid oxidation was MDA. MDA compound was formed as a result of lipid peroxidation at cell membranes. Measurement of MDA content can be used as indication stress oxidative. Increase of lipid peroxide indicated by level of MDA in the livers can be used an indication that fishes treated without heat stress and supplemented with jaloh leaves powder can be toxic in the liver (P2, P3 and P4). On the other hand, fishes were treated with heat stress and supplemented with jaloh leaves powder reduced level of MDA in the livers. Gab (2011) stated that several toxic compounds resulted in increasing of free radical activities indicated by increasing of MDA level.

MDA content was relative lower in the treatment P6 and P7 due to antioxidant content of jaloh leaves. Commercial feed supplemented with jaloh leaves reduced lipid oxidation and oxidation stress as a result of environmental temperature increase. Bioactive compounds in plants were able to protect cells from oxidative stress. According to Enayat and Benerjee (2009) jaloh plant (Salix sp.) was identified and had several strong antioxidant compounds such as compounds of flavonoid and several lipid derivatives. Asparpanah (2012) informed that some compounds at salix plants have big potency as antioxidant such as ; galic acid, caffeine acid, catechin and quercetin. Other compunds were carvone, citronellol, methyleugenol, dan eugenol. Ahmed and Shah 92012) reported that bioactive compounds contained in salix plants and had strong antioxidant were luteolin, dihydrokaempferol, quercetin, resorcin, dihydrobenzofuran**,** para-hydroxyacetaphenone.

Malonaldehida (MDA) was end product of lipid oxidation. Formation of MDA at normal condition and followed by antioxidant formation on the body. Therefore it will balance between free radical and antioxidant. However, it will produce more than protective limited antioxidant cellular, some parts of free radicals have bad effects on the cells (Lefrina, 2009).

Conclusions

Nila fishes treated with heat stress in the aquariums (temperature 35 ± 1^{o}C) and fed with commercial feed supplemented with powder of jaloh leaves (5-10%) increased body weight and MDA content of the liver tissues. Feeding of commercial feed supplemented with the powder of jaloh leaves (5-15%) to the nila fishes without heat stress treatment decreased body weight and stimulated lipid peroxide.

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