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# FATS IN PRODUCTS FEED SUPPLEMENTS AND EFFECT ON RETENTION OF PROTEIN IN NILEM FISH (*Osteochilus hasseltii*)

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***Abstract***

*Nilem fish (Osteochilus hasseltii) has a great potential as product of baby fish (three months old), and fish eggs (caviar) and that is now introduced in the floating cages started nursery phase. Optimization of cultivation through input-protein energy efficient as well as enrichment efforts fatty acids in the diet is very urgent. This study aims to get the kind of fat supplement of alternative materials which added to the feed to improve growth and protein retention in nilem fish reared in Cirata. The research was conducted in two stages: (1) Phase 1, scale up alternative sources of fats from hazelnut and meal worm (Tenebrio molitor); added in the feed formulation; (2) Phase 2, growth, feed conversion, and retention of protein on fish meat. The experimental design in biological assay using a completely randomized design (6x3); with standard ration treatment (Rs), then Rs with the addition of animal fat supplements (meal worm) and vegetable oils (hazelnut) in the form of flour and oil extraction, as well as a commercial feed controls. The results showed that: 1) Yield of pure oil of hazelnut and meal worm results flouring solvent extraction followed respectively by 27.3% and 12.15% of the fresh weight. 2) Feeding for four weeks showed that growth rate between 3.72 g - 5.82 g, with meal worm feed supplements 2% was highest absolute growth rate, and no significant with high protein on control, and so was hazelnut 2%. 3) Retention of protein obtained in supplement worm oil and hazelnut oil 1%, respectively 33.42 and 33.46%.*

***Key words:*** *fat supplements, growth rate, retention of protein, nilem fish.*

## INTRODUCTION

The main feed materials are often used in fish feed is fish meal which has undergone the process so that the pressing and drying fish oil out. Until now, fish oil is a major supplier of the fatty acids ω3 good for fish cultivation and for human consumption of the fresh especially fish herbivore / omnivore like fish nilem able to synthesize omega-3 (EPA and DHA) fatty acids C-18, so it does not depend on the fish oil and fish meal in feed.

Sources of fat used as a supplement in unsaturated fatty acids other than fish oil is beef tallow, linseed oil and corn oil. Because these oils are generally imported it is necessary to look for another alternative feed ingredients based on local ingredients. Alternative sources of fatty acids that potentially are pecan seeds, and larvae of *Tenebriomolitor*. Pecan seed (*Alleurites mollucana*) is known as one of the original Indonesian spice recommended as a source of unsaturated fatty acid linseed oil substitute for part of the fruit (seed) oil content of 55-65%, and oil content in the shell by 60%.Based on this background, it is very important to do research on the utilization of fat supplements exploration results from the fatty acid source alternative materials in order to improve the growth performance, feed conversion of nilem fish and their effects on fish meat protein retention.

The purpose of this study is to determine the type of fat (vegetable and animal fat) best in fish feed formulations on fingerling of nilem fish in the floating net Cirata effect on growth, feed conversion, and protein retention in meat of nilem fish.

## MATERIALS AND METHODS

Materials research was pecan seed and mealworm (*Tenebrio molitor*), nilem fish, feed raw materials and commercial feed,and floating net cages. The chemicals used include chemicals for extraction and chemicals for proximate analysis (protein, fat components, energy, ash, crude fiber). The treatment is based on the addition of a fat source (flour and oil) from animal and vegetable fat source in artificial feed. The feed material consists of fish meal, soybean meal and white bran.

The study consisted of two phases: separation and multiplication of feed supplements of fat extraction, analysis of nutrient composition, and formulation of nilem fish feed. The second stage was the Feeding Trial on nilem fish to obtain type supplement(flour and oil extract). Fat supplement added to low protein feed (20%), to see the value of the benefits and efficiency by measuring the growth, feed conversion continued the third phase measurement of protein retention in nilem.

**Research procedure**

The process of making fat supplements includes the following stages:

1) Preparation of materials including water level mealworm and hazelnut oil, performed with the following stages: solving or downsizing, then drying, followed by immersion in an organic solvent, then extraction and evaporation. Mealworm turn off the process by means of watering in hot water of 80°C is also intended as steaming process (provision of hot steam), then performed the drying process at a temperature of about 80° C and to reduce discoloration done by flipping back the biomass of mealworm as often as possible. Hazelnut is *Euperbiaceae* plant seeds, which initially had a hard seed should undergo the process of solving (crushed) shell beans, in order to obtain a yellow pecan seed round and intact.Formulation and manufacture of fat-supplemented feed for fish nilem stadia enlargement. The composition of the basal feed ingredients were Soybean 10%, rice bran 40%, fish meal 8% flour 5%; White bran 34%, and vitamin mix and mineral mix, respectively 1.5% . The addition of sources of fat in the form of flour by 2%, while in the form of oil by 1%, respectively mixed into the basal ration of raw materials, and made into pellets.
2) Biological Test Phase (feeding trials) include:
   a. Stage adaptation of fish to the container trial and media maintenance,
   b. Stage adaptation of fish to feed the test, for 1 week, and the determination of the number of feeding.
   c. Stage of collecting and recording data growth during maintenance, carried out for a month (30 days). Measurement of feed consumption, sampling body weight gain and feed conversion is done weekly maintenance.
3) The fish meat from fish sampling dissected, separated and in fillets, then weighed and the fresh weight aside into wrap clipping and temporarily stored in the refrigerator, and then analyzed the protein content.

The study was conducted experimentally by using a completely randomized design of six treatments and three replications. The treatment is based on the addition of fatty acids (from vegetable oils and fat animal), namely:

Feed A: basal feed (standard ration) protein 20%;
Feed B: Feed basal + 2% hazelnut flour;
Feed C: basal feed + 2% flour mealworm;
Feed D: basal feed + 1% bv/bw hazelnut oil extraction;
Feed E: Feed basal + 1% bv/bwmealworm oil extraction;
Feed F: commercial feed (ration control).

Treatment effect was tested by F test and statistical analysis to determine the differences of each used Duncan Multiple Range Test (Steel and Torrie, 1980).

## RESULTS AND DISCUSSIONS

The results of the crude protein and extract ether analysis were 19% and 55% for hazelnut meal and 55.11% and 15.51% for mealworm. The results obtained by the mechanical extraction of materials for seed yield hazelnut 60%, while the mealworm 70% from fresh

weight. The reduced weight of the material ingredients of feed supplement is caused mainly by the loss of moisture, lost in the drying process and flouring. However, pressing and flouring still needed to gain fat. The components of macromolecules nutrients, valuable biological contents of cells located inside the plant so that treatment to remove the contents of a cell of the plant cell walls necessary, by breaking or damaging the cell walls so that the desired components can be taken. Cooking on mealworm through flouring and extraction is also intended to improve the digestibility of theshell mealworm and should be done peeling shell/skin from the body parts. The analysis showed that the fat content is greater hazelnut flour at 55%, compared with 15.51% fat mealworm. Protein content of mealworm greater than fat's. Inseparability of fat in the extraction process either mechanically or chemically called leaching (washing). In extraction plants, extraction or leaching is a process that is preceded by contact between the phases followed by diffusion of solution phase (solute) from the solid phase and liquid phase, so that the components are dissolved.

From the research results to the manufacture of mechanical extraction of hazelnut oil do Estrada (2011), walnut oil in the process of flouring do pressing and generate yield too little hazelnut oil. So pressing mechanical extraction results in this study was not done. To get more fat components followed by solvent extraction process. The results of the analysis of the fat content of grains hazelnut (hazelnut flour) large enough that 55%, with a calorific value of 6260 kcal/ kg., But the yield obtained from the flouring by 60%, so the content of dry hazelnut flour drying by 33%. After going through the stages of mechanical extraction with flouring, followed by solvent extraction stages or chemically using a solvent n-hexane with immersion techniques. According to Hartadi (1986) Steep extracted (extraction immersion) is the treatment of the material (raw material) resources containing fat in water or solvent hexane to remove dissolved materials. The materials are expected extracted substances such as oils, fats, or fatty acids and others more quickly and perfectly.

Table 1. Yield of Oil and Fat after extraction solvents of Hazelnut and Mealworm

| | Hazelnut | Mealworm* |
|---|---|---|
| Extract ether (%) | 55 | 15.51 |
| Yield flour | 60 | 70 |
| Solvent : material ratio | 2 : 1 | 2 : 1 |
| Yield of extraction | 30% | 35% |
| Yield of oil/100 g raw material**) | 27.3% | 12.15% |

Note: *) The results of solvent extraction with hexan solvent using techniques of maceration (soaking 24 hours) the hazelnut flour and mealworm
**)Yield of oil = (fat content (BK100%) x yield x preparation stage solvent extraction yield) of the initial weight (fresh).

Extraction of the fat that comes from animal should be preceded by a process called Rendering, a process that begins with the destruction of body tissues of animals such as bone, fat deposits by heating vacuum wet method, and then to separate the oil component by way of centrifuge (Gurr, 2002). The effectiveness of the process of dissolving fat can be determined from the agitation process, the large number of solvents and solvent type. According to Estrada (2011), on the whole seed extraction, leaching process efficiency is determined by the contact between the solvent and solute-containing solids to be separated. Leching Speed shows the speed rate leching depending on: the size of the particles, the type and speed of solvent flow, and temperature. Solvents are chosen to be selective for the separation of solutes concerned and low viscosity makes it easier to circulate.

The molecular weight was86.18 g/gmol, density 0.6548 g / ml, the freezing point of -95°C, and the boiling point of 69°C. These solvents are not polar, inert so it does not react with the components of oil and the price

is cheap, not flammable and non-toxic. In this study treatment with pressing (result precondition with flouring) continued extraction of oil cake. Hazelnut fatty acids are predominantly oleic which is a component of omega 9 fatty acid (C18: 1W9) similarly mealworm. Although the fat content of hazelnut greater than, the results of this study saphonification number (KOH) fat hazelnut higher, indicating smaller molecular weight. The higher the saphonification of vegetable fat has also been confirmed from the results of research Mathyazhagan (2011) that are generally non-food vegetable oils such as Jathropa, pongamin, containing volatile fatty acids that are quite high. The high content of constituent soap VFA will increase, thus increasing the numbers saponification and susceptible to oxidation. Although the variation of the fatty acid component is quite a lot but the fatty acids they need to be purified and further bindering, as well as additional treatment added antioxidants to improve stability, so that the molecules can be bound in glycerides and not easily evaporate or turn into free fatty acids.

The ratio of the use of polar solvent used in this study is a 2: 1 or 50g / 100ml n-hexane. The ratio of oil cake mass/volume n-hexan conducted to determine the yield (fat produced). It obtained from the extraction of oil produced by flouring stage followed by solvent extraction with a maceration technique using a solvent n-hexan. According to Estrada (2011), to produce a higher oil yield required processing techniques with expeller on the precondition phase and continued with chemical engineering. From the results showed that the yield resulting from stage expeller less but produce better quality oil than the immersion technique. This is because the use of solvent immersion technique more and can be repeated so as to produce greater oil yield.

Oil extraction solvent undergo a process of separation by heating to a temperature of about 70°C, which can cause the oil is oxidized to form aldehydes and ketones and free fatty acids. Besides oxidation also causes the breakdown of the double bonds that lack saturated degree decreases and the iodine numbers to be down. The decline in the degree of unsaturation causes the refractive index decreases. The disintegration of the oil with the oxidation process has caused the amount of triglycerides is reduced, which causes the saphonification is reduced. The time required for the extraction process takes quite a long time so that the solvent can dissolve the solute to reach a point of equilibrium.

In the extraction of fat hazelnut, pressing method only by mechanical means, resulting in a higher oil quality but lower the net yield of oil (yields) than pressing a mechanical way, followed by extraction with a solvent (oil cake) (Estrada, 2011). The decline in oil quality is shown by an increase in free fatty acid content (free fatty acid) and colors as well as a decrease in the number of iodine and saphonification.

Nilem fish used comes from local farmers with the initial weight of about 5 grams. Growth is defined as the change of fish in weight, size and volume in line with the changing times (Effendie 1997). According Djajasewaka and Djajadireja (1980), the growth rate will be different levels depending on the ability to digest and utilize the feed as optimally as possible.Analysis of variance showed that the growth rate of fish nilem treated feed supplements increase by 1-2% indicates real. Feeding with the addition of flour feed supplements mealworm 2% highest absolute growth rate that is equal to 5.82 grams of 4 weeks of the study. Daily growth in each treatment has a value that is not much different and it ranged between 3.72 g-5.82 g.

According to Gurretal (2002) proximate composition of the body can be affected by the fatty acid composition because its permeability properties support the overall cell metabolism. The cell membrane is not only covers the outside of the cells that wrap around the cell, but also covers the outside of the cell organelles such as ribosomes. Cell membrane permeability was influenced by the lipid active role influenced by unsaturated fatty acids compounds such phospholipids (Bellet al.,1986). Changes in the permeability of cell membranes can interfere with the activity of the enzymes o that it does not directly interfere with protein synthesis in cells.

Table 2. Growth absolute and Protein Retention

| Treatment | Growth Absolute (g) | Protein Retention (%) |
|---|---|---|
| A (Basal Feed) | 3.72 a | 22.96 a |
| F (Control) | 4.61 b | 27.75 b |
| B (Hazelnut 2%) | 4.75 bc | 28.73 b |
| C (Mealworm 2%) | 4.80 c | 33.19 c |
| D (Hazelnut oil 1%) | 5.28 cd | 33.42 c |
| E (Mealworm oil 1%) | 5.82 d | 33.48 c |

In Table 2 it appears that produced the lowest protein retention in treatment without the fat supplement (Feed A), while the retention of the protein with the addition of hazelnut oil and oil supplements mealworm higher.

Retention of higher protein with the addition of oil showed a positive effect on protein synthesis. The cell membrane permeability of phospholipid that is influenced by lipid active role influenced by unsaturated fatty acids compounds such phospholipids (Bell et al., 1986).

Then were changes in cell membrane permeability that can disrupt the activity of enzymes in mitochondria that are rich in unsaturated fatty acids (Fleischer et al., 1962 in Mokoginta, 1986). If this happens, then indirectly interfere with protein synthesis in cells. These events seen on treatment a deficiency in fatty acids n-3 and n-6, so that the protein content be low at 53.06%.

Ensminger et al. (1990), state that fish that lack essential fatty acids, free water content and body fat will increase, but the protein content will decrease.

The same thing happened in the feed is too high fatty acids, the body becomes low protein, high levels of body fat into a situation were allegedly closely associated with protein and body fat as a result of fatty acids in the diet. This happens because the protein is a molecule that is polar and can bind water molecules while the fat is non-polar and does not bind water.

Then, when the review of energy, allegedly part of the energy from fat in feed D used efficiently for growth in addition to metabolism, whereas most proteins utilized by the fish for growth well because fat is a protein sparing effect in the use of energy for the body.

## CONCLUSIONS

The results showed that:

1) Yield of pure oil of hazelnut and meal worm results flouring solvent extraction followed respectively by 27.3% and 12.15% of the fresh weight.

2) Feeding for four weeks showed that growth rate between 3.72 g - 5.82 g, with meal worm feed supplements 2% was highest absolute growth rate, and no significant with high protein on control, and so was hazelnut 2%.

3) Retention of protein obtained in supplement worm oil and hazelnut oil 1%, respectively 33.48% and 33.42%.

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