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Digestibility of tarum leaf flour (*Indigofera zollingeriana*) the fermented *Aspergillus niger* as a diet ingredients jaya sakti carp larvae (*Cyprinus carpio*, L)

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Abstract
Tarum leaves (Indigofera zollingeriana) is an alternative local raw ingredients that can be used as fish diet ingredients. *I. zollingeriana* has a fairly good nutritional content, namely 29.06% protein. Utilization of *I. zollingeriana* is faced with the constraint of a high fiber content of 15.25% which reduces the digestibility of the diet. Efforts to overcome this problem are through a fermentation process, one of which uses Aspergillus niger. This research aims to look at the digestibility of *I. zollingeriana* leaf flour fermented by *A. niger* as a diet ingredient for the Jaya Sakti Carp larvae. This study consisted of two treatments and each with four replicates, commercial diet as a reference diet, non-fermented *I. zollingeriana* leaf flour and fermented *I. zollingeriana* leaf flour as test diet and added Cr_2O_3 as an indicator of digestibility. The test fish used were Carp larvae, 7-8 cm long, 8-9 grams in weight, kept in a 60x50x40 cm aquarium. Each aquarium was filled with 90 fish or at a density of 83 fish/cm2 and fed at satiation 3 times a day. Based on the results of research that has been done, the use of Indigofera zollingeriana leaf flour fermented by Aspergillus niger as a diet ingredient for carp larvae gives a digestibility value of 42.53%, protein digestibility 77.19% and energy digestibility 66.21% the best compared to leaf flour diet. *I. zollingeriana* is non-fermented so it can be used in fish enlargement diet formulations and is expected to improve fish growth performance.

Keywords: Digestibility, Indigofera zollingeriana, Aspergillus Niger, Carp

Introduction

Carp (*Cyprinus carpio*, L) is a species of freshwater fish that is in great demand and has the potential to be developed. Increasing production from Carp farming certainly requires diet with good quantity and quality. Diet costs consume 60-70% of the total production costs (Nurhayati and Nazlia, 2019) [21]. In an effort to overcome this, fish cultivators need to look for alternative local raw ingredients that have nutritional content according to the needs of fish, are available in large quantities, are relatively cheap and do not compete with humans (Aida *et al.*, 2020).

One of the alternative local raw ingredients that can be used as a diet ingredient is tarum leaves (Indigofera zollingeriana). As a tropical tree leguminous forage, *I. zollingeriana* has a high protein content, 29.06% (Pangentasari *et al.*, 2018) [22]; 25.17% - 26.44% (Kumalasari *et al.*, 2017), 22-28% (Santi, 2017). *I. zollingeriana* also contains the amino acid histidine 0.67%, threonine 1.14%, arginine 1.67%, tyrosine 1.05%, methionine 0.43%, valine 1.56%, phenylalanine 1.60%, isoleucine 1.35%, leucine 2.26%, and lysine 1.57 (Marzuqi *et al.*, 2015). In addition, *I. zollingeriana* also contains minerals, namely: Ca 1.16%, P 0.26%, Mg 0.46% (Abdullah, 2010). The content and composition of vitamin A in *I. zollingeriana* is more complete compared to soybean flour which is equal to 3828.79 IU/100g. The content of β-carotene in Indigofera sp leaves (507.6 mg/kg) has the potential as an antioxidant for livestock (Palupi *et al.*, 2015) so that it has great potential to be developed as a local diet raw ingredients that can replace soybean flour (Mukti, 2019).

The use of *I. zollingeriana* as a raw ingredients for diet has problems, namely the high crude fiber content which will interfere with the digestion of diet in fish (Hansen and Hemre, 2013) [9], the digestibility of fish becomes low (Pandey, 2013) as well as limiting the use of diet by fish (Caruso, 2015). Crude fiber in diet ingredients contains acid detergent fiber (ADF), neutral detergent fiber (NDF), cellulose, hemicellulose and lignin fractions (Melati and Suranto, 2016). Efforts to reduce high levels of crude fiber and toxic content in diet ingredients and increase crude protein content are carried out by means of fermentation (Jakobsen *et al.*, 2015) [11].

Fermentation is a ingredients processing activity using micro-organisms as the main actor in a process. Fermentation activities produce enzymes to break down crude fiber and increase protein content, where a number of proteins, carbohydrates and fats are broken down into smaller fractions to facilitate digestion and absorption of nutrients (Liwe *et al.*, 2014). The fermentation process also aims to increase protein content and quality, maintain nutritional value during storage and reduce anti-nutrients (Restiningtyas *et al.*, 2015); (Clude *et al.*, 2016); (Daud *et al.*, 2020). One of the microorganisms used in fermentation is *A. niger*, which is a species of Aspergillus that can grow fast, is easily available at a low price, is able to grow in relatively inexpensive media and does not produce mycotoxins (Maryanty *et al.*, 2010). *A. niger* can grow at temperatures of 35°C - 37°C (optimum), 6°C - 8°C (minimum), 45°C - 48°C (maximum) and requires sufficient oxygen (Sinaga *et al.*, 2012). *A. niger* is a species of mold that has the ability to secrete the enzymes cellulase, chitinase, amylase, glucoamylase, catalase, pectinase, lipase, lactase, invertase, and acid protease (Purkan *et al.*, 2016); (Djunaidi *et al.*, 2020).

A. niger is more suitable for fermentation of leaf ingredients than bacteria because of its higher level of lignocellulase secretion (Wang *et al.*, 2013). Utilization of *A. niger* at a dose of 0.3%, fermentation time of 96 hours with a temperature of 30-35°C in *I. zollingeriana* leaves was able to reduce the crude fiber of the substrate from 15.1% to 3.6% (Puspitasari *et al.*, 2019) [24]. Supplementation of 12% *A. niger* on water hyacinth leaves increased crude protein from 6.56 to 18.15% (176.67%) and decreased crude fiber content from 26.47 to 11.95% (54.93%) so it is good as a fish diet ingredient (Suharman *et al.*, 2021) [29]. *A. niger* has been widely used in fermenting fish diet raw ingredients and gives the best results on ingredients digestibility. This research aims to evaluate the digestibility effectiveness of turum leaf flour (*I. zollingeriana*) fermented by *A. niger* as a diet ingredient for Jaya Sakti Carp (C. carpio, L.).

Metode Research

Research Tools and Ingredients

The tools used in this research were a pellet molding machine with a 3 mm die size, an aquarium measuring 60x50x40 cm3, a hose and aeration stone, a siphon hose with a diameter of 4.5 mm, a petri dish, a faecal drying oven, a sample bottle with a volume of 25 grams, a digital scale with accuracy of 0.1 mg, a digital thermometer with an accuracy of two decimal places, a digital pH meter with an accuracy of 0.01, and a DO meter with an accuracy of 0.1 mg/L.

The fish used in this research were test fish (Jaya Sakti carp larvae) with a total length ranging from 7-8 cm and body weight ranging from 8-9 grams, commercial diet in the form of pellets (PF 500), non-fermented *I. zollingeriana* leaf flour, fermented *I. zollingeriana* leaf flour, tapioca flour, 0.3% *A. niger* and 0.5% Cr_2O_3 as digestibility indicators.

Research Design

This Research used a completely randomized design, in which the treatments used were fermented *I. zollingeriana* leaf flour and non-fermented *I. zollingeriana* leaf flour with 4 (four) replications for each treatment. In more detail, this treatment refers to the basis for measuring the digestibility of ingredients indirectly according to Watanabe (1988), namely:

Treatment 1 is commercial diet as a reference diet.

Treatment 2 was non-fermented *I. zollingeriana* leaf flour (diet test)

Treatment 3 was leaf flour of *I. zollingeriana* fermented test diet (diet test)

Preparation of Containers, Media and Test Fish

The aquarium was placed on an iron rack horizontally and labeled for each treatment and replication and then filled with water with a volume of 90 liters with a water level of 30 cm. Each aquarium is equipped with a hose and aeration stone as a supply of oxygen which is placed in the middle of the long side of the aquarium and half the depth of the aquarium water. The water used comes from well water which is deposited in a reservoir for 48 hours. The water quality conditions in the aquarium are well maintained by cleaning the aquarium every day and changing the water ± 30-50% of the total volume of aquarium water. Each aquarium was filled with 90 fish or with a density of 83 fish/cm2 and adapted for 7 days. Then the fish is fasted for 1x24 hours to empty the fish stomach. Fish were given the test diet at satiation with a frequency of dieting 3 times a day, namely at 08.00 WIB, 12.00 WIB and 16.00 WIB.

Manufacture of Test Diet

The leaves of *I. zollingeriana* which are used as raw ingredients for diet are taken from trees aged 5 months with a height of ± 1 meter, separated from the branches and then dried in the sun to dry and then made into flour. For the test diet ingredients in the form of fermented *I. zollingeriana* leaf flour, steamed *I. zollingeriana* leaf flour in boiling water 100°C for 30 minutes, cooled and given an *A. niger* fermenter with a dose of 0.3% and stirred thoroughly then put into polyethylene plastic, perforated and fermented for 7 days with temperatures ranging from 35°C - 40°C. Then steamed for 10 minutes to stop the fermentation process. Making digestibility test diet using the Watanabe formulation (1988) and can be seen in Table 1.

Table 1: Formulation of diet digestibility test for diet ingredients Jaya Sakti Carp Larvae

No	Ingredients name	Test Diet (%)		
		Commercial	Indigofera leaf flour	
			Non fermented	Fermentation
1	commercial diet	96.5	66.5	66.5
2	Non-fermented *I. zollingeriana* leaf flour	0	30.0	0
3	Fermented *I. zollingeriana* leaf flour	0	0	30.0
4	Cr_2O_3	0.5	0.5	0.5
5	Tapioca flour	3.0	3.0	3.0
	Total	100	100	100

Source: Watanabe (1988)

Observation of Test Parameters

Collection of faeces from test fish will be carried out after 30-60 minutes after dieting. Fish faeces are taken fresh using a small hose and fine filter then put in a sample bottle that has been labeled and stored in the freezer. Furthermore, the collected faeces were placed in a petri dish and dried using an oven at 65°C for 12 hours to remove the water content in the feces. The dried faeces are stored in sample bottles labeled according to treatment in the freezer until the faecal sample weight reaches 10-15 grams.

Proximate analysis was carried out on test diets (commercial diet as reference diet, non-fermented *I. zollingeriana* leaf flour, and fermented *I. zollingeriana* leaf flour as test diet) and fish feces including measurement of water content, crude protein, crude fat, crude fiber and ash. Analysis of crude protein using the Kjeldahl method, water content and ash content was carried out using the Gravimetric method and crude fat using the Soxhlet method (AOAC, 2005) and Cr_2O_3 content analysis (Takeuchi, 1988). Observations of water quality parameters including temperature, pH and DO were measured 3 times a day.

Data analysis

Data tabulated from research results include: digestibility of ingredients, digestibility of protein, digestibility of fat, and digestibility of energy as well as water quality data which will be tabulated in the form of tables and graphs which will then be analyzed descriptively.

1. Digestibility of Diet Ingredients (KB)

The digestibility of raw ingredients is calculated using the formula (Watanabe 1988)

$$KB\ (\%) = \frac{ADT - 0.7\ AD}{0.3} X\ 100$$

Information

ADT = Digestibility value of the test ingredients diet (%)

AD = Digestibility value of reference diet (%)

2. Digestibility of Protein (KP) calculated using a formula (Taukechi 1988)

$$KP\ (\%) = 100 - \left[100\ x \frac{\%Cr_2O_3\ diet\ X\ \%\ faecal\ protein}{\%Cr_2O_3\ feces\ X\ \%\ Diet\ proteins}\right]$$

3. Digestibility of Fat (KL) calculated using a formula (Taukechi 1988)

$$KL\ (\%) = 100 - \left[100\ x \frac{\%Cr_2O_3\ diet\ X\ \%\ faecal\ fat}{\%Cr_2O_3\ feces\ X\ \%\ diet\ fat}\right]$$

4. Digestibility of Energy (KE) calculated using a formula (Taukechi 1988)

$$KE\ (\%) = 100 \left[100\ x \frac{\%Cr_2O_3\ diet\ x\ \%\ faecal\ energy}{\%Cr_2O_3\ feces\ X\ \%\ diet\ energy}\right]$$

Results and Discussion

The main factors limiting the use of vegetable raw ingredients as raw ingredients for fish diet are the presence of anti-nutrients and high crude fiber. To overcome this, further processing is needed, one of which is through the fermentation process. The use of 0.3% *A. niger* for 6 days with a temperature of 35-40 °C in fermenting I. zoligeriana leaf flour was able to reduce the crude fiber content and increase the protein in I. zoligeriana leaf flour so that it could be used as a fish diet ingredient. Proximate analysis of I. zoligeriana leaf flour before and after fermentation is presented in Table 2.

Table 2: Proximate Analysis of Diet Ingredients Before and After Fermentation

Nutritional content	commercial diet	*I. zollingeriana* leaf flour	
		Before fermentation	After fermentation
Coarse fiber (%)	4	10.17	4.23
Proteins (%)	39 - 41	27.94	31.40

Fermentation of I. zoligeriana leaf flour with A. niger of 0.3% for 6 days at 35-40 °C was able to reduce the crude fiber of the substrate from 10.17% to 4.23%. The incubation time of days increased the chance for *A. niger* to grow and ferment. The longer the incubation time, the higher the opportunity for *A. niger* to degrade the substrate ingredients (Tampoebolon, 2009) [18]. Fermentation of *I. zollingeriana* leaf flour for 6 days is thought to reduce the content of Acid Detergent Fiber (ADF). In the fermentation process there is activity of *A. niger* which produces cellulase enzymes to digest fiber components in the form of cellulose, where cellulose is a part of ADF so that *A. niger*'s activity in digesting cellulose can reduce ADF levels (Dewi *et al.*, 2019) [5]. *A. niger* is a microbe that tends to break down simple sugars, where the cellulase enzyme produced from *A. niger* inoculum is able to break down complex bonds from fibers into simpler components (Soares *et al.*, 2018) [23].

The decrease in the levels of Acid Detergent Fiber (ADF) was in line with the decrease in Neutral Detergent Fiber (NDF). *A. niger* utilizes cell content (NDS) to support its growth, where NDS consists of protein, carbohydrates and soluble minerals and fat. The decrease in NDF content is due to the production of a lot of cellulase enzymes so that the cell walls are broken down more and can degrade the crude fiber contained in the substrate, in the end the crude fiber in ingredients will decrease (Nurdin *et al.*, 2019) [20]. The decrease in NDF content was caused by a decrease in hemicellulose, where hemicellulose and cellulose are cell wall components that can be digested by microbes. The high level of lignin causes microbes to be unable to master hemicellulose and cellulose perfectly. The lower the NDF and ADF fractions, the higher the digestibility of the diet, so that the diet given is beneficial for fish growth, optimal ADF and NDF content is needed (Sudirman *et al.*, 2015) [25]. Furthermore, Amanda (2021) [1] in his research stated that the fermentation process for 6 days after adding *A. niger* significantly affected the Acid Detergent Fiber (ADF) content of sago pulp, which was 15.67% and the Nutrient Detergent Fiber (NDF) content was 2.07 %.

Fermentation process of *I. zollingeriana* leaf flour with *A. niger* of 0.3% for 6 days with a temperature of 35-40 ºC besides reducing crude fiber was also able to increase the crude protein of the substrate ingredients from 27.94% to 31.40%. This is also reinforced by the statement of Jakobsen *et al.*, (2015) [11] namely, in addition to reducing crude fiber content, fermentation can increase crude protein content. The increase in protein is thought to have come from the contribution of protein from the mycelia of the mold, where the mold has a PK content of 31 ± 50% which contributes to the addition of protein substrate after fermentation (Yohanista *et al.*, 2014) [61]. Extracellular enzyme secretion by *A. niger* plays a role in increasing the crude protein content (Indariyanti and Rakhmawati, 2013) [10].

The decrease in crude fiber content and the increase in protein content of I. zoligeriana leaf flour through *A. niger* makes I. zoligeriana leaf flour suitable for use as raw ingredients for fish diet, where the fiber of fish diet is not more than 8%. Based on the results of the proximate analysis, both reference and test diets have different nutritional content. The test diet using *I. zollingeriana* leaf flour which was fermented by *A. niger* had a lower crude fiber content value while the protein content was higher compared to diet with non-fermented *I. zollingeriana* leaf flour. For more details see Table 3.

Table 3: Proximate Analysis of Diet Ingredients Digestibility Test on Jaya Sakti Carp larvae (% dry matter)

Nutrient Content (%)	Test Diet		
	Commercial	*I. zollingeriana* leaf flour	
		Non fermented	Fermentation
Ash	11.42	10.71	9.91
Proteins	41.04	38.57	39.47
Fat	6.10	4.21	4.02
Coarse Fiber	1.93	4.72	2.56
BETN *	39.90	41.79	44.04
Digestible Energy** (kcal/kg diet)	291.82	273.59	280.82

Information

* extract ingredients without nitrogen are calculated by the formula: 100-(protein content + fat + fiber + ash.

** digestible energy refers to NRC (1993) [18] with fat conversion of 8.1 Kcal/kg; protein of 3.5 Kcal/kg and carbohydrates of 2.5 Kcal/kg.

The ash content of the test diet using fermented *I. zollingeriana* leaf flour was lower at 9.91% compared to the ash content of the tested diet using non-fermented *I. zollingeriana* leaf flour at 10.71%. Changes in the ash content of the substrate during the fermentation process are caused by changes in organic matter during the bioconversion process. In the process of fermenting organic matter in *I. zollingeriana* leaf flour, the number of fermented leaves is increasing due to the use of carbohydrates as an energy source for *A. niger*. A niger utilizes organic matter in the form of crude fiber and BETN through degradation by enzymes to produce glucose compounds for growth and development. The more organic matter in the fermentation process, the lower the ash content proportionally (Kusumaningrum *et al.*, 2012) [14]. Changes in the ash content of the substrate during the fermentation process are caused by changes in organic matter during the bioconversion process (Haddadin *et al.*, 2009) in (Yohanista *et al.*, 2014) [61].

The protein content of *I. zollingeriana* leaf flour test diet which was fermented by *A. niger* was higher, namely 39.47%, compared to the non-fermented *I. zollingeriana* leaf flour protein, which was 38.57%. A decrease in crude fiber content can also increase the crude protein content proportionally. This increase in protein comes from mushrooms containing protein, enzymes produced by fungi contribute to the amount of protein in the fermented ingredients. In addition, duration and dose also affect the increase in protein content in the substrate because it is related to the number of fungi that grow and develop in the substrate (Puspitasari *et al.*, 2019) [24].

The increase in crude protein substrate ingredients after fermentation was caused by the ability of *A. niger* to produce proteolytic enzymes to hydrolyze proteins into amino acids and contributions of protein originating from the microorganism itself in the form of single cell protein (PST) and enzymes produced by microbes resulting in increase in protein ingredients. Besides that, *A. niger* also produces cellulolytic enzymes and mannase which can release the bonds of cellulose or other fiber fractions with proteins. The use of *A. niger* in fermenting *I. zollingeriana* leaf flour has a low protein increase value compared to coconut cake fermentation with *A. niger* being able to increase protein from 22.41% to 35.27% (Mairizal, 2009) [16].

The fat content of *I. zollingeriana* leaf flour fermented by *A. niger* was lower, namely 4.02%, compared to non-fermented *I. zollingeriana* leaf flour fat, namely 4.21%. The decrease in the fat content of the substrate is due to the use of fat as a source of energy. Increased development of mold along with increased utilization of fat (Yohanista *et al*., 2014) [41]. In the fermentation process, there is an ov erhaul of fat by the enzyme lipase which is used as energy for its growth. The initial crude fat content of the substrate stimulated lipase enzyme activity and lipase enzyme production which was influenced by the growth of mold biomass (Kusumaningrum *et al*., 2012) [14]. The more use of diet ingredients containing glucose in the substrate can stimulate the growth of mold biomass which results in more lipase enzyme production (Nurhayati *et al*., 2006) [21].

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The content of the fermented non-nitrogen-free extract (BETN) of *I. zollingeriana* leaf flour was 44.04% higher than the non-fermented *I. zollingeriana* leaf flour (41.79%). The low crude fiber content can increase BETN. The increase in the BETN value of fermented *I. zollingeriana* leaf flour was due to the addition of carbohydrates to the substrate during the fermentation process and the ability of *A. niger* to break down cellulose (Pangestusari *et al*. 2018) [22]; (Kusumaningrum *et al*., 2012) [14]. The energy value of fermented *I. zollingeriana* leaf flour was higher, namely 280.82 kcal/kg of diet compared to the energy value of non-fermented *I. zollingeriana* leaf flour, namely 273.59 kcal/kg of diet. Energy is not a nutrient, it is released during the oxidation metabolism of carbohydrates, fats and amino acids (NRC, 1993) [18].

Digestibility of fish is the ability of fish to digest an ingredient, while digestible ingredients is part of the diet that is not excreted in feces. High digestibility indicates a high amount of certain nutrients absorbed by livestock, whereas low digestibility indicates that the diet ingredients are less able to provide nutrients for primary needs or production needs. Digestibility value is a value that describes the amount of nutrients in the diet that can be digested by fish (Putra *et al*., 2015) [25].

The nutritional value of diet for fish depends on how far the fish are able to digest the diet. To determine the digestibility of fish to diet, it is done by using Chromix Oxide (Cr_2O_3) as an indicator. After knowing the value of the Cr_2O_3 indicator, we can use this value to see the value of digested diet in the digestion process of a type of diet. Cr_2O_3 concentration values in diet and feces in each treatment are presented in Table 4.

Table 4: Value of Cr_2O_3 concentration in diet and feces on Jaya Sakti Carp larvae (% dry weight)

Treatment	Cr_2O_3 Concentration (%)	
	In Diet	**In Faeces**
Commercial diet	0.59	1.26
Non-fermented leaf flour of *I. zollingeriana*	0.53	0.99
Fermented *I. zollingeriana* leaf flour diet	0.54	1.08

The concentration value of Cr_2O_3 in this study showed that the concentration of Cr_2O_3 in feces (reference diet and treatment) was higher than the concentration of Cr_2O_3 in diet (reference diet and treatment). The high value of Cr_2O_3 concentration in feces (reference diet and treatment) indicates that the diet can be digested by fish. Cr_2O_3 is an indigestible ingredients (Nugraha and Khazaidan, 2017) [19]. Diet digestibility values consist of digestibility of ingredients, digestibility of protein, digestibility of fat and digestibility of energy, can be seen in Table 5.

Table 5: Digestibility Value of *I. zollingeriana* Leaf Flour on Jaya Sakti Carp Larvae

Parameter	Commercial Diet	Test Diet (%)	
		Non fermented	**Fermentation**
Digestibility of Ingredients	53.20	30.87	42.53
Digestibility of Protein	66.95	55.62	77.19
Digestibility of Fat	70.59	99.52	40.09
igestibility of Energy	65.24	43.41	66.21

Digestibility of Ingredients

The digestibility value of a diet ingredient is a reflection of the high or low value of the benefits of a diet ingredient, describing the portion of nutrients or diet energy that is digested by fish and not excreted through feces. The diet given to fish is not only assessed from the chemical composition of the nutrients it contains but from how much of the nutrients contained in the diet can be absorbed and utilized by fish.

Based on the results of the research that has been done, the digestibility value of each test diet is as follows: commercial diet 53.20%, non-fermented *I. zollingeriana* leaf flour 30.87% and fermented *I. zollingeriana* leaf flour 42.53%. The digestibility value of commercial diet ingredients was higher than non-fermented *I. zollingeriana* leaf flour and fermented *I. zollingeriana* leaf flour. However, the digestibility of fermented *I. zollingeriana* leaf flour was higher than that of non-fermented *I. zollingeriana* leaf flour. Commercial diet had the highest ingredients digestibility value compared to non-fermented *I. zollingeriana* leaf flour and fermented *I. zollingeriana* leaf flour. The high digestibility value of commercial diet ingredients is because the diet has a lower fiber value (1.93%) than the fiber value of non-fermented *I. zollingeriana* leaf flour (4.72%) and the fiber value of fermented *I. zollingeriana* leaf flour (2.56%). This research also found that the digestibility value of fermented *I. zollingeriana* leaf flour was higher than the digestibility value of non-fermented *I. zollingeriana* leaf flour, this was because the fiber value of fermented *I. zollingeriana* leaf flour was smaller than the value of fermented *I. zollingeriana* leaf flour. Non-fermented *I. zollingeriana* leaf flour.

The low crude fiber of fermented *I. zollingeriana* leaf flour is because in the fermentation process, the mold utilizes the nutrients contained in the substrate for development and increases the activity of cellulase enzymes which will degrade complex fiber components into simpler components (Yohanista *et al*., 2014) [43]; (Zuraida *et al*., 2013) [42]. The lower the value of crude fiber in the diet, the higher the digestibility value of the diet ingredients, where the diet given can be absorbed properly by fish. Fiber content that is too high in diet ingredients will interfere with digestion in fish (Hansen and Hemre, 2013) [9].

The high digestibility value of commercial diet ingredients and fermented *I. zollingeriana* leaf flour compared to non-fermented *I. zollingeriana* leaf flour is also due to the presence of enzymes in the digestion of fish in this treatment which are able to absorb the diet given properly. This is supported by the opinion of Suprayudi *et al*., (2011) [30], where the high digestibility value of diet ingredients is due to the support of enzymes in the digestive tract of fish so that the digestibility value of diet is higher.

The digestibility of fish for a type of diet depends on the quality and quantity of diet, the type of diet ingredients, the nutritional content of the diet, the type and activity of digestive enzymes in the fish digestive system, the size and age of the fish and the physical and chemical characteristics of the waters (NRC, 1993) [18]; (Mayulu, 2014) [17]. Furthermore Hepher (1990) in (Siahaan, *et al*., 2021) [28], (Liao *et al*., 2015) explained that diet digestibility is influenced by the presence of enzymes in the digestive tract of fish, the level of activity of digestive enzymes and the length of contact of the diet eaten with digestive enzymes. The role of digestive enzymes is very dominant in hydrolyzing complex compounds into simple compounds that are readily absorbed by fish. In line with this, (Salgado *et al*., 2015; Wang *et al*., 2013) [24] stated that fermentation can help improve odor, increase crude protein content and produce digestive enzymes to improve the quality of animal diet.

The high digestibility value of a diet ingredient indicates that the beneficial value of the diet is also high. Conversely, if the digestibility of a diet ingredient is low, the beneficial value of the diet is also low (Sakaryana *et al*., 2011) [25]. Digestibility value has a positive correlation to fish growth. Where the higher the digestibility value of fish diet ingredients, the better the diet digested by fish as a source of energy and growth (Hassaan *et al*., 2019) [8]; (Azaza *et al*., 2020) [3].

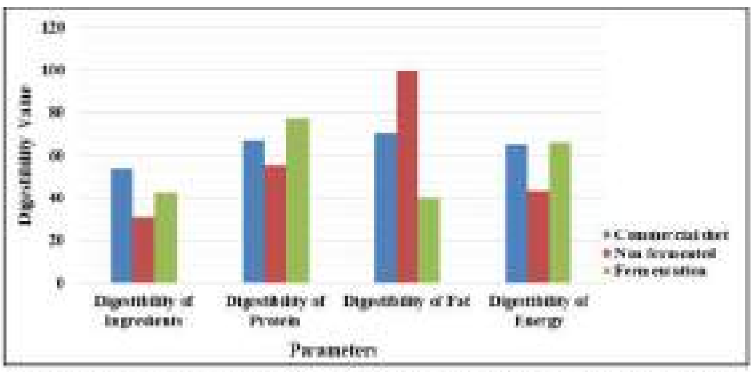

Fig 1: Percentage Digestibility Value of *I. zollingeriana* leaf flour on Jaya Sakti Carp larvae

Digestibility of Protein

Based on the research that has been done, the protein digestibility value of each treatment is: protein digestibility using commercial diet 66.95%, protein digestibility using non-fermented *I. zollingeriana* leaf flour 55.62% and protein digestibility using fermented *I. zollingeriana* leaf flour 77 .19%. The highest protein digestibility value was found in *I. zollingeriana* leaf flour.

Protein digestibility is the protein content obtained from the reduction of protein intake and protein wasted in the feces. Protein digestibility greatly determines the efficiency of protein utilization by the body, this is because protein digestibility will affect the amount of digested protein which will affect wasted protein and retained protein. (Kurniaji, 2013) [12]. According to (NRC, 1993) [18], protein digestibility of fish is generally 75-95%.

Based on the results of research that has been done, the highest protein digestibility value in Jaya Sakti Carp larvae is found in the use of fermented *I. zollingeriana* leaf flour. The high digestibility of protein in this treatment was due to the high total digestibility of 70.04%, this is supported by the opinion (Kurniaji, 2013) [13] that total digestibility affects protein digestibility, where the percentage of protein digestibility in fish will increase if the total digestibility of fish also increases. Protein digestibility will affect the ratio of protein that is utilized later in the metabolic process, so Carp have a higher ratio of protein utilized by the fish's body at the metabolic level.

Digestibility is the part of the diet consumed and not excreted as feces (Affandi *et al*., 1992) in (Kurniaji, 2013) [13]. Digestibility value states the amount of nutritional composition of a ingredients or energy that can be absorbed and used by fish (NRC, 1993) [18], while according to Silva (1989), digestibility is a quantitative evaluation of the utilization of diet and nutritional components. The level of digestibility of diet is divided into two, namely total and protein digestibility.

According to (Cho *et al*., 1985) [4], the digestibility of protein in fish diet varies depending on the crude fiber content, where high crude fiber causes a larger portion of excreta, thus causing a reduction in digestible protein input. In this study, fermented *I. zollingeriana* leaf flour had a low fiber content of 2.56%, where the crude fiber content of the carp rearing diet was a maximum of 8% (SNI, 2006). The low crude fiber will make it easier for fish to digest and absorb the nutrients contained in the diet (Suprayudi *et al.*, 2012) [37].

In line with this, (Wooton, *et al.*, 1980) [40] states that, fish size also affects the fish's ability to digest protein, the bigger the fish, the better the digestibility of fiber components. In addition to the fish size factor, the digestibility value is influenced by the composition of the diet, the amount of consumption, namely protein, physiological status, and the method of dieting. Fermented *I. zollingeriana* leaf flour diet has a relatively high protein content of 39.47%, where the protein content of diet for growing Carp is a minimum of 25% (SNI, 2006). The increased digestibility value of fermented *I. zollingeriana* leaf flour protein is a reflection of the breakdown of the easily digestible crude protein component (Sukaryana *et al.*, 2011) [35]. The high digestibility value of fermented *I. zollingeriana* leaf flour protein is due to the availability of enzymes that help break down nutrients into simpler ones (Silaban *et al.*, 2021) [9]. The more enzymes added to the diet, the more protein will be hydrolyzed into amino acids, which will increase the digestibility of fish for diet protein (Silvia and Suharman, 2016) [30].

The high protein digestibility value of the diet ingredients using fermented *I. zollingeriana* leaf flour indicates that the Jaya Sakti Carp is able to digest and hydrolyze protein in the diet optimally so that the protein digestibility in this treatment is high. The higher the protein digestibility, the greater the protein that can be utilized by fish for growth (Andriani *et al.*, 2018) [2]; (Sianturi *et al.*, 2021) [28]. The use of diets with good protein digestibility is especially important in high-density culture conditions because the accumulation of undigested diet can be detrimental to water quality. Protein that fish cannot digest will cause a high ammonia composition in the waters (Fang *et al.*, 2015) [8].

Digestibility of Fats

Based on the research that has been done, the fat digestibility value of each treatment is fat digestibility using commercial diet 70.59%, fat digestibility using non-fermented *I. zollingeriana* leaf flour 99.52% and fat digestibility using fermented *I. zollingeriana* leaf flour 40, 09%. The fat digestibility value using fermented *I. zollingeriana* leaf flour was lower than the fat digestibility value using non-fermented *I. zollingeriana* leaf flour.

Fat is an important source of energy and essential fatty acids (EFA) needed for fish growth and development (NRC, 1993) [18]. Based on proximate analysis, it is known that the fat content of fermented *I. zollingeriana* leaf flour ingredients is lower, namely 4.02% compared to other treatment diets so that it has an impact on lower fat absorption by fish compared to other treatments. This is supported by NRC (1993) [18] which states that, digestibility value is the amount of nutrient composition of a ingredients as well as energy that can be absorbed and used by fish.

Furthermore, Suprayudi *et al.*, (2012) [37] in his research stated that the digestibility value of fat is related to the ability of fish to utilize energy sources other than protein, namely carbohydrates and fats. In this study, the test fish used more carbohydrates as a source of energy. In fermented *I. zollingeriana* leaf flour diet, there is a change in the structure of the ingredients due to fermentation so that it is easier to digest and there is the ability of fish to utilize carbohydrates as an energy source..

Digestibility of Energi

Based on the research that has been done, the energy digestibility value of each treatment is energy digestibility using commercial diet 65.24%, energy digestibility using non-fermented *I. zollingeriana* leaf flour 43.41% and energy digestibility using fermented *I. zollingeriana* leaf flour 66, 21%. The energy digestibility value using *I. zollingeriana* leaf flour was higher than the energy digestibility value using non-fermented *I. zollingeriana* leaf flour.

Energy is not a nutrient; it is released during the oxidation metabolism of carbohydrates, fats and amino acids. Digestibility of energy in fish is influenced by several factors, including stadia, activity, temperature and species. The energy digestibility value in fish is 70-85% (NRC, 1993) [18], the energy digestibility value of fish is in grain ingredients and 85% in animal ingredients Halver (1989) in (Suprayudi *et al.*, 2012) [37].

The high value of energy digestibility using fermented *I. zollingeriana* leaf flour is thought to be related to the ability of fish to utilize non-protein energy sources, namely carbohydrates and fats. Suprayudi *et al.*, (2012) [37] in their research stated that the digestibility value of fat is related to the ability of fish to utilize energy sources other than protein, namely carbohydrates and fats. In this study, the test fish used more carbohydrates as a source of energy. In fermented *I. zollingeriana* leaf flour diet, there is a change in the structure of the ingredients due to fermentation so that it is easier to digest and there is the ability of fish to utilize carbohydrates as an energy source. Carp are known to have the ability to utilize protein more effectively as an energy source. NAS (1983) in (Suprayudi *et al.*, 2012) [37].

The energy digestibility value in this research is still low compared to that found (NRC, 1993) [18], this is presumably due to the method of making the test diet, namely in the form of sinking pellets. Diet digestibility is also determined by the way of making diet, where diet made by the extrusion method (floating diet) has a higher energy digestibility than in the form of sinking pellets (NRC, 1993; Gunadi *et al.*, 2010) [18].

Conclusion

Based on the research results, it can be concluded that the utilization of tarum leaf flour (*I. zollingeriana*) fermented by *A. niger* as a diet ingredient for the Jaya Sakti carp larvae gives a ingredients digestibility value of 42.53%, protein digestibility 77.19% and energy digestibility 66. 21% best compared to non-fermented leaf flour of *I. zollingeriana*.

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