Potential addition of black soldier fly carcass meal in sangkuriang catfish (Clarias gariepinus) feed formulation

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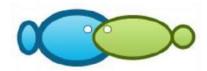
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Abstract. The purpose of this study was to determine the potential addition of black soldier fly (Hermetia illucens) carcass meal in the formulation of sangkuriang catfish (Clarias gariepinus) feed. H. illucens carcass is an alternative raw material that can be used to replace the main raw material, namely fish meal. The formulated test feed contains 37-38% protein. Performances of t test feed control were compared using DMRT (Duncan Multipkle Range Test). C. gariepinus measuring 5-7 cm were randomly stocked into 15 aquariums with an aquarium size of 100 x 50 x 51 cm. Each aquarium contained 25 C. gariepinus, which were fed as much as 6% of the total weight of all C. gariepinus tested during 35 days of maintenance. The results showed that the growth of C. gariepinus was significantly different between the test feed formulation with 50% carcass meal and the control feed (P<0.05). Based on these results, 50% concentration of H. illucens carcass meal has the potential to be used as raw material for C. gariepinus feed.

Key Words: commercial feed, DMRT, fish meal, alternative raw material, protein.

Introduction. Fish meal is the main raw material in making fish food. Almost all feeds, both animal feed and fish feed, use raw materials in the form of fish meal, which is a relatively expensive source of protein. Aulia et al (2013) stated that shortages and prohibitive prices are the new fish meal challenges to be addressed.

Ac25 ding to Houlihan et al (2001), fish meal is generally considered the best source of protein, due to its essential amino acid profile, covering the needs of most fish species, and to its high nutrients bio-availability. The protein contained in fish meal ranges from 58-68% with a moisture content of 5.5-8.5% (Boniran 1998). According to Lestari et al (2013), the nutritional content of fish meal in dry weight is 46.87% protein, 5.08% fat, 3.54% fiber, 33.92% ash and 10.56% extract material without nitrogen.

Black soldier fly (Hermetia illucens) is a possible alternative to fish meal, which is currently underexploited, despite its nutritional qualities. The proximate analyzes conducted by Mawadda et al (2018) indicated that the H. illucens carcass has the following composition: 7.05% moisture, 9.52% ash, 42.65% crude protein, 17.95% crude fat, and 6.98% crude fiber. Huis (2013) highlighted the economic advantages of the proteins extracted from insects. In addition, according to Wang et al (2005), the use of insects as a source of protein has been widely used.

H. illucens carcass is a type of fish meal substitute. The protein content of H. illucens carcass is 42.65% (Lestari et al 2013), compared to the fish meal, which has 46.87% protein content (Mawaddah et al 2018), while the fat content of H. illucens carcasses is relatively high, 17.95%, compared to 5.08% in the fish meal. However, in the utilization of nutrients, protein content can support growth in fish. Aside from the nutrient aspect, H. illucens carcasses are also more economical and accessible, compared to fish meal. H. illucens carcasses are easy to produce, especially in tropical regions such as Indonesia.

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The purpose of this study was to examine the potential for adding *H. illucens* carcass meal in the formulation of sangkuriang catfish feed (*Clarias gariepinus*).

Material and Method

Research site. This research was conducted from August to December 2019, at the Laboratory of the Research Center for Freshwater Aquaculture Fisheries and Extension of Fisheries, Bogor, West Java, Indonesia.

Experimental design. This research used a complete random design with 5 treatment as a replications. The treatment used was *H. illucens* (BSF) carcass meal (CM) as a substitute for fish meal (FM) in the test feed formulation, as follows:

A = 100% FM + 0% CM-BSF B = 75% FM + 25% CM-BSF C = 50% FM + 50% CM-BSF D = 25% FM + 75% CM-BSF E = 0% FM + 100% CM-BSF

Tools and materials. The equipment used during the study consisted of: aquarium with acclimation, aeration systems such as aeration hose, aeration stones and blowers, heaters, alcohol thermometers, nets, analytical scales, rulers, plastic buckets and ovens. The materials used in this research were: salt, potassium permanganate, *C. gariepinus* and *H. illucens* carcass meal.

Procedure

H. illucens carcass. The test material used in this research was the *H. illucens* carcass originating from the abattoir. A quantity of 15 kg of *H. illucens* carcass in a wet state was put into a plastic container, and then dried in an oven (Hock, Indonesia) at a temperature of 60°C, for 24 hours. After drying, the black soldier fly carcass was weighed using an analytical balance (Ohaus, USA). After the *H. illucens* carcasses were weighed, the dry biomass weight was 9.2 kg. After being weighed, the *H. illucens* carcasses were processed into coarse meal. The process consisted of carcass sieving using a blender and milling. The resulting meal weight was of 8.5 kg.

Test fish. The fish specimens used in this study were of *C. gariepinus*, with a size of 5-7 cm. Grading was done to get relatively uniform size. The aquarium was prepared for the acclimation process. The aquarium medium was water at a level of 30 cm and a volume of 150 L.

Acclimatization was done for 4-7 days. Acclimatized fish were fed with commercial pellets ad libitum, with a feeding frequency of 2 times a day, at 7-8 am and 3-4 pm. The fish were used in the trial if they were healthy and agile. In the preparation of the test fish, 50 specimens of *C. gariepinus* were weighed and measured in length.

Test feed. Weighted raw materials were mixed evenly according with predetermined formulations, and then disposed in 5 containers (buckets). Boiled water was added to the raw material mixture, in proportion of 40% (800 mL) of the total volume, using a measuring cup, and then stirred until evenly distributed. The dough was placed into a pellet printing machine, sized at 2 mm and then dried in the sun for 2 hours. If not dry, the pellet was put in an oven at 52°C for 4 hours. 20 g samples of dry pellets were used for proximate testing. The results can be seen in Table 1.

Table 1
The composition of *Hermetia illucens* carcass meal, as a substitute for fish meal nutrition

1	15				
Raw material	A (0%)	B (25%)	C (50%)	D (75%)	E (100%)
Fish meal	35.000	26.250	17.500	8.750	
Soybean meal	25.000	25.000	25.000	25.000	25.000
Black soldier fly carcass	-	8.750	17.500	26.250	35.000
Fish oil	3.500	2.700	1.910	1.120	0.330
Crude palm oil (CPO)	3.500	2.700	1.910	1.120	0.330
Vitamin-premix	2.000	2.000	2.000	2.000	2.000
Mineral	1.000	1.000	1.000	1.000	1.000
Distillers dried grains solubles	3.000	3.000	3.000	3.000	3.000
Bran	15.000	15.000	15.000	15.000	15.000
Palm oil cake	5.000	5.000	5.000	5.000	5.000
Tapioca meal	3.290	4.310	5.330	6.340	7.350
Supplement*	3.210	3.210	3.210	3.210	3.210
Corn gluten meal (CGM)	-	0.580	1.150	1.720	2.290
Cr_2O_3	0.500	0.500	0.500	0.500	0.500
Total	100.00	100.00	100.00	100.00	100.00
Prox	kimate (%	dry ingredi	ents)		
Protein	32.57	32.59	32.16	33.01	33.00
Fat	13.25	12.49	12.33	12.62	13.20
Ash	11.41	10.22	9.31	8.99	7.58
EMWN	33.63	35.75	37.50	34.16	34.47
Energy (kcal g ⁻¹)**	337.965	337.199	338.343	336.167	341.595
Ratio E/P (kcal g ⁻¹)***	10.38	10.52	10.52	10.18	10.35

^{*}The supplement consists of Monosodium glutamate, Chlorine chloride, Dicalcium phosphate, NaCl and Chlorella: EMWN-extract material witho introgen; **Calculated based on digestibility energy according to NRC (2011): 6 g of protein is 4.5 kcal, fat is 8.1 kcal, and 1 g of carbohydrate is 2.5 kcal; ***According to NRC (2011), the optimal E/P value for fish growth is between 8-9 kcal g⁻¹.

Containers and media experiment. The process of preparing an experimental container supposed to wash the aquarium first using clean water and then a brush with a sponge. After being brushed, the aquarium was allowed to stand for 2 hours. After settling, the research equipment was sanitized with sterile sponge to be free of potentially harmful germs and bacteria. The aquarium was disposed horizontally and the top of the aquarium was closed using a net against dirt and animals.

Once the preparation of the container ended, the media was prepared, using water from reservoirs pretreated for 3-4 days. The volume of 90 L was used in the experimental aquarium, at a salinity of 3 ppt.

Pisciculture. Fish specimens were graded to the same weight and size. All systems were inspected to be set at the expected parameters of aeration and water level. Calibration was performed: the accuracy of the gauge was set at 0.1 cm and the accuracy of the analytical balance at 0.01 g. The aeration bucket was checked. Afterwards, *C. gariepinus* were released into the aquarium by 5-10 specimens, once weighed using analytical scales, until 25 specimens were stocked in each aquarium.

In the maintenance profisss, *C. gariepinus* seeds were fed at 6% of their total weight, with a frequency of 3 times a day, at 7-8 am, 12-1 pm and 5-6 pm. Feed was first prepared and weighed using analytical scales and put into plastic containers (5 containers), in doses for 1 day, morning, afternoon and evening.

In the maintenance process, fatalities were recorded every day. Sampling for measuring fish weight was performed every 7 days and the data was used to determine the feeding rate for the next feed. Sampling was carried out by turning off all cultivation systems such as the aeration and by reducing the water level by 50% to facilitate fish specimens capture. The total weight and fish number were determined for each aquarium, in order to calculate the daily amount of feed to be administered. The amount of feed obtained is then distributed in doses, as follows: 30% in the morning, 35% at noon and 35% in the afternoon. The results were used for the next week.

Observation parameters

Protein retention (PR). Protein retention can be calculated by using the formula of Watanabe (1988):

PR (%) =
$$\frac{p'}{p} x 100$$

Where:

P - increased body protein weight;

p - total weight of protein consumed (gram).

Fat retention (FR). Fat retention can be calculated by using the formula of Watanabe (1988):

FR (%) =
$$\frac{m^{f}}{m}$$
 x 100

Where:

m' - increased body fat weight;

p - total weight of fat consumed (gram).

Feed consumption (FC). The formula used to calculate the amount of feed consumption is (Watanabe 1988):

$$FC\left(g/fish\right) = \frac{Final\ fseed\ amount\left(g\right) - Amount\ of\ initial\ (g)}{Final\ fish\ number}$$

Absolute weight growth (AWG). Weight gain is calculated based on the weight or length difference at the beginning and at end of maintenance. Weight or length gain is calculated according to the formula (Watanabe 1988):

$$AWG (g) = Wt-W0$$

Where:

ΔW - absolute weight gain;

Wt - weight of fish at the end of the experiment (gram);

WO - fish weight at the beginning of the experiment (gram).

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Feed efficiency (FE). Efficiency of feed is the increase in fish weight per unit of feed consumed. Feed efficiency is used to compare the amount of feed consumption to fish weight gain (Watanabe 1988):

$$FE(\%) = \frac{Wight\ gain\ (g)}{Total\ feed\ consumption\ (g)} \times 100$$

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Specific growth rate (SGR). Specific growth rate or often called daily weight growth rate using the formula according to Halver & Hardy (2002):

SGR (%/day) =
$$\frac{L_n W_t - Ln W_0}{\Delta t} x$$
 100

Where:

SGR 23 pecific growth rate (%/day);

Wt - end of maintenance fish weight (gram);

WO - initial weight before maintenance (gram);

Δt - duration of maintenance (days).

Statistical analysis. The data obtained were analyzed using ANOVA statistical analysis (Analysis of Variance). If the effect was significantly different, it was flab wed by further Duncan Multiple Range Test (DMRT) tests using calculations based on a 95% confidence level.

Results and Discussion

Results. The research results for a maintenance of 35 days showed that the individual weight growth of *C. gariepinus* ranged from 3.26 g to 5.22 g (Table 2). Individual consumption of feed based on black soldier fly carcass meal ranged from 4.56 g to 5.49 g. Protein retention and fat retention in the black soldier fly carcass meal ranges between 1.00% and 4.35%, and between 14.70% and 49.20%, respectively. Specific growth rate in *C. gariepinus* ranges from 4.02% to 5.10% per day with a feed efficiency ranging from 71.92% to 95.08%.

Table 2
Growth performance of *Clarias gariepinus* using black soldier fly carcasses based feed,
with different compositions, for 35 days of maintenance period

Parameters	Percentage of black soldier fly carcass (%)					
Parameters	0	25	50	75	100	
Absolute weight growth (g)	4.69±0.28 ^{bc}	4.17±0.20 ^{ab}	5.22±0.14°	3.80±0.15 ^{ab}	3.26 ±0.55 ^a	
Feed consumption (g/fish)	4.83 ± 0.36^{ab}	5.04±0.09 ^b	5.49±0.20°	4.90±0.22 ^{ab}	4.56±0.17ª	
Protein retention (%)	3.75±1.61ª	3.88±1.80°	2.13±0.51 ^a	4.35±0.86°	1.00±0.41ª	
Fat retention (%)	16.07±6.18 ^a	14.70±1.96 ^a	49.20±4.23 ^c	54.52±4.59°	29.24±3.10 ^b	
Specific growth rate (%/day)	4.86±0.28 ^{bc}	4.59±0.20 ^{abc}	5.10±0.14°	4.39±0.15 ^{ab}	4.02±0.55ª	
Feed efficiency (%)	97.46±14.83 ^b	82.66±6.27 ^{ab}	95.08±4.64 ^b	77.48±2.62 ^{ab}	71.92±18.61 ^a	

Values listed are average values \pm standard deviation. The superscript letter behind the different standard deviation values in each row shows a significantly different effect (P<0.05).

Discussion. According to Effendie (1997) growth is the increase in weight and length of fish in a certain period of time, under food, temperature, fish age and fish size influence. The statistical test results showed that the treatments with CM of 25%, CM of 50% and CM of 75% were significantly different (P<0.05) when compared to the control (CM 0%). The 50% CM based feed formulation treatment showed the best performance in *C. gariepinus* weight gains. The difference in weight gains between treatments is due to diffe 10 ces in nutrients content (Arifin & Rumondang 2017). For a CM 100% treatment, the results were not significantly different (P>0.05) when compared with CM 0% treatment.

Khairuman & Amri (2002) stated that appropriate feed criteria are: ingredients availability, low price, nutritional quality, easily digestible and being in accordance with fish mouth openings. The *H. illucens* carcass meal meets these criteria in the demonstrated feed formulation for *C. gariepinus*.

According to Madinawati et al (2011), the protein content is the fish growth promoter. Feed conversion (to meat) and efficiency (given feed consumption) ratios are negatively correlated (Effendie 1979), which confirms that farmed animals consume more food than they produce. This can indicate that the *H. illucens* carcass meal by feeding as much as 6% contains a fairly high nutritional value.

The treatment at 50% CM resulted in a higher level of feed consumption in $\it C. gariepinus$, reaching 5.49±0.20 g. This is presumably due to a distinctive odor due to the addition of a dose level of black soldier fly carcass meal to the feed formula. Besides, the the dark coloured feed is also preferred by $\it C. gariepinus$. These suppositions are supported by Pamungkas (2013) and Abidin et al (2015), who note that differences in feed consumption levels can be influenced by feed content and palatability, determined by taste, odor and color.

The lowest level of feed consumption in $\it C. gariepinus$ was found in the 0% CM treatment, namely 4.56 ± 0.17 g. The feed in this treatment was not added by fish meal, a preferred ingredient, therefore the smell of feed was less favored by $\it C. gariepinus$. This is consistent with the statement of Noviyana et al (2014) that an attractive aroma can stimulate fish to approach and consume the feed given. The amount of feed consumption influences the weight gain of $\it C. gariepinus$, which can be seen in the 50% CM treatment, with the higher feed consumption, where the weight of $\it C. gariepinus$ had the highest increase.

Protein retention results from digested food into the simpler nutrients, which can be used for body metabolism. Proteins are digested into amino acids, a source of metabolic and biological functions energy, the rest being stored by the body (Suprayudi et al 2013). Statistical analysis showed that protein retentation of each treatment carried out, when compared with controls, showed results that were noting infinitely different (P>0.05) (Table 2). According to Suprayudi et al (1999, 2000), the protein retention in fish is influenced by the digestibility of the raw materials, feed protein levels, feed energy content, and by the equilibrium of essential acid content in feed.

From the results of the research conducted, the protein retention values ranged 1.00% to 4.35%, the highest amount of protein retention was in the 75% CM treatment and the lowest protein retention was in the 100% CM treatment. The difference in the amount of protein retention per treatment is due to a change in the equilibrium of the essential amino acid content, following an increase in the content of the black soldier fly carcass meal in the feed (Suprayudi et al 2013). The low protein retention in the treatment is due to the presence of chitin content in the pure black soldier fly carcass meal, interfering with the digestive ability of fish (Priyadi et al 2009). This is supported by the studies of Fickler (2002) and Mokoginta et al (1999), stating that more than 1.5% chitin binding cysteine amino acids in fish meal can affect the digestive system and in particular the absorption of other amino acids, which can affect the availability of protein and non-protein, causing growth in fish to be disrupted.

Statistical analysis showed that fat retention when compared with controls was significantly different (P<0.05) (Table 2). The highest fat retention was found in the 50% CM and 75% CM treatments, with values of 49.20±4.23% and 54.52±4.59%, respectively, while the lowest fat retention value was found in the 25% CM treatment, i.e. 14.70%.

The higher the use of *H. illucens* carcass meal in the feed formula, the higher the fat retention value. Setiawan et al (2003) stated that when fat retention decreases, it causes protein feed to be more efficient in fish body weight increasing. However, the research concluded that decreasing the value of fat retention did not cause an increase in body weight. This happens because the use of carbohydrates in feed, as a source of energy. This opinion is supported by Syamsunamo et al (2011), who state that high levels of fat in feed raise the energy content, causing less feed consumption. Fat content in black soldier fly meal ranges from 29-32% (Bosch et al 2014). From the above statement it can be seen that the weight value of the 75% CM treatment is only 3.80 g which is due to the high fat retention in the treatment.

Decreasing the use of black soldier fly carcasses in feed determines lower fat retention values, causing high protein retention, which can increase the weight of C.

gariepinus. In a study conducted for CM 50%, fat retention has a value of $49.20\pm4.23\%$. For the CM 50% treatment, the body weight gain has the highest value, equal to 5.22 g, among all treatments. The amount of fat retention is suspected, in the 50% CM treatment, of favorably affecting the body weight. The fat content in the feed ratio meets the fish's needs for the fat and non-fat synthesis, according to Syamsunarno et al (2011).

Fat retention also varies. According to Panagan et al (2011), fat retention concerns unsaturated fatty acids such as Eicosapentaenoic acid (EPA) and omega-3, obtained from fish oil, as stated in his research related to the unsaturated fatty acids from catfish (Pangasius sp.). Unlike the case developed by Arief et al (2015) in their study, that fat retention comes from probiotics whose function is to help the absorption of nutrients to the maximum. If nutrients can be properly digested, then fat retention will increase.

The results from Table 2 show that the specific growth rate for CM 25% and CM 50% treatments are significantly different when compared to the CM 0% (control). The CM 50% treatment are optimal for C. gariepinus (P<0.05), while the treatment of CM 75% and CM 100% showed no significant difference when compared with CM 0%. CM 100% gives unfavorable results in C. gariepinus (Table 2). The highest specific growth rate was found with the 50% CM treatment, with a value of $5.10\pm0.14\%$ day⁻¹. According to Dewi et al (2018), the specific growth rate is influenced by several factors such as feed, heredity, parasites and water conditions. The highest specific growth rate in this study was thought to be due to the high digestibility value of the protein in the 50% CM treatment, compared to its digestibility value in the other treatments. Protein is the most important macronutrient for fish growth: the higher the protein level, the higher the fish growth rate (Harver & Hardy 2002).

The higher specific growth rate is also suspected to be due to the high level of at protein, 40%-50%, in the *H. illucens* carcass (Bosch et al 2014) so that it helps the growth of *C. gariepinus*. This presumption is in contrast with the opinion of Ahmadi et al (2012), who stated that the specific growth rate is influenced by the energy in the feed consumed, when exceeding the energy requirements needed for the body maintenance and other activities, resulting in an excess energy use for growth.

The lowest specific growth rate was found in the CM treatment 100% with a value of 4.02±0.55% day-1, due to the amount of protein in the dose given to the C. gariepinus, which does not meet its growth requirements, thus causing stunted growth. In addition, the composition of 100% CM treatment only consists of black soldier fly carcass meal, without fish meal addition. Is causes stunted growth in the CM 100% treatment, where the nutrient composition of fish meal and black soldier fly meal differ mainly in protein concentrations: 46.87% [5] estari et al 2013) and 42.65% (Mawaddah et al 2018), respectively. Statistical analysis showed that the substitution efficiency of black soldier fly carcass was significantly different (P<0.05) for the treatments. The highest feed efficiency was found in the treatments of CM 0% and CM 50%, with values of 97.46±14.83% and 95.08±4.64%, respectively. Although in the treatment with pure fish meal the percentage of feed efficiency is higher, this is due to the fish meal recognized nutritional qualities. But high feed efficiency found in the 50% CM treatment is presumably due also to high nutritional values. The growth is directly proportional to the feed efficiency (Putra et al 2020). This position is supported by Kurniawan et al (2017), who conclude that high feed efficiency is caused mainly by protein and amino acids high contents. In the 25% CM, TC 75% and CM 100% treatments there was no significant difference (P>0.05) in the efficiencies, while the CM treatment 100% gave the poorest feed efficiend results.

Amin et al (2010) stated that the increased metabolic processes in the body will spur fish to consums more feed.

The lowest feed efficiency was found in the 100% CM treatment with a value of 71.92±18.61, probably due to a sub-optimal feed absorption and conversion into energy, specific to *C. gariepinus* (Yespus et al 2018). This position is also supported by Mutiasari et al (2017), who concluded that the low feed efficiency is suspected to be due to the sub-optimal ability of fish to digest and absorb feed. Feeding is efficient if more than 50%

of the given feed is consumed. This is still the case of the 100% CM treatment, although it has the lowest value of feed efficiency (Warsono et al 2017).

Conclusions. According to our research, the use of formula feed with raw materials of *H. illucens* carcass flour provides an optimal growth response for *C. gariepinus*. The best treatment to increase the growth of *C. gariepinus* is 50% in replacing fish meal in the feed formulation.

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