Submission letter

Article title : **The Effects of Dietary Carbohydrate Level on The Growth Performance, Body Composition and Feed Utilization of Juvenile Kelabau (*Osteochilus melanoplueura)***

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Hereby I would like to submit the manuscript entitled "**article title**" to Aquaculture, Aquarium, Conservation & Legislation - International Journal of the Bioflux Society.

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I am submitting a manuscript for consideration of publication in **Aquaculture, Aquarium, Conservation & Legislation - International Journal of the Bioflux Society**. The manuscript is entitled "**The Effects of Dietary Carbohydrate Level on The Growth Performance, Body Composition and Feed Utilization of Juvenile Kelabau (*Osteochilus melanoplueura)***"

It has not been published elsewhere and that it has not been submitted simultaneously for publication elsewhere.

Indonesian freshwater is rich in herbivorous fish species, especially the Cyprinidae family, including the kelabau *O. melanopleurus* Bleeker. In 2013, The Kelabau began to be cultivated until now. This fish ecologically play an important role in river and lake ecosystems because they are classified as herbivorous fish. As herbivorous fish, in the digestive tract of Kelabau, more plants and phytoplankton and algae are found. In young fish of size 200 - 299 mm in the intestine there are 83.3% of plants, whereas in larger fish (300 mm) the intestine content is 100% of plants (Aizam et al 1983). Freshwater fish and sea fish have different abilities in digesting carbohydrates. The ability of sea fish to digest carbohydrates is around 20 %, while freshwater fish can digest above 20 % such as 30-40 % . Research on the carbohydrate requirements of kelabau (*O.melanopleurus*) has never been done. Therefore, research on the carbohydrate requirements of kelabau (*O.melanopleurus*) is very necessary. *O. melanopleurus* that consume 32.76 % CHO provides weight growth, relative growth, feed efficiency, protein efficiency ratio, protein retention, lipid retention, and the best energy retention compared to other treatments. Increasing carbohydrate levels up to 34.82 % does not increase liver and muscle glycogen levels and liver lipid content.

Thank you very much for your consideration.

Sincerely,
Adi Susanto

The Effects of Dietary Carbohydrate Level on The Growth Performance, Body Composition and Feed Utilization of Juvenile Kelabau (*Osteochilus melanoplueura* Bleeker)

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Abstract This study was conducted to evaluate the effect of feeding with different carbohydrate (CHO) content on growth performance and body composition in kelabau (*O. melanopleura* Bleeker). Five experimental diets iso-nitrogenous, iso-lipid, and different CHO levels. A, B, and C feeds contained 30.50 %, 32.76 %, and 34.83 % CHO levels respectively. Kelabau obtained from the cultivation in Balai Benih Air Tawar Mandiangin with an average weight of 3.18 ± 0.26 g with a density of 20 individuals maintained in plastic tubs measuring 54.3 cm x 38 cm x 31.5 cm and filled 40 liters of water. Kelabau fed 2 times a day on *at satiation* for 60 days. The result showed that fish fed diet B (32.76 %) had higher growth performance and feed utilization compare to the other groups (C and A) ($P < 0.05$). The increase in carbohydrate administration in kelabau from 30.50 % CHO to 34.82 % CHO does not affect the liver and muscle glycogen levels but does affect the lipid liver content.
Key Words*: Growth Performance, Feed Utilization, Glycogen, Osteochilus melanopleura*

Introductions. Indonesian freshwater is rich in herbivorous fish species, especially the Cyprinidae family, including the kelabau *Ostechilus melanopleurus* Bleeker. In 2013, The Kelabau began to be cultivated until now. This fish ecologically play an important role in river and lake ecosystems because they are classified as herbivorous fish. As herbivorous fish, in the digestive tract of kelabau, more plants and phytoplankton and algae are found. In young fish of size 200 - 299 mm in the intestine there are 83.3% of plants, whereas in larger fish (300 mm) the intestine content is 100% of plants (Aizam et al 1983). Fish that consume feed from complex sources with the carbohydrate content of 31 % produce the best relative growth (RGR) of 49.45% compared to other treatments (Mardani 2014). Susanto et al (2019), reported that kelabau *O. melanopleurus* Bleeker that consumed feed with a protein content of 31.88 % with 30.53 % carbohydrate content gave the best growth.

Fish have a lower ability to utilize carbohydrates than land animals, but carbohydrates must be available in fish feed, because if carbohydrates are not available then other nutrients such as protein and lipid will be metabolized to be used as energy so that fish growth will be slow (Wilson 1994). Yamada (1983), further explained that the use of carbohydrate-containing feeds in Fish is differ, depending on the complexity of carbohydrates. Carnivorous fish are unable to utilize complex carbohydrates in their feed at high levels. However carnivorous fish can utilize simple carbohydrates such as glucose, sucrose, and lactose as the main energy source. Furthermore, Furuichi (1988) added that carnivorous fish can utilize optimum carbohydrates at the level of 10-20 % in their feeds and omnivorous fish needs optimum at the level of 30-40 % in their feeds. The results of the Senappa and Devaraj (1995) experiments using three levels of carbohydrates (15, 25, and 35 %) on indian major carps *Catla catla* showed that the best fish growth was the use of 35% carbohydrates.

Freshwater fish and sea fish have different abilities in digesting carbohydrates. The ability of sea fish to digest carbohydrates is around 20 %, while freshwater fish can digest above 20 % such as 30-40 % for *Cyprinus carpio* (Satoh 1991 *in* Wilson 1994), 25-30 % for *Ictalurus punctatus* (Wilson 1991) and about 40 % for *Tilapia* sp (Luquet 1991 *in* Wilson 1994). Hernandez et al (1995), states that the administration of carbohydrates to tambaqui which is 0.5 g in size is as effective as lipids as an energy source. Gunther (1996) further stated that tambaqui can efficiently utilize carbohydrates and produce the best growth by feeding with the carbohydrate content of 38 %, whereas, in grass carp *Ctenopharybgodon idella*, optimal growth can occur in fish that consume feed with carbohydrate levels of 27.5 % (Gau et al 2010). In rohu *Labeo rohita*, which consumes feed with an increase in carbohydrates from 30 % to 40 % along with a decrease in protein content from 40 % to 30 % causes an increase in Protein Efficiency Ratio (PER) (Erfanullah and Jafri 1995). Research on the carbohydrate requirements of Kelabau *O. melanopleura* has not been conducted so research needs to be doing to find the right carbohydrate levels to produce the best growth of Kelabau *O. melanopleura*.

Material and Method

Diets. This study uses 3 kinds of artificial feed which are isonitrogenous (32.1 %) and isolipid (9.1 %) with different carbohydrate content, namely feed A (30.5 %), feed B (32, 7 %), and C feed (34.8 %) with a CP ratio ranging from 8.0 to 8.3 kcal. Feed formulations can be seen in Table 1.

Table 1

Treatment feed composition (g) and feed nutrient content *

Ingredients	*Ingredient percentage in the trial feed (% dry matter)*		
	A(30.50 % CHO)	B(32.76 % CHO)	C(34.82 % CHO)
Fish meal	29.1	28.7	28.7
Soybean meal	25.8	25.7	25.2
Wheat meal	13.0	15.3	17.9
Brand meal	9.0	9.2	9.1
Fish Oil	2.5	2.5	2.5
Corn Oil	2.5	2.5	2.5
Vitamin Mix	3.0	3.0	3.0
Mineral Mix	3.0	3.0	3.0
Choline Chloride	2.0	2.0	2.0
CMC[1]	2.0	2.0	2.0
Filler	8.1	6.1	4.1
Proximate analysis result			
Protein (%)	31.26	31.38	31.29
NFE (%)	30.50	32.76	34.82
LIPID (%)	9.11	9.54	9.57
FIBER (%)	15.55	12.61	10.59
Total Energy (Kcal g^{-1})[4)]	259.46	269.01	274.13
E/P (Kcal g^{-1} Protein)	8.30	8.57	8.76

[*)] - Calculation based on dry weight.

[1] - Carboxymethyl cellulose.

[2] - In mg/kg feed: vit.B_1 60; vit. B_2 100; vit. B_{12} 100; vit.C 2000; vit. K_3 50; vit. A/$D_3$400; vit. E 200; Ca pantothenate 100; inositol 2000; biotin 300; folic acid 15; niasin 400.

[3] - In mg/kg feed: $MgSO_4.7H_20$ 7.5; NaCl 0.5;$NaH_2PO_4.2H_2O$ 12.5;KH_2PO_4 16.0; $CaHPO_4.2H_2O$ 6.53; Fe citric 1.25; $ZnSO_4.7H_2O$ 0.1765; $MnSO_4.4H_2O$ 0.081; $CuSO_4.5H_2O$ 0.0155; KIO_3 0.0015; $CoSO_4$ 0.0003.

[4] - Protein = 3.5 kcal g^{-1}; Nitrogen Free Extract (NFE) = 2.5 kcal g^{-1}; Lipid = 8.1 kcal g^{-1}.

Fish culture management. The Kelabau were obtained from the hatchery of Freshwater Aquaculture Center (BBAT), Mandiangin, South Kalimantan. Fish were reared in a plastic tank containing 40 L water with a density of 20 fish per tank with an average weight of 3.18 ± 0.26 g. Fish are kept for 60 days by feeding twice a day in the morning and evening at satiation. Kelabau *O. melanopleura* is kept in a semi-closed circulation system. Stools are carried out in the morning. Water lost due to the siphon is replaced by new water up to the same volume. The filter is washed every day and the filter bath is washed and replaced with new water every 1 week. During the study, the average water temperature was 28.5 ± 1.0 oC, dissolved oxygen was 4.50-6.10 mg L^{-1}, pH was between 6.80-6.95, total ammonia nitrogen was between 0.382-0.623 mg L^{-1}. This shows that the water conditions during the study were at optimum conditions. (Tebbut, 1992; Effendie, 1997)

***Data Collection and Chemical Analysis*.** Initial and final body weight was measured upon anesthetized fish. The fish were anesthetized using MS 222. Weighing is done to determine the Daily Growth Rate (SGR) (De Silva & Anderson 1995). Feed consumed during the study was recorded to determine the Total Feed Consumption (Pereira et al 2007), and Feed Efficiency, Protein Retention, Lipid Retention, and Energy Retention (NRC 2011), and Protein Efficiency Ratio (PER) (Bake et al 2014). Proximate body analysis was carried out at the beginning and end of the study used to determine the nutrient composition of fish (Takeuchi 1988). Analysis of liver and meat glycogen, as well as Liver lipid content, was carried out at the beginning and end of the study to determine the reserve energy (Takeuchi 1988).

Statistic Analysis. The design of this study was an experimental laboratory model, using a completely randomized design (CRD) consisting of three treatments and five replications. The data of feed efficiency (FE), weight growth, total feed consumption (TFC), protein retention (PR), lipid retention (LR), energy retention (ER) and protein efficiency ratio (PER) were analyzed for diversity with ANOVA and followed by Tukey test at 95% confidence interval using the SPSS program, while liver and muscle glycogen levels, as well as Liver lipid levels, are analyzed descriptively in tabular form.

Result and Discussion

Growth Performance and Feed Utilization Efficiency. The values of various parameters of feed use which include weight gain, specific growth rate, relative growth rate, protein retenstion, lipid retention, energy retention and the ratio of protein efficiency as well as feed efficiency from fishes after being reared for 60 days by feeding different carbohydrate-containing are presented in Table 2.

Fish that are fed with different carbohydrate content have a significant influence on weight growth, relative growth rate, specific growth rate, protein efficiency ratio (PER), feed efficiency, protein retention, lipid retention, and energy retention (P <0.05) and did not significantly affect the level of feed consumption (P> 0.05). The best weight growth was obtained in the treatment of feed B (32.76% CHO) and then followed by fish that consumed feed C (34.82% CHO). The lowest weight growth was obtained in the group of fish that consumed feed A (30.50% CHO) (P <0.05).

The best relative growth rate was also obtained in the group of fish fed B (32.76% CHO) which was 0.84% per day significantly different in fish that consumed other feed (P <0.05). The same phenomenon was also seen in the specific growth rate, where the group of fish that consumed feed B had the best specific growth rate of 1.04% per day significantly different from the group of fish that consumed feed C and A (P <0.05).

Table 2

The average value of initial weight, final weight, growth weight, relative growth rate (RGR), specific growth rate (SGR), total feed consumption (TFC), protein efficiency ratio (PER), feed efficiency (FE), protein retention (PR), lipid retention (LR), energy retention (ER) and total ammonia nitrogen (TAN) obtained in *O. melanopleura* Bleeker which is maintained for 60 days by feeding with different carbohydrates.

Parameters	**CHO (%)**		
	A(30,50)	**B(32,76)**	**C(34,82)**
Initial Weight(g)	3,17 ± 0,32[a]	3,08 ± 0,23[a]	3,24 ± 0,29[a]
Final Weight(g)	4,86 ± 0,14[a]	5,65 ± 0,09[c]	5,14 ± 0,06[b]
Weight Growth(g)	33,71 ± 5,14[a]	51,39 ± 5,49[b]	37,48 ± 4,90[a]
RGR (%)	0,54 ± 0,12[a]	0,84 ± 0,14[b]	0,60 ± 0,15[a]
SGR(%)	0,72 ± 0,14[a]	1,04 ± 0,08[b]	0,80 ± 0,06[a]
TFC(%)	121,26 ± 4,57[a]	127,52 ± 3,82[a]	125,89 ± 3,16[a]
PER(%)	0,89 ± 0,12[a]	1,28 ± 0,11[b]	0,95 ± 0,13[a]
FE(%)	27,74 ± 3,69[a]	40,23 ± 3,34[b]	29,80 ± 4,13[a]
PR(%)	61,64 ± 7,33[a]	84,17 ± 7,91[b]	66,19 ± 8,05[a]
LR(%)	62,51 ± 4,51[a]	91,06 ± 8,09[b]	76,41 ± 13,19[ab]
ER(%)	45,00 ± 4,13[a]	63,34 ± 3,45[b]	49,40 ± 7,01[a]
TAN (mg $g^{-1}body^{-1}h^{-1}$)	0,00170 ± 0,00004[c]	0,00124 ±0,00007[a]	0,00154 ±0,00012[b]

Note: The average followed by different superscript letters in the same row shows significant difference (P< 0.05).

O. melanopleura that is maintained by feeding B shows no more feed consumption levels than fish that are kept by feeding C and A (P> 0.05). Fish that consume feed B have the highest protein efficiency ratio (PER) compared to fish that consume feed A and C (P <0.05), which is 1.28%. The best value of feed efficiency was obtained in fish that consumed feed B which was 40.23 ± 3.34% different from the group of fish that consumed feed C with a value of feed efficiency of 29.80 ± 4.13%, while fish that consumed feed A produced efficiency the lowest feed with a level of feed efficiency of 27.74 ± 3.69%.

Fish that consume fish feed B has the highest protein retention value of 84.17 ± 7.91% compared to fish that consume feed A and C (P <0.05) respectively 66.19 ± 8.05% and 61 64 ± 7.33%. The lipid retention value in fish that consumed B feed was also the highest with a value of 91.06 ± 8.09% followed by the group of fish that consumed feed C and A. The same thing was also seen in the energy retention value in the group of fish that consumed B feed tended to be higher, with a value of 63.34 ± 3.45%, while fish that consume feed A produce a low energy retention value with an energy retention value of 45.00 ± 4.13%. The group of fish that consumed feed B produced TAN excretion of 0.00124 ± 0.00007 mg/g/body/hour lower than the group of fish that consumed feed C followed by group A (P <0.05).

Initial and Final Proximate Analysis, Liver and Muscle Glycogen Content, and Liver Lipid Content. Initial and Final Proximate composition after being reared for 60 days by feeding different carbohydrate contents is presented in Table 3.

The protein level of the fish body at the end of the study seemly decrease but however not different statistically along with the increase in feed carbohydrates to the level of 34.82% CHO and has no significant effect on fish body protein levels (P> 0.05). The same phenomenon was also found in the ash content of the fish body at the end of the study but the administration of different carbohydrate levels significantly affected the ash content (P <0.05). Different things happen to NFE content. The NFE content of fish that consume diets with different carbohydrate levels does not have a significant effect with an increase in carbohydrate levels (P> 0.05).The increase in feed carbohydrates significantly affected the body lipid content of fish and the best body lipid content of fish was obtained in the group of fish that consumed 32.76 % CHO (P <0.05).

Table 3

Initial and Final Proximate body composition of *O. melanopleura* maintained for 60 days by feeding with different carbohydrates (% dry weight)

Parameters	**Level CHO (%)**		
	A(30,50)	**B(32,76)**	**C(34,82)**
Initial Body Composition (%):			
Protein	62,41	62,41	62,41
Lipid	5,87	5,87	5,87
Ash	21,04	21,04	21,04
NFE	9,43	9,43	9,43
Final Body Composition (%):			
Protein	64,86 ±0,62^{a}	63,87 ±1,13^{a}	64,64 ±1,11^{a}
Lipid01	10,94 ±0,49^{a}	13,01 ±0,95^{b}	12,63 ±1,17^{b}
Ash	15,11 ±0,46^{b}	13,62 ±0,46^{a}	14,29 ±0,55^{ab}
NFE	7,77 ±0,55^{a}	8,57 ±0,59^{a}	7,73 ±0,47^{a}

Note: The average followed by different superscript letters in the same row shows significant difference (P< 0.05).

Liver and muscle glycogen levels, as well as initial and final liver lipid content *O. melanopleura* maintained for 60 days by feeding with different carbohydrates, are presented in Table 4.

Maintenance of fish for 60 days with feed containing different carbohydrates does not have a significant effect on the liver and muscle glycogen levels. Liver glycogen levels of fish fed C (34.82 % CHO) had liver glycogen levels of 2.14 ± 0.11 $\mu g\ g^{-1}$ which were relatively similar compared to fish consuming B and A feeds with consecutive liver glycogen levels according to 2.11 ± 0.10 $\mu g\ g^{-1}$ and 2.09 ± 0.13 $\mu g\ g^{-1}$. Glycogen levels of fish muscle fed C (34.82 % CHO) of 0.178 ± 0.009 $\mu g\ g^{-1}$ were the same as fish that consumed feed B and C with muscle glycogen levels respectively 0.176 ± 0.008 $\mu g\ g^{-1}$ and 0.174 ± 0.010 $\mu g\ g^{-1}$.

Feeding with carbohydrate content in *O. melanopleura* for 60 days also showed an increase in liver lipid levels to a level of 32.76% CHO. The highest level of liver lipid was found in the group of fish that consumed feed B which was 6.50 ± 0.47%, followed by fish that consumed feed C and feed A with a consecutive liver lipid content of 6.31 ± 0.58% and 5.47 ± 0.25%.

Table 4

Levels of the liver and muscle glycogen and liver lipid content *O. melanopleura* were maintained for 60 days by giving feed containing different carbohydrates.

Parameters	**Levels CHO (%)**		
	A(30,50)	**B(32,76)**	**C(34,82)**
Glycogen Content ($\mu g\ g^{-1}$)			
Liver	2,09±0,13^{a}	2,11±0,10^{a}	2,14±0,11^{a}
Muscle	0,174±0,010^{a}	0,176±0,008^{a}	0,178±0,009^{a}
Lipid Liver Content (%)			
Initial	2,94	2,94	2,94
Final	5,47±0,25^{a}	6,50±0,47^{b}	6,31±0,58^{b}

Note: The average followed by different superscript letters in the same row shows significant difference (P< 0.05).

Feeding with carbohydrate content in *O. melanopleura* for 60 days also showed an increase in liver lipid levels to a level of 32.76% CHO. The highest level of liver lipid was found in the group of fish that consumed feed B which was 6.50 ± 0.47%, followed by fish that consumed feed C and feed A with a consecutive liver lipid content of 6.31 ± 0.58% and 5.47 ± 0.25%.

Discussion. Feeding with an increase in carbohydrate levels up to 32.76% levels significantly increases the growth performance of Kelabau, and decreases with increasing carbohydrate content up to 34.82%. This indicates that Kelabau that consume feed B (32.76% CHO) are better able to utilize non-protein feed energy sources for their activities and are more efficient in utilizing protein for growth compared to fish groups that consume C feed (34.82% CHO) and fish groups that consumed feed A (30.50% CHO).

Bray & Lawrence (1992), states that the availability of energy mainly from carbohydrates as an energy source other than lipids and proteins is mainly used for metabolism, both for growth and subsequently for reproduction in nature. Therefore, if the energy needed for metabolism Is sufficient, then growth, excessed nutrients, or energy will be stored or used for reproduction. Wilson (1994), added that if carbohydrates are deficiency then other nutrients such as protein and lipids will be metabolized to be used as energy so that fish growth will be slow.

Feed efficiency and protein efficiency ratio is the parameters of feed utilization to determine the effectiveness of feed-in growth. The fish group that consumed feed B (32.76% CHO) had a higher feed efficiency and protein efficiency ratio than the other groups. This indicates that the fish can utilize the nutrients they consume, especially carbohydrates and lipids as a source of energy and protein for growth. The results of this study also illustrate the importance of the presence of carbohydrates in the feed. The importance of providing carbohydrates at certain levels was also reported by Castro et al (2016), that in gilthead seabream juveniles. Fish fed with carbohydrates as much as 20% at different lipid sources did not cause differences in growth but caused high PER and lipid retention in the group of fish that consumed feed with carbohydrates. Conversely, fish that are fed without carbohydrates, cause lower lipid retention.

Retention value describes the number of nutrients stored in the body that come from the feed nutrients consumed. The results of this study indicate that the fish group fed B (32.76% CHO), produced Protein Retention (PR), Lipid Retention (LR), and Energy Retention (ER) higher than the C and A fish groups. This illustrates that the nutrients consumed besides being used for activities are also stored in the muscles as a source of energy reserves. The high value of nutrient retention (PR, LR, and ER), in group B fish also indicates that the feed energy consumed is balanced, so that the portion of protein for growth is not disrupted.

Conversely, an increase in CHO levels to 34.82% CHO caused a decrease in nutrient retention in Kelabau *O. melanopleura*. The low value of nutrient retention in the C fish group is closely related to the decreased level of feed consumption. The high energy in the feed causes the fish to limit the amount of feed consumed, thus affecting the number of feed nutrients consumed.

In group A fish (30.50% CHO), the low value of nutrient retention is due to the low energy consumed by the feed which is not enough for standard activities, and to fulfill it, protein and body lipids are metabolized. Mokoginta et al (1995) explained that if the energy content of the feed were too low, then most of the feed protein would be catabolized to meet energy needs so that fish consume a lot of food to meet their needs. Fish will limit the amount of feed consumption if the feed contains too high energy because basic energy needs have been met. Indicate the presence of several proteins catabolized for energy in both groups of fish (C and A) as seen from the higher total ammonia nitrogen (TAN) excretion value of 0.00170 ± 0.00004 mg/g/body/hour and 0.00154 ± 0.00012 mg/g/body/hour compared to group B fish which was only 0.00124 ± 0.00007 mg/g/body/hour.

Carbohydrates administration that is higher will cause decreased growth. Li et al. (2019), stated that the growth performance and utilization of feed decreased in groupers with the increasing carbohydrate content of the feed, as seen from glucose intolerance in these fish. The same thing was also found by Zhao et al (2013) in *Megalobrama amblycepahla*; Wu et al (2016) in juvenile black carp *Mylopharyngodon piceus*; Dong et al (2016) on golden pompano *Trachinotus ovatus*; Wang et al (2016) in juvenile grouper *Epinephelus akaara* and Xie et al (2017) in Tilapia *Oreochromis niloticus*. Ren et al (2011) also stated that increasing levels of carbohydrate to 18.4 % CHO for juvenile cobia *Rachycentron canadum* L) will increase SGR, FER, and PER.

The provision of carbohydrate feed, which is increasing in fish from 30.50 % CHO to 34.82 % CHO, does not affect the level of liver glycogen and muscle but does affect the level of liver lipid. Similar results were obtained by Wang et al (2016), which states that an increase in feed carbohydrates will lead to the accumulation of liver glycogen in carnivorous fish such as juvenile groupers *E. akaara* and large yellow croaker juveniles *Larimichthys crocea* (Xing et al 2016). Guo et al (2015) stated that grass carp herbivorous fish *Ctenopharyngodon idella* Val, fed with carbohydrate content increased from 30.94 % to 42.31 % did not affect the growth performance but increased the level of liver lipid and liver glycogen. In omnivores such as gibel carp *Carassius auratus gibelio,* high levels of carbohydrates with low lipid (45.0 % CHO and 2.0 % lipid) also did not cause differences in specific growth rates but increased HIS, liver lipid content and lipid retention efficiency (Li et al 2019). The same thing also delivered by Mozanzabeh et al (2016) on juvenile silvery-black-porgy *Sparidentex hasta* which was fed with increased carbohydrate content and reduced dietary lipid content did not influence growth performance such as weight growth, condition factors, and rate specific growth and feed consumption and feed conversion rates.

Based on growth performance and feed utilization, the optimum level of carbohydrate for kelabau *O. melanopleura* is 32.76 %. These results are almost the same as reported by Booanuntanasarn et al (2018) in tilapia *O. niloticus* where the optimal level of carbohydrates is 32.6 %. Lower yields have been reported in other fish such as silver barb *Puntius gonionotus* (29.3 - 29.8 %) (Mohanta et al 2009); juvenile cobia *R. candum* L (21.1 % CHO) (Ren et al 2011); Wuchang Bream *M. amblycepahla* (31.0 %) (Zhou et al 2015); golden pompano *T. ovatus* (16.93 - 20.64 %) (Dong et al 2016); juvenile large yellow croaker, *L. crocea* (21.29%) (Xing et al 2016); and juvenile black carp *M. piceus* (24, 98%) (Wu and Ye, 2016). The optimum level of carbohydrate 32.76 % in this study was also higher than the optimum value obtained by Xie et al (2017) in juvenile tilapia *O. niloticus* (28.87 %), and striped catfish *Pangasianodon hypophthalamus* fingerlings (30.81 - 31.13 %) (Asemani et al 2019), and *Barbonymus schawenfeldii* (22.89 g %) (Yanto et al 2019).

Senappa and Devaraj (1995) found higher carbohydrate levels, in major carp *Catla catla* with optimum levels of 35 %; *Tilapia* sp 40.0 % CHO (Luquet 1991 *in* Wilson 1994), gourami *Ospronemus gouramy* measuring 78.7 g requires 47.5 % CHO (Mokoginta et al 1995). Mohapatra et al (2003) reported that rohu (*Labeo rohita*) that consumed feed with a carbohydrate content of 45.0 % at a protein content of 30 % was more efficient in using the feed.

Based on the results of the research above, it is necessary to further research to find out the optimal carbohydrate and lipid ratio so that the growth of *O. melanopleura* can be maximized.

Conclusion. Kelabau *O. melanopleurus* that consume 32.76 % CHO provides weight growth, relative growth, feed efficiency, protein efficiency ratio, protein retention, lipid retention, and the best energy retention compared to other treatments. Increasing carbohydrate levels up to 34.82 % does not increase liver and muscle glycogen levels and liver lipid content.

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Submission Paper Adi Susanto

Adi Susanto <adisusanto73@gmail.com> Sat, Jun 27, 2020, 5:52 PM
to Mag

Dear Mr. Ioan Valentin Petrescu-Mag

I would like to publish my article entitled " **The Effects of Dietary Carbohydrate Level on The Growth Performance, Body Composition and Feed Utilization of Juvenile Kelabau (*Osteochilus melanopleura*)** " in the AACL BIOFLUX journal.

I submit herewith also the manuscript, submission letter, and ethical statements.

I will be waiting for the next information.

Best regards,
Adi Susanto

3 Attachments

AACL Bioflux

Eni Kovacs <ek.bioflux@gmail.com> Sun, Jun 28, 2020, 10:27 PM
to me

Dear Adi Susanto,

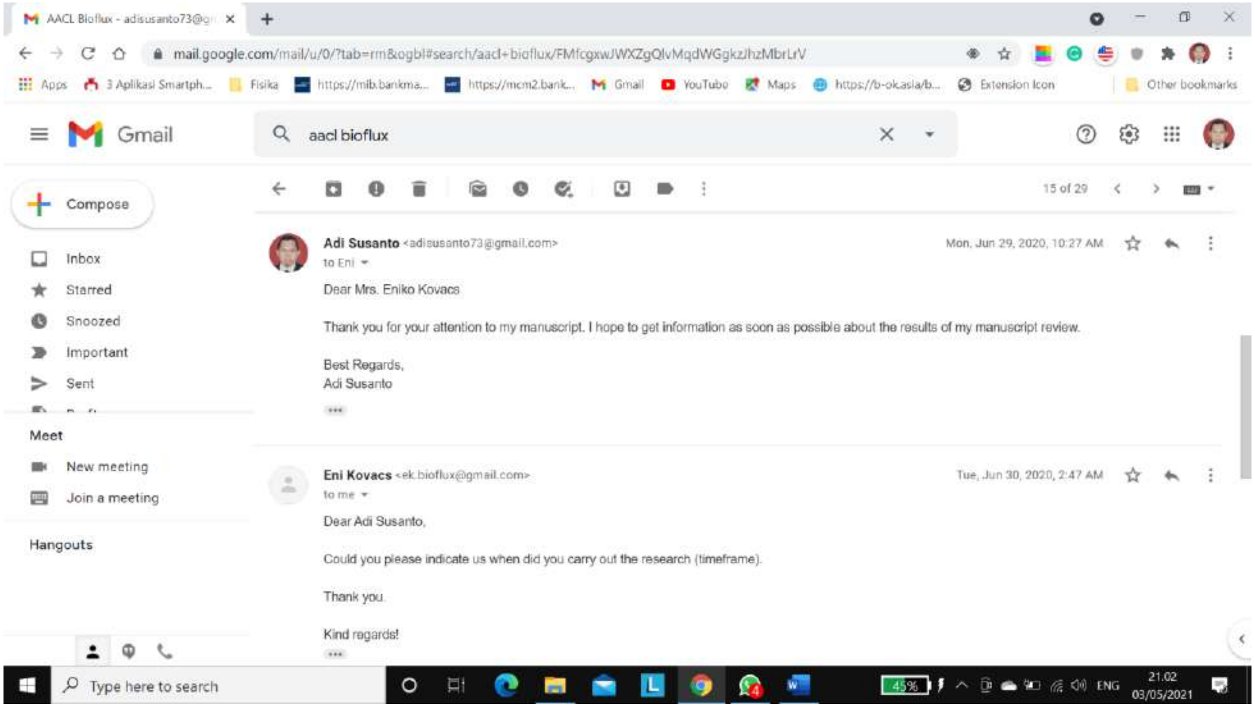
Thank you for your interest in publishing with us.

I will be your editor and I will stay at your disposition for all the aspects concerning your manuscript.

The editorial team is assessing your manuscript, we will get back to you as soon as possible with the result.

Kind regards,
Editor AACL Bioflux
Eniko Kovacs

Adi Susanto <adisusanto73@gmail.com> Mon, Jun 29, 2020, 10:27 AM
to Eni



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15 of 29

Adi Susanto <adisusanto73@gmail.com> Mon, Jun 29, 2020, 10:27 AM
to Eni

Dear Mrs. Eniko Kovacs

Thank you for your attention to my manuscript. I hope to get information as soon as possible about the results of my manuscript review.

Best Regards,
Adi Susanto

Eni Kovacs <ek.bioflux@gmail.com> Tue, Jun 30, 2020, 2:47 AM
to me

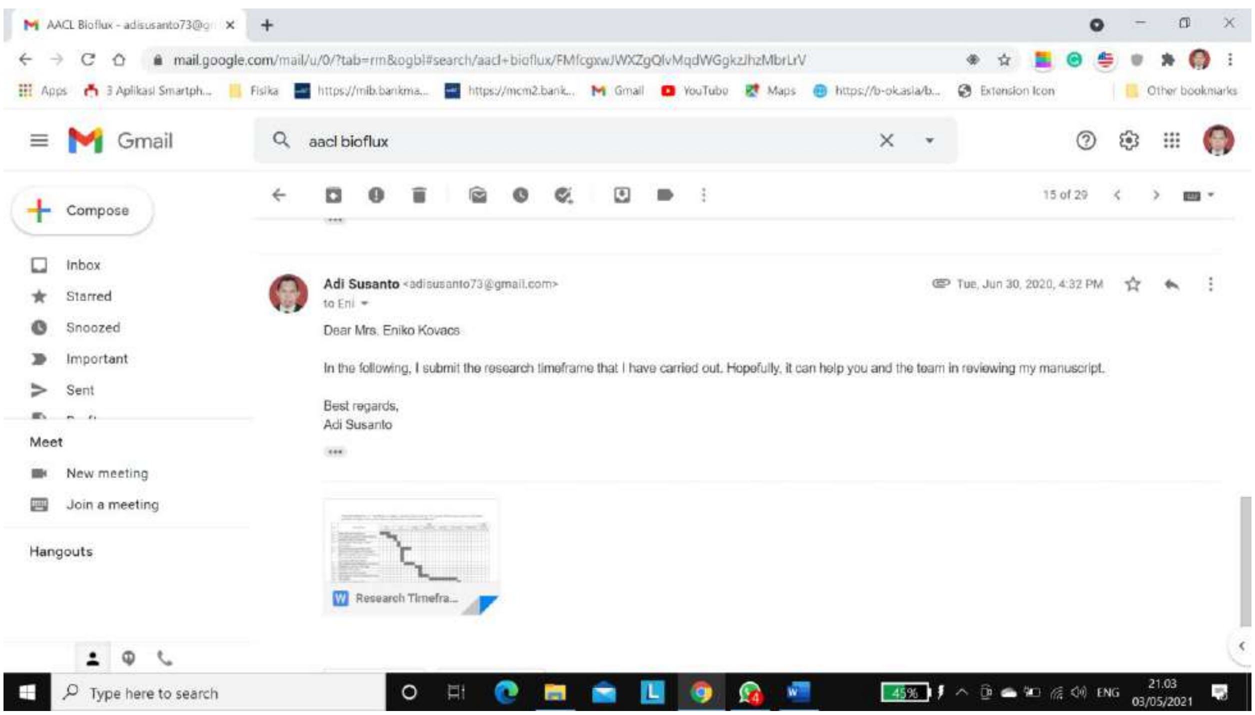
Dear Adi Susanto,

Could you please indicate us when did you carry out the research (timeframe).

Thank you.

Kind regards!

21.02 03/05/2021



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15 of 29

Adi Susanto <adisusanto73@gmail.com> Tue, Jun 30, 2020, 4:32 PM
to Eni

Dear Mrs. Eniko Kovacs

In the following, I submit the research timeframe that I have carried out. Hopefully, it can help you and the team in reviewing my manuscript.

Best regards,
Adi Susanto

Research Timefra...

21.03 03/05/2021

Preliminary acceptance

Eni Kovacs <ek.bioflux@gmail.com>
to me
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We would like to inform you that after the preliminary evaluation your paper is qualified for processing (reviewing).

Before proceeding to the review please consider that a processing publication fee of 250 USD is required. The average duration of the publication process is 10 weeks, but it can be reduced in exchange of a priority tax of 50 USD (http://www.bioflux.com.ro/journal/).

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2 Attachments

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11 of 29

Eni Kovacs <ek.bioflux@gmail.com> Jul 8, 2020, 2:16 AM

to me

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Thank you very much for the payment. We will further proceed with the review of your manuscript.

Kind regards!

--

Adi Susanto <adisusanto73@gmail.com> Aug 5, 2020, 9:00 PM

to Eni

Dear Mrs. Eniko Kovacs

I want to ask about my manuscript. Is my manuscript review process complete?

Best Regard!

Adi Susanto

21.05 03/05/2021

Acceptance for publishing - adis

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7 of 29

Acceptance for publishing

Inbox

Eni Kovacs <ek.bioflux@gmail.com> Thu, Aug 13, 2020, 12:55 AM

to me

Dear Dr. Susanto,

Could you please check the final form of the article attached, some adjustments were operated.

If you have any final observations and instructions prior to the article publishing, please inform us, any changes should be clearly highlighted. Change requests cannot be considered after publishing the material.

If all the authors agree with the final form of the article, could you please send us your acceptance for publishing, signed by all the authors.

Kind regards,
Editor AACL Bioflux
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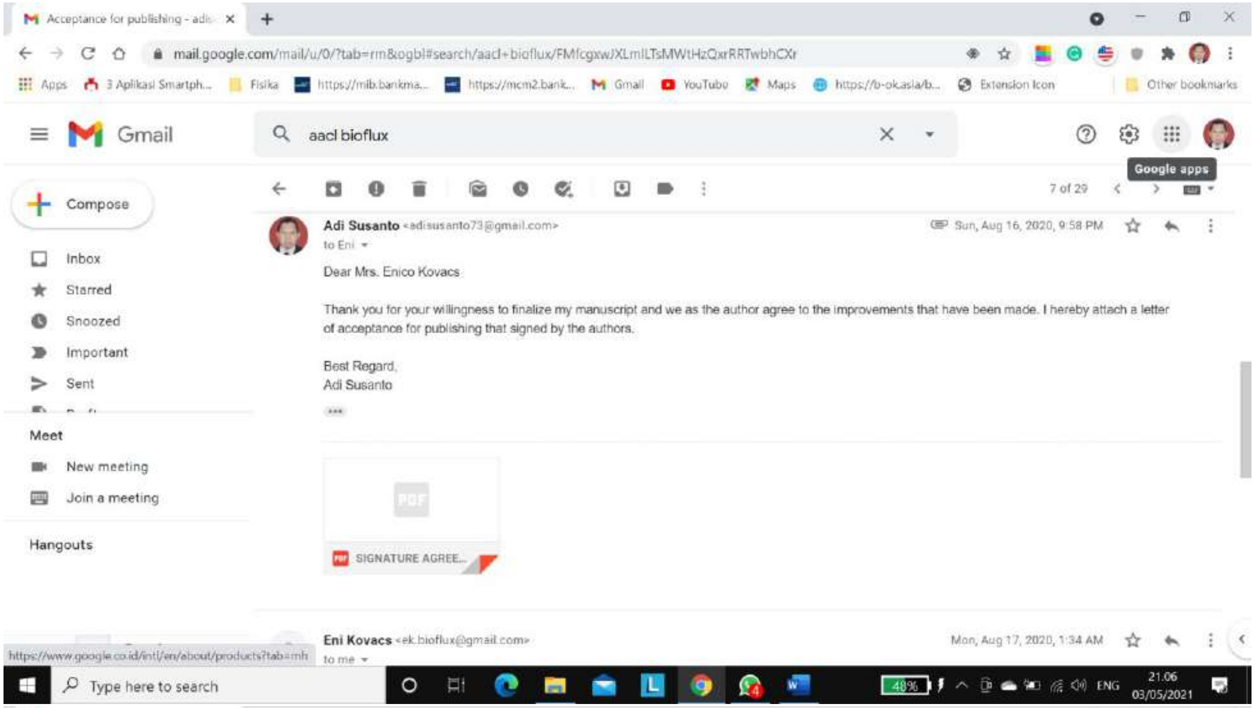
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7 of 29
Adi Susanto <adisusanto73@gmail.com>
Sun, Aug 16, 2020, 9:58 PM
to Eni
Dear Mrs. Enico Kovacs
Thank you for your willingness to finalize my manuscript and we as the author agree to the improvements that have been made. I hereby attach a letter of acceptance for publishing that signed by the authors.
Best Regard,
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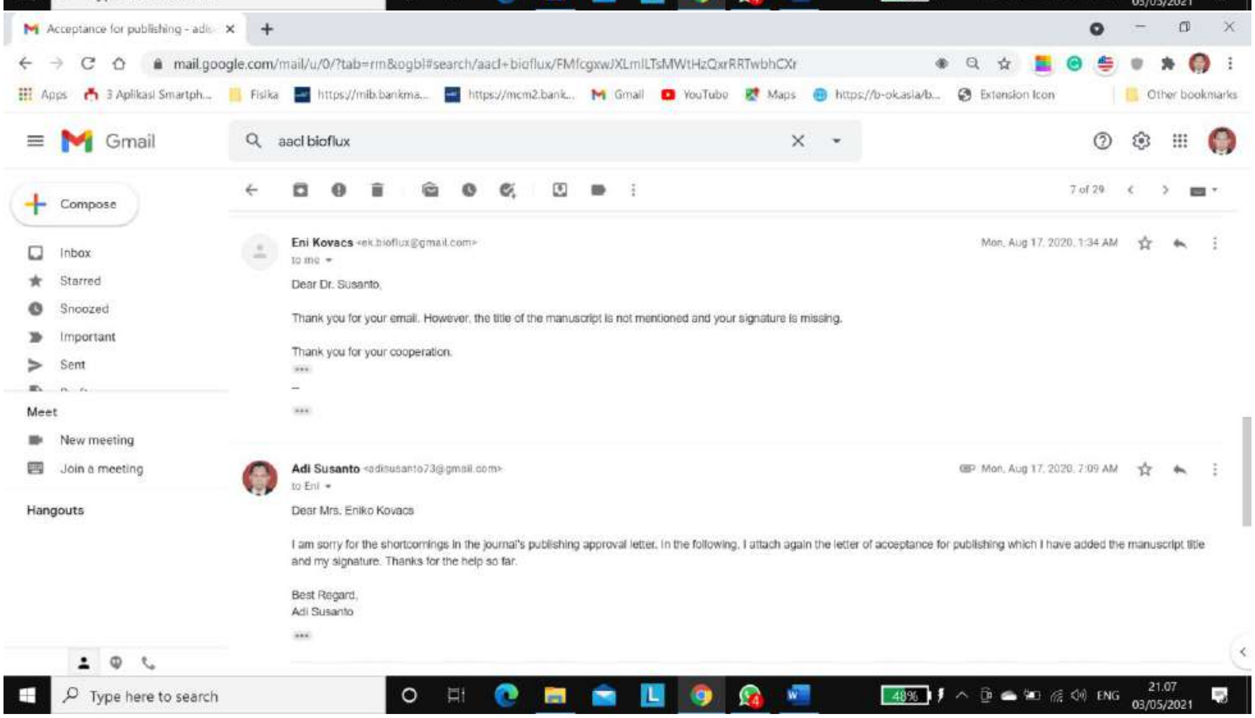
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Eni Kovacs <ek.bioflux@gmail.com>
Mon, Aug 17, 2020, 1:34 AM
to me
Dear Dr. Susanto,
Thank you for your email. However, the title of the manuscript is not mentioned and your signature is missing.
Thank you for your cooperation.
Adi Susanto <adisusanto73@gmail.com>
Mon, Aug 17, 2020, 7:09 AM
to Eni
Dear Mrs. Eniko Kovacs
I am sorry for the shortcomings in the journal's publishing approval letter. In the following, I attach again the letter of acceptance for publishing which I have added the manuscript title and my signature. Thanks for the help so far.
Best Regard,
Adi Susanto
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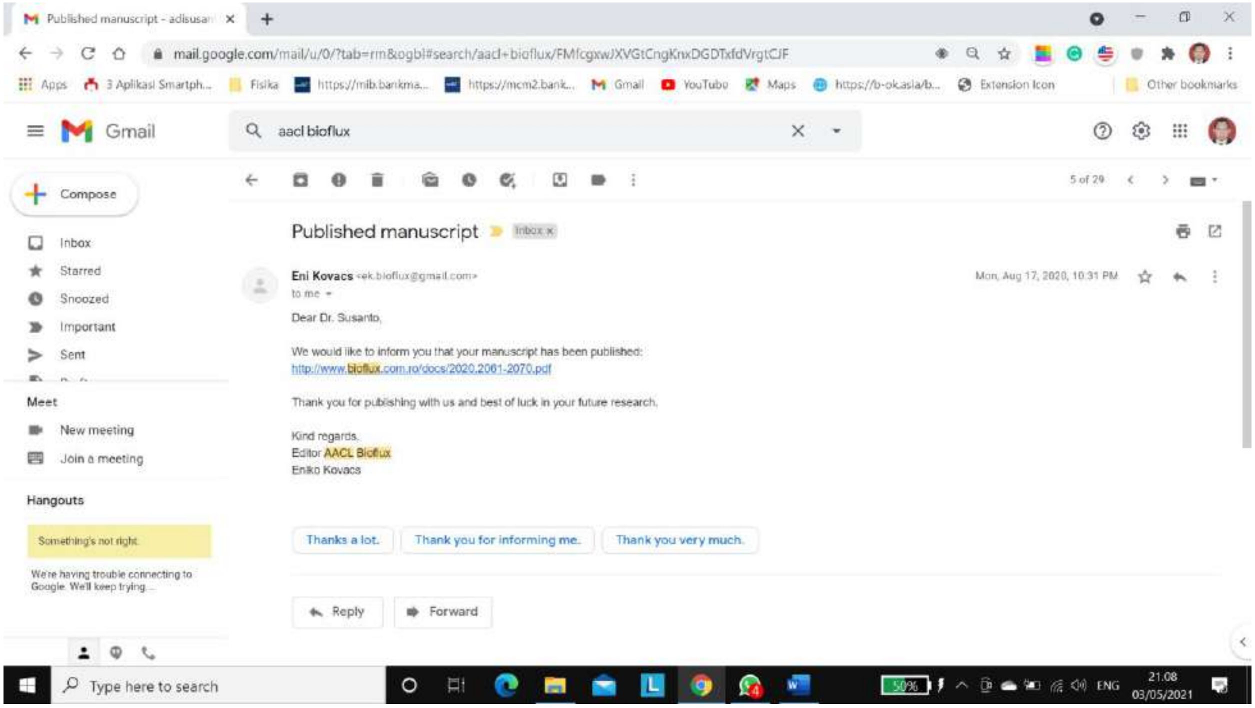

Published manuscript

Eni Kovacs <ek.bioflux@gmail.com>
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Mon, Aug 17, 2020, 10:31 PM

Dear Dr. Susanto,

We would like to inform you that your manuscript has been published:
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Thank you for publishing with us and best of luck in your future research.

Kind regards,
Editor AACL Bioflux
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Certificate/Letter of preliminary acceptance

This certificate shows that your paper:

The Effects of Dietary Carbohydrate Level on The Growth Performance, Body Composition and Feed Utilization of Juvenile Kelabau (*Osteochilus melanoplueura* Bleeker)

Authors:

Adi Susanto, Johannes Hutabarat, Sutrisno Anggoro, Subandiyono

was preliminary accepted for publication, with revision, in volume 13 (2020) of the scientific/academic journal: Aquaculture, Aquarium, Conservation & Legislation – International Journal of the Bioflux Society.

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The effects of dietary carbohydrate level on the growth performance, body composition and feed utilization of juvenile Kelabau (*Osteochilus melanopleurus*)

[1]Adi Susanto, [2]Johannes Hutabarat, [3]Sutrisno Anggoro, [2]Subandiyono

[1] Department of Aquaculture, Faculty of Fisheries and Marine Science, Mulawarman University, Samarinda, East Kalimantan, Indonesia; [2] Department of Aquaculture, Faculty of Fisheries and Marine Science, Diponegoro University, Tembalang Semarang, Central Java, Indonesia; [3] Department of Aquatic Resources Management, Faculty of Fisheries and Marine Science, Diponegoro University, Tembalang Semarang, Central Java, Indonesia. Corresponding author: A. Susanto, adisusanto@fpik.unmul.ac.id; adisusanto73@gmail.com

Abstract. This study was conducted to evaluate the effect of feeding with different carbohydrate (CHO) content on growth performance and body composition in kelabau (*Osteochilus melanopleurus*). Treatments consisted in 3 experimental iso-nitrogenous and iso-lipid diets, at different CHO levels: 30.50% (A), 32.76% (B), and 34.83% (C). Treatments were applied to *O. melanopleurus* culture specimens, with an average weight of 3.18±0.26 g and a stocking density of 20 individuals, maintained in plastic tubs measuring 54.3 x 38 x 31.5 cm and filled with 40 L of water. *O. melanopleurus* was fed 2 times a day at satiation basis, for 60 days. The result showed that fish fed with diet B (32.76%) had higher growth performance and feed utilization compared to the other groups (A and C) ($P<0.05$). The increase in carbohydrate administration in *O. melanopleurus* from 30.50% to 34.82% CHO does not affect the liver and muscle glycogen levels, but does affect the lipid liver content.
Key Words: herbivorous fish, freshwater, lipid content, protein retention, energy retention.

Introduction. Indonesian freshwater is rich in herbivorous fish species, especially the Cyprinidae family, including the kelabau, *Ostechilus melanopleurus* (Bleeker). In 2013, *O. melanopleurus* began to be cultivated until nowadays. This fish ecologically plays an important role in river and lake ecosystems, because they are classified as herbivorous fish. In the digestive tract of *O. melanopleurus*, several species of plants and phytoplankton and algae are found. In young fish of a size between 200 and 299 mm, the intestine contains 83.3% plants, whereas in larger fish (300 mm) the intestine content is 100% plants (Aizam et al 1983). Fish that consume feed from complex sources with the carbohydrate content of 31% produce the best relative growth (RGR), reaching 49.45%, compared to other treatments (Mardani 2014). Susanto et al (2019) reported that *O. melanopleurus* that consumed feed with a protein content of 31.88% and a carbohydrate content of 30.53% gave the best growth.

Fish have a lower ability to utilize carbohydrates than land animals, but carbohydrates must be available in fish feed, because if carbohydrates are not available then other nutrients such as protein and lipid will be metabolized to be used as energy so that fish growth will be slow (Wilson 1994). Yamada (1983) further explained that the carbohydrates concentrations in fish feed depend on the complexity of the carbohydrates. Carnivorous fish are unable to utilize complex carbohydrates in their feed at high levels. However carnivorous fish can utilize simple carbohydrates such as glucose, sucrose, and lactose as the main energy source. Furthermore, Furuichi (1988) stated that carnivorous fish can optimize carbohydrates use at the level of 10-20% in their feed and omnivorous fish reaches the optimal efficiency at the level of 30-40% carbohydrates in their feed. The results of the Seenappa & Devaraj (1995) experiments, using three levels of carbohydrates (15, 25 and 35%) on Indian major carps *Catla catla*, showed that the best fish growth was recorded at 35% carbohydrates.

Freshwater fish and sea fish have different abilities in digesting carbohydrates. The ability of sea fish to digest carbohydrates is around 20%, while freshwater fish reach 30-40%, in the case of *Cyprinus carpio* (Satoh 1991), 25-30% in the case of *Ictalurus punctatus* (Wilson 1991) and about 40% in the case of *Tilapia* sp. (Luquet 1991). Hernandez et al (1995) observed that the administration of carbohydrates to tambaqui (*Colossoma macropomum*) juveniles at a weight of 0.5 g is as effective as lipids, in terms of energy sources. Gunther (1996) further stated that *C. macropomum* can efficiently use carbohydrates and produce the best growth by feeding with the carbohydrate content of 38%, whereas, in grass carp (*Ctenopharyngodon idella*), the optimal growth occurs in fish that consume feed at a 27.5% carbohydrate level (Gau et al 2010). In roho labeo (*Labeo rohita*), which consumes feed with an increase in carbohydrates from 30% to 40% along with a decrease in protein content from 40% to 30%, an increase in Protein Efficiency Ratio (PER) is observed (Erfanullah & Jafri 1995). Research on the carbohydrate requirements of *O. melanopleurus* has not yet been conducted, which is the rationale for the current research aiming to determine the carbohydrate levels corresponding to an optimal growth of *O. melanopleurus*.

Material and Method

Diets. This study used 3 kinds of artificial feed which are isonitrogenous (32.1%) and isolipid (9.1%) with different carbohydrate content, namely feed A (30.5%), feed B (32.7%), and C feed (34.8%) with a calorie to protein (CP) ratio ranging from 8.0 to 8.3 kcal. Feed formulations can be seen in Table 1.

Table 1

Treatment feed composition (g) and feed nutrient content based on dry weight

Ingredients	*Ingredient percentage in the trial feed (% dry matter)*		
	A (30.50% CHO)	*B (32.76% CHO)*	*C (34.82% CHO)*
Fish meal	29.1	28.7	28.7
Soybean meal	25.8	25.7	25.2
Wheat meal	13.0	15.3	17.9
Brand meal	9.0	9.2	9.1
Fish oil	2.5	2.5	2.5
Corn oil	2.5	2.5	2.5
Vitamin mix*	3.0	3.0	3.0
Mineral mix**	3.0	3.0	3.0
Choline chloride	2.0	2.0	2.0
CMC	2.0	2.0	2.0
Filler	8.1	6.1	4.1
Proximate analysis result			
Protein (%)	31.26	31.38	31.29
NFE (%)	30.50	32.76	34.82
Lipid (%)	9.11	9.54	9.57
Fiber (%)	15.55	12.61	10.59
Total energy (Kcal g^{-1})***	259.46	269.01	274.13
E/P (Kcal g^{-1} protein)	8.30	8.57	8.76

CMC-carboxymethyl cellulose; NFE-nitrogen free extract; E/P-energy protein ratio; *in mg kg^{-1} feed: vit. B_1 60; vit. B_2 100; vit. B_{12} 100; vit. C 2000; vit. K_3 50; vit. A/$D_3$400; **in mg kg^{-1} feed: $MgSO_4.7H_20$ 7.5; NaCl 0.5; $NaH_2PO_4.2H_2O$ 12.5; KH_2PO_4 16.0; $CaHPO_4$.2H2O 6.53; Fe citric 1.25; $ZnSO_4.7H_2O$ 0.1765; $MnSO_4.4H_2O$ 0.081; $CuSO_4.5H_2O$ 0.0155; KIO3 0.0015; CoSO4 0.0003; ***protein=3.5 kcal g^{-1}; NFE=2.5 kcal g^{-1}; lipid=8.1 kcal g^{-1}.

Fish culture management. *O. melanopleurus* specimens were obtained from the hatchery of a Freshwater Aquaculture Center (Balai Benih Air Tawar), in Mandiangin, South Kalimantan. Fish were reared in a plastic tank containing 40 L water with a density of 20 fish per tank with an average weight of 3.18±0.26 g. Fish were cultured for 60 days in a semi-closed circulation system, being fed twice a day in the morning and evening, at satiation. Dejections were collected in the morning. Water lost due to the siphon was replaced by new water up to the same volume. The filter was washed every day and the filter bath was washed and replaced with new water every week. During the study, the average water temperature was 28.5±1.0°C, dissolved oxygen was 4.50-6.10 mg L^{-1}, pH was between 6.80 and 6.95, total ammonia nitrogen was between 0.382 and 0.623 mg L^{-1}. This shows that the water conditions during the study were optimal (Tebbut 1992; Effendie 1997).

Data collection and chemical analysis. Initial and final body weights were measured upon anesthetized fish (using MS 222). Weighing was performed daily to determine the specific growth rate (SGR) according to De Silva & Anderson (1995). During the study, the following data were recorded: feed consumed, to determine the Total Feed Consumption (TFC), according to Pereira et al (2007); feed efficiency, protein retention, lipid retention and energy retention, according to NRC (2011); Protein Efficiency Ratio (PER), according to Bake et al (2014). Proximate body analysis was carried out at the beginning and at the end of the study, to determine the nutrient composition of fish (Takeuchi 1988). Analysis of liver and meat glycogen, as well as liver lipid content, was carried out at the beginning and end of the study to determine the reserve energy (Takeuchi 1988).

Statistic analysis. The design of this study followed an experimental laboratory model, using a completely randomized design (CRD) consisting of three treatments and five replications. The data of feed efficiency (FE), weight growth, total feed consumption (TFC), protein retention (PR), lipid retention (LR), energy retention (ER) and protein efficiency ratio (PER) were tested for variance with ANOVA, followed by a Tukey test at 95% confidence interval, using the SPSS program, while liver and muscle glycogen levels, as well as liver lipid levels, were analyzed descriptively in tabular form.

Results and Discussion

Growth performance and feed utilization efficiency. The values of various parameters of feed use, including: weight gain, specific growth rate, relative growth rate, protein retention, lipid retention, energy retention and the ratio of protein efficiency. as well as feed efficiency, were determined for a 60 days rearing period and three feed treatments with different carbohydrate-concentrations, as presented in Table 2.

The change in the carbohydrate concentration levels have a significant influence on the weight growth, relative growth rate, specific growth rate, protein efficiency ratio (PER), feed efficiency, protein retention, lipid retention and energy retention ($P<0.05$), and did not significantly affect the level of feed consumption ($P>0.05$). The best weight growth was obtained in the treatment B (32.76% CHO), followed by C (34.82% CHO). The lowest weight growth was obtained in the group of fish that consumed feed A (30.50% CHO) ($P<0.05$).

The best relative growth rate was also obtained in the group of fish fed with B (32.76% CHO) which was 0.84% per day statistically higher than in fish that consumed other feed ($P<0.05$). The same phenomenon was also seen in the specific growth rate, where the group of fish that consumed feed B had the best specific growth rate of 1.04% per day statistically higher than the group of fish that consumed feed C and A ($P<0.05$).

Table 2

The average value of initial weight, final weight, growth weight, relative growth rate (RGR), specific growth rate (SGR), total feed consumption (TFC), protein efficiency ratio (PER), feed efficiency (FE), protein retention (PR), lipid retention (LR), energy retention (ER) and total ammonia nitrogen (TAN) obtained in *Osteochilus melanopleurus* maintained for 60 days by feeding with different carbohydrates

Parameters	*CHO (%)*		
	A(30.50)	*B(32.76)*	*C(34.82)*
Initial weight (g)	3.17±0.32[a]	3.08±0.23[a]	3.24±0.29[a]
Final weight (g)	4.86±0.14[a]	5.65±0.09[c]	5.14±0.06[b]
Weight growth (g)	33.71±5.14[a]	51.39±5.49[b]	37.48±4.90[a]
RGR (%)	0.54±0.12[a]	0.84±0.14[b]	0.60±0.15[a]
SGR (%)	0.72±0.14[a]	1.04±0.08[b]	0.80±0.06[a]
TFC (%)	121.26±4.57[a]	127.52±3.82[a]	125.89±3.16[a]
PER (%)	0.89±0.12[a]	1.28±0.11[b]	0.95±0.13[a]
FE (%)	27.74±3.69[a]	40.23±3.34[b]	29.80±4.13[a]
PR (%)	61.64±7.33[a]	84.17±7.91[b]	66.19±8.05[a]
LR (%)	62.51±4.51[a]	91.06±8.09[b]	76.41±13.19[ab]
ER (%)	45.00±4.13[a]	63.34±3.45[b]	49.40±7.01[a]
TAN (mg (g body hour)$^{-1}$)	0.00170±0.00004[c]	0.00124±0.00007[a]	0.00154±0.00012[b]

The average followed by different superscript letters in the same row shows significant difference (P<0.05).

O. melanopleurus that was maintained by feeding B showed the same feed consumption levels as the fish fed with C and A (P>0.05). Fish that consumed feed B have the highest protein efficiency ratio (PER), compared to fish that consumed feed A and C (P<0.05), which is 1.28%. The best value of feed efficiency was obtained in fish that consumed feed B, which was 40.23±3.34%, higher than in the group of fish that consumed feed C, with a value of feed efficiency of 29.80±4.13%, while fish that consumed feed A produced the lowest feed efficiency, at a level of 27.74±3.69%.

Fish that consumed feed B had the highest protein retention value (84.17±7.91%), compared to the specimens that consumed feed A and C (66.19±8.05% and 61.64±7.33%, respectively). The lipid retention value in fish that consumed feed B was also the highest, with a value of 91.06±8.09%, followed by the group of fish that consumed feed C and A. The same observation stands for the energy retention value in the group of fish that consumed feed B, which was higher, with a value of 63.34±3.45%, than in specimens that consumed feed A (45.00±4.13%). The group of fish that consumed feed B produced a TAN excretion of 0.00124±0.00007 mg (g body hour)$^{-1}$, lower than the group of fish that consumed feed C, followed by group A (P<0.05).

Initial and final proximate analysis, liver and muscle glycogen content, and liver lipid content. Initial and final proximate composition, after being reared for 60 days by feeding with different carbohydrate contents, is presented in Table 3.

The protein level of the fish body at the end of the study decreased, but it was not influenced by the increase in feed carbohydrates (to the level of 34.82% CHO) and had no significant effect on fish body protein levels (P>0.05). The same phenomenon was also found in the ash content of the fish body at the end of the study, but the administration of different carbohydrate levels significantly affected the ash content (P<0.05). At the opposite, increasing the carbohydrate levels does not have a significant effect on the NFE content (P>0.05). The increase in feed carbohydrates significantly affected the fish body lipid content, the highest value being obtained in the group of fish that consumed feed with 32.76% CHO (P<0.05).

Table 3
Initial and final proximate body composition of *Osteochilus melanopleurus* maintained for 60 days by feeding with different carbohydrates (% dry weight)

Parameters	*Level CHO (%)*					
	A (30.50)		*B (32.76)*		*C (34.82)*	
Initial body composition (%)						
Protein	62.41		62.41		62.41	
Lipid	5.87		5.87		5.87	
Ash	21.04		21.04		21.04	
NFE	9.43		9.43		9.43	
Final body composition (%)						
Protein	64.86	±0.62[a]	63.87	±1.13[a]	64.64	±1.11[a]
Lipid 01	10.94	±0.49[a]	13.01	±0.95[b]	12.63	±1.17[b]
Ash	15.11	±0.46[b]	13.62	±0.46[a]	14.29	±0.55[ab]
NFE	7.77	±0.55[a]	8.57	±0.59[a]	7.73	±0.47[a]

The average followed by different superscript letters in the same row shows significant difference (P<0.05).

Liver and muscle glycogen levels, as well as initial and final liver lipid content in *O. melanopleurus* maintained for 60 days by feeding with different carbohydrates, are presented in Table 4.

Maintenance of fish for 60 days with feed containing different carbohydrates does not have a significant effect on the liver and muscle glycogen levels. Liver glycogen levels of fish fed with C (34.82% CHO) had liver glycogen levels of 2.14±0.11 μg g^{-1} which were relatively similar compared to fish consuming B and A feeds with liver glycogen levels of 2.11±0.10 μg g^{-1} and 2.09±0.13 μg g^{-1}, respectively. Glycogen levels of fish muscle fed with C (34.82% CHO), reaching 0.178±0.009 μg g^{-1}, were the same as in fish that consumed feed B and C with muscle glycogen levels of 0.176±0.008 μg g^{-1} and 0.174±0.010 μg g^{-1}, respectively.

The highest level of liver lipid was found in the group of fish that consumed feed B, reaching 6.50±0.47%, followed by fish that consumed feed C and feed A with liver lipid contents of 6.31±0.58% and 5.47±0.25%, respectively.

Table 4
Levels of the liver and muscle glycogen and liver lipid content *Osteochilus melanopleurus* were maintained for 60 days by giving feed containing different carbohydrates

Parameters	*Levels CHO (%)*					
	A (30.50)		*B (32.76)*		*C (34.82)*	
Glycogen content (μg g^{-1})						
Liver	2.09	±0.13[a]	2.11	±0.10[a]	2.14	±0.11[a]
Muscle	0.174	±0.010[a]	0.176	±0.008[a]	0.178	±0.009[a]
Liver lipid content (%)						
Initial	2.94		2.94		2.94	
Final	5.47±0.25[a]		6.50±0.47[b]		6.31±0.58[b]	

The average followed by different superscript letters in the same row shows significant difference (P<0.05).

Discussion. Feeding with an increase in carbohydrate levels up to 32.76% significantly increases the observed specimens' growth performance. Conversely, the latter decreases by rising the carbohydrate content up to 34.82%. This indicates that in the treatment B scenario (32.76% CHO), non-protein feed energy sources are better processed and more efficient in growth, compared treatments C (34.82% CHO) and A (30.50% CHO).

Bray & Lawrence (1992), states that the availability of energy mainly from carbohydrates as an energy source other than lipids and proteins is mainly used for metabolism, both for growth and subsequently for reproduction in nature. Therefore, if the energy needed for metabolism and growth is sufficient, the exceeding nutrients or energy will be stored or used for reproduction. Wilson (1994) added that if carbohydrates are deficient, then other nutrients such as protein and lipids will be metabolized to energy, therefore fish growth will be slower.

Feed efficiency and protein efficiency ratio are the determinant parameters for evaluating the effectiveness of feed in growth. The fish group that consumed feed B (32.76% CHO) had a higher feed efficiency and protein efficiency ratio than the other groups. This indicates that the fish can utilize the nutrients they consume, especially carbohydrates and lipids as a source of energy for protein synthesizing and for growth. The results of this study also illustrate the importance of the presence of carbohydrates in the feed. The importance of providing carbohydrates at certain levels was also reported by Castro et al (2016), in gilthead seabream (*Sparus aurata*) juveniles. Fish fed with 20% carbohydrates and different lipid concentrations did not experience differences in growth but higher PER and lipid retention were observed in the group of fish that consumed feed with carbohydrates. Conversely, fish that are fed without carbohydrates, experience lower lipid retention.

Retention ratio describes the stored nutrients fraction of the total intake of nutrients of the same type, during a reference period. The results of this study indicate that the fish group fed B (32.76% CHO), produced Protein Retention (PR), Lipid Retention (LR), and Energy Retention (ER) higher than the C and A fish groups. This illustrates that the consumed nutrients, besides being used for activities are also stored in the muscles as energy reserves. The high value of nutrient retention (PR, LR, and ER), in group B fish also indicates that the consumed feed is balanced, so that the portion of protein for growth is not disrupted.

Conversely, an increase in CHO levels to 34.82% CHO caused a decrease in nutrient retention in *O. melanopleurus*. The low value of nutrient retention in the fish group corresponding to treatment C is closely related to the decreased level of feed consumption. The high energy in the feed causes the fish to limit the amount of feed consumed, thus affecting the number of feed nutrients consumed.

In the fish group corresponding to treatment A (30.50% CHO), the low value of nutrient retention is due to the suboptimal use of the feed, which does not provide enough energy for standard activities, and requires compensation via protein and lipids metabolizing. Mokoginta et al (1995) explained that if the energy content of the feed was too low, then most of the feed protein would be catabolized to meet energy needs, so that fish consumes larger amounts of food. Fish will limit the amount of feed consumption if the feed contains too high energy because basic energy needs have been met. An indicator of the presence of proteins catabolized for energy in both groups of fish corresponding to treatments C and A is the higher level of total ammonia nitrogen (TAN) excretion value of 0.00170±0.00004 mg (g body hour)$^{-1}$ and 0.00154±0.00012 mg (g body hour)$^{-1}$, respectively, compared to fish group of treatment B, with only 0.00124±0.00007 mg (g body hour)$^{-1}$.

Carbohydrates administration in higher proportions will inhibit growth. Li et al (2019) stated that the growth performance and utilization of feed decreased in groupers by increasing carbohydrate content of the feed, as seen from glucose intolerance in these fish. The same phenomenon was observed by Zhou et al (2013) in *Megalobrama amblycephala*, by Wu et al (2016) in juvenile black carp *Mylopharyngodon piceus*, by Dong et al (2016) on golden pompano *Trachinotus ovatus*, by Wang et al (2016) in juvenile grouper *Epinephelus akaara* and by Xie et al (2017) in Nile tilapia *Oreochromis niloticus*. Ren et al (2011) also stated that increasing levels of carbohydrate to 18.4% CHO for juvenile cobia *Rachycentron canadum* will increase SGR, FER, and PER.

The provision of carbohydrate feed, which is increasing in fish from 30.50% CHO to 34.82% CHO, does not affect the level of liver glycogen and muscle but does affect the level of liver lipid. Similar results were obtained by Wang et al (2016), who stated that an increase in feed carbohydrates will lead to the accumulation of liver glycogen in carnivorous fish such as juvenile groupers *E. akaara* and large yellow croaker juveniles *Larimichthys crocea* (Xing et al 2016). Guo et al (2015) stated that grass carp *C. idella* herbivorous fish, fed with carbohydrate content increased from 30.94% to 42.31%, did not experience alterations of the growth performance, but only an increased level of liver lipid and liver glycogen. In omnivores such as Prussian carp, *Carassius gibelio,* high levels of carbohydrates with low lipid (45.0% CHO and 2.0% lipid) also did not cause differences in specific growth rates but increased Hepatosomatic Index (HSI), liver lipid content and lipid retention efficiency (Li et al 2019). Similar observations were done by Mozanzadeh et al (2016) on juvenile Sobaity seabream *Sparidentex hasta* fed with increased carbohydrate content and reduced dietary lipid content, which did not influence the growth performance, in particular the weight growth, condition factors, specific growth rate , feed consumption and feed conversion rates.

Based on the growth performance and feed utilization, the optimum level of carbohydrate for *O. melanopleurus* is 32.76%. These results are almost the same as reported by Booanuntanasarn et al (2018) in tilapia *O. niloticus* where the optimal level of carbohydrates is 32.6%. Lower yields have been reported in other fish such as: the silver barb *Puntius gonionotus* (29.3-29.8%) by Mohanta et al (2009); the juvenile cobia *Rachycentron candum* L (21.1% CHO), by Ren et al (2011); the Wuchang Bream *Megalobrama amblycepahla* (31.0%), by Zhou et al (2015); the golden pompano *T. ovatus* (16.93-20.64%), by Dong et al (2016); the juvenile large yellow croaker, *L. crocea* (21.29%), by Xing et al (2016) and 16.27% CHO by Zhou et al (2016), and the juvenile black carp *M. piceus* (24.98%), by Wu et al (2016). The optimum level of carbohydrate 32.76% in this study was also higher than the optimum value obtained by Xie et al (2017) in juvenile tilapia *O. niloticus* (28.87%), by Asemani et al (2019) in striped catfish *Pangasianodon hypophthalmus* fingerlings (30.81-31.13%) and by Yanto et al (2019) in *Barbonymus schwanenfeldii* (22.89 g%).

Higher optimal carbohydrate levels in feed were found by Seenappa & Devaraj (1995) in major carp *Catla catla* (35% CHO), by Luquet (1991) in *Tilapia* sp. (40.0% CHO) and by Mokoginta et al (2004) in gourami *Osphronemus goramy* measuring 78.7 g (47.5% CHO). Mohapatra et al (2003) reported that roho labeo (*Labeo rohita*) that consumed feed with a carbohydrate content of 45.0% at a protein content of 30% was more efficient in using the feed.

Based on the results of the research above, further research is necessary to discover the optimal carbohydrate and lipid ratio so that the growth of *O. melanopleurus* can be maximized.

Conclusions. *O. melanopleurus* that consume 32.76% CHO provided the best weight growth, relative growth, feed efficiency, protein efficiency ratio, protein retention, lipid retention and energy retention compared to other treatments. Increasing carbohydrate levels up to 34.82% did not increase liver and muscle glycogen levels and liver lipid content.

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