Submission letter

Article title: **THE EFFECTS OF DIETARY PROTEIN LEVEL ON THE GROWTH, PREOTEN EFFICIENCY RATIO AND BODY COMPOSITION OF JUVENILE KELABAU (*Osteochilus melanopleurus*)**

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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.

This manuscript was not submitted or published to any other journal. The authors declare that the manuscript is an original paper and contain no plagiarised text. All authors declare that they are not currently affiliated or sponsored by any organization with a direct economic interest in subject of the article. My co-authors have all contributed to this manuscript and approve of this submission.

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THE EFFECTS OF DIETARY PROTEIN LEVEL ON THE GROWTH, PROTEIN EFFICIENCY RATIO AND BODY COMPOSITION OF JUVENILE KELABAU (*Osteochilus melanopleurus*)

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Abstract This study was conducted to evaluate the effect of feeding with different protein levels on growth performance and body composition of kelabau fish (*O. melanopleurus* Bleeker). Four experimental feeds with different protein levels but same fat content was carried out. Feed A, B, C and D contain protein levels, respectively 25.14 %, 28.26 %, 31.88 % and 34.73 %. Kelabau fish was obtained from breeding unit of the Freshwater Aquaculture Center (BBAT) Mandiangin within an average of weight: 2.09 ± 0.16 g with a density of 20 fish maintained in a plastic box size 54.3 cm x 38 cm x 31.5 cm and filled with 40 litres of water. Kelabau fish were given experimental feed 2 times a day at satiation for 60 days. Kelabau fish feed C (31 % protein) showed higher growth rate, relative growth rate, total feed consumed, feed utilization efficiency were higher when compared to other treatments ($P < 0.05$). The highest mean growth rate was in the treatment of feed C (31 %) with an average of 4.14 ± 0.54%, while the lowest relative growth in the treatment of feed A (25 %) with an average of 2.92 ± 0.54%. Protein efficiency ratio (PER) group of fish that consumed feed C = 1.50 ± 0.06%, feed A = 1.45 ± 0.13%, feed B = 1.40 ± 0.04% and significantly different from in group fish that consumed feed D = 1.16 ± 0.07%.
Keywords: *Relative Growth Rate, Feed Consumption Level, Feed Efficiency, At Satiation*.

Introductions. Kelabau fish (*Osteochilus melanopleurus*) is a freshwater fish belonging to the order Cypriniformes, sub-order Cyprinoidae, Cyprinidae family, genus *Osteochilus* and *O. melanopleurus* species (Kottelat et al 1993). Kelabau fish has been cultivated since 2013. As a local fish with high economic value, lives in public waters found in Kalimantan and Sumatera unfortunately is still not cultured and the production is still limited depending on the fishing seasons. However, kelabau fish should be cultured like other species of Cyprinid fishes, and the culture should be improved by optimizing the maintenance of both the maintenance of culture system and the quality of the feed provided. Some researches of nutritional feed are under go in marine species, but still only a little. Mardani (2014) conducted a study of different food sources for the growth of fish in the sea. Black fish that consume feed with a composition of 29.3% protein content provides the best relative growth (RGR) of 49.45% compared to other treatments.

Each fish species needs different levels of protein for its growth and is influenced by fish size, but generally fish need around 35-50% protein in their feed (Hepher, 1990). Research on the need for protein in omnivorous fish has been widely carried out as in tilapia (*Oreocromis niloticus*) by Teshima et al (1985) in Merola and Cantelmo (1987); pacu (*Colossoma macropomum*) by Eckmann (1987); and Carp (*Cyprinus carpio*) (Shimeno, Kheyyali and Shikata, 1995). In herbivorous fish such as gourami (*Ospronemus gouramy*) (Mokoginta, et al 1995); on mashseer fish (*Tor putitora* (Hemilton)) (Hossain, et al 2002); Rohu fish (*Labeo rohita*) (Satpathy, et al 2003); Silver Barb (*Puntius gonionotus*) (Mohanta, et al 2008). Furthermore Mansour, et al (2017) and Dewantoro, et al (2018), on Tinfoil barb (*Barbonymus schwanenfeldii*). Research on protein requirements in kelabau fish (*O. melanopleurus*) has never been done. Research to find the right protein content to produce the best growth of kelabau fish (*O. melanopleurus*) is needed.

MATERIALS AND METHODS

Diets. This study used 4 types of artificial feed with different protein content, namely feed A (25 %), feed B (28 %), feed C (31 %) and feed D (34 %)) with a CP ratio ranging from 8-10 kcal. Feed formulations are presented in Table 1.

Table 1. Treatment feed composition (gr) and feed nutrient content *

Ingredient Composition	Ingredient percentage in the trial feed (% dry matter)			
	A (25 %)	B (28 %)	C (31 %)	D (34 g %)
Fish Meal	29.0	34.0	37.0	39.3
Soybean Meal	15.5	15.0	17.0	20.2
Wheat Flour	6.5	10.0	12.3	13.0
Bran Meal	15.0	12.0	10.0	10.2
Fish Oil	2.5	2.5	2.5	2.5
Corn Oil	2.5	2.5	2.5	2.5
Vitamin Mix[2)]	3.0	3.0	3.0	3.0
Mineral Mix[3)]	3.0	3.0	3.0	3.0
Coline Chlorida	2.0	2.0	2.0	2.0
CMC[1)]	2.0	2.0	2.0	2.0
Filler	19.0	14.0	8.7	2.3
Proximate analysis results				
Protein (%)	25,14	28,26	31,88	34,73
NFE (%)	34.34	31.75	30.53	31.84
FAT (%)	11.55	11.37	11.59	11.55
Fiber (%)	2.35	2.00	1.85	1.99
Total Energi (KKal g^{-1})[4)]	267.40	270.38	281.78	294.71
E/P (KKal g^{-1} Protein)	10.64	9.57	8.84	8.49

Remarks :

*) : Calculation based on dry weight.

1 : Carboxymethil cellulose.

2 : In mg kg^{-1} : 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 pantotenat 100; inositol 2000; biotin 300; asam folat 15; niasin 400.

3 : in mg kg^{-1} : $MgSO_4.7H_20$ 7.5; NaCl 0.5;$NaH_2PO_4.2H_2O$ 12.5;KH_2PO_4 16.0; $CaHPO_4.2H_2O$ 6.53; Fe sitrat 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 kkal g^{-1}; NFE = 2.5 kkal g^{-1}; Fat = 8.1kkal g^{-1}.

Fish Culture Management. The Kelabau fish used comes from hatchery of Freshwater Aquaculture Center (BBAT), Mandiangin, South Kalimantan. Kelabau fish were reared in plastic tank containing 40 L water with a density of 20 fish per tank with an average weight of 2.09 ± 0.16 g. Fish reared for 60 days by feeding twice a day in the morning and in the afternoon *at satiation*. Kelabau fish reared in semi-closed circulation system. Siphoning the fish faeces was done every morning. The filter washed every day and the filter tub is washed and replaced with new water every 1 week. During the study, the water temperature averaged 30.0 ± 1.0 ^oC, dissolved oxygen between 4.60 to 6.20 mgL^{-1}, pH between 6.70-6.80, TAN between 0.398 to 0.721 mgL^{-1}. This shows that the condition of the water during the study was at optimum conditions. (Tebbut, 1992; Effendie, 1997)

Data Collection and Chemical Analysis. Weighing body was done at the beginning and end of the study in the state of anesthetized fish. The fish is anesthetized using MS222. Weighing is done to determine the relative growth rate (De Silva & Anderson, 1995). Feed consumed during the study was recorded to determine Total Feed Consumption (TFC) (Pereira et al 2007), and Feed Utilization Efficiency (FUE) (NRC, 1993), 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 nutrient composition in fish (Takeuchi, 1988).

Statistical Analysis. The design of this study is a laboratory experimental model, using a completely randomized design (CRD) consisting of 4 treatments and 4 replications. Data of Feed Utilization Efficiency (FUE), Weight Growth Rate, Specific Growth Rate (SGR),

Relative Growth Rate (RGR) and Protein Efficiency Ratio (PER), were analysed for diversity with ANOVA and continued with the Tukey test at a 95% confidence interval using the SPSS version 11.5 program.

RESULTS AND DISCUSSION

Results

Weight Growth, Relative Growth and Total Feed Consumption and Feed Utilization Efficiency (FUE). The values of various parameters of feed use which include weight growth rate, specific growth rate, relative growth rate, and protein efficiency ratio as well as feed efficiency of the fish after being kept for 60 days by feeding different proteins are presented in Table 2.

Kelabau fish fed with different protein levels had a significant effect on weight growth, relative growth rate, specific growth rate, feed consumption level, and protein efficiency ratio and feed efficiency (*P <0.05*). The best growth of weight was obtained in the treatment of feed C (31.0 %) then fed by fish that consumed feed D (34.0 %). The lowest weight growth was obtained in the group of fish that consumed feed A (25.0 %) and B (28.0 %) (*P <0.05*). The best relative growth rate was obtained in the group of fish fed C (31.0 %), which was 4.07% day^{-1} which was significantly different from fish consuming other feeds (*P <0.05*). The same phenomenon was also seen in the specific growth rate, where the group of fish consuming C feed had the best specific growth rate of 2.06% per day which was significantly different from the group of fish that consumed feed D, B and A (*P <0.05*).

Kelabau fish which is maintained by feeding C and D shows more levels of feed consumption compared to fish that are maintained by feeding A and B (*P <0.05*). Fish, which consume D feed, have the lowest protein efficiency ratio (PER) compared to fish that consume feed A, B and C (*P <0.05*). The best feed utilization efficiency (FUE) value was obtained for fish that consumed C feed which was 47.26 ± 2.18% which was different from the group of fish that consumed feed B with a feed utilization efficiency (FUE) value of 39.16 ± 0.91% (*P<0.05*). Fish that consume feed A produce a low value of feed utilization efficiency (FUE) with a feed utilization (FUE) efficiency value of 35.62 ± 2.51%.

Table 2.

The average value of the initial weight, final weight, weight growth, relative growth rate (RGR), specific growth rate (SGR), total feed consumption (TFC), protein efficiency ratio (PER) and feed utilization efficiency (FUE), obtained from Kelabau Fish (*O. melanopleurus*) which was kept for 60 days by feeding different proteins.

Biology Parameters	Diet (%)			
	A(25,0)	B(28,0)	C(31,0)	D(34,0)
Initial weight(g)	2,02 ± 0,21	2,02 ± 0,12	2,10 ± 0,12	2,21 ± 0,16
Final Weight (g)	5,42 ± 0,06	5,73 ± 0,06	7,23 ± 0,13	6,39 ± 0,02
Weight growth (g)	68,00 ± 4,52^{a}	74,30 ± 1,81^{a}	102,48 ± 3,78^{c}	83,77 ± 2,93^{b}
RGR (%)	2,92 ± 0,54^{a}	3,11 ± 0,30^{a}	4,14 ± 0,54^{b}	3,37 ± 0,25^{a}
SGR(%)	1,68 ± 0,20^{a}	1,75 ± 0,10^{a}	2,07 ± 0,15^{b}	1,84 ± 0,08^{a}
TFC%)	191,13 ± 9,66^{a}	189,79 ± 4,38^{a}	217,20 ± 13,73^{b}	220,57 ± 10,91^{b}
PER(%)	1,45 ± 0,13^{b}	1,40 ± 0,04^{b}	1,50 ± 0,06^{b}	1,16 ± 0,07^{a}
FUE(%)	34,36 ± 7,68^{a}	34,86 ± 3,57^{a}	54,44 ± 5,64^{b}	43,77 ± 3,68^{a}

Remarks : Numbers followed by the same letters on the same lane show no significant difference (P> 0.05).

Proximate Composition of the Initial and Final Bodies

The proximate composition of fish body kelabau both at baseline and at the end of the study and after the fish reared for 60 days by feeding different protein content are presented in Table 3.

Protein levels of fish body at the end of the study tend to increase with increasing protein feed up to protein content 31.0% but did not significantly affect the protein content of fish bodies (P> 0.05). Likewise, the body fat content of fish, ash content and NFE content, showed no significant difference (P> 0.05).

Table 3.

The proximate composition of the initial and final body of Kelabau fish (*O. melanopleurus*) were maintained for 60 days by giving feed containing different proteins (% dry weight)

Diet	Protein Lavel (%)			
	A(25,0)	B(28,0)	C(31,0)	D(34,0)
Initial Body Composition (%):				
Protein	56.42	56.42	56.42	56.42
Fat	23.75	23.75	23.75	23.75
Ash	13.37	13.37	13.37	13.37
NFE	6.03	6.03	6.03	6.03
Finel Body Composition (%):				
Protein^{ns}	58.62 ±2.56	59.73 ±2.29	61.69 ±0.87	60.89 ±1.98
Fat^{ns}	20.78 ±1.41	21.51 ±2.65	21.77 ±2.94	19.83 ±1.03
Ash^{ns}	14.76 ±1.15	14.47 ±0.54	15.15 ±1.01	13.84 ±0.73
NFE^{ns}	2.19 ±0.80	2.25 ±0.47	1.77 ±0.32	2.51 ±1.28

Remarks: ns: not significantly different (P> 0.05).

Discussion. Feeding with increased protein levels to levels of 31.0% will significantly improve the growth performance of Kelabau fish, and decrease with increasing protein levels to 34.0%. Kelabau fish that consume feed with a protein content of 31.0% are better able to utilize feed protein sources for growth compared to fish that consume D feed which is higher in protein (34.0%). This shows that the protein consumed is not necessarily used for growth. In general, nutrition must be available first for metabolism, and second for growth and third for reproduction in nature (Bray & Lawrence, 1992). This means that, once the nutrients or energy needed for metabolism and growth are met, excess nutrients or energy will be stored or used for reproduction.

The group of fish that consumed C feed had a relatively high growth compared to other feed groups. Many authors have reported that there is an increase in protein levels that will not support further growth and may even affect lowering growth due to insufficient energy availability (McGoogan & Gatlin 1999). This might explain that the proportion of protein will be degraded, where the carbon skeleton is used as an energy source at high dietary protein levels. Excreted ammonia nitrogen causes damage to water quality, so an increase in food protein levels does not increase production so it must be avoided (El-Sayed & Kawanna 2008).

Based on growth and feed efficiency, the optimum level of protein feed for Kelabau fish was 31.0%. These results are lower than those reported in other fish, such as *Oncorhynchus nerka* (45.0%) (NRC, 1993), masu salmon (*Oncorhynchus masou* Brevoort) (40.0 g 100g -1, 16.2 g 100g-1 lipids)) (Lee & Kim 2001) and Atlantic salmon (39.0%, 25.5% lipids) (Hillestad & Johnsen 1994), and Pacific salmon *Oncorhynchus* spp (55.0%) (NRC, 2011). The optimum diet protein level of 31.0% in this study was also lower than the 48.0% protein diet value with 20.0 ~ 25.0% lipids for Manchurian trout larvae (0.15 g) reported by Zhang et al (2009). The difference between the two studies can be explained by different fish sizes. Evidence shows that protein requirements decrease with increasing fish size (NRC, 2011).

Higher yields also found in fingerling / juvenile tilapia (*Oreocromis niloticus*) requires 35.0% protein (Abdel-Tawwab, et al 2010), Gourami (*Ospronemus gouramy*) measuring 0.27 g requires 43.29% protein with an energy protein (C / P) ratio of 8 kcal DE / gram (Mokoginta, et al 1995). Hossain, et al (2002), stating that the mashseer fish (*Tor putitora* (Hemilton)) grows well on feed containing 40% Protein. Rohu Fish (*Labeo rohita*) measures an average of 4.3 ± 0.02 g which consumes feed with a protein content of 45.0% and fat content of 10% or 15% gives the best growth (Satpathy, et al 2003). Similar results were found in carp (*Cyprinus carpio*) measuring 121 g requiring 31.6% protein and 11.9% fat (Shimeno, Kheyyali and Shikata, 1995). Silver Barb (*Puntius gonionotus*) grows well at a protein content of 31.77% with a P / E ratio of 21.1 g of MJ-1 protein (Mohanta, et al 2008). Furthermore Mansour, et al (2017), showed that the best growth of tinfoil barb (*Barbonymus schwanenfeldii*) fish was fed with 32% protein content. Dewantoro, et al (2018) obtained lower protein requirements at 30% protein content and C / P ratio of 10 Kcal / g protein which was able to increase specific growth rate (SGR), protein retention and fat retention in the same fish.

The value of feed utilization efficiency (FUE) is the ratio between body weight gain and the amount of feed consumed. In the group of fish that consumed C food had the highest efficiency value 47.26 ± 2.18%, compared to the group of fish that consumed feed B (39.16 ± 0.91%) and D (38.08 ± 2.18%) and A (35.62 ± 2.91%). The group of fish that consumed C food had a higher weight gain while the food consumed was less than the fish in group D (Table 2). Protein efficiency ratio (PER) group of fish that consumed feed C = 1.50 ± 0.06%, feed A = 1.45 ± 0.13%, feed B = 1.40 ± 0.04% and better than in group fish that consumed feed D = 1.16 ± 0.07%. This illustrates that the protein consumed by fish in group C can be used efficiently to increase growth compared to other group fish.

Further research should be conducted to determine the optimal carbohydrate levels so that Kelabau fish growth can be maximized.

Conclusion. Kelabau fish that consume 31.88% of protein-containing feed were provide excelen weight growth rate, relative and specific growth rate, protein efficiency ratio, total feed consumption and the best feed utilization efficiency compared to other treatments.

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November 28, 2018

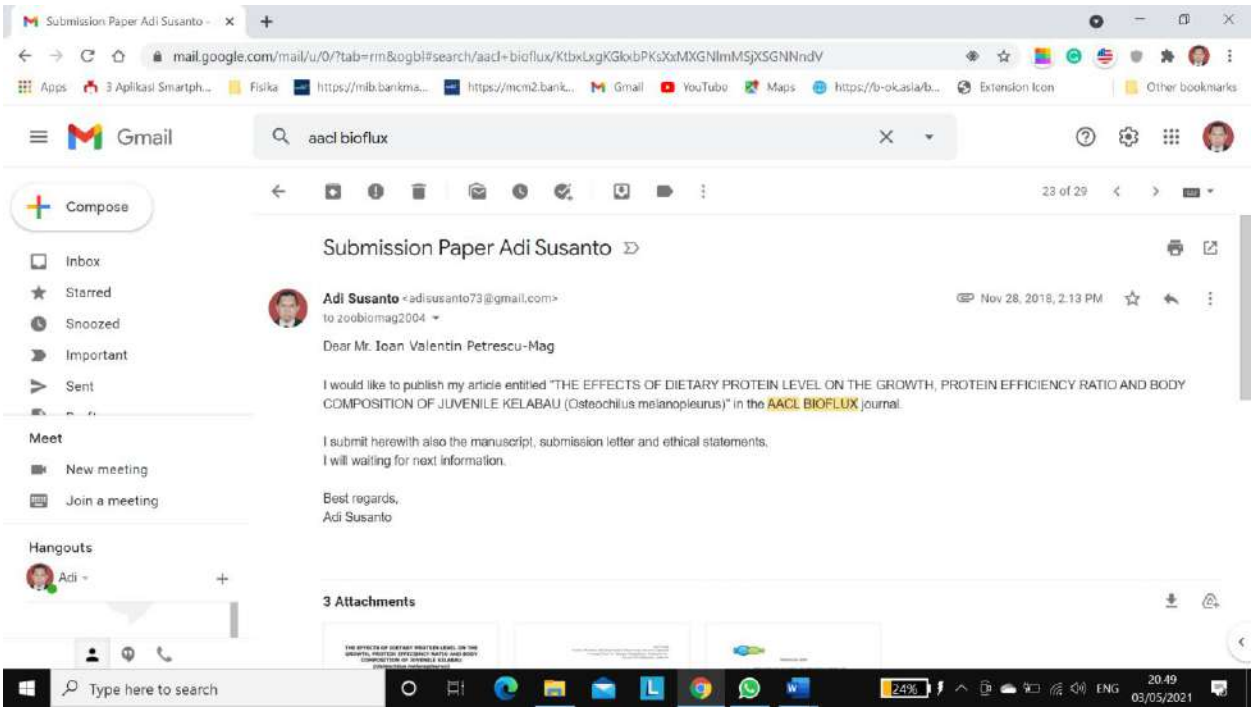
I am submitting a manuscript for consideration of publication in **Aquaculture, Aquarium, Conservation & Legislation - International Journal of the Bioflux Society**. The manuscript is entitled "**The Effects Of Dietary Protein Level On The Growth, Protein Efficiency Ratio And Body Composition Of Juvenile Kelabau (*Osteochilus melanopleurus*)**"

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

Kelabau fish (*Osteochilus melanopleurus*) is a freshwater fish belonging to the order Cypriniformes, sub-order Cyprinoidae, Cyprinidae family, genus *Osteochilus* and *O. melanopleurus* species (Kottelat et al 1993). Kelabau fish has been cultivated since 2013. As a local fish with high economic value, lives in public waters found in Kalimantan and Sumatera unfortunately is still not cultured and the production is still limited depending on the fishing seasons. However, Kelabau fish should be cultured like other species of Cyprinid fishes, and the culture should be improved by optimizing the maintenance of both the maintenance of culture system and the quality of the feed provided. Each fish species needs different levels of protein for its growth and is influenced by fish size, but generally fish need around 35-50% protein in their feed. Research on the protein requirements of kelabau fish (*O.melanopleurus*) has never been done. Therefore, research on the protein requirements of kelabau fish (*O.melanopleurus*) is very necessary. Kelabau fish feed C (31 % protein) showed higher growth rate, relative growth rate, total feed consumed, feed utilization efficiency were higher when compared to other treatments.

Thank you very much for your consideration.

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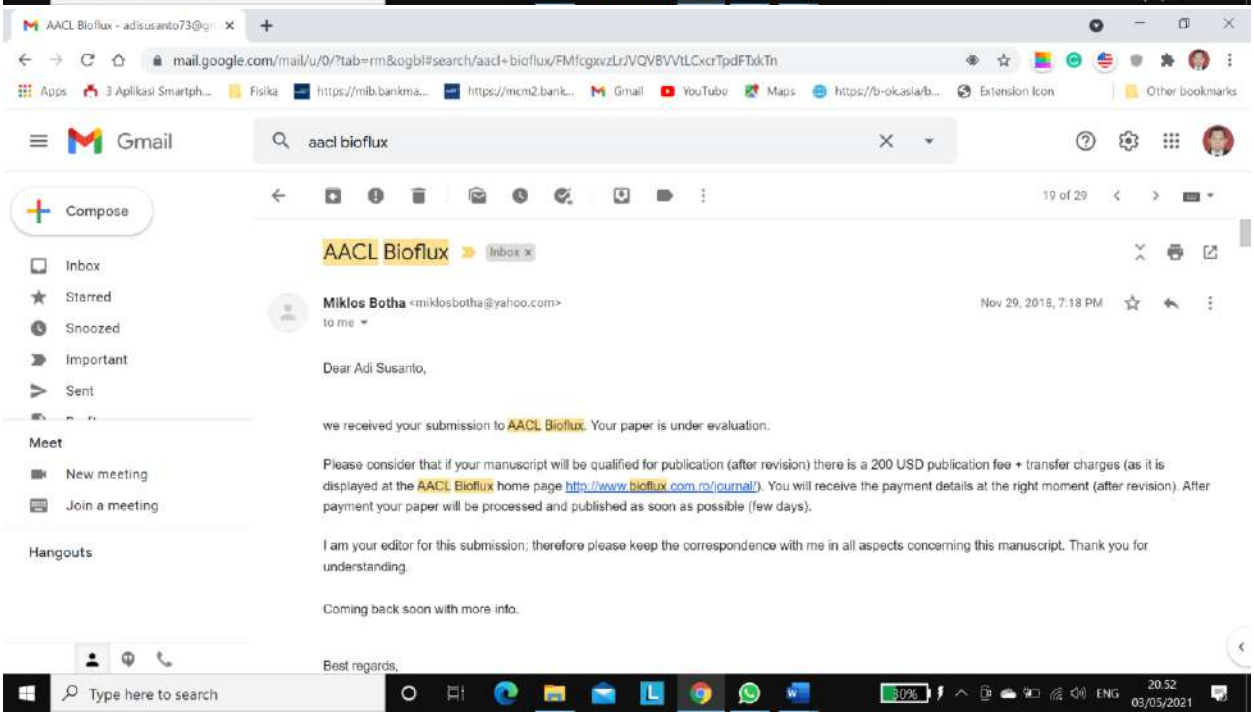
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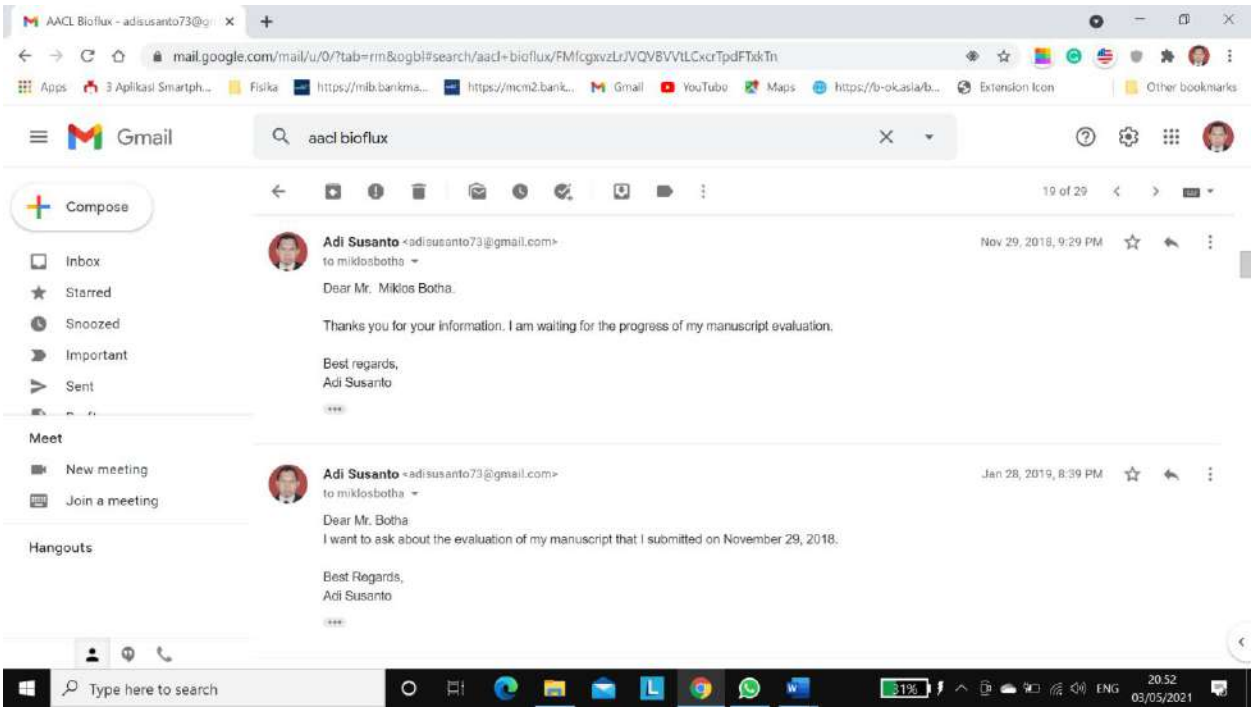
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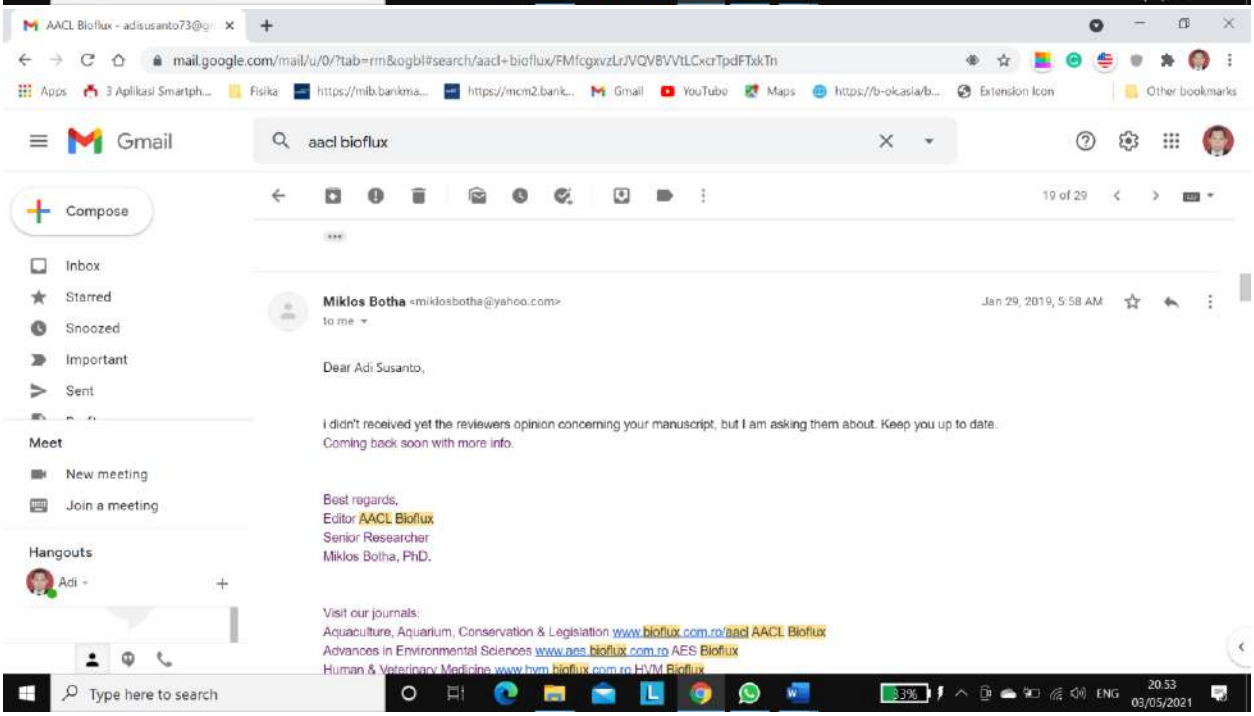
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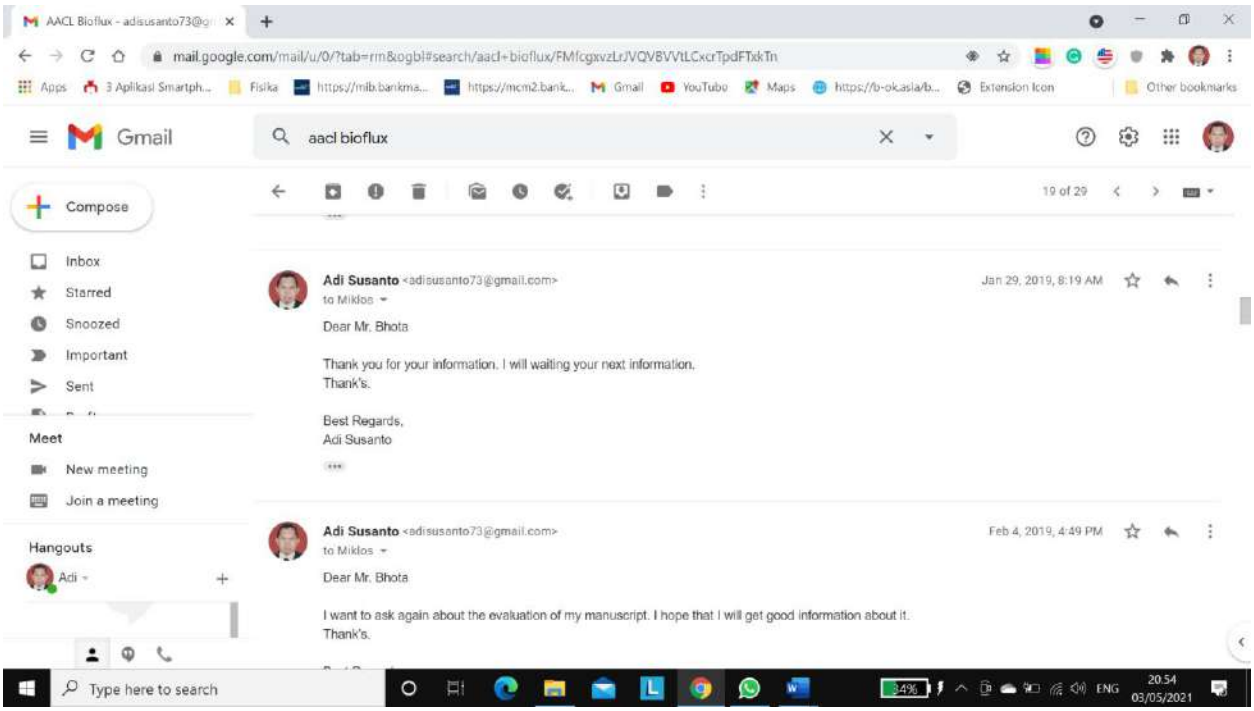
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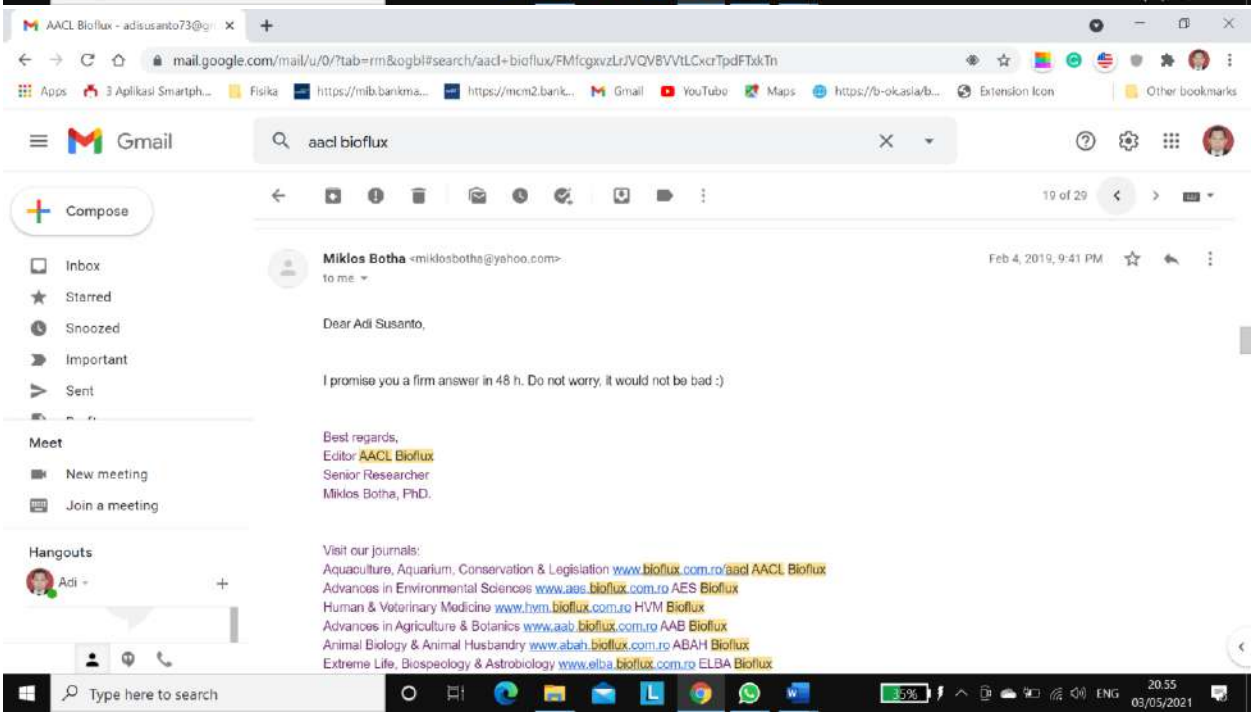
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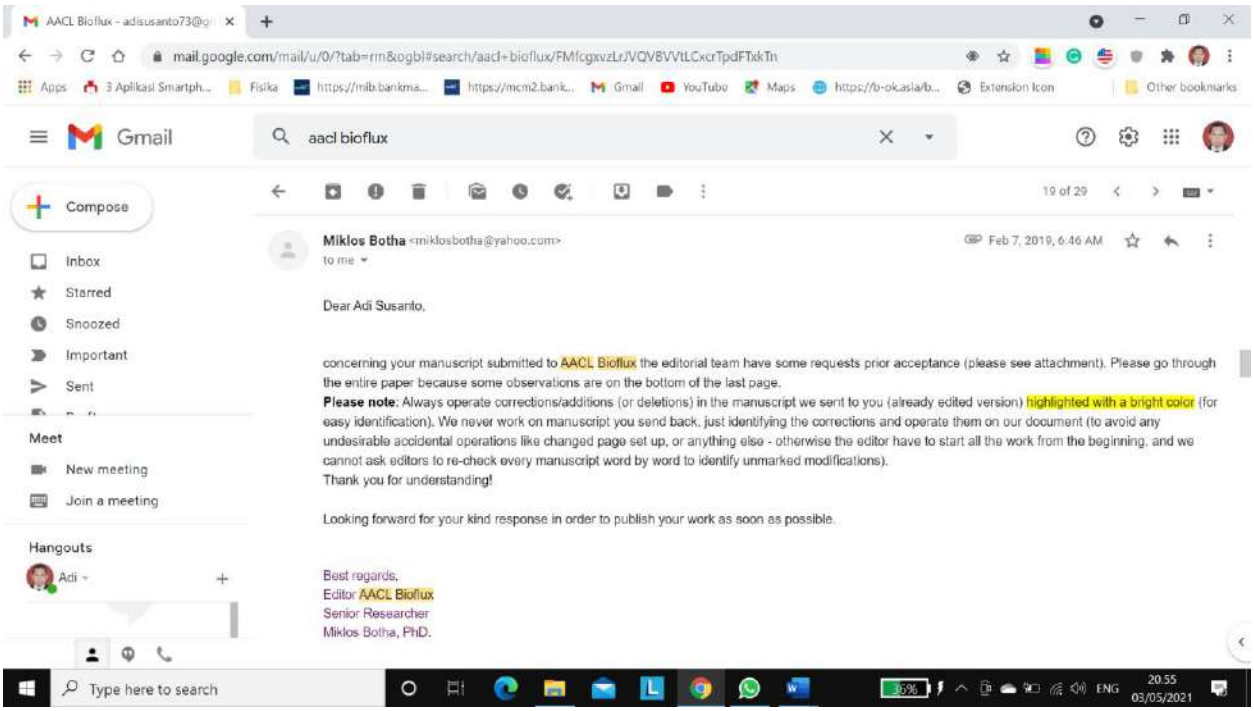
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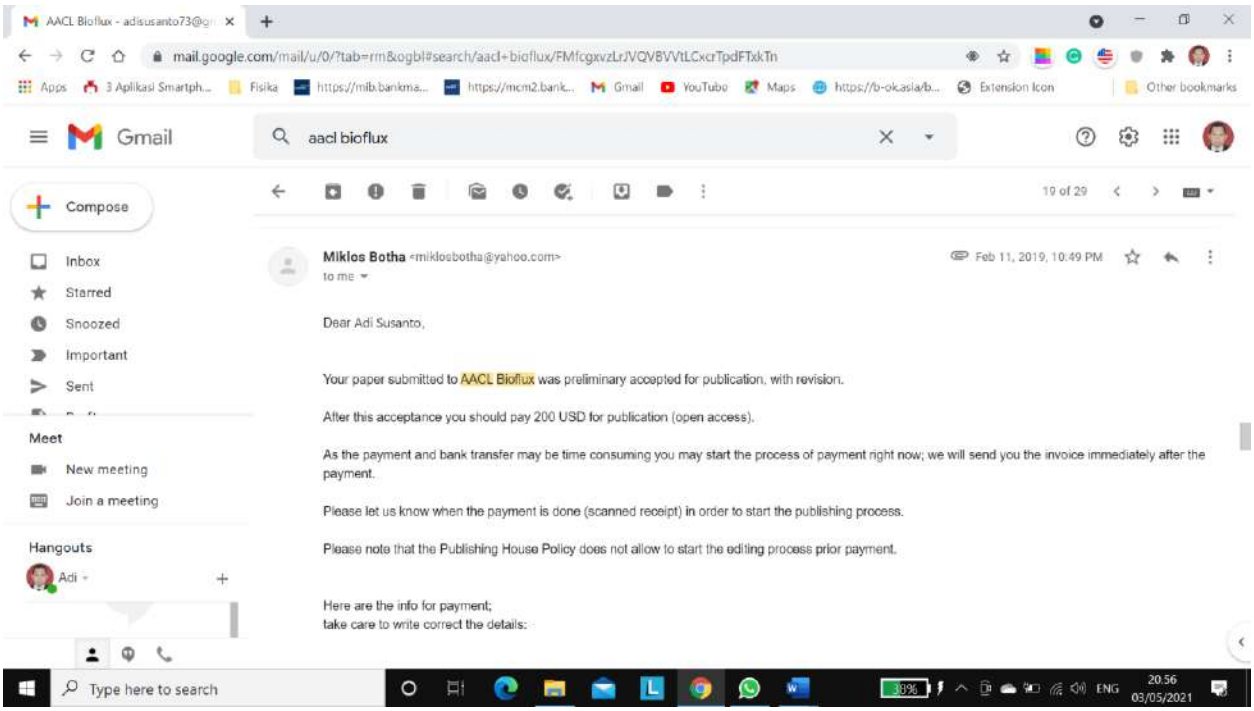
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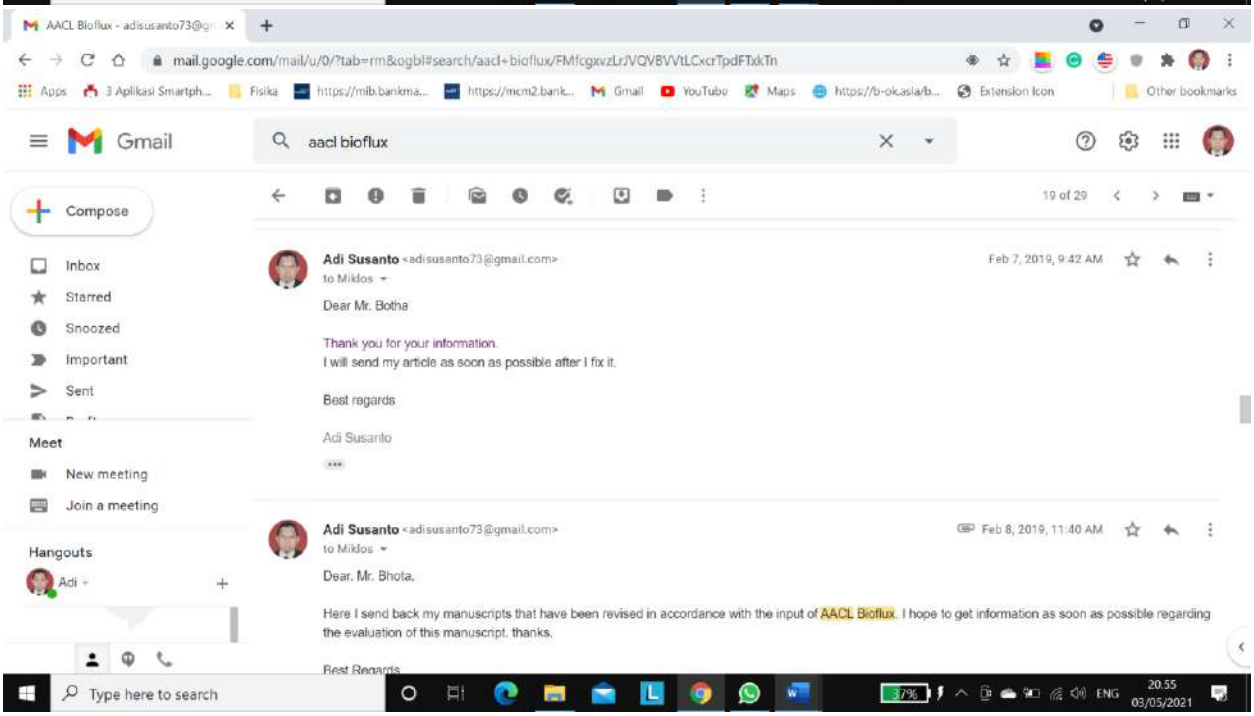
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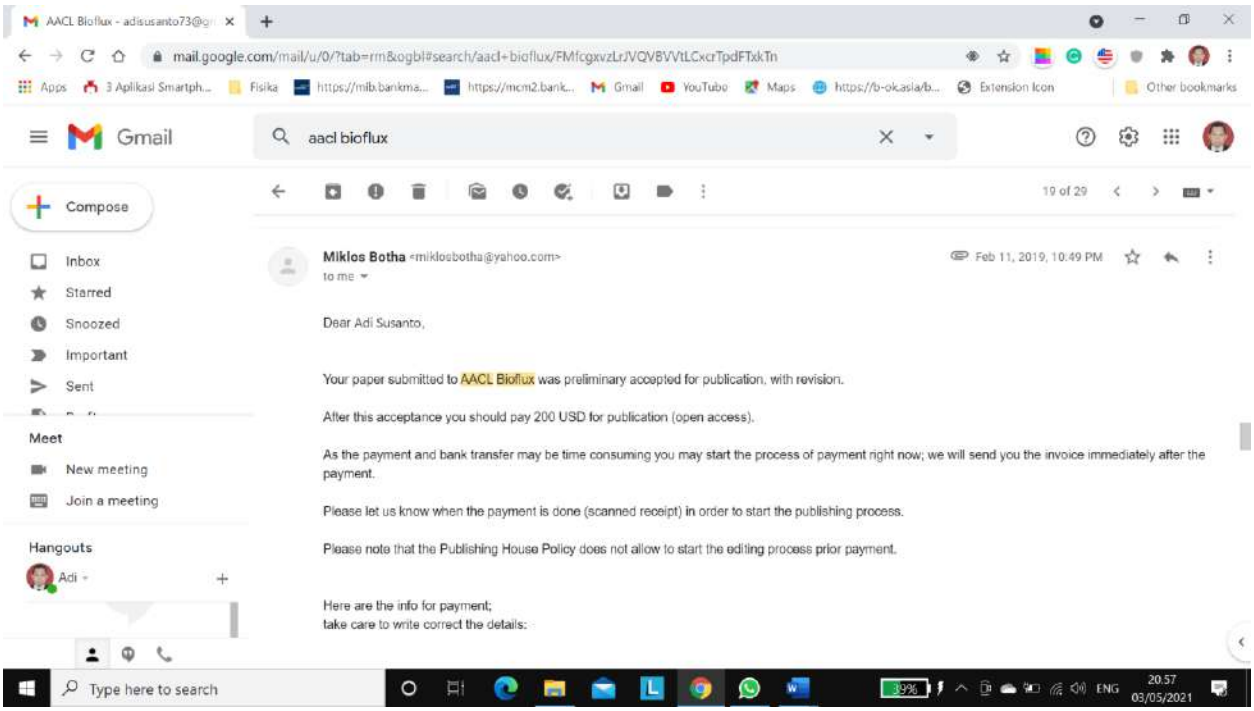
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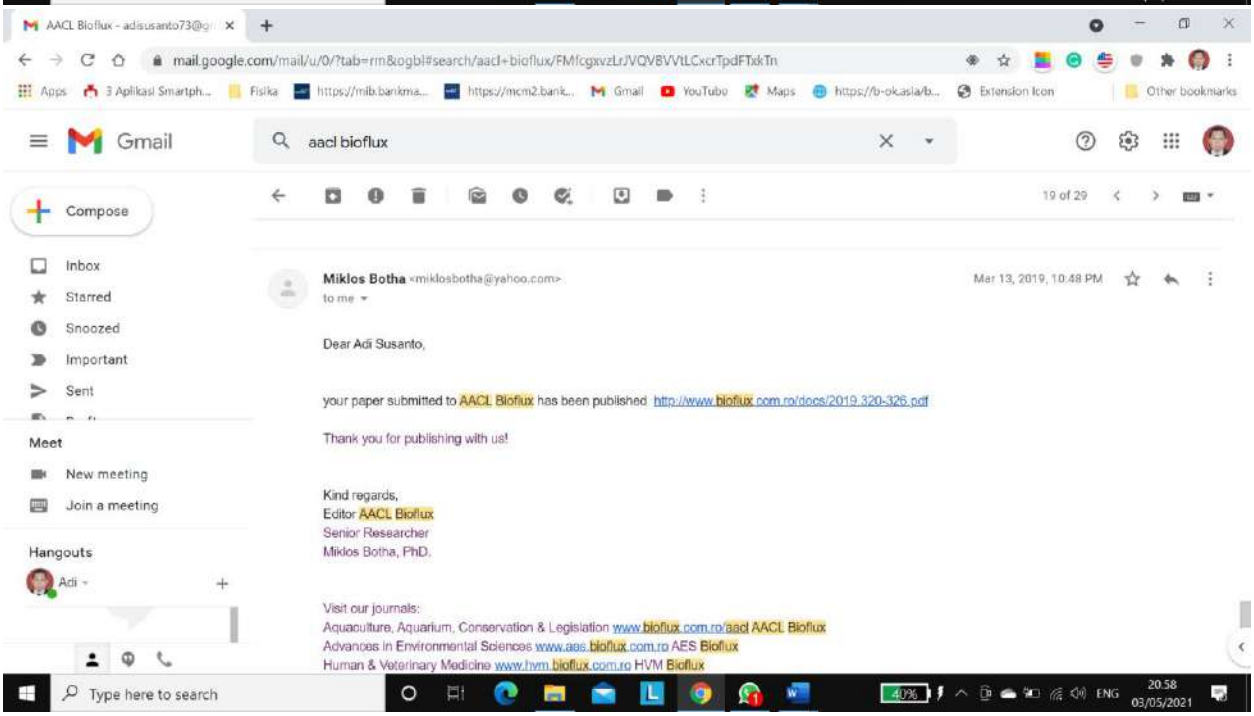
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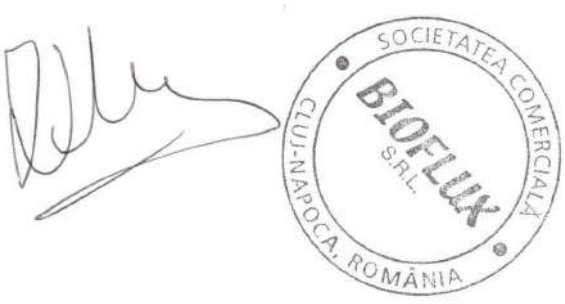

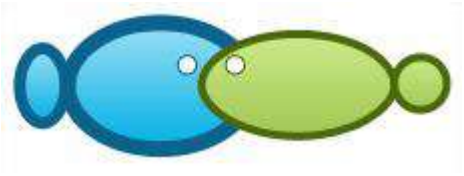

The effects of dietary protein level on the growth, protein efficiency ratio and body composition of juvenile kelabau (*Osteochilus melanopleurus*)

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Abstract. This study was conducted to evaluate the effect of feeding with different protein levels on growth performance and body composition of kelabau fish (*Osteochilus melanopleurus*). Four experimental feeds with different protein levels but same fat content were formulated. The experimental variants were A, B, C and D containing protein levels of 25.14, 28.26, 31.88 and 34.73% respectively. *O. melanopleurus* was obtained from breeding unit of the Freshwater Aquaculture Center (BBAT) Mandiangin within an average weight of 2.09±0.16 g with a density of 20 fish maintained in plastic box sized 54.3 x 38 x 31.5 cm and filled with 40 L of water. *O. melanopleurus* were given experimental feed 2 times a day at satiation for 60 days. *O. melanopleurus* feed C (31% protein) showed higher growth rate, relative growth rate, total feed consumed, feed utilization efficiency were higher compared to other treatments ($P<0.05$). The highest mean growth rate was in the C feed treatment (31%) with an average of 4.14±0.54%, while the lowest relative growth in the feed treatment A (25%) with an average of 2.92±0.54%. Protein efficiency ratio (PER) of fish group which consumed feed formulation C = 1.50±0.06%, feed A = 1.45±0.13%, feed B = 1.40±0.04% and significantly different from fish group fish that consumed feed D = 1.16±0.07%.
Key Words: relative growth rate, feed consumption level, feed efficiency, at satiation.

Introduction. Kelabau fish (*Osteochilus melanopleurus*) is a freshwater fish belonging to the order Cypriniformes, sub-order Cyprinoidae, Cyprinidae family, genus *Osteochilus* (Kottelat et al 1993). As a local fish with high economic value, lives in public waters found in Kalimantan and Sumatera unfortunately is still not cultured and the production is still limited depending on the fishing seasons. However, *O. melanopleurus* should be cultured like other species of Cyprinid fishes, and the culture should be improved by optimizing the maintenance of both the maintenance of culture system and the quality of the feed provided. Some researches of concerning feed formulation for marine species are in progress, but still only a limited scale. Mardani (2014) conducted a study of different food sources for the growth of *O. melanopleurus*. *O. melanopleurus* that consumed feed with a composition of 29.3% protein content provided the best relative growth (RGR) of 49.45% compared to other treatments.

Each fish species needs different levels of protein for its growth and it is influenced by fish size, but generally fish need is around 35-50% protein in their feed (Hepher 1990). Research on the need for protein in omnivorous fish has been widely carried out as in Nile tilapia (*Oreochromis niloticus*) by Teshima et al (1985) in Merola and Cantelmo (1987), in cachama (*Colossoma macropomum*) by Eckmann (1987), and in common carp (*Cyprinus carpio*) (Shimeno et al 1995), and also in herbivorous fish such as giant gourami (*Osphronemus goramy*) (Mokoginta et al 1995), putitor mahseer (*Tor putitora* (Hemilton, 1822)) (Hossain et al 2002), roho labeo (*Labeo rohita* (Hemilton, 1822)) (Satpathy et al 2003), silver barb (*Barbonymus gonionatus*) (Mohanta et al

2008); furthermore Mansour et al (2017) and Dewantoro et al (2018), on tinfoil barb (*Barbonymus schwanenfeldii* (Bleeker, 1854)).

Research on protein requirements in *O. melanopleurus* has never been done. Therefore research to find the right protein content to produce the best growth of *O. melanopleurus* is needed.

Material and Method

Diets. The present study used 4 types of artificial feed with different protein content, namely feed A (25%), feed B (28%), feed C (31%) and feed D (34%)) with a E/P ratio ranging from 8 to 10 Kcal. Feed formulations are presented in Table 1.

Table 1

Treatment feed composition (g) and feed nutrient content*

Ingredients	*Ingredient percentage in the trial feed (% dry matter)*			
	A (25 %)	*B (28 %)*	*C (31 %)*	*D (34 g %)*
Fish meal	29.0	34.0	37.0	39.3
Soybean meal	15.5	15.0	17.0	20.2
Wheat flour	6.5	10.0	12.3	13.0
Bran meal	15.0	12.0	10.0	10.2
Fish oil	2.5	2.5	2.5	2.5
Corn oil	2.5	2.5	2.5	2.5
Vitamin mix[2)]	3.0	3.0	3.0	3.0
Mineral mix[3)]	3.0	3.0	3.0	3.0
Choline chloride	2.0	2.0	2.0	2.0
CMC[1)]	2.0	2.0	2.0	2.0
Filler	19.0	14.0	8.7	2.3
Proximate analysis results				
Protein (%)	25.14	28.26	31.88	34.73
NFE (%)	34.34	31.75	30.53	31.84
FAT (%)	11.55	11.37	11.59	11.55
Fiber (%)	2.35	2.00	1.85	1.99
Total Energy (Kcal g^{-1})[4)]	267.40	270.38	281.78	294.71
E/P (Kcal g^{-1} Protein)	10.64	9.57	8.84	8.49

* - Calculation based on dry weight.
1 - Carboxymethil cellulose.
2 - In mg kg^{-1} : 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 pantotenat 100; inositol 2000; biotin 300; asam folat 15; niasin 400.
3 - in mg kg^{-1} : $MgSO_4.7H_20$ 7.5; NaCl 0.5;$NaH_2PO_4.2H_2O$ 12.5;KH_2PO_4 16.0; $CaHPO_4.2H_2O$ 6.53; Fe sitrat 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}; NFE = 2.5 Kcal g^{-1}; Fat = 8.1 Kcal g^{-1}.

Fish culture management. The *O. melanopleurus* were obtained from hatchery of Freshwater Aquaculture Center (BBAT), Mandiangin, South Kalimantan. *O. melanopleurus* were reared in plastic tank containing 40 L water with a density of 20 fish per tank with an average weight of 2.09±0.16 g. Fish were reared for 60 days by feeding twice a day in the morning and in the afternoon at satiation. The experiment was carried out in semi-closed circulation system. Faeces were removed by siphoning every morning. The water filter was washed every day and the filter tub was washed and replaced with new water every 1 week. During the study, the water temperature averaged 30.0±1.0°C, dissolved oxygen between 4.60 and 6.20 mg L^{-1}, pH between 6.70 and 6.80, TAN between 0.398 and 0.721 mg L^{-1}. This shows that the condition of the water during the study was at optimum conditions (Tebbut 1992; Effendie 1997).

Data collection and chemical analysis. Body weight was measured at the beginning and at the end of the study upon anesthetized fish. The fish were anesthetized using

MS222. Weighing was performed in order to determine the relative growth rate (De Silva & Anderson 1995). Feed consumed during the study was recorded to determine Total Feed Consumption (TFC) (Pereira et al 2007), and Feed Utilization Efficiency (FUE) (NRC 1993), 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 nutrient composition in fish (Takeuchi 1988).

Statistical analysis. The design of the present study is a laboratory experimental model, using a completely randomized design (CRD) consisting of 4 treatments and 4 replications. Data of Feed Utilization Efficiency (FUE), Weight Growth Rate, Specific Growth Rate (SGR), Relative Growth Rate (RGR) and Protein Efficiency Ratio (PER), were analyzed for diversity with ANOVA and continued with the Tukey test at a 95% confidence interval using the SPSS program version 11.5.

Results and Discussion

Weight growth, relative growth, total feed consumption and feed utilization efficiency. The values of various parameters of feed use which include weight growth rate, specific growth rate, relative growth rate, and protein efficiency ratio as well as feed efficiency of the fish after being kept for 60 days by feeding different proteins are presented in Table 2.

Table 2

The average value of the initial weight, final weight, weight growth, relative growth rate (RGR), specific growth rate (SGR), total feed consumption (TFC), protein efficiency ratio (PER) and feed utilization efficiency (FUE), of *Osteochilus melanopleurus* kept for 60 days fed different protein levels

Parameters	*Diet protein level*			
	A (25.0%)	*B (28.0%)*	*C (31.0%)*	*D (34.0%)*
Initial weight(g)	2.02±0.21	2.02±0.12	2.10±0.12	2.21±0.16
Final weight (g)	5.42±0.06	5.73±0.06	7.23±0.13	6.39±0.02
Weight growth (g)	68.00±4.52^{a}	74.30±1.81^{a}	102.48±3.78^{c}	83.77±2.93^{b}
RGR (%)	2.92±0.54^{a}	3.11±0.30^{a}	4.14±0.54^{b}	3.37±0.25^{a}
SGR (%)	1.68±0.20^{a}	1.75±0.10^{a}	2.07±0.15^{b}	1.84±0.08^{a}
TFC (%)	191.13±9.66^{a}	189.79±4.38^{a}	217.20±13.73^{b}	220.57±10.91^{b}
PER (%)	1.45±0.13^{b}	1.40±0.04^{b}	1.50±0.06^{b}	1.16±0.07^{a}
FUE (%)	34.36±7.68^{a}	34.86±3.57^{a}	54.44±5.64^{b}	43.77±3.68^{a}

Values followed by the same letters on the same row show no significant difference (P>0.05).

O. melanopleurus fed with different protein levels exhibited significant effect on weight growth, relative growth rate, specific growth rate, feed consumption level, protein efficiency ratio and feed efficiency (P<0.05). The best weight growth was obtained in the treatment of feed C (31.0%) followed by feed D (34.0%). The lowest weight growth was obtained in the group of fish that consumed feed A (25.0%) and B (28.0%) (P<0.05). The best relative growth rate was obtained in the group of fish fed experimental feed C (31.0%), with 4.07% day^{-1} which was significantly different from fish consuming other feed treatments (P<0.05). The same phenomenon was also seen in the specific growth rate, where the group of fish consuming C feed had the highest specific growth rate of 2.06% per day which was significantly different from the group of fish that consumed feed D, B and A (P<0.05).

O. melanopleurus which was fed experimental diets C and D showed higher levels of feed consumption compared to fish that were administered diets A and B (P<0.05). Fish, which consumed D feed, has the lowest protein efficiency ratio (PER) compared to fish that consume feed A, B and C (P<0.05). The best feed utilization efficiency (FUE) value was obtained for fish that consumed C feed which was 47.26±2.18% which was different from the group of fish that consumed feed B with a feed utilization efficiency

(FUE) value of 39.16±0.91% (P<0.05). Fish which consumed feed A produced a low value of feed utilization efficiency (FUE) with a feed utilization (FUE) efficiency value of 35.62±2.51%.

Initial and final proximate composition. The proximate composition of *O. melanopleurus* both at baseline and at the end of the study and after the fish reared for 60 days by feeding different protein content are presented in Table 3.

Protein levels of fish body at the end of the study tended to increase with increasing protein feed up to protein content 31.0% but did not significantly affect the protein content of fish bodies (P>0.05). Likewise, the body fat content of fish, ash content and NFE content, showed no significant difference (P>0.05).

Table 3

The initial and final body proximate composition of *Osteochilus melanopleurus* in a 60 days trial by giving feed containing different protein levels (% dry weight)

Diet	*Protein level (%)*			
	A (25.0)	*B (28.0)*	*C (31.0)*	*D (34.0)*
Initial body composition (%)				
Protein	56.42	56.42	56.42	56.42
Fat	23.75	23.75	23.75	23.75
Ash	13.37	13.37	13.37	13.37
NFE	6.03	6.03	6.03	6.03
Final body composition (%)				
Protein^{ns}	58.62±2.56	59.73±2.29	61.69±0.87	60.89±1.98
Fat^{ns}	20.78±1.41	21.51±2.65	21.77±2.94	19.83±1.03
Ash^{ns}	14.76±1.15	14.47±0.54	15.15±1.01	13.84±0.73
NFE^{ns}	2.19±0.80	2.25±0.47	1.77±0.32	2.51±1.28

ns - not significantly different (P> 0.05).

Discussion. Feeding with increased protein levels up to 31.0% will significantly improve the growth performance of *O. melanopleurus*, and will decrease with increasing protein levels to 34.0%. *O. melanopleurus* that consume feed with a protein content of 31.0% are able to utilize more efficiently the feed protein sources for growth compared to fish that consumed D feed which is higher in protein (34.0%). This shows that the protein consumed is not necessarily used for growth. In general, nutrition must be available first for metabolism, and second for growth and third for reproduction biology (Bray & Lawrence 1992). This means that, once the nutrients or energy needed for metabolism and growth are met, excess nutrients or energy will be stored or used for reproduction.

The group of fish that consumed C feed had a relatively high growth compared to other feed groups. Many authors have reported that an increase in protein levels will not support further growth and may even affect lowering growth due to insufficient energy availability (McGoogan & Gatlin 1999). This might explain that the proportion of protein will be degraded, where the carbon skeleton is used as an energy source at high dietary protein levels. Excreted ammonia nitrogen causes damage to water quality, so an increase in food protein levels above a certain level, does not increase production so it must be avoided (El-Sayed & Kawanna 2008).

Based on growth and feed efficiency, the optimum level of feed protein for *O. melanopleurus* was 31.0%. These results are lower than those reported in other fish, such as *Oncorhynchus nerka* (45.0%) (NRC 1993), masu salmon (*Oncorhynchus masou* (Brevoort, 1856)) (40.0%) (Lee & Kim 2001) and Atlantic salmon (*Salmo salar*) (39.0%) (Hillestad & Johnsen 1994), and Pacific salmon *Oncorhynchus* sp. (55.0%) (NRC 2011). The optimum diet protein level of 31.0% in this study was also lower than the 48.0% protein diet value with 20.0 ~ 25.0% lipids for Manchurian trout larvae (0.15 g) reported by Zhang et al (2009). The difference between the two studies can be explained by different fish sizes. Evidence shows that protein requirements decrease with increasing fish size (NRC 2011).

Higher yields also found in fingerling/juvenile *O. niloticus* requires 35.0% protein (Abdel-Tawwab et al 2010), (*O. goramy*) measuring 0.27 g requires 43.29% protein with an energy protein (C/P) ratio of 8 kcal DE/g (Mokoginta et al 1995). Hossain et al (2002), stating that the *T. putitora* grows well on feed containing 40% protein. *L. rohita* with an average weight of 4.3±0.02 g which consumes feed with a protein content of 45.0% and fat content of 10-15% gives the best growth (Satpathy et al 2003). Similar results were found in *C. carpio* a 121 g individual requires 31.6% protein and 11.9% fat (Shimeno et al 1995). *B. gonionatus* grows well at a protein content of 31.77% with a P/E ratio of 11.32 g of Kcal g^{-1} protein (Mohanta et al 2008). Furthermore Mansour et al (2017), showed that the best growth of *B. schwanenfeldii* was fed with 32% protein content. Dewantoro et al (2018) obtained lower protein requirements of 30% and C/P ratio of 10 Kcal/g protein which was able to increase specific growth rate (SGR), protein retention and fat retention in the same species.

The value of feed utilization efficiency (FUE) is the ratio between body weight gain and the amount of feed consumed. In the group of fish that consumed C food had the highest efficiency value 47.26±2.18%, compared to the group of fish that consumed feed B (39.16±0.91%), D (38.08±2.18%) and A (35.62±2.91%). The group of fish that consumed C food had a higher weight gain while the food consumed was less than the fish in group D (Table 2). Protein efficiency ratio (PER) group of fish that consumed feed C = 1.50±0.06%, feed A = 1.45±0.13%, feed B = 1.40±0.04% and better than in group fish that consumed feed D = 1.16±0.07%. This illustrates that the protein consumed by fish in group C can be used efficiently to increase growth compared to other fish group.

Further research should be conducted to determine the optimal carbohydrate levels so that *O. melanopleurus* growth could be maximized.

Conclusions. *O. melanopleurus* that consumed 31.88% of protein-containing feed provided an excellent weight growth rate, relative and specific growth rate, protein efficiency ratio, total feed consumption and realized the best feed utilization efficiency compared to other treatments.

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