EFFECTS OF COMMERCIAL FEEDING WITH DIFFERENT PROTEINS ON THE GROWTH OF G6 TRANSGENIC MUTIARA CATFISH IN BUDIKDAMBER REARING

ABSTRACT

This research was conducted in the Hatchery Building 4 Faculty of Fisheries and Marine Science, Padjadjaran University. The implementation started from July to September 2023. The purpose of this study was to test the growth performance of G6 transgenic mutiara catfish by feeding commercial feed containing different proteins to induce growth rate and protein use efficiency. The treatment design used the complete randomized design (CRD) method with four treatments (A: 39% feed protein; B: 32% feed protein; C: 20% feed protein; and D: 37% feed protein (mixture of pindangtongkol and PF.1000)) with three replications. Data were analysed using Sigmaplot 15.0 for absolute weight gain (Wg), feed conversion ratio (FCR), protein retention (RP), and protein productive value (PPV) and using Duncan Multiple Range Test (DMRT) with 95% confidence level. The results showed that treatment A gave the best results on the value of Wg (average weight gain of 4619 g ± 344.3 g) and the lowest FCR (0.63 ± 0.058), and the best protein retention and PPV values were shown in treatment B, as follows 19.24% and 17.4%.

Keywords:Transgenic Fish, Feed Protein, WG, FCR, RP, PPV

1. INTRODUCTION

Catfish is one of the carnivorous freshwater fish. Carnivorous species basically require feed with high protein content [1]. Protein is the most expensive component in most fish feeds and accounts for as much as 40-70% of the cultivation process [2], The development of catfish farming in recent years, has been intensified by increasing stocking densities and feeding rates [3]. The main biological function of feed in fish as a source of energy used for growth, activity, and reproduction. Therefore, the feed given must fulfil the standard energy requirements for fish such as protein, fat, and carbohydrates [4]. Most fish will use energy derived from protein as basic needs and growth [5].

Optimal dietary protein requirements for several catfish species have been determined at different growth stages and under various conditions. Protein is one of the main substances that are essential for achieving optimal growth [6]. In addition, protein is also the main source of energy for fish [7]. Growth is a complex process and involves a number of hormones such as growth hormone. A comprehensive approach is needed to improve growth that involves various factors, both endogenous and exogenous factors, including genetic factors, nutrition, hormones, cultivation techniques, pests and diseases and the environment [8].

Fish growth is also influenced by genetic factors, which are genes that come from the fish itself which will describe the growth rate of fish. Each fish has a growth hormone that functions to spur the process of protein synthesis [9]. Therefore, it is necessary to conduct a study that tests the acceleration of growth of transgenic catfish to reach the size of consumption using commercial feed with different protein content. The best growth will be determined by the value of Wg, FCR, RP and PPV in the growth test.

2. MATERIALSANDMETHODS

This research was conducted from July to September 2023, at the Hatchery Building 4, Faculty of Fisheries and Marine Science, Padjadjaran University. The materials used in this study were G6 transgenic mutiara catfish fingerlings measuring 8.5-10.5 cm (45 days old), using commercial feed (Prima Feed-1000 with a protein content of 39%, Hi ProVite 781-2 with a protein content of 32%, Turbo 78-3A with a protein content of 20%, and a mixed feed of pindangtongkol and PF-1000 with a protein content of 37%).

2.1 Research Design

The study was conducted experimentally with four treatments and three replications, the treatments given were: Prima Feed 1000 commercial feed with 39% protein (treatment A), Hi ProVite 781-2 commercial feed with 32% protein (treatment B), Turbo 78-3A commercial feed with 20% protein (treatment C), and mixed feed of pindangtongkol and Prima Feed 1000 with 37% protein (treatment D). Feed was given as much as 5% of the total biomass weight and given twice a day. If the treatment is significantly different, at the 95% confidence level it is continued by conducting Duncan's Multiple Range Test (DMRT) analysis. The research design used was a completely randomised design (CRD).

2.1.1 Preparation for implementation

Fill each bucket with a water capacity of 60 L, followed by setting up an aeration installation and heating installation at 28 °C, then put 60 test fish into each bucket of each treatment that has been labeled, covered with a bucket cover.

2.1.2 Implementation of research

The research was conducted for 56 days and observations were made every 7 days, each bucket was filled with 60 fish and initial weight measurements were taken before being put into the bucket, feeding was done twice a day at 08.00 and 16.00 WIB. Water quality measurements were taken every day including pH, temperature, and DO (dissolved oxygen) parameters. Parameters observed to assess growth performance and feed efficiency were as follows:

a. Absolute Weight Gain

Absolute growth is measured by calculating the body weight of the fish and measuring the length of the fish body every 7 days, the calculation of absolute growth is done using the absolute weight gain formula [10].

$$\Delta W = Wt - W_0$$

ΔW = Growth in absolute weight (g)
Wt = Fish weight at the end of rearing (g)
W_0 = Initial fish weight rearing (g)

b. Feed Conversion Ratio (FCR)

Feed conversion ratio is measured by calculating the amount of feed consumption during maintenance divided by the total weight gain at the end of the study (if there is a dead weight added to the final weight) using the feed conversion ratio formula, namely [11].

$$FCR = \frac{F}{(Wt + D) - Wo}$$

FCR = Feed Conversion Ratio
F = Amount of food eaten by fish
Wt = Final weight of fish (g)
IN_0 = initial weight of fish (g)
D = Weight of dead fish (g)

c.Protein retention

Protein retention can be known by analyzing the proximate protein of the fish body at the beginning and end of maintenance and divided by the amount of protein consumed during maintenance. The formula for protein retention is as follows [12] :

$$RP = \frac{(Fp - Lp)}{P} \text{ x } 100\%$$

PR = Protein retention
Fp =Amount of fish body protein at the beginning of rearing (g)
Lp =Amount of body protein at the end of maintenance (g)
P =Total protein consumption during rearing

d. Protein Productive Value

Protein productive value (PPV) is also known as protein utilization efficiency [13]. protein productive value is used to evaluate the protein in feed by the ratio between the protein retained in the fish tissue and the dietary protein fed. PPV is determined by carcass analysis of fish samples taken before and after feeding with the evaluated protein, and is generally expressed as the percentage of protein retained from the feed given. The protein productive value formula is as follows [14]:

$$PPV = \frac{((\bar{x}Wt \; x \; Fp) - (\bar{x}W0 \; x \; Ip))}{Ft} \; x \; Mix \; Protein \; (\%)$$

PPV =Protein Productive Value (%)
$\bar{x}W_0$ = Initial fish weight means(g)
$\bar{x}Wt$ = Fish weight final means(g)
Ip = Amount of fish body protein at the beginning of rearing (g)
Fp = Amount of body protein at the end of maintenance (g)
Ft = Total Feed
Mix Protein =Amount of mixed protein

e.Water Quality

During maintenance, water quality measurements were taken, namely acidity (pH), dissolved oxygen (DO) and temperature. These measurements were taken every day.

2.2 Data Analysis

The data obtained were analyzed with Analysis Of Variance (ANOVA) with a confidence level of 95% and if there is a significant difference, it will be continued with the Duncan Multiple Range Test (DMRT) using Sigmaplot 15.0 software

3. RESULTS AND DISCUSSION

3.1 Absolute Weight Gain

Absolute weight gain was significantly different between the treatments which represents the final biomass weight and the initial biomass due to the provision of different prtoein. The value of absolute weight gain(Figure 1).

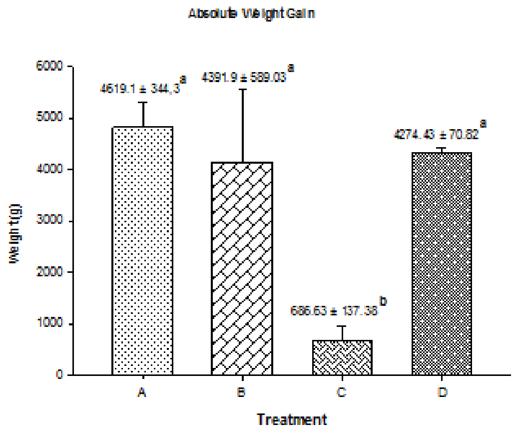

Figure 1. Absolute weight gain. A (39% feed protein); B (32% feed protein); C (20% feed protein); D (37% mixed feed protein).

The results of the analysis showed that the absolute weight gain of G6 transgenic Mutiara catfish fingerlings reared for 56 days increased in each treatment between 686.63 and 4619.1 g (Figure 1). The highest absolute weight gain value was in treatment A at 4619.1 g (39% feed protein), followed by treatment B with a value of 4391.9 g (32% feed protein), Treatment D with a value of 4274.4 g (37% feed protein). ; a mixture of pindangtongkol 35% and commercial feed 65%), and treatment C with a value of 686.63 g (feed protein 20%).

.

Table 1. Growth of G6 transgenic mutiara catfish (weight gain)which conducted during the 56-day rearing.

Treatment	Initial Biomass (g)	Initial Fish Size	Final Biomass (g)	Final Fish Size
A	483.57		5102.67	
B	507.83		4899.73	
C	436.03		1122.67	
D	390.57		4665.00	

Description: Weight gain of transgenic G6 mutiara catfish during 56 days of rearing. Treatment A (feed protein 39%); B (feed protein 32%); C (feed protein 20%); D (mixed feed protein pindangtongkol and PF.1000 (37%))

Fish growth is influenced by the level of feed protein content and complete nutrition including the availability of essential amino acids that are important for fish growth [15]. The absolute weight gain rate is obtained by calculating the total fish biomass at the end of rearing minus the total fish biomass at the beginning of rearing expressed in grams. According to the data presented in (Table.1), an observable escalation in fish biomass weight is evident from the initial stagesand end during the study in each treatment. In treatment A, the increase was quite high, namely (483.57 g to 5102.67 g); Treatment B (507.83 g to 4899.73 g); (treatment C of 436.03 to 1122.67); and treatment D (390.57 g to 4665 g). The weight gain data presented in (Table.1) reveals a notable increase in absolute weight gain, and this can be attributed to the elevated protein content in the feed. This increase in protein content is identified as a contributing factor to the enhanced growth observed in G6 transgenic Mutiara catfish. Effective and optimal utilisation of feed will make the feed consumed by the fish is actually utilised as the intake of fish nutrients needed for its growth [16].It can be seen in (table.1) that transgenic fish fed with 39% protein content can optimise protein intake in feed and can maximise fish growth rate. When compared to feeding with 20% protein content, fish tend to grow slowly and are less able to optimise the intake of feed nutrients used for growth.

The presence of the CgGH hormone insert promotes increased growth of transgenic catfish. fed with test feed with protein content >30% showed significant growth compared to test fish fed with 20% protein content. High protein content in the feed promotes increased expression of the CgGH gene which stimulates IGF-I production. The high growth performance in transgenic fish is a compensation for the presence of exogenous GH (CgGH) that activates increased feed protein synthesis which is converted into increased growth of transgenic fish [17]. Research results on transgenic coho salmon have a higher ability to use carbohydrates as energy to store proteins and lipids, thus supporting higher growth [18]. The induction of increased growth in CgGH transgenic catfish is mainly due to increased expression of exogenous GH, which stimulates IGF-1 production in the liver. Increased GH secretion plays an important role in stimulating fish growth, improving feed conversion, and increasing protein utilisation efficiency. This increased protein use efficiency favours faster growth. Growth hormones, including transgenic GH, also function to increase amino acid uptake, especially essential amino acids that cannot be intrinsically synthesised in the fish body [19].

3.2 Feed Conversion Ratio (FCR)

The higher feed protein content will affect the FCR value, where treatments A (39% protein), B (32% protein) and D (37%) show the best feed efficiency value (lower FCR value which is less than 1) while low feed protein of 20% shows feed efficiency value of more than 1.5 indicating inefficient fish in utilising feed for growth. FCR value in each treatment can be beseen in Figure 2.

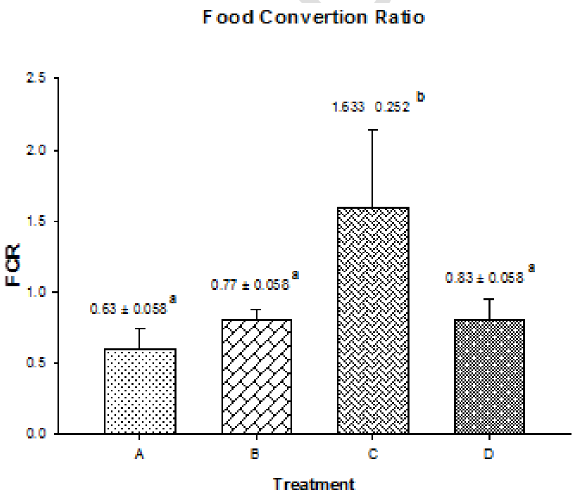

Figure 2. Feed Conversion Ratio. Treatment A (feed protein 39%); B (feed protein 32%); C (feed protein 20%); and D (mixed feed protein 37%)

Food convertion ratio is a parameter to determine and measure how efficiently the feed given can be converted into growth. There is obtained by knowing the amount of feed given divided by the final weight of the fish plus the amount of dead fish weight during maintenance minus the weight of the fish at the beginning of maintenance. The observation data listed in Figure 2 shows that there is a significant difference in the feed conversion ratio of each treatment (P<0,05). The value of the feed conversion ratio of transgenic mutiara catfish treatment (C) of 1.63 is significantly different from treatment A of 0.63, B of 0.77, and treatment D of 0.83. The results of the analysis showed that the lowest FCR value was obtained in treatment A which was 0.63, while the highest FCR value was found in treatment C which was 1.63. The lower the feed conversion ratio value obtained, the better the optimisation of the feed utilisation given.

The high FCR value is due to the feed used in treatment C is a feed with low protein (20% protein), while the optimal fish need for protein to meet the normal growth of catfish is 30 - 36% [20]. The lower the feed conversion ratio value illustrates that the quality of the feed provided is getting better, so that it will have an impact on the efficiency of feed utilisation [21]. This is inseparable from the addition of hormone inserts in transgenic fish which have an impact on the feed consumed by fish. Exogenous GH can affect lipolysis and gluconeogenesis. [22], GH also has an effect in promoting protein synthesis and converting lipids into proteins. As a result, transgenic fish treated with high protein feed have a greater ability to convert feed protein into growth (body weight gain). As a consequence, the weight gain of the fish is greater than the amount of feed consumed and leads to a low FCR value (high feed efficiency value).

In the previous study showed that the FCR value of G5 transgenic mutiara catfish showed (average 1.25-2.27), while the feed conversion value of G6 transgenic mutiara catfish showed a value (average 0.63-1.63) these results showed that G6 transgenic fish experienced better feed conversion ratio development and were more optimal when compared to previous generations. This is influenced by the expression of GH transgenic fish can increase the efficiency of feed use, in accordance with research on transgenic salmon showing that GH overexpression can generally increase fish growth, increase feed use efficiency, and increase body protein [23].

3.3 Protein Retention

Protein retention represent the protein stored in the body which is measured based on the average initial weight which shows the individual weight in each treatment will affect the results of protein retention carried out, High and low individual weight of fish at the beginning of maintenance will affect the value of protein retention obtained, it can be seen in treatment B that the value of protein retention is higher when compared to other treatments. The value of protein retention in each treatment showed in Figure 3.

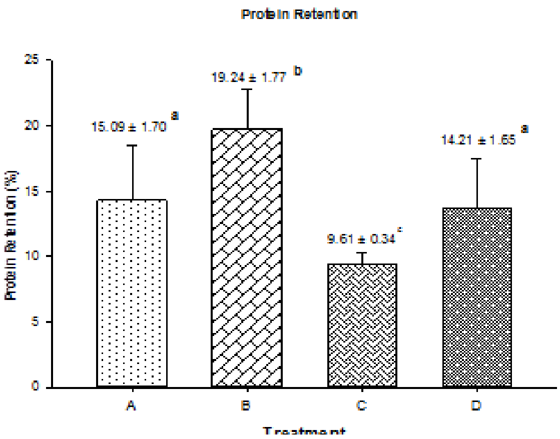

Figure 3. Protein retention. Treatment A (39% feed protein); B (32% feed protein); C (20% feed protein); and D (37% mixed feed protein)

The protein retention shows the percentage of protein weight stored by the body. As the results of the Duncan's Multiple Range Test (DMRT), treatment B exhibited the highest protein retention value at 19.24%, whereas treatment C displayed the lowest retention value at 9.61%.The findings suggest that treatment B (32%) demonstrates a more efficient utilization of protein. This indicates that the higher the protein retention value obtained, it shows the percentage of protein that is stored and made into new tissues by fish during maintenance [24].

The low value of protein retention in treatment C is due to the low level of protein contained in the feed. Previous research stated that the retention value of feed protein is determined by the source of protein used in the feed and is closely related to the quality of protein which is determined by the composition of amino acids and fish needs for these amino acids [25]. The presence of non-protein energy (carbohydrate and fat) reduces the breakdown of protein into energy (protein sparring effect) so that the value of protein retention increases and ammonia excretion decreases. Exogenous hormone administration can increase protein retention. The value of protein retention in transgenic fish given exogenous GH inserts shows a higher value compared to nontransgenic, it can be seen that the provision of exogenous GH inserts can increase the utilisation of non-protein nutrients as a source of energy (protein sparring effect) [26].

Protein retention is used as one of the parameters that can describe the effectiveness of the use of feed protein to be converted into growth and obtained by analysing the protein content of fish at the beginning and end of the study [27].Through the conducted calculations, it is evident that the diminished protein retention in treatment C can be attributed to the feed protein content falling below 20%. This indication illustrates that the feed protein absorbed by the fish is lower than treatments A (39% feed protein), B (32% feed protein) and D (37% mixed feed protein). Previous research indicates that feeds with low protein content lead to a deficiency in the protein acquired by fish from their diet, prompting a tendency for fish to convert non-protein nutritional components, such as carbohydrates and fats, into protein. [28]. Increased feed efficiency in transgenic mutiara catfish leads to an increase in final biomass weight. This effect represents the relationship between CgGH overexpression and protein, where exogenous GH can convert feed fat and carbohydrates into metabolic energy sources to replace protein (protein sparring effect) [29]. Therefore, feed protein can be optimally utilised to promote the growth of transgenic fish. The indication of final biomass weight gain was associated with a significant increase in body protein content and a decrease in FCR values in transgenic fish compared to non-transgenic fish. The positive effect of GH transgenesis is related to the increase in body protein content of fish resulting from the conversion of non-protein feed nutrients into protein (protein sparing effect).

3.4 Protein Productive Value

Protein productive value is shown to evaluate how efficiently the protein contained in feed is used for growth. It can be seen that in each treatment the ppv value does not differ much from the protein retention value, which means that the ability of the fish to utilize protein used for growth is not only viewed from the protein retention value, but an evaluation of the ppv value calculation is carried out. It was proven that treatments A and D showed relatively similar PPV values, but this was not the case in treatment B, which shows that high protein retention values do not always cause an increase in PPV values. The protein productive values of each treatment can be seen in Figure 4.

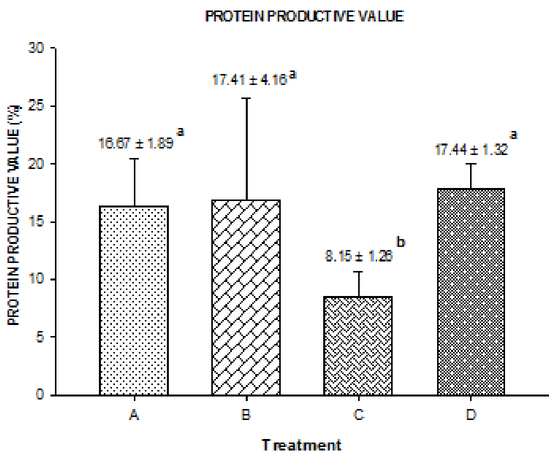

Figure4.Protein Productive Value. Treatment A (feed protein 39%); B (feed protein 32%); C (feed protein 20%); D (mixed feed protein 37%)

Protein Productive Value is the ratio of fish body protein at the end of maintenance to feed protein consumed to be utilised for growth [30]. The average protein productive value in all treatments ranged from 8.15-17.44% (Figure 4).The analysis test showed that G6 transgenic mutiara catfish in treatment C had the lowest protein productive value of 8.15% with 20% feed protein and the highest in treatment D worth 17.44% with 37% feed protein. Meanwhile, the PPV value of G6 transgenic Mutiara catfish in treatment A with 39% feed protein and treatment B with 32% feed protein were 16.67% and 17.41%, respectively.

The PPV value for G3 transgenic mutiara catfish is 6.94-12.3%, while for non-transgenic catfish it is 4.39% [31] and for transgenic tilapia 15-20% [32]. In G6 transgenic mutiara catfish, there was an increase in PPV value (8.15-17.44%) when compared to G3 transgenic mutiara catfish. This increase in PPV is thought to be related to increased expression of the CgGH gene, which is associated with the promotion of protein synthesis. This indication is reinforced by the results of a previous study that exogenous GH induces glycolytic enzymes in transgenic coho salmon, which causes catabolism of carbohydrates and lipids to produce metabolic energy and leads to increased protein storage in the body [33].

The higher the PPV value, the more efficient the fish is in using protein for growth. show in Figure 4 that the higher the protein content in the feed, the more efficient the fish's ability to convert protein in the feed into body protein. The results of research on asela fish also show that the percentage of PPV will increase with increasing protein in the feed [34].

3.5 Water Quality

Water quality plays an important role in supporting the survival of catfish. Water quality measured for 56 days in the study were temperature, pH, and dissolved oxygen (DO) levels. The average value of water quality can be seen in Table 2.

Table 2.Water Quality

Water Quality	Observation Results
Suhu (^{o}C)	28 – 30
pH	6,5 – 8,0
DO (mg/L)	3,6 – 5,4

Water quality affects the growth process of fish. When the temperature increases, it will increase the appetite of the fish. However, an increase in temperature that is too high will also cause an increase in ammonia toxicity. When ammonia increases, dissolved oxygen levels become low, the presence of ammonia in water can also cause reduced oxygen binding capacity by blood grains, this will cause fish appetite to decrease and inhibit fish growth [35]..

4 CONCLUSION

The use of different commercial feeds has a significant effect on the analytical tests carried out, the higher the protein value contained in the feed will make a difference to the value of the analytical tests carried out. It is proven that the best treatment for the growth of G6 transgenic mutiara catfish is obtained at high protein, namely treatment A shows the best results for biomass weight gain, protein retention and the best ppv.

REFERENCES

1. Wee K.L. & Tacon A.G.J. A preliminary study on the dietary protein requirement of juvenile snakehead. Bulletin of the Japanese Society of Scientific Fisheries. 1982; 48(1463-1470).
2. Pathmasothy S. A review of feed, their processing and feeding techniques in Malaysian aquaculture system. In: Proceedings of the international Conference on Development and Management of Dopical Living Aquatic Resources (ed. by H.H. Chan. K.J. Ang. H.M. Ibrahim & M.A. Ambak). pp. 126-131. UniversitiPertanian Malaysia. Serdang. Selangor. Malaysia;1983.
3. Li M. Effect of dietary protein content on weight gain of channel eatfish stocked intensively in earthen ponds. MS thesis. Auburn University. Auburn. AL.1989; 48 pp.
4. Mudjiman, A. MakananIkanEdisiRevisi, PenebarSwadaya. 2004. Depok.
5. National Research Council. Nutrient RequirmentsOf Warm water Fishes and Shellfishes. National Academy Press.1983; Washington DC.
6. Cech, J.J and Moyle, P.B., Fishes: an introduction to ichthyology. PrenticeHall; 2000.
7. Halver, J.E. Fish Nutrition. Second Edition. Academic Press Inc. 1989; California.
8. Pillay, T.V.R. and M.N. Kutty. Aquaculture: Principles and Practices. Second Edition. USA: Blackwell Publishing Ltd;2005.
9. Widiyanto, W. Latihan Dan SekresiHormonPertumbuhan. Medikora, 3(2), 156391
10. Hidayat, D., &Sasanti, A. D. Kelangsunganhidup, pertumbuhandanefisiensipakanikangabus*(channastriata)* yang diberipakanberbahanbakutepungkeong mas *(pomaceasp)*. Jurnalakuakulturrawa Indonesia. 2013; 1(2), 161-172.
11. Djarijah, A.S.PakanAlami. Kanisius, Yogyakarta. 1995; 87 hlm.
12. Takeuchi, T. Laboratory work chemical evaluation of dietary nutrients.p.179-233. In: Watanabe, T. (Ed). Fish nutrition and maricultureJICA.The general aquaculture course. Kanagawa international fisheries training centre. Japan International

Cooperation Agency (JICA). 1988; 23 pp. Tokyo.
13. Gerking, S.D. Influence of rate of feeding and body weight on protein metabolism of bluegill sunfish. Physiological Zoology. 1971; 44:9–19.
14. Buwono, I. D., I. Iskandar dan R. Grandiosa. Growth Hormone Transgenesis and Feed Composition Influence Growth and Protein Amino Acid Content in Transgenic G3 Mutiara Catfish (*Clariasgariepinus*). *Aquaculture International*. 2021; 29: 431-451.
15. Li, M. H., Bruce B. M. and Robinson, E. H. Effect of Daily Feed Intake on Feed Efficiency of Juvenile Channel Catfish, North American Journal of Aquaculture. 2004; 66:2, 100-104.
16. Ihsanudin, I., S. Rejekidan T. Yuniarti. PengaruhPemberianRekombinanHormonPertumbuhan (rGH) melaluiMetode Oral dengan Interval Waktu yang BerbedaterhadapPertumbuhandanKelulushidupan BenihIkanNilaLarasati (Oreochromisniloticus). Journal of Aquaculture Management and Technology.2014;3(2); 94-102.
17. Raven PA, Devlin RH, Higgs DA.Infuence of dietary digestible energy content on growth, protein and energy utilization and body composition of growth hormone transgenic and non-transgenic coho salmon (Oncorhynchus kisutch). Aquaculture. 2006;254:730–747. https://doi.org/10.1016/j.aquaculture.2005.11.009
18. Leggatt RA, Raven PA, Mommsen TP, Sakhrani D, Higgs D, Devlin RH. Growth hormone transgenesis influences carbohydrate, lipid and protein metabolism capacity for energy production in coho salmon (Oncorhynchus kisutch). Comp BiochemPhysiol Part B. 2009; 154:121–133. https://doi.org/10.1016/j.cbpb.2009.05.010
19. Farmanfarmaian A, Sun LZ. Growth hormone effects on essential amino acid absorption, muscle amino acid profile, N retention and nutritional requirements of striped bass hybrids. Genetic Analysis. 1999; 15(3-5):107-113.
20. Frikardo.Teknologi pembuatan pakan buatan.2009.http://afsaragih.wordpress.com
21. Halver, J. E. dan Hardy, R. W. Fish Nutrition. Academy Press inc. 1972; New York.
22. Alimuddin, B. Handoyo, N. B. P. Utomo. Efektivitaspemberianhormonpertumbuhanrekombinanikankerapukertang (Epinepheluslanceolatus, Bloch 1790) melaluiperendamandan oral terhadappertumbuhanelverikansidat (Anguilla bicolor bicolor). JurnalIktiologi Indonesia. 2014; 14(3): 179-189.
23. Oakes JD, Higgs DA, Eales JG, Devlin RH.Infuence of ration level on the growth performance and body composition of non-transgenic and growth-hormone-transgenic coho salmon (Oncorhynchus kisutch). Aquaculture. 2007; 265:309–324. https://doi.org/10.1016/j.aquaculture.2007.01.015.
24. Samsudin, R., N. Suhenda dan M. Sulhi. Evaluasi Penggunaan Pakan dengan Kadar Protein yang Berbeda terhadap Pertumbuhan Benih Ikan Nilem (Osteochilus hasseltii). Prosiding Forum InovasiTeknologiAkuakutur. 2010; Hal 697-701.
25. Webster, C. D., and C. E. Lim. Nutrient requirements and feeding of finfish for aquaculture. CABI Publishing. New York.2002.
26. Kurniawan A., Basuki F., Nugroho R.A. PengaruhPemberianRekombinanHormonPertumbuhan (rGH) MelaluiMetode Oral Dengan Interval Waktu Yang BerbedaTerhadapPertumbuhan Dan KelulushidupanBenihIkanBawal Air Tawar (Colossomamacropomum). Journal of Aquaculture Management and Technology.2017; Vol.8. hal 20-29
27. Viola, S., & Rappaport. The extra caloric effect of oil in the nutrition of carp. Bamigdeh. 1979; 31(3), 51-69;
28. Melzer, K.Carbohydrate and Fat Utilization during Rest and Physical Activity. European e-Journal of Clinical Nutrition and Metabolism. 2011; Vol 6: 45 – 52.
29. Leggatt RA, Raven PA, Mommsen TP, Sakhrani D, Higgs D, Devlin RH. Growth hormone transgenesis influences carbohydrate, lipid and protein metabolism capacity for energy production in coho salmon (Oncorhynchus kisutch). Comp BiochemPhysiol Part B, 2009; 154:121-133.
30. Hepher. B. Nutrition of pond fishes. Cambrige university Press. Cambrige. New York.1988; 388 p.
31. Buwono, I. D., I. Iskandar dan R. Grandiosa. Growth Hormone Transgenesis and Feed Composition Influence Growth and Protein Amino Acid Content in Transgenic G3 Mutiara Catfish (*Clariasgariepinus*). *Aquaculture International*.2021; 29: 431-451.
32. Rahman MA, Ronyai A, Engidaw BZ, Jauncey K, Hwang GL, Smith A, Roderick E, Penman D, Varadi L, Maclean N. Growth and nutritional trials on transgenic Nile tilapia containing an exogenous fish growth hormone gene. J Fish Biol. 2001; 59:62–78. https://doi.org/10.1111/j.1095-8649.2001.tb02338.x
33. Levesque HM, Shears MA, Fletcher GL, Moon TW. Myogenesisdanmetabolismeototpada salmon Atlantikmuda (Salmosalar) yang

dibuattransgenikuntukhormonpertumbuhan. J Exp Biol. 2008; 211:128-137.https://doi.org/10.1016/j.cbpb. 2009.05.010
34. Suresh K W., Madhav K S., Sunila R., Narayan P P. 2016. Effect of different protein levels on feed utilization, growth and body composition on fry of snowtroutSchizothoraxrichardsonii. International Journal of Fisheries and Aquatic Studies. 2016; 4(5): 89-95
35. Tokah, C., Undap, S. L., &Longdong, S. N. J. KajianKualitas Air Pada Area BudidayaKurunganJaringTancap (KJT) di DanauTutudDesaTombatuTigaKecamatanTombatuKabupatenMinahasa Tenggara. BudidayaPerairan.2017; 5(1), 1–11