



Engineering Technology Fish Farming of Snakeheads *Channa striat* ,Bloch, 1793) Based Feed Vitamin C Increase to Superior Quality Using Microsatellite

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ABSTRACT

Objectives for snakeheads farming Engineered to produce superior products based artificial diet enrichment vitamin C with different doses and the use of microsatellites to increase growth and determine the superior quality. Methods February to November 2017 Examined the Effect of artificial feed Enriched with vitamin C doses per 100 g feed were given 0 mg (T1), 12 mg (T2), 24 mg ,and 36 mg (T3), 48 mg (T4) were using a completely randomized design (CRD) and 4 treatments and 3 replications. Furthermore, to determine the superior quality using microsatellite genetic markers to see the genetic code snakeheads. Then the treatment was analyzed by ANOVA and data arrangement of molecular weight DNA, polymorphism were analyzed by Cco-stat and Minitab version 11. The data collected observations of the size of the weight absolute, survival, FCR, molecular weight DNA composition, heterogeneity genotype, polymorphism. The results showed that highest of polymorphism were T3 (weight absolute 169.18 mg) and lowest polymorphism treatment T1 (weight absolute 149.25 mg) .Next analysis of polymorphism results using a micro-satellite in treatment (T2, ribbon 3,4), and (T3, ribbons 5,6) and (T4, ribbons 7 and 8) respectively using IS-GB1F 5-CCC TGT ATT TCA TTT CTC CA-3 and IS-GB 1 R 5-ACC AAC ACT GCA ATC TCT CT-3 bright ribbons found in treatment T4 (ribbons 7,8) snakeheads are the results showed that the growth of snakeheads were highest in T3 (with ribbons 205bp allele ladder with 215 bps and 225 bps on the right, Addition as well as having polymorphism is high and. variation of different types also found the number of alleles and heterozygotes.

Keywords: *microsatellite analysis, DNA, snakeheads, engineering culture*

1. Introduction

Chemical Snakeheads is the current high mortality 90% due to unaccounted-based aquaculture technology engineering enrichment artificial diet enriched with vitamin C and before used of microsatellites to increase growth and determine the superior quality and biofiltersystem that can improve water quality.Because because with vitamin C-enriched feed, it will cause the snakeheads fish to grow quickly and be more resistant to disease, so the quality increases and excels. To find out the advantages of snakeheads fish one of them is detecting DNA through genetic markers using microsatellite.

One way to improve efforts to accelerate the growth in snakeheads fish reproduction that with the addition of vitamin C on artificial feed. The addition of vitamin C in the diet may play a role as an antioxidant, which is able to maintain the availability of

enriched in feed plays an important role in the growth and survival of fish snakeheads that can help in the process of metabolism (Halver, 1989).Using microsatelite and used in research, because of the will be better and easier to do, and can be known quickly and accurately genetic variation such as polymorphic heterozigot. Objectives for snakeheads farming engineered to produce superior products based artificial diet enrichment vitamin C with different doses and the use of microsatellites to increase growth and determine the superior quality.

2. Materials and Method

Methods February to November 2017 examined the effect of artificial feed enriched with vitamin C doses per 100 g feed were given 0 mg (T1), 12 mg (T2), 24 mg (T3), and 36 mg (

T4), were using a completely randomized design (CRD) and 4 treatments and 3 replications. Furthermore, to determine the superior quality using microsatellite genetic markers to see the genetic code snakeheads. Then the treatment was analyzed by ANOVA and data arrangement of molecular weight DNA, polymorphism were analyzed by Cco-stat and Minitab version 11. The data collected observations of the size of the weight absolute, survival, FCR, molecular weight DNA composition, heterogeneity genotype, polymorphism.

2.2. Ingredients for Mixture.

The materials used for the development of fish farming snakeheads broodstock mikrosatelite analysis sample extract fish snakeheads, reagents such as PCR Kit: 10 x PCR buffer, 2.5 mM dNTP mix, primer IS-GB1F 5-ATT TGT CCC TCA TTT CTC CA-3 and used GB 1 R 5-ACC ATC AAC ACT GCA TCT CT-3 (INTEGRATED DNA TECHNOLOGIES SINGAPORE), and primer using GB2 IS F oligo BASE TYPE 5-AGA AGA AGA AGC CGA GT-3 and IS -GB2 R 5-AAA GAA AGG AGC CAG AAC AC-3 (INTEGRATED DNA TECHNOLOGIES SINGAPORE) and primary, as well as, 5 uL. This primer is design from to use tag Polymerase, akuadest and mt-genome DNA in 0.2 ml PCR Tube, Universal primer OPA 4, 1% agarose gel in 1 x TBE (tris boric acid EDTA) buffer, Lodder 100 bp DNA, ethidium bromide, UV transilluminator, enzyme Hind III restriction (A'AGCTT); Bam HI (G'GATCC); EcoR V (GAT'ATC) and HAE III (GG'CC the reason choose this restriction enzyme better good and result is better to use Lart 10 x buffer, 100 x BSA, restriction enzymes and akuadest and mt-DNA template, 1.5% agarose gel in 1 x TBE buffer.

2.3. Equipment.

The equipment used in the development of snakeheads fish farming using mikrosatelite analysis including surgical tools (tweezers, knives, scissors, watch glass, petri dish, glass objects, pipette, hotplate, tissue paper, a microscope (magnification 1000x, 1500 x) and the extraction and purification equipment mt-DNA, mt-genome PCR amplification of DNA, Restriction Fragment Length Polymorphism. equipment such as: eppendorf tube, water bath, heating equipment, centrifuges with size capacity was more than 13,000 rpm, PCR amplification, 0.2 ml PCR tube, UV Transilluminator, cameras gel, electrophoresis.

2.5. Microsatellite analysis method was performed as follows method and data collection instruments and extraction and purification of mt-DNA.

2.6. Method and data collection instruments.

Strategy 1 Using Snakeheads holding elections on the basis of genetic markers. This research used samples of the parent fish with a length of 31.5 snakeheads to 50.3 cm and weight 262-1037 g original from Central Java waters (Gajah Mungkur, Rawa Pening, Solo River) Each sample group was 20 head.

2.7. Extraction and Purification of mt-DNA.

Method of extraction and purification of mt-DNA of fish snakeheads based method Jamsari et al (2011) was conducted by means of genome mikrosatelite obtained through modification of the method of extraction followed Ovendem (2000). Network snakeheads fish (fins, meat fish snakeheads) made an extraction destroyed in 500 mL of 10% Chelex-100 were included in the eppendorf tube and add 5 mL proteinase kinase (10 mg / mL) and heated in a 55 ° C water bath for 3-4 hours. Furthermore, the solution was heated again at a temperature of 89 ° C for 8 minutes and cooled at room temperature to cool before adding 55 mL of TE (Tris-EDTA) buffer pH 8.0. Mt-DNA genome could be obtained by centrifugation for 5 minutes at 13,000 rpm. Solution in the upper layer and a clear colorless genomic DNA was transferred into a new eppendorf tube and stored at -20 ° C for further analysis so the result are good.

2.8. Genomic DNA PCR 3. Amplifikasi.

PCR Amplification of Genomic DNA by PCR amplification of the genomic DNA samples of snakeheads each treatment began with mixing multiple PCR reagent kit (Qiagen) consisting of 10 x PCR buffer, 2.5 mM dNTP mix, primer-primer IS GB1F 5- ATT TGT CCC TCA TTT CTC CA-3 and IS-GB 1 R 5-ACC ATC AAC ACT GCA TCT CT-3 (INTEGRATED DNA TECHNOLOGIES SINGAPORE), and primary -GB2 IS F oligo BASE TYPE 5-AGA AGA AGA AGC CGA GT-3 and IS -GB2 R 5-CAG GAA AAA AGC AGG AAC AC-3 (INTEGRATED DNA TECHNOLOGIES SINGAPORE), 0.5 uL Taq polymerase, akuadest and genomic DNA in 0.2 mL PCR tube and incubated in a PCR machine with 38 cycles. In this amplification used an initial denaturation temperature of 94 ° C for 2 min and a final temperature of 94 ° C denaturation for 40 seconds. For the 60 ° C annealing

temperature used for 1 min and followed by the initial extension temperature 72 ° C for 5 min and final extension temperature of 72 ° C for 5 minutes. Universal primers used in the amplification of DNA was determined based snakeheads species, to determine the banding pattern resulting from the use of DNA amplification poly acrylamide and 1% agarose gel in 1 x TBE (tris boric acid EDTA) electrophoresis buffer with 25-30 minutes long. As molecular markers used for the analysis of micro-satellite by using primer BP6-2 4% (W / V) agarose gel metaphore (CAMBREX, USA), a 100 bp DNA ladder, used for ethidium bromide staining by soaking for 10 minutes and wash with water for 10 minutes. The results observed under UV Transilluminator and documented by gel camera.

2.9. Microsatellite Polymorphism

Microsatellite polymorphism determined by using restriction enzymes on the DNA template PCR amplification product was cut with the restriction enzyme Hind III (A'AGCTT); Bam HI (G'GATCC); EcoR V (GAT'ATC) and HAE III (GG'CC). Cutting the DNA template starts with preparing a solution of 10 x buffer, 100 x BSA, and akuadest restriction enzymes and DNA template PCR amplification products with a certain concentration. Subsequently incubated in a water bath with a temperature of 37 ° C for 2.5 - 3 hours. The use of 1.5% agarose gel in 1 x TBE buffer and electrophoresis process for 30-35 minutes and staining with ethidium bromide for 10 min, then obtained pieces of fragments of each DNA template. Used as a molecular marker 100 bp DNA ladder, whereas for the control of DNA that do not use templates to experience cuts. The results observed under UV-Transilluminator at 320 nm and documented by gel camera. Confirmation of genotype

frequency analysis performed by the GEN-POP Program. Parameters measured include total length and weight of snakeheads, composition and molecular weight of the DNA, heterogeneity, genotype and allele frequencies.

Statiscal analysis. The data include the growth of the total fish length and weight of snakheads were analyzed by F test (ANOVA) (Hadi 2004), and knowing the length weight relationship using soft ware Minitap 11, while data on the composition of the molecular weight of the DNA, genotypic heterogeneity, the calculation of the diameter and color eggs, gonadal development (Istiyanto & Sardiyatmo 2007) is done by analysis ekostat and descriptive.

2.10. Absorption spectrometry (AAS) analysis

Atomic Absorption Spectrometry (AAS) (GBC® 902)/Perkin Elmer for analysis heavy metals in mussels with gas flow condition contain of air- acetilene and wave long (λ) were measured Ni(II) (λ = 232.0 nm), Zn(II) (λ =213.9 nm), Cu(II) (λ = 321.47 nm) and Cd(II) (λ = 228.8 nm).

3. Results and Discussion

3.1. Absolute growth, survival snakeheads

The results showed that artificial feeding C enriched with vitamine C with different doses significantly ($p < 0.05$) on the absolute growth, survival of snakeheads. Growth snakeheads absolute highest weight obtained from treatment C (5% perbiomass / day), ie 169.18 mg \pm 0.25^a and lowest T1 (weight absolute 149.25 mg) he survival of 95.5 \pm 1.07^a. Water quality during maintenance still within the reasonable range for fish farming snakeheads for use biofiltersystem well controlled (Table.3)..

Tabel.3. Growth in absolute weight. Survival, conversion ratio in aquaculture snakeheads (*Channa striata*)

	Treatment*)			
	T1 (0 mg)	T2 (12 mg)	T3 (24 mg)	T4 (36mg)
1. Absolute growth of the rearing snakeheads (g)	149.25 ^c	164.75 \pm 0.67 ^b	169.18 \pm 0.51 ^a	163.25 \pm 0.60 ^b
2. Survival rate of the snakeheads (%)	75.29 \pm 1.48 ^c	84.25 \pm 1.24 ^b	95.5 \pm 1.07 ^a	78.75 \pm 1.26 ^b
3. Food Covertion Ratio (FCR)	3.15 \pm 0.04 ^b	1.97 \pm 0.02 ^b	1.76 \pm 0.04 ^c	2.28 \pm 0.14 ^a

Information :

There were dose 0 mg (T1), 12 mg (T2), 24 mg (T3) ,and 36 mg (T4). At the same superkrip sign showed no significantly different ($P < 0.05$),.

Furthermore, from Table 1 do variance analysis showed significant effect ($P < 0.05$) on the growth of absolute weight, survival rate and feed conversion ratio snakeheads. Then to find out the difference in value between treatments middle'S Tukey test showed significant differences between treatment C-B, C-D, C-A, B-D, B-A.

Tukey test showed significant differences between treatment C-B, C-D, C-A, B-D, B-A.

The growth of the absolute weight of snakeheads

At The same Superkrip (Table 1) showed no significant effect ($P < 0.05$ and analysis of variance in a completely randomized design shows a comparison of male and female parent of fish snakeheads in different waters was highly significant ($P < 0.01$) on the absolute weight (Table.1). because the growth of the absolute weight of the fish snakeheads because the fish snakeheads can utilize feed so well that grow well too. This is in accordance with the opinion of Huet (1971), Istiyanto et al (2012) physical growth occurs with the change in the number or size of the cells making up the body's tissues, morphologically visible growth of body shape changes. Growth will occur when the Cnergy needs for the metabolism and maintenance of body tissues has been fulfilled in accordance with the needs of fish (Hepher, 1988, Yuvaraj et al.2015). Also described by other researchers that snakeheads an grow well when fed on feed containing protein in accordance with the needs of the body both for Cnergy and growth, as well as feed consumed shrimp wind is greater than the amount needed for the maintenance of the body and used as a source Cnersinya (Bautista, 1986, BPPT.2007, Djajasewaka.1985, De Silva and Anderson, 1995).

The success in farming of snakeheads (*C striatus*) (Table 1), is determined among other environmental factors of feeds. The environmental factors and enriched artificial feed used vitamine C can increase growth of the absolute weight of snakeheads. The snakeheads fish better higher are environment among other fish live in freshwater, especially in rivers, lakes, wetlands, waterways, also in the brackish water from lowlands up to a height of more than 1000 meters above sea level. These fish are able to live in the muddy area without water for a long time, as long as the skin and divetikulumnya still wet. Cooked eggs at the age of approximately 2 years. At the time of spawning fish snakeheads make a nest of aquatic plants to lay their eggs at a depth of 30-100 cm from the surface of the water (DG fisheries, 1990).

Snakeheads living in fresh waters with a pH of 4.5 to 6 and not so deep, there is also live in brackish water (Wise, 2003 2006.20012 Makmur, 2006^{a, b}), the Director General of fisheries, 1990). Makmur (2006^c) suggested that the snakeheads has two varieties, namely fast-growing and slow-growing. Snakeheads fast growing generally live around the lake, and their characteristics are, color scales back light gray, chest colored silvery-white, and at the same age in total length and width of the body is greater than the varieties are slow growing. Snakeheads nest in the banks of the waters filled with aquatic plants. eggs fertilized egg will float around the nest, and maintained with care by both parents of snakeheads fish until the larvae are about 50 mm (Berra, 2001).

Snakeheads spawning season in Thailand between May to October with a peak in July through September. While fish snakeheads in a swamp area in South Sumatra Musi River flood may spawn throughout years to size of mature female fish for 180 mm and 154 mm male (Makmur, 2006^{a, b}).

Furthermore, from Table 1 do variance analysis showed significant effect ($P < 0.05$) on the growth of absolute weight, survival rate and feed conversion ratio snakeheads fish. Then to find out the difference in value between treatments middle'S Tukey test showed significant differences between treatment were C-B, C-D, C-A, B-D, B-A.

Survival rate of snakeheads

The same Superkrip (Table 1) showed no significant effect ($P < 0.05$), and analysis of variance in a completely randomized design shows a comparison of male and female parent fish in different waters snakeheads was highly significant ($P < 0.01$) in the survival of snakeheads fish.

Then to find out the difference in value between treatments middle'S Tukey test showed significant differences between treatment were C-B, C-D, C-A, B-D, B-A. The Cxistence of a significant influence on fish snakeheads for maintenance of water quality set by the administration and using aeration biofilter system so that the water quality was always good. This is supported by the opinions Berra (2001) Wise, (2003, 2006.20012), Makmur, (2006^{a, b}), the Director General of the

fishery, (1990) the role of water management snakeheads fish culture media is very important because it can improve the survival of snakeheads fish.

Food Covertion Ratio (FCR) of snakeheads

The results showed that the food conversion ratio is the lowest in treatment T3 FCR (Food Conversion Ratio) of 1.76 ± 0.04^c (Tabel.3). Based on the analysis of variance with the differences in male and female parent of snakeheads fish comparisons very significant effect on FCR ($P < 0.01$) in and by Tukey test showed significant differences between means treatment A-D, A-B, A-C, , D-B, D-C, B-C.

The same Superkrip showed no significant Cffect ($P < 0.05$), and analysis of variance in a completely randomized design shows a comparison of male and female parent snakeheads fish in different waters was highly significant ($P < 0.01$) in the feed conversion. This is in accordance with the opinion of Istiyanto et al. (2010-2012), Istiyanto and Rachmawati (2016), Tacon, (1987) states that the feed conversion ratio is a very important role to see whether the feed is able to increase the growth of catfish with growth better or whether feed given more efficient. Feed conversion values can also see how much feed is given enhances growth with better / faster growth. There is a tendency feed conversion rate (FCR) lower ($T3 = 1.76 \pm 0.04^c$) provides absolute weight higher growth, which means more efficient feed given.

Genetic analysis of snkeheads with microsatellites

The results showed the analysis of polymorphism results using a micro-satellite in treatment (T2, ribbon 3,4), and (T3, ribbons 5.6) and (T4, ribbons 7 and 8) respectively using the primer IS-GB1F 5-CCC TGT ATT TCA TTT CTC CA-3 and IS-GB 1 R 5-ACC AAC ACT GCA ATC TCT CT-3.bright ribbons

found in treatment T4 (ribbons 7.8) snakeheads are the results showed that the growth of snakeheads were highest in T3 (with ribbons 205bp allele ladder with 215 bps and 225 bps on the right, as well as having polymorphism is high. variation of different types.

Heterogeneity genotype, polymorphism

The results showed that highest of polymorphism were T3 (weight absolute 169.18 mg) and lowest polymorphism are treatment T1 (weight absolute 149.25 mg) . analysis of polymorphism results using a micro-satellite in treatment (T2, ribbon 3,4), and (T3, ribbons 5.6) and (T4, ribbons 7 and 8) respectively using the primer IS-GB1F 5-CCC TGT ATT TCA TTT CTC CA-3 and IS-GB 1 R 5-ACC AAC ACT GCA ATC TCT CT-3 (.INTEGRATED DNA SINGAPORE TECHNOLOGIES), bright ribbons found in treatment T4 (ribbons 7.8) snakeheads are the results showed that the growth of snakeheads were highest in T3 (with ribbons 205bp allele ladder with 215 bps and 225 bps on the right, as well as having polymorphism is high. variation of different types, the treatment T4 is also found the number of alleles and heterozigotes different, so it will produce superior seeds that have good genetic code.(Figure 1).

Based on the analysis of micro satellite using a primer IS-GB1F 5-CCC TGT ATT TCA TTT CTC CA-3 and IS-GB 1 R 5-ACC AAC ACT GCA ATC TCT CT-3 (INTEGRATED DNA TECHNOLOGIES SINGAPORE), indicating that the tape allele 200bp ladder with 214 bps and 224 bps on the right,based on the results of the study showed that the use of vitamin C in snakeheads fish feed can cause fish to be more resistant to disease attacks and more immune, as well as improving growth so that the quality is superior. Microsatellite method so that genetic variation can be known including heterezigot, polymorphism, DNA weight, DNA sequence (Jamsari,et al. 2011, Almaniar et al. 2012 Haitham et al.2017).

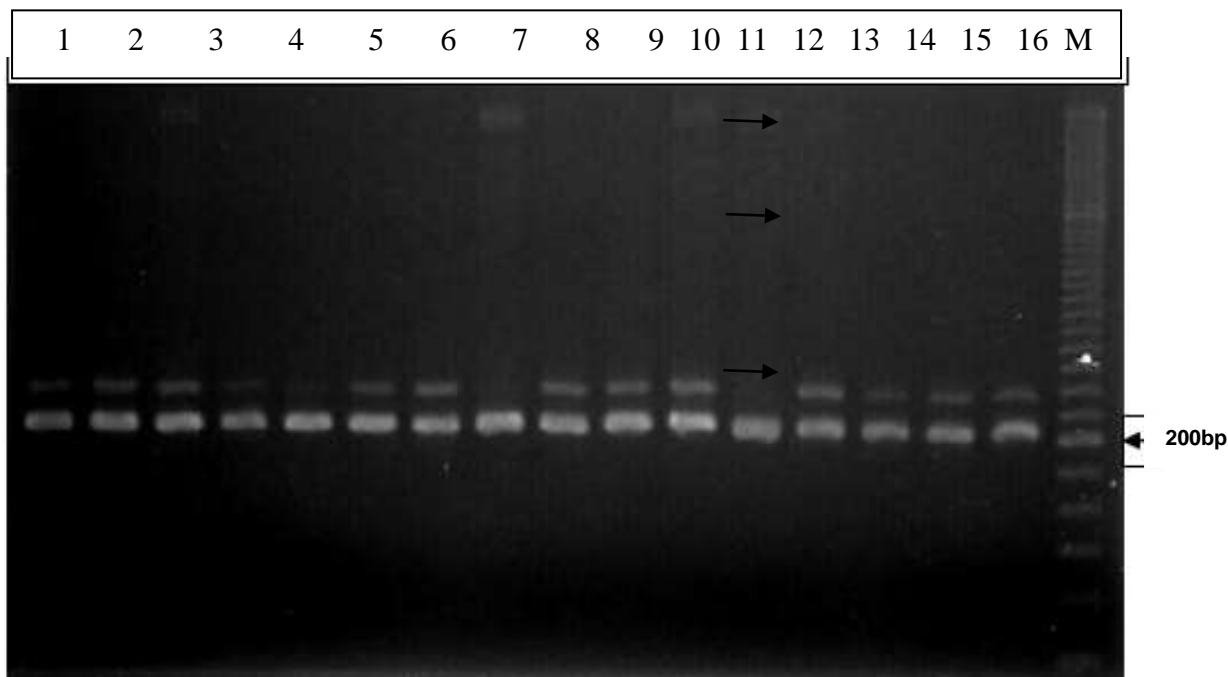


Figure.1. The results showed that of the maintenance T3 (169.18 ± 0.51^a mg) and lowest treatment T1(145.27 mg) .Next polymorphism analysis results using a micro-satellite in treatment (T2, ribbon 3,4), and (T3, ribbons 5.6) and (T4, ribbons 7 and 8) hereinafter respectively using the primer IS-GB1F 5-CCC TGT ATT TCA TTT CTC CA-3 and IS-GB 1 R 5-ACC AAC ACT GCA ATC TCT CT-3 (INTEGRATED DNA SINGAPORE TECHNOLOGIES), bright ribbons found in treatment T4 (ribbons 7.8) snakeheads are the results showed that the growth of snakeheads were highest in T3 (with ribbons 205bp allele ladder with 215 bps and 225 bps on the right, as well as having polymorphism is high. variation of different types, the treatment T4 is also found the number of alleles and heterozigotes different, so it will produce superior seeds that have good genetic code

4. Conclusions

The results showed that highest of polymorphism were T3 (weight absolute 169.18 ± 0.51^a mg), survival rate (95.5 ± 1.07^a %), Feed conversion ratio ($FCR = 1.76 \pm 0.04^c$) and lowest polimorpsame treatment T1 (weight absolute 149.25 mg) .Next analysis of polymorphism results using a micro-satellite in treatment (T2, ribbon 3,4), and (T3, ribbons 5.6) and (T4, ribbons 7 and 8) respectively using the primer IS-GB1F 5-CCC TGT ATT TCA TTT CTC CA-3 and IS-GB 1 R 5-ACC AAC ACT GCA ATC TCT CT-3.bright ribbons found in treatment T4 (ribbons 7.8) snakeheads are the results showed that the growth of snakeheads were highest in T3 (with ribbons 205bp allele ladder with 215 bps and 225 bps on the right, as well as having polymorphism is high. variation of different types. the treatment T4 is also found the number of alleles and heterozigotes different, so it will produce superior seeds that have good genetic code.

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