UNIVERSITI PUTRA MALAYSIA

ASSESSMENT OF MORPHOMETRIC VARIATION, GROWTH PERFORMANCE, DISEASE RESISTANCE AND NUTRITIONAL VALUE OF CLARIID CATFISH HYBRID (Clarias macrocephalus GUNTHER, 1864 x Clarias gariepinus BURCHELL, 1822)

NORA FATEN AFIFAH BINTI MOHAMAD

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BERILMU BERBAKTI

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By

NORA FATEN AFIFAH BINTI MOHAMAD

Thesis Submitted to the School of Graduate Studies, Universiti Putra Malaysia, in Fulfilment of the Requirements for the Degree of Doctor of Philosophy

November 2020

Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfilment of the requirement for the degree of Doctor of Philosophy

ASSESSMENT OF MORPHOMETRIC VARIATION, GROWTH PERFORMANCE, DISEASE RESISTANCE AND NUTRITIONAL VALUE OF CLARIID CATFISH HYBRID (*Clarias macrocephalus* GUNTHER, 1864 x *Clarias gariepinus* BURCHELL, 1822)

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NORA FATEN AFIFAH BINTI MOHAMAD

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Chair : Assoc. Prof. Hassan Bin Haji Mohd Daud, PhD
Faculty : Veterinary Medicine

The present study was carried out to access the morphometric variation and performance of crossbreed of *Clarias macrocephalus* (♀) and *Clarias gariepinus* (♂), (hybrid CMxCG) and its parental species; female, CM (*C. macrocephalus*♀ x *C. macrocephalus* ♂); male, CG (*C. gariepinus*♀ x *C. gariepinus*♂). The first objective was conducted to evaluate the effect of hybridization on the breeding performance, growth and survival of hybrid CMxCG and its parental species as a control for a period of 60 days, with fortnightly sampling. For the purposes, broodstock fishes were induced to spawn by intramuscular injection of Ovaprim hormone at the dose of 0.5 mL kg^{-1} body weight (female) and 0.25 mL kg^{-1} body weight (male). The result revealed that hybrid CMxCG achived better in breeding performance than *C. macrocephalus* in regard to fertilization and hatching rate as well as growth and survival. Percentage of fertilization and hatching rate of hybrid CMxCG were 72.62±2.51% and 64.01±9.17%, respectively, which were higher than in *C. macrocephalus* which recorded 60.76±0.64 % and 28.14±6.51 %, respectively. As for growth performance, the body weight and total lenght of hybrid CMxCG showed significantly larger and longer (p<0.05) than *C. macrocephalus* at the end of the study period which resulted in 1.54±0.48 g and 5.81±1.09 cm, respectively for hybrid CMxCG and 0.98±0.28 g and 4.77±0.47 cm for *C. macrocephalus*. Higher survival rate of 58% which significantly different (p<0.05) was noticed in hybrid CMxCG followed by *C. macrocephalus* (49%) and *C. gariepinus* (41%).

The second objective of this study employed the morphological variation of hybrid CMxCG and its parental species. A total of 30 morphometric measurements and 5 meristic counts were carried out on each specimen using method from Teugels (1986) and Agnese et al. (1997) with slight modification. For the morphometric characters in this present study, eight new measurements were added with one new measurement in the head and seven new meaasurement in toothplate. The results emphasized that the shape of the occipital process and the shape of the premaxillary as well as vomerine toothplate appears to be highly reliable index of morphological discrimination because it's showed high phenotypic differentiation among all three fish species. Most of the hybrids also showed intermediate morphological characteristics and exhibits more similarities of phenotypic featured to their paternal species after juvenile stage which might be due to paternal dominating characteristics.

The third objective of this study was to evaluate the nutritional composition of hybrid CMxCG, *C. gariepinus* and *C. macrocephalus*. Analyses on proximate composition revealed that hybrid CMxCG had the highest protein content (40.07±0.09%) than both parental species (CM: 39.84±0.07%; CG: 38.54± 0.05%). Fatty acid analysis was also done in triplicate consisted of two consecutive steps which were preparations of fatty acid methyl ester (FAME) and gas liquid chromatography. In this study, fatty acids varied between species, demonstrating high polyunsaturated fatty acids (PUFA), EPA, DHA in hybrid CMxCG with the value of 31.41±0.94%, 2.02±0.09 and 2.42±0.40%, respectively. However, no significant difference ($p>0.05$) was observed in total amino acids (EAA) content of hybrid CMxCG.

Apart from that, this study also was conducted to assess the resistance of hybrid CMxCG and its parental species towards bacteria, *Aeromonas hydrophila.* The PCR primers used for specific detection of *A. hydrophila* was tested successfully and a desired PCR product of 685 bp was obtained. For aerolysin gene detection, bacteria isolates produced a 309 bp amplicon as expected. In the next phase of the study, fishes were intraperitoneally injected (0.1 mL) with different *A. hydrophila* concentrations; T_0= control (0.85% saline solution); and five serial dilutions (10^{-9}, 10^{-8}, 10^{-7}, 10^{-6}, and 10^{-5}) of *A. hydrophila* into juveniles of hybrid CMxCG, *C. gariepinus* and *C. macrocephalus*. The last objective of this present study revealed that 96 h-LD_{50} calculated using the Reed and Muench (1938) method was higher in hybrid CMxCG (x$10^{6.09}$ cfu mL^{-1}) than *C. macrocephalus* (x$10^{5.36}$ cfu mL^{-1}) but lower than *C. gariepinus* (x$10^{6.33}$ cfu mL^{-1}). Intraperiteonal injection resulted in 100% mortality at the highest concentration of bacteria (10^9 cfu mL^{-1}). Lesions scores were observed to be more severe in *C. macrocephalus* particularly in the kidney tubules with significantly higher ($p<0.05$) mean lesion score as compared to *C. gariepinus* and hybrid CMxCG.

In conclusion, the present study has provided important information on morphometric variation which can be used as quick and cheap method in identifying and distinguishing the parental species and the hybrids especially for field application. Hybrid CMxCG also have showed good performance of important traits which is beneficial for future aquaculture industry in regard to growth and disease resistance. Moreover, hybrid CMxCG revealed better performance of flesh quality content such as protein, PUFA, EPA and DHA, hence, aquaculturing of hybrid can contribute to a better source of protein diet for human being.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk ijazah Doktor Falsafah

PENILAIAN VARIASI MORFOMETRIK, PRESTASI PERTUMBUHAN, RINTANGAN PENYAKIT DAN NILAI PEMAKANAN IKAN KELI CLARIID HIBRID (*Clarias macrocephalus* GUNTHER, 1864 x *Clarias gariepinus* BURCHELL, 1822)

Oleh

NORA FATEN AFIFAH BINTI MOHAMAD

November 2020

Pengerusi : Prof. Madya Hassan Bin Haji Mohd Daud, PhD
Fakulti : Perubatan Veterinar

Kajian ini dijalankan untuk menilai variasi morfometrik dan prestasi kacukan *Clarias macrocephalus* (♀) dan *Clarias gariepinus* (♂), (hibrid CMxCG) dan spesies induk; betina, CM (*C. macrocephalus*♀ x *C. macrocephalus* ♂); jantan, CG (*C. gariepinus*♀ x *C. gariepinus*♂). Objektif pertama dijalankan untuk menilai kesan hibridisasi terhadap prestasi pembiakan, pertumbuhan dan kelangsungan hidup hibrid CMxCG dan spesies induk sebagai kawalan untuk tempoh 60 hari, dengan persampelan dua minggu. Untuk tujuan itu, induk ikan telah diransang melalui suntikan intramuskular dengan menggunakan hormon Ovaprim pada dos 0.5 mL kg-1 berat badan (betina) dan 0.25 mL kg-1 berat badan (jantan). Hasil menunjukkan bahawa hibrid CMxCG mencapai prestasi yang lebih baik dalam pembiakan berbanding *C. macrocephalus* berkaitan dengan persenyawaan dan kadar penetasan serta pertumbuhan dan kelangsungan hidup. Peratus persenyawaan dan penetasan kadar hibrid CMXCG adalah masing-masing 72.62 ± 2.51% dan 64.01 ± 9.17%, yang mana lebih tinggi daripada *C. macrocephalus* yang mencatatkan 60.76 ± 0.64% dan 28.14 ± 6.51%. Bagi prestasi pertumbuhan, berat badan dan jumlah keseluruhan hibrid CMXCG menunjukkan secara signifikan lebih besar dan lebih lama ($p <0.05$) daripada *C. macrocephalus* pada akhir tempoh kajian yang menghasilkan 1.54 ± 0.48 g dan 5.81 ± 1.09 cm, masing-masing untuk hibrid CMXCG dan 0.98 ± 0.28 g dan 4.77 ± 0.47 cm untuk *C. macrocephalus*. Kadar kelangsungan hidup yang lebih tinggi sebanyak 58% yang mana kadar perbezaan adalah ketara (p<0.05) telah dilihat di hibrid CMxCG diikuti oleh *C. macrocephalus* (49%) dan *C. gariepinus* (41%).

Objektif kedua kajian ini menggunakan variasi morfologi hibrid CMxCG dan spesies induk. Sebanyak 29 pengukuran morfometrik dan 5 kiraan meristik dilakukan pada setiap spesimen menggunakan kaedah dari Teugels (1986) dan Agnese et al. (1997) dengan sedikit pengubahsuaian. Untuk watak morfometrik dalam kajian ini, lapan pengukuran baru ditambah dengan satu pengukuran baru di kepala dan tujuh pengukuran baru dalam plat gigi. Hasilnya menekankan bahawa bentuk proses occipital dan bentuk premaxillary serta vomerine gigi-palatal ternyata menjadi indeks diskriminasi morfologi yang sangat boleh dipercayai kerana menunjukkan perbezaan fenotipik yang tinggi di antara ketiga spesies ikan ini. Kebanyakan hibrid juga menunjukkan ciri-ciri morfologi perantaraan dan mempamerkan lebih banyak persamaan fenotip yang diketengahkan kepada spesies induk jantan mereka selepas peringkat juvenil yang mungkin disebabkan oleh ciri-ciri keturunan induk jantan.

Objektif ketiga kajian ini adalah untuk menilai komposisi nutrisi hibrid CMxCG, *C. gariepinus* dan *C. macrocephalus*. Analisis komposisi menunjukkan bahawa CMxCG hibrid mempunyai kandungan protein tertinggi (40.07 ± 0.09%) daripada kedua-dua spesies induk (CM: 39.84 ± 0.07%; CG: 38.54 ± 0.05%). Analisis asid lemak bervariasi antara spesis juga dilakukan sebanyak tiga kali ulangan terdiri daripada dua langkah berturut-turut iaitu penyediaan metil ester asid lemak (FAME) dan kromatografi cecair gas. Dalam kajian ini, asid lemak berbeza antara spesies, menunjukkan asid lemak tidak tepu (PUFA), EPA, DHA dalam hibrid CMxCG dengan nilai masing-masing 31.41 ± 0.94%, 2.02 ± 0.09 dan 2.42 ± 0.40%. Walau bagaimanapun, tidak perbezaan yang signifikan ($p > 0.05$) diperhatikan dalam jumlah kandungan asid amino (EAA) CMxCG hibrid.

Selain itu, kajian ini juga dilakukan terhadap ketahanan hibrid CMxCG dan spesies induknya terhadap bakteria, *Aeromonas hydrophila*. Primer PCR yang digunakan untuk pengesanan spesifik *A. hydrophila* diuji dengan jayanya dan produk PCR yang diinginkan sebanyak 685 bp diperoleh. Untuk pengesanan gen aerolysin, isolat bakteria menghasilkan amplikon 309 bp seperti yang diharapkan. Dalam fasa kajian seterusnya, ikan disuntik secara intraperitoneal (0.1 mL) dengan penggunaan kepekatan *A. hydrophila* yang berbeza; T_0 = kawalan (larutan garam 0.85%); dan lima pencairan bersiri (10^{-9}, 10^{-8}, 10^{-7}, 10^{-6}, dan 10^{-5}) *A. hydrophila* terhadap hibrid CMxCG, *C. gariepinus* dan *C. macrocephalus*. Objektif terakhir dari kajian ini menunjukkan 96j LD_{50} yang dikira dengan kaedah Reed dan Muench (1938) lebih tinggi pada hibrid CMxCG ($x10^{6.09}$ cfu mL^{-1}) daripada *C. macrocephalus* ($x10^{5.36}$ cfu mL^{-1}) tetapi lebih rendah daripada *C. gariepinus* ($x10^{6.33}$ cfu mL^{-1}). Kaedah suntikan intraperiteonally mengakibatkan kematian 100% pada kepekatan bakteria tinggi (10^{9} cfu mL^{-1}). Luka juga dilihat lebih teruk pada *C. macrocephalus* dengan skor luka yang lebih tinggi ($p < 0.05$) berbanding dengan *C. gariepinus* dan CMxCG hibrid.

Kesimpulannya, kajian ini telah memberikan maklumat penting tentang variasi morfometrik yang boleh digunakan sebagai kaedah yang cepat dan murah dalam mengenalpasti dan membezakan spesies induk dan hibrid mereka terutama untuk aplikasi di ladang. Hibrid CMxCG juga telah menunjukkan prestasi yang baik terhadap ciri-ciri penting yang bermanfaat untuk industri akuakultur di masa depan mengenai pertumbuhan dan rintangan penyakit. Selain itu, hibrid CMxCG menunjukkan prestasi yang lebih baik terhadap kandungan kualiti daging seperti protein, PUFA, EPA dan DHA, oleh itu, akuakultur hibrid boleh menyumbang kepada sumber makanan protein yang lebih baik untuk manusia.

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This thesis was submitted to the Senate of Universiti Putra Malaysia and has been accepted as fulfilment of the requirement for the degree of Doctor of Philosophy. The members of the Supervisory Committee were as follows:

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Date: 11 November 2021

Declaration by graduate student

I hereby confirm that:

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°C	Degree celcius
m	Meter
cm	Centimeter
mg	Miligram
mM	Milimolar
mL	Mililiter
µl	Microliter
bp	Base pair
kb	Kilobase
rpm	Revolution per minute
V	Volt
$MgCl_2$	Magnesium chloride
NCBI	National Center for Biotechnology Information
Sp. Or spp.	Species or species (plural)
Cfu	Colony-forming unit
dH_2O	Distilled water
DNA	Deoxyribonucleic acid
dpi	dpi
EDTA	Ethylenediaminetetraacetic acid
H&E	Hematoxylin and eosin
HCL	Hydrochloric acid
LD_{50}	Median lethal dose
NaOH	Sodium hydroxide

NaCl	Sodium chloride
PCR	Polymerase chain reaction
TBE	Tris-borate-EDTA
TSA	Trypticase soy agar
RSA	Rimler-Shott Agar
UV	Ultra violet
h	Hour
EFA	Essential fatty acid
EAA	Essential amino acid
MUFA	Monounsaturated fatty acid
PUFA	Polyunsaturated fatty acid

CHAPTER 1

INTRODUCTION

1.1 World Fisheries and Aquaculture

Human population is increasing rapidly where it expected to reach 9.6 billion people by 2050 (FAO 2014). Reports of large numbers of people that are facing social problems such as poverty and chronic malnourishment has made the need for food production one of the worldwide issues of concern. Fisheries is one of the activities that contribute to major food production since people have depended so greatly on fish and other aquatic food as the primary sources of dietary animal protein for their well-being. Apart from being a economical source of animal protein of the household compare to poultry and red meat, fish also contains high amounts of vitamins and good quality of amino acids such as lysine, methionine and tryptophan as well as high polyunsaturated fatty acids (PUFA) omega three ω-3 (n-3) and omega six ω-6 (n-6) which are good for human health (Simopoulos, 2004; Sidhu, 2003; Lovell, 1989; Benitez, 1999).

According to FAO (2014), world per capita apparent fish consumption increased each year and it is in fact highly concentrated in Asia. Total food fish consumption demand particularly in South-East Asia is expected to increase by 36.3% from 14.2 million tonnes in 2010 to 19.3 million tonnes in 2030 (Worldbank, 2014). Apparently however, recent reports obviously shows that fish supplies from global captured fisheries are declining rapidly due to several factors including over-fishing, habitat destruction, and pollution (Eyo 2001; Dunham et al 2001; Olufeagba et al 2007; Adewumi & Olaleye 2011). Due to this current issues, it is undisputable that fish supplies from capture fisheries sector solely will not able to meet the heavy demand fish and fish based products in the near future (FAO, 2006). Hence, aquaculture or fish farming became one of the recommended activities that can fits exactly into the role as an alternative fish production system and satisfy the high domestic demand for fish and export demand of fish products as well.

Generally, there are several species of catfish belong to the family *Clariidae* are present in Malaysia. They are *Clarias anfractus*, *Clarias leiacanthus*, *Clarias planiceps*, *Clarias sulcatus*, *Clarias teijsmanni*, *Clarias batrachus* and *Clarias macrocephalus*. The English "walking catfish", or "Ikan Keli" in English and Malay respectively, is a generic name for a group of catfish species belongs to the family *Clariidae*. Among them,*Clarias batrachus,* or also known as "keli kayu" and *Clarias macrocephalus,* which is locally known as "keli bunga"are the most common and important native species in Malaysia.

Clarias macrocephalus is found to be generally restricted to the northern region of Peninsular Malaysia, particularly in Kedah and Perlis which are famous with paddy fields as their natural environment and habitat (Mohsin and Ambak, 1983; Lee at al., 1993). On the other hand, *C. macrocephalus* also can be found in irrigation canals, stagnant pools or streams. As an air-breathing fish, this species of catfish is tolerant to harsh environmental conditions such as extreme dry season and consequently have a capability to move to another secure habitats using their pectoral fins especially during spawning season, feeding and seeking for shelter (Pouyaud et al., 2009).

On the other hand, *Clarias gariepinus* (Burchell, 1822), which is another common species of *Clarias* catfish, was introduced to Malaysia from North Africa. This African catfish was introduced to Malaysia from Thailand in the early 1980's which is between 1986-1989 (Csavas I, 1995) and is currently represents as dominant finfish culture not only in Malaysia but also in various regions in the world (Akeem et al., 2018). The first report of *C. gariepinus* culture in Malaysia can be traced back to 1987 with an annual output of 6.46 metric tonnes, and this has developed and grown quickly since then (Akeem et al. 2018). Available official statistical data was obtained from Department of Fisheries (DOF), Ministry of Agriculture and Food Industries, Malaysia, showed that freshwater catfish, *Clarias* sp. had the highest total production of 555, 822 MT between 2007 and 2018 (DOF, 2018; Saba et al., 2020). However, according to FAO (2017), the statistics of total production of African catfish especially in Malaysia is currently reported inaccurate production amount. This inconsistency was said to be due to farming of African catfish hybrids and it was difficult to separate the data obtained from farmers for pure African catfish and that of hybrid. Other than that, misclassifications also were found between *C. gariepinus* and hybrids. Therefore, FAO did not capture the output under the name African catfish but were usually reported as *Clarias* sp. for both hybrid and nonhybrid (Xiaowei Zhou, 2017).

Furthermore, the success in gonadal maturation in captivity followed by induced spawning and mass seed production resulted in the rapid expansion of the hybrid catfish culture by local hatcheries. In fact, the catfish that is broadly cultivated now is the hybrid between local female clariid species, *Clarias macrocephalus* and exotic male, *Clarias gariepinus*. The *Clarias* hybrid with fast growth, high disease resistance and environmental conditions (from paternal genes), high meat quality and good taste (from maternal genes), are attractive to farmers and has become one of the most popular freshwater fishes by consumers (Senanan et al., 2004). As a result, hybrid Clariid catfishes almost completely replaced the native clariid catfish aquaculture in our countries and other countries such as Thailand (Poompuang and Na-Nakorn, 2004). In addition, it has given a great potential to aquaculture of clariid catfishes in many Asian countries and positively impacted the livelihoods of many catfish farmers. Since Clariid catfishes has become a great economic importance in Malaysia, especially as a food fish and vital in the local sustainability of the aquaculture activity, it has been widely cultures uncontrollably both in ponds and artificial tanks without considering the proper husbandry and management practices of culture systems (Akeem et al., 2018; FAO, 2010).

Likewise, the culture methods also have become more intensive in order to produce higher yields which simultaneously caused disease problems in the aquaculture industry. Disease issues has become a great concern in aquaculture production because it can impacts negatively on market value and lead to great monetary losses which is related to increase production costs such as high mortality of cultured animals, increase cost of treatment, decreased quality and quantity of yield and loss of the opportunity to sell the fish due to undesirable appearance which affects customer's choice (Idowu et al., 2017; Akoll and Mwanja, 2012; Subasinghe et al., 2002). Studies showed that almost fifty percent of production loss is because of diseases-related problems and become more severe in developing countries (Ayalew and Fufa, 2018). In addition to disease problems, abiotic stresses such as water quality factors including high water temperature and low levels of dissolved oxygen also can reduce the effectiveness of the fishes immune systems and then lead to significant economic losses (Welker et al., 2007). Stressors can be defined as the sum of the physiological responses the fish makes both physiologically and behaviourally to adapt with deterioration of environmental conditions and then regain its normal balance (Chris et al., 2003). However, if a fish is constantly exposed to any stressor which is exceeds the fish's ability to adapt, it may be lethal or will facilitate the infection by opportunistic pathogens or parasite which may present in the water.

1.2 Statement of Problems and Significance of Study

Genetic improvement of economically important traits of farm-raised catfish using interspecific hybridization has become one of a short-cut approach to increase the production of fish, lower the production costs and improve profitability for catfish farmers. Attributes that have been associated with hybrid catfish include faster growth rates, higher survival rate, improved yield and meat quality and improve disease resistance. These attributes would motivates the community and catfish farmers to breed this type of fish and will contribute to the increased of inland fisheries production through aquaculture (Bartley et al., 2001; Aminur Rahman, 2013; Omeji et al., 2013).

However, to date, as the production of *Clarias* hybrids draw attention among catfish farmers. In Thailand, hybrid catfish production was introduced since 1988 by artificially crossing males of African catfish (*Clarias gariepinus*), with females of the Thai walking catfish (*Clarias macrocephalus*). These hybrid catfish is already adopted for commercial aquaculture in Thailand since 1996 with more than 80% of catfish farmers raise hybrid catfish (Na-Nakorn, 1999). Although hybrid catfish is currently became the dominant fish culture in Thailand, this present study must be done particularly in Malaysia to develop better understanding on the gene interactions and degrees of genetic differences (Falconer, 1989) contributed by two distinct populations, species or inbred lines produced by the hybrid catfish in the future. Additionally, differences of strains used could effects the superior performance of the interspecific catfish hybrids despite the large genetic differentiation between the parental species (Koolboon et al., 2014).

On the other hand, since culture of the hybrids catfish are widely used in aquaculture, the possibilities to increase production through inappropriate hybridization will also increase. Some hybrids are accidentally produced through mixed spawning of different species in a hatchery, misidentification of species by hatchery personnel, or by contamination of the aquaculture facility with wild fish (Senanan et al., 2004; Akeem et al., 2018). Such uncontrolled and unintentional hybridization could decrease the performance of cultured stocks and produce unrecognized introgressed individuals, thus, make future use of the contaminated stocks as broodstock questionable (Senanan et al., 2004). Other problem that hybrids presently cause with regard to their inclusion in FAO database due to the incorporation of production information on hybrids by catfish farmers or aquaculturist (Bartley et al., 2001). Consequently, most of the hybrid catfish production is simply list and reported to the taxonomic level of genera only which is *Clarias* spp. The report with incompletely identified species reflected official FAO statistics and reduces the effectiveness of the data for monitoring the utilization of aquatic biodiversity for aquaculture. On the other hand, many fish geneticists and molecular biologists realized that the use of inter-specific hybrid is not well-reported (Aminur Rahman et al., 2013). The lack of constant reporting hybrid species may be due to the difficulty of assessing parental origins of inter-specific hybrids.

Therefore, since catfish hybrids (*C. macrocephalus* x *C. gariepinus*) are produced from the Clariid species that belong to the same genus and the external features of hybrids show very similar in appearance with their parental species, identifying these individuals become critical not only for development of management strategies in aquaculture and fisheries industry but also to allow for a better understanding of biodiversity among aquaculturist. It can reduce the potential of ecological problems by preventing the risk of fish from hybridizing in nature which is considered as one of good efforts to conserve native catfish as well. A morphological analysis and molecular genetics markers such as microsatellite DNA are important methods to differentiate inter-specific hybrids species (Chaiparinya, 1996; Na-Nakorn, 1999; Senanan et al., 2004; Nazia and Siti Azizah, 2014).

Other than that, since interest in hybridization of Clariid catfish in aquaculture has been purely for genetic and economic importance, this current study aimed to evaluate the successful of hybridizations between *Clarias gariepinus* and *Clarias macrocephalus*, and demonstrated the possible reproductive performance such as growth and survival, disease resistance and proximate composition of hybrids of this species compared to their parental lines crosses. Assessment of morphometric variation of the parental species amd its hybrids also is considered as critical for identification purposes. Hence, the hypotheses and research objectives were as follows:

1.3 Research Hypothesis

Hybridization between African catfish, *C. gariepinus* and Asian catfish, *C. macrocephalus* holds high potential for global aquaculture production in the coming years due to general achievement of better growth rate, survival rate, high disease resistance, flesh quality and good nutritional value. The offspring of this *Clarias* sp. is considered exhibit positive heterosis for culture traits as they can perform better than both or at least either one of the parental lines. Other than that, there is a variation in the morphometric characteristics of hybrid CMxCG and its parental species.

1.4 Objectives of the study

1. To determine the growth rates and survival rates of the hybrid of CMxCG in comparison with its parental lines as a control
2. To examine the differentiation of morphological characteristic of hybrid CMxCG and its parental species
3. To compare the nutritional value of hybrid CMxCg and its parental species
4. To determine the 96 h-LD_{50} values of hybrid CMxCG and its parental species towards *A. hydrophila*
5. To examine the clinical signs and histopathological changes in kidney, liver and spleen of hybrid CMxCG and its parental species

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