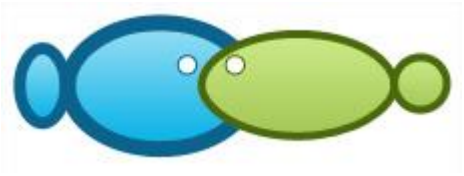

The effect of salinity on the body chemical composition and RNA/DNA ratio of the hybrid brackishwater Nile tilapia *Oreochromis niloticus*

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Abstract. The hybrid brackish water tilapia is a cross-breeding between female GIFT (Genetic Improvement of Farmed Tilapias) with local male tilapia (*Oreochromis mossambicus*) which has potency for further development. The research aim is to analyze the effect of salinity on the chemical composition of the body and the ratio of RNA/DNA. The research was conducted in Brackishwater Aquaculture Development Centre (BADC), Takalar, South Sulawesi, Indonesia. The experiment was carried out in 18 plastic buckets with a capacity 15 L of water each. The test animal was three days old of hybrid brackishwater tilapia seed with 0.02 g average of body weight and it reared maintained for 30 days with stocking density of 75 fish per bucket. The juvenile was fed with rotifer S type (140-200 µm), and artificial feed. Salinity treatments were 0, 10, 16, 22, 28 and 34 ppt. Analysis of variance showed that salinity affected significantly ($p<0.01$) on the protein content, fat, energy and RNA/DNA ratio of the hybrid brackishwater juvenile tilapia. The best protein content, fat, energy and RNA/DNA ratio performance were observed in the salinity concentration of 16 ppt and the lowest performance was observed in the salinity of 0 ppt.

Key Words: protein, fat, body energy, genetic improvement, *Oreochromis mossambicus*.

Introduction. The hybrid brackishwater tilapia is a cross-breeding between female GIFT tilapia with local male tilapia (*Oreochromis mossambicus*) in South Sulawesi brackishwater areas developed by BADC Takalar, 2011. The advantages of this species are: ability to live, grow and develop very well in culture environment with high salinity and persistent in a low level of dissolved oxygen. Furthermore, in a brackish water pond, it has high survival rate, fast growth and tasty with thick texture of flesh. However, there are several constraints related to seedling which is one of the limitations is the optimum level of salinity in their culture stage.

The salinity is one of the environmental factors that affect aquatic organism. In the coastal areas and estuaries, organism encounter fluctuated salinities that generate osmotic stress which leads to low survival and low growth rate. These effects may vary on physiologic adaptation such as osmoregulation ability. In addition, salinity changes affect osmoregulation (Sterzelecki et al 2013; de Azevedo et al 2015), physiologic process (Guner et al 2005; Kaneko & Hiroi 2008), modification on chemical composition (Bakhiet & Khogalie 2011; Lisboa et al 2015; Anthony et al 2016), and fish RNA/DNA ratio (Kim et al 2008; O'Neil et al 2011).

One of the chemical compositions affected by intracellular osmotic pressure adjustment is the body protein. Besides that, extracellular osmoregulation correlates with energy utilization for active ion transportation, which involves degradation of productive/rich compound energy such as lipids. This mechanism should produce biochemical changes in response to salinity fluctuation.

Researches concerning changes of chemical composition of the body affected by salinity were conducted by Rooker & Holt (1996), Pepin et al (1999), Permana et al

(2011) and Parenrengi et al (2013). The results of these research show that salinity affects body chemical composition and RNA/DNA ratio.

This research aims to analyze the effect of salinity on body chemical composition and the ratio of RNA/DNA of hybrid brackishwater tilapia.

Material and Method. The research was conducted in Brackishwater Aquaculture Development Center (BADC) Takalar regency, South Sulawesi Province, Indonesia.

Testing organism for this research was hybrid brackishwater tilapia from cross-breeding between female GIFT tilapia and local tilapia (*Oreochromis mossambicus*). The fish was three days old, with 1.76±0.21 cm body length and average biomass of 0.028±0.01 g. The tested fishes were reared in 15 L black plastic buckets with a density of 75 individuals/bucket for 22 days.

The water used was sea water of 34 ppt and fresh water. Seawater was taken from the beach next to the BADC, while fresh water was from an artesian well at BADC Takalar. Dilution was conducted to obtain appropriate salinity for the treatment. To maintain water quality, water exchange at 35% in each bucket was conducted weekly.

The fish was feed by rotifer S type size of 140-200 µm and combination with artificial feed, commercial feed with 30 to 32% protein content. Rotifer was applied from day 1 to day 5 with density of 25 individuals/mL, continued with a combination between rotifer and artificial feed from day 5 to day 10, and from day 11 until the end of the experiment 100% artificial feed was administered. Feeding frequency was three times a day at 08:00, 12:00 and 15:00 h.

Design of the experiment was a completely randomized design consisted of six salinities treatments (0, 10, 16, 22, 28 and 34 ppt) with three replications.

Parameters observed were protein and fat content, energy and ratio of RNA/DNA of the tested specimens. The protein content was analyzed by Kjeldahl method, fat was analyzed by soxhlet method, and the energy was measured by Colorimeter (AOAC 2005).

DNA and RNA extraction was conducted followed guidance from Gwak et al (2003). Calculation of RNA and DNA concentration was performed according to Fatchiyah (2011):

$$RNA = Å260 \times 40 \times \text{dilution factors}$$

Where: 40 = 40ug/mL single-stranded RNA (ssRNA).

$$DNA = Å260 \times 50 \times \text{dilution factor}$$

Where: Å260 = absorbance value on 260 nm.

50 = Solutions with absorbance value 1.0 equal with 50 ug double stranded DNA per mL (dsDNA).

Results and Discussion

Chemical composition. The values of body chemical composition of hybrid brackishwater tilapia juvenile in various salinities are presented in Table 1.

Table 1

Chemical composition of the tilapia seedling in various salinities

Salinity (ppt)	*Protein (%)*	*Fat (%)*	*Energy (Kal/g)*
0	51.43±0.39^{e}	14.78±0.52^{d}	3.16±0.24^{e}
10	52.47±0.03^{d}	13.2±0.06^{e}	3.59±0.01^{de}
16	57.62±0.03^{a}	17.58±0.05^{a}	4.34±0.01^{a}
22	56.50±0.08^{b}	16.47±0.05^{b}	4.11±0.01^{ab}
28	54.15±0.26^{c}	15.83±0.04^{bc}	3.88±0.01^{bc}
34	52.45±0.54^{d}	15.38±0.45^{cd}	3.75±0.05^{cd}

Different letters in each column indicates significant differences between treatments at 5% level ($p<0.05$).

Analysis of variance showed that the differentiation of salinity had significant effect on the body chemical composition (protein, fat, and energy) of hybrid brackishwater tilapia seedling ($p<0.01$). The highest content of protein, fat, and energy were observed in the salinity of 16 ppt, while the lowest values were in the salinity of 0 ppt (Table 1). These results indicated that physiological condition of the tilapia seedling preferred to live in salinity of 16 ppt compared to other salinity concentrations. Hasbullah et al (2018) found that optimum salinity for hybrid brackishwater tilapia was 16 ppt. Ali et al (2005), notify that the differentiation of body chemical composition of the fish was related to the physiological condition and it was affected by several environmental factors especially by salinity. Castillo-Vargasmachuca et al (2017) also reported that highest body chemical composition values of *Lutjanus guttatus* juvenile was observed in the salinity of 15 ppt. In addition, high salinity level generates a detrimental effect on the chemical composition.

The lowest content of protein, fat and energy of hybrid brackishwater tilapia in salinity of 0 ppt was caused by high osmotic activity that leads to alteration of energy used for osmoregulation and consequently affects body chemical composition. Maicá et al (2014), obtained that cultured sea organism at low salinity utilized high energy. Energy was used to increase biomass and it also for osmotic pressure maintenance. Furthermore, Fu et al (2017) explained that the energy utilized by an organism to maintain osmotic pressure, mainly came from fat and protein of the body. Therefore, salinity affected the body chemical composition and survival rate of the organism.

Body chemical composition found in this study was similar to other studies. For example, El-Zaeem et al (2012) reported protein and fat contents were 57.10% and 28.72% in the tilapia kept in salinity of 16 ppt, and 56.73% and 14.87% in 32 ppt. Another study also reported that the protein and fat contents at 16 and 32 ppt were 57.10%, 28.72% and 56.73% and 14.87%, respectively (Sampaio & Bianchini 2002; Jandal & Wilson 2011).

RNA/DNA ratio. The RNA/DNA ratios of hybrid brackishwater tilapia seedlings in different salinity treatments are presented in Table 2.

Table 2

Average ratio of RNA/DNA in salinities treatment

Salinity (ppt)	*RNA*	*DNA*	*RNA/DNA*
0	0.106±0.02	0.107±0.01	$0.98±0.04^{c}$
10	0.237±0.07	0.233±0.07	$1.02±0.01^{c}$
16	0.218±0.03	0.125±0.02	$1.73±0.08^{a}$
22	0.138±0.02	0.108±0.01	$1.31±0.04^{b}$
28	0.173±0.06	0.170±0.06	$1.2±0.02^{c}$
34	0.209±0.24	0.191±0.21	$1.04±0.07^{c}$

Different letters in the RNA/DNA column indicates significant differences between treatments at 5% level ($p<0.05$).

Analysis of variance revealed that different salinities affected significantly ($p<0.01$) on RNA/DNA ratios of hybrid brackishwater tilapia. The highest ratio of RNA/DNA was observed at salinity of 16 ppt. This result indicated that in the culture media with salinity of 16 ppt RNA synthesis increased as shown also in the protein content. The ratio alteration of RNA/DNA affected by enhancement of RNA synthesis as well as protein synthesis. According to Chicharo & Chicharo (2008) the amount of DNA as a primary carrier for genetic information is stable even though under environmental changes, whereas RNA amount fluctuate depends on the rate of protein synthesis. Our results affirms Chicharo & Chicharo (2008) statement that RNA/DNA ratio based on assumption, the amount of DNA as a primary carrier for genetic information is stable under environmental changes in a somatic cell, whereas RNA amount is directly involved in protein synthesis. Our results indicate that salinity also affects protein metabolism.

Another finding of the present research is that RNA/DNA ratio at low salinity (0 ppt) exhibited the protein synthesis the lowest level. This result is in accordance with a

previous study of Glémet & Rodriguez (2007) who stated that when the level of cell division and the level of protein synthesis are low, then high ratio of RNA/DNA is produced. The study reveals that the RNA/DNA ratio of Sultana tilapia is 0.57, Nirwana 0.47, Srikandi 0.47, and Red tilapia 0.24.

Conclusions. The highest values of protein contents, fat, energy and RNA/DNA ratio of hybrid tilapia juvenile were produced at salinity level of 16 ppt and the lowest values at salinity of 0 ppt.

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