

Growth performance of tilapia (*Oreochromis niloticus* Linnaeus, 1758) larvae with feeding *Tubifex tubifex* (Müller, 1774) from different fermentation of animal manures

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Abstract

Tubifex tubifex is a natural feed for many fish species, which still relies on natural catches. High nutrient contents culture media is needed to increase the availability and quality of *T. tubifex*. The aim of this study was to investigate growth performance of Nile tilapia (*Oreochromis niloticus*) larvae fed with *T. tubifex* cultured using different animal manures. Some profiles of *T. tubifex* nutrition (N, P, and K), Nile tilapia composition (proximate, essential amino acids, and fatty acids) fed with *T. tubifex*, Relative Growth Rate (RGR), Survival Rate (SR), biomass, feed intake, protein efficiency ratio, and water quality were analyzed. The results showed that tilapia fed with *T. tubifex* which cultured using various animal manures had a significant effect ($p<0.05$) on its nutrition, growth, biomass, and feed intake level compared to tilapia fed with *T. tubifex* which cultured without animal manures. However, it did not have significant effect in survival rate ($p>0.05$). The best treatment was obtained at *T. tubifex* cultured using 50 g/L of quail manure + 100 g L⁻¹ of rice bran + 50 g L⁻¹ of tofu waste (T₂).

Keywords: Growth rate, *Oreochromis niloticus*, Tilapia larvae, *Tubifex tubifex*

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Introduction

The 23% of total Indonesia's aquaculture production is Nile tilapia (*Oreochromis niloticus*), this makes the need for Nile tilapia seeds to support the aquaculture production of Nile tilapia is also increasing (Fish Stat, 2016). The quality of Nile tilapia seed depends on nutrition and water quality in the aquaculture system (Hamre, 2016). The suitable feed both in size and nutrient contents play an important role in the early phase of tilapia larvae. During larvae phase, Nile tilapia larvae need natural feeds such as *Tubifex tubifex*. Nutrient content of *T. tubifex* are protein (50.23%), fat (20.9%), crude fiber (1.3%), and ash (6.7%) (Herawati *et al.*, 2015). Protein was an important nutrient item needed for tilapia's growth because all its cells require a continuous supply of protein to meet their metabolic demands (Guimarães *et al.*, 2008). Natural feeds have an important role to fish larvae; therefore, the development of research in this field makes many researchers develop alternative sources of natural feed (Liti *et al.*, 2006; Chepkirui-Boit *et al.*, 2011; Herawati *et al.*, 2015; Devic *et al.*, 2017). In terms of quality, the natural feed is better to utilize for fish larvae rearing compared to artificial feed (Faruque *et al.*, 2010).

The availability of *T. tubifex* is commonly found by filtering mud in rivers, gutters, ditches, or other places as it has the same habitat as silkworm. *T. tubifex* prefers habitats with high conductivity, low depth, and rich in organic materials (Anggraeni and Abdulgani, 2013; Elissen *et al.*, 2015).

Production of *T. tubifex* is seasonally dependent, making it more difficult to find during rainy season, compared to dry season. The development of *T. tubifex* culture is therefore deemed essential to maintain and produce enough natural feed (in terms of both quantity and quality) to obtain maximum growth rate and survival rate of Nile tilapia. Both the quality and quantity of *T. tubifex* highly rely on the medium on which it is cultured.

The culture medium has an important role for high quality *T. tubifex* culture production. The fermented organic wastes were used in different culture media aims to increase the nutrient content of *T. tubifex*. Various wastes, such as quail manure, goat manure, chicken manure, rice bran, and tofu waste, are readily available and abundant. These wastes are highly rich in nutrients. However, when they are not utilized, the wastes are emerged as an environmental pollutant. The nutrient in manures was claimed contains N, P, and K which could be obtained from fermentation process (Damle and Chari, 2011; Herawati and Agus, 2014; Pilot *et al.*, 2014). The fermentation process of organic material usually involves a microbe or a probiotic activator. One of the functions of the activator is to accelerate the process of decomposition and to improve the quality of the product. The probiotic activator used in this study was the fermentation of vegetable waste extract. The type of bacteria found in the vegetable waste extract was *Lactobacillus* sp. (Utama *et al.*, 2013). The aim of this study was to evaluate

the growth rate and nutrition profiles of tilapia larvae fed with *T. tubifex* mass cultured using fermentation of various wastes and animal manures.

Materials and methods

Fermentation of media

The first stage of fermentation consisted of the preparation of molasses, water, and probiotic activator. The ratio used were at 1:1 (1 mL of both molasses and probiotic bacteria, and 100 mL of water) (Herawati and Agus, 2014). The organic materials used were quail manure, goat manure, chicken manure, rice bran, and tofu waste. All of the wastes were dried. The bacteria from the vegetable waste extract were used as the probiotic activator for the fermentation process. The treatments were divided into 10 (T₀-T₉), they were:

1. 100 g L⁻¹ of rice bran+50 g L⁻¹ of tofu waste (T₀)
2. 25 g L⁻¹ of quail manure+100 g L⁻¹ of rice bran+50 g L⁻¹ of tofu waste (T₁)
3. 50 g L⁻¹ of quail manure+100 g L⁻¹ of rice bran+50 g L⁻¹ of tofu waste (T₂)
4. 75 g L⁻¹ of quail manure+100 g L⁻¹ of rice bran+50 g L⁻¹ of tofu waste (T₃)
5. 25 g L⁻¹ of goat manure + 100 g L⁻¹ of rice bran+50 g L⁻¹ of tofu waste (T₄)
6. 50 g L⁻¹ of goat manure+100 g L⁻¹ of rice bran+50 g L⁻¹ of tofu waste(T₅)
7. 75 g L⁻¹ of goat manure + 100 g L⁻¹ of rice bran + 50 g L⁻¹ of tofu waste (T₆)
8. 25 g L⁻¹ of chicken manure+100 g L⁻¹ of rice bran+ 50 g L⁻¹ of tofu waste (T₇)
9. 50 g L⁻¹ of chicken manure+100 g L⁻¹ of rice bran+50 g L⁻¹ of tofu waste (T₈)
10. 75 g L⁻¹ of chicken manure+100 g L⁻¹ of rice bran+50 g L⁻¹ of tofu waste (T₉)

Tilapia larvae culture

Three-days-old tilapia larvae were cultured for 14 days with stock densities of 50 individuals L⁻¹. *T. tubifex* was given at *ad libitum*, 5 times in a day. The water quality during the study was maintained at 28–29°C temperature, 0.3 ppm dissolved oxygen (DO) and 8.1–8.2 pH. The study was conducted within the optimal water quality for tilapia (25–30°C, 0.3–0.6 ppm DO, and a pH of 6.5–9), as suggested by previous studies (Lim *et al*, 2011; Nina *et al*, 2012; Herawati *et al*, 2015).

T. tubifex population and biomass

The dispersion of *T. tubifex* worms had a stock density of 10 g for each container. The number of worms was determined by counting those found in a 1 g sample.

Proximate analysis

Proximate analysis was done based on AOAC (2000) procedure, the proximate analysis consist of crude ash content was determined by incineration at 550°C over night, crude lipids was determined by soxhlet apparatus method, crude protein was determined by kjeldhal method; crude fiber was

determined by enzymatic-gravimetric method and crude carbohydrate content was determined by Gravimetric method.

Essential amino acids profile

The essential amino acids profile of *T. tubifex* and tilapia larvae samples were determined by examining the essential amino acids content. The essential amino acids analysis was conducted using High Performance Liquid Chromatography (HPLC) type 1100 with a Eurospher 100-5 C18, with a 250 x 4.6 mm column and a P/N: 1115Y535 precolumn. The effluents included: A) 0.01 M acetate buffer at pH 5.9 and B) 0.01 M MeOH acetate buffer at pH 5.9: THF>80:15:5 A Fluorescence: Ext: 340 nm Em: 450 nm. 2.5 g of sample was put into a sealed glass (AOAC, 1999) and 15 mL of HCl 6N were added. This mixture was vortexed for homogeneity and underwent hydrolysis using an autoclave at 110°C for 12 hours. It was then cooled down at a room temperature and neutralized with NaOH 6N. After the addition of 2.5 mL of 40% lead(II) acetate and 1 mL of 15% oxalate acid, around 3 mL of the mixture were filtered using a 0.45 µm millex. For the injection into the HPLC, 25 µL of the filtered mixture and 475 µL of *ortho*-phthalaldehyde (OPA) solution was vortexed and incubated for 3 minutes. Finally, 30 µL of the final mixture were put into the HPLC (Herawati *et al.*, 2015).

Fatty acids profile

The fatty acids profile of *T. tubifex* was determined by analyzing its total fatty

acids content. The equipment used for this purpose was a gas chromatograph mass spectrophotometer (GCMS-QP-2010) with a WCOT fused silica counting CP-SIL-88 column of 50 m length, 0.22 mm in diameter and column temperature of 120–200°C. The method employed was *in situ* transesterification. 100 mg of the sample was homogenized using 4 mL of water. 100 µL of sample homogenate was then transferred to a test tube (Park and Goins, 1994).

Data analysis

All samples were analyzed by three replications; obtained data were analyzed by using the statistical software (SPSS 13.0). All the data were subjected one-way analysis of variance (ANOVA) followed by a comparison of means using the Least Significant Different (LSD) and Duncan Test. All differences were known as significantly different at $p < 0.05$ among treatments.

Results

Based on the results, the highest nutrient content of N, P, and K were 2.40%, 1.67%, and 1.95% respectively, obtained in quail manure (Table 1). Overall different culture media from animal manures fermentation showed significantly different ($p < 0.05$) for N, P and K content, except for P content from quail manure and goat manure. K content from goat manure and chicken manure had not significantly different ($p > 0.05$). The population density and biomass of *T. tubifex* cultured through fermentation of various animal manures were found to be highest at 50 g L⁻¹ of

quail manure + 100 g L⁻¹ of rice bran + 50 g L⁻¹ of tofu waste feed formulation (T₂). Results showed that the treatments gave significant different ($p < 0.05$) for population density. However, the biomass weight of *T. tubifex* had not significantly different ($p > 0.05$) (Table 2). Tilapia which fed with *T. tubifex* mass cultured using various animal manures (quail, goat, and chicken) showed a significantly different ($p < 0.05$) on growth and biomass weight compared to tilapia fed with *T. tubifex* cultured without the addition of animal manures. Tilapia fed with *T. tubifex* mass cultured using goat manure had not significantly different ($p > 0.05$) in growth compared to the other treatments. The highest level of Tilapia's growth rate and biomass weight was found at 50 g L⁻¹ of quail manure+100 g L⁻¹ of rice bran+50 g L⁻¹ of tofu waste feed formulation (T₂), and the lowest level of tilapia's growth rate and biomass weight was found at 100 g L⁻¹ of rice bran+50 g L⁻¹ of tofu waste treatment (T₀). The proximate content of *T. tubifex* and proximate in body composition of Nile tilapia larvae fed with *T. tubifex* showed in Table 3. The highest protein and lipid content were 65.30% ± 0.23 and 12.29% ± 0.01 at 50 g L⁻¹ of quail manure+100 g L⁻¹ of rice bran+50 g L⁻¹ of tofu waste feed formulation (T₂). T₂ had significant different ($p < 0.05$) to lipid content of Nile tilapia larvae. Overall ash and carbohydrate content decreased in every treatment except control treatment (T₀).

The positive correlation of proximate content in *T. tubifex* and body composition of Nile tilapia larvae were found at T₂, with 15.29 ± 0.11 for lipid and 67.53 ± 0.13 for protein (Table 3).

The profile of amino acids and fatty acids both in *T. tubifex* and body composition of Nile tilapia larvae were presented in Table 4, Table 5, and 7. Table 4 shown the highest essential amino acids provided by T₂, which were dominated by essential amino acids like alanine, cysteine, valine, methionine, isoleucine, leucine, tyrosine, phenylalanine, lysine, and histidine. It is correlated with essential amino acids which were absorbed by Nile tilapia larvae (Table 6), the highest essential amino acids content were found on T₂, which dominated by aspartic acid, threonine, serine, glutamic, glycine, alanine, valine, methionine, isoleucine, tyrosine, lysine, histidine, arginine, and tryptophan. The different of animals manures fermentation resulted significant different ($p < 0.05$) to the essential amino acids both on *T. tubifex* and body composition of Nile tilapia larvae fed with *T. tubifex*. Quality of protein from *T. tubifex* which absorbed by tilapia was showed by PER (Protein Efficiency Ratio) and NPU (Net Protein Utility) (Table 6). The highest value of PER and NPU showed by T₂ treatment, this is directly proportional with the highest of protein also showed by T₂.

Table 1: The content of N, P and K media mass culture *Tubifex tubifex* using various fermented livestock wastes.

Nutrient	poultry dung	quail dung	goat dung	chicken dung
N	1.08±0.08 ^a	2.40±0.02 ^b	1.53±0.02 ^c	1.88±0.05 ^d
P	0.37±0.09 ^a	1.67±0.06 ^b	0.35±0.07 ^a	1.19±0.05 ^c
K	0.65±0.06 ^a	1.95±0.09 ^b	1.14±0.02 ^c	1.23±0.07 ^c

Noted: values are means ± SD for triplicate and values in same row were followed by different letters are significantly different ($p < 0.05$).

Table 2: Growth population and biomass production in *Tubifex tubifex* mass cultured by utilization of various fermented livestock wastes.

	T ₀	T ₁	T ₂	T ₃	T ₄	T ₅	T ₆	T ₇	T ₈	T ₉
Population of <i>T. tubifex</i>	29692.38±889.06 ^a	40957.35±769.48 ^b	45109.16±901.66 ^c	36028.92±527.98 ^d	30961.91±263.18 ^e	35216.24±495.85 ^f	25923.47±204.37 ^g	33081.77±394.36 ^h	37830.93±657.61 ⁱ	32103.14±2106.90 ^j
Biomass of <i>T. tubifex</i>	124.59±0.05 ^a	146.29±0.03 ^a	152.19±0.02 ^a	142.17±0.05 ^a	114.58±0.08 ^a	123.81±0.05 ^a	131.51±0.01 ^a	138.69±0.02 ^a	149.27±0.04 ^a	132.44±0.06 ^a

Noted: Values are means ± SD for triplicate and values in same row were followed by different letters are significantly different ($p < 0.05$).

T₀ (100 g L⁻¹ of rice bran + 50 g L⁻¹ of tofu); T₁ (25 g L⁻¹ of quail manure + 100 g L⁻¹ of rice bran + 50 g L⁻¹ of tofu waste); T₂ (50 g L⁻¹ of quail manure + 100 g L⁻¹ of rice bran + 50 g L⁻¹ of tofu waste); T₃ (75 g L⁻¹ of quail manure + 100 g L⁻¹ of rice bran + 50 g L⁻¹ of tofu waste); T₄ (25 g L⁻¹ of goat manure + 100 g L⁻¹ of rice bran + 50 g L⁻¹ of tofu waste); T₅ (50 g L⁻¹ of goat manure + 100 g L⁻¹ of rice bran + 50 g L⁻¹ of tofu waste); T₆ (75 g L⁻¹ of goat manure + 100 g L⁻¹ of rice bran + 50 g L⁻¹ of tofu waste); T₇ (25 g L⁻¹ of chicken manure + 100 g L⁻¹ of rice bran + 50 g L⁻¹ of tofu waste); T₈ (50 g L⁻¹ of chicken manure + 100 g L⁻¹ of rice bran + 50 g L⁻¹ of tofu waste); T₉ (75 g L⁻¹ of chicken manure + 100 g L⁻¹ of rice bran + 50 g L⁻¹ of tofu waste).

Table 3: Proximate of *Tubifex tubifex* in mass culture using the different animal waste fermentation and proximate in body composition of Nile tilapia larvae with feeding *T. tubifex*.

Proximate (%)	Poultry dung (T ₀)	Quail dung (%)			Goat dung (%)			Chicken dung (%)		
		T ₁	T ₂	T ₃	T ₄	T ₅	T ₆	T ₇	T ₈	T ₉
Proximate in <i>T. tubifex</i>										
Crude ash	11.82 ±0.05 ^a	5.98±0.07 ^b	5.11±0.08 ^b	4.31±0.06 ^b	10.01±0.09 ^{ac}	7.57±0.09 ^{abc}	9.38±0.23 ^d	9.75±0.09 ^d	7.31±0.09 ^a	9.99±0.23 ^{de}
Crude lipid	7.62±0.06 ^a	11.24±0.08 ^b	12.29±0.01 ^b	11.03±0.09 ^b	10.85±0.02 ^c	11.67±0.03 ^b	10.30±0.06 ^c	11.25±0.02 ^b	12.04±0.03 ^b	10.78±0.06 ^{bc}
Crude fiber	9.55±0.10 ^a	5.01±0.03 ^b	4.07±0.02 ^b	6.98±0.07 ^c	6.73±0.01 ^c	9.13±0.05 ^a	8.89±0.19 ^d	9.37±0.01 ^a	6.54±0.05 ^c	8.89±0.19 ^d
Crude protein	52.11±0.17 ^a	61.16±0.04 ^b	65.30±0.23 ^c	60.17±0.01 ^b	54.80±0.03 ^a	60.86±0.06 ^b	53.54±0.03 ^a	56.75±0.03 ^d	62.13±0.06 ^b	59.04±0.03 ^a
Carbohydrate	18.90±0.05 ^a	13.17±0.10 ^b	11.82±0.19 ^c	15.71±0.07 ^d	17.61±0.09 ^a	10.77±0.10 ^c	17.41±0.05 ^a	12.88±0.09 ^f	11.98±0.10 ^c	11.78±0.05 ^c
Proximate in body composition of Nile tilapia larvae with feeding <i>T. tubifex</i>										
Crude ash	10.82±0.15 ^a	3.98±0.02 ^b	2.11±0.23 ^b	5.03±0.09 ^c	9.01±0.06 ^d	7.57±0.06 ^a	9.38±0.03 ^f	8.75±0.19 ^d	7.31±0.10 ^a	9.99±0.03 ^f
Crude lipid	10.62±0.08 ^a	13.24±0.18 ^b	15.29±0.11 ^c	12.03±0.23 ^d	11.15±0.01 ^a	11.67±0.01 ^f	10.78±0.05 ^a	12.25±0.26 ^d	13.04±0.11 ^b	11.30±0.02 ^a
Crude fiber	7.55±0.17 ^a	4.17±0.09 ^b	4.23±0.06 ^b	5.98±0.07 ^c	7.73±0.09 ^a	9.13±0.08 ^d	8.89±0.11 ^d	8.57±0.17 ^a	6.54±0.15 ^f	7.89±0.20 ^a
Crude protein	53.16±0.03 ^a	65.13±0.14 ^b	67.53±0.13 ^c	62.02±0.12 ^b	56.40±0.01 ^d	62.43±0.04 ^b	55.04±0.01 ^d	56.75±0.03 ^d	63.93±0.16 ^b	60.74±0.03 ^b
Carbohydrate	17.85±0.05 ^a	13.48±0.11 ^b	10.84±0.10 ^c	14.94±0.05 ^d	15.71±0.03 ^a	10.77±0.10 ^c	9.20±0.05 ^f	13.68±0.09 ^d	9.18±0.17 ^f	10.08±0.07 ^e

Noted: Values are means ± SD for triplicate and values in same row were followed by different letters are significantly different ($p < 0.05$). Values with bold show the highest value in every treatment.

T₀ (100 g L⁻¹ of rice bran + 50 g L⁻¹ of tofu); T₁ (25 g L⁻¹ of quail manure + 100 g L⁻¹ of rice bran + 50 g L⁻¹ of tofu waste); T₂ (50 g L⁻¹ of quail manure + 100 g L⁻¹ of rice bran + 50 g L⁻¹ of tofu waste); T₃ (75 g L⁻¹ of quail manure + 100 g L⁻¹ of rice bran + 50 g L⁻¹ of tofu waste); T₄ (25 g L⁻¹ of goat manure + 100 g L⁻¹ of rice bran + 50 g L⁻¹ of tofu waste); T₅ (50 g L⁻¹ of goat manure + 100 g L⁻¹ of rice bran + 50 g L⁻¹ of tofu waste); T₆ (75 g L⁻¹ of goat manure + 100 g L⁻¹ of rice bran + 50 g L⁻¹ of tofu waste); T₇ (25 g L⁻¹ of chicken manure + 100 g L⁻¹ of rice bran + 50 g L⁻¹ of tofu waste); T₈ (50 g L⁻¹ of chicken manure + 100 g L⁻¹ of rice bran + 50 g L⁻¹ of tofu waste); T₉ (75 g L⁻¹ of chicken manure + 100 g L⁻¹ of rice bran + 50 g L⁻¹ of tofu waste).

Table 4: Amino acid profile of *Tubifex tubifex* in mass culture using different animal waste fermentation.

Amino	Poultry dung (T ₀) (%)	Quail dung (%)				Goat dung (%)				Chicken dung (%)			
		T ₁ (%)	T ₂ (%)	T ₃ (%)	T ₄ (%)	T ₅ (%)	T ₆ (%)	T ₇ (%)	T ₈ (%)	T ₉ (%)	T ₁₀ (%)	T ₁₁ (%)	T ₁₂ (%)
Aspartic Acid	1.07 ± 0.05 ^a	1.18 ± 0.07 ^a	1.38 ± 0.08 ^b	4.31 ± 0.06^c	0.15 ± 0.09 ^d	0.82 ± 0.09 ^a	1.97 ± 0.23 ^f	1.18 ± 0.18 ^a	1.06 ± 0.03 ^a	2.04 ± 0.08 ^f			
Threonine	0.56 ± 0.06 ^a	0.93 ± 0.08 ^b	2.97 ± 0.01 ^c	2.71 ± 0.09 ^d	0.70 ± 0.02 ^a	0.59 ± 0.03 ^a	0.63 ± 0.06 ^a	0.62 ± 0.01 ^a	1.64 ± 0.05 ^f	3.64 ± 0.06^g			
Serine	0.78 ± 0.10 ^a	0.96 ± 0.03 ^a	1.33 ± 0.02 ^b	1.99 ± 0.07^c	0.32 ± 0.01 ^d	0.83 ± 0.05 ^a	0.92 ± 0.19 ^a	0.82 ± 0.03 ^a	0.84 ± 0.04 ^a	0.94 ± 0.13 ^a			
Glutamic acid	1.87 ± 0.17 ^a	1.66 ± 0.04 ^b	2.09 ± 0.23 ^c	1.66 ± 0.01 ^b	1.48 ± 0.03 ^b	1.13 ± 0.06 ^c	2.36 ± 0.03^d	1.51 ± 0.06 ^b	2.43 ± 0.13 ^d	1.60 ± 0.19 ^b			
Glycine	0.85 ± 0.05 ^a	1.44 ± 0.10 ^b	1.41 ± 0.19 ^b	1.08 ± 0.07 ^c	0.93 ± 0.09 ^a	0.91 ± 0.10 ^a	0.98 ± 0.05 ^a	1.03 ± 0.23 ^a	1.96 ± 0.25^d	1.04 ± 0.17 ^a			
Alanine	1.02 ± 0.03 ^a	1.15 ± 0.23 ^a	3.44 ± 0.02^b	1.29 ± 0.10 ^c	1.22 ± 0.01 ^d	0.94 ± 0.17 ^a	0.92 ± 0.01 ^a	1.07 ± 0.26 ^a	2.97 ± 0.19 ^a	1.17 ± 0.03 ^a			
Cysteine	0.08 ± 0.23 ^a	0.16 ± 0.26 ^a	1.16 ± 0.03^b	0.11 ± 0.13 ^a	0.10 ± 0.04 ^a	0.10 ± 0.03 ^a	0.10 ± 0.09 ^a	0.09 ± 0.02 ^a	0.09 ± 0.17 ^a	0.10 ± 0.01 ^a			
Valine	0.46 ± 0.26 ^a	1.53 ± 0.19 ^b	2.62 ± 0.17^c	1.42 ± 0.09 ^b	0.51 ± 0.01 ^a	1.42 ± 0.19 ^b	0.39 ± 0.01 ^a	1.44 ± 0.19 ^b	2.43 ± 0.23 ^c	1.48 ± 0.23 ^b			
Methionine	0.35 ± 0.17 ^a	0.64 ± 0.10 ^b	3.63 ± 0.10^c	0.48 ± 0.08 ^a	0.42 ± 0.09 ^a	1.38 ± 0.25 ^d	0.36 ± 0.07 ^a	0.44 ± 0.23 ^a	2.40 ± 0.25 ^a	0.48 ± 0.06 ^a			
Isoleucine	0.28 ± 0.03 ^a	2.36 ± 0.03 ^b	3.45 ± 0.26^c	2.29 ± 0.03 ^b	0.31 ± 0.03 ^a	0.29 ± 0.02 ^a	0.27 ± 0.07 ^a	1.30 ± 0.17 ^d	2.32 ± 0.03 ^b	0.38 ± 0.10 ^a			
Leucine	0.89 ± 0.06 ^a	1.94 ± 0.01 ^b	4.37 ± 0.45^c	2.88 ± 0.09 ^d	1.02 ± 0.06 ^a	1.89 ± 0.20 ^b	0.98 ± 0.08 ^a	1.28 ± 0.35 ^a	3.97 ± 0.09 ^f	1.15 ± 0.25 ^a			
Tyrosine	0.45 ± 0.10 ^a	0.88 ± 0.19 ^b	1.83 ± 0.15^c	1.48 ± 0.01 ^d	0.42 ± 0.01 ^a	0.40 ± 0.31 ^a	1.43 ± 0.03 ^d	0.55 ± 0.12 ^a	0.44 ± 0.06 ^a	0.43 ± 0.07 ^a			
Phenylalanine	0.58 ± 0.01 ^a	1.06 ± 0.03 ^b	3.08 ± 0.12^c	2.79 ± 0.05 ^d	0.68 ± 0.06 ^a	0.64 ± 0.05 ^a	0.64 ± 0.05 ^a	0.76 ± 0.25 ^a	1.66 ± 0.10 ^f	0.74 ± 0.09 ^a			
Lysine	0.96 ± 0.23 ^a	3.05 ± 0.12 ^b	4.53 ± 0.11^c	3.69 ± 0.01 ^d	1.92 ± 0.07 ^a	2.81 ± 0.09 ^f	0.97 ± 0.01 ^a	2.74 ± 0.38 ^f	3.93 ± 0.13 ^g	2.13 ± 0.10 ^b			
Histidine	0.36 ± 0.34 ^a	0.62 ± 0.09 ^b	2.64 ± 0.17^c	1.53 ± 0.10 ^d	0.39 ± 0.09 ^a	0.36 ± 0.03 ^a	0.40 ± 0.09 ^a	0.39 ± 0.14 ^a	1.39 ± 0.19 ^d	1.42 ± 0.15 ^d			
Arginine	0.74 ± 0.53 ^a	1.43 ± 0.45 ^b	1.46 ± 0.09 ^b	2.94 ± 0.45^c	0.71 ± 0.03 ^a	2.66 ± 0.06 ^d	0.81 ± 0.13 ^a	0.99 ± 0.19 ^a	2.77 ± 0.03 ^c	0.88 ± 0.31 ^a			
Proline	0.43 ± 0.12 ^a	0.49 ± 0.12 ^a	0.72 ± 0.07 ^b	0.53 ± 0.30 ^a	0.43 ± 0.23 ^a	1.44 ± 0.09^c	0.43 ± 0.30 ^a	0.44 ± 0.13 ^a	0.46 ± 0.06 ^a	0.49 ± 0.02 ^a			
Tryptophan	0.23 ± 0.02 ^a	0.23 ± 0.23 ^a	3.23 ± 0.15^b	1.19 ± 0.12 ^c	0.10 ± 0.26 ^a	1.93 ± 0.03 ^d	0.23 ± 0.26 ^a	0.17 ± 0.05 ^a	2.66 ± 0.01 ^a	2.35 ± 0.09 ^f			
EAA	5.12 ± 0.01 ^a	13.24 ± 0.05 ^b	36.35 ± 0.16^c	26.46 ± 0.19 ^d	6.47 ± 0.19 ^a	11.71 ± 0.01 ^b	6.30 ± 0.19 ^a	9.69 ± 0.25 ^a	22.84 ± 0.10 ^d	14.20 ± 0.05 ^b			
TAA	11.96 ± 0.03 ^a	21.71 ± 0.01 ^b	45.34 ± 0.08^c	33.37 ± 0.23 ^d	11.81 ± 0.34 ^a	20.54 ± 0.06 ^b	14.79 ± 0.19 ^a	16.82 ± 0.23 ^a	35.42 ± 0.06 ^d	22.46 ± 0.04 ^b			

Noted: Values are means ± SD for triplicate and values in same row were followed different letters are significantly different ($p < 0.05$). Values with bold show the highest value in every treatment.

T₀ (100 g L⁻¹ of rice bran + 50 g L⁻¹ of tofu); T₁ (25 g L⁻¹ of quail manure + 100 g L⁻¹ of rice bran + 50 g L⁻¹ of tofu waste); T₂ (50 g L⁻¹ of quail manure + 100 g L⁻¹ of rice bran + 50 g L⁻¹ of tofu waste); T₃ (75 g L⁻¹ of quail manure + 100 g L⁻¹ of rice bran + 50 g L⁻¹ of tofu waste); T₄ (25 g L⁻¹ of goat manure + 100 g L⁻¹ of rice bran + 50 g L⁻¹ of tofu waste); T₅ (50 g L⁻¹ of goat manure + 100 g L⁻¹ of rice bran + 50 g L⁻¹ of tofu waste); T₆ (75 g L⁻¹ of goat manure + 100 g L⁻¹ of rice bran + 50 g L⁻¹ of tofu waste); T₇ (25 g L⁻¹ of chicken manure + 100 g L⁻¹ of rice bran + 50 g L⁻¹ of tofu waste); T₈ (50 g L⁻¹ of chicken manure + 100 g L⁻¹ of rice bran + 50 g L⁻¹ of tofu waste); T₉ (75 g L⁻¹ of chicken manure + 100 g L⁻¹ of rice bran + 50 g L⁻¹ of tofu waste).

Table 5: Profile fatty acid of *Tubifex tubifex* in mass culture using different animal waste fermentation.

Fatty Acids	T ₀	T ₁	T ₂	T ₃	T ₄	T ₅	T ₆	T ₇	T ₈	T ₉
Meristic	1.07 ± 0.05 ^a	3.65 ± 0.03 ^b	9.25 ± 0.05^c	4.25 ± 0.02 ^d	1.45 ± 0.03 ^a	4.45 ± 0.01 ^d	1.23 ± 0.08 ^a	1.65 ± 0.05 ^a	6.65 ± 0.07 ^f	3.23 ± 0.04 ^b
Pentadecanoic	2.56 ± 0.04 ^a	2.12 ± 0.02 ^a	6.12 ± 0.07^b	2.02 ± 0.03 ^c	0.24 ± 0.02 ^d	1.24 ± 0.09 ^a	2.24 ± 0.03 ^c	1.16 ± 0.02 ^a	3.16 ± 0.09 ^f	1.17 ± 0.02 ^a
Palmitic	2.36 ± 0.06 ^a	7.52 ± 0.01^b	5.52 ± 0.03 ^c	3.23 ± 0.01 ^d	3.47 ± 0.05 ^d	2.47 ± 0.02 ^a	2.47 ± 0.03 ^a	6.26 ± 0.01 ^a	6.26 ± 0.01 ^a	4.26 ± 0.08 ^f
Stearic	1.87 ± 0.01 ^a	5.50 ± 0.03 ^b	6.50 ± 0.05^c	1.75 ± 0.05 ^a	2.07 ± 0.03 ^a	5.23 ± 0.01 ^b	0.23 ± 0.05 ^d	4.57 ± 0.05 ^a	5.57 ± 0.05 ^b	1.23 ± 0.06 ^f
Arachnid	0.85 ± 0.04 ^a	1.04 ± 0.08 ^a	0.04 ± 0.06 ^b	0.23 ± 0.08 ^b	0.07 ± 0.08 ^b	1.16 ± 0.05^a	1.16 ± 0.09^a	0.75 ± 0.04 ^a	0.75 ± 0.02 ^a	0.75 ± 0.02 ^a
SAFA	8.71 ± 0.03 ^a	19.83 ± 0.09 ^b	27.43 ± 0.01^c	11.48 ± 0.04 ^d	7.30 ± 0.01 ^a	14.55 ± 0.03 ^d	7.33 ± 0.06 ^a	14.39 ± 0.06 ^d	22.39 ± 0.02 ^c	10.64 ± 0.09 ^d
Palmitoleic	2.08 ± 0.02 ^a	3.38 ± 0.02^b	3.38 ± 0.08^b	3.38 ± 0.03^b	1.83 ± 0.05 ^c	2.83 ± 0.07 ^d	1.83 ± 0.05 ^c	3.09 ± 0.04 ^b	3.09 ± 0.05 ^b	3.09 ± 0.01 ^b
Oleic	3.46 ± 0.01 ^a	14.78 ± 0.01 ^b	17.26 ± 0.04^c	6.26 ± 0.05 ^d	9.03 ± 0.06 ^a	13.01 ± 0.03 ^f	5.01 ± 0.03 ^g	10.91 ± 0.05 ^h	12.10 ± 0.09 ⁱ	6.10 ± 0.05 ^d
eikosenoic	1.35 ± 0.08 ^a	2.19 ± 0.03^b	0.17 ± 0.08 ^c	0.23 ± 0.01 ^c	1.84 ± 0.02 ^d	0.26 ± 0.08 ^c	0.05 ± 0.06 ^c	1.13 ± 0.01 ^a	1.10 ± 0.03 ^a	0.17 ± 0.08 ^c
behenic	0.28 ± 0.09 ^a	1.19 ± 0.05 ^b	3.19 ± 0.05^c	1.19 ± 0.07 ^b	3.23 ± 0.07 ^c	1.03 ± 0.01 ^b	0.03 ± 0.07 ^a	2.35 ± 0.07 ^d	2.35 ± 0.05 ^d	0.23 ± 0.02 ^a
MUFA	7.17 ± 0.05 ^a	21.54 ± 0.07 ^b	24.45 ± 0.03^b	11.06 ± 0.04 ^c	15.93 ± 0.04 ^d	16.13 ± 0.03 ^d	7.92 ± 0.01 ^a	17.48 ± 0.08 ^d	18.64 ± 0.02 ^d	10.59 ± 0.01 ^c
linoleic	0.45 ± 0.04 ^a	5.25 ± 0.05 ^b	10.25 ± 0.08^c	3.17 ± 0.02 ^d	1.26 ± 0.03 ^a	5.26 ± 0.02 ^b	1.26 ± 0.05 ^a	3.17 ± 0.02 ^d	7.10 ± 0.01 ^f	1.10 ± 0.08 ^a
arachidonic	0.58 ± 0.02 ^a	0.08 ± 0.05 ^b	1.26 ± 0.01 ^c	0.26 ± 0.08 ^b	0.75 ± 0.09 ^d	1.75 ± 0.07^a	0.75 ± 0.04 ^d	0.23 ± 0.09 ^b	0.53 ± 0.08	0.53 ± 0.06
linolenic	0.96 ± 0.01 ^a	6.59 ± 0.06^b	6.19 ± 0.04 ^c	3.29 ± 0.09 ^d	2.23 ± 0.02 ^a	4.23 ± 0.02 ^f	1.23 ± 0.02 ^g	4.61 ± 0.03 ^h	4.61 ± 0.02 ^h	2.23 ± 0.09 ^a
EPA / eicosapentanoic acids (ω-3)	1.71 ± 0.03 ^a	0.68 ± 0.08 ^b	2.18 ± 0.09^c	1.18 ± 0.03 ^d	0.22 ± 0.01 ^a	1.05 ± 0.01 ^f	1.65 ± 0.01 ^a	0.51 ± 0.01 ^b	1.92 ± 0.06 ^c	1.19 ± 0.02 ^d
DHA / Docosahexaenoic acid (ω-6)	0.74 ± 0.02 ^a	0.14 ± 0.02 ^b	1.17 ± 0.01^a	0.17 ± 0.09 ^b	0.35 ± 0.06 ^b	0.35 ± 0.02 ^b	0.35 ± 0.06 ^b	0.19 ± 0.02 ^b	0.10 ± 0.02 ^b	0.17 ± 0.01 ^b
PUFA	4.44 ± 0.01 ^a	12.74 ± 0.08 ^b	21.05 ± 0.03^c	8.07 ± 0.01 ^d	4.81 ± 0.09 ^a	12.64 ± 0.03 ^b	5.24 ± 0.03 ^a	8.71 ± 0.09 ^f	14.26 ± 0.03 ^g	5.22 ± 0.03 ^a

Noted: Values are means ± SD for triplicate and values in same row were followed different letters are significantly different ($p < 0.05$). Values with bold show the highest value in every treatment.

T₀ (100 g L⁻¹ of rice bran + 50 g L⁻¹ of tofu); T₁ (25 g L⁻¹ of quail manure + 100 g L⁻¹ of rice bran + 50 g L⁻¹ of tofu waste); T₂ (50 g L⁻¹ of quail manure + 100 g L⁻¹ of rice bran + 50 g L⁻¹ of tofu waste); T₃ (75 g L⁻¹ of quail manure + 100 g L⁻¹ of rice bran + 50 g L⁻¹ of tofu waste); T₄ (25 g L⁻¹ of goat manure + 100 g L⁻¹ of rice bran + 50 g L⁻¹ of tofu waste); T₅ (50 g L⁻¹ of goat manure + 100 g L⁻¹ of rice bran + 50 g L⁻¹ of tofu waste); T₆ (75 g L⁻¹ of goat manure + 100 g L⁻¹ of rice bran + 50 g L⁻¹ of tofu waste); T₇ (25 g L⁻¹ of chicken manure + 100 g L⁻¹ of rice bran + 50 g L⁻¹ of tofu waste); T₈ (50 g L⁻¹ of chicken manure + 100 g L⁻¹ of rice bran + 50 g L⁻¹ of tofu waste); T₉ (75 g L⁻¹ of chicken manure + 100 g L⁻¹ of rice bran + 50 g L⁻¹ of tofu waste).

Table 6: Growth performance and feed utilization of Tilapia larvae fed with *Tubifex tubifex* mass cultured using various of fermented wastes.

Para meters	Treatment									
	T ₀	T ₁	T ₂	T ₃	T ₄	T ₅	T ₆	T ₇	T ₈	T ₉
IBW	0.05±0.03 ^a	0.05±0.03 ^a	0.05±0.03 ^a	0.05±0.03 ^a	0.05±0.03 ^a	0.05±0.03 ^a	0.05±0.03 ^a	0.05±0.03 ^a	0.05±0.03 ^a	0.05±0.03 ^a
FBW	0.52±0.13 ^a	1.32±0.15 ^a	1.83±0.04 ^{ab}	1.05±0.23 ^a	0.89±0.11 ^a	0.98±0.01 ^a	0.76±0.04 ^a	1.21±0.06 ^a	1.66±0.06 ^{ab}	1.13±0.06 ^a
WG	0.47±0.05 ^a	1.27±0.03 ^a	1.78±0.02 ^a	1.00±0.01 ^a	0.64±0.09 ^a	0.93±0.07 ^a	0.71±0.08 ^a	1.16±0.02 ^a	1.61±0.01 ^a	1.08±0.07 ^a
RGR	39.95±0.02 ^a	149.41±0.03 ^{ab}	209.41±0.03 ^{ab}	117.64±0.03 ^{ab}	75.29±0.03 ^a	109.41±0.03 ^{ab}	83.53±0.01 ^a	136.47±0.13 ^a	189.41±0.10 ^{ab}	14.75±0.09 ^a
FI (first week)	104.47±0.03 ^a	106.23±0.15 ^a	110.26±0.17 ^b	106.45±0.23 ^a	106.26±0.08 ^a	106.17±0.01 ^a	106.19±0.01 ^a	107.19±0.10 ^a	108.11±0.15 ^{ab}	127.05±0.02 ^a
FI (second week)	160.88±0.23 ^a	165.19±0.03 ^a	167.23±0.10 ^{ab}	164.17±0.03 ^a	162.75±0.08 ^a	163.77±0.09 ^a	162.26±0.03 ^a	165.03±0.03 ^a	165.78±0.17 ^a	166.81±0.07 ^a
PER	0.90±0.08 ^a	2.07±0.05 ^a	2.72±0.06 ^a	1.66±0.04 ^a	1.17±0.05 ^a	1.52±0.01 ^a	1.33±0.09 ^a	2.04±0.02 ^a	2.59±0.07 ^a	1.82±0.03 ^a
NPU	1.05±0.01 ^a	1.97±0.06 ^a	2.23±0.05 ^{ab}	1.85±0.07 ^a	1.60±0.03 ^a	1.57±0.03 ^a	1.50±0.06 ^a	1.65±0.06 ^a	1.80±0.03 ^a	1.70±0.04 ^a
SR	96.57±0.09 ^b	97.46±0.08 ^b	98.55±0.03 ^b	98.23±0.02 ^b	96.77±0.16 ^b	97.09±0.26 ^b	96.89±0.08 ^b	98.03±0.13 ^b	98.10±0.04 ^b	97.15±0.08 ^b

Values are mean ± standard error. Values in the same row with different superscripts are significantly different ($p<0.05$)

Initial Body Weight (IBW) (g); Final body weight (FBW) (g); Weight gain (g) = final body weight (FBW) - initial body weight (IBW); Relative growth rate (RGR) (%/day); Feed intake (FI) (G fish)= (Total feed consumption (g)/ number of fish); Protein efficiency ratio (PER)(%); Net Protein Utility (NPU) (%) = Weight gain (Wg)/ Protein fed x 100% Survival Rate (SR) (%)

Table 7: Body composition of tilapia larvae fed with *Tubifex tubifex* mass cultured using various fermented wastes.

Crude fiber	7.55±0.17	4.17±0.09	4.23±0.06	5.98±0.07	7.73±0.09	9.13±0.08	8.89±0.11	8.57±0.17	6.54±0.15	7.89±0.20
Crude protein	53.16±0.03	65.13±0.14	67.53±0.13	62.02±0.12	56.40±0.01	62.43±0.04	55.04±0.01	56.75±0.03	63.93±0.16	60.74±0.03
Carbohydrate	17.85±0.05	13.48±0.11	10.84±0.10	14.94±0.05	15.71±0.03	10.77±0.10	9.20±0.05	13.68±0.09	9.18±0.17	10.08±0.07
Fatty acid Profile										
Meristic	2.12± 0.05	6.35±0.07	10.15±0.04	5.75±0.12	3.15±0.13	5.75±0.11	3.26±0.06	4.35±0.15	7.81±0.07	5.23±0.04
pentadecanoic	1.56±0.04	3.03±0.09	6.23±0.07	3.19±0.08	1.17±0.12	3.19±0.04	2.12±0.01	1.26±0.04	5.25±0.08	3.17±0.07
Palmitic	3.26±0.08	5.12±0.08	7.02±0.03	6.26±0.11	4.17±0.09	3.23±0.03	3.09±0.05	6.86±0.11	6.91±0.21	5.15±0.02
Stearic	2.17±0.09	4.55±0.07	5.75±0.15	3.25±0.02	3.09±0.03	3.02±0.11	2.23±0.08	3.17±0.15	4.28±0.02	2.09±0.08
arachidat	0.15±0.14	1.39±0.06	1.90±0.02	2.23±0.04	0.89±0.05	1.17±0.03	1.35±0.10	1.75±0.09	0.65±0.05	0.25±0.03
Σ SAFA	9.26±0.07	20.44±0.03	31.05±0.08	20.68±0.02	12.47±0.06	16.36±0.03	12.05±0.09	17.39±0.06	24.90±0.04	15.89±0.02
Palmitoleic	4.17±0.08	5.29±0.07	6.16±0.03	5.95±0.02	3.17±0.07	4.19±0.15	3.76±0.06	4.90±0.03	5.75±0.06	4.15±0.03
Oleic	10.16±0.09	17.08±0.02	20.26±0.06	16.07±0.02	12.12±0.07	14.06±0.06	13.35±0.04	15.11±0.07	17.10±0.06	17.06±0.05
eikosenoic	0.23±0.08	0.89±0.03	0.45±0.08	0.23±0.01	0.19±0.02	0.16±0.09	0.29±0.03	0.75±0.08	0.44±0.09	0.26±0.04
Behenat	1.19±0.06	1.90±0.15	3.25±0.09	2.88±0.05	0.23±0.02	1.43±0.03	0.96±0.05	0.76±0.02	0.59±0.04	1.19±0.04
Σ MUFA	15.75±0.09	25.16±0.06	30.12±0.08	25.13±0.04	15.71±0.01	19.84±0.06	18.36±0.09	21.52±0.08	23.88±0.03	22.92±0.01
Linoleic	3.45±0.04	6.08±0.03	11.33±0.06	5.23±0.07	3.26±0.03	3.26±0.02	2.17±0.07	3.86±0.08	9.44±0.08	4.58±0.09
Arachidonat	0.58±0.5	1.28±0.02	1.43±0.08	0.89±0.04	0.66±0.02	0.98±0.09	0.81±0.07	1.26±0.02	1.55±0.06	1.33±0.05
Linolenic	0.48±0.09	3.19±0.06	3.23±0.04	2.90±0.02	0.23±0.01	1.26±0.08	1.01±0.03	1.61±0.13	2.68±0.04	1.70±0.06
EPA	0.67±0.09	1.13±0.07	2.09±0.08	1.95±0.02	0.45±0.05	0.52±0.08	1.26±0.09	0.17±0.11	1.99±0.01	0.49±0.01
DHA	0.04±0.02	0.49±0.12	1.55±0.01	0.87±0.05	0.63±0.08	0.47±0.02	0.42±0.05	0.59±0.06	0.67±0.08	0.51±0.07
Σ PUFA	5.22±0.08	12.17±0.09	19.63±0.05	11.84±0.03	5.23±0.07	6.49±0.02	5.67±0.03	7.49±0.04	16.33±0.01	8.61±0.03
Amino acid										
Aspartic	2.37 ±0.03	2.45±0.06	3.04±0.07	2.39±0.04	1.23±0.03	1.98±0.08	1.19±0.03	2.08±0.06	2.96±0.03	2.14±0.03
Threonine	1.17±0.01	1.90±0.08	2.99±0.09	2.78±0.05	1.73±0.05	2.26±0.09	1.53±0.04	1.16±0.02	2.75±0.03	2.04±0.07
Serine	2.10±0.09	1.08±0.03	2.86±0.01	2.07±0.08	1.02±0.01	1.93±0.05	1.16±0.09	1.89±0.05	2.54±0.02	1.94±0.03
Glutamic	2.88±0.17	2.90±0.05	3.07±0.02	2.86±0.11	2.48±0.09	2.83±0.02	2.09±0.01	2.35±0.05	2.98±0.01	2.60±0.09
Glycine	0.15±0.02	1.46±0.08	2.08±0.09	1.97±0.05	1.23±0.05	1.18±0.09	1.98±0.06	1.13±0.03	2.06±0.05	1.98±0.07
Alanine	1.28±0.01	1.89±0.02	3.98±0.04	2.26±0.08	1.32±0.07	2.26±0.09	1.08±0.06	2.17±0.06	3.17±0.09	2.97±0.05
Cystein	1.35±0.03	1.79±0.06	2.23±0.02	1.95±0.03	1.10±0.09	1.81±0.04	1.54±0.04	1.39±0.03	2.24±0.07	1.75±0.01
Valine	2.35±0.26	3.98±0.09	5.17±0.07	3.42±0.04	2.75±0.01	3.94±0.19	3.35±0.08	3.44±0.09	4.73±0.03	3.28±0.03
Methionine	1.15±0.17	1.75±0.02	3.25±0.01	2.26±0.01	1.12±0.06	2.78±0.05	1.45±0.08	1.74±0.02	3.08±0.05	1.48±0.05
Isoleucine	4.35±0.03	4.97±0.03	5.96±0.03	4.76±0.06	4.11±0.03	2.59±0.07	4.37±0.04	4.93±0.07	5.32±0.09	4.28±0.07
Leucine	2.88±0.09	3.73±0.09	5.66±0.06	5.01±0.04	2.52±0.01	3.89±0.08	3.68±0.04	3.28±0.05	4.97±0.09	3.55±0.05
Tyrosine	1.25±0.10	1.82±0.05	2.03±0.02	1.96±0.07	1.52±0.01	1.94±0.03	1.13±0.07	1.55±0.02	1.84±0.04	1.23±0.01
Phenylalanine	2.19±0.02	2.90±0.01	3.56±0.05	2.86±0.02	1.98±0.02	2.64±0.09	2.09±0.07	2.76±0.05	3.66±0.03	2.98±0.08
Lysine	1.16±0.09	3.97±0.08	5.97±0.08	3.96±0.06	2.19±0.02	3.89±0.01	2.96±0.07	3.74±0.08	4.91±0.17	3.89±0.01
Histidine	2.07±0.02	2.85±0.07	2.93±0.07	2.08±0.07	1.98±0.06	2.13±0.01	1.74±0.08	2.39±0.04	2.98±0.04	2.32±0.05
Arginine	1.75±0.05	2.06±0.09	2.67±0.01	2.13±0.05	1.17±0.09	1.98±0.09	1.54±0.01	1.99±0.09	2.37±0.03	1.88±0.01
Proline	0.23±0.03	1.09±0.15	2.35±0.09	1.98±0.01	0.93±0.03	1.74±0.08	1.65±0.13	1.44±0.03	2.64±0.06	2.49±0.09
Tryptophan	0.15±0.03	0.19±0.03	0.20±0.06	0.17±0.02	0.10±0.06	0.03±0.08	0.13±0.08	0.19±0.08	0.15±0.02	0.12±0.09
EAA	18.72±0.08	28.06±0.02	37.72±0.06	29.26±0.09	20.00±0.01	26.09±0.09	22.43±0.07	25.18±0.05	34.30±0.01	25.17±0.09
TAA	30.83±0.07	42.78±0.03	60.00±0.09	44.48±0.02	30.48±0.04	41.80±0.02	35.74±0.04	39.62±0.12	55.26±0.07	42.92±0.05

Profile of fatty acids both in *T. tubifex* and body composition of Nile tilapia larvae were shown in Table 5 and 7. The T₂ treatment was gave higher value of fatty acids on *T. tubifex*, with the dominance fatty acids were meristic acid, pentadecanoic acid, stearic acid, palmitoleic acid, oleic acid, behenic acid, linoleic acid, EPA (ω-3) and DHA (ω-6) (Table 5). The different of animal manure fermentation had significant differences ($p<0.05$) to the total saturated fatty acids (SAFA) except T₄

and T₆; and total monounsaturated fatty acids (MUFA) except T₆. Transfer nutrition especially fatty acids into Nile tilapia larvae were evaluated (Table 7), the highest level of total SAFA, MUFA and polyunsaturated fatty acids (PUFA) were obtained on T₂ which dominated by meristic acid, pentadecanoic acid, palmitic acid, stearic acid, palmitoleic acid, oleic acid, behenic acid, linoleic acid, linolenic acid, EPA, and DHA.

The survival rate of tilapia fed with *T. tubifex* mass cultured using animal

manures did not have significant differences ($p>0.05$) compared to the larvae that were fed with *T. tubifex* without any animal manures in its culture media (Table 6). The biomass, growth, and survival rate were found to be highest at 18.25%, 2.78 g and 98.55%, respectively in the tilapia fed with *T. tubifex* cultured with 50 g L⁻¹ of quail manure+100 g L⁻¹ of rice bran+ 50 g L⁻¹ of tofu waste (T₂). The level of feed intake and PER of tilapia fed with *Tubifex* sp. cultured using the same treatment (T₂) was at 110.26% in the first week, 167.23% in the second week, and 2.73% for PER (Table 6).

Discussion

The use of quail manure could provide the highest level of tubifex growth and biomass due to the higher level of nutrients (N: 2.4%, P: 1.67%, and K: 1.95%), compared to goat or chicken manure. Based on Damle and Chari (2011), the N, P, and K content in chicken manure were 2.86%, 0.31% and 0.23%; quail manure it is 3.19%, 1.37% and 0.21%, while goat manure contains 2.38%, 0.07% and 0.18% respectively. Pilot *et al.* (2014) reported that the N, P, and K content of rice bran were 1.71%, 1.10%, and 0.26–0.27%, respectively. Based on Saravanan *et al.* (2015) stated that the high levels of nutrients such as N, P, and K in culture media could increase the nutrition supply, which later on, will affect the growth rate and biomass weight of tilapia. This statement was supported with study conducted by Yuniwati *et al.* (2012) investigating the ability of the nutrient in the culture

media to determine the quality of nutrition and the quantity of *T. tubifex* as the source of natural feed in Nile tilapia hatchery production. An important factor determining the nutrition quality of *T. tubifex* is the media which used for its mass culture. The fermentation process aims to produce high quality, nutrition, and biomass weight of *T. tubifex*. This is achieved with the help of probiotic bacteria that shorten the long chain of C and N in the nutrient media, to make it easier to be absorbed and used by *T. tubifex*. This statement is in line with previous study conducted by Nwachi (2013) and Abu *et al.* (2013) stated that the fermentation is used to obtain higher nutritional quality of feed. Reported by Zahidah and Subhan (2012) and Elissen *et al.* (2015), the culture media that experiences a fermentation process will have higher nutrient content and will be more easily taken up by zooplanktons. Nutrients in the culture media may include N and P that can increase the number of probiotic bacteria as the source of feed for *T. tubifex*. This, in turn, may improve the quality of nutrients, fulfilling the nutrient requirements for tilapia larvae and improving their growth. Pandriyani *et al.* (2012) and Elissen *et al.* (2015) stated that the organic substance in culture media can increase the number of bacteria and organic particles, and the decomposition by these bacteria can increase the nutrient supply in culture media. Elissen *et al.* (2015), showed that the feed supply in the culture container would affect the growth rate of *T.*

tubifex. Conversely, the lack of feed supply in the media can hinder reproduction. Damle and Chari (2011) and Yuniwati *et al.* (2012) stated that the process of fermentation in culture media can affect both the density and biomass.

The nutrients of culture media determine both the quality of the nutrition and the quantity of *T. tubifex*. The result showed that *T. tubifex* cultured using animal manures provides a higher quantity of *T. tubifex*, both in population density and the biomass weight, compared to *T. tubifex* cultured without animal manures. This were supported with a study conducted by Yuniwati *et al.* (2012) that investigating the ability of the nutrient in the culture media to determine the quality of nutrition and the quantity of *T. tubifex* as the source of natural feed in hatchery production. Utilization of *T. tubifex* as a natural feed has recently been applied to catfish (*Clarias gariepinus*). In this research, the *T. tubifex* had been cultured using the fermentation of various animal manures as its culture media for the tilapia larvae feed. The highest growth and biomass increase (18.25%/day and 2.78g) was found in tilapia fed with *T. tubifex* cultured with 50 g L⁻¹ of quail manure+100 g L⁻¹ of rice bran+50 g L⁻¹ of tofu waste (T₂). This result showed a higher increase, compared to Herawati *et al.* (2015), which conducted a research about tilapia fed with *Daphnia magna*, cultured using the organic fertilizer fermented with probiotic bacteria. They showed a growth rate of 10.98%/day and 0.32 g weight (Herawati *et al.*,

2015). In the study of Anggraeni and Abdulgani (2013) an increase in length and weight of 0.271%/day and 1.59 g was noted in the marble goby (*Oxyeleotris marmorata*) fed with fresh *Tubifex* sp. cultured in a media without any fermentation. Mahfuj *et al* (2012) stated that growth rate of 56.66 ± 2.29% was noted in the Carp (*Cyprinus carpio*) larvae fed with fresh *Tubifex* sp.

Proximate composition of animal manures and body composition of Nile tilapia larvae fed with *T. tubifex* was shown in Table 3. The similar report has been found by Damle and Chari (2011), with dried tofu waste contains 27.09% crude protein, 22.85% crude fiber, 7.37% fat, 35.02% ash, 6.87% nitrogen-free extract, 0.5% calcium, and 0.2% P (Damle and Chari (2011), and also Herawati and Agus (2014), showed that rice bran contains 13% crude protein, 4.7% crude fiber, 3.1% K, and 0.65% P.

The improvement of the nutrition quality that occurred in the body composition of tilapia fed with *T. tubifex* cultured without animal manures was 53.16% protein, ∑ PUFA 5.22%, and 18.72% of essential amino acids. Meanwhile, the body composition of tilapia fed with *T. tubifex* cultured using various animal manures was in the range of 56.75–67.53% for protein, 5.23–19.63% for ∑ PUFA, and 20.00–37.72% for essential amino acids. The improvement of nutrition quality in the body composition was due to the quality of the nutrition in *T. tubifex* suitable with which is needed by tilapia. It can improve the nutrition quality of its

body. Birol *et al.* (2015) explained that the feed intake suitable for the needs can increase the nutrition of the body composition – thus causes optimal growth achieved by fish. This statement is in line with Pimolrat *et al.* (2015) and Saravan *et al.* (2015) stated that the feed suitable with the nutritional needs as well as optimal water quality can improve both growth and the quality of the nutrition in the body composition of tilapia.

The growth of tilapia can be affected by the nutritional quality of feed suitable for the needs of the larvae. Thus, the nutrition intake can be optimally used for growth (Asadi *et al.*, 2012; Pilot *et al.*, 2014). Nutrition from the natural feed (*T. tubifex*) consumed by the larvae will be absorbed as an energy source for metabolism and to repair damaged body tissue. This statement is supported by the result showing that the level of feed consumption and the protein efficiency ratio (PER) of tilapia larvae fed with *T. tubifex* cultured using fermentation of various animal manures had a significant effect ($p < 0.05$) on both growth and survival rate of the larvae.

The survival rate of tilapia fed with *T. tubifex* mas cultured using the fermentation of various animal manures provided no effect ($p > 0.05$) compared to the ones given with *T. tubifex* with mass culture without using the fermented animal manures. The range of the survival rate ranged from 96.57% to 98.55% in Herawati *et al.* (2015), which the highest survival rate was 98% at 50 g L⁻¹ of quail manure+100 g L⁻¹ of rice bran+50 g L⁻¹ of tofu waste

treatment (T₂). This was highly related to the suitable water quality and the environmental surroundings, thus not giving any influence in the research process. The water quality under optimal conditions could be helpful in the growth process of tilapia (Nina *et al.*, 2012). Mahfuj *et al.* (2012) also explained that a suitable water quality can accelerate the growth process, while an unsuitable water quality could hinder the growth process of tilapia larvae.

The high level of feed consumption and PER showed a correlation with high growth and biomass among treatments. This was due to the increased nutrient quality of the *T. tubifex*, as well as its size, which was both suitable for tilapia larvae, enabling an adequate deposition of nutrients. This can be seen in the increased growth rate and biomass. Pilot *et al.* (2014), stated that the level of feed intake in the fish feed is the most important factor in determining the growth rate and the biomass weight. This statement is supported by Melianawati *et al.* (2012) and Herawati *et al.* (2015) who showed that a number of essential factors, which impact the level of natural feed, are related to the form of suitable natural feed for larvae, the species, the size of the natural feed for fish, nutrient values and the feed dosage.

The high growth rate and biomass of tilapia fed with *T. tubifex* cultured using 50 g L⁻¹ of quail manure+100 g L⁻¹ of rice bran+50 g L⁻¹ of tofu waste (T₂) were due to the high content of nutrition of *T. tubifex* in the culture

media. The nutrition in the form of high protein and fat is used as a source of energy as well as to repair damaged cell tissue during growth. This further resulted in increased growth and survival rate of the tilapia larvae. Nina *et al.* (2012), and Ovie and Eze (2013) stated that protein is very important for the larvae, especially at the initial stage, because it has function as a source of energy and has an important role in improving the body tissue. Gao *et al.* (2011) and Pilot *et al.* (2014) stated that the nutritional needs of *O. niloticus* larvae for protein are 50% and 7% for fat. Similarly, Ovie and Ezze (2013) stated that the need for protein was 55% and that for fat was 8%.

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References

- Abu-Elala, N. and Marzuok, Moustafa. 2013. Use of different *Saccharomyces cerevisiae* biotic form as immune modulator and growth promoter for *Oreochromis niloticus* challenged with some fish pathogens. *International Journal of Veterinary Science and Medicine*, 1, 21-29.
- Anggraeni, N.M. and Abdulgani, N., 2013. Effect of natural feed and artificial feed on the growth of Marble Goby fish (*Oxyeleotris marmorata*) on a laboratory scale. *ITS Science and Art Journal*, 2(2), pp. E197-E201.
- AOAC. 1999. Association of official analytical chemists. Official methods of analysis of the association of official analytical chemists. Washington. Method No. 92307.
- AOAC, W. Horwitz. (Ed.), 2000. Official methods of analysis, 13th Ed. Academic Press. Washington DC. USA. 925.09.
- Asadi, Rad., Zakeri, M.M., Vahid, Y. and Seyed, S.M., 2012. Effect of dietary levels of dietary supplementation of *Saccharomyces cerevisiae* on growth performance, food utilization and body biochemical composition of Nile tilapia (*Oreochromis niloticus*) fingerlings. *Journal of the Persian Gulf*, 3, 15-24
- Birol, B., Sedat, G. and Dilara, K., 2015. Comparison of food, amino acid and fatty acid composition of wild and cultivated bass (*Dicentrarchus labrax* L. 1758). *Turkish. Journal of Fisheries and Aquatic Science*, 15, 175-179.
- Chepkirui-Boit, V., Ngugi, C.C., Bowman, J., Oyoo-Okoth, E., Rasowo, J., Mugo-Bundi, J. and Cherop, L., 2011. Growth performance, survival, feed utilization and nutrient utilization of African catfish (*Clarias gariepinus*) larvae co-fed Artemia and a micro-diet containing freshwater atyid shrimp (*Caridina nilotica*) during weaning. *Aquaculture Nutrition*, 17, 82-89.
- Damle, and Chari, M.S., 2011. Performance evaluation of different animal residue on culture of

- Daphnia* sp. *Journal of Fisheries and Aquatic Science*, 6, 57-61.
- Devic, E., Leschen, W., Murray, F. and Little, D.C., 2017.** Growth performance, feed utilization and body composition of advanced nursing Nile tilapia (*Oreochromis niloticus*) fed diets containing black soldier fly (*Hermetia illucens*) larvae meal. *Aquaculture Nutrition*, 2017, 1-9.
- Elissen, T.L.G., Hendrickx, H., Temmink, B., Laarhoven, C.J.N. and Buisman, 2015.** Worm-it: converting organic wastes into sustainable fish feed by using aquatic worms. *Journal of Insects as Food and Feed*, 1(1), 67-74.
- Faruque, M.M., Ahmed, M.K. and Quddus, M.M.A., 2010.** Use of live food and artificial diet supply for the growth and survival of African catfish (*Clarias gariepinus*) larvae. *World Journal of Zoology*, 5(2), 82-89.
- Fish Stat , 2016.** Database and software for fishery statistical analysis. United Nations FAO. Accessed from <<http://www.fao.org/fishery/statistics/software/fishstatj/en>>.
- Gao, W., Tian, L., Liang, M., Huai, Y. and Luo., 2011.** Protein sparing capability of dietary lipid in herbivorous and omnivorous freshwater finfish: a comparative case study on *Ctenopharyngodon idella* and *Oreochromis niloticus*. *Journal of Aquatic Nutrition*, 17, 2-12.
- Guimarães, I.G., Pezzato, L.E. and Barros, M.M., 2008.** Amino acid availability and protein digestibility of several protein sources for Nile tilapia, *Oreochromis niloticus*. *Aquaculture Nutrition*, 14, 396-404.
- Hamre, K., 2016.** Nutrients profiles of rotifers (*Brachionus* sp.) and rotifer from four different marine fish hatcheries. *Aquaculture*, 450, 136-142.
- Herawati, V.E. and Agus, M., 2014.** Analysis growth and survival of catfish larvae feed *Daphnia* sp. in mass culture using fermented organic fertilizer. *Journal of Science and Technology*, 26, 1-11.
- Herawati, V.E., Pinandoyo, Hutabarat, J. and Radjasa, O.K., 2015.** Growth and survival rate of tilapia (*Oreochromis niloticus*) larvae fed by *Daphnia magna* cultured with organic fertilizer resulted probiotic bacteria fermentation. *HAYATI Journal Bioscience*, 22, 123-129.
- Lim, C.M., Aksoy, Y. and Klesius, P., 2011.** Lipid and fatty acid requirements of tilapia, North Amerika. *Journal Aquatic International*, 73, 188-195.
- Liti, D.M., Mugo, R.M., Munguti, J.M. and Waidbacher, H., 2006.** Growth and economics performance of Nile tilapia (*Oreochromis niloticus* L.) fed on three brans (maize, wheat and rice) in fertilized ponds. *Aquaculture Nutrition*, 12, 239-245.
- Mahfuj, M.A., Hossain, M.G. and Sarower., 2012.** Effect of different feeds on larval development and survival of ornamental koi carp, *Cyprinus carpio* (Linnaeus, 1758) larvae in laboratory condition.

- Journal of the Bangladesh Agricultural University*, 10(1), 179–183.
- Melianawati, R., Astuti, N.W.W. and Slamet, B., 2012.** The growth pattern of blacksaddled coralgrouper larvae (*Plectropoma laevis* Lacepède, 1801) and their consumption rate to zooplankton rotifer (*Brachionus rotundiformis*). *Journal of Tropic Marine Science Technology*, 4(2), 217-228.
- Nina, S., Givskov, J. and Martin, D., 2012.** The potential of dietary polyunsaturated fatty acid to modulate eicosanoid synthesis and reproduction in *Daphnia magna*. *Journal Physiology*, 162, 449-454.
- Nwachi, O.F. 2013.** An Overview of the importance of pro-biotic in aquaculture. *Journal of Fish Aquatic Science*, 8, 30-32.
- Ovie, S.O and Eze, S.S. 2013.** Lysine requirement and its effect on body composition of *Oreochromis niloticus* fingerlings. *Journal of Fish Aquatic Science*, 8, 94-100.
- Pandriyani., Yanetri, A.N. and Hadi, D.A., 2012.** Effectiveness doses trichompos dregs know and manure. *Journal Industria*, 13, 15-25.
- Park, P.W. and Goins, R.E. 1994.** In situ preparation of fatty acids methyl ester for analysis of fatty acids composition. *Food Science Journal*, 59, 122-136.
- Pilot, A.I., Sumi, K.R., Sharker, R., Mandal., S.C., Rahman, M.R. and Alam, M.R., 2014.** Effect of different live feeds on the growth performance and flesh quality of Nile tilapia (*Oreochromis niloticus*) fingerling. *International. Journal of Life Science Bt and Pharm Res*, 3(2), 180-188.
- Pimolrat, P., Whangchai, N., Chitmanat, C., Itayama, T. and Lebel, L., 2015.** Off-flavor characterization in high nutrient load tilapia ponds in northern Thailand. *Turkish Journal of Fish and Aquatic Science*, 15, 275-278.
- Saravanan, R., Samyappan, K., Gnanavel, K. and Purushothaman, K., 2015.** Studies on the Nutritive value and fatty acid contents in fingerling of *Catlacatla* fed on traditional diet and freeze dried *Tubifex*. *International Journal of Information Research and Review*, 2(5), 726-733.
- Utama, C.S., Nyoman, S., Bambang S. and Bhakti, E.S., 2013.** Utility of rice bran mixed with fermentation extract of vegetable waste unconditioned as probiotics from vegetable market. *International Journal of Science and Engineering*, 4(2), 97-102.
- Yuniwati, M., Iskarima, F. and Padulemba, A., 2012.** Optimization of compost production process condition from organic waste by fermentation using EM4. *Journal of Technology*, 5, 172-181.
- Zahidah., Gunawan, W. and Subhan, V. 2012.** Analysis of population and growth of *Daphnia* sp. in floating cages culture at Cirata reservoirs with waste fertilizers fermented EM4. *Journal of Aquatic Science*, 3, 84-94.