

## Profile of amino acids, fatty acids, proximate composition and growth performance of *Tubifex tubifex* culture with different animal wastes and probiotic bacteria

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Abstract. Culture media will play an essential role towards the quality and the biomass production of Tubifex tubifex. The abundance of livestock wastes cannot be utilized but with the fermentation of various livestock wastes it is possible to be used as the cultivation media of *T. tubifex*. The aim of this research is to find out the best culture medium with different animal wastes fermented using the probiotic bacteria to increase the profile amino acid, fatty acid, proximate composition and growth of T. tubifex. The method used was the complete randomized design. Treatment in the study used 10 treatments with three replications, T<sub>0</sub> is the comparator treatment. Media culture used chicken manure, goat manure, guail manure, rejected bread and tofu waste fermented with the probiotic bacteria; early planting with 10 g/container and maintenance of T. tubifex within 50 days. The result of this research showed a significant effect (p < 0.05) on the growth and biomass of T. tubifex. The most significant growth was found through the dense population and the biomass of T. tubifex at 42306.16 ind  $L^{-1}$  and 172.19 gr, respectively cultured with the mixture of fermentation of media of 50 gr L<sup>-1</sup> of quail manure, rejected bread, and 50 gr L<sup>-1</sup> tofu waste. The highest protein and fat were at 66.26% and 100 g L<sup>-</sup> 12.79% in the same medium, respectively and finally the highest profile of amino acid and fatty acid was found in lysine at 3.63%; linoleic acid at 7.25% and linolenic acid at 6.19%. In general, the content of ammonia, DO, temperature, and pH during the research were in the reasonable ranges of the T. tubifex life.

Key Words: nutrient value, growth, *Tubifex*, animal waste, probiotic bacteria, culture.

**Introduction**. *Tubifex tubifex* is the best natural food for fish and prawn due to its size and nutritional content that is appropriate with their needs. As revealed from the study by Rech et al (2013) the nutritional content of *T. tubifex* included protein (50-55%), fat (8-10%), crude fibre (2-5%), ash level (4-7%) and water (8-10%). Compared to other natural food such as *Daphnia magna*, the nutrient content of *T. tubifex* is higher and has an equal nutritional quality compared to *Artemia* sp. (Oplinger et al 2011).

Today, the needs for *T. tubifex* as a natural food are relied highly upon the natural and seasonal capture. The existence of *T. tubifex* could be found in gutters, trenches and farmland and obtained by filtering the mud of river, gutters and trenches purposely to maintain the sustainable quality and the quantity of *T. tubifex* for fulfilling any demand as the natural food (Bruggemann 2012). In this case, the culture media will play an essential role towards the quality and the biomass production of *T. tubifex*.

The use of organic fertilizers in culture media including the wastes/faeces of quail, goat and chicken mixed with the rejected bread and tofu waste fermented with the probiotic bacteria has not so far been conducted as the use of organic fertilizer could impact the growth performance and content of *T. tubifex.* The highest nutrients - particularly for the content of N, P and Ca in organic fertilizer are the food sources of *T. tubifex.* Damle & Chari (2011) in their study explained the chicken waste containing N

(2.86%); P (0.31%) and Ca (0.23%); quail waste containing N (3.19%); P (1.37%) and Ca (0.21%); and goat waste/faeces containing N (2.38%); P (0.07%) and Ca (0.18%). Furthermore, the analysis on the dried materials of tofu waste based upon the research by Liswahyuningsih et al (2011) contained crude protein (27.09%), crude fibre (22.85%), fat (7.37%), ash (35,02%), and extract material without nitrogen/BETN (6.87%). Purbowati et al (2007) explained that the rejected bread contained the crude protein (12.63%), crude fibre (0.13%); crude fat (4.63%); ash (4.19%) and the extract material without nitrogen (58.42%.)

The fermentation of the fertilizer has been proven to be effective in the increase of the nutrient of culture media. Nwachi (2013) in their research stated that the fermented organic fertilizer would be easily digested and used for having experienced any alteration by the probiotic bacteria. The process of fermentation as stated by Zahidah et al (2012) and Nwaichi (2013) refers to a process of breaking the organic compound into the simple compound involving a microorganism. The aims of the fermentation are to produce a product (food materials) that contains the nutrients, to have a longer storage time, and to have a better organoleptic characteristics and nutritional components.

The aim of this research is to find out the performance of *T. tubifex* growth through a dense population, biomass production and proximate composition, profile of amino acid and fatty acid of *T. tubifex* massively cultured and fermented by the probiotic bacteria as the organic fertilizers.

The benefit of this research was to find out the best nutritional content and the performance of *T. tubifex* growth massively cultured using fermentation as the different animal waste.

**Material and Method**. The first phase consists of the preparation of fermented molasses, water, and probiotic activator which are bacteria *Sacharomyces cerevisae* and *Lactobacillus* sp. contained therein. The ratio used was 1 mL of both molasses and probiotic bacteria, and 100 mL of water. The organic materials used were quail manure, goat manure, chicken manure, expired bread, and tofu waste and all of these were dried. The 10 treatments ( $T_0$ - $T_9$ ) included:  $T_0$  - 100 gr L<sup>-1</sup> of expired bread, 50 gr L<sup>-1</sup> of tofu ;  $T_1$  - 25 gr L<sup>-1</sup> of quail manure, 100 gr L<sup>-1</sup> of expired bread, 50 gr L<sup>-1</sup> of tofu waste;  $T_2$  - 50 gr L<sup>-1</sup> of quail manure, 100 gr L<sup>-1</sup> of expired bread, 50 gr L<sup>-1</sup> of tofu waste;  $T_3$  - 75 gr L<sup>-1</sup> of quail manure, 100 gr L<sup>-1</sup> of expired bread, 50 gr L<sup>-1</sup> of tofu waste;  $T_4$  - 25 gr L<sup>-1</sup> of goat manure, 100 gr L<sup>-1</sup> of expired bread, 50 gr L<sup>-1</sup> of tofu waste;  $T_5$  - 50 gr L<sup>-1</sup> of goat manure, 100 gr L<sup>-1</sup> of expired bread, 50 gr L<sup>-1</sup> of tofu waste;  $T_6$  - 75 gr L<sup>-1</sup> of goat manure, 100 gr L<sup>-1</sup> of expired bread, 50 gr L<sup>-1</sup> of tofu waste;  $T_7$  - 25 gr L<sup>-1</sup> of chicken manure, 100 gr L<sup>-1</sup> of expired bread, 50 gr L<sup>-1</sup> of tofu waste;  $T_7$  - 25 gr L<sup>-1</sup> of chicken manure, 100 gr L<sup>-1</sup> of expired bread, 50 gr L<sup>-1</sup> of tofu waste;  $T_7$  - 25 gr L<sup>-1</sup> of chicken manure, 100 gr L<sup>-1</sup> of expired bread, 50 gr L<sup>-1</sup> of tofu waste;  $T_7$  - 25 gr L<sup>-1</sup> of chicken manure, 100 gr L<sup>-1</sup> of expired bread, 50 gr L<sup>-1</sup> of tofu waste;  $T_8$  - 50 gr L<sup>-1</sup> of chicken manure, 100 gr L<sup>-1</sup> of expired bread, 50 gr L<sup>-1</sup> of tofu waste;  $T_8$  - 50 gr L<sup>-1</sup> of chicken manure, 100 gr L<sup>-1</sup> of expired bread, 50 gr L<sup>-1</sup> of tofu waste;  $T_9$  - 75 gr L<sup>-1</sup> of chicken manure, 100 gr L<sup>-1</sup> of expired bread, 50 gr L<sup>-1</sup> of tofu waste;  $T_9$  - 75 gr L<sup>-1</sup> of chicken manure, 100 gr L<sup>-1</sup> of expired bread, 50 gr L<sup>-1</sup> of tofu waste;  $T_9$  - 75 gr L<sup>-1</sup> of chicken manure, 100 gr L<sup>-1</sup> of expired bread, 50 gr L<sup>-1</sup> of tofu waste;  $T_9$  - 75 gr L<sup>-1</sup> of chicken manure, 100 gr L<sup>-1</sup> of expired bread, 50 g

**T. tubifex population and biomass.** The dispersion of *T. tubifex* worms had a density of 10 gr container. The container used in this study consist of 30 trays with area size (height) of each container is  $0.044 \text{ m}^2$  (4 cm). The number of worms was determined by extrapolating the number of worms found in a 1 g sample.

**Proximate analysis.** The proximate chemical composition of the samples was determined using a standart procedure (AOAC 2005). The crude protein content was calculated by multiplying the total nitrogen factor. The carbohydrate content was estimated by the difference.

*Essential amino acid profile.* The amino acid composition of the sample was determined using HPLC (Shimadzu LC-6A) (AOAC 2005).

*Fatty acid profile*. The fatty acid composition of the sample was determined using a gas cromatograph (Shimadzu) (AOAC 2005).

*Water quality*. The water quality during the study was maintained at  $28-29^{\circ}$ C temperature, 0.3 mg L<sup>-1</sup> dissolved oxygen (DO) and 8.1-8.2 pH. This is within the optimal water quality parameters for *T. tubifex* (25-30°C, 0.3-0.6 mg L<sup>-1</sup> DO, and a pH of 6.5-9), as suggested by Oplinger et al (2011); Schlotz et al (2012) and Elissen et al (2015).

*Statistical analysis.* Each batch of products was prepared twice. Determinations were carried out in triplicate and errors were reported as the standard deviation from the mean. The experimental design of this study was completely randomized design and a relevant statistically significant and additional analysis was done using the advanced Duncan test.

**Results and Discussion**. The culture medium is an important factor for the improvement of nutrient quality of *T. tubifex*. We found that the nutrient content in the culture medium, using the fermentation of different animal wastes, mixed with the tofu and expired bread, based on the highest N, P, and K in quail manure, showed values of 8.70%, 6.26%, and 5.75% for N, P, and K, respectively (Table 1).

Table 1

The content of N, P and K in media mass culture of *T. tubifex* using a variety of livestock wastes

	Poultry manure	Quail manure	Goat manure	Chicken manure
N (%)	$2.08^{a} \pm 0.08$	$8.70^{ab} \pm 0.02$	$4.53^{a}\pm0.02$	$5.88^{a} \pm 0.05$
P (%)	$1.37^{a} \pm 0.09$	$6.26^{a} \pm 0.06$	$2.35^{a}\pm0.07$	$4.19^{a} \pm 0.05$
K (%)	$1.65^{a} \pm 0.06$	$5.75^{a} \pm 0.09$	$2.14^{a}\pm0.02$	4.23 <sup>a</sup> ±0.07

The result of the research showed that T. tubifex cultured massively using the culture media of T2 provided a growth performance through the higher population and biomass that were 42306.16 ind L<sup>-1</sup> and 172.19 gr, respectively (Table 2) compared to *T. tubifex* cultured massively in the culture media not using the animal waste  $(T_0)$ . The use of quail waste has provided the higher growth and biomass as N: 8.70%; P: 6.26%; and Ca: 5.75% (Table 1) in the media were higher than the other culture media. The elements of N, P and Ca in the culture media were able to increase the number of probiotic bacteria as the food source of T. tubifex for the enhancement of the nutritional quality and the process of its propagation. This statement is in line with the result of the research by Elissen et al (2015), stating that N is the essential nutrient playing a role in the formation of amino acid, protein, and other compounds. T. tubifex can grow in a media containing the sufficient nutrients such as N, P, Ca and other micro elements. The population density and biomass of T. tubifex cultured through fermentation of various animal manures, were found to be highest (42306.16 ind  $L^{-1}$  and 172.19 g of biomass) in treatment 2 (T<sub>2</sub>). The results for the population density and biomass weight of T. tubifex are presented in Table 2, respectively.

The result of the research showed that the biomass of *T. tubifex* with the fermentation of various animal wastes was able to provide a significant effect (p < 0.05) on the biomass and the density of the *T. tubifex* population. The factors influencing the biomass and the density of the *T. tubifex* population include the food supply, quality of fertilizer and environment. The organic substances in the form of mixture of quail manure, rejected bread and fermented tofu waste can increase the number of bacteria and the organic particles as a result of the decomposition by the probiotic bacteria, thus being able to increase the nutrition supply in the media that will influence the population and the biomass production of silkworm. As stated by Rech et al (2013), and Ellissen et al (2015), food supply in the culture media will influence the growth rate of *T. tubifex*. In contrast, the lack of food supply in media can make the production ability hindered. The food supply in the treatment media was conducted by fertilization aimed to add the food source in the media of *T. tubifex* treatment.

Table 2

Treatments	Population of T. tubifex (ind $L^{-1}$ )	Biomass of T. tubifex (g)
TO	19692.38 <sup>a</sup> ±889.06	$124.59^{a}\pm0.05$
T1	31957.35 <sup>a</sup> ±769.48	156.29 <sup>a</sup> ±0.03
Τ2	42306.16 <sup>ab</sup> ±901.66	172.19 <sup>ab</sup> ±0.02
Т3	36028.92 <sup>a</sup> ±527.98	$163.17^{a} \pm 0.05$
Τ4	30961.91 <sup>a</sup> ±263.18	$119.58^{a} \pm 0.08$
Τ5	37216.24 <sup>a</sup> ±495.85	$143.8^{a}1\pm0.05$
Т6	$30823.47^{a} \pm 204.37$	$134.5^{a}1\pm0.01$
Τ7	$37080.57^{a} \pm 394.36$	$148.69^{a} \pm 0.02$
Т8	40830.93 <sup>a</sup> ±657.61	169.67 <sup>a</sup> ±0.04
Т9	35103.14 <sup>a</sup> ±2106.90	$152.44^{a}\pm0.06$

Growth population and biomass production in *T. tubifex* mass cultured by utilization of a variety of livestock wastes

Damle & Chari (2011) stated that the fertilizing process can bring an effect of the density and its biomass. Oplinger et al (2011) stated that the high level of N, P and Ca in media can increase the availability of nutrition; thus it can bring an effect on its population and biomass. This statement is supported in the results of the research by Elissen et al (2015), explaining that the nutrient in a culture media can determine the quality of the nutrient and the quantity of *T. tubifex* as the natural food source.

The fermentation of the fertilizer in media has been proven effective in the culture of *T. tubifex*. The process of the fermentation aims to increase the quality of the nutrient and biomass with the help of probiotic bacteria in the process of reorganizing the nutrient media to make it possible to be easily absorbed and used for improving the nutrient quality. Nwachi (2013) argued that the aim of the fermentation is to obtain the new food with higher quality of the nutrient. Zahidah et al (2012) stated that the culture media experiencing the fermentation process has higher nutrient content and will be easily absorbed by zooplankton, diatom and detritus using it as food. The result of this research is supported by the research of Bruggemann (2012) stating that the use of the fermented organic fertilizer has been proven to significantly increase the number of population and biomass of silkworms in comparison to the fertilizer of chicken waste without any fermentation. Another factor is the water quality during the maintenance period in a sufficient volume to make it possible to improve the guality of the nutrient and the performance of the T. tubifex growth through its population density and biomass (Elissen et al 2015). The results of the proximate composition of T. tubifex in mass culture using the different animal wastes fermented are presented in Table 3.

From the result of the research, the highest content of nutrient based upon the protein was 66.26% higher, that the result of the research conducted by Rech et al (2013) that was 44.33% protein. The high protein content is inversely proportional with the content of fat in this research as the higher contents of nitrate and phosphate can make its protein content higher and its lipid content lower. The result of the research is supported by the statement of Oplinger et al (2011); Mandila & Hidajati (2013) saying that the higher the concentration of nitrate and phosphate, the higher the protein content in it. The highest profiles of amino acids of *T. tubifex* were found in quail manure ( $T_2$ ) with the total amount of essential amino acid being 30.35% and the highest licine amino acid 3.63%. The profile of amino acid of *T. tubifex* is presented in Table 4.

The highest profile of essential amino acid in *T. tubifex* massively cultured in the culture media of T2 was lysine amino acid by 3.63%. According to Ovie & Eze (2013), and Baki et al (2015) lysine functions as the frame of forming B1 vitamin, antivirus, helping in calcium absorption, stimulating the appetite, assisting the carnitine production to change the fatty acid into energy. The most essential amino acids for the larvae of fish and prawn in accordance with the research of Ovie & Eze (2013) and Valverde et al (2013) included leucine, isoleucine, valine and lysine. The highest non-essential amino acid based upon the research was found in glutamic acid by 2.19%. This amino acid has an important function in the metabolism of sugar and fat, in the transportation of the

high amount of potassium and glutamine in blood and used as an energy source. This statement is in line with the result of the research of Valverde et al (2013) saying that the glutamate is the energy source and plays an essential role in the metabolism process to support the growing process.

Meanwhile, the highest profiles of fatty acid of T. tubifex were found in  $(T_2)$ , where the total of PUFA was 16.79%, the highest linoleic fatty acid was 7.25%, and the lowest was 1.45%; total MUFA 30.64% and total PUFA is 40.13% (Table 5). The highest profiles of fatty acid in a similar media with the palmitic, oleic and linoleic fatty acid were 17.52%; 27.26% and 7.25%, respectively. The palmitic fatty acid is a place of the energy source storage. This statement is in line with the result of the researches by Santoso et al (2015), and Saravanan et al (2015) saying that the largest energy source is in the palmitic fatty acid. Meanwhile, the oleic fatty acid is a substrate to form the long chain of PUFA as supported by the statement of Pratiwi et al (2009); Karakatsouli (2012) and Schlotz et al (2012) that the oleic fatty acid is the substrate in the process of denaturalization and the catalyst prolongation. The biosynthesis of PUFA is started from the oleic fatty acid then turning into the linoleic fatty acid as the basic substrate of the formation of the long chain of omega-6 and the linolenic acid as the basic substrate of formation of the long chain of omega-3. The analysis result of this research showed the highest range of linoleic acid and the linolenic fatty acid at 7.25% and 6.19%, respectively compared to the result of the research showing the range of 0.97% and 3.74%, respectively (Oplinger et al 2011; Elissen et al 2015).

**Conclusions**. The use of the culture media of 50 gr L<sup>-1</sup> quail manure, 100 gr L<sup>-1</sup> rejected bread, 50 gr L<sup>-1</sup> tofu waste ( $T_2$ ) for the mass culture of *T. tubifex* provided the quality of the nutrient and the growth performance through the highest level of population and biomass. The highest contents of the nutrient based upon the protein and fat were at 66.26% and 12.79%; based upon profile of fatty acid, the highest level of the palmitic, oleic and linoleic fatty acid were at 17.52%; 27.26% and 7.25%, respectively. Further, the highest profile of amino acid found in lysine was at 3.63%, the highest density of population was 42306.16 ind. L<sup>-1</sup> and biomass at 172.19 g.

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The result of the proximate composition of *T. tubifex* in mass culture using the different animal wastes

Proximate (%)	Poultry		Quail manure (%)			Goat manure (%)			Chicken manure (%)		
	manure (T <sub>0</sub> ) (%)	$T_1$	$T_2$	Τ <sub>3</sub>	$T_4$	$T_5$	Τ <sub>6</sub>	<i>T</i> <sub>7</sub>	T <sub>8</sub>	Τ <sub>9</sub>	
Crude ash	$7.82 \pm 0.05$	$5.98 \pm 0.07$	$5.06 \pm 0.08$	$4.31 \pm 0.06$	$9.01 \pm 0.09$	$7.57 \pm 0.09$	9.38±0.23	7.75±0.09	4.11±0.09	7.99±0.23	
Crude lipid	$8.62 \pm 0.06$	$10.24 \pm 0.08$	12.79±0.01	$11.03 \pm 0.09$	$10.85 \pm 0.02$	$11.67 \pm 0.03$	$11.30 \pm 0.06$	$11.25 \pm 0.02$	$12.14 \pm 0.03$	10.78±0.06	
Crude fiber	$8.55 \pm 0.10$	$6.45 \pm 0.03$	$4.07 \pm 0.02$	$6.98 \pm 0.07$	7.73±0.01	$7.13 \pm 0.05$	7.89±0.19	$5.37 \pm 0.01$	$4.54 \pm 0.05$	6.89±0.19	
Crude protein	56.11±0.17	$63.16 \pm 0.04$	66.26±0.23	64.17±0.01	$59.80 \pm 0.03$	$62.86 \pm 0.06$	$60.54 \pm 0.03$	62.75±0.03	$65.13 \pm 0.06$	$63.04 \pm 0.03$	
Carbohydrate	18.90±0.05	$14.17 \pm 0.10$	11.82±0.19	11.71±0.07	$12.61 \pm 0.09$	$10.77 \pm 0.10$	$10.89 \pm 0.05$	$12.88 \pm 0.09$	$13.98 \pm 0.10$	$11.30 \pm 0.05$	

Table 4

The result of the amino acid profile of *T. tubifex* in mass culture using the different animal wastes

Amino acid	Poultry	C	Quail manure (	(%)	Goat manure (%)			Chicken manure (%)		
	manure (T <sub>o</sub> ) (%)	$T_1$	$T_2$	Τ <sub>3</sub>	$T_4$	$T_5$	Τ <sub>6</sub>	<i>T</i> <sub>7</sub>	T <sub>8</sub>	T9
Aspartic acid	1.17±0.05	1.18±0.07	1.38±0.08	4.31±0.06	0.15±0.09	0.82±0.09	1.97±0.23	1.18±0.18	1.06±0.03	2.04±0.08
Serine	0.78±0.10	$0.96 \pm 0.03$	$1.33 \pm 0.02$	1.99±0.07	$0.32 \pm 0.01$	$0.83 \pm 0.05$	0.92±0.19	0.82±0.03	$0.84 \pm 0.04$	0.94±0.13
Glutamic acid	$1.57 \pm 0.17$	$1.76 \pm 0.04$	2.19±0.23	$1.81 \pm 0.01$	$1.58 \pm 0.03$	$1.63 \pm 0.06$	$1.98 \pm 0.03$	$1.71 \pm 0.06$	2.13±0.13	1.89±0.19
Glycine	$0.85 \pm 0.05$	$1.44 \pm 0.10$	$1.41 \pm 0.19$	$1.08 \pm 0.07$	$0.93 \pm 0.09$	$0.91 \pm 0.10$	$0.98 \pm 0.05$	$1.03 \pm 0.23$	1.96±0.25	$1.04 \pm 0.17$
Alanine	$1.02 \pm 0.03$	$1.15 \pm 0.23$	$3.44 \pm 0.02$	1.29±0.10	$1.22 \pm 0.01$	$0.94 \pm 0.17$	$0.92 \pm 0.01$	$1.07 \pm 0.26$	2.97±0.19	$1.17 \pm 0.03$
Cysteine	0.08±0.23	$0.16 \pm 0.26$	$1.16 \pm 0.03$	$0.11 \pm 0.13$	$0.10 \pm 0.04$	$0.10 \pm 0.03$	$0.10 \pm 0.09$	$0.09 \pm 0.02$	$0.09 \pm 0.17$	$0.10 \pm 0.01$
Arginine	$0.74 \pm 0.53$	$1.43 \pm 0.45$	1.46±0.09	$2.94 \pm 0.45$	$0.71 \pm 0.03$	$2.66 \pm 0.06$	$0.81 \pm 0.13$	0.99±0.19	$2.77 \pm 0.03$	0.88±0.31
Proline	$0.43 \pm 0.12$	$0.49 \pm 0.12$	$0.72 \pm 0.07$	$0.53 \pm 0.30$	$0.43 \pm 0.23$	$1.44 \pm 0.09$	$0.43 \pm 0.30$	$0.44 \pm 0.13$	$0.46 \pm 0.06$	$0.49 \pm 0.02$
Threonine	$0.56 \pm 0.06$	1.93±0.08	2.97±0.01	2.71±0.09	1.70±0.02	1.59±0.03	2.63±0.06	1.62±0.01	$2.55 \pm 0.05$	2.64±0.06
Valine	$0.46 \pm 0.26$	1.83±0.19	$2.62 \pm 0.17$	$2.42 \pm 0.09$	$1.51 \pm 0.01$	$2.35 \pm 0.19$	$2.19 \pm 0.01$	$2.14 \pm 0.19$	2.43±0.23	1.98±0.23
Methionine	$0.35 \pm 0.17$	$2.64 \pm 0.10$	$3.53 \pm 0.10$	2.48±0.08	$1.42 \pm 0.09$	`2.38±0.25	$1.36 \pm 0.07$	$2.44 \pm 0.23$	$3.40 \pm 0.25$	2.48±0.06
Isoleucine	1.28±0.03	$2.36 \pm 0.03$	$3.45 \pm 0.26$	2.29±0.03	$2.31 \pm 0.03$	3.29±0.02	$2.27 \pm 0.07$	$2.30 \pm 0.17$	3.12±0.03	2.38±0.10
Leucine	0.89±0.06	$1.94 \pm 0.01$	$3.37 \pm 0.45$	2.88±0.09	$1.02 \pm 0.06$	1.89±0.20	1.18±0.08	$1.28 \pm 0.35$	2.97±0.09	1.15±0.25
Tyrosine	$0.45 \pm 0.10$	0.88±0.19	1.83±0.15	$1.48 \pm 0.01$	$0.42 \pm 0.01$	$0.40 \pm 0.31$	$1.43 \pm 0.03$	$0.55 \pm 0.12$	$0.44 \pm 0.06$	0.43±0.07
Phenylalanine	$0.58 \pm 0.01$	$1.06 \pm 0.03$	$3.08 \pm 0.12$	$2.79 \pm 0.05$	$0.68 \pm 0.06$	$1.64 \pm 0.05$	$0.64 \pm 0.05$	$0.76 \pm 0.25$	$1.66 \pm 0.10$	$0.74 \pm 0.09$
Lysine	1.96±0.23	$2.05 \pm 0.12$	$3.63 \pm 0.11$	2.69±0.01	$1.92 \pm 0.07$	$2.81 \pm 0.09$	1.97±0.01	$2.14 \pm 0.38$	2.93±0.13	2.53±0.10
Histidine	$0.36 \pm 0.34$	$0.62 \pm 0.09$	$2.64 \pm 0.17$	$1.53 \pm 0.10$	$0.39 \pm 0.09$	$0.36 \pm 0.03$	$0.80 \pm 0.09$	$0.39 \pm 0.14$	$1.39 \pm 0.19$	1.42±0.15
Tryptophan	$0.23 \pm 0.02$	1.93±0.23	$3.23 \pm 0.15$	2.19±0.12	2.10±0.26	2.93±0.03	2.23±0.26	$2.17 \pm 0.05$	$3.06 \pm 0.01$	$2.35 \pm 0.09$
EAA	7.12±0.01	$17.24 \pm 0.05$	$30.35 \pm 0.16$	23.46±0.19	$13.47 \pm 0.19$	$19.64 \pm 0.01$	16.70±0.19	15.79±0.25	$24.39 \pm 0.10$	$18.10 \pm 0.05$
TAA	13.76±0.03	25.81±0.01	$43.44 \pm 0.08$	37.52±0.23	18.91±0.34	28.97±0.06	24.81±0.07	23.12±0.23	$36.67 \pm 0.06$	26.65±0.04

## Table 5

Profile of fatty acid of *T. tubifex* in mass culture using the different animal wastes

	Poultry	Q	uail manure (S	%)	G	Goat manure (%)			Chicken manure (%)		
Fatty acid	manure (T <sub>o</sub> ) (%)	$T_1$	<i>T</i> <sub>2</sub>	<i>T</i> <sub>3</sub>	$T_4$	$T_5$	Τ <sub>6</sub>	<i>T</i> <sub>7</sub>	T <sub>8</sub>	T <sub>9</sub>	
Myristic	$1.07 \pm 0.05$	$3.65 \pm 0.03$	$9.25 \pm 0.05$	4.25±0.02	$1.45 \pm 0.03$	$4.45 \pm 0.01$	1.23±0.08	$1.65 \pm 0.05$	$6.65 \pm 0.07$	$3.23 \pm 0.04$	
Pentadecanoic	$2.56 \pm 0.04$	$2.12 \pm 0.02$	$6.12 \pm 0.07$	$2.02 \pm 0.03$	$0.24 \pm 0.02$	$1.24 \pm 0.09$	$2.24 \pm 0.03$	$1.16 \pm 0.02$	$3.16 \pm 0.09$	$1.17 \pm 0.02$	
Palmitic	$11.36 \pm 0.06$	$12.52 \pm 0.01$	$17.52 \pm 0.03$	$13.23 \pm 0.01$	$13.57 \pm 0.05$	$14.47 \pm 0.02$	$12.47 \pm 0.03$	$16.26 \pm 0.01$	$16.26 \pm 0.01$	$14.26 \pm 0.08$	
Stearic	$3.87 \pm 0.01$	$5.50 \pm 0.037$	$6.50 \pm 0.05$	$4.75 \pm 0.05$	$3.07 \pm 0.03$	$5.23 \pm 0.01$	$4.23 \pm 0.05$	$4.57 \pm 0.05$	$5.57 \pm 0.05$	$4.23 \pm 0.06$	
Arachidic	$0.85 \pm 0.04$	$1.04 \pm 0.08$	$0.74 \pm 0.06$	$1.23 \pm 0.08$	$1.07 \pm 0.08$	$1.16 \pm 0.05$	$2.16 \pm 0.09$	$0.75 \pm 0.04$	$1.75 \pm 0.02$	$0.55 \pm 0.02$	
SAFA	19.71±0.03	24.83±0.09	$40.13 \pm 0.01$	$25.48 \pm 0.04$	$19.40 \pm 0.01$	$26.55 \pm 0.03$	$22.33 \pm 0.06$	$24.39 \pm 0.06$	$33.39 \pm 0.02$	$23.44 \pm 0.09$	
Palmitoleic	$2.08 \pm 0.02$	$3.38 \pm 0.02$	$3.38 \pm 0.08$	$3.38 \pm 0.03$	$1.83 \pm 0.05$	$3.83 \pm 0.05$	$2.83 \pm 0.07$	$3.09 \pm 0.04$	$3.09 \pm 0.05$	$3.09 \pm 0.01$	
Oleic	$13.46 \pm 0.01$	24.78±0.01	$27.26 \pm 0.04$	$25.26 \pm 0.05$	19.03±0.06	$23.01 \pm 0.03$	21.01±0.03	$20.91 \pm 0.05$	22.10±0.09	$21.10 \pm 0.05$	
Eicosenoic	$1.35 \pm 0.08$	2.19±0.03	$0.17 \pm 0.08$	$0.23 \pm 0.01$	$1.84 \pm 0.02$	$0.26 \pm 0.08$	$2.05 \pm 0.06$	$1.13 \pm 0.01$	$1.10 \pm 0.03$	$0.17 \pm 0.08$	
MUFA	16.89±0.05	$30.35 \pm 0.07$	$30.64 \pm 0.03$	$28.87 \pm 0.04$	$22.70 \pm 0.04$	27.10±0.03	25.89±0.01	25.13±0.08	$26.29 \pm 0.02$	$24.36 \pm 0.01$	
Linoleic	$1.45 \pm 0.04$	$5.25 \pm 0.05$	$7.25 \pm 0.08$	$3.77 \pm 0.02$	$3.26 \pm 0.03$	$5.26 \pm 0.02$	$4.26 \pm 0.05$	$5.17 \pm 0.02$	$7.10 \pm 0.01$	$4.10 \pm 0.08$	
Linolenic	$3.96 \pm 0.01$	4.59±0.06	$6.19 \pm 0.04$	$3.29 \pm 0.09$	$4.23 \pm 0.02$	$5.23 \pm 0.02$	$3.23 \pm 0.02$	$4.61 \pm 0.03$	$5.61 \pm 0.02$	$4.23 \pm 0.09$	
EPA	$1.71 \pm 0.03$	0.68±0.08	$2.18 \pm 0.09$	$1.18 \pm 0.03$	$0.22 \pm 0.01$	$1.05 \pm 0.01$	$1.65 \pm 0.01$	$0.51 \pm 0.01$	$1.92 \pm 0.06$	$1.19 \pm 0.02$	
DHA	$0.74 \pm 0.02$	$1.14 \pm 0.02$	$1.17 \pm 0.01$	$1.17 \pm 0.09$	$0.35 \pm 0.06$	$0.35 \pm 0.02$	$0.35 \pm 0.06$	$0.19 \pm 0.02$	$0.10 \pm 0.02$	$0.17 \pm 0.01$	
PUFA	$7.86 \pm 0.01$	11.66±0.08	$16.79 \pm 0.03$	$9.41 \pm 0.01$	$8.06 \pm 0.09$	$11.89 \pm 0.03$	$10.48 \pm 0.03$	$8.71 \pm 0.09$	$14.73 \pm 0.03$	$9.69 \pm 0.03$	

SAFA - saturated fatty acid; MUFA - mono unsaturated fatty acid; EPA - eicosapentaenoic acid; DHA - docosahexaenoic acid; PUFA - polyunsaturated fatty acids.

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