Potential of maggot and earthworm meals as protein sources for the growth of ~~Nirwana~~ Nile tilapia (*Oreochromis niloticus*)

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Abstract. ... The purpose of this study was to determine whether maggot flour and earthworm flour have the potential to replace fish meal as a protein source in artificial feed formulations, including the assess~~ing~~ment of their effect on the specific growth rate (SGR), rate of relative growth (RRG), ratio of feed conversion (RFC), feed efficiency (FE), and survival rate (SR) of the ~~nirvana~~ Nile tilapia (*Oreochromis niloticus*). This study used an experimental method with a completely randomized design (CRD), 4 treatments (F1, F2, F3, and F4) and commercial feed (CF) as a comparison. ~~Nthe nirwana tilapia~~ *O. niloticus* sample specimens, ~~were the experimental fish, with~~had an average weight of 30.14±0.02 g. The treatment applied was the use of maggot meal for the fish meal substitution ~~of~~, at concentrations from 5~~%~~ to 20%, together with ~~and~~ 4% earthworm meal. The treatments are F1 = 30% fish meal (FM) + 5% maggot meal (MM) + 4% earthworm meal (EM), F2 = 25% fish meal (FM) + 10% maggot meal (TM) + 4% earthworm meal (EM), F3 = 20% fish meal (FM) + 15% maggot meal (MM) + 4% earthworm meal (EM), and F4 = 15% fish meal (FM) + 20% maggot meal (MM) + 4% earthworm meal (EM). ~~Rthe relative growth rate (RRG), specific growth rate (SGR), survival rate (SR), feed conversion ratio (RFC), and feed efficiency (FE) are the variablesindicators identifiedused in this study (FE).~~ The SGR, RRG, FE, and SR values of *O. niloticus* ~~nirvana tilapia~~ can increase, while the RFC value decreases with the addition of animal protein sourced feed containing maggot flour and earthworms. In fact, the use of maggot meal and earthworm meal ~~at~~in the treatment F3 (20% FM + 15% MM + 4% EM), with 25.32 protein, produced the following effect: RRG ~~=~~ = ~~(~~2.81%, ~~) and~~ SGR= ~~(~~1.65%~~)~~, RFC ~~(~~=2.89%~~)~~, FE= ~~(~~34.58~~)~~ and SR= ~~(~~95%~~)~~ which was almost the same as in the use of commercial feed (CF), with 26% protein, which produced the following effect: RRG =~~(~~2.87%,~~)~~ and SGR ~~(~~=1.67%~~)~~, RFC ~~(~~=2.86%~~)~~, FE ~~(~~=34.74~~)~~ and SR ~~(~~=95%~~)~~. ~~W~~The water quality in *O. niloticus* ~~nirwana tilapia~~ culture media (temperature, dissolved oxygen (DO), pH (power of hydrogen), ammonia) was also ~~concluded~~found to be within the appropriate range for *O. niloticus* ~~nirwana tilapia~~ cultivation.

Key Words: maggot meal, earthworm meal, nirwana tilapia, growth, feed conversion.

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Introduction. Indonesia is the second largest producer of tilapia in the world, with 6.3 million tonnes produced globally in 2018. Nile t~~T~~ilapia (*Oreochromis niloticus*) was widely cultivated worldwide (Suhermanto et al 2019). *O. niloticus* ~~Tilapia~~ could be adapted to aquaculture environments, traditional, semi or intensive scale cultivation systems, has a high economic value~~,~~ and was not affected by the market price fluctuations, ~~contributes to~~is a significant source of animal protein, and its production had experienced a significant increase in ~~production~~ in recent years (Wang & Lu 2016).

The increasing demand for *O. niloticus* ~~tilapia~~ commodities was related to the intensification of aquaculture, so that it will have an impact on the need for feed, which was one of the inhibiting factors for growth. The cost of feed needs for cultured fish was around 60-70% of the total production cost, so the development of feed with sustainable local raw materials was needed, and becomes a challenge for farmers (Hamid et al 2016; Sarker et al 2018). The main cause of the high price of fish feed was the increase~~d~~ ~~in~~of the price of feed raw materials. The main protein source of commercial fish feed still comes from imported raw materials such as fish meal. In addition, excessive use of fish meal causes the supply of fish resources to be depleted (Pucher et al 2014). In addition, there is a fairly high pressure on the sustainability of fishery resources due to the excessive use of fish meal. As a result, a breakthrough in the quest for substitute protein sources was required to support the availability of feed that is sustainable, accessible, and must be environmentally friendly.

Maggots and earthworms are sources of animal protein, that have a high nutritional value, could reduce~~d~~ the need for fish meal, and have positive ecological effects (Li et al 2019). Black soldier fly maggots or larvae have the potential to serve as an alternate source of protein for fish food, whether processed into fresh (live maggots) or dried into meal. The protein content of maggot was 42.1% (in dry weight), moisture (7.9%), fat (24.8%), ash (10.3%), and crude fiber (7%), so that it can meet the protein needs of livestock (Park 2016). The amino acid content in maggot contains arginine (2.29%), methionine (0.66%), phenylalanine (1.63%), threonine (1.70%), tryptophan (0.55), valine (2.56%), histidine (1.50%), isoleucine (1.87%), lysine (2.71%), and leucine (3.23%) and can support fish development (Djissou et al 2015).

The ~~high~~ protein content was also relatively large (approximately 54-66%) in dry matter) carbohydrates, fats and ash (Musyoka et al 2019). According to a research study (Parolini et al 2020), earthworm meal (*Lumbricus rubellus*) contains 63.0% protein, 5.9% crude fat, 8.9% ash, and 14.76 kJ g^{-1} of energy for metabolism. T~~ogether with~~he amino acid profile found in earthworm flour was: arginine (2.83 g kg^{-1}), phenylalanine (6.26 g kg^{-1}), tryptophan (4.43 g kg^{-1}), valine (4.43 g kg^{-1}), histidine (1.47 g kg^{-1}), isoleucine (2.04 g kg^{-1}), leucine (4.11 g kg^{-1})~~,~~ and lysine (6.35 g kg^{-1}) ~~were the amino acid profiles found in earthworm flour~~.

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~~Utilizing maggots~~The suitability of other animal protein sources for~~to~~ replac~~e~~ing fish meal ~~as a source of animal protein~~ was studied for the ~~*Oreochromis*~~ *O. niloticus*, *with maggot* (Ezewudo et al 2015),~~maggot and earthworm meal~~ for *gariepinus*~~-~~(Djissou et al 2016a), ~~for~~ juvenile turbot (*Psetta maxima*) and white shrimp*, with maggot and earthworm* (Kroeckel et al 2012; Cummins et al 2017), ~~earthworm~~ for *Clarias gariepinus* (Dedeke et al 2013), ~~earthworms for~~ *Cyprinus carpio* (Pucher et al 2014)~~,~~ and *Oreochromis* sp. juvenile (Jabir 2012), with earthworms. In general, substituting fish meal could reduce~~d~~ feed consumption while boosting growth and feed effectiveness (Rachmawati ~~and~~ & Nurhayati 2022). There hadn't been any investigation on the effects of mixing maggot flour with earthworm flour on the growth of nirwana tilapia, according to some earlier literature, so the current study ~~it~~ is a novelty ~~in the study~~. This study aims to determine the effect of using maggot flour and earthworm flour ~~on~~ as fish meal substitution, in artificial feed formulations, on the rate of relative growth (RRG), specific growth rate (SGR), ratio of feed conversion (RFC), feed efficiency (FE), and survival rate (SR) of nirvana tilapia.

Material and Method

Experimental materials. The raw material for fish meal was obtained from the fish meal industry, Sari Ulam, Tegal City. Maggot and earthworms were obtained from local maggot and worm farms, Tegal agrofarm Indonesia. The manufacture of maggot meal refers to (Ahmad et al 2022) with a slight modification.~~,~~ A hot air oven was used to dry the maggot for around 36 hours at 60 ºC, then mashed using a grinder and filtered through a sieve. The manufacture of earthworm meal refers to an existing method (Parolini et al 2020), with a slight modification:~~, by~~ steaming the earthworms at a temperature of 70 ºC for 10 minutes and grinding them with a grinder until the particles are finer, then~~. After that,~~ bak~~e~~ing for 10 to 12 hours at 60 ºC. The dry materials were crushed and put through a sieve with a mesh size of 50 (…).

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Experimental fish. *O. niloticus* ~~The nirwana tilapia~~ utilized in this ~~investigation~~ research had an average ~~beginning~~ weight of 30.14±0.05 g at the beginning. The number of specimens of *O. niloticus* ~~tilapia fish~~ used in the study ~~were~~was 100 ~~-~~individuals,~~fish~~ obtained from the Fish Seed Center of Tegal City, Central Java, Indonesia. ~~The~~20 ~~-~~test tilapia specimens were stocked as ~~many as 20 fish~~ in each maintenance tarpaulin container. The completeness of the organs, physical health, size, and weight were taken into consideration when choosing the test fish. The fish are fed ~~until~~ at ~~they were completely~~ sati~~sfied~~ation twice daily, at 8:00 and 16:00, to ensure that they have truly adapted to the new surroundings and the feed provided. The test fish were reared for 60 days and during the rearing the tilapia were fed according to the treatment to determine the weight growth from May to July 2022. Sampling ~~of test samples~~ was carried out every 10 days to determine the increase in the weight of the test fish and to adjust the weight of the feed to be given.

Diet experiment. The ingredients for the feed composition include fish meal, earthworm meal, maggot meal, corn meal, rice bran, copra meal, tapioca meal, fish oil, premix and commercial feed from PT Matahari Sakti, Surabaya, Indonesia, with 26% protein. As much as 5% to 20%~~The use~~ of maggot flour was used as a substitute for fish meal ~~as much as 5% to 20%~~.~~,~~ ~~while~~4% earthworm meal was ~~4%~~ ~~for~~added to all 4 treatments (F1, F2, F3, and F4). ~~b~~The treatments ~~ased on the~~had differen~~ce~~t ~~in the percentages~~concentrations of fish meal (FM), maggot meal (MM)~~, and meal.~~ and earthworm meal (EM):~~.~~ F1 = 30% fish meal (FM) + 5% maggot meal (MM) + 4% earthworm meal (EM), F2 = 25% fish meal (FM) + 10% maggot meal (TM) + 4% earthworm meal (EM), F3 = 20% fish meal (FM + 15% maggot meal (MM) + 4% earthworm meal (EM), and F4 = 15% fish meal (FM) + 20% maggot meal (MM) + 4% earthworm meal (EM). ~~and c~~Commercial feed (CF) was also used, ~~as~~for comparison. Previously, proximate analyses of feed components like fish meal, maggot meal, and earthworm meal had been performed (Table 1) for determining their composition before the experimental diet was used.~~, and~~ Table 2 presents the proximate results of the experimental feed formulation. ~~Provided~~ F~~f~~ood, up to 5% of their body weight, was provided at a frequency of two meals per day~~ during maintenance, and two meals per day~~, at 8:00 and 17:00 WIB~~.~~.

Table 1

Nutrient composition (% dry weight) i~~o~~n fish meal, maggot meal, and earthworm meal

Ingredient (%)	*Protein*	*Moisture*	*Ash*	*Fat*	*Fiber*
Fish meal	40.43±0.09^{c}	8.67±0.01^{a}	16.48±0.27^{a}	8.41±0.01^{b}	4.40±0.12^{b}
Maggot meal	44.63±0.02^{b}	6.34^{c}±0.06^{b}	12.53±0.03^{b}	11.50±0.11^{a}	10.19±0.04^{a}
Earthworm meal	54.46±0.09^{a}	8.18^{b}±0.02^{c}	5.61±0.03^{c}	7.43^{c}±0.02^{c}	6.39±0.14^{b}

*analysis outcome from the University of Diponegoro's Laboratory of Animal Nutrition, Faculty of Animal Sciences and Agriculture (2022). The number after ± i~~wa~~s the standard error value;~~,~~ different~~unequal~~ superscript letters on the same line indicate significantly unequal treatment effects ($P<0.05$).

Table 2

Experimental diet composition in each formula

Materials	*Feed formulation (% ~~/~~100 g^{-1})*			
	F1	*F2*	*F3*	*F4*
Fish meal	30.00	25.00	20.00	15.00
Maggot meal	5.00	10.00	15.00	20.00
E~~e~~arthworm meal	4.00	4.00	4.00	4.00
Tapioca meal	10.00	10.00	10.00	10.00
Bran meal	18.00	18.00	18.00	18.00
Copra meal	10.00	10.00	10.00	10.00
Corn meal	20.00	20.00	20.00	20.00
Vitamin-mineral mix	1.00	1.00	1.00	1.00
Fish oil	2.00	2.00	2.00	2.00
Total	100	100	100	100

F1 (30% FM, ~~and~~ 5% MM and~~,~~ 4% EM); F2 (25% FM, 10% MM and 4% EM); F3 (20% FM, 15% MM and 4% EM); F4 (15% FM, 20% MM and 4% EM) and CF (Commercial feed).

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Experimental containers. In this study, the container used was a plastic pool with a length, width and height of 1~~,~~.2 m x 1 m x 0.70 m, then the pool was filled with water ~~with~~until a height of 50 cm was reached. The maintenance container was equipped with an aeration and circulation system for changing water using a pipe~~ on the~~ inside of the pond. The test *O. niloticus* ~~tilapia~~ were stocked into the plastic pool and acclimatized first. Feed was ~~given little by little~~administered progressively, ~~if there was feed that was not~~the uneaten ~~by the fish then the~~ feed ~~was taken so that~~being removed, avoiding turbidity in the water ~~in~~of the test container~~ was not cloudy~~. Water quality was maintained by siphoning the remaining feed and feces at the bottom of the container, as well as by changing the water every 7 days~~ with the volume of water replaced, which was~~ (25% of the initial volume was replaced at once).

Research methods. Completely Randomized Design (CRD) was used in the experimental methodology of this study, with 4 trials, and the use of commercial feed (CF) ~~as a comparison~~as control sample. Microsoft Excel 2013 was used to determine the mean difference between treatments: a~~A~~nalysis of variance (ANOVA) was employed in statistical data analysis to ~~establish the significance level~~ ($p < 0.05$), followed by ~~and continued using the~~ a Tu~~e~~key test. ~~if there was a difference~~. ~~Descriptive analysis to analyze water quality, Microsoft Excel 2013 was used to determine the mean difference between treatments.~~

Research variable. Several variables were observed in this study, including the ~~rate of relative growth~~ ~~(~~RRG~~)~~, and ~~specific growth rate (~~SGR~~)~~ (Katya et al 2017), ~~ratio of feed conversion~~ ~~(~~RFC~~)~~ (Selvam et al 2018), ~~feed efficiency (~~FE~~)~~, and ~~survival rate (~~SR~~)~~. The formula equations applied in this study are presented as follows:

$$\text{Rate of relative growth (RRG) (\%)} = \frac{\text{final weight-initial weight}}{\text{Fish rearing time in days-initial weight}} \times 100$$

$$\text{Spesific growth rate (SGR) (\%)} = \frac{\text{ln (final weight-initial weight)}}{\text{Fish rearing time in days}} \times 100$$

$$\text{RFC} = \frac{\text{Amount of dry feed intake}}{\text{(final weight in days+dead fish weight in days)- initial weight in days}} \times 100$$

$$\text{FE} = \frac{\text{(final weight in days+dead fish weight in days)- initial weight in days}}{\text{Amount of dry feed intake}} \times 100$$

$$\text{SR (\%)} = \frac{\text{final fish count}}{\text{Initial Fish count}} \times 100$$

Chemical and microbiological analysis. Protein, fat, water, ash, and fiber proximate chemical analyses were performed in accordance with the AOAC's recommended procedure (Latimer 2016). The Kjeldahl method was used for protein proximate analysis (Kusnadi et al 2022). The solution of the destroyed sample was placed in a steam distillation apparatus and three drops of phenolphthalene indicator were added before the distillation was completed. When the dripping distillate reacts neutrally to red litmus and the color of the reservoir solution changes to green, the reservoir solution ~~wa~~is determined as a solution of the destroyed sample (pink). Fat analysis was performed using Soxhlet extraction. The a~~A~~mino acids profile of feed was determined using the HPLC method~~ (HPLC) applied by~~ (Nik Sin et al. 2021) with slight modifications~~ was used for amino acid analysis~~. A total of 30 mg of protein hydrolyzate was then added with 4 ml of 6 N HCl which had been heated at 110 °C for 24 hours, ~~. C~~cooled ~~at~~to room

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temperature, neutralized (pH-=-7) with 6 N NaOH, added with the sample to 10 µl µL of distilled water, then filtered using 0.2 m of Whatman filter paper. Add 10 µl of (...) were added through the HPLC injector to 50 µl of sample and 300 µl of OPA (Orthophalaldehyde) solution after stirring for 5 minutes. Feed microbiological tests include analyzing aflatoxin contamination using the HPLC method (María et al 2022) and Salmonella, with ISO 6579-1, which were evaluated in the laboratory center for agro-based industry (Mooijman et al 2019).

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Facets of wWater quality parameters. Measurements of temperature (°C), pH (hydrogen power), and dissolved oxygen (DO) were measuredperformed to determine the for water quality supporting parameters in this study, every two days at 08.00 and 16.00 WIB, in each unit experiment. At the start, midpoint, and end of the study, UV-Vis Spectrophotometry was used to analyze the ammonia (NH_3) content.

Results. Table 3 displays the findings of the proximate and microbiological examination of feed on 4 formulations using earthworm meal and maggot meal as well as 1 commercial feed. The protein and fat content (%) for treatments F1 to F4 ranged from 23.65%, 24.74%, 25.32%, and 26.60%, respectively. The protein content in each formula increased from F1 to F4, in line with the 5% increase inof the maggot meal in each formula.

Table 3

Proximate and microbiological analysis (% dry weight) on each feed formulation

Proximate analysis	*F1*	*F2*	*F3*	*F4*	*CF*
Protein (%)	23.65±0.02^{d}	24.74±0.02^{c}	25.32±0.03^{b}	26.66±0.03^{a}	26.00
Moisture (%)	9.87±0.02^{a}	9.75±0.01^{b}	9.66±0.01^{bc}	9.55±0.02^{c}	10.00
Ash (%)	12.76±0.03^{a}	12.24±0.02^{b}	11.63±0.25^{c}	10.88±0.02^{d}	12.00
Fat (%)	5.14±0.01^{d}	5.64±0.01^{c}	6.60±0.02^{b}	7.66±0.01^{a}	5.00
Fiber (%)	7.19±0.04^{d}	7.88±0.02^{c}	8.35±0.01^{b}	8.83±0.04^{a}	8.00
Energy (kcal)	3818.2	3853.7	3899.3	3936.5	3906.8
Microbiological analysis					
Aflatoxin (µg kg^{-1})	6.79	6.18	4.68	3.81	3.76
Salmonella	Negative	Negative	Negative	Negative	Negative

The number after ± was the standard error value; unequaldifferent superscript letters on the same line indicate significantly unequal treatment effects (P <0.05). F1 (30% FM and 5% MM, and 4% EM); F2 (25% FM, 10% MM and 4% EM); F3 (20% FM, 15% MM and 4% EM); F4 (15% FM, 20% MM and 4% EM) and CF (Commercial feed).

Table 4 lists the essential amino acid (EAA) composition of the feed formulation, rations as well as the necessary EAA amounts for *O. niloticus*nirwana tilapia.

Table 4

Profile of essential amino acids (EAA) in each feed treatment

Asam amino	*Experimental diets formulation*				
	F1	*F2*	*F3*	*F4*	*CF*
Histidine	0.34	0.36	0.36	0.46	0.43
Threonin	0.94	1.00	1.08	1.30	1.07
Arginin	2.00	2.16	2.26	2.72	2.76
Tyrosin	1.62	2.12	2.46	2.98	2.20
Methionin	0.2	0.22	0.24	0.3	0.37
Valin	1.08	1.24	1.36	1.68	1.25
Phenylalanin	0.82	0.9	0.94	1.16	1.14
Isoleuscin	0.70	0.82	0.90	1.12	0.95
Leuscin	1.70	1.86	1.96	2.36	1.99
Lysin	1.90	1.90	2.18	2.52	2.33

F1 (30% FM ~~and~~ 5% MM, and 4% EM); F2 (25% FM, 10% MM and 4% EM); F3 (20% FM, 15% MM and 4% EM); F4 (15% FM, 20% MM and 4% EM) and CF (Commercial feed).

Table 4 shows ~~that~~ the experimental feed's amino acids~~ content and~~ profile~~ increased~~ from the formulations F1 to F4, wh~~ich~~ose pattern was consistent with the rise in the total protein content in each feed formulation.

Table 5 displays the findings from the examination of the variables ~~rate of relative growth (~~RRG~~)~~, ~~specific growth rate (~~SGR~~)~~, ~~ratio of feed conversion (~~RFC~~)~~, ~~feed efficiency (~~FE~~)~~, and ~~survival rate (~~SR~~)~~. The results of observations on all variables show the experimental feed the F3 formula showed almost the same results as the use of ~~commercial feed (~~CF~~)~~. Commercial feed showed the best results of RRG, SGR, RFC, FE, and SR compared to the experimental formula,~~ then~~ followed by the treatment~~ of~~ s F3 and F~~5~~4~~ feed~~, while the results of the treatments F2 and F1 ~~treatments~~ showed lower ~~results~~values. Measurements and observations of water quality during the study are presented in Table 6.

Table 5

Data replication of rate of relative growth (RRG), specific growth rate (SGR), ratio of feed conversion (RFC), feed efficiency (FE), and survival rate (SR) of *Oreochromis niloticus*~~nirwana tilapia~~

Parameter	*Experimental diet formulation*				
	F1	*F2*	*F3*	*F4*	*CF*
RRG (% day^{-1})	2,35±0.01^{d}	2,58±0.02^{c}	2.81±0.02^{ab}	2.76±0.03^{b}	2.87±0.03^{a}
SGR (%)	1.46±0.02^{b}	1.56±0.01^{b}	1.65^{c}±0.01^{a}	1.63^{d}±0.02^{a}	1.67±0.02^{a}
RFC	3.30±0.01^{d}	3.07±0.02^{c}	2.89±0.03^{a}	2.98±0.02^{b}	2.86±0.01^{a}
FE	30.23±0.02^{e}	32.49±0.02^{d}	34.58±0.01^{b}	33.47±0.01^{c}	34.74±0.02^{a}
SR (%)	90.00±0.00	95.00±0.00	95.00±0.00	90.00±0.00	95.00±0.00

The number after ± was the standard error value; ~~unequal~~different superscript letters on the same line indicate significantly unequal treatment effects (P<0.05).

Table 6

~~Data replication of Facets of w~~Water quality parameters ~~in nirwana tilapia culture~~for each experimental treatment (the intervals correspond to the values measured during the replications)

~~Facet~~Parameters of water quality	*Treatment*					*Ref. range*
	F1	*F2*	*F3*	*F4*	*CF*	
Temperature (°C)	27.2-28.6	27.2-28.7	27.3-28.5	27.2-28.6	27.2-28.8	28.5-30.55*
DO (mg L^{-1})	5.8-6.8	5.7-6.9	5.8-6.7	5.7-7.0	5.7-6.8	> 3*
pH	6.6-7.8	6.5-7.7	6.5-7.5	6.6-7.6	6.7-7.7	6.5-8.5*
Ammonia (mg L^{-1})	0.006-0.02	0.006-0.02	0.006-0.02	0.006-0.06	0.006-0.04	< ~~-~~0.1*

*Boyd (1992). F1 (30% FM ~~and~~ 5% MM, and 4% EM); F2 (25% FM, 10% MM and 4% EM); F3 (20% FM, 15% MM and 4% EM); F4 (15% FM, 20% MM and 4% EM) and CF (Commercial feed).

During the study, the value~~ of facets~~ of water quality parameters in the shrimp culture medium was still within the desired range, thus ensuring the growth of *O. niloticus*~~nirwana tilapia~~.

Discussion. ~~T~~In the results of the proximate test, the protein values from treatment F1 to F4 were 24.65~~%~~ to 26.60%~~, while the different test results were different in water content, fat, ash and fiber content~~ (Table 3). The increase in protein content in the feed formulation was influenced by the maggot meal, which had a protein content ~~(~~of 44.13%,~~)~~ ~~greater~~higher than in fish meal 40.43%. The combination of maggot meal and

Commented [11]: Please respect the scientific rigors and deliver a sound text earthworm meal atin F1 (30% FM and 5% MM, 4% EM) showed the lowest protein level. While tThe microbiological examination of aflatoxin contamination in all formulations revealed that aflatoxin levels decreased with an increased usage of maggot meal. This was because maggot hasdue to its antimicrobial and antifungal activity, so that it could reduceding the microbial contamination in feed formulations (Valachova et al 2014; Pöppel et al 2015). The results of aflatoxin content for all feed formulas were still relatively safe for fish consumption, because they were still below the threshold, which was less than 20 µg kg^{-1} and also do not contain salmonella bacteria.

Maggot and earthworms were good sources of animal protein for *O. niloticus* Tilapia because they contain essential amino acids required for the fish growth. Protein requirements for fish growth vary:, the younger the age of the fish, the greater the protein needs. Nguyen et al (2020a) in his research stated in his research that the need and balance of feed protein was 24-30%, for *O. niloticus* a Nile tilapia measuring 7.90 g, for its growth, up to 10 weeks. Another study stated that the protein requirement of feed for juvenile *O. niloticus* Nile tilapia measuring 12.7 g in an eight-week period was 22.2% to 29.7%, for proteins sourced from soybean meal, fish meal, and corn meal protein (Nguyen et al 2020b). The protein content of the formulas F1 through F4 was shown in this study based on the literature, and it still met the suggested levels of fish feed protein for tilapia growth.

Amino acid content in the treatments F4 and F3 treatment feeds with ratios (FM)/(MM)/(EM) of (15:20:4)(%)%, respectively and (20:15:4)(%), respectively, alwayswere sistematically higher occupied the highest position, while the treatment F1, combination with a ratio(FM)/(MM)/(EM) with a ratio of (30:5:4)(%) was almost always in the lowest place during the F1 treatment (Table 3). This suggests that the value of theits amino acids it contains content could be improved by adding natural feed sources of maggot meal to the amino acid profilefeed combination, in amounts ranging from 5% to 20%. Table 4 shows that there are nine profiles of essential amino acids out of ten that are required to be present in the five types of feed, namely arginine, histidine, isoleucine, leucine, lysine, threonine, valine, methionine, and phenylalanine. Lysine was one of the 1is an0 necessaryessential amino acids that can serve to evaluate the feed effects on the fish growth as a benchmark amino acid (Hamid et al 2016). This wais because the amino acid lysine in the animal body canexplicitly stimulatecontributes to the fish growth and protein deposits protein in the tissues, because its needs are not influenced by since it has no other metabolic roles (Marchão et al 2020; Cai et al. 2018). The requirement for the amino acid lysine in the growth phase of lysine tilapia can reach 1.55% (Diógenes et al 2016). The values of amino acid levels, are larger than 1.55% according to determined in the formulastions F1 through F4 and commercial feed (CF) content, are larger than this threshold value, i.e.: F1 (1.90% in)F1 , F2 (1.90%) in F2, F3 (2.18%) in F3, F4 (2.52%)F4, and CF (2.33% in CF). This shows that the requirement for the amino acid lysine for *O. niloticus* tilapia is fully met. Diets with an unbalanced amino acid profile can lead to less food intake and less effective utilization of essential amino acids (Prabu et al 2020).

The results of feed treatment on formula F3, F4 and commercial feed (CF) with 25.32%, 26.66% and 26% protein, respectively, for *O. niloticus* Nirwana tilapia resulted in RRG, SGR, RFC, feed efficiency (FE), and Survival rate values (SR) values which wasere better than in the experimental feed treatments F1, F2, and F4. It was anticipated that the inclusion of earthworm meal and maggot meal in experimental feeds F3 and F4 will provide *O. niloticus* nirwana tilapia with a rich source of essential amino acids. The analysis findings revealed that CF treatment caused the highest rate of relative growth (RRG) and specific growth rate (SGR), of 2.87% and 1.67%, respectively, followed. By F3, with RRG (2.81%) and SGR (1.65%) is the next highest result. There were differences based onamong the RRG and SGR values for all formulastions, although for CF and F3 they were not statistically differentsignificant (p > 0.05). The ratio of feed nutrients (protein, fat, and fiber) affected relative and specific growth rates to promote fish growth (Eriegha & Ekokotu 2017). Growth happens when there was extra metabolic energy left over after it had been consumed for bodily upkeep and activities. The majority of the feed consumed was used for growth after first being used to maintain the

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body and replace damaged cells (Adeniyi et al 2020). Increased feed protein does not always lead to increased growth. Increasing feed protein without being followed by a balance with non-protein energy sources will cause protein to be used as an energy source (Samuelsen et al 2022).

The ~~results of proximate analysis,~~ substitution of fish meal with 15% maggot flour in the F3 feed formula (25.33% protein) resulted in a higher ~~rates of relative growth (~~RRG~~)~~ and ~~specific growth rate (~~SGR~~)~~, than in F4 feed with 20% substitution (26.66% protein). This was presumably because the nutritional content of the feed used for growth was sufficient. This was also in accordance with the study of Rachmawati & Samidjan (2013), which s~~tat~~uggested that the substitution of fish meal with maggot flour with 25.13% protein could provide~~d~~ more optimal growth, compared to protein~~ with~~ levels of 26.94~~%~~ and 27.79% respectively. If their protein requirements are met, fish can grow well if their food intake is appropriate. The amount of protein in the meal has an impact on *O. niloticus* ~~the tilapia's~~ ~~high and low~~ growth rates (Ngugi et al 2017). Due to the consumption of protein from bodily tissues to maintain important processes, a lack of protein in the diet could resulted in stunted growth, ~~which was~~ followed by weight loss. The quality of the protein, the energy content of the feed, the nutritional balance, and the level of feeding all had a significant impact on how well the fish used the protein for growth (Samuelsen et al 2022). If the amount of protein consumed from the feed was too high, only a portion of it would be absorbed and used for cell formation and repair, and the remainder would be expelled (Prangnell et al 2022). High protein intake had the effect of increasing the amount of energy needed for protein catabolism, which results in nitrogen being excreted in the form of ammonia through the kidneys. This was due to fish's limited ability to store protein (Silva et al 2022).

The findings of assessing the ratio of feed conversion (RFC) in F1 to F4 and CF treatments in *O. niloticus* ~~Nirwana tilapia (*O. niloticus*)~~ for 60 days likewise revealed significant variations between treatments ($p < 0.05$). The RFC values in the F3 and CF treatments also ~~produced~~recorded the smallest values, ~~and~~ which were not significantly different ($p > 0.05$), compared to the CF, i.e. 2.89 and 2.86, respectively. Table 5 shows that the F1 treatment ~~shows~~had the highest RFC value, of 3.30, ~~and~~while the lowest was observed in the F4 treatment: ~~of~~ 2.89. Therefore, it could be seen that the feed that has the highest utilization efficiency was observed in the F3 treatment (15% maggot meal in ~~place~~stead of fish meal), namely ~~of~~ 2.89. The higher the use of ~~maggot mea~~MM~~l~~ (20%) in the formulation, the higher the value of the ~~ratio of feed conversion (~~RFC~~) value of~~ (3.30). This was most likely owing to the chitin content in maggot meal, which reduced the ability of *O. niloticus* ~~tilapia~~ seeds to digest feed when fish meal was substituted more frequently. Because chitin was crystalline and soluble only in strong acid solutions, the body was unable to properly digest it (Cummins et al 2017). The quality of the water and the feed utilized had an impact on the RFC value, which was also strongly connected with the fish diet (Rachmawati & Samidjan 2019). The smaller the RFC value, the better the level of feed efficiency (FE), and vice versa. Compared to the control treatment, the use of feed was smaller, ~~this~~which can be seen from the fish's low appetite (Rachmawati & Nurhayati 2022). Tayebi & Sobhanardakani (2020)~~,~~ stated that *O. niloticus* ~~Tilapia~~ had an omnivorous nature, so that aquaculture was efficient with low feed costs. The higher the quality of the feed delivered, the lower the RFC value~~.~~ (Selvam et al 2018). Meanwhile, if the feed conversion value was high, it means that the quality of the feed provided was not good.

Table 5 demonstrates that *O. niloticus*'s ~~the nirwana tilapia's~~ survival value differed when fish food was substituted with maggot meal and earthworm meal. The mean ~~survival rate (~~SR~~)~~ in *O. niloticus* ~~Nirwana tilapia~~ cultivation in the F2, F3, and CF treatments was 95%, while in the F1 and F4 treatments were 90%. The results of observations during the study~~,~~ showed that tilapia fed the test feed (using ~~maggot meal in place of fish meal~~ 5~~%~~, 10~~%~~, 15~~%~~, and 20% of maggot meal instead of fish meal) and 4% earthworm meal had a higher survival value. The high survival value ~~of fish The tilapia obtained in this study~~ was suspected to be also related to the water quality parameters during the maintenance, that were still within the limits of optimum conditions for cultivation purposes so that it was feasible for the survival of tilapia

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(Diógenes et al 2016). The size of the survival rate was affected by both internal and external parameters, such as water quality, stocking density, amount of complete amino acids in feed, and gender, heredity, age, reproduction, and disease resistance (Obirikorang et al 2022).

According to Djissou et al (2016a), growth performance and survival rate of *O. niloticus* tilapia were influenced by feed and water quality. Cthe constraints faced in aquaculture were caused by the mainly the components, namely quality seeds, water quality management, feed management, and fish pest management. Provision of quality feed with the required quantity, use of seeds and professional cultivation management were factors that support the success of cultivation (Djissou et al 2016b). Mthe measurement of the study water quality parameters showed that the dissolved oxygen (DO) level in *O. niloticus* tilapia water was between 5.8 and 6.9 mg /L-1 , which was a level that is still good for fish maintenance and survival. The results of temperature measurements obtained resultsshowed values of 27.2-28.8ºC. It was also stated thatby Rachmawati & Samidjan (2019) that *O. niloticus*tilapia's could lived well tolerance is within a temperature tolerance valuerange of 25-30ºC, to stay in a comfort zone. Ttilapia can survive in a low oxygen content of up to 3 mg L-1, but a goodsuitable oxygen concentration range value for cultivation is between 5-7 mg L-1 and the levels of ammonia (NH_3) should be <-0.1 mg L-1.

Conclusions. According to the study's findings, replacing fish meal with a protein source consisting of 4% earthworm meal and 15% maggot meal could raise the *O. niloticus*nirwana tilapia's SGR, RRG, FE, and SR values, while decreasing the RFC. The use of maggot meal and earthworm meal on F3 (20% FM + 15% MM + 4% EM) with 25.32 protein resulted in: RRG (=2.81%), and SGR (=1.65%), RFC (=2.89), FE (=34.58) and SR= (95%,) which was almost the same as the use of commercial feed (for the CF), with 26% protein, which produces an RRG (of 2.87%), and an SGR of (1.67%), an RFC (of 2.86), an FE (of 34.74) and an SR (of 95%). Usinge of earthworm meal and maggot meal as a sources of protein can help minimizeing the reliance on fish meal in fish feed.

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Conflict of interest. The authors declare no conflict of interest.

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Potential of Maggot and Earthworm Meals as Protein Sources for the Growth of Nile tilapia (*Oreochromis niloticus*)

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Abstract. Maggots and earthworms are sources of animal protein that have a high nutritional value, could reduce the need for fish meal, and have positive ecological effects. Maggot and earthworms have the potential as alternative protein sources for fish feed, either processed into fresh or dried into flour. The purpose of this study was to determine whether maggot flour and earthworm flour have the potential to replace fish meal as a protein source in artificial feed formulations including the assessment their effect on the specific growth rate (SGR), rate of relative growth (RRG), ratio of feed conversion (RFC), feed efficiency (FE), and survival rate (SR) of the *O. niloticus* (*Oreochromis niloticus*). This study used an experimental method with a completely randomized design (CRD), 4 treatments (F1, F2, F3, and F4) and commercial feed (CF) as a comparison. *O. niloticus* sample specimens, an average weight of 30.14±0.02 g. The treatment applied was the use of maggot meal for the fish meal substitution, at concentrations from of 5 to 20%, together with and 4% earthworm meal. The treatments are F1 = 30% fish meal (FM) + 5% maggot meal (MM) + 4% earthworm meal (EM), F2 = 25% fish meal (FM) + 10% maggot meal (TM) + 4% earthworm meal (EM), F3 = 20% fish meal (FM + 15% maggot meal (MM) + 4% earthworm meal (EM), and F4 = 15% fish meal (FM) + 20% maggot meal (MM) + 4% earthworm meal (EM). The SGR, RRG, FE, and SR values of *O. niloticus* can increase, while the RFC value decreases with the addition of animal protein sourced feed containing maggot flour and earthworms. In fact, the use of maggot meal and earthworm meal the treatment F3 (20% FM + 15% MM + 4% EM) with 25.32% protein, produced the following effect: RRG = 2.81%, SGR = 1.65%, RFC = 2.89%, FE = 34.58, and SR = 95% which was almost the same as in the use of commercial feed (CF), with 26% protein, which produced the following effect: RRG = 2.87%, SGR = 1.67%, RFC = 2.86%, FE = 34.74, and SR = 95%. The water quality in *O. niloticus* culture media (temperature, dissolved oxygen (DO), pH (power of hydrogen), ammonia) was also concluded to be within the appropriate range for *O. niloticus* cultivation.
Key Words: artificial feed, fish feed, water quality, earthworm, maggot.

Introduction. Indonesia is the second largest producer of tilapia in the world, with 6.3 million tonnes produced globally in 2018. Nile tilapia (*Oreochromis niloticus*) was widely cultivated worldwide (Suhermanto et al 2019). *O. niloticus* could be adapted to aquaculture environments, traditional, semi or intensive scale cultivation systems, has high economic value, and was not affected by the market price fluctuations, is a significant source of animal protein, and its production had experienced a significant increase in recent years (Wang & Lu 2016).

The increasing demand for *O. niloticus* commodities was related to the intensification of aquaculture so it will have an impact on the need for feed which was one of the inhibiting factors for growth. The cost of feed needed for cultured fish was around 60-70% of the total production cost, so the development of feed with sustainable local raw materials was needed, and becomes a challenge for farmers (Hamid et al 2016; Sarker et al 2018). The main cause of the high price of fish feed was the increased of the price of feed raw materials. The main protein source of commercial fish feed still comes from imported raw materials such as fish meals. In addition, excessive use of fish meals causes the supply of fish resources to be depleted (Pucher et al 2014). In addition, there is fairly high pressure on the sustainability of fishery resources due to the excessive use of fish meals. As a result, a breakthrough in the quest for substitute protein sources was required to support the availability of feed that is sustainable, accessible, and must be environmentally friendly.

Maggots and earthworms are sources of animal protein that have a high nutritional value, could reduce the need for fish meal, and have positive ecological effects (Li et al 2019). Black soldier fly maggots or larvae have the potential to serve as an alternate source of protein for fish food whether processed into fresh (live maggots) or dried into the meal. The protein content of maggot was 42.1% (in dry weight), moisture (7.9%), fat (24.8%), ash (10.3%), and crude fiber (7%), so that it can meet the protein needs of livestock (Park 2016). The amino acid content in maggot contains arginine (2.29%), methionine (0.66%), phenylalanine (1.63%), threonine (1.70%), tryptophan (0.55), valine (2.56%), histidine (1.50%), isoleucine (1.87%), lysine (2.71%), and leucine (3.23%) and can support fish development (Djissou et al 2015).

Nutrients contained in earthworms such as protein (approximately 54-66% in dry matter), carbohydrates, fats and ash (Musyoka et al 2019). According to a research study (Parolini et al 2020), earthworm meal (*Lumbricus rubellus*) contains 63.0% protein, 5.9% crude fat, 8.9% ash, and 14.76 kJ g^{-1} of energy for metabolism. The amino acid profile found in earthworm flour was: arginine (2.83 g kg^{-1}), phenylalanine (6.26 g kg^{-1}), tryptophan (4.43 g kg^{-1}), valine (4.43 g kg^{-1}), histidine (1.47 g kg^{-1}), isoleucine (2.04 g kg^{-1}), leucine (4.11 g kg^{-1}), and lysine (6.35 g kg^{-1}).

The suitability of other animal protein sources for replacing fish meal was studied for the *O. niloticus,* with maggot (Ezewudo et al 2015), for *gariepinus* (Djissou et al 2016), juvenile turbot (*Psetta maxima*) and white shrimp, with maggot and earthworm (Kroeckel et al 2012; Cummins et al 2017), for *Clarias gariepinus* (Dedeke et al 2013), *Cyprinus carpio* (Pucher et al 2014), and *Oreochromis* sp. juvenile (Jabir 2012), with earthworms. In general, substituting fish meals could reduce feed consumption while boosting growth and feed effectiveness (Rachmawati & Nurhayati 2022). There hadn't been any investigation on the effects of mixing maggot flour with earthworm flour on the growth of *O. niloticus*, according to some earlier literature, so the current study is a novelty. This study aims to determine the effect of using maggot flour and earthworm flour as as fish meal substitution, in artificial feed formulations, on the rate of relative growth (RRG), specific growth rate (SGR), ratio of feed conversion (RFC), feed efficiency (FE), and survival rate (SR) of Nile tilapia.

Material and Method

Experimental materials. The raw material for fish meal was obtained from the fish meal industry, Sari Ulam, Tegal City. Maggots and earthworms were obtained from local maggot and worm farms, Tegal agrofarm Indonesia. The manufacture of maggot meal refers to (Ahmad et al. 2022) with a slight modification, A hot air oven was used to dry the maggot for around 36 hours at 60°C, then mashed using a grinder and filtered through a sieve. The manufacture of earthworm meal refers to an existing method (Parolini et al 2020), with a slight modification: steaming the earthworms at a temperature of 70°C for 10 minutes and grinding them with a grinder until the particles are finer. Then, baking for 10 to 12 hours at 60°C. Next, the dry ingredients are done milling process with flour milling machine, then continued the sifting process to get flour more refined.

Experimental fish. The *O. niloticus* utilized in this research had an average weight of 30.14±0.05 g at the beginning. The number of specimens of *O. niloticus* used in the study was 100 individuals, obtained from the Fish Seed Center of Tegal City, Central Java, Indonesia. 20 test tilapia specimens were stocked as in each maintenance tarpaulin container. The completeness of the organs, physical health, size, and weight were taken into consideration when choosing the test fish. The fish are fed satiation twice daily, at 8:00 and 16:00 to ensure that they have truly adapted to the new surroundings and the feed provided. The test fish were reared for 60 days and during the rearing the tilapia were fed according to the treatment to determine the weight growth from May to July 2022. A sampling was carried out every 10 days to determine the increase in the weight of the test fish and to adjust the weight of the feed to be given.

Diet Experiment. The ingredients for the feed composition include fish meal, earthworm meal, maggot meal, corn meal, rice bran, copra meal, tapioca meal, fish oil, premix, and commercial feed from PT Matahari Sakti, Surabaya, Indonesia with 26% protein. As much as 5% to 20% of maggot flour was used as a substitute for fish meal. Earthworm meal was added to all 4 treatments (F1, F2, F3, and F4). The treatments had different concentrations of fish meal (FM), maggot meal (MM), and earthworm meal (EM): F1 = 30% fish meal (FM) + 5% maggot meal (MM) + 4% earthworm meal (EM), F2 = 25% fish meal (FM) + 10% maggot meal (TM) + 4% earthworm meal (EM), F3 = 20% fish meal (FM + 15% maggot meal (MM) + 4% earthworm meal (EM), and F4 = 15% fish meal (FM) + 20% maggot meal (MM) + 4% earthworm meal (EM). Commercial feed (CF) was also used, for comparison. Previously, proximate analyses of feed components like fish meal, maggot meal, and earthworm meal had been performed (Table 1) for determining their composition before the experimental diet was used. Table 2 presents the proximate results of the experimental feed formulation. Food, up to 5% of their body weight, was provided at a frequency of two meals per day, at 8:00 and 17:00 WIB.

Table 1

Nutrient composition (% dry weight) in fish meal, maggot meal, and earthworm meal

Ingredient (%)	Protein	Moisture	Ash	fat	fiber
Fish meal	40.43 ± 0.09^{c}	8.67 ± 0.01^{a}	16.48 ± 0.27^{a}	8.41 ± 0.01^{b}	4.40 ± 0.12^{b}
Maggot meal	44.63 ± 0.02^{b}	$6.34^{c} \pm 0.06^{b}$	12.53 ± 0.03^{b}	11.50 ± 0.11^{a}	10.19 ± 0.04^{a}
Earthworm meal	54.46 ± 0.09^{a}	$8.18^{b} \pm 0.02^{c}$	5.61 ± 0.03^{c}	$7.43^{c} \pm 0.02^{c}$	6.39 ± 0.14^{b}

Note: The number after ± is the standard error value, different superscript letters on the same line indicate significantly unequal treatment effects ($P<0.05$).

Table 2

Experimental diet composition in each formula

Materials	Feed formulation (% 100 g^{-1})			
	F1	F2	F3	F4
Fish meal	30.00	25.00	20.00	15.00
Maggot meal	5.00	10.00	15.00	20.00
Earthworm meal	4.00	4.00	4.00	4.00
Tapioca meal	10.00	10.00	10.00	10.00
Bran meal	18.00	18.00	18.00	18.00
Copra meal	10.00	10.00	10.00	10.00
Corn meal	20.00	20.00	20.00	20.00
Vitamin-mineral mix	1.00	1.00	1.00	1.00
Fish oil	2.00	2.00	2.00	2.00
Total	100	100	100	100

Note: F1 (30% FM, 5% MM, and 4% EM); F2 (25% FM, 10% MM, and 4% EM); F3 (20% FM, 15% MM, and 4% EM); F4 (15% FM, 20% MM, and 4% EM) and CF (Commercial feed).

Experimental Containers. In this study, the container used was a plastic pool with a length, width, and height of 1.2 m x 1 m x 0.70 m, then the pool was filled with water until a height of 50 cm was reached. The maintenance container was equipped with an aeration and circulation system for changing water using a pipe inside of the pond.

The test *O. niloticus* were stocked into the plastic pool and acclimatized first. Feed was administered progressively, the uneaten feed being removed, avoiding turbidity in the water of the test container. Water quality was maintained by siphoning the remaining feed and feces at the bottom of the container, as well as by changing the water every 7 days (25% of the initial volume was replaced at once).

Research methods. Completely Randomized Design (CRD) was used in the experimental methodology of this study, with 4 trials and the use of commercial feed (CF) control sample. Microsoft Excel 2013 was used to determine the mean difference between treatments: analysis of variance (ANOVA) was employed in statistical data analysis to ($p<0.05$), followed by a Tekey test.

Research variable. Several variables were observed in this study, including the RRG, SGR (Katya et al 2017), RFC (Selvam et al 2018), FE, and SR. The equation formula applied in this study refers to Rachmawati & Nurhayati (2022), which was presented as follows:

$$\text{Rate of relative growth (RRG) (\%)} = \frac{\text{final weight-initial weight}}{\text{Fish rearing time in days-initial weight}} \times 100$$

$$\text{Specific growth rate} = \frac{\text{ln (final weight-initial weight)}}{\text{Fish rearing time in days}} \times 100$$

$$\text{RFC} = \frac{\text{Amount of dry feed intake}}{\text{(final weight in days+dead fish weight in days)- initial weight in days}} \times 100$$

$$\text{FE} = \frac{\text{(final weight in days+dead fish weight in days)- initial weight in days}}{\text{Amount of dry feed intake}} \times 100$$

$$\text{SR (\%)} = \frac{\text{final fish count}}{\text{Initial Fish count}} \times 100$$

Chemical and microbiological analysis. Protein, fat, water, ash, and fiber proximate chemical analyses were performed in accordance with the AOAC's recommended procedure (Latimer 2016). The Kjeldahl method was used for protein proximate analysis (Kusnadi et al 2022). The solution of the destroyed sample was placed in a steam distillation apparatus and three drops of phenolphthalene indicator were added before the distillation was completed. When the dripping distillate reacts neutrally to red litmus and the color of the reservoir solution changes to green, the reservoir solution is determined as a solution of the destroyed sample (pink). Fat analysis was performed using Soxhlet extraction. The amino acids profile of feed was determined using the HPLC method (Nik Sin et al. 2021) with slight modifications. A total of 30 mg of protein hydrolyzate was then added with 4 ml of 6 N HCl which had been heated at 110^{o}C for 24 hours cooled at room temperature, neutralized (pH=7) with 6 N NaOH, added with 10 μL of distilled water, then filtered using 0.2 m of Whatman filter paper. Take a 50μL sample plus 300μL of OPA (Orthophalaldehyde) solution, stir for 5 minutes and then put it into the HPLC injector as much as 10μL. Feed microbiological tests include analyzing aflatoxin contamination using the HPLC method (María et al. 2022) and Salmonella with ISO 6579-1, which were evaluated in the laboratory center for agro-based industry (Mooijman et al 2019).

Water quality parameters. Measurements of temperature (^{o}C), pH (hydrogen power), and dissolved oxygen (DO) were performed to determine water quality, every two days at 08.00 and 16.00 WIB in each unit experiment. At the start, midpoint, and end of the study, UV-Vis Spectrophotometry was used to analyze the ammonia (NH_3) content.

Results. Table 3 displays the findings of the proximate and microbiological examination of feed on 4 formulations using earthworm meal and maggot meal as well as 1 commercial feed.

The protein and fat content (%) for treatments F1 to F4 ranged from 23.65, 24.74, 25.32, and 26.60%, respectively. The protein content in each formula increased from F1 to F4, in line with the 5% increase of the maggot meal in each formula.

Table 3

Proximate and microbiological analysis (% dry weight) on each feed formulation

Proximate analysis	F1	F2	F3	F4	CF
Protein (%)	23.65±0.02^{d}	24.74±0.02^{c}	25.32±0.03^{b}	26.66±0.03^{a}	26.00
Moisture (%)	9.87±0.02^{a}	9.75±0.01^{b}	9.66±0.01^{bc}	9.55±0.02^{c}	10.00
Ash (%)	12.76±0.03^{a}	12.24±0.02^{b}	11.63±0.25^{c}	10.88±0.02^{d}	12.00
Fat (%)	5.14±0.01^{d}	5.64±0.01^{c}	6.60±0.02^{b}	7.66±0.01^{a}	5.00
Fiber (%)	7.19±0.04^{d}	7.88±0.02^{c}	8.35±0.01^{b}	8.83±0.04^{a}	8.00
Energy (kcal)	3818.2	3853.7	3899.3	3936.5	3906.8
Microbiological analysis					
Aflatoxin (μg kg^{-1})	6.79	6.18	4.68	3.81	3.76
Salmonella	Negative	Negative	Negative	Negative	Negative

Note: the number after ± was the standard error value; different superscript letters on the same line indicate significantly unequal treatment effects (P<0.05). F1 (30% FM, 5% MM, and 4% EM); F2 (25% FM, 10% MM, and 4% EM); F3 (20% FM, 15% MM, and 4% EM); F4 (15% FM, 20% MM, and 4% EM) and CF (Commercial feed).

Table 4 lists the essential amino acid (EAA) composition of the feed formulation, as well as the necessary EAA amounts for *O. niloticus*.

Table 4

Profile of essential amino acids (EAA) in each feed treatment

Asam amino	Experimental diets formulation				
	F1	F2	F3	F4	CF
Histidine	0.34	0.36	0.36	0.46	0.43
Threonin	0.94	1.00	1.08	1.30	1.07
Arginin	2.00	2.16	2.26	2.72	2.76
Tyrosin	1.62	2.12	2.46	2.98	2.20
Methionin	0.2	0.22	0.24	0.3	0.37
Valin	1.08	1.24	1.36	1.68	1.25
Phenylalanin	0.82	0.9	0.94	1.16	1.14
Isoleucin	0.70	0.82	0.90	1.12	0.95
Leucin	1.70	1.86	1.96	2.36	1.99
Lysin	1.90	1.90	2.18	2.52	2.33

Note: F1 (30% FM, 5% MM, and 4% EM); F2 (25% FM, 10% MM, and 4% EM); F3 (20% FM, 15% MM, and 4% EM); F4 (15% FM, 20% MM, and 4% EM) and CF (Commercial feed).

Table 4 shows the experimental feed's amino acids profile from the formulations F1 to F4, whose pattern was consistent with the rise in the total protein content in each feed formulation.

Table 5 displays the findings from the examination of the variables RRG, specific SGR, RFC, FE, and SR. The results of observations on all variables show the experimental feed the F3 formula showed almost the same results as the use of CF. Commercial feed showed the best results of RRG, SGR, RFC, FE, and SR compared to the experimental formula, followed by the treatments F3 and F4 feed, while the results of the treatments F2 and F1 showed lower values. Measurements and observations of water quality during the study are presented in Table 6.

Table 5

Data replication of rate of relative growth (RRG), specific growth rate (SGR), ratio of feed conversion (RFC), feed efficiency (FE), and survival rate (SR) of *Oreochromis niloticus*

Parameter	Experimental diet formulation				
	F1	F2	F3	F4	CF
RRG (% day^{-1})	$2,35 \pm 0.01^{d}$	$2,58 \pm 0.02^{c}$	2.81 ± 0.02^{ab}	2.76 ± 0.03^{b}	2.87 ± 0.03^{a}
SGR (%)	1.46 ± 0.02^{b}	1.56 ± 0.01^{b}	$1.65^{c} \pm 0.01^{a}$	$1.63^{d} \pm 0.02^{a}$	1.67 ± 0.02^{a}
RFC	3.30 ± 0.01^{d}	3.07 ± 0.02^{c}	2.89 ± 0.03^{a}	2.98 ± 0.02^{b}	2.86 ± 0.01^{a}
FE	30.23 ± 0.02^{e}	32.49 ± 0.02^{d}	34.58 ± 0.01^{b}	33.47 ± 0.01^{c}	34.74 ± 0.02^{a}
SR (%)	90.00±0.00	95.00±0.00	95.00±0.00	90.00±0.00	95.00±0.00

Note: the number after ± was the standard error value; differentl superscript letters on the same line indicate significantly unequal treatment effects ($P<0.05$).

Table 6

Water quality parameters for each experimental treatment (the intervals correspond to the values measured during the replications)

Parameters of water quality	Treatment					Ref. range
	F1	F2	F3	F4	CF	
Temperature (°C)	27.2-28.6	27.2-28.7	27.3-28.5	27.2-28.6	27.2-28.8	28.5-30.55*
DO (mg L^{-1})	5.8-6.8	5.7-6.9	5.8-6.7	5.7-7.0	5.7-6.8	> 3*
pH	6.6-7.8	6.5-7.7	6.5-7.5	6.6-7.6	6.7-7.7	6.5-8.5*
Ammonia (mg L^{-1})	0.006-0.02	0.006-0.02	0.006-0.02	0.006-0.06	0.006-0.04	< 0.1*

Note: *Boyd (1992). F1 (30% FM, 5% MM, and 4% EM); F2 (25% FM, 10% MM, and 4% EM); F3 (20% FM, 15% MM, and 4% EM); F4 (15% FM, 20% MM, and 4% EM) and CF (Commercial feed).

During the study, the value of facets of water quality in the shrimp culture medium was still within the desired range, thus ensuring the growth of O. niloticus .

Discussion. In the results of the proximate test, the protein values from treatment F1 to F4 were 23.65 to 26.66% (Table 3). The increase in protein content in the feed formulation was influenced by the maggot meal, which had a protein content 44.13%, higher than in fish meal 40.43%. The combination of maggot meal and earthworm meal in F1 (30% FM and 5% MM, 4% EM) showed the lowest protein level. The microbological examination of aflatoxin contamination in all formulations revealed that aflatoxin levels decreased with increased usage of maggot meal. Due to its antimicrobial and antifungal activity, reduce the microbial contamination in feed formulations (Valachova et al 2014; Pöppel et al 2015). The results of aflatoxin content for all feed formulas were still relatively safe for fish consumption, because they were still below the threshold, which was less than 20 μg kg^{-1} and also do not contain salmonella bacteria.

Maggot and earthworms were good sources of animal protein for *O. niloticus* because they contain essential amino acids required for the fish growth. Protein requirements for fish growth vary, the younger of the fish, the greater the protein needs.

Nguyen et al (2020) stated in his research that the need and balance of feed protein was 24-30% for *O. niloticus* measuring 7.90 g for its growth up to 10 weeks. Another study stated that the protein requirement of feed for juvenile *O. niloticus* measuring 12.7 g in an eight-week period was 22.2% to 29.7%, for proteins sourced from soybean meal, fish meal, and corn meal (Nguyen et al 2020). Based on the research report presented, the range of protein use for tilapia growth is between 22.2 to 30%, so that alternative feed products in this study which have a protein content of 23.65 to 26.66% can already be used as a reference for tilapia cultivation.

Amino acid content in the treatments F4 and F3 with ratios FM/MM/EM of 15:20:4(%), respectively and (20:15:4)%, respectively, were sistematically higher, while the treatment F1 with a ratio (FM)/(MM)/(EM) of 30:5:4(%) was almost always the lowest (Table 3). This suggests that the value of it amino acids content could be improved by adding natural feed sources of maggot meal to the feed combination, in amounts ranging from 5% to 20%. Table 4 shows that there are nine essential amino acids out of ten that are required to be present in the five types of feed, namely arginine, histidine, isoleucine, leucine, lysine, threonine, valine, methionine, and phenylalanine. Lysine is essential amino acids that can serve to evaluate the feed effects on the fish growth (Hamid et al 2016). This is because the amino acid lysine in the animal body explicitly contributes to the fish growth and protein deposits in the tissues, since it has no other metabolic roles (Marchão et al 2020; Cai et al 2018). The requirement for the amino acid lysine in the growth phase of lysine tilapia can reach 1.55% (Diógenes et al 2016). The values of amino acid levels, determined in the formulations F1 through F4 and commercial feed (CF) content, are larger than this threshold value, i.e. 1.90% in F1, 1.90% in F2, 2.18% in F3, 2.52% in F4, and 2.33% in CF. This shows that the requirement for the amino acid lysine for *O. niloticus* is fully met. Diets with an unbalanced amino acid profile can lead to less food intake and less effective utilization of essential amino acids (Prabu et al 2020).

The results of feed treatment on formula F3, F4 and commercial feed (CF) with 25.32%, 26.66% and 26% protein respectively for *O. niloticus* resulted in RRG, SGR, RFC, FE, and SR value which were better than in the experimental feed treatments F1, F2, and F4. It was anticipated that the inclusion of earthworm meal and maggot meal in experimental feeds F3 and F4 will provide *O. niloticus* with a rich source of essential amino acids. The analysis findings revealed that CF treatment caused the highest RRG and SGR of 2.87% and 1.67%, respectively, followed by F3 with RRG (2.81%) and SGR (1.65%). There were differences among the RRG and SGR values for all formulations, although for CF and F3 they were not statistically significant ($p>0.05$). The ratio of feed nutrients (protein, fat, and fiber) affected relative and specific growth rates to promote fish growth (Eriegha & Ekokotu 2017). Growth happens when there was extra metabolic energy left over after it had been consumed for bodily upkeep and activities. The majority of the feed consumed was used for growth after first being used to maintain the body and replace damaged cells (Adeniyi et al 2020). Increased feed protein does not always lead to increased growth. Increasing feed protein without being followed by a balance with non-protein energy sources will cause protein to be used as an energy source (Samuelsen et al 2022).

The substitution of fish meal with 15% maggot flour in the F3 feed formula (25.33% protein) resulted in a higher RRG and SGR, than in F4 feed with 20% substitution (26.66% protein). This was presumably because the nutritional content of the feed used for growth was sufficient. This was also in accordance with the study of Rachmawati & Samidjan (2013), which suggest that the substitution of fish meal with maggot flour with 25.13% protein could provided more optimal growth compared to protein levels of 26.94 and 27.79% respectively. If their protein requirements are met, fish can grow well if their food intake is appropriate. The amount of protein in the meal has an impact on the *O. niloticus* growth rates (Ngugi et al 2017). Due to the consumption of protein from bodily tissues to maintain important processes, a lack of protein in the diet could resulted in stunted growth, followed by weight loss. The quality of the protein, the energy content of the feed, the nutritional balance, and the level of feeding all had a significant impact on how well the fish used the protein for growth

(Samuelsen et al 2022). If the amount of protein consumed from the feed was too high, only a portion of it would be absorbed and used for cell formation and repair, and the remainder would be expelled (Prangnell et al 2022). High protein intake had the effect of increasing the amount of energy needed for protein catabolism, which results in nitrogen being excreted in the form of ammonia through the kidneys. This was due to fish's limited ability to store protein (Silva et al 2022).

The findings of assessing the ratio of feed conversion (RFC) in F1 to F4 and CF treatments in *O. niloticus* for 60 days likewise revealed significant variations between treatments ($p<0.05$). The RFC values in the F3 and CF treatments also recorded the smallest values and which were not significantly different ($p>0.05$), compared to the CF, i.e. 2.89 and 2.86, respectively. Table 5 shows that the F1 treatment had the highest RFC value of 3.30 and while the lowest was observed in the F4 treatment 2.89. Therefore, it could be seen that the feed that has the highest utilization efficiency was observed in the F3 treatment (15% maggot meal in stead of fish meal), namely 2.89. The higher the use of (20%) in the formulation, the higher the value of the RFC 3.30. This was most likely owing to the chitin content in maggot meal, which reduced the ability of *O. niloticus* seeds to digest feed when fish meal was substituted more frequently. Because chitin was crystalline and soluble only in strong acid solutions, the body was unable to properly digest it (Cummins et al 2017). The quality of the water and the feed utilized had an impact on the RFC value, which was also strongly connected with the fish diet (Rachmawati & Samidjan 2019). The smaller the RFC value, the better the level of feed efficiency (FE), and vice versa. Compared to the control treatment, the use of feed was smaller, which can be seen from the fish's low appetite (Rachmawati and Nurhayati 2022). Tayebi & Sobhanardakani (2020), stated that *O. niloticus* had an omnivorous nature, so that aquaculture was efficient with low feed costs. The higher the quality of the feed delivered, the lower the RFC value (Selvam et al 2018). Meanwhile, if the feed conversion value was high, it means that the quality of the feed provided was not good.

Table 5 demonstrates that the *O. niloticus*'s survival value differed when fish food was substituted with maggot meal and earthworm meal. The mean SR in *O. niloticus* cultivation in the F2, F3, and CF treatments was 95%, while in the F1 and F4 treatments were 90%. The results of observations during the study, showed that *O. niloticus* fed the test feed (using 5, 10, 15, and 20%) and 4% earthworm meal had a higher survival value. The high survival value was suspected to be also related to the water quality parameters during the maintenance, that were still within the limits of optimum conditions for cultivation purposes so that it was feasible for the survival of tilapia (Diógenes et al 2016). The survival rate was affected by both internal and external parameters, such as water quality, stocking density, amount of complete amino acids in feed, and gender, heredity, age, reproduction, and disease resistance (Obirikorang et al 2022).

According to Djissou et al (2016), growth performance and survival rate of *O. niloticus* were influenced by feed and water quality. The constraints faced in aquaculture were mainly the quality seeds, water quality management, feed management, and fish pest management. Provision of quality feed with the required quantity, use of seeds and professional cultivation management were factors that support the success of cultivation (Djissou et al. 2016). The measurement of the water quality parameters showed that the dissolved oxygen (DO) level in *O. niloticus* water was between 5.8 and 6.9 mg L^{-1}, still good for fish maintenance and survival. The results of temperature measurements obtained results of 27.2-28.8^{o}C. It was stated by Rachmawati & Samidjan (2019) *O. niloticus* tolerance is with in a temperature range of 25-30^{o}C, to stay in a comfort zone. Tilapia can survive in a low oxygen content of up to 3 mg L^{-1}, but a suitable oxygen concentration range for cultivation is between 5-7 mg L^{-1} and the levels of ammonia (NH_3) should be < 0.1 mg L^{-1}.

Conclusions. According to the study's findings, replacing fish meal with a protein source consisting of 4% earthworm meal and 15% maggot meal could raise *O. niloticus* SGR, RRG, FE, and SR values, while decreasing the RFC. The use of maggot meal and earthworm meal on F3 (20% FM + 15% MM + 4% EM) with 25.32% protein resulted in RRG = 2.81%, and SGR = 1.65%, RFC = 2.89, FE = 34.58, and SR = 95%, which was almost the same as for CF with 26% protein, which produces an RRG of 2.87%, an SGR of 1.67%, an RFC of 2.86, an FE of 34.74) and an SR of 95%. Using of earthworm meal and maggot meal as sources of protein can help minimizeing the reliance on fish meal in fish feed.

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Potential of maggot and earthworm meals as protein sources for the growth of Nile tilapia (*Oreochromis niloticus*)

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Abstract. Maggots and earthworms are sources of animal protein that have a high nutritional value, could reduce the need for fish meal, and have positive ecological effects. Maggot and earthworms have the potential as alternative protein sources for fish feed, either processed into fresh or dried into flour. The purpose of this study was to determine whether maggot flour and earthworm flour have the potential to replace fish meal as a protein source in artificial feed formulations, including the assessment of their effect on the specific growth rate (SGR), rate of relative growth (RRG), ratio of feed conversion (RFC), feed efficiency (FE), and survival rate (SR) of the Nile tilapia (*Oreochromis niloticus*). This study used an experimental method with a completely randomized design (CRD), 4 treatments (F1, F2, F3, and F4) and commercial feed (CF) as a comparison. *O. niloticus* sample specimens had an average weight of 30.14±0.02 g. The treatment applied was the use of maggot meal for the fish meal substitution, at concentrations from 5 to 20%, together with 4% earthworm meal. The treatments are F1=30% fish meal (FM)+5% maggot meal (MM)+4% earthworm meal (EM), F2=25% fish meal (FM)+10% maggot meal (TM)+4% earthworm meal (EM), F3=20% fish meal (FM)+15% maggot meal (MM)+4% earthworm meal (EM), and F4=15% fish meal (FM)+20% maggot meal (MM)+4% earthworm meal (EM). The SGR, RRG, FE, and SR values of *O. niloticus* can increase, while the RFC value decreases with the addition of animal protein sourced feed containing maggot flour and earthworms. In fact, the use of maggot meal and earthworm meal in the treatment F3 (20% FM+15% MM+4% EM), with 25.32 protein, produced the following effect: RRG=2.81%, SGR=1.65%, RFC=2.89%, FE=34.58 and SR=95% which was almost the same as în the use of commercial feed (CF), with 26% protein, which produced the following effect: RRG=2.87%, and SGR=1.67%, RFC=2.86%, FE=34.74 and SR=95%. The water quality in *O. niloticus* culture media (temperature, dissolved oxygen (DO), pH (power of hydrogen), ammonia) was also found to be within the appropriate range for *O. niloticus* cultivation.

Key Words: artificial feed, fish meal, commercial feed, feed efficiency, water quality.

Introduction. Indonesia is the second largest producer of tilapia in the world, with 6.3 million tonnes produced globally in 2018. Nile tilapia (*Oreochromis niloticus*) was widely cultivated worldwide (Suhermanto et al 2019). *O. niloticus* could be adapted to aquaculture environments, traditional, semi or intensive scale cultivation systems, has a high economic value and was not affected by the market price fluctuations, is a significant source of animal protein, and its production had experienced a significant increase in in recent years (Wang & Lu 2016).

The increasing demand for *O. niloticus* commodities was related to the intensification of aquaculture, so that it will have an impact on the need for feed, which was one of the inhibiting factors for growth. The cost of feed needs for cultured fish was around 60-70% of the total production cost, so the development of feed with sustainable local raw materials was needed, and becomes a challenge for farmers (Ngugi et al 2017; Sarker et al 2018). The main cause of the high price of fish feed was the increase of the price of feed raw materials. The main protein source of commercial fish feed still comes from imported raw materials such as fish meal. In addition, excessive use of fish meal causes the supply of fish resources to be depleted (Pucher et al 2014). In addition, there is a fairly high pressure on the sustainability of fishery resources due to the excessive use of fish meal. As a result, a breakthrough in the quest for substitute protein sources was required to support the availability of feed that is sustainable, accessible, and must be environmentally friendly.

Maggots and earthworms are sources of animal protein, that have a high nutritional value, could reduce the need for fish meal, and have positive ecological effects (Li et al 2019a). Black soldier fly maggots or larvae have the potential to serve as an alternate source of protein for fish food, whether processed into fresh (live maggots) or dried into meal. The protein content of maggot was 42.1% (in dry weight), moisture (7.9%), fat (24.8%), ash (10.3%), and crude fiber (7%), so that it can meet the protein needs of livestock (Park 2016). The amino acid content in maggot contains arginine (2.29%), methionine (0.66%), phenylalanine (1.63%), threonine (1.70%), tryptophan (0.55), valine (2.56%), histidine (1.50%), isoleucine (1.87%), lysine (2.71%), and leucine (3.23%) and can support fish development (Djissou et al 2015).

Earthworm meal has a considerably high nutrient content, is easily digested, and contains high protein (approximately 54-66%) that is relatively similar to that in fish meal (Musyoka et al 2019). According to a research study conducted by Parolini et al (2020), earthworm meal (*Lumbricus rubellus*) contains 63.0% protein, 5.9% crude fat, 8.9% ash, and 14.76 kJ g^{-1} of energy for metabolism. The amino acid profile found in earthworm flour was: arginine (2.83 g kg^{-1}), phenylalanine (6.26 g kg^{-1}), tryptophan (4.43 g kg^{-1}), valine (4.43 g kg^{-1}), histidine (1.47 g kg^{-1}), isoleucine (2.04 g kg^{-1}), leucine (4.11 g kg^{-1}) and lysine (6.35 g kg^{-1}).

The suitability of other animal protein sources for replacing fish meal was studied for the *O. niloticus*, with maggot (Ezewudo et al 2015), for gariepinus (Djissou et al 2016a), juvenile turbot (*Psetta maxima*) and white shrimp, with maggot and earthworm (Kroeckel et al 2012; Cummins et al 2017), for *Clarias gariepinus* (Dedeke et al 2013), *Cyprinus carpio* (Pucher et al 2014) and *Oreochromis* sp. juvenile (Jabir 2012), with earthworms. In general, substituting fish meal could reduce feed consumption while boosting growth and feed effectiveness (Rachmawati & Nurhayati 2022). There hadn't been any investigation on the effects of mixing maggot flour with earthworm flour on the growth of *O. niloticus*, according to some earlier literature, so the current study is a novelty. This study aimed to determine the effect of using maggot flour and earthworm flour as fish meal substitution, in artificial feed formulations, on the rate of relative growth (RRG), specific growth rate (SGR), ratio of feed conversion (RFC), feed efficiency (FE), and survival rate (SR) of *O. niloticus*.

Material and Method

Experimental materials. The raw material for fish meal was obtained from the fish meal industry, Sari Ulam, Tegal City. Maggot and earthworms were obtained from local maggot and worm farms, Tegal agrofarm Indonesia. The manufacture of maggot meal refers to (Ahmad et al 2022) with a slight modification. A hot air oven was used to dry the maggot for around 36 hours at 60°C, then mashed using a grinder and filtered through a sieve. The manufacture of earthworm meal refers to an existing method (Parolini et al 2020), with a slight modification: steamed earthworms at 70°C for 10 min and grinded with a grinder until the particles were finer, then at 60°C in a hot air oven. The earthworm were taken out of the oven and placed in plastic bags after 10-12 h.

Experimental fish. *O. niloticus* utilized in the current research had an average weight of 30.14±0.05 g at the beginning. The number of specimens of *O. niloticus* used in the study was 100 individuals, obtained from the Fish Seed Center of Tegal City, Central Java, Indonesia. 20 test tilapia specimens were stocked as in each maintenance tarpaulin container. The completeness of the organs, physical health, size, and weight were taken into consideration when choosing the test fish. The fish were fed at satiation twice daily, at 8:00 and 16:00, to ensure that they have truly adapted to the new surroundings and the feed provided. The test fish were reared for 60 days and during the rearing the tilapia were fed according to the treatment to determine the weight growth from May to July

2022. Sampling was carried out every 10 days to determine the increase in the weight of the test fish and to adjust the weight of the feed to be given.

Diet experiment. The ingredients for the feed composition include fish meal, earthworm meal, maggot meal, corn meal, rice bran, copra meal, tapioca meal, fish oil, premix and commercial feed from PT Matahari Sakti, Surabaya, Indonesia, with 26% protein. As much as 5 to 20% of maggot flour was used as a substitute for fish meal. 4% earthworm meal was added to all 4 treatments (F1, F2, F3, and F4). The treatments had different concentrations of fish meal (FM), maggot meal (MM) and earthworm meal (EM): F1=30% fish meal (FM)+5% maggot meal (MM)+4% earthworm meal (EM), F2=25% fish meal (FM)+10% maggot meal (TM)+4% earthworm meal (EM), F3=20% fish meal (FM+15% maggot meal (MM)+4% earthworm meal (EM), and F4=15% fish meal (FM)+20% maggot meal (MM)+4% earthworm meal (EM). Commercial feed (CF) was also used, for comparison. Previously, proximate analyses of feed components like fish meal, maggot meal, and earthworm meal had been performed (Table 1) for determining their composition before the experimental diet was used. Table 2 presents the proximate results of the experimental feed formulation. Food, up to 5% of their body weight, was provided at a frequency of two meals per day, at 8:00 and 17:00 WIB.

Table 1

Nutrient composition (% dry weight) in fish meal, maggot meal, and earthworm meal

Ingredient (%)	*Protein*	*Moisture*	*Ash*	*Fat*	*Fiber*
Fish meal	40.43 ± 0.09^{c}	8.67 ± 0.01^{a}	16.48 ± 0.27^{a}	8.41 ± 0.01^{b}	4.40 ± 0.12^{b}
Maggot meal	44.63 ± 0.02^{b}	$6.34^{c} \pm 0.06^{b}$	12.53 ± 0.03^{b}	11.50 ± 0.11^{a}	10.19 ± 0.04^{a}
Earthworm meal	54.46 ± 0.09^{a}	$8.18^{b} \pm 0.02^{c}$	5.61 ± 0.03^{c}	$7.43^{c} \pm 0.02^{c}$	6.39 ± 0.14^{b}

The number after ± is the standard error value; different superscript letters on the same line indicate significantly unequal treatment effects (P<0.05).

Table 2

Experimental diet composition in each formula

Materials	*Feed formulation (% 100 g^{-1})*			
	F1	*F2*	*F3*	*F4*
Fish meal	30.00	25.00	20.00	15.00
Maggot meal	5.00	10.00	15.00	20.00
Earthworm meal	4.00	4.00	4.00	4.00
Tapioca meal	10.00	10.00	10.00	10.00
Bran meal	18.00	18.00	18.00	18.00
Copra meal	10.00	10.00	10.00	10.00
Corn meal	20.00	20.00	20.00	20.00
Vitamin-mineral mix	1.00	1.00	1.00	1.00
Fish oil	2.00	2.00	2.00	2.00
Total	100	100	100	100

F1 (30% FM, 5% MM and 4% EM); F2 (25% FM, 10% MM and 4% EM); F3 (20% FM, 15% MM and 4% EM); F4 (15% FM, 20% MM and 4% EM) and CF (Commercial feed).

Experimental containers. In this study, the container used was a plastic pool with a length, width and height of 1.2 m x 1 m x 0.70 m, then the pool was filled with water until a height of 50 cm was reached. The maintenance container was equipped with an aeration and circulation system for changing water using a pipe inside of the pond. The test *O. niloticus* were stocked into the plastic pool and acclimatized first. Feed was administered progressively, the uneaten feed being removed, avoiding turbidity in the water of the test container. Water quality was maintained by siphoning the remaining feed and feces at the bottom of the container, as well as by changing the water every 7 days (25% of the initial volume was replaced at once).

Research methods. Completely Randomized Design (CRD) was used in the experimental methodology of this study, with 4 trials, and the use of commercial feed (CF) as control sample. Microsoft Excel 2013 was used to determine the mean difference between treatments: analysis of variance (ANOVA) was employed in statistical data analysis to ($p<0.05$), followed by a Tukey test.

Research variable. Several variables were observed in this study, including the RRG and SGR (Katya et al 2017), RFC (Selvam et al 2018), FE, and SR. The formula equations applied in this study are presented as follows (Rachmawati & Nurhayati 2022):

$$\text{Rate of relative growth (RRG) (\%)} = \frac{\text{final weight-initial weight}}{\text{Fish rearing time in days-initial weight}} \times 100$$

$$\text{Specific growth rate} = \frac{\ln\text{(final weight-initial weight)}}{\text{Fish rearing time in days}} \times 100$$

$$\text{RFC} = \frac{\text{Amount of dry feed intake}}{\text{(final weight in days+dead fish weight in days)- initial weight in days}} \times 100$$

$$\text{FE} = \frac{\text{(final weight in days+dead fish weight in days)- initial weight in days}}{\text{Amount of dry feed intake}} \times 100$$

$$\text{SR (\%)} = \frac{\text{final fish count}}{\text{Initial Fish count}} \times 100$$

Chemical and microbiological analysis. Protein, fat, water, ash, and fiber proximate chemical analyses were performed in accordance with the AOAC's recommended procedure (Latimer 2016). The Kjeldahl method was used for protein proximate analysis (Kusnadi et al 2022). The solution of the destroyed sample was placed in a steam distillation apparatus and three drops of phenolphthalene indicator were added before the distillation was completed. When the dripping distillate reacts neutrally to red litmus and the color of the reservoir solution changes to green, the reservoir solution is determined as a solution of the destroyed sample (pink). Fat analysis was performed using Soxhlet extraction. The amino acids profile of feed was determined using the HPLC method (Nik et al 2021) with slight modifications. 30 mg of protein hydrolyzate was placed into a tube, then 4 mL of 6 N HCl was added which has been heated at 110°C for 24 hours, cooled at room temperature, neutralized (pH=7) with 6 N NaOH, then the sample was added with distilled water to a volume of 10 mL, filtered using 0.2 μm Whatman filter paper. The tube was rinsed for 30 seconds with nitrogen gas and immediately covered with a layer of Teflon hat. The tubes were placed in an electric oven for 24 hours at 110°C for samples hydrolysis. The tubes were then cooled at room temperature for 30 minutes. Feed microbiological tests include analyzing aflatoxin contamination using the HPLC method (María et al 2022) and Salmonella, with ISO 6579-1, which were evaluated in the laboratory center for agro-based industry (Mooijman et al 2019).

Water quality parameters. Measurements of temperature (°C), pH (hydrogen power), and dissolved oxygen (DO) were performed to determine the water quality, every two days at 08.00 and 16.00 WIB, in each unit experiment. At the start, midpoint, and end of the study, UV-Vis Spectrophotometry was used to analyze the ammonia (NH_3) content.

Results. Table 3 displays the findings of the proximate and microbiological examination of feed on 4 formulations using earthworm meal and maggot meal as well as 1 commercial feed. The protein and fat content (%) for treatments F1 to F4 ranged from

23.65, 24.74, 25.32, and 26.60%, respectively. The protein content in each formula increased from F1 to F4, in line with the 5% increase of the maggot meal in each formula.

Table 3

Proximate and microbiological analysis (% dry weight) on each feed formulation

Proximate analysis	*F1*	*F2*	*F3*	*F4*	*CF*
Protein (%)	23.65±0.02[d]	24.74±0.02[c]	25.32±0.03[b]	26.66±0.03[a]	26.00
Moisture (%)	9.87±0.02[a]	9.75±0.01[b]	9.66±0.01[bc]	9.55±0.02[c]	10.00
Ash (%)	12.76±0.03[a]	12.24±0.02[b]	11.63±0.25[c]	10.88±0.02[d]	12.00
Fat (%)	5.14±0.01[d]	5.64±0.01[c]	6.60±0.02[b]	7.66±0.01[a]	5.00
Fiber (%)	7.19±0.04[d]	7.88±0.02[c]	8.35±0.01[b]	8.83±0.04[a]	8.00
Energy (kcal)	3818.2	3853.7	3899.3	3936.5	3906.8
Microbiological analysis					
Aflatoxin (μg kg^{-1})	6.79	6.18	4.68	3.81	3.76
Salmonella	Negative	Negative	Negative	Negative	Negative

The number after ± was the standard error value; different superscript letters on the same line indicate significantly unequal treatment effects (P<0.05). F1 (30% FM 5% MM, and 4% EM); F2 (25% FM, 10% MM and 4% EM); F3 (20% FM, 15% MM and 4% EM); F4 (15% FM, 20% MM and 4% EM) and CF (Commercial feed).

Table 4 lists the essential amino acid (EAA) composition of the feed formulation, as well as the necessary EAA amounts for *O. niloticus*.

Table 4

Profile of essential amino acids (EAA) in each feed treatment

Asam amino	*Experimental diets formulation*				
	F1	*F2*	*F3*	*F4*	*CF*
Histidine	0.34	0.36	0.36	0.46	0.43
Threonin	0.94	1.00	1.08	1.30	1.07
Arginin	2.00	2.16	2.26	2.72	2.76
Tyrosin	1.62	2.12	2.46	2.98	2.20
Methionin	0.2	0.22	0.24	0.3	0.37
Valin	1.08	1.24	1.36	1.68	1.25
Phenylalanin	0.82	0.9	0.94	1.16	1.14
Isoleucin	0.70	0.82	0.90	1.12	0.95
Leucin	1.70	1.86	1.96	2.36	1.99
Lysin	1.90	1.90	2.18	2.52	2.33

F1 (30% FM 5% MM, and 4% EM); F2 (25% FM, 10% MM and 4% EM); F3 (20% FM, 15% MM and 4% EM); F4 (15% FM, 20% MM and 4% EM) and CF (Commercial feed).

Table 4 shows the experimental feed's amino acids profile from the formulations F1 to F4, whose pattern was consistent with the rise in the total protein content in each feed formulation.

Table 5 displays the findings from the examination of the variables RRG, SGR, RFC, FE, and SR. The results of observations on all variables show the experimental feed the F3 formula showed almost the same results as the use of CF. Commercial feed showed the best results of RRG, SGR, RFC, FE, and SR compared to the experimental formula, followed by the treatments F3 and F4, while the results of the treatments F2 and F1 showed lower values. Measurements and observations of water quality during the study are presented in Table 6.

Table 5

Data replication of rate of relative growth (RRG), specific growth rate (SGR), ratio of feed conversion (RFC), feed efficiency (FE), and survival rate (SR) of *Oreochromis niloticus*

Parameter	*Experimental diet formulation*				
	F1	*F2*	*F3*	*F4*	*CF*
RRG (% day^{-1})	$2,35 \pm 0.01^{d}$	$2,58 \pm 0.02^{c}$	2.81 ± 0.02^{ab}	2.76 ± 0.03^{b}	2.87 ± 0.03^{a}
SGR (%)	1.46 ± 0.02^{b}	1.56 ± 0.01^{b}	$1.65^{c} \pm 0.01^{a}$	$1.63^{d} \pm 0.02^{a}$	1.67 ± 0.02^{a}
RFC	3.30 ± 0.01^{d}	3.07 ± 0.02^{c}	2.89 ± 0.03^{a}	2.98 ± 0.02^{b}	2.86 ± 0.01^{a}
FE	30.23 ± 0.02^{e}	32.49 ± 0.02^{d}	34.58 ± 0.01^{b}	33.47 ± 0.01^{c}	34.74 ± 0.02^{a}
SR (%)	90.00±0.00	95.00±0.00	95.00±0.00	90.00±0.00	95.00±0.00

The number after ± was the standard error value; different superscript letters on the same line indicate significantly unequal treatment effects (P<0.05).

Table 6

Water quality parameters for each experimental treatment (the intervals correspond to the values measured during the replications)

Parameters of water quality	*Treatment*					*Reference range*
	F1	*F2*	*F3*	*F4*	*CF*	
Temp. (°C)	27.2-28.6	27.2-28.7	27.3-28.5	27.2-28.6	27.2-28.8	28.5-30.55*
DO (mg L^{-1})	5.8-6.8	5.7-6.9	5.8-6.7	5.7-7.0	5.7-6.8	>3*
pH	6.6-7.8	6.5-7.7	6.5-7.5	6.6-7.6	6.7-7.7	6.5-8.5*
Ammonia (mg L^{-1})	0.006-0.02	0.006-0.02	0.006-0.02	0.006-0.06	0.006-0.04	<0.1*

*Boyd (1992). F1 (30% FM 5% MM, and 4% EM); F2 (25% FM, 10% MM and 4% EM); F3 (20% FM, 15% MM and 4% EM); F4 (15% FM, 20% MM and 4% EM) and CF (Commercial feed).

During the study, the value of water quality parameters in the shrimp culture medium was still within the desired range, thus ensuring the growth of *O. niloticus*.

Discussion. In the results of the proximate test, the protein values from treatment F1 to F4 were 24.65 to 26.60% (Table 3). The increase in protein content in the feed formulation was influenced by the maggot meal, which had a protein content of 44.13%, higher than in fish meal 40.43%. The combination of maggot meal and earthworm meal in F1 (30% FM and 5% MM, 4% EM) showed the lowest protein level. The microbological examination of aflatoxin contamination in all formulations revealed that aflatoxin levels decreased with an increased usage of maggot mealdue to its antimicrobial and antifungal activity, reducing the microbial contamination in feed formulations (Valachova et al 2014; Pöppel et al 2015). The results of aflatoxin content for all feed formulas were still relatively safe for fish consumption, because they were still below the threshold, which was less than 20 μg kg^{-1} and also do not contain salmonella bacteria.

Maggot and earthworms were good sources of animal protein for *O. niloticus* because they contain essential amino acids required for the fish growth. Protein requirements for fish growth vary: the younger the fish, the greater the protein needs. Nguyen et al (2020a) stated in his research that the need and balance of feed protein was 24-30%, for *O. niloticus* measuring 7.90 g, for its growth, up to 10 weeks. Another study stated that the protein requirement of feed for juvenile *O. niloticus* measuring 12.7 g in an eight-week period was 22.2% to 29.7%, for proteins sourced from soybean meal, fish meal, and corn meal (Nguyen et al 2020b). Based on the research report presented, the range of protein used for *O. niloticus* growth is between 22.2 to 30%, so that alternative feed products in this study which have a protein content of 23.65 to 26.66% can be used as a reference for *O. niloticus* cultivation. Amino acid content in the treatments F4 and F3 with ratios FM/MM/EM of 15:20:4(%), respectively and 20:15:4(%), respectively, were sistematically higher, while the treatment F1, with a ratio(FM)/(MM)/(EM) of 30:5:4(%) was almost always the lowest (Table 3). This suggests that the value of its amino acids content could be improved by adding natural feed sources of maggot meal to the feed combination, in amounts ranging from 5% to 20%. Table 4 shows that there are nine essential amino acids out of ten that are required to be present in the five types of feed, namely arginine, histidine, isoleucine, leucine, lysine, threonine, valine, methionine, and phenylalanine. Lysine is an essential amino acid that can serve to evaluate the feed effects on the fish growth (Wu et al 2022). This is because the amino acid lysine in the animal body explicitly contributes to the fish growth and protein deposits in the tissues, since it has no other metabolic roles (Li et al 2019b; Marchão et al 2020). The requirement for the amino acid lysine in the growth phase of lysine tilapia can reach 1.55% (Diógenes et al 2016). The values of amino acid levels, determined in the formulations F1 through F4 and commercial feed (CF) content, are larger than this threshold value, i.e.: 1.90% in F1, 1.90% in F2, 2.18% in F3, 2.52% F4, and2.33% in CF. This shows that the requirement for the amino acid lysine for *O. niloticus* is fully met. Diets with an unbalanced amino acid profile can lead to less food intake and less effective utilization of essential amino acids (Prabu et al 2020).

The results of feed treatment on formula F3, F4 and commercial feed (CF) with 25.32, 26.66 and 26% protein, respectively, for *O. niloticus* resulted in RRG, SGR, RFC, FE, and SR values which were better than in the experimental feed treatments F1, F2, and F4. It was anticipated that the inclusion of earthworm meal and maggot meal in experimental feeds F3 and F4 will provide *O. niloticus* with a rich source of essential amino acids. The analysis findings revealed that CF treatment caused the highest RRG and SGR, of 2.87 and 1.67%, respectively, followed By F3, with RRG (2.81%) and SGR (1.65%). There were differences among the RRG and SGR values for all formulations, although for CF and F3 they were not statistically significant ($p>0.05$). The ratio of feed nutrients (protein, fat, and fiber) affected relative and specific growth rates to promote fish growth (Eriegha & Ekokotu 2017). Growth happens when there was extra metabolic energy left over after it had been consumed for bodily upkeep and activities. The majority of the feed consumed was used for growth after first being used to maintain the body and replace damaged cells (Adeniyi et al 2020). Increased feed protein does not always lead to increased growth. Increasing feed protein without being followed by a balance with non-protein energy sources will cause protein to be used as an energy source (Samuelsen et al 2022).

The substitution of fish meal with 15% maggot flour in the F3 feed formula (25.33% protein) resulted in a higher RRG and SGR, than in F4 feed with 20% substitution (26.66% protein). This was presumably because the nutritional content of the feed used for growth was sufficient. This was also in accordance with the study of Rachmawati & Samidjan (2013), which suggested that the substitution of fish meal with maggot flour with 25.13% protein could provide more optimal growth, compared to protein levels of 26.94 and 27.79% respectively. If their protein requirements are met, fish can grow well if their food intake is appropriate. The amount of protein in the meal has an impact on *O. niloticus* growth rates (Ngugi et al 2017). Due to the consumption of protein from bodily tissues to maintain important processes, a lack of protein in the diet could resulted in stunted growth, followed by weight loss. The quality of the protein, the energy content of the feed, the nutritional balance, and the level of feeding all had a significant impact on how well the fish used the protein for growth (Samuelsen et al 2022). If the amount of protein consumed from the feed was too high, only a portion of it would be absorbed and used for cell formation and repair, and the remainder would be expelled (Prangnell et al 2022). High protein intake had the effect of increasing the amount of energy needed for protein catabolism, which results in nitrogen being excreted in the form of ammonia through the kidneys. This was due to fish's limited ability to store protein (Silva et al 2022).

The findings of assessing the ratio of feed conversion (RFC) in F1 to F4 and CF treatments in *O. niloticus* for 60 days likewise revealed significant variations between treatments ($p<0.05$). The RFC values in the F3 and CF treatments also recorded the smallest values, which were not significantly different ($p>0.05$), compared to the CF, i.e. 2.89 and 2.86, respectively. Table 5 shows that the F1 treatment had the highest RFC value, of 3.30, while the lowest was observed in the F4 treatment: 2.89. Therefore, it could be seen that the feed that has the highest utilization efficiency was observed in the

F3 treatment (15% maggot meal instead of fish meal), namely 2.89. The higher the use of MM (20%) in the formulation, the higher the value of the RFC (3.30). This was most likely owing to the chitin content in maggot meal, which reduced the ability of *O. niloticus* to digest feed when fish meal was substituted more frequently. Because chitin was crystalline and soluble only in strong acid solutions, the body was unable to properly digest it (Cummins et al 2017). The quality of the water and the feed utilized had an impact on the RFC value, which was also strongly connected with the fish diet (Rachmawati & Samidjan 2019). The smaller the RFC value, the better the level of feed efficiency (FE), and vice versa. Compared to the control treatment, the use of feed was smaller, which can be seen from the fish's low appetite (Rachmawati & Nurhayati 2022). Suhermanto et al (2019) stated that *O. niloticus* had an omnivorous nature, so that aquaculture was efficient with low feed costs. The higher the quality of the feed delivered, the lower the RFC value (Selvam et al 2018). Meanwhile, if the feed conversion value was high, it means that the quality of the feed provided was not good.

Table 5 demonstrates that *O. niloticus*'s survival value differed when fish food was substituted with maggot meal and earthworm meal. The mean SR in *O. niloticus* cultivation in the F2, F3, and CF treatments was 95%, while in the F1 and F4 treatments were 90%. The results of observations during the study showed that tilapia fed the test feed (using 5, 10, 15, and 20% of maggot meal instead of fish meal) and 4% earthworm meal had a higher survival value. The high survival value was suspected to be also related to the water quality parameters during the maintenance, that were still within the limits of optimum conditions for cultivation purposes so that it was feasible for the survival of tilapia (Diógenes et al 2016). The survival rate was affected by both internal and external parameters, such as water quality, stocking density, amount of complete amino acids in feed, and gender, heredity, age, reproduction, and disease resistance (Obirikorang et al 2022).

According to Djissou et al (2016a), growth performance and survival rate of *O. niloticus* were influenced by feed and water quality. the constraints faced in aquaculture were mainly the quality seeds, water quality management, feed management, and fish pest management. Provision of quality feed with the required quantity, use of seeds and professional cultivation management were factors that support the success of cultivation (Djissou et al 2016b). the measurement of the water quality parameters showed that the dissolved oxygen (DO) level in *O. niloticus* water was between 5.8 and 6.9 mg L^{-1}, still good for fish maintenance and survival. The temperature measurements showed values of 27.2-28.8^{o}C. It was stated by Rachmawati & Samidjan (2019) that *O. niloticus*'s tolerance is within a temperature range of 25-30^{o}C, to stay in a comfort zone. Tilapia can survive in a low oxygen content of up to 3 mg L^{-1}, but a suitable oxygen concentration rangefor cultivation is between 5-7 mg L^{-1} and the levels of ammonia (NH_3) should be <0.1 mg L^{-1}.

Conclusions. According to the study's findings, replacing fish meal with a protein source consisting of 4% earthworm meal and 15% maggot meal could raise the *O. niloticus*'s SGR, RRG, FE, and SR values, while decreasing the RFC. The use of maggot meal and earthworm meal on F3 (20% FM + 15% MM + 4% EM) with 25.32 protein resulted in: RRG=2.81%, SGR=1.65%, RFC=2.89, FE=34.58 and SR=95%, which was almost the same as for the CF, with 26% protein, which produces an RRG of 2.87%, an SGR of 1.67%, an RFC of 2.86, an FE of 34.74 and an SR of 95%. Using earthworm meal and maggot meal as sources of protein can help minimizing the reliance on fish meal in fish feed.

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