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The effect of the incorporation of the flour of the worm of compost (*Eisenia foetida*) (Savigny, 1928) in the feed of tilapia *Oreochromis niloticus* high out in basin

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Abstract
The present study aims at determining the optimal and maximum quantity of the flour of worm of manure *E. foetida* in the feed of fingerlings of *O niloticus.*
The experiment has been carried out of basins square of 1m^3 of volume during 45 days from October at November 2016. During the period of acclimatization which lasted three days the fingerlings of initial average weight 4.79 ± 0.23 g have been nourished with Coppens. They have been then subjected to five treatments (T0; T5; T10; T15 and T20 corresponding to contents of 0%; 5%; 10%; 15% and 20% of flour of worm of manure in feed.
At the end of the experiment the final average weights calculated was in the between 33.18 ± 13.71 g and 56.70 ± 14.65 g according to treatments'.
Thus, the variance analysis with single direction (Anova1) with Statview 5.1 indicates that no significant difference exists between the various rates of survival. However, a significant difference between the various specific growth rates (from 3.45 ± 0.31%/day to 6.67 ± 0.30%/day) was observed.
According to the mathematical model (amount-answer) based on the polynomial regression of the second degree, the maximum rate of incorporation of the flour of worm of manure in feed of fingerlings of *O niloticus* high out of basin under the conditions of this study was 13,82%.

Keywords: Content, worm of manure, maximum, Optimum, *O. niloticus*

1. Introduction
In the intensive systems of breeding, several factors like the quantity and quality of the food [1] as well as the density of loading [2-4] influence the survival rate, the growth, the food effectiveness and the production of the fishes.
Indeed, the determination of the suitable rations through the optimum and maximum of incorporation of food ingredients for cultivated fishes are significant to carry out a better production. The determination of the optimum and maximum rate is of an immutable utility because it makes it possible to make an efficient management of the resources available. Moreover this practice also allows avoiding the presence of limiting factor observed in the food formulations and of this fact correct use of the nutriments by the subjects of breeding.
Oreochromis niloticus is a preferred fish for food and constitutes an extremely significant part of principal food for Africans because of its tasty flesh, it remarkable growth and it high economic value [5].
This fish species was nourished a long time with fish forages or of waste of fishing and canning facilities for imitate the supply natural environment (phytoplankton, zooplankton, macro-invertebrates, etc). This practice, which generates a significant quantity of waste, was neither compatible with an intensification of pisciculture (fluctuating provisioning), nor with the respect of the environment. The manufacture of made up food starting from nonconventional local by-products whose worm of manure will make it possible to reach a decisive stage in the breeding of fish.
It reacts correctly in the presence of a feed containing the worm of compost [6]. However, there do not exist data on the amount of worm of manure having to be built-in in food of this species.

This study gives itself for principal objective the determination not only of the optimum but also of the maximum manure's worm flour (*E. foetida*) in feed of fingerlings of *O. niloticus* by the mathematical model (amount-answer) of Brett and Grove [7].

2. Materials and methods

2.1 Experimental procedure

This study has been realized at the experimental station of the National Institute of Agricultural Research of Benin and precisely at the level of halieutic program production. The soya bean oil cake, the fish meal and the maize yellow corn sound are the principal conventional ingredients used. They have been obtained from in the local market of Abomey-Calavi. The other by products except the complex-vitaminized has been collected in nature to minimize the cost of food and to as make sure as the ingredients are free from contaminants related to the transformation of the products from which they resulted. The manure's worm used has been produced at the test center of the Agronomic Science Faculty of the University of Abomey-Calavi [8]. The raw ingredients have been crushed and filtered with a sieve of 400 micrometers. The mixture of the ingredients and the setting in pellet have been made according to Kpoguè *et al.* [9] ; Chikou *et al.* [10] and Houndonougbo *et al.* [11]

The pellets were sun dried, bagged and stored in a room with 20 ˚C until the distribution. The table 1 below presents the analysis of the biochemical composition of the ingredients used for the manufacture of food.

Table 1: Biochemical composition of the ingredients (expressed in % of the matter dries).

Ingredients	Fish meal	Flour of sound of soya	Flour of yellow maize	Flour of worm of manure (*Eisenia foetida*)	Flour of sheet of moringa
Dry maters [1]	91.47	88.96	86.12	nc	25
Proteins	47.21	43.50	7.84	59	6.7
Lipids	1.62	1.38	1.43	9	1.7
Fibres	0.95	6.60	6.59	<5	0.9
Ashes	28.14	5.80	1.32	17	nc
ENA[2]	13.55	31.68	68.94	15	nc
Calcium (g/Kg)	6.31	0.29	0.03	nc	440 mg
Phosphorus (g/Kg)	3.89	0.71	0.25	nc	70 mg
AAE (g/100 g DM)[3]					
Lysin	5.05	2.79	0.17	6.8	+4.3
Valin	3.91	1.41	0.30	4.7	7.1
Leucine	4.62	3.53	0.80	7.2	9.3
Histidine	1.48	0.98	0.17	2.60	+2.1
Arginine	3.15	3.48	0.30	6.00	6.0 g/16g
Threonine	3.32	1.68	0.20	5.2	4.9
Isoleucine	2.11	1.61	0.25	4.3	6.3
Methionine + Cysteine	2.31	1.08	0.28	nc	2.0
Tryptophan	0.91	0.69	0.06	nc	+1.9
Phenylalanine	2.73	1.83	0.58	3.8	+6.4

1: compared to the fresh matter, 2:Extractive Not Nitrogenized = 100% - (% lipid+ % water content + % proteins+ % fibres + % ashes) and 3: Essential amino acids.

nc = unknown, 1 compared to the fresh matter, 2 Extractive Not Nitrogenized = 100% - (% lipid+ % water content + % protens+ % fibres + % ashes), 3 essential amino Acids.

Source : [11-17] ; [10 ; 6].

2.2 Feed's analysis formulated

Five feed treatment T0; T5; T10; T15 and T20 have been elaborated for tilapia's feed of the in phase of pre-enlargement. In the treatments, the manure's worm flour is gradually built-in at respective rates of 0%, 5%, 10%, 15% and 20% in progressive substitution of the fish meal.The chemical analysis realized has been related primarily to the diets. Whole fish taken at the beginning as at the end of the experiment were analyzed for comparison.

Fish carcasses have been beforehand crushed in the form of fine powder in order to facilitate the analysis and to obtain homogeneous samples as well as a good reproducibility of the results. The analysis of the various samples has been realized at the Laboratory of Valorization and Management of the quality of the Bio-ingredients feeds (LABIO) of the department of Nutrition and Food Sciences of the Agronomic Faculty of Science of the University of Abomey-Calavi.

The analysis of the water content, proteins, lipids, ashes and out of raw fibres, are realized out by using standard procedures [18].

Table 2 shows the centesimal composition of the experimental modes and the results of the analysis.

Table 2: Centesimal composition and bromatologic analysis of experimental feed.

Ingredients	Treatments				
	T0	**T5**	**T10**	**T15**	**T20**
Fish meal	25	20	15	10	5
Flour of soya	25	25	25	25	25
Flour of yellow corn	38	38	38	38	38
Flour of worm of manure (*Eisenia foetida*)	0	5	10	15	20
Flour of sheet of moringa	10	10	10	10	10
Palm oil	1	1	1	1	1
Methionine	0.5	0.5	0.5	0,5	0.5
Lysin	0.5	0.5	0.5	0.5	0.5
Total	100	100	100	100	100
Results of the analysis (g/100g) of feed					
Dry matter	85.51	90.06	89.52	90.03	90.12
Proteins	27.41	35.50	46.25	35.34	40.01
Lipids	17.23	18.15	18.04	18.14	18.16
Starch	38.92	31.32	20.03	35.62	27.97
Ashes	13.25	12.11	12.63	8.78	11.17
Fibre	3.19	2.92	3.05	2.12	2.69
Energy (g/100g)	481.72	491.94	488.80	508.43	496.69

(T0: food containing 0% of flour of worm of manure; T5: food containing 5% of flour of worm of manure; T10: food containing 10% of flour of worm of manure; T15: food containing 15% of flour of worm of manure and T20: food containing 20% of flour of worm of manure)

2.3 Experimental devices

In total 15 square basins of 1 m^3 of volume, laid out in three series of five have been used in open circuit for the pre-enlargement of 450 fingerlings of initial average weight 4.79 ± 0.23g given starting from the morphometric measurements taken on 50 individuals chosen randomly in the starting population seen that the fingerlings were same troop and that the apparent variation of size was not large. Then the fingerlings have been distributed by chance in the basins, that is to say 30 fish per basin of 400 liters of useful volume, thus forming five treatments in triplicate corresponding each one to a food. All the fishes came from a single source. They were stored in the basins 3 days before the beginning of the experiment in order to acclimatize them to the new ecological conditions.

The feed rations applied were 10% during the first 4 weeks and 5% the 2 last weeks.

From beginning till the end of the experiment, the fishes have been nourished manually, with experimental food, at a rate of three meals per day (8 AM, 1EM and 6 PM) except the days of growth control. They were considered to satiety when they did not pay any more attention to the pellets. Every 7 days, the fishes are taken into account and weighed individually by rotation of basin to remove the basin effect and to carry out a readjustment of the ration.

During the experiment, the physicochemical parameters (temperature, dissolved oxygen and with saturation, pH, TDS, Conductivity and Salinity) of water were measured three times per day and during three days in the week in order to appreciate their impact on the of fishes growth. The temperature and dissolved oxygen have been measured respectively with 0.1 ˚C close and with 0.01mg.L-1 near using a oxythermometer WTW Oxi 197. The pH has been measured to 0.1 unit of pH near using a pH-meter PIERRON HI 1290.Various measurements were taken at 7:30 AM; 12:30 PM and 17:30 PM.

2.4 Data statistical analysis and treatment

The specific growth rate (SGR), the effectiveness of food (EF), the rate of survival, the factor of condition (k) and the proteinic coefficient of effectiveness has been calculated starting from the Excel spreadsheet at base of the following formulas:

- Specific Growth Rate SGR (%/j) = 100 X [ln (final body weight) - ln (initial body weight)]/d.
- EF = (Final biomass + Initial biomass - Biomass of death)/Quantity of food distributed.
- Survival Rate (%) = 100 x FN/IN (IN and FN = initial and final number of fish).
- Factor of Condition K = final body weight x 100/L3 where L is the length of fish in centimeter.
- Introduced Voluntary IV (%/d) = 100 x Q/ [(IB + FB)/2)]/lasted of the experiment (days).With Q: quantity cumulated of food distributed (g), IB and FB, initial and final biomass (g).

The average values of the morphometric and physicochemical parameters have been compared inside and between the treatments by Analysis of the Variance with single direction (Anova1) after checking of the homogeneity of the dissension with the test of "Hartley". Significant differences between the averages of treatments (P<0.05) have been determined by using the test of Fisher [19]. Results are given accompanied by standard deviation.

The level for the statistical significance has been placed at 5%.

The mathematical model (amount - answer) of Brett and Grove [7] has been applied to the polynomial regression of the second degree between the concentration of the manure's worm flour in the rations and the specific growth rate SGR for the determination of the maximum rate of the flour of earthworm (*E. foetida)*, corresponding to the maximum specific growth rate while taking first derived from the equation polynomial of the second degree) and the optimum (obtained graphically)

3. Results

3.1 Physicochemical parameters

The physicochemical parameters of the area of breeding have been compared at the end of the experiment by the variance analysis to a criterion and the average values accompanied by their standard deviation appear in the table 3 below.

Table 3: Average values of the physicochemical parameters of breeding area.

Treatments	T°C water	O_2 (mg/l)	pH	Cond	TDS	Sal (g/l)
T0	26.07 ± 0.78 [a]	7.04 ± 2.14 [a]	7.10 ± 0.31 [a]	575.40[a] ± 23.00	395.74[a] ± 4.40	0.28 ± 0.14 [a]
T5	25.76 ± 0.68 [a]	7.18 ± 2.24 [a]	7.11 ± 0.29 [a]	560.81[a] ± 16.34	385.84[a] ±7,11	0.28 ± 0.01 [a]
T10	25.60 ± 0.67 [a]	7.38 ± 2.11 [a]	6.78 ±0.43 [a]	547.83[a] ± 17.87	375.26[a] ± 8,34	0.27 ± 0.11 [a]
T15	25.76 ± 0.70 [a]	7.11 ± 2.33 [a]	6.78 ±0.43 [a]	545.75[a] ±11.23	375.00[a] ± 8,45	0.31 ± 0.30 [a]
T20	25.72 ± 0.71 [a]	6.99 ± 2.05 [a]	7.02 ± 0.15 [a]	571.03[a] ± 6.12	375.00[a] ± 6,98	0.22 ± 0.10 [a]

For the same parameter, the averages carrying the same letters indicate that there was no significant difference between these values (p>0.05).
T: temperature; O2: dissolved oxygen; pH: hydrogen potential; Sal: salinity.
T0:food containing 0% of flour of worm of manure; T5:food containing 5% of flour of worm of manure; T10: food containing 10% of flour of worm of manure; T15: food containing 15% of flour of worm of manure and T20: food containing 20% of flour of worm of manure.

The probability associated with Least Significant Difference test of Fisher indicates that there is not a significant difference between the values of the physicochemical parameters of the area of breeding (P<0.05). The theoretical variation observed was not sufficient to induce a significant difference. This fact would be caused by the single source of supply water of the basins of breeding. It is what justifies averages carrying the same letters while exposing for the same parameter. It is what justifies averages carrying the same letters while exposing for the same parameter in the table three.

3.2 Influences of treatments

The table 4 shows the effect of the various treatments on the principal zootechnical parameters and figure 1 the evolution of the weights of fingerlings according to the treatments. From the beginning until the end of the test, the fish presented an increase in average weight for all the treatments. The highest average final weight has been obtained with the T15 treatment where the fishes have been nourished with a ration containing 15% of worm of manure's flour.

Fingerlings subjected to the treatments T20, T10 and T5 containing respectively 20%; 10% and 5% of worm of compost's flour present a relatively weak growth. The increase in body weight is weaker for fingerlings nourished with the T0 treatment deprived of flour of earthworm.

The survival rate (%) and the specific growth rate (%) varied respectively from 99.63 ± 0.50 to 100 ± 0.00 and from 3.45 ± 0.31 to 6.67 ± 0.30 for all the treatments. The food conversion rate, the voluntary introduced and the proteinic coefficient of effectiveness respectively varied from 1.02 ± 0.09 to 1.99 ± 0.07; from 3 ± 03 to 3 ± 0.82; and of 1.50 ± 0.04 to 2.11 ± 0.11 for all the treatments.

Table 4: Morphometric Parameters and feed valorization T0: food containing 0% of flour of worm of manure;T5: food containing 5% of flour of worm of manure; T10: food containing 10% of flour of worm of manure; T15: food containing 15% of flour of worm of manure and T20: food containing 20% of flour of worm of manure.

Parameters		T0	T5	T10	T15	T20
Number	Initial	90	90	90	90	90
	Final	89	89		90	90
Average weight (g)	Initial	4.79 ± 0.23	4.79 ± 0.23	4.79 ± 0.23	4.79 ± 0.23	4.79 ± 0.23
	Final	33.18±13,71[a]	41.37±14,24[b]	44.02±14,64[c]	56.70±14,65[d]	48.50±15.5[e]
SGR (%/jour)		3.45 ± 0.31[a]	5.79 ± 0.32[b]	5.90 ± 0.30[c]	6.67 ± 0.30[d]	5.97 ± 0.30[c]
SR (%)		99.63 ± 0.50[a]	99.63 ± 0.50[a]	99.63 ± 0.50[a]	100 ± 0.00[b]	100 ± 0.00[b]
PCE		2.11 ± 0.11[a]	2.04 ± 0.07[b]	1.96 ± 0.08[c]	1.50 ± 0.04[d]	1.78 ± 0.04[e]
TCA (g/g)		1.99 ± 0.07[a]	1.58 ± 0.05[b]	1.02 ± 0.09[c]	1.49 ± 0.07[d]	1.28 ± 0.08[e]
IV (%/j)		3.10 ± 0.82[a]	3.02 ± 0.23[b]	3.01 ± 0.40[b]	3.03 ± 0.30[b]	3.06 ± 0.11[a]

PCE: Proteinic Coefficient of effectiveness, FGR: Food growth rate, IV: Introduced voluntary, SR: Survival rate.

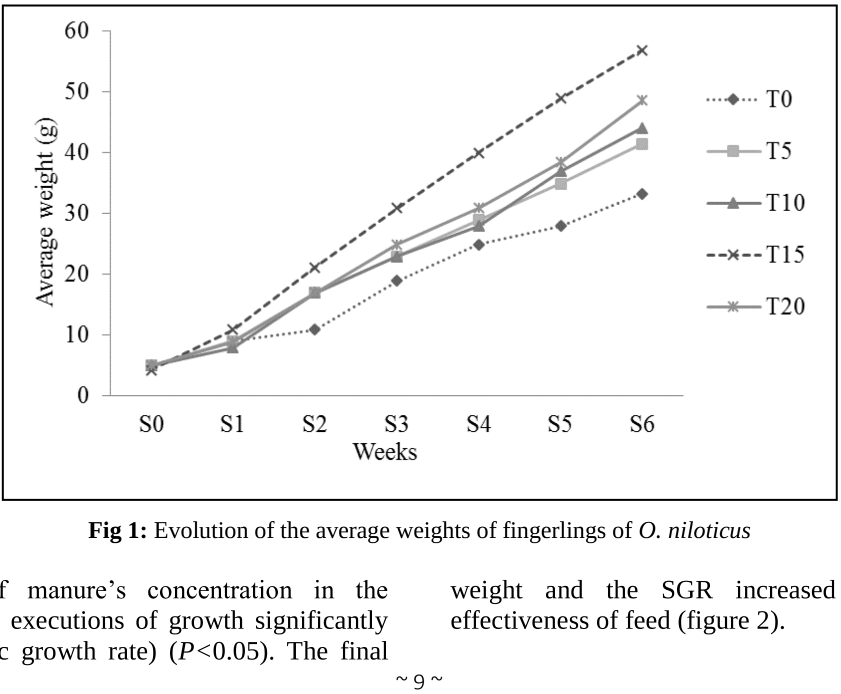

Fig 1: Evolution of the average weights of fingerlings of *O. niloticus*

The flour of worm of manure's concentration in the treatments influenced the executions of growth significantly (final weight and specific growth rate) (P<0.05). The final weight and the SGR increased significantly with the effectiveness of feed (figure 2).

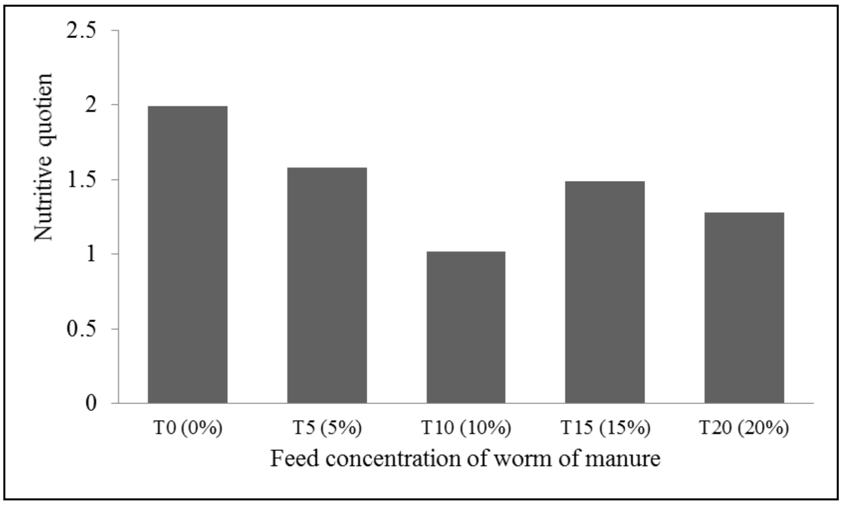

Fig 2: Variation of the food effectiveness according to the concentration of the flour of worm of manure in the various rations.

From an economic standpoint, the optimal ration is that which associates the minimal food at the best speed of growth and which is in lower than maximum ration. With this approach, the optimum of the ration in the flour of compost worm for the youthful of *O. niloticus* of initial average weight 4.07 ± 0.07g, breed in basin was 10%. With the mathematical model (amount answer) of Brett, the maximum quantity of worm of manure corresponding to the maximum growth rate was 13.82% (fig 3).

The determination of these quantities of flour of worm of manure makes it possible to make an efficient management of this local resource highly rich in protein in fish feed thus reducing the problem involved in the high cost of the feed of the aquacoles subjects.

The observation of fig 3 also shows that a rate of incorporation of 15% didn't affect negatively the growth of the high subjects. Thus, the use of such quantity with an aim of balancing the feed formula would not carry damages on the zoo technical performances of fishes. First derived from the equation polynomial of the second degree obtained (fig3) confirms this maximum rate of worm of manure to introduce into food of this species when it is cancelled for X = 0.1382 or 13.38%. Mathematically we have:

Y = - 154.9X2 + 42.84X + 3.598 = > Y ' = - 309.8X + 42.84;
Y' = 0 <=> - 309.8X + 42.84 = 0.
We have - 309.8X = - 42.84 what gives *X = 0.1382, expressed as a percentage*
X = 13.82% with X: maximum quantity of worm of manure; Y': the first derived.

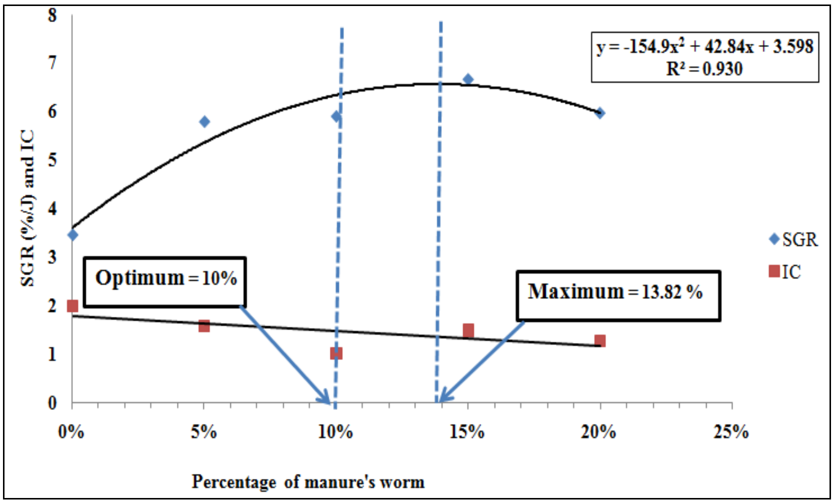

Fig 3: Variation of the Specific Growth Rate (SGR) and Index of consumption of fingerlings of *O. niloticus* nourished during 45 days according to the feed content of manure's worm flour according to the model of Brett and Grove [7].

4. Discussion

The physicochemical parameters of the water of breeding of fish are in the standards indicated by Mélard *et al.* [20], Kestemont and *al.* [21], Tossavi *et al.* [22] then Houndonougbo *et al.* [8] for fish breeding under similar conditions. They thus did not influence the growth of fingerlings.

The values of the physicochemical parameters presented in table 3 are compatible with those indicated by Mélard *et al.* [20], Kestemont *et al.* [21], Tossavi *et al.* [22] then Houndonougbo *et al.* [8] for the breeding of fish. These parameters negatively did not have affected the growth of fingerling of *O niloticus* having made objects of this study.

The survival rates varying between 99.63% and 100% are better. These survival rates are largely higher than those from 22.5% to 49.4%; from 67.1% to 70.5% and 86.67% and 97.78% obtained respectively by Schouveller [23], Abou [24] then Fiogbé *et al.* [25] thus stating that the food was not toxic for fingerlings of *O. niloticus* breed in basins.

The various specific growth rates shown by fishes (from 3.45 ± 0.31% to 6.67 ± 0.30%) are also better than those (of 2.01 ±

0.04 to 2.46 ± 0.05) found by Azaza *et al*. [26] which have tested the effect of the incorporation of the tomato flour in the food of the same species of fish in breeding in geothermal water in south Tunisian then to those (1.24 ± 0.10 to 1.82 ± 0.06) obtained by Kpoguè *et al.* [20]. Similar values (5.19 ± 0.08% to 5.887 ± 0.04%) of the SGR have been reported by Azaza *et al*. [27] followed making of dry food for the tilapia of the Nile always in breeding in geothermal water in south Tunisian. These results corroborate those (5.90 ± 0.15% to 7.60 ± 0.39%) reported by Houndonougbo *et al*. [6] through the study compared of two modes riches with the worms of manure fresh or dried built-in on the performances of growth of *O. niloticus* elevated in basin. The mathematical model (amount-answer) of Brett and Grove [7] proposed to determine the optimum and the maximum of the flour of ground worm in the food of *O. niloticus* has been already used by Kpoguè [28] and Tossavi *et al.* [22] with satisfactory results.

Beyond this threshold of 15%, a clear reduction of the growth and a bad food transformation increase the food conversion rate. Richter *et al.* [29] succeeded in incorporating, without negative consequences on the growth of *Oreochromis niloticus*, a threshold of 10% of the flour of moringa sheet. These same authors showed that the incorporation of 20% of that ingredient decreases the growth of 73% compared to pilot food. According to Keembiyehetty and De Silva [30] and Youssuf *et al*. [31], a weak food transformation would be due to certain mechanisms determining the effectiveness of digestibility and assimilation caused by the strong content of fibres (cellulose). By same manner Azaza *et al.* [26] have showed that beyond 20% of the tomato flour in substitution of the oil cake soya bean and of the maize cornstarch in the food of *O. niloticus* the growth of fingerlings is negatively affected.

The conversion rates (of 1.48 ± 0.08 to 2.11 ± 0.12) reported by Kpoguè [32] are similar to those found in this study.

5. Conclusion

The results obtained during this study highlight, under the realized experimental conditions, the possibility of incorporating the flour of worm of compost (*Eisenia foetida*) up to 13.82% in a basic diet for the enlargement of Tilapia. Indeed the substitution of the fish meal by the flour of manure worm in such a proportion does not affect the performances of growth at all nor the food effectiveness of transformation in this fish.

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