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Growth Performances of *Clarias gariepinus* Fry Fed *Hermetia illucens* Based Diet Supplemented with Synthetic Amino Acids (Methionine and Lysine)

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

Objective: this study focused on the effect of a local feed supplement with synthetic amino acids (methionine and lysine) on the survival and growth of *Clarias gariepinus* fry in happas.
Study Design: the study took place at the DJOKWE aquaculture farm located in the Cameroon Littoral Region, Moungo Department, Nkongsamba 1st District.

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Methodology: Six (06) local T0- diets without synthetic amino acid negative control, T0+ coppens imported feed positive control, T1 (L1.5/M1) containing 1.5% lysine and 1% methionine, T2 (L1, 5/M1.5) containing 1.5% lysine and 1.5% methionine, T3 (M1/L2) containing 1% methionine and 2% lysine, T4 (M1/L2.5) containing 1% methionine and 2.5% lysine were tested in triplicate on 540 fry of 10± 3.1g distributed in 18 happas of 0.5 m^2. The fish were fed 3 times per day at a rate of 8% of the ichthyo biomass. Every 14 days, zootechnical parameters were evaluated.

Results: depending on the level of supplementation, the survival rate was higher for the T0+ coppens treatment, 96.66%, and lower for T4 (M1/L2.5) supplemented with 1% methionine and 2.5% of lysine with 80%. The significantly highest average weight gain (p<0.05) was recorded with the T0+coppens treatment (89.67±1.52g) compared to the T1 (L1.5/M1) and low (66. 33 ±00g) compared to treatment T3 (M1/L2). The specific growth rate was higher (p>0.05) or (2.83%±0.16g/d) with T2(L1.5/M1.5) and lower (0.78%±0 .02%g/d) for T0+ coppens. As for the condition factor K, it is significantly higher for the T0+coppens treatment (0.46%±0.030) and lower for the T4 treatment (0.29±0.023).

Conclusion: the incorporation of 5% of black soldier fly larva into local feed supplemented with 1.5% lysine and 1% methionine makes it possible to improve the survival of *Clarias gariepinus* fry but also their growth as well as the production cost.

Keywords: Maggot meal; synthetic amino acids; local feed; Clarias gariepinus; growth.

1. INTRODUCTION

Fishing and aquaculture remain a vital resource for feed, income and livelihoods for around a hundred million people around the world. On a global scale, the supply of fish has reached a record figure of 20.3 kg/inhabitant/year [1] and it remains one of the most consumed feeds in Africa. It thus contributes approximately 50% of protein intake of animal origin in Sub-Saharan Africa [2] and covers almost 50% of the demand for animal proteins of populations in Cameroon. National demand for fish products has increased considerably in recent years following the growing population. It increased from 409,519 tones in 2013 to an average of 433,616 tones in 2015 and the forecast for 2020 is estimated at 496,891 tones according to the Ministry of Livestock, Fisheries and Animal Industries in 2018. However, fishing alone is no longer enough to sustainably satisfy the ever-increasing demand, due to overfishing, climate change and environmental degradation [2]. Faced with this drastic situation, aquaculture is becoming an essential alternative for fish production. But its emergence is faced with several problems, so food remains one of the main challenges [3] due to the unavailability of food, the high cost of inputs and their quality on the health of the final consumer [4,5] and more importantly the use of fish meal from the natural environment as the main source of proteins and amino acids, which leads to a degradation of biodiversity. To address this problem, other sources of animal protein can be used, such as *Hermetia illucens* larvae, but their limiting amino acid intake is only half of what fish meal provides. The use of synthetic products such as amino acids is one of the ways to resolve this problem. Feed represents a significant part of the cost of fish production [5]. In intensive aquaculture, the feed component represents 55 to 60% of the cost of fish production [6]. Reducing feed costs, and consequently reducing the total cost of fish production, is one of the priorities in Aquaculture [3].

Because the use of imported feed in livestock farming is a common practice in most developing countries. The economic interest of this type of breeding is therefore very dependent on the availability and cost of feed [7]. Thus, research in Aquaculture must use other alternative sources by producing quality local feeds at a lower cost and which will not be a source of depletion of biodiversity in the natural environment with the use of fish flour. maggots and synthetic amino acids. One of the ways to achieve this is the use of local by-products, alternative sources to fish meal and oil, and finally synthetic products. Hence the importance of this study based on the effect of the supplementation of two synthetic amino acids (Methionine and lysine) in the local food formulated based on the lava flour of *Hermetia illucens* on some zootechnical performances of the fry of *Clarias gariepinus*. The general objective of this study was to contribute to improving the production of *Clarias gariepinus* through the use of feeds made from local inputs and synthetic products.

2. MATERIALS AND METHODS

2.1 Study Zone

The DJOKWE fish farm is located in the Cameroon Littoral Region, Moungo Department, Nkongsamba 1st District located in the Lonako village. Following 4°27' and 4°30' North Latitude and 9°57' and 10°20' East Longitude at an average altitude of 17.5 m. The climate is the same throughout the city. It is an equatorial type climate with 02 distinct seasons, namely: a rainy season going from March to October and a dry season going from November to February.

2.2 Biological Material

The experiment lasted 56 days, with the aim of evaluating the tracking rate, growth parameters as well as the cost of producing the feed. 540 *Clarias gariepinus* fry with an average weight of 10±3.1g were taken from the fry production of the DJOKWE farm where they were all fed with the same coppens feed.

2.3 Non-Biological Material and Experimental Device

Twelve happas with a mesh diameter of 0.2mm and a dimension of 0.4x0.4x1m, previously sewn, were installed in a 24m^2 concrete tank. 540 fry were distributed in triplicates in 6 treatments of 90 individuals with an average weight of 10±3.1g following a completely randomized design (3 repetitions x 6 treatments). Each replicate contained 30 fry and each treatment 90 fry. Every two weeks (14 days), control fishing was carried out and the weight and height of each individual was taken.

2.4 Origin of Methionine and Lysine

The amino acids were purchased from a local feed store.

2.5 Experimental Diets

For this experimental study, the ingredients for the production of our feed were purchased in a local feed mill, a feed with 50% protein was formulated. Once the purchasing operation was completed, the ingredients were weighed according to the previously established formula, then ground to obtain a fine powder, after grinding they were mixed and extruded. Then the feed from the excavator was dried in the sun in order to eliminate impurities and increase the feed conditioning time. Once dry, it was packaged in a hermetically sealed bag and stored in the store.

A total of six diets were used.

T0- (ss): without supplement (negative control);
T0+ : imported feed coppens (positive control);
T1(L1.5/M1): feed supplemented with 1.5% lysine and 1% methionine;
T2(L1.5/M1.5), feed supplemented with 1.5% lysine and 1.5% methionine;
T3 (M1/L2): feed supplemented with 1% methionine and 2% lysine;
T4 (M1/L2.5): feed supplemented with 1% methionine and 2.5% lysine.

2.6 Conduct of the Test

Each treatment thus contained a total of 90 fry, therefore 3 happas of 30 fry each. These fry were fed at a frequency of four times (4) per day at a regular interval of 3 hours including 7 a.m., 10 a.m., 1 p.m. and 4 p.m. with quantities of feed equivalent to 11% of their biomass during the first month then 8 % over 6 weeks of experience for all treatments. A control fishing was carried out every two weeks (after 14 days) and at the end of the fishing, the growth characteristics such as weight were measured using a balance sensitive to 0.001g when loaded. then with an SF-400 balance with a sensitivity of 1g, the size (total length) of the fry was measured using graph paper. The fry were placed in a bucket containing water and were handled so as not to leave them out of the water for long. Before distribution of the different diets, the quantities proportional to the densities of the fry for each happa were calculated, weighed and crumbled. These quantities of feed distributed to the fry were adjusted according to their development. A TDS/EC/PH/SALT/SG/ORP brand multi-parameter was used for taking the temperature and the JBL brand analysis kit for taking the physicochemical parameters.

2.7 Zootechnical Parameters and Characteristics Studied

- **Survival rate (SR)**

SR ($\mathbf{en}$%) = 100 × NF/Ni
NF = number of fish at the end of the experiment and Ni = number of fish at the start of the experiment.
Growth characteristics

Table 1. Centesimal composition of the experimental feed

Ingredients	**Percentages (%)**
Fishmeal	17,6
Soy flour	16
Maggot meal	3,2
Peanut cakes	16
Cotton cakes	14 ,4
wheat four	12
Corn four	12
Rice	4,8
Premix 10%*	3,2
Rafined oil	0,8
TOTAL	100
Calculated chemical composition (% of dry matter)	
Proteins	50,47
Lipids	8,2
Carbohydrates	12
Ash	9,18
Energy (Kcal)	2554,80
Chemical composition analyzed (% of dry matter)	
Proteins	40,75
Lipids	4 ,36
Carbohydrates	49,70
Dry matter	97,01
Ash (%)	2,05
Energy (Kcal)	49,70

**Premix 10%; Metabolizable energy = 2300 Kcal/Kg; Raw protein = 43,8%; Lysine = 3.1%; Methionine = 1.5; Calcium = 8%; Phosphorus = 3,1%*

- **Live weight**

At the start of the test and every 14 days thereafter, fish from each experimental unit were weighed. The weekly weight gain was obtained by taking the difference between 2 consecutive average weekly live weights

- **Average weight gain (AWG in g)** = final average fish weight (FAFW in g) - initial average fish weight (IAWG in g);
- **Average daily weight gain (AWDG in g/day)** = (FAWG-IAWG)/t With IAW = initial average weight (g), FAW = final average weight (g), t = duration of the experiment (in days);
- **Specific growth rate (SGR in %day)** = [(ln Pmf –ln Pmi)/ rearing time (day)] x 100; Pmi = initial average weight (g), Pmf = final average weight (g);
- **Feed conversion ratio (FCR)** = Quantity of feed distributed / Body mass gain;
- **Condition factor (K)** = W×100/ LT3 with W: weight (g), LT: Total length (cm).

Data on survival rate, growth characteristics were subjected to one-way analysis of variance (ANOVA 1). In the event of significant differences between the means of the treatments, Duncan's test was applied to separate at the significance level of 5%. SPSS 20.0 (Statistical Package for Social Sciences) statistical software was used for these analyses.

3. RESULTS

3.1 Survival Rate

Fig. 1 shows the variation in the survival rate depending on the treatments. It appears from this figure that the significantly ($p<0.05$) higher rate was observed with the fry fed with imported feed T0+ coppens, (96.66%) and the lowest rate of 80% was observed. to treatment T4 (L1/M2) which was fed with feed supplemented with 1% methionine and 2% lysine.

3.2 Zootechnical Performances

It appears from Table 2 that whatever the treatment considered over the entire period of the trial, with the exception of the specific growth rate and the consumption index all other parameters (final average weight, weight gain, average daily gain and condition factor K) were significantly ($p<0.05$) affected by local feed supplementation with lysine and methionine.

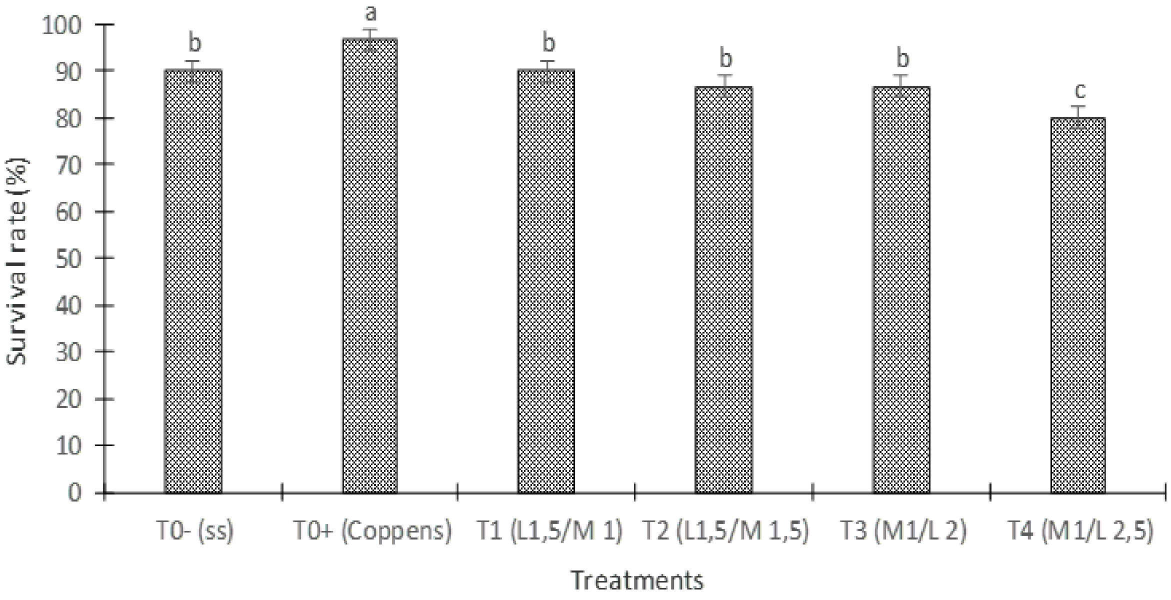

Fig. 1. Variations in the survival rate depending on the treatments

T0- (ss) no supplement Corresponding respectively to the local feed with 0% supplementation; T0+ coppens= imported food; T1 (L1.5/M1)= feed containing 1.5% lysine and 1% methionine; T2(L1.5/M1.5)= feed containing 1.5% lysine and 1.5% methionine; T3 (M1/L2)= feed containing 1% methionine and 2% lysine; T4 (M1/L2.5) = feed containing 1% methionine and 2.5% lysine. P= probability; M=methionine; L=Lysine

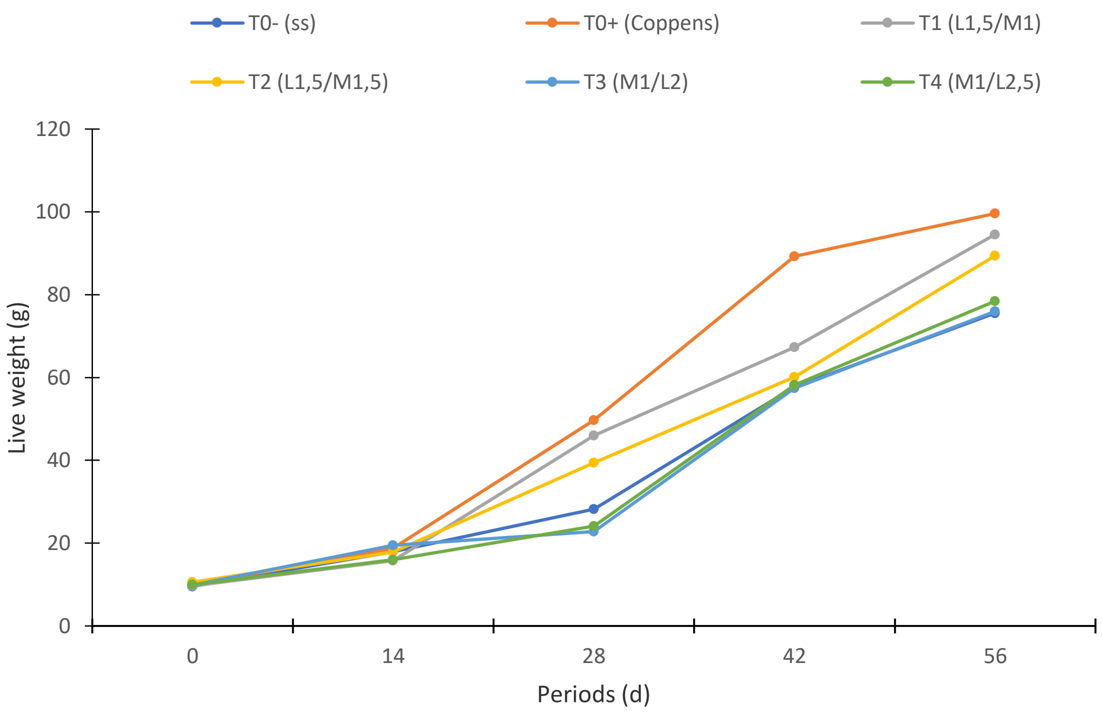

Fig. 2. Evolution of average weight gain depending on the breeding periods

T0-no supplement Corresponding respectively to the local food with 0% supplementation; T0+ coppens= imported feed; T1 (L1.5/M1)= feed containing 1.5% lysine and 1% methionine; T2(L1.5/M1.5)= feed containing 1.5% lysine and 1.5% methionine; T3 (M1/L2.5) = food containing 1% methionine and 2.5% lysine; T4 (M1/L2) = feed containing 1% methionine and 2% lysine. P= probability; M=methionine; L=Lysine; d= day

Table 2. Variations in growth characteristics of *Clarias gariepinus* fry fed local feed supplemented with lysine and methionine

	Treatments						
Caracteristics studied	**T0- ss**	**T0+ coppens**	**T1 (L1,5/M1)**	**T2 (L1,5/M1,5)**	**T3 (M1/L2)**	**T4 (M1/L2,5)**	**P**
Ni	**90**	**90**	**90**	**90**	**90**	**90**	
Nf	**81**	**87**	**81**	**78**	**78**	**72**	
IAW (g)	10±3	10±3	10±3	10±3	10±3	10±3	
FAW(g)	75,66±4,16[b]	99,33±1,154[a]	94,66±0,57[a]	89,33±2,08[a]	78,66±6,50[b]	76±11[b]	**0,001**
AWG (g)	66±4,358[c]	89,67±1,52[a]	84,67±0,57[a, b]	78,67±3,21[b]	68,33±6,50[c]	66±10,00[c]	**0,000**
ADWG (g/j)	1,33±0,577[ab]	1±0,000[b]	2±0,000[a]	2±0,000[a]	1,33±0,577[a, b]	1,33±0,577[a, b]	**0,055**
SGR (%/j)	1,91±0,35[a]	0,78±0,02[a]	2,43±0,19[a]	2,83±0,16[a]	2,12±0,80[a]	1,95±1,02[a]	**0,456**
FCR	1,62[a]	2,53 [a]	1,5 [a]	1,86 [a]	2,42 [a]	2,68 [a]	**0,201**
K (%)	0,32±0,021[c]	0,46±0,030[a]	0,40±0,043[a, b]	0,35±0,060[b, c]	0,29±0,023[c]	0,32±0,014[c]	**0,001**

a, b, c,: Values with the same letter on the same line are not significantly different (p>0.05). T0-no supplement Corresponding respectively to the local feed with 0% supplementation; T0+ coppens= imported feed; T1 (L1.5/M1)= feed containing 1.5% lysine and 1% methionine; T2(L1.5/M1.5)= feed containing 1.5% lysine and 1.5% methionine; T3 (M1/L2.5) = feed containing 1% methionine and 2.5% lysine; T4 (M1/L2) = food containing 1% methionine and 2% lysine. P= probability; M=methionine; L=Lysine. IAW = Initial average weight; FAW= Final average weight; AWG= Average weight gain; ADWG=Average daily weight gain; SGR= Specific Growth Rate; K= Condition Factor; FCR= Feed conversion ratio

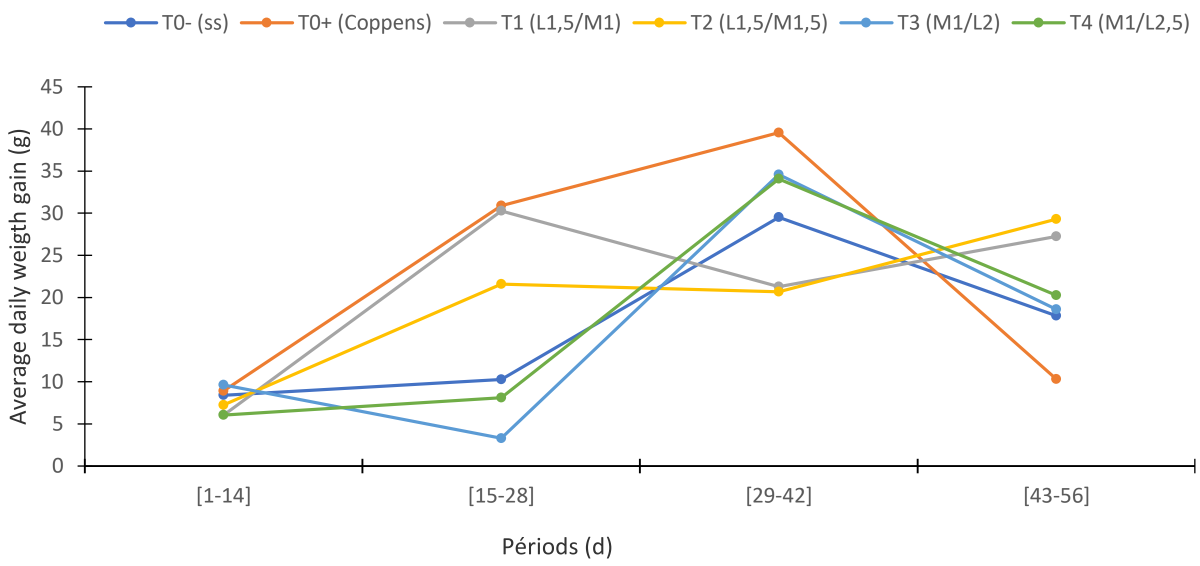

Fig. 3. The evolution of Average weight gain depending on the breeding periods

T0- no supplement Corresponding respectively to the local feed with 0% supplementation; T0+ coppens= imported feed; T1 (L1.5/M1)= feed containing 1.5% lysine and 1% methionine; T2(L1.5/M1.5)= feed containing 1.5% lysine and 1.5% methionine; T3 (M1/L2.5) = feed containing 1% methionine and 2.5% lysine; T4 (M1/L2) = feed containing 1% methionine and 2% lysine. P= probability; M=methionine; L=Lysine; d= day

3.3 Live Weight

The evolution of the live weight according to the breeding periods is illustrated in Fig. 2. It appears that all the curves have a regular and increasing appearance, whatever the period of the study. From the 14th day, the weight gain curve of fish fed imported feed (T0+ coppens) remained above all the other curves with a significantly higher average value ($p>0.05$) at 56 days of 89.67±1.52 compared to other treatments. The live weight curves of fish fed with supplemented food T3 (M1/L2) supplemented with 1% methionine and 2% lysine, and T4 (M1/L2.5) supplemented with 1% methionine and 2.5% lysine remained confused and below the other curves throughout the trial period, with a significantly lower mean value at 56 days ($p<0.05$) of 68.33±6.50g and 66.00±10g respectively compare to other treatments.

3.4 Average Daily Gain

The evolution of the average daily gain as a function of the rearing periods is illustrated in Fig. 3. Whatever the period of the test, the weight gain varied in all the treatments and evolved in a sawtooth pattern. From the 1st – 42nd days the weight gain curve of fish fed imported feed (T0+ coppens) was significantly ($p<0.05$) above all the other curves. From the 43rd – 56th days the treatment curve T2 (L1.5/M1.5), containing 1.5% lysine and 1.5% methionine, made it possible to record the highest weight gain followed by the treatment T1 (L1.5/M1%) or the feed was supplemented with 1.5% lysine and 1% methionine.

3.5 Specific Growth Rate

The evolution of the specific growth rate as a function of the breeding period is illustrated in Fig. 4. All the curves in this figure have a sawtooth evolution. However, the highest value was recorded from the 1st to the 14th day in fish fed with feed T1 (L1.5/M1) followed by T0+ coppens. In addition, at the end of the trial, from the 43rd to the 56th day, the highest rate was recorded with treatment T2 (L1.5/M1.5%) followed by T1 (L1.5/M1%) and weaker with T0+ coppens.

3.6 Condition Factor K

From Fig. 5, we observe a sawtooth evolution for all treatments. This while the highest value (0.49±0.03) was obtained by the subjects fed with the imported feed coppens followed by T1 (L1.5/M1) (0.40±0.04) and the lowest values (0.29±0.02); (0.32±0.01) were obtained respectively in fish fed with supplemented food at T4 (M1/L2) followed by T4 (M1/L2.5).

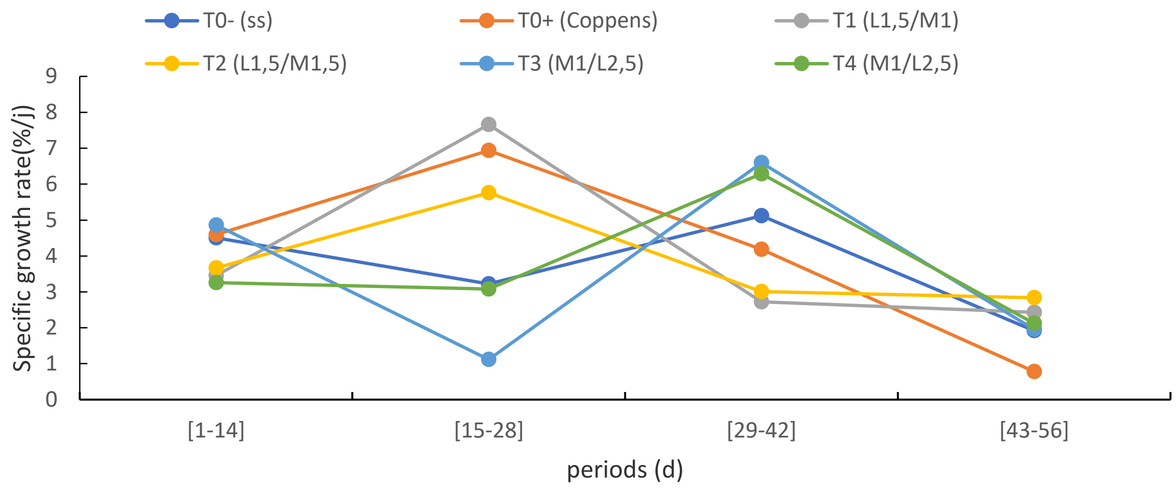

Fig. 4. Evolution of the Specific Growth Rate at different treatments depending on the breeding period

T0-no supplement Corresponding respectively to the local feed with 0% supplementation; T0+ coppens= imported feed; T1 (L1.5/M1)= feed containing 1.5% lysine and 1% methionine; T2(L1.5/M1.5)= feed containing 1.5% lysine and 1.5% methionine; T3 (M1/L2.5) = feed containing 1% methionine and 2.5% lysine; T4 (M1/L2) = feed containing 1% methionine and 2% lysine. P= probability; M=methionine; L=Lysine; d= day

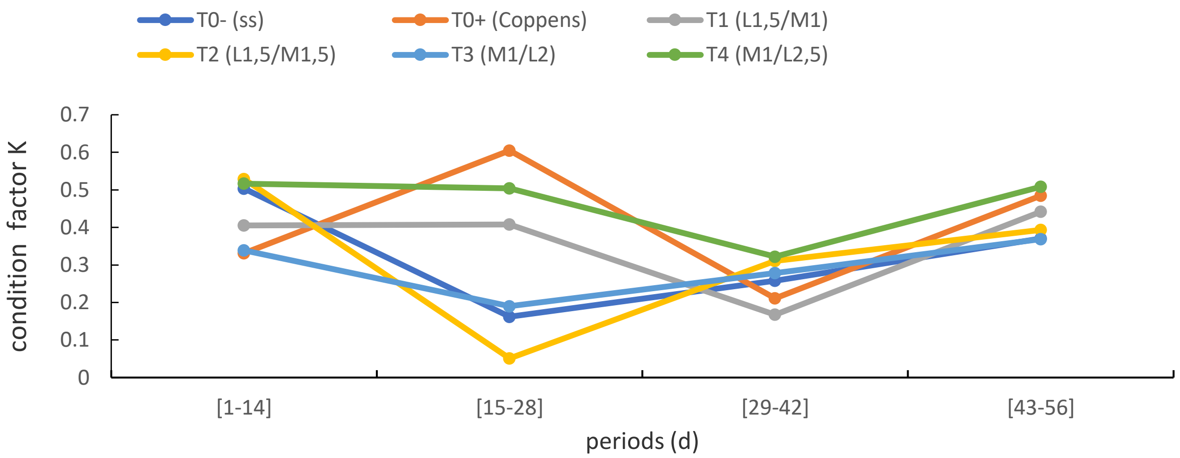

Fig. 5. Evolution of the condition factor K at different treatments.

T0-no supplement Corresponding respectively to the local feed with 0% supplementation; T0+ coppens= imported food; T1 (L1.5/M1)= feed containing 1.5% lysine and 1% methionine; T2(L1.5/M1.5)= feed containing 1.5% lysine and 1.5% methionine; T3 (M1/L2.5) = feed containing 1% methionine and 2.5% lysine; T4 (M1/L2) = feed containing 1% methionine and 2% lysine. P= probability; M=methionine; L=Lysine; d= day

4. DISCUSSION

The survival rate ranges from 80 to 96%. These values are higher than that obtained by Burchell in 1822 after 50 days of rearing fry in aquariums and that of [3] after 8 weeks of rearing with a feed containing 20% protein (47,87-66 ,62). These differences in mortality observed between these authors could be attributed to the rearing environment which could be a source of stress for individuals during acid rain.

The average daily gain was highest (2g/d) at T1(L1.5/M1) and lowest (1g/d) in T0+ coppens. These values are greater than 0.05 g/day obtained by [8] in 5 g fry of the same species and 0.24 g/day recorded by [9] with an incorporation rate of 50% flour. maggots in *Clarias gariepinus*. This difference observed would be due to the effect of amino acids which would have improved the dietary balance, promoting better digestion and use of nutrients. Regarding the consumption index (CI), the values found in this study (1.¬50 to 2.68) are elevated to 1.59 obtained by [9] in 5 g fry of the same species compared to those obtained by [10] (1.95 to 2.08) and [5] (1.51 to 2.11). These differences could be attributed to underconsumption of the food distributed. Likewise, the differences between these values could also be linked to the variation in the physicochemical characteristics of the water during the test, creating stress and thus reducing feed intake.

The specific growth rate obtained in this study varies between (3.67 ± 0.5%/day and 4%/day) and remains higher than that obtained by [10] at the IRAD fish farming station in Foumban which varied from 0.04 to 0.18%/day and those presented by [11] in juveniles of *Clarias gariepinus* (3.60%/day). On the other hand, it is lower than the results obtained by [10] for *Clarias gariepinus*, which are between 4.78 and 5.8%/d and those of 4.26; 4.05 and 3.85%/day obtained respectively for fish fed with feed containing 30 and 50% Azolla [12]. This difference in performance could be justified by the density and size of the fish used by the latter during their tests.

The condition factor K varied with the treatments. In fact, the values obtained were between 0.29 ± 0.02 and 0.46 ± 0.03 respectively of the T0+ coppens and T4 (M1/L2%), 1% methionine and 2% lysine treatments. These values were lower than those reported by [13] and [14] recorded 0.79 to 0.83% in *Clarias gariepinus* reared at several densities and fed a complete diet or those reported by [12] (0, 06 to 0.74).

5. CONCLUSION

At the end of this study on the effect of the supplementation of two synthetic amino acids (Methionine and lysine) in the local feed formulated based on the lava flour of *Hermetia illucens* on the survival and some growth performances of the fry of *Clarias gariepinus* in tanks, it appears from the results obtained that the food supplemented with amino acids had no effect on the survival rate of the fish. Growth and feed utilization characteristics remained superior in fish that were fed the feed containing 5% black soldier fly larvae in the local feed supplemented with 1.5% lysine and 1% methionine.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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