**Combined effect of two feed additives (Complex Enzyme CE805\* and Seafood Flavor II\*) in local feed on some zootechnical characteristics the fry of *Oreochromis niloticus*.**

**ABSTRACT**

**Objective :** this study aimed to evaluate the combined effect of two feed additives (Complex Enzyme CE805\* and Seafood Flavor II\*) in the manufactured feed on some zootechnical characteristics of *Oreochromis niloticus* fry.

**Study area :** the study was carried out at the Dibamba aquaculture farm in the locality of Logbadjeck located in the Littoral Region of Cameroon, Sanaga maritime Department, Dibamba district.

**Methodology :** A total of 600 single-sex fry of *Oreochromis niloticus* with an average weight of 0.95 ± 0.01 g were distributed in 24 happas of dimensions 0.3\*0.5\*0.5m3 each and were installed in a circular tank of dimensions 4m3 operating in open circuit. Eight (8) feed rations were used, one of which is a commercial feed available on the local market T0+: commercial feed and the other seven all lipoprotein, iso-lipidic and iso-energetic were formulated T(E0%; A0%): local feed formulated with 0% enzyme and 0% flavor, T(E0.125%; A0.01%): local feed formulated with 0.125% enzyme and 0.01% flavor, T(E0.125%; A0.0125%): local feed formulated with 0.125% enzyme and 0.0125% flavor, T(E0.125%; A0.0150): local feed formulated with 0.125% enzyme and 0.0150% flavor, T(E0.1%; A0.0125%): local feed formulated with 0.1% enzyme and 0.0125% flavor, T(E0.150%; A0.0125%): local feed formulated with 0.150% enzyme and 0.0125% flavor, T(E0.175%; A0.0125%): local feed formulated with 0.2% enzyme and 0.0125% flavor, in a completely randomized design repeated three times.

**Results :** the following results were recorded: the significantly highest survival rate (P > 0.05) (81.33 ± 2.30%) was recorded with subjects fed T(E0.150%; A0.0125%). The mean daily weight gain (0.16 ± 0.06 g/d), specific growth rate (2.21 ± 0.05%/j) and condition factor K (1.76 ± 0.09) were significantly higher (P > 0.05) and the lowest feed conversion ratio (1.69 ± 0.07) (P < 0.05) were recorded with diet T(E0.175%; A0.0125%).

**Conclusion :** enzymes and flavors combined at respective doses of 0.175% and 0.0125% can be used to improve the digestibility and attractiveness of locally produced feeds**.**

***Keywords:*** combined effect, feed additives, local feed, zootechnical characteristics, *Oreochromis niloticus*.

1. **INTRODUCTION**

One of the challenges of the current century is to solve the problem of food insecurity, which is a growing problem, hence the mission of FAO which is to fight against hunger and poverty in the world [1]. Aquaculture is one of the fastest growing aquatic food production sectors and is expected to play a key role in meeting the growing demand for fishery products due to the increasing human population and declining natural fishery resources (FAO, 2018) [2]. According to [3], "the absence of fish feed industries, difficult access to credit, the absence and/or insufficiency of quality fry, the lack of qualified personnel, the lack of mastery of production technologies and the poor development of fish ponds are all constraints which continue to hinder the development of the sector in Cameroon." Despite the various efforts made by the Cameroonian government to intensify the aquaculture sector, feeding fish species remains a major difficulty faced by fish farmers; limited access to quality, available and inexpensive exogenous food is therefore highlighted by breeders [4]. Cameroon is full of many potentials such as the availability of a good range of products and by-products of plant origin with very good nutritional values ​​likely to be used for the manufacture of high-performance local foods. There is also the emergence on the market of many natural feed additives that have the potential to improve the performance of animal production. These additives include probiotics, prebiotics, trace elements, plant extracts, enzymes, and flavorings [5]. That being said, paying particular attention to the formulation of a feed that can meet production expectations remains essential for the improvement of the aquaculture sector and fish farming in particular. Tilapia, being one of the species of piscicultural interest prized by the population, and whose breeding requires a nutritional, ecological and biological contribution [6]. The Complex Enzyme CE805 and Seafood Flavor II which are feed additives contain active molecules whose functions allow improving the nutritional properties, the texture, digestibility, attractiveness, palatability of local formula feeds. It is in this perspective that this study was initiated with the general objective of contributing to the search for substitutes for enzymes and aromas that can promote animal growth and are safe for humans and the environment.

1. **MATERIALS AND METHODS**
	1. **Study area**

The study was carried out in the Littoral Region, Sanaga Maritime Department, Dibamba District, Logbadjeck Village. The geographical coordinates are between 3º48ʹ39ʹʹ and 3°58'42"North 10°06'0" and 10º06ʹ04ʹʹ East and the average altitude is 42 meters. The objective was to evaluate the combined effect of two feed additives (Complex Enzyme CE805 and Seafood Flavor II) in the manufactured feed on some zootechnical characteristics of Oreochromis niloticus fry in tanks.

**Biological material and duration of the study :** The study lasted 63 days, with the aim of evaluating the survival rate, growth parameters as well as the cost of production of the feed. 600 mono-sex *Oreochromis niloticus* fry with an average weight of 0.95 ± 0.01 g of *Oreochromis niloticus* were collected from the fry production of Mungo Fish Farm.

**Origin of food additives :** Complex Enzyme CE805 and Seafood Flavor II were purchased from the local market, weighed and incorporated into the feed at different rates.

**Non-biological material :** Tt consisted of 24 happas of dimensions 0.3\*0.5\*0.5m3 each were installed in a circular tank of dimensions 4m3 operating in open circuit.

**Feeding ration :** An imported Skreting brand feed containing 35% protein was purchased from the local market, the other seven, all lipoprotein, iso-lipid and iso-energetic, were formulated.

T0+: commercial feed (Skretting);

T (E0%; A0%) : local feed containing 0% enzyme and 0% flavor;

T (E0.125%; A0.01%) : local feed containing 0.125% enzyme and 0.01% flavor;

T (E0.125%; A0.0125%) : local feed containing 0.125% enzyme and 0.0125% flavor;

T (E0.125%; A0.0150) : local feed containing 0.125% enzyme and 0.0150% flavor;

T (E0.1%; A0.0125%) : local feed containing 0.1% enzyme and 0.0125% flavor;

T (E0.150%; A0.0125%) : local feed containing 0.150% enzyme and 0.0125% flavor;

T (E0.175%; A0.0125%) : local feed containing 0.2% enzyme and 0.0125% flavor.

* 1. **Experimental design**

600 fry were distributed in triplicate in 8 treatments of 75 individuals, or 25 individuals per happa with an average weight of 0.95 ± 0.01g and total length 3.2 ± 0.2 mm after a randomized design (3 repetitions x 8 treatments). Eight (08) rations were formulated and distributed randomly.

**Test procedure :** Each treatment thus contained a total of 75 fry, so 3 happas of 25 fry each. These fry were fed at a frequency of four times (4) per day at regular intervals of 3 hours including 8 a.m., 11 a.m., 2 p.m. and 5 p.m. with food quantities equivalent to 11% of their biomass during the first month after control fishing. Control fishing was carried out every three weeks (after 21 days) and at the end of fishing, Growth characteristics such as weights were measured using a SF-400 scale with a sensitivity of 1g, The size (total length) of the fry was measured using graph paper and a ruler. The fry were placed in a bucket of water and handled so as not to leave them out of the water for long. Before distributing the different diets, the quantities proportional to the fry densities for each happa were calculated, weighed and crumbled. These quantities of food distributed to the fry were adjusted according to their development. A TDS/EC/PH/SALT/SG/ORP brand multiparameter was used for temperature measurement and the JBL brand analysis kit for measurement of physicochemical parameters" [7].

Table 1 : Centesimal composition of the experimental feed

|  |  |
| --- | --- |
| **Ingredients** | **Percentage (%)**  |
| Fish meal | 35 |
| Soybean meal | 13 |
| Peanut meal | 21 |
| Cassava flour | 2 |
| Corn flour | 2 |
| Wheat bran | 16 |
| Shrimp | 3 |
| Palm oil | 2 |
| Premix 5%\* | 2 |
| Lysine | 2 |
| Methionine | 2 |
| Total | 100 |
| **Chemical composition analyzed (%)** |
| Protein  | 43,45 |
| Lipid  | 13,7 |
| Ash | 9,9 |
| Fiber | 11,3 |
| Carbohydrate  | 9,65 |

\*Premix 5%; Metabolizable energy = 2078 Kcal/Kg; Crude protein = 40%; Lysine = 3.3%; Methionine = 2.40; Calcium = 8%; Phosphorus = 2.05%

**Zootechnical parameters and characteristics studied :**

**• Survival rate (SR)**

SR (%) = 100 × NF/Ni

Nf = number of fish at the end of the experiment and Ni = number of fish at the beginning of the experiment.

**Growth characteristics**

• **Live weight**

At the beginning of the trial and every 21 days thereafter, fry from each treatment were weighed. Weekly weight gain was obtained by taking the difference between 2 consecutive weekly average live weights.

• **Average daily gain (ADG)** (g/day) = (Pmf – Pmi) / Δt

Pmf: final average weight (g); Pmi: initial average weight (g); Δt: rearing duration in days

• **Specific growth rate (SGR) :**

SGR (%day) = [(ln Pmf – ln Pmi)/ Δt x 100 ; Pmf: final average weight (g); Pmi: initial average weight (g); ln: natural logarithm; Δt: rearing duration in days.

**• 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).

* 1. **Statistical Analyzes**

Data on survival rate, average daily gain, specific growth rate, feed conversion ratio, condition factor k and feed cost of production were processed and subjected to analysis of variance (1-way ANOVA). When there were significant differences between the means, the Duncan test was applied to separate the means at the 5% significance level. SPSS (Statistical Package of Social Science) software version 25.0 was used for the analyses.

1. **RESULTS**

Table 2 : Variation in zootechnical parameters of fry of Oreochromis niloticus depending on the treatments

|  |  |
| --- | --- |
| **Characteristics** |  **Treatments** |
| **T0+**  | **T (E0% ; A0%)**  | **T(E0,125% ;A0,01%)**  | **T(E0,125% ;A0,0125%)**  | **T(E0,125% ;A0,0150%)**  | **T(E0,1% ;A0,0125%)**  | **T(E0,150% ;A0,0125%)**  | **T(E0,175% ;A0,0125%)** | ***p***  |
| IW (g) | 0,95 ± 0,01 | 0,95 ± 0,01 | 0,95 ± 0,01 | 0,95 ± 0,02 | 0,95 ± 0,01 | 0,95 ± 0,0 | 0,9 ± 0,02 | 0,95 ± 0,0 | / |
| FW (g) | 12,60 ± 0,27a | 8,09 ± 1,15d | 7,03 ± 0,65e | 8,42 ± 0,15d | 9,06 ± 0,62cd | 9,91 ± 0,12bc | 10,41 ± 0,12b | 10,38 ± 0,37b | 0,000 |
| SR (%) | 75,00±6,24abc |  72,00 ± 4,58bc | 70,66 ± 2,51c | 74,66 ± 4,16abc | 78,67 ± 2,30ab | 78,67 ± 2,30ab | 81,33 ± 2,30a | 80 ± 0,00a | 0,016 |
| AWG (g) | 249,0 ± 17,77a | 147,33 ± 0,00e | 116,33 ± 5,29e | 169,00 ± 7,49d | 143,33± 7,63d | 193,00±3,57c | 202,33±8,02b | 207,33±7,50bc |  0,000 |
| DWG (g/d) | 0,19 ± 0,025a | 0,10 ± 0,027d | 0,11 ± 0,03cd | 0,15 ± 0,047bc | 0,14 ± 0,11bc | 0,15 ± 0,07ab | 0,16± 0,06ab | 0,15 ± 0,012ab | 0,005 |
| SGR (%J) | 1,85 ± 0,045a |  1,40 ± 0,046d | 1,96 ± 0,059e | 2,21 ± 0,05c | 1,87 ± 0,12d | 1,88 ± 0,07b | 1,88 ± 0,06b | 1,69 ± 0,055b | 0,000 |
| FCR | 1,33 ± 0,13f | 2,65 ± 0,00a | 2,46 ± 0,12c | 2,36 ± 0,14b | 2,43 ± 0,11d | 1,95 ± 0,04d | 2,01 ± 0,09d | 1,69 ± 0,07e | 0,000 |
|  K  | 2,08 ± 0,15a | 1,22 ±0,04d | 1,69 ± 0,20b | 1,44 ± 0,03c | 1,37 ± 0,27cd | 1,40 ± 0,03cd | 1,31 ± 0,03cd | 1,76 ± 0,09b | 0,000 |
| FCP (fcfa) | 2793 | 1947,271 | 1804,232 | 1736,899 | 1784,309 | 1432,153 | 1473,765 | 1224,778 |  |

a, b, c, d, e, f, :(P˃0.05) =values with the same letter are not significantly different; T0+ = Commercial feed (Skretting); T (E0%; A0%) = Local feed 0% enzyme and 0% flavor; T(E0.125%; A0.01%) = Local feed 0.125% enzyme and 0.01% flavor; T(E0.125%; A0.0125%) = Local feed 0.125% enzyme and 0.0125% flavor; T(E0.125%; A0.0150%) = Local feed 0.125% enzyme and 0.0150% flavor; T(E0.1%;A0.0125%) = Local feed 0.1% enzyme and 0.0125% flavor; T(E0.150%;A0.0125%) = Local feed 0.150% enzyme and 0.0125% flavor; T(E0.175%;A0.0125%) = Local feed 0.175% enzyme and 0.0125% flavor; E = Enzyme; A= Flavor; d = day; IW = Initial Weight; FW= Final Weight; SR = Survival rate; AWG = Average weight gain; DWG = Average daily weight gain; FCR = Feed conversion ratio; P = probability; SGR = Specific Growth Rate; K = Condition factor K; FCP= feed cost of production.

**Survival rate :** The survival rate significantly (P < 0.05) varied depending on the treatments (Fig.1) and the highest value (81.33 ± 2.30%), was obtained with fry fed with T(E0.150%; A0.0125%) compared to fry fed with the rations.

**Average daily gain :** Average daily gain increased significantly (P < 0.05) with combined levels of both food additives in the diet (Fig.2). However, the combined levels of both additives were significantly low compared to the T0+ commercial feed treatment, but comparable between treatments supplemented with the additives. Moreover, with treatment T(E0.175%;A0.0125%), the highest value of weight gain was recorded (0.16 ± 0.06g) but significant compared to the other treatments.

**Specific growth rate :** The specific growth rate varied significantly (P < 0.05) with increasing levels of the combination of the two feed additives in the diet (Fig.3). However, with varying levels of additives in the diet, growth rates were significantly low compared to the T0+ commercial diet treatment, but otherwise comparable between treatments supplemented with the additives. With treatment T(E0.150%;A0.0125%), having the highest value of the specific growth rate recorded of (2.21 ± 0.05%/d).

**Feed conversion ratio:** Feed conversion ratio varied significantly (P < 0.05) between treatments during the experiment with increasing levels of the two feed additives in the diet (Fig.4). The significantly lowest feed conversion ratio was recorded with the T0+ commercial feed treatment, but otherwise comparable between treatments containing combined levels of the enzyme and flavour in the feed. With treatment T(E0.175%;A0.0125%), having the lowest value (1.67 ± 0.07) but not significant compared to the other treatments.

T0+ = Commercial feed (Skretting); T (E0%; A0%) = Local feed 0% enzyme and 0% flavor; T(E0.125%; A0.01%) = Local feed 0.125% enzyme and 0.01% flavor; T(E0.125%; A0.0125%) = Local feed 0.125% enzyme and 0.0125% flavor; T(E0.125%; A0.0150%) = Local feed 0.125% enzyme and 0.0150% flavor; T(E0.1%;A0.0125%) = Local feed 0.1% enzyme and 0.0125% flavor; T(E0.150%;A0.0125%) = Local feed 0.150% enzyme and 0.0125% flavor; T(E0.175%;A0.0125%) = Local feed 0.175% enzyme and 0.0125% flavor.

Figure 1: Variation in survival rate over the entire experimental period depending on the treatments

T0+ = Commercial feed (Skretting); T0- = T (E0%; A0%) = Local feed 0% enzyme and 0% flavor; T1 = T(E0.125%; A0.01%) = Local feed 0.125% enzyme and 0.01% flavor; T1 = T(E0.125%; A0.0125%) = Local feed 0.125% enzyme and 0.0125% flavor; T1 = T(E0.125%; A0.0150%)= Local feed 0.125% enzyme and 0.0150% flavor; T1 = T(E0.1%; A0.0125%) = Local feed 0.1% enzyme and 0.0125% flavor; T1 = T(E0.150%;A0.0125%) = Local feed 0.150% enzyme and 0.0125% flavor; T1 = T(E0.175%;A0.0125%) = Local feed 0.175% enzyme and 0.0125% flavor.

Figure 2 : Evolution of average daily weight gain as a function of the duration of the experiment

T0+ = Commercial feed (Skretting); T (E0%; A0%) = Local feed 0% enzyme and 0% flavor; T(E0.125%; A0.01%) = Local feed 0.125% enzyme and 0.01% flavor; T(E0.125%; A0.0125%) = Local feed 0.125% enzyme and 0.0125% flavor; T(E0.125%; A0.0150%) = Local feed 0.125% enzyme and 0.0150% flavor; T(E0.1%;A0.0125%) = Local feed 0.1% enzyme and 0.0125% flavor; T(E0.150%;A0.0125%) = Local feed 0.150% enzyme and 0.0125% flavor; T(E0.175%;A0.0125%) = Local feed 0.175% enzyme and 0.0125% flavor.

Figure 3 : Variation of the specific growth rate as a function of treatments over the entire duration of the experiment

T0+ = Commercial feed (Skretting); T (E0%; A0%) = Local feed 0% enzyme and 0% flavor; T(E0.125%; A0.01%) = Local food 0.125% enzyme and 0.01% flavor; T(E0.125%; A0.0125%) = Local feed 0.125% enzyme and 0.0125% flavor; T(E0.125%; A0.0150%) = Local feed 0.125% enzyme and 0.0150% flavor; T(E0.1%;A0.0125%) = Local feed 0.1% enzyme and 0.0125% flavor; T(E0.150%;A0.0125%) = Local feed 0.150% enzyme and 0.0125% flavor; T(E0.175%;A0.0125%) = Local feed 0.175% enzyme and 0.0125% flavor.

Figure 4: Variation of the consumption index according to the treatments over the entire duration of the experiment

1. **Discussion**

Survival refers to the ability of fish to remain alive in the face of fluctuations in the physicochemical parameters of the culture water [8]. During the whole experimental period, the survival rate varied significantly (P < 0.05), and the survival rate was observed to range from 70.66 ± 2.51 to 81.33 ± 2.30%. The values ​​corroborated those obtained by [9] who had a survival rate of *Oreochromis niloticus* fry of 74.67 ± 10.06%, 77.33 ± 12.87%, 82.67 ± 12.22 99%. The recorded survival rate is also lower than that obtained by [10] who obtained survival rates of 89.33 ± 0.44% of *Oreochromis niloticus* fry with ingredients. The survival rate could be attributed to the immune boost from the additives.

The mean daily weight gain of fish was significant (P < 0.05), evolving more and more with the increase in the combined rate of addictives and this gain varied between 0.10 ± 0.02g/d to 0.16 ± 0.06g/d depending on the treatments. These gains are lower than those obtained by [9] who obtained the values ​​of the average daily weight gain of e 0.59 ± 0.02g/d, and 0.63 ± 0.35g/d, and 0.64 ± 0.38g/d and 0.68 ± 0.64g/d in *Oreochromis sp*. in 94 days with a local diet supplemented with *Afrostyrax lepidophyllus* seed powder and those obtained by [11] in Tilapia fry from 5 commercial feeds of 0.30 ± 0.15 (g/d); 0.37 ± 0.23 (g/d); 0.28 ± 0.35 (g/d); 0.27 ± 0.14 (g/d); 0.28 ± 0.35 (g/d); 0.28 ± 0.12 (g/d) depending on the treatments. The increase in progressive daily weight gain induced by the inclusion of increasing levels of aroma and enzyme compared to the performance of those fed the negative control diet could arise from the presence of pancreatic enzymes (lipases, amylases and proteases) and from increasing the activity of digestive enzymes of the gastric mucosa.

The specific growth rates (1.40 ± 0.04 and 2.21 ± 0.05) observed during the experiment varied significantly (P < 0.05) among treatments. They were higher with treatment T(E0.175%;A0.0125%) with a value of 2.21 ± 0.05%/day. These values ​​are lower than those obtained by [9] who studied the effect of supplementing *Afrostyrax lepidophyllus* seed powder in the diet of red tilapia fry (*Oreochromis Sp*.). After 94 days he obtained a specific growth rate between 2.52 ± 0.17%/d and 2.77±0.02%/d. Similarly [12] after 185 days of experiment obtained a low specific growth rate of 1.48 ± 0.01(%/d) and 1.46 ± 0.0(%/d). The variation in specific growth rate would be due to the combined effect of the two food addictives at different levels.

The feed conversion ratio varied significantly (P < 0.05) during the experiment between treatments, with values ​​ranging from 1.69 ± 0.07 to 2.65 ± 0.00 a being obtained. These feed conversion indices are higher compared to those obtained by [13] who had a better feed conversion index of around 1.55±0.05 to 1.68±0.19 on *Oreochromis sp*. and lower compared to those recorded by [12] which were 1.23 ± 0.030, 1.31 ± 0.01, 1.32 ± 0.02. The low feed conversion index of treatment T(E0.175%;A0.0125%) is close to the positive control treatment and will demonstrate the good level of digestibility of the feed.

The condition factor K during the experimental period varied significantly (P < 0.05). These values ​​varied between 1.22 ± 0.04 and 1.76 ± 0.15. These values ​​of the condition factor K obtained at the end of the test were lower than 1.51 and 1.70 reported by [7] by incorporating respectively 0.1% and 0.2% of A. lepidophyllus fruit powder in Oreochromis sp feed and comparable to those obtained by [9] on *Oreochromis sp.* fry which are respectively 1.19; 1.20 and 1.08 for the incorporation levels of 0.3%, 0.4% and 0.5% of *A. lepidophyllus* fruit powder.

The higher cost of production of the feed was recorded in subjects fed with the imported skreting feed (2793 fcfa) compared to the local ration T0- (1947.271 fcfa) and to the supplemented rations different levels of enzyme and aromas T(E0.125%;A0.01%), T(E0.125%;A0.0125%), T(E0.125%;A0.0150%), T(E0.1%;A0.0125%), T(E0.150%;A0.0125%) and T(E0.175%;A0.0125%) (1804,232, 1736,899, 1784,309, 1432,153, 1473,765 and 1224,778 CFA francs respectively) which were also comparable with each other. Feed production costs were significantly (P < 0.05) affected. That of the imported food is significantly higher compared to those of the other treatments which were comparable with each other, showing that the combination of these two additives in the feed induces a reduction in the production cost.

1. **Conclusion**

At the end of the experiment which focused on evaluating the combined effect of two food additives (Complex Enzyme CE805\* and Seafood Flavor II\*) in the feed manufactured on some zootechnical characteristics of Oreochromis niloticus fry in tanks, It appears that zootechnical performances were relevant with fish fed with a feed containing the two additives combined. Treatment T(E0.175%;A0.0125%) obtained the most satisfactory results.

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