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RESEARCH ARTICLE

PROFILE FATTY ACID OF CHIRONOMIDAE LARVAE PRODUCED FROM RABBIT MANURES FISH FARMS IN THE GUINEAN FORESTED REGION

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Abstract

This study confirms the richness in fatty acids of Chironomidae larvae produced from rabbit droppings. These Chironomidae larvae can be used in aquaculture for more environmentally friendly and less costly larval production for fry rearing in fish farms. This simple technique for producing Chironomidae larvae would reduce environmental impact while strengthening the resilience of aquaculture to climate change.

Introduction:-

Use live feed in hatcheries in Africa general and in Guinea particular is a promising alternative following the various failures of using dry feed during larval and fry rearing, as well as its unavailability and high cost (Adande et al. 2025). Live feeds contain essential compounds beneficial to fish growth and survival (Iraj et al., 2024). Similarly, the effectiveness of live prey depends on the supply sufficient quantities amino acids (AA) and unsaturated fatty acids (UFA) for larval growth and development (Samat et al., 2020). Similarly, according to Arts et al. (2001), benthic macroinvertebrates are one the most important components freshwater ecosystems and a primary food source for fish. Through the process energy transfer (Eddy et al., 2021), the fatty acid richness of fish could play an important role in men's need for essential fatty acids. Improving the nutritional quality of live fatty acid foods is therefore essential. The aim this study was to evaluate the different proportions essential fatty acids in Chironomidae produced from rabbit manure.

Materials and Methods:-

Producing Chironomidae from rabbit manure

Chironomidae larvae were produced using an optimal dose of 600 g/m^3 or 25 g/dm^2 of rabbit droppings, according to Adandé et al. (2018). These rabbit manure consist of 15%, 1.3%, and 0.8% N, P, and K, respectively. The characteristics are presented in Table 1. The first step in this production technique involves setting up a medium composed of sixteen (16) liters of borehole water and a dose of 600 g/m^3 or 25 g/dm^2 of rabbit. The second step consists of adding four (04) liters of pond water, previously filtered using a 200µm metal sieve, three (03) days after fertilization, in order to eliminate any macroinvertebrates and allow phytoplankton, a source of food, to develop.

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The third step, three (03) days after fertilization, involves seeding the media with macroinvertebrates (Chironomidae larvae) at an initial density of at least 6 to 12 individuals per 20 liters. The production areas are covered with mosquito netting to prevent potential predators of macroinvertebrates from entering the culture environment.

Parameters	Averages
Temp (°C)	31.51±0.63
pH	7.12±0.10
Cond (µs/cm)	621.06±14.53
OD (mg/l)	3.28±0.46
TDS	318.46±8.58
Sal (mg/l)	0.34±0.00
Transp (cm)	16.85±1.51
Turb (NTU)	84.77±4.01
$N\text{-}NH_3$ (mg/l)	0.98±0.09
$N\text{-}NO^2$ (mg/l)	0.29±0.05
$N\text{-}NO^3$ (mg/l)	0.19±0.01
$P\text{-}PO_4$ (mg/l)	1.21±0.11

Table 1:- Production medium.

Chemical analyses

Physicochemical parameters (temperature, pH, conductivity, salinity (from rabbit manure), TDS, dissolved oxygen) were measured in situ every 7 days (at 8 a.m. and 5 p.m.) using the CALYPSO multi-parameter probe (softer version/2015 2138 SN-ODEON CALYPSO; ± 0.1C sensitivity). Turbidity measurements were taken in the various production environments using a turbidimeter (EUTECH TN-100). Five hundred milliliters of water from the production environment were sampled every seven days and stored in a refrigerator (at 4°C) for the measurement of nitrates, nitrites, orthophosphates, and ammonium using a molecular absorption spectrophotometer (HACH DR/2800) according to Rodier & Merlet (2012).

Fatty acid analysis.

To assess the fatty acid profile, Chironomidae (bloodworms) produced from rabbit droppings were harvested from the production buckets and sorted using a 200 µm sieve. Harvested Chironomidae worms were placed in microtubes and rapidly frozen in liquid nitrogen. These samples were then immediately transferred to the Aquatic Animal Nutrition Laboratory at the Regional Laboratory for Health Safety Expertise and Analysis (LSSEA/IRGIB) and stored at -80°C until analysis.

Measurement fatty acid composition involved two steps: a) fat extraction and esterification according to Folch et al. (1957) and Firston (1998). Fat extraction was performed using the methanol-chloroform extraction method, followed by fat esterification using 2% methanolic sodium and BF3 (boron trifluoride). b) Fatty acid samples were then analyzed using a gas chromatograph (Philips, Sussex, England) equipped with an SGE BPX70 capillary column (ID: 0.25 mm × 0.22 µm × 30 m). We used a flame ionization detector at a temperature of 300°C and an injector set at 250°C. A volume of 0.2 µL of the ester sample was injected into the gas chromatograph for analysis. The initial column temperature was set at 160°C and gradually adjusted up to 230°C and maintained for 5 minutes until all compounds were eluted. In addition, helium was used as carrier gas, hydrogen as fuel, nitrogen as auxiliary gas and synthetic air. By comparing the inhibition times of chromatograms from unknown samples with those obtained from a standard solution, the fatty acids present in Chironomidae produced from rabbit manure were identified and the results expressed as a percentage.

Results and Discussion:-

Physicochemical parameters

Temperature is an important parameter for optimizing the production of freshwater Chironomidae larvae (Table 1). The average value recorded during this study is comparable to those obtained by Williams (1985) during his work on the drift of Chironomidae egg masses. The neutral pH obtained in this study is similar to those obtained by Sotaro et al. (2023) during the conservation of Chironomidae larvae in the laboratory for DNA sequencing. The production of Chironomidae larvae at a neutral pH would allow for the conservation of their genetic material (Bisthoven et al., 2003). Conductivity, salinity, turbidity, and transparency (Table 1) are values selected for the successful production of Chironomidae larvae (Adandé et al., 2018). The average values for ammonia, nitrite, and nitrate are close to those obtained by Poulsen et al. (2014). Chironomidae are capable of mobilizing and denitrifying ammonium (Samuiloviene et al., 2019). The average orthophosphate level obtained during this study is higher than those obtained by Biswas et al. (2009), which ranged from 0.03 to 0.189 mg/l. Chironomidae larvae are capable of increasing orthophosphate levels during the bioturbation process (Biswas et al., 2009). All of these average values for the various physical and chemical parameters would allow for the production of good-quality Chironomidae larvae for fish larvae.

Table 2:- Fatty acid profile Chironomidae larvae produced from rabbit manure (% of total proportions).

Fatty Acid	Names	%
C14 :0	Myristic acid	1,65
C15 :0	Pentadecanoic acid	1,08
C16 :0	Palmitic acid	19,67
C17 :0	Heptadecanoic acid	1,85
C18 :0	Stearic acid	4,14
C20 :0	Arachidic acid	0,23
C21 :0	Heneicosanoic acid	0,78
C24 :0	Tetracosanoic acid	0,18
Total saturated fatty acids	TOTAL SFA	**29,58**
C14 :1	Myristoleic acid	0,17
C15 :1	Pentadecanoic acid	0,39
C16 :1	Palmitoleic acid	13,49
C17 :1	Heptadecanoic acid	0,53
C18 :1	Oleic acid	16,93
C20 :1	Gondoic acid	1,82
C24 :1	Nervonic acid	0,51
Total monosaturated fatty acids	**TOTAL MsFA**	**33,84**
C18 :2 ω6	Linoleic acid	14,86
C18 :3 ω6	α-linolenic acid	0,52
C20 :3 ω6	Dihomolinolenic acid	0,54
C20 :4 ω6	Arachidonic acid	4,97
Total polysaturated fatty acids ω6 (PFGA ω6)	**TOTAL:** PsFGA ω6	**20,89**
C18 :3 ω3	γ-Linolenic Acid	3,03
C18 :4 ω3	Stearidonic Acid	4,29
C20 :5 ω3	Eicosapentaenoic Acid (EPA)	5,79
C22 :5 ω3	Docosapentaenoic Acid	1,06
C22 :6 ω3	Docosanoic Acid	1,52
Total polyunsaturated fatty acids ω3	Total: PsFGA ω3	**15,69**
PsFGA ω6/ PsFGA ω3		**1,33**

T: temperature, pH: Hydrogen potential, OD: dissolved oxygen, TDS: Total dissolved solids, Cond: conductivity and sal: salinity, Turb: turbidity, Transp: transparence

Quantity and quality of Chironomidae fatty acids produced from rabbit manure

Chironomidae larvae produced from rabbit manure have the right fatty acid proportions in terms of quantity and quality. Such larvae are likely to meet the needs of fish larvae. Indeed, the ratio of total long-chain polysaturated fatty acids ω6 to polysaturated fatty acids ω3 (TAGP) ω6/TAGP ω3) is equal to 1.33 (Table 2). This ratio highlights the richness of larvae in long-chain plolysaturated fatty acids, and perfectly meets the needs of fish larvae and fry.

However, this ratio is lower than those obtained by Iraj et al. (2024), which is 2.81 in the Chironomidae. This difference may be due to the production environments and species used. On the other hand, these results corroborate those obtained by Kiyashko et al. (2004) in the larvae Stictochironomus pictulus (Diptera: Chironomidae). One the most important factors is the nutritional quality of the live feed for rearing larvae and fry, including the content essential fatty acids, in particular eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA).

They have values 5.79 and 1.52% respectively and are referred to as highly unsaturated fatty acids (HUFA) (Table 2). The long-chain polyunsaturated fatty acids in the (ω3) series are 3.03% higher than those obtained in the fish larvae feed formulation by Zambonino (2000). According to Michaïl et al (2013), these fatty acids are physiologically indispensable to animals, including humans.

Conclusion:-

This study of the fatty acid profile Chironomidae freshwater produced from rabbit droppings, shows that these fatty acids can be used in hatchery larval production for more environmentally friendly, efficient, and less costly production for rural fish farmers. However, an optimization technique in a healthily controlled environment is needed.

Conflict of interest:

The authors declare that they have no conflict of interest.

Author contributions:

ADANDE Richard: Conceived the study, directed the research writing, editing and interpretation of the results. KEITA Oumar and BAKAYOKO Ibrahima: Conceived the study, directed the research writing, editing and interpretation of the results. SANGARE Aboubacar: Editing and directed the research.

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