Effects of dietary inclusion of *Lannea microcarpa* oil on the growth and the whole body composition of Nile tilapia (*Oreochromis niloticus*)

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Abstract

This study aimed to determine the effects of *L. microcarpa* oil on the growth and the whole body composition of Nile tilapia. For that, five (5) iso-nitrogeneous diets were formulated and used as treatments. This oil was included in the diet at 0%, 2%, 4%, 6% and 8%, noted as R0, HL2, HL4, HL6 and HL8, respectively. Each treatment was used on triplicate groups of fish (6.04± 0.2 g) stocked in cages at a density of 15 fish per cage. These cages were submerged in a 10m³ fiberglass tank. Fish were fed with experimental diets for 8 weeks. All data were subjected to One-way analysis of variance (ANOVA) using R Software. Results showed that fish fed control diet (R0) had the highest final weight (FW), weight gain (WG), daily weight gain (DWG), and specific growth rate (SGR). The highest feed conversion ratios (FCR) were observed in fish fed HL6 and HL8. Regarding the body composition, fish fed R0 had the highest dry matter (97%), lipids (15%), and energy (392 kcal/100g) while those fed with diet HL2 showed the highest protein content (64%). In terms of protein utilization, low protein efficiency ratio (PER) and protein productive value (PPV) were found in fish fed diet with high oil inclusion level. The highest protein growth rate (PGR) (1.93%) was observed in fish fed R0 and this rate appeared to decrease with higher oil incorporation levels. In conclusion, *L. microcarpa* oil had no positive effects on Nile tilapia growth but its inclusion in diets at 2% contributed to improved fish body protein composition.

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Keywords: Nile tilapia, *Lannea microcarpa*, oil, incorporation, growth

Effets d'incorporation de l'huile de *Lannea microcarpa* dans l'aliment sur la croissance et la composition biochimique de la chair du tilapia du Nil (*Oreochromis niloticus*)

Résumé

Cette étude détermine les effets de l'huile de *L. microcarpa* dans l'aliment sur la croissance et la composition de la chair du tilapia du Nil. Ainsi, 5 aliments iso-protéiques ont été formulés et utilisés comme traitements. Cette huile a été incluse dans l'aliment à 0%, 2%, 4%, 6% et 8%, notés R0, HL2, HL4, HL6 et HL8, respectivement. Chaque traitement a été répété trois fois. Les poissons (6,04±0,2g) sont stockés dans des cages submergées dans un bac en fibre de verre ($10m^3$) à 15 alevins /cages et nourris pendant 8 semaines. Les données ont été soumises à une analyse de variance (ANOVA) à un facteur en utilisant le logiciel R. Les résultats indiquent des poids moyen final, gain en poids, gain moyen quotidien et taux de croissance spécifique plus élevés chez les poissons du traitement R0. Des taux de conversion alimentaire ont été plus élevés chez les poissons des traitements HL6 et HL8. Concernant la composition biochimique, les poissons nourris avec R0 ont présenté les teneurs élevées en matière sèche (97%), lipides (15%) et énergie (392 Kcal/100g) tandis que ceux du traitement HL2 avaient la teneur protéique plus élevée (64%). On note que des coefficients d'efficacité protéique et valeur productive protéique étaient plus faibles chez les poissons des traitements à teneurs élevées d'huile. Le taux de croissance protéique (1,93%) le plus élevé a été observé chez les poissons du traitement R0 et celui-ci décroit avec l'augmentation du niveau d'inclusion de l'huile. En définitive, l'huile de *L. microcarpa* ne présente pas d'effet positif sur la croissance des poissons mais une inclusion de 2% dans l'aliment a permis d'améliorer la teneur en protéine de la chair.

Mots clés : Tilapia du Nil, *Lannea microcarpa*, huile, incorporation, croissance.

Introduction

Aquaculture plays a crucial role in alleviating food and nutritional insecurity by providing animal protein for populations. It is a fast growing food producing sector with an annual growth rate of 6.6% (FAO, 2024). However, the production of aquatic animal species in general, and fish farming in particular, depends on the utilisation of a number of ingredients provided by wild fish stocks. These ingredients are primarily fishmeal and fish oil, the supply of which is becoming increasingly difficult due to the scarcity of these marine resources. For example, the fish oil production process uses one ton of pelagic fish to produce 50 kg of fish oil (Hua *et al.*, 2019). In addition to this, import costs lead to higher fish oil prices and consequently increased

aquaculture feed costs (Fry *et al.*, 2016; Apraku *et al.*, 2017; Sankian *et al.*, 2019). For this reason, feed becomes the determining factor in the economic profitability of fish farms, representing approximately 60 to 70% of production costs (Am *et al.*, 2023). Dietary lipids are essential in aquaculture feeds due to their important role in the organism. Indeed, these lipids are a source of energy and essential fatty acids for growth and proper body function, as well as for sparing proteins from being used as an energy source (NRC, 1993; Kim and Lee, 2005). Therefore, to contribute to the sustainable development of the entire aquaculture industries, it is necessary to find alternatives to imported fish oil. Vegetable oils are cost-effective, readily available, and rich in essential fatty acids and energy. Furthermore, the inclusion of various vegetable oils, such as those of soybean, coconut, corn, caraway, sunflower, and palm in diets has improved growth and the whole body composition in Nile tilapia, especially protein, lipids and minerals (John *et al.*, 2022; Mehrim *et al.*, 2024; Nguyen and Tran, 2025). Several aquatic species require polyunsaturated fatty acids from oils but are unable to biosynthesize them (Lim *et al.*, 2009). These needs can therefore be only met through diet being consumed. This has raised interest in supplementing aquafeeds with oils. In Burkina Faso, oilseed oils are available and can be utilised as feed supplements. Among these, *Lannea microcarpa* oil, due to its fatty acid composition, could be a good candidate for formulating tilapia feed. Previous studies have shown that *L. microcarpa* seeds contain 40 to 60% oil with important fatty acids such as palmitic (23%), oleic (12%), and linoleic (13%) acids (Glew *et al.*, 1997; Yunus *et al.*, 2013; Bazongo *et al.*, 2014). These fatty acids appeared to be beneficial for tilapia growth by providing digestible energy, contributing to cell membranes building and serving as precursors for several hormones. Based on these important data on nutritional value, the use of this oil in fish feed appeared to be promising. However, information is lacking on the specific effectiveness of its use in tilapia feed. Furthermore, the lack of control over oil inclusion levels in aquaculture feeds leads to reduced feed intake and growth in fish as high lipid/protein ratio is associated with poor fish growth (Ghanawei *et al.*, 2011). In fact, vegetable oil inclusion levels have been shown to affect fish growth through direct impact on feed palatability, acceptability and energy, which could interfere with lipid utilisation and accumulation (Azevedo *et al.* 2013). Therefore, for the rational use of these oils in fish feed, lipid requirements and oil inclusion rates must be considered in feed formulations. In this context,

this study was designed, aiming to determine the effects of *L. microcarpa* oil inclusion in diets on the growth performances and the biochemical composition of Nile tilapia.

I. Material and methods

I.1. Presentation of the study area

This experiment was conducted in the Oubri region (former Central Plateau region) and in the municipality of Loumbila as shown in Figure 1. The experimental site was located at the "Ferme Agricole Palmier-Dattier " (*12°31'0.85836 N and 1°23'10.4408 W*). This site is about 25 km far away from Ouagadougou, capital city of Burkina Faso. This area is under Sudano-Sahelian climate with an average annual temperature ranging from 18°C to 39°C (INSD, 2022).

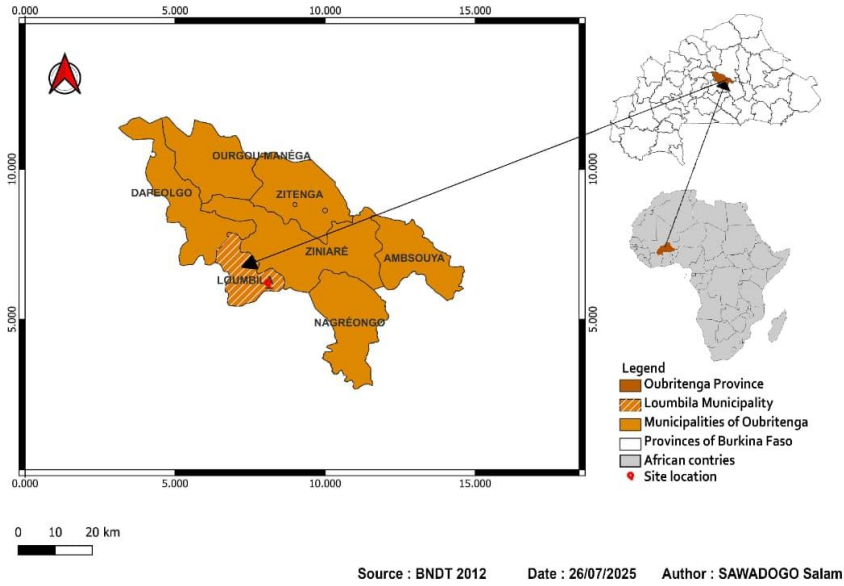

Figure 1: Location of the study site

I.2. Material

During this experiment, biological material and technical rearing equipment were used. Nile tilapia (*Oreochromis niloticus*) of the Bouaké strain was used as biological material. The technical equipment includes the rearing infrastructures which consisted of a 10 m^3 circular fiberglass tank and a set of 0.03m^3 circular cages. It also includes an

electronic balance (Notebook, 0.1) and a water analysis toolkit (HANA multi-parameter analyzer).

I.3. Methods

I.3.1. Experimental design

A supplementation of *L. microcarpa* oil was performed at five inclusion levels (0%, 2%, 4%, 6% and 8%), forming five experimental diets as treatments. These inclusion levels were chosen to be graded as to cover the requirement range for tilapia. This will allow identifying the optimal inclusion level for tilapia growth. Treatments with three replicates each, were randomly assigned to 15 cages as experimental units. Each cage contained 15 fish (6.04±0.2g). Then, a total of 225 fish were stocked. Fish were reared for 8 weeks (56 days) and fed with experimental diets twice a day. This study duration was chosen to ensure a substantial growth of experimental fish. Parameters such as the number of dead fish, the quantity of consumed feed and the weight of the specimens were measured during the trial period.

In the beginning and end of the experiment, 4 fish and 4fish per experimental units (12 fish/treatments), respectively were used for the whole body composition analysis.

I.3.2. Set up of the rearing facilities

This experiment was conducted using a circular fiberglass tank measuring 3 m in diameter, 1.5 m in height, and 10 m^3 of volume. This tank was set in an outdoor system and exposed to the full sunlight photoperiod from morning to the evening. Fifteen (15) cages made of white plastic material (4 mm mesh size) were placed in this tank. This material was purchased locally. The cages were circular, with a radius of 0.15 m, a depth of 0.4 m, and a volume of 0.03 m^3. They were submerged and randomly distributed throughout the tank, allowing them to move freely in the water. Each cage was stabilized by floats attached to its top, ensuring free movement of each cage and maintaining a constant volume. The upper layer of each cage was also closed to avoid fish from escaping. Water was supplied to the system from a borehole (water flow rate of 10 m^3/hour) equipped with a water storage tank.

I.3.3. Experimental diets

Five (5) iso-nitrogeneous experimental diets corresponding to treatments were formulated by incorporating *L. microcarpa* oil (HL)

into the feed. These diets were formulated to contain 28% crude protein and graded lipid content ranging from 8 to 14% due to the oil inclusion rates. The ingredient composition and the proximate composition are recorded in Table I. Experimental diets are noted as R0, HL2, HL4, HL6 and HL8 based on the inclusion rate of 0%, 2%, 4%, 6%, and 8%, respectively. R0 represents the control diet formulated without oils. Soybean meal and fishmeal were the main protein sources, while the energy sources were rice bran and brewer grains. These feedstuffs were purchased from the local market in Ouagadougou, Burkina Faso. Mineral and vitamin premixes were also acquired from Yamba-Yamba, Sarl, Burkina Faso. Oil of *L. microcarpa* was provided by Laafinat Group. Diet formulation was performed using "Diet formulator" software (MS Excel, version 2016, CSIRO Marine Research database). First, the nutritional requirements (protein, fat) are determined. Then, available raw materials are identified as protein and energy sources. Finally, the proportions of the ingredients are typed into the software, which then calculates the ideal proportions of each ingredient to meet the required nutrient content of the diet. This allowed calculation of the gross composition of ingredients along with the required dietary protein level for tilapia.

Table I: Gross composition of ingredients (g/kg of diet) and proximate composition of experimental diets

Treatment [1]	R0	HL2	HL4	HL6	HL8
L. microcarpa oil inclusion level (%)	0	2	4	6	8
Ingredients (kg)					
Fishmeal	25	25	25	25	25
Soybean meal	40	38	36	34	32
Rice bran	16	16	16	16	16
Wheat flour	2	2	2	2	2
Brewer grains	15	15	15	15	15
L. microcarpa oil	0	2	4	6	8
Vitamin premix	1	1	1	1	1
Mineral Premix	1	1	1	1	1
Total	100	100	100	100	100
Proximate composition[2] (%)					
DM	95.11±0.08	94.98±0.07	95.34±0.07	94.85±0.07	95.12±0.07
CP	29.28±0.09	28.29±0.02	28.05±0.09	28.46±0.09	28.80±0.08
CL	8.40±0.09	11.10±0.08	12.92±0.07	13.40±0.06	14.86±0.06
Ash	21.34±09	21.26±0.1	20.74±0.2	20.21±0.08	18.7±0.08
GE (Kcal/100g)	337.1±0.1	350.4±1.1	363±0.6	365.5±0.3	379.6±0.9

The values are means of three repetitions ± standard deviation; means on the same line without common superscripts are significantly different;[1]R0 = Control diet (without oil); HL2 = Diet incorporated with 2% of *L. microcarpa* oil; HL4 = Treatment incorporated with 4% *L. microcarpa* oil; HL6 = Treatment

incorporated with 6% *L. microcarpa* oil; HL8 = Treatment incorporated with 8% *L. microcarpa* oil;[2]DM = dry matter; CP = crude protein; CL = crude lipid; GE = gross energy.

I.3.3.1. Ingredients processing

In the diets production process, the various ingredients must be well processed in order to get suitable powder. For this purpose, raw ingredients such as soybean meal, fishmeal, rice bran, and brewer grains were purchased locally and kept for feed manufacturing. Then, all ingredients were ground using a grain mill (MORIFLOR) to produce flours made of fine particles. This was accomplished to facilitate ingredients mixing for obtaining a homogeneous mixture to be used in the production of pellets. These prepared ingredients were then weighed, packaged in plastic bags, and stored at -4°C until the experimental diet preparation.

I.3.3.2. Diets preparation

Experimental diets were manufactured using the prepared ingredients. The process involved mixing of these different ingredients using a mini-mixer for 3 to 5 mn to achieve a homogeneous texture. This was realised following these steps: First, the major ingredients (soybean meal and fishmeal) were well mixed together until a homogeneous powder was obtained. Then, rice bran and brewer grains were added and mixed, followed by the minor ingredients (mineral and vitamin premix). Rice bran was also considered to be a binder for diets. Finally, oils were incorporated into the mixed ingredients based on the specified inclusion rates. For that, the oils were weighed to determine the correct proportions, then poured and mixed with the other ingredients. The final step involved adding water (about 20%) to form a moist dough which was then used to make the pellet through an extruder (SANEYOO model ZS115, ROC) equipped with a 2 mm mesh. During extrusion, all extruder columns were cleaned with distilled water after manufacturing of any type of experimental diet in order to avoid contamination. Diets were sinking pellets with a pellet size of 2 mm and were dried in the shade under a fan for 24 hours, packaged in sealed and airtight small plastic bags to prevent moisture and oxidation. Finally, these diets were stored in a fridge at -4°C for two weeks until feeding.

I.3.4. Fish rearing and feeding

Fish were purchased from the Bagrépole Fish Farming Center (CEP) in the Centre-East region of Burkina Faso. These fish were brought to the study site in Loumbila. During transportation, fish were packaged in

plastic bags containing one-third water and two-thirds oxygen. Before the start of the experiment, fish were acclimated to the experimental conditions for one week, during which they were kept in cages submerged in the fiberglass tank. They were fed with commercial diet "Ranan fish feed" containing 40% crude protein. The experiment lasted for eight weeks (56 days). In the beginning of the trial, 225 fish of homogenous size (6.04±0.2 g) were selected and distributed among 15 cages. Each cage contained 15 fish. After stocking and acclimation, the fish were fed twice a day (8:00 AM and 4:00 PM) to apparent satiation throughout the experiment. During feeding time, a phytoplankton net was attached to each cage to collect uneaten feed as to avoid feed for leaching and mixed with other diets in surrounding cages. Then, all collected uneaten feeds were weighed and subtracted from the distributed feed to ensure recording of consumed feed. The rearing water quality was monitored daily by siphoning debris from the tank bottom and by replacing 50% of the water to ensure adequate aeration.

I.3.5. Monitoring of the physico-chemical parameters of rearing water

Water quality was monitored by tracking the physicochemical parameters such as pH, temperature, and dissolved oxygen. Over the eight weeks of the experiment, values of these parameters were recorded daily at three different times: morning (6:00 AM), noon (1:00 PM), and evening (5:00 PM). A HANNA multiparameter analyzer was used to record the water temperature, dissolved oxygen, and pH. For that, calibration was performed by using a buffer solution (with a known pH) to calibrate once. This was accomplished by cleaning the prove with distilled water, placing it in the buffer solution (pH =7) until stabilisation. Then, the process is repeated using solution (pH= 4.001 or 10.01).

I.3.6. Sampling and data collection

In the beginning of the experiment, four (04) fish were selected from the purchased fish, anesthetised, gutted, ground, and stored at -4°C till laboratory analysis. This involved determining the initial whole body composition of fish. At the end of the experiment, four fish per cage were also sampled, anesthetized, gutted, ground, and stored for the same chemical analyses. The grinding was performed using a mixer-grinder.

During the experiment, growth parameters were evaluated. For this purpose, sampling was carried out every two-week. During each sampling, fish were weighed as a group using an electronic balance (Notebook, 0.1). The total weight of the group was then divided by the number of fish in each treatment to determine the individual weight of the experimental fish. In the beginning of the trial, fish were individually sorted and weighed to select those with a homogeneous initial size. Every day, the number of fish in each cage was checked, and the number of dead fish was recorded in case of any observed mortality.

I.3.7. Chemical analysis

Experimental diets and fish were analyzed for their proximate composition. For this purpose, previously collected, prepared, and packaged samples were used. Chemical analyses focused on parameters such as dry matter, crude protein, crude lipid, ash, and energy. Analysis was performed following the procedures described by the International Organization for Standardization (ISO). All samples were analysed in triplicate groups through ISO protocols designed for proximate analysis of feed and fish. Briefly, the diets and fish were analyzed for dry matter by placing samples in a Fisherbrand oven at 103°C for 2 hours until a constant weight was obtained (ISO, 2020). These samples were then removed and placed in a desiccator for 30 minutes before being weighed to calculate the moisture content. Crude protein was determined using the Kjeldahl method (ISO, 2013). Thus, 5 g of diets and fish samples were weighed in a Kjeldahl flask, to which Kjeltabs catalyst pellets and 10 mL of concentrated sulfuric acid (98%) were added. The samples were mineralized at progressively increasing temperatures of 90,120 and 400 °C for 4 hours until the solution was completely decolorized. The resulting mineralized solution was diluted with distilled water to 50 mL, and the distillate (150 mL) was collected in a beaker containing 5 mL of indicator. The mixture was titrated with 0.1 N sulfuric acid until the indicator changed its color from green to pink. The resulting titration volume was used to determine the nitrogen (N) content of the samples. Protein content was calculated using the formula %P = N * 6.25. Lipid content was determined by the Soxhlet method according to ISO (1998) by performing a hot extraction for 4 hours, followed by soaking and rinsing in 200mL of hexane. The content was determined by weighing after hexane was evaporated. Ash content was determined by differential weighing after the samples were heated in an oven at 600 °C overnight (ISO, 2023). Carbohydrate

content was determined by subtracting from 100%, the content of moisture, ash, lipid and protein (100-(%Moisture+%Ash+%Lipid+%Protein)). Finally, energy was calculated according to Merrill and Watt (1975) by multiplying the protein, carbohydrate, and lipid content of the samples by their respective energy values.

I.3.8. Calculation of the studied parameters

The assessment of the effects of *L. microcarpa* oil on Nile tilapia was realised based on the growth and nutrient utilisation parameters. For the growth performance, variables were Average Daily Weight Gain, Weight Gain, Specific Growth Rate, Survival Rate, Feed intake, Feed Conversion Ratio, and Feed Efficiency. These variables were calculated using the following formulas:

Daily Weight Gain, **DWG (g/day)** $=(\mathbf{Pmf - Pmi})/\boldsymbol{T}$ where Pmf and Pmi are final and initial individual weight, respectively and T = duration of the experiment (days)

Weight Gain, **WG (%)** $= 100 \times \mathrm{Pmf} - \mathrm{Pmi})/\mathrm{Pmi}$ where Pmf and Pmi denote final and initial individual weights, respectively

Specific Growth Rate, **SGR (%/day)** $= 100 \times (\ln P mf - \ln \mathrm{Pmi})/\mathrm{T}$ where T is the time (days)

Survival Rate, **SR (%)**$= 100 \times (\text{Number of survived fish})/(\text{Initial number of fish})$

Feed intake, **FI(g/fish)** $= \text{Total consumed feed (g)}/\text{Number of fish}$

Feed Conversion Ratio, **FCR** $= \text{Total consumed feed(g)}/\text{FBW} - \text{IBW (g)}$ where FBW and IBW are final and initial body weight, respectively

Feed Efficiency, **FE** $= (\mathrm{FBW} - \mathrm{IBW})/\text{Total feed intake}$ Where FBW and IBW are final and initial body weight, respectively

Regarding the protein utilisation parameters, variables such as Protein Efficiency Ratio (PER), Protein Productive Value (PPV**)** and the Protein Growth Rate (PGR) were calculated based on the following formulas:

PER$= (\mathrm{FBW} - \mathrm{IBW})(\mathrm{g}) / (\text{ protein fed(g)})$, where Protein fed = Total consumed feed (g)×Feed protein content (%).

PPV (%) $=100 \times (Retained\ protein\ (g)/Protein\ fed(g))$, Where Retained Protein =Fish final protein content (g)- Fish initial protein content (g).

PGR(%/day)$=100 \times$ (Log Fish final protein content $-$ Log Fish initial protein content) /T Where T is the experimental period (days)

I.3.9. Data processing and statistical analysis

The collected data were typed using Excel. For statistical analysis, all data were analysed using R statistics software (version 4.6.1, Posit, PBC). These data were subjected to Shapiro-Wilk and Bartett's tests to verify normality and homogeneity, respectively. Then, a one-way analysis of variance (ANOVA) was performed using data found to be normally distributed. A comparison of treatment means was realised using Tukey test, and means were considered significantly different at 5% significance level.

II. Results

II.1. Growth performance, survival and feed efficiency

During the experimental period, water temperature in the rearing tank varied significantly between morning and evening. A variation in the water pH was also observed. Thus, the average values for temperature, pH, and dissolved oxygen ranged between 30°C and 34°C, 7 and 8, and 3 and 4 mg/l, respectively (Table II).

Table II: Physico-chemical parameters of the culture water during 56 days of experiment

Variables	Period		
	Morning	Mid-day	Afternoon
T(°C)	30.28 ±1.04[b]	33.69±1.82[a]	34.07±1.51[a]
pH	7.98±0.62[b]	8.27±0.46[a]	8.28±0.53[a]
DO (mg/l)	3.28 ±1.04[a]	3.86±2.05[a]	3.84±2.26[a]

T: temperature; DO: Dissolved oxygen; values are means of 56 records± standard deviation; means on the same line without common letters are significantly different.

Results on the growth of Nile tilapia fed diets containing different levels of *L. microcarpa* oil inclusions are presented in Table III. The initial body weight (IBW) of the experimental fish were similar (ranging from 5.8 to 6.23 g). At the end of the 8-week experimental period, fish fed the control diet showed the highest values for final mean weight (FW), weight gain (WG), daily gain (DWG), and specific growth rate (SGR),

while the lowest values for these variables were observed in fish fed diets HL6 and HL8. Considering the *L. microcarpa* oil treated diets, the highest values for FW, WG, DWG, and SGR were obtained in fish fed diets HL2 and HL4.

Regarding feed utilisation parameters, the highest feed conversion ratio (FCR) was observed in fish fed with diet HL8. The lowest feed intake (FI) and feed efficiency (FE) were also observed in fish fed diet HL8. The best FI and FE were found in fish fed control diet. Considering the oil treated diets, the highest FI, FCR, and FE values were found in fish fed diets HL2 and HL4.

Table III: Zootechnical performance of Nile Tilapia fed with experimental diets

Treatment[1]	R0	HL2	HL4	HL6	HL8
Inclusion level (%)	0	2	4	6	8
Parameter[2]					
IW (g)	6.00±0.08^{a}	6.23 ± 0.28^{a}	6.16 ± 0.10^{a}	6.04 ± 0.28^{a}	5.8 ± 0.10^{a}
FW (g)	14.50±0.68^{a}	10.31±1.07^{b}	11.28 ± 0.56^{b}	8.00±0.18^{c}	6.03 ± 0.20^{d}
WG(%)	141.66±14.85^{a}	65.43±16.63^{b}	83.18±8.78^{b}	32.66±3.67^{c}	3.96±1.68^{c}
SR (%)	95.56±3.85^{a}	91.11±10.18^{ab}	75.56±10.18^{ab}	71.11±3.85^{c}	91.11±10.18^{ab}
DWG (g/day)	0.152 ±0.013^{a}	0.073±0.018^{b}	0.091±0.010^{b}	0.035±0.002^{c}	0.004±0.002^{d}
SGR (%/day)	1.57±0.11^{a}	0.89 ± 0.19^{b}	1.08 ± 0.09^{b}	0.50±0.05^{c}	0.07±0.03^{d}
FI (g/fish)	19.06±0.04^{a}	11.72 ± 0.87^{b}	12.83 ± 0.96^{b}	9.13±0.61^{c}	6.22 ± 0.17^{d}
FCR	2.26 ± 0.19^{b}	2.98±0.62^{b}	2.51 ± 0.08^{b}	4.66 ± 0.41^{ab}	11.20 ± 6.74^{a}
FE	0.45±0.04^{a}	0.34 ± 0.06^{b}	0.40± 0.001^{ab}	0.22 ± 0.02^{c}	0.04±0.02^{d}

Values are means of three replicates ± standard deviation; means on the same line without common letters are significantly different.

[1]R0 = Control feed (without oil); HL2 = Treatment incorporated with 2% *L. microcarpa* oil; HL4 = Treatment incorporated with 4% *L. microcarpa* oil; HL6 = Treatment incorporated with 6% *L. microcarpa* oil; HL8 = Treatment incorporated with 8% *L. microcarpa* oil;

[2] IW=initial weight; FW=final weight; WG=Weight Gain; SR =Survival Rate; SGR =Specific Growth Rate; DWG: Daily Weight Gain; FCR =Feed Conversion Ratio; FI=Feed Intake; FE=Feed Efficiency.

II.2. Fish whole body composition and protein utilisation

II.2.1. Fish whole body composition

The proximate composition of the fish in the beginning and end of the experiment is shown in Table IV. Results show that the inclusion of *L. microcapa* oil in diet had a significant effect on the whole body

composition of the fish. Fish fed the control diet showed high content of dry matter (DM), crude lipid (CL), and gross energy (GE). The highest crude protein (CP) content was obtained in fish fed diet HL2, while the highest ash and carbohydrate levels were found in fish fed diets HL4 and HL8, respectively. Further, based on the *L. microcarpa* oil treated diets, fish fed with diet HL2 had a higher dry matter (DM) and crude protein (CP) content compared to those fed other treatments. Similar results were found when compared with the initial fish body composition. Fish fed diet containing 6% *L. microcarpa* oil showed the highest lipid content. As for the ash content of the fish body, it was higher in fish fed diet HL8. In terms of carbohydrates, the highest content was observed in the fish fed diet containing 4% *L. microcarpa* oil.

Table IV: Whole body composition of Nile tilapia fed experimental diets

Treatments	TI	R0	HL2	HL4	HL6	HL8
Inclusion level (%)		0	2	4	6	8
Nutrients (%)						
DM	94.48±0.09^{e}	97.1±0.01^{a}	96.15±0.06^{b}	95.8±0.03^{c}	96.15±0.06^{b}	95.3±0.08^{d}
CP	59.81±0.08^{e}	62.73±0.06^{c}	64.60±0.07^{a}	63.5±0.07^{b}	60.43±0.06^{d}	56.69±0.10^{f}
CL	7.14±0.07^{e}	15.06±0.05^{a}	10.13±0.08^{c}	8.51±0.10^{d}	11.93±0.08^{b}	8.49±0.09^{d}
Ash	26.94±0.06^{b}	17.9±0.09^{e}	20.52±0.10^{d}	16.6±0.08^{f}	23.27±0.09^{c}	29.3±0.09^{a}
Glu	0.59±0.30^{c}	1.44±0.09^{b}	0.90±0.17b^{c}	7.2±0.28^{a}	0.53±0.20^{c}	0.83±0.01^{bc}
GE (Kcal/100g)	305.9±0.27^{e}	392.2±0.2^{a}	353.2±0.28^{c}	359.4±0.1^{b}	351.2±0.65^{d}	306.5±0.4^{e}

Values are means of three replicates ± standard deviation; means on the same line without common letters are significantly different. [1] TI = initial content; R0 = Control feed (without oil); HL2 = Treatment incorporated with 2% *L. microcarpa* oil; HL4 = Treatment incorporated with 4% *L. microcarpa* oil; HL6 = Treatment incorporated with 6% *L. microcarpa* oil; HL8 = Treatment incorporated with 8% *L. microcarpa* oil; [2] DM = Dry matter; CP = Crude protein; CL = Crude lipid; Glu = Carbohydrates; GE = Gross energy.

II.2.2. Protein utilisation in the fish body

Table V presents the results on the protein utilisation. Statistical analysis revealed significant differences among treatments in terms of protein efficiency ratio (PER), protein productive value (PPV), and protein growth rate (PGR). Specifically, the lowest PER and PPV values were observed in fish fed diets HL6 and HL8, but no difference was found among fish fed diets HL2 and HL4 compared to those fed control diet. Similarly, treatments HL6 and HL8 showed the lowest PGR values. Fish fed the control diet had the highest PGR values. Among fish fed oil treated diets, HL2 and HL4 showed the highest PER, PPV and PGR values. Overall, an incorporation level of *L. microcarpa* oil above 4% leads to reduced PER, PPV and PGR.

Table V: Protein utilisation in the body of Nile tilapia fed *L. microcarpa* oil-based diets

Treatment[1]	R0	HL2	HL4	HL6	HL8
Inclusion level (%)	0	2	4	6	8
Parameter[2]					
PER	0.0152±0.002[a]	0.0136±0.003[a]	0.0142±0.001[a]	0.008±0.001[b]	0.0015±0.001[c]
PPV (%)	98.30±8.55[a]	98.06±15.72[a]	96.49 ± 2.64[a]	47.24±3.97[b]	3.07±3.29[c]
PGR(%/day)	1.93±0.13[a]	1.20±0.22[b]	1.38 ± 0.10[b]	0.61±0.06[c]	0.03±0.03[d]

The values are means of three repetitions ± standard deviation; means on the same line with no letters in common are significantly different. [1]R0 = Control feed (without oil); HL2 = Treatment incorporated with 2% *L. microcarpa* oil; HL4 = Treatment incorporated with 4% *L. microcarpa* oil; HL6 = Treatment incorporated with 6% *L. microcarpa* oil; HL8 = Treatment incorporated with 8% *L. microcarpa* oil; [2]PER = Protein Efficiency Ratio; PPV= Protein Productive Value; PGR = Protein Growth Rate

III. Discussion

III.1. Growth performance, survival and feed efficiency

During the experiment, pH and temperature varied only slightly. These parameters were within the optimal growth range for tilapia (Lacroix, 2004; Azaza *et al.*, 2008; Rebouças *et al.,* 2016). Similarly, dissolved oxygen levels were within the growth range for tilapia as evidenced by studies of Kestmont *et al.* (1989). Based on the fish growth data, it was observed that a high inclusion of *L. microacarpa* oil in diets led to poor growth in tilapia specimens under the rearing conditions of the present study. This could be explained by the fact that the high inclusion rate was responsible for an excessive increase in the rate of available energy and thus caused fat storage rather than promoting muscle growth. This observed poor growth phenomenon could result from a decrease in the fish ability to digest lipids when the level of incorporation in the feed is high and a reduction in feed consumption (NRC, 1993; Wang *et al.*, 2005). Furthermore, similar results were found when palm oil and *Balanites aegyptiaca* oil were incorporated into feed of Nile tilapia and *Clarias anguillaris*, respectively (Ayisi *et al.,* 2017; Sagne *et al.,* 2019). According to these authors, an increase in the content of these oils in the feed led to poor fish growth. In contrast, Nobrega *et al.* (2017) found higher growth performance than that observed in our study when linseed oil was included in tilapia diet. On the contrary, another study showed that including sunflower oil in diet did not affect growth and survival of *Cyprinus carpio* (Sonu *et al.,* 2014). In terms of feed utilisation, low food consumption and feed rejection by fish were observed when oil was included at levels between 6 and 8% in the current study. This could be explained by the fact that feed acceptability

depends on its palatability or attractiveness, as demonstrated by previous studies which found that feed intake was strongly influenced by the palatability of diets containing vegetable oils (Rodríguez-Serna, 1996; Azevedo *et al.*, 2013). Furthermore, as highlighted by previous studies, a high rate of dietary lipid incorporation caused low feed intake and reduced growth in several species such as Nile tilapia, Gibel carp, and Chinese catfish. *Leiocassis longirostris* (Pei *et al*., 2004; El-Marakby, 2006).

III.2. Whole body composition of fish and protein utilisation

In the present study, the results showed a significant improvement in the chemical whole body composition of Nile tilapia fed graded inclusion levels of *L. microcarpa* oil-based diets. Generally, feeds reflect the chemical composition of fish through a modification of the nutritional properties of the body (Ayisi *et al.*, 2019). In this study, high body protein contents were observed in tilapia when *L. microcarpa* oil was incorporated at 2% in diets. This could be explained by physiological conditions of digestion according to the protein-sparing effect, as highlighted by Gao *et al.* (2011). Indeed, according to this phenomenon, when the lipid level is adequate for the fish, dietary proteins are no longer used as an energy source but rather for building the fish body muscles. Therefore, this could promote better flesh quality in terms of protein suitable for human consumption. Our results contradict those of Taşbozan et *al*. (2015), who showed that the protein content of tilapia whole body increased with increasing canola oil inclusion rates in the feed. In a comparative study, Zagre (2025) showed that incorporating *L. microcarpa* oil into diets for Nile tilapia resulted in higher whole body protein levels than it was observed when incorporating *B. aegyptiaca* oil. Regarding other nutrients, the results of the present study showed a higher lipid content in fish fed diet containing 6% *L. microcarpa* oil compared to those fed diets from other treatments. This is consistent with the findings of Ayisi *et al.* (2017) who showed that high lipid contents in tilapia were observed with feed inclusion rates of 6% and 8% palm oil. Furthermore, high lipid values in the fish body have been found in other freshwater species such as *beluga sturgeon* fed diets containing canola oil (Falahatkar *et al.*, 2018) and rainbow trout fed cottonseed oil-based diets (Güler and Yıldız, 2011). Thus, fat accumulation in the flesh appeared to be related to the level of ingested lipids (Erondu *et al.*, 2021; Zhou *et al.*, 2024). This was not observed in the present study, which showed that the digestion

of lipids from *L. microcarpa* oil is not directly related to the inclusion level.

With regards to the protein utilisation, it was shown in the present study that PER, PPV and PGR were the lowest at high incorporation rates of *L. microcarpa* oil in the fish diets. These results were found to be consistent with those of Ayisi *et al*. (2017), who showed low PER values when palm oil was incorporated at 8% in diet for Nile tilapia. However, contrary results were found by Fayza (2007), who showed that these parameters were improved with increased dietary inclusion level of corn oil. It should be noted that the fish body composition and nutrient utilisation can change with the physiological stage of the fish (Johansen and Overturf, 2006). This could explain the differences in results observed between this study and other studies. This physiological stage includes age as well as the growth stage of experimental fish.

Conclusion

The present study aimed to determine the effects of *L. microcarpa* oil in diets on the growth and the whole body composition of Nile tilapia. The results obtained in this study highlighted the potential for using this oil in Nile tilapia feed. Indeed, the inclusion of this oil did not show a positive effect on tilapia growth performance or protein utilisation. However, supplementing the feed with this oil improved the whole body composition of tilapia, particularly the protein content of tilapia fed diet containing 2% of this oil. Based on these results and considering the nutritional quality of the fish body composition, a 2% inclusion level of this oil in the feed was found to be optimal in terms of enhancement of the fish body composition. Thus, *L. microcarpa* oil could be recommended in diet for Nile tilapia as it will allow cost effective feed formulations. However, future research is needed in order to allow its better use in fish feed. Thus, it is essential to study the effect of mixing *L. microcarpa* oil with other oils in diets for Nile tilapia as to improve its effectiveness. Further, it is essential to conduct in-depth further studies for determining the effect of *L. microcarpa* oil in diets on the fatty acid composition of Nile tilapia.

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Conflict of interest

The authors declare no conflict of interest for the paper.

Authors contribution

SS realised the concept and design, data acquisition, data analysis / interpretation, drafting manuscript, CI participated to the concept and design, critical revision of manuscript, supervision, final approval, ZAR conducted data acquisition, critical revision of manuscript, SF participated to the critical revision of manuscript, BSF participated to the critical revision of manuscript.

Ethical approval

This study conformed to the guidance of animal ethical treatment for the care and use of experimental animals

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