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Using blood metabolites to identify nutrient disbalances in Nile tilapia (Oreochromis Niloticus) fed waste streams in pond culture

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Abstract

Due to sustainability concerns the use of local waste streams in pond aquaculture is on the rise. The study aimed to demonstrate whether a targeted profile of blood metabolites could indicate how Nile tilapia (*Oreochromis niloticus*) responds to a particular feed application. Three diets prepared from local waste streams were randomly assigned to six earthen ponds, each stocked with 60 fingerlings and fed twice daily for 120 days. Every 14 days, 20 fish per pond were sampled to measure body weight and length. At the end of the experiment dried bloodspot samples of twenty individual fish were collected for the analysis of free amino acids and acylcarnitines. The results show suboptimal growth across treatments, with blood metabolite profiling pointing to protein deficiency as a key limiting factor. Among metabolite analyses, the higher ratios of valine:leucine and C4DC:(methionine+valine) showed a high use of dietary protein as a glucogenic energy source compared to observations in nature. The inverse association of growth performance with ketone formation further demonstrates that inefficient energy metabolism, caused by a lack of glucogenic supply to the citric acid cycle, is likely the main cause for the poor growth. Our study demonstrates the utility of dried blood spot metabolite analysis as an indicator for metabolic changes and to identify limiting nutrients in aquaculture trials. This approach could support the development of more efficient, locally adapted feeds for sustainable fish production.

Introduction

Because of sustainability concerns, the use of local, sustainable resources for pond aquaculture needs to be stimulated. It is important to evaluate which local, unutilized waste streams are useful as nutrient sources for aquaculture (Kasahun et al., 2012). Testing new raw materials in aquaculture is typically done through trials in ponds or tanks where the growth performance of fish or other aquatic organisms is monitored in response to a particular raw material (Ayisi et al., 2017; Guo et al., 2020; Pius Mmanda et al., 2020). However, performance only does not show the underlying causes of malperformance. A targeted panel of blood metabolites may identify the mechanisms of suboptimal performance due to nutrient imbalances or inhibition. This has been demonstrated by our team in a study in free-living Nile tilapia, using the profiling of carnitine esters and selected free amino acids (Bayissa et al., 2021) but has not been evaluated in pond systems. In many low-income regions earthen ponds are often the only affordable aquaculture system, hence the relevance of studying the metabolic response of fish in earthen ponds to waste streams as feed sources.

Carnitine esters and free amino acid profiles have been used in several species to reveal differences in nutrient metabolism (Geda et al., 2017; Li et al., 2017; Worku et al., 2021; Xu et al., 2025). By using this method, Worku et al. (2021) estimated the impact of seasons and agro-ecological zones on the nutritional status of dairy cows. Here, we set up a proof of concept study using Nile tilapia in earthen ponds fed a variety of mixtures based on locally available waste streams in Jimma, Ethiopia. The idea was to test if a targeted panel of blood metabolites (carnitine esters and free amino acids) would be able to identify nutrient disbalances in fish pond systems when challenged with a variety of waste streams with expected low nutritive quality. Due to the very different types of substrates, we anticipated clear differences in responses of the fish.

Materials and Methods

Concept and substrates

We looked for available waste streams in the neighbourhood of the test site, i.e. Jimma, Ethiopia. The ingredients assumed suitable for the study were cow dung, rumen liquor, poultry manure, wheat bran, brewery waste, and noug cake *(Guizotia abyssinica)*. The collected cow dung and rumen liquor were sun-dried and ground. The raw materials of the other ingredients like wheat bran, brewery waste, and noug cake, were used as provided. Using these ingredients, three mixtures were composed by persons with aquaculture experience but without prior knowledge of the nutrient composition of the ingredients. This was done with the aim to obtain a real-life simulation of a low-cost aquaculture setting, yet creating a variation in nutrients and energy.

To obtain the actual nutritive value, the substrates were analysed for dry matter content (DM), crude protein (CP), crude fat (CFat), crude fibre (CF), and ash content, following the standard procedure of the Association of Official Analytical Chemists (AOAC, 1990) (Table 1). The moisture content was determined by oven-drying at 105°C until constant weight and the ash content was determined by combustion of the ingredients in a muffle furnace at 550°C. The crude fat was analysed with the Soxhlet method (ISO 1443, 1973). The crude protein (6.25 × N) was determined by using the Kjeldahl method (ISO 5983–1, 2005). Mineral concentrations were analysed via inductively coupled plasma optical emission spectrometry (Ca, P, K, Na, Mg, Cu, Fe, Mn, Zn) or inductively coupled plasma mass spectrometry (Se, I). Neutral detergent fiber and Acid detergent fiber was analyazed by using filter bag technique (ANKOM200 fiber analyzer 220V). Amino acid profiles were analyzed by using LC-MS/MS (Liquid Chromatography–Tandem Mass Spectrometry). While Gas Chromatography-Mass Spectrometry (GC-MS) was used for fatty acid analysis.

Table 1
Macronutrient and energy concentrations in three waste stream mixtures used as substrates for ponds with Nile tilapia (on fresh matter basis of the dried material as used in the study).

Ingredients	**unit**	**T1**	**T2**	**T3**	
Cow manure	%	49.8	-	-	
Rumen liquor	%	50.2	-	42.8	
Brewery waste	%	-	45.4	-	
Wheat bran	%	-	27.3	-	
Poultry manure	%	-	27.3	28.6	
Noug cake		%	-	-	28.6
Nutrients					
Dry matter		%	88.8	90.0	90.7
Crude protein	%	18.0	25.0	25.0	
Ash	%	13.3	9.3	17.5	
Crude fat	%	0.9	5.9	4.0	
Crude fibre	%	16.9	12.5	9.5	
Acid-detergent fibre	%	23.9	26.3	15.7	
Neutral-detergent fibre	%	29.9	35.7	26.1	
Nitrogen-free extract	%	39.6	37.3	34.7	
Gross energy	MJ/kg	14.9	17.3	15.5	

Ponds and fish

The study was conducted in earthen ponds with a 4 m width x 5 m length area and 70 cm depth at Jimma University's main campus, in Ethiopia. Ponds were filled with water and fertilized using wet cow dung and rumen liquor for about two weeks to boost plankton growth before fingerling introduction. Nile tilapia (*Oreochromis niloticus*) fingerlings were obtained from Sebeta Fishery and Aquatic Life Research Centre. Fish with an average of 7.5 cm in length and an average of 8.5 g weight were selected, and a total of 60 fingerlings were randomly stocked in each pond.

Water quality of the ponds was monitored daily basis in which data for temperature, pH, dissolved oxygen, and electric conductivity were collected three times daily (morning, mid-day and evening) by using multi-probe meter (HQ40D Single-Input Multiparameter Digital Meter; Hach Company, Loveland, CO, USA). The concentration of nitrate, nitrite and ammonia were measured daily (Palintest multiparameter photometer 7500; Tyne and Wear, UK, NE11 0NS).

Set-up

The three substrate combinations (composed as explained higher) were each applied to two ponds. On as-fed basis, treatment 1 (T1) contained 49.8% cow manure and 50.2% rumen liquor; treatment 2 (T2) consisted of 45.4% brewery waste, 27.3% wheat bran, and 27.3% poultry manure; treatment 3 (T3) contained 42.8% rumen liquor, 28.6% noug cake, and 28.6% poultry manure (Table 1).

Before the start of the experiment, the fish were acclimatized to the experimental ponds for two weeks during which they were provided a 50:50 (w:w) combination of cow dung and rumen liquor. During the experiment, fish were fed between 9:00–10:30 am and 3:00–4:00 pm for 120 days. Twenty fish from each pond were randomly sampled every fourteen days to measure their weight and length.

Blood sampling and analysis

At the end of the experiment, individual fish were randomly collected for blood sampling (seven per substrate mixture, except for T1, where one fish was accidently discarded). The weight and length of each fish were measured. Then the fish were washed with tap water to remove any contaminants before the blood collection. The fish were euthanized by pitching the brain (Bayissa et al., 2021). Blood samples were collected from the caudal vein by puncturing with a 1 mL syringe (Becton Dickinson S.A., Madrid, Spain) and 26-G needle (Becton Dickinson, Drogheda, Ireland) rinsed with heparin (LEO Pharma, Ballerup, Denmark). (Ferguson, 2005) on the filter paper (Whatman 903, LOT, 7067117W161, UK) and dried. Finally, the dried blood spots (DBS) were sent to Ghent University, Belgium, for the analysis of acylcarnitines and selected free amino acid profile by using electrospray tandem mass spectrometry following the standard (Ferguson, 2005; Vieira et al., 2012) at the Laboratory for Metabolic Diseases at Ghent University Hospital.

A priori, specific metabolite ratios were calculated as proxies for specific metabolic traits:

- the ratio of all measured carnitine esters to free carnitine as a proxy for metabolic rate;
- the ratio of Propionylcarnitine to acetyl carnitine (C3:C2) indicates how well the citric acid cycle is provided with glucogenic substrate
- the ratio of 3-hydroxybutyryl carnitine to acetyl carnitine (C4OH:C2) as proxy for ketone production, hence inefficient energy metabolism;
- the ratio of methylmalonylcarnitine to propionylcarnitine (C4DC:C3) suggests the rate of glucogenic substrate use from amino acids and propionate.
- the ratio of malonyl carnitine to acetyl carnitine (C3DC:C2) as proxy for lipogenesis;
- the ratio of methylmalonyl carnitine to the sum of valine and methionine (C4DC:(val + met)) as proxy for the use of amino acids as glucogenic substrate for the citric acid cycle;
- the ratio of valine to leucine (val:leu), as proxy for the use high demand of glucogenic amino acids to supply the citric acid cycle;
- the ratio of the 3-hydroxy forms of long-chain acylcarnitines (> C10) to their respective acylcarnitines, as proxy for lipolysis.

Statistical analysis

Data were collected in Microsoft Excel and uploaded in SPSS version 29 for statistical analysis. Normality of the data was checked through boxplot analysis. To evaluate the associations between growth performance (weight, length and Fulton's factor) and blood metabolites, bivariate correlations (Pearson) were calculated. Significant differences were accepted at $P < 0.05$.

Results

The ingredient and macronutrient composition of the three waste stream mixtures used as substrates are presented in Table 1. Their mineral, fatty acid and amino acid concentrations are presented in Tables 2, 3 and 4 respectively. The pond water quality is presented in Table 5. The correlation between growth performance and selected acylcarnitine and free amino ratios are presented in Table 6.The acylcarnitine:free carnitine ratio only showed a tendency to positively correlate with the performance parameters ($r \sim 0.4$; $p > 0.05$). Ketone production (C4OH:C2) had the strongest negative correlation with weight ($r = -0.631$; $p = 0.004$), than with length ($r = -0.486$; $p = 0.030$), and a tendency with fulton factors ($r = 0.054$ $p = 0.825$). Fat synthesis (C3DC:C2) also showed negative correlations with the fish growth parameters. By contrast, no significant correlations were seen between fish performance on the one hand and fat combustion (3OH-LCFA:LCFA) and the rate of amino acid use in the citric acid cycle, although the valine:leucine ratio did show negative correlations with fish performance. Ratios of selected acylcarnitines and free amino acids in dried blood spots of Nile tilapia in ponds with different waste stream mixtures as substrates (n = 6 for T1; n = 7 for T2 andT3) is presented in Table 7. The changes in body weight, body length, and body condition of Nile tilapia during the experimental period are presented in Figs. 1, 2, and 3, respectively.

Table 2
Mineral concentrations in three waste stream mixtures used as substrates for ponds with Nile tilapia (on fresh matter basis).

Ca	Unit	T1	T2	T3
	g/kg	**4.5**	**7.2**	**8.8**
P	g/kg	6.6	4.8	7.1
K	g/kg	9.6	6.9	11.1
Na	g/kg	11.6	1.9	8.1
Mg	g/kg	2.4	2.3	2.8
Cu	mg/kg	13	24	25
Fe	mg/kg	17227	4343	8504
Mn	mg/kg	947	392	621
Zn	mg/kg	104	120	97
Se	µg/kg	708	226	910
I	µg/kg	2196	696	1511

Table 3
Fatty acid profile of three waste stream mixtures used as substrates for ponds with Nile tilapia.

Fatty acid (mg/100 g)	*T1*	*T2*	*T3*
C10:0	5.25	9.12	8.55
C12:0	5.29	1.79	4.82
C14:0	11.40	17.18	9.27
C15:0	8.02	7.78	6.14
C16:0	82.13	1241.61	352.08
C17:0	5.08	7.65	6.35
C18:0	51.14	136.8	190.25
C20:0	11.49	19.99	28.41
C22:0	0.34	1.11	0.42
C23:0	1.04	3.36	1.02
C24:0	5.25	9.12	8.55
∑SFA	**186.43**	**1455.51**	**615.86**
C14:1	0.21	0.44	0.46
C16:1	15.28	9.91	10.75
C17:1	2.52	3.61	2.79
c9C18:1	45.73	676.23	861.90
c11C18:1	22.05	37.82	32.19
C20:1	1.79	35.29	18.68
C22:1	-	-	-
C24:1	-	-	-
∑MUFA	**87.58**	**763.3**	**926.77**
C18:2n-6	41.62	2452.20	802.99
C18:3n-6	0.97	1.15	1.64
C20:2n-6	1.47	3.94	2.78
C20:3n-6	0.35	0.34	0.65
C20:4n-6	2.74	5.37	2.61
∑n-6 PUFA	**47.15**	**2,463.00**	**810.67**
C18:3n-3	8.31	242.21	744.19
C20:3n-3	0.36	1.21	0.88
C20:4n-3	2.04	3.64	2.95
C20:5n-3	-	-	-
C22:5n-3	-	-	0.39
C22:6n-3	0.18	-	-
∑n-3 PUFA	**10.89**	**247.06**	**748.41**
Sum	**332.05**	**4928.87**	**3101.71**

∑SFA is the sum of saturated fatty acid; ∑MUFA is the sum of mono-unsaturated fatty acid; ∑n-6 PUFA is the sum of omega six polyunsaturated fatty acid; ∑n-3 PUFA is the sum of omega three polyunsaturated fatty acid.

Table 4
Amino acid profile of three waste stream mixtures used as substrates for ponds with Nile tilapia.

Variables (g/kg)	***T1***	***T2***	***T3***
Aspartic acid	8.9	11.0	14.6
Threonine	4.5	5.7	6.0
Serine	4.3	6.8	7.3
Glutamic acid	10.5	30.2	24.5
Glycine	5.5	7.8	9.5
Alanine	6.0	7.9	8.1
Valine	4.9	8.1	7.5
Isoleucine	4.0	6.0	6.0
Leucine	6.5	10.8	9.8
Tyrosine	2.9	4.8	4.5
Phenylalanine	6.1	8.0	8.3
Histidine	2.2	3.9	3.8
Lysine	3.7	6.5	6.0
Arginine	3.9	8.8	11.4
Proline	4.2	13.2	6.6
Cystine	1.5	3.5	2.6
Methionine	1.8	2.7	2.7
Tryptophan	0.6	0.3	0.4

Table 5
Physicochemical condition of the ponds in study (n = 2).

Parameter		***T1***	***T2***	***T3***	***SEM***
Temperature (°C)	Morning	19.05	19.25	19.3	0.18
	Mid-day	24.95	25.15	24.85	0.19
	Evening	23.95	24.00	23.55	0.18
Dissolved oxygen (mg/L)	Morning	3.1	2.7	2.5	0.3
	Mid-day	6.4	6.0	6.6	0.3
	Evening	5.4	4.5	5.0	0.3
pH	Morning	7.10	7.20	7.15	0.07
	Mid-day	7.95	7.85	7.85	0.06
	Evening	7.45	7.4	7.45	0.08
Electric conductivity (µS/cm)	Morning	134	134	143	0.4
	Mid-day	132	135	143	0.4
	Evening	125	128	137	0.4
Nitrite-N (mg/L)		0.04	0.05	0.16	0.03
Nitrate-N (mg/L)		0.23	0.06	0.37	0.05
Ammonia-N (mg/L)		0.13	0.21	0.58	0.11

Table 6
Correlation coefficients (p-values between brackets) between growth performance and selected acylcarnitine and free amino ratios in dried blood spots of Nile tilapia in ponds with different waste stream mixtures as substrates.

Acylcarnitine:free carnitine	**Weight**	**Length**	**Fulton factors**
	0.402 (0.079)	**0.388 (0.091)**	**-0.271 (0.276)**
3OHC4:acetyl carnitine	-0.486 (0.030)	-0.613 (0.004)	0.054 (0.825)
C3:C2	-0.358 (0.12)	-0.397 (0.083)	0.319 (0.184)
C4DC:C3	-0.353 (0.138)	-0.268 (0.267)	-0.121 (0.633)
C3DC:acetyl carnitine	-0.525 (0.017)	-0.386 (0.093)	-0.024 (0.924)
3-OH LCFA:LCFA	0.183 (0.439)	0.113 (0.636)	-0.475(0.825)
C4DC:(methionine+valine)	-0.070 (0.769)	-0.039 (0.871)	0.836 (0.133)
Valine:leucine	-0.450 (0.046)	-0.479 (0.033)	0.059 (0.81)

C3:C2 (propionylcarnitine : acetylcarnitine); C4DC:C3 (methylmalonylcarnitine : propionylcarnitine); 3OHC4: 3-hydroxybutyryl carnitine; C3DC: malonyl carnitine; (3-OH) LCFA: (3-hydroxy) long-chain acyl carnitines; C4DC: methylmalonyl carnitine.

Table 7
Ratios of selected acylcarnitines and free amino acids in dried blood spots of Nile tilapia in ponds with different waste stream mixtures as substrates (n = 6 for T1; n = 7 for T2 andT3).

Metabolite ratio (mmol/mol)	***T1***	***T2***	***T3***	***P***	***SEM***	**Bayissa et al., 2021**
Acylcarnitines:carnitine	976	3560	3850	0.216	0.68	840–1030
C3:C2	12.0	9.4	7.0	0.700	2.0	70–160
3OHC4:C2	14.9	14.1	3.5	0.720	2.2	6–18
C4DC:C3	990	450	404	0.133	132	20–60
C3DC:C2	3.8^{b}	$2.9^{a\,b}$	1.5^{a}	0.046	0.4	1–2
3-OHLCFA:LCFA	811	986	1040	0.178	51	270–490
C4DC: (Met + Val)	0.046	0.047	0.038	0.897	0.0078	0.21–0.32
Valine:leucine	2260	1420	1260	0.146	217	700–800

^{a,b} Different superscripts indicate significant differences within a row at $P < 0.05$.

C3:C2 (propionylcarnitine : acetylcarnitine); 3OHC4:C2 (3-hydroxybutyrylcarnitine : acetylcarnitine); C4DC:C3 (methylmalonylcarnitine : propionylcarnitine); C3DC:C2 (malonylcarnitine : acetylcarnitine); 3-OHLCFA:LCFA (sum of 3-hydroxy-long-chain fatty acids: sum of long-chain fatty acids); C4DC: (Met + Val) (methylmalonylcarnitine : sum of methionine and valine).

Discussion

The suboptimal performance of the fish in terms of growth performance was expected (Fig. 1–3), knowing that the substrates were not completely fitting all diet requirements for Nile tilapia according to NRC (2011). Below, we list the potential dietary causes of poor performance and will then evaluate if the blood metabolites were able to identify the limiting causes.

Based on the analytical results of the treatments, several nutrients can have contributed to the overall low performance: to start with, protein and fat were low (especially in T1) compared to standard requirements for which protein ranges from 28% to 35% and fat ranges from 5% to 15%(Al Hafedh, 1999; Ng, 2005; NRC, 2011) whereas ash and fibre were high compared with standard requirements in which ash ranges from 6% to 12% while fibres should be below 8% (NRC, 2011), together leading to low metabolizable energy; the potential imbalances in minerals may have added to that, such as the inverse Ca:P ratio and the excessive amounts of Fe and Mn; irrespective of the total amount of fat, the fatty acid profile was relatively deficient of essential fatty acids; finally, the amino acid profile was not balanced for fish production.

In addition, the treatments exerted different changes in water quality. The differences in water quality and nutrient supply to the ponds will have affected the invertebrate and microbial life in the ponds, which will have contributed to the final diet of the fish as well (Mondal et al., 2014; Meng et al., 2022). At least, the higher nitrates seemed rather a consequence than a cause because high nitrates were positively associated with performance. This aspect of “indirect feeding” adds to the importance of looking into the fish’ nutrient metabolism since what is added to the pond is not necessarily what the fish are eating. The above explains how apparently simple treatments can exert highly complex conditions, for which it is a challenge to identify which aspects are most limiting the performance of the fish.

This was the reason for evaluating the use of selected metabolite ratios in dried bloodspots as a tool to unravel the causes underlying the low growth performance.

The metabolite ratios at least point to protein lack as an important explanation for lowest growth performance in the T1 fish (Fig. 1), with in particular the need for protein to be used as glucogenic energy source. In aquatic environments, enzymatically digestible carbohydrates such as starch are scarce, hence leaving only protein (and in few species propionate fermentation) as the main glucogenic energy sources for the citric acid cycle (Maas et al., 2020). This partially explains the high protein requirements of fish.

The much higher ratios of valine:leucine, C4DC:(methionine+valine), C4DC:C3 (Table 7) confirm the higher use of glucogenic precursors in the pond fish compared to nature (Bayissa et al., 2021). Propionylcarnitine (C3) needs some further consideration because it can be generated from several amino acids (isoleucine, valine, methionine, and threonine) (Wongkittichote et al., 2017) but can also be derived from propionic acid, generated through microbial fermentation in the gut, or even from organisms in the pond that are then eaten by the fish. In that perspective, the much higher C3:C2 ratio and much lower C4DC:C3 ratio in the wild may point to a relatively higher availability of propionic acid as compared to glucogenic amino acids. This warrants further study as not much is known about the contribution of fermenting organisms in ponds to aquaculture. We recently identified that feeding a fibrous ingredient such as beet pulp to another herbivorous species, tambaqui (*Colossoma macropomum*) translates into increased use of water organisms by the fish (Awot, 2023).

On the other hands, the valine:leucine ratio showed a tendency to negatively correlate with fish performance (Table 6). The 3-hydroxybutyryl-to-acetylcarnitine ratio (3OHC4) is a marker of the proportion of acetyl-CoA diverted from entry into the citric acid cycle towards ketone body synthesis, which may occur when the availability of glucogenic substrates is relatively limited. Therefore, negative correlation between growth performance and ketone body formation further suggests that inefficient energy metabolism, caused by an insufficient supply of glucogenic substrates to the citric acid cycle, is likely the main cause of poor growth.

A lack of fat could also have contributed to the poor growth performance but this is unlikely as the rate of fat synthesis (C3DC:C2) was even significantly higher in T1, the treatment with the lowest fat concentration in the substrate, and without apparent differences in the use of fat as energy source (3-OHLCFA:LCFA).

Although the direct effects of for instance the lack of essential fatty acids and imbalances of minerals cannot be identified through acylcarnitine and amino acid profiling, there are specific ratios that could be affected. As an example, the conversion of propionyl coenzyme A (C3 as carnitine ester) to methylmalonyl coenzyme A (C4DC as carnitine ester) requires biotin as cofactor that on its turn requires zinc (Tong, 2013). In case of a primary or secondary deficiency (for instance caused by excess Fe antagonism), the ratio of C4DC:C3 would be low. This was not the case, and the ratio was even numerically highest in the treatment with the highest Fe, i.e., T1.

In conclusion, the present study shows the potential and limitations of using the blood profile of acylcarnitines and free amino acids to allow a more mechanistic and early identification of limiting nutrients in fish trials, for instance when exploring novel feed ingredients in the search for sustainable aquaculture systems in the Global South.

Declarations

Author Contributions

T.N.B., G.P.J.J., S.G.G and M.W. designed and conceptualized the study; T.N.B., S.G.G and B.A collected the samples; T.N.B., G.P.J.J., and S.G.G analyzed samples and data; T.N.B., M.W. and G.P.J.J. supervised the study; S.G.G drafted the manuscript; G.P.J.J. revised and edited the manuscript. All authors have read and agreed to sent for a publication to Journal.

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Animal ethics statement.

This study was checked and given approval by Jimma University, College of Natural Sciences Research and Ethical Review Board Committee (Jimma, Ethiopia). The committee has followed the standard procedures written on Article 6 (methods of killing) and Article 9 (Animals are taken from the wild), of directive 2010/63/EU of the European parliament and of the council of 22 September 2010 on the protection of animals used for scientific purposes and certified this study with the letter referenced (Ref. No:RPG/165/2019). This study was carried out in compliance with the ARRIVE guidelines.

Conflict of Interest: The authors declare that they have no conflict of interest.

Data Availability Statement: The data that support the findings of this study are available from the corresponding author upon reasonable request.

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Figures

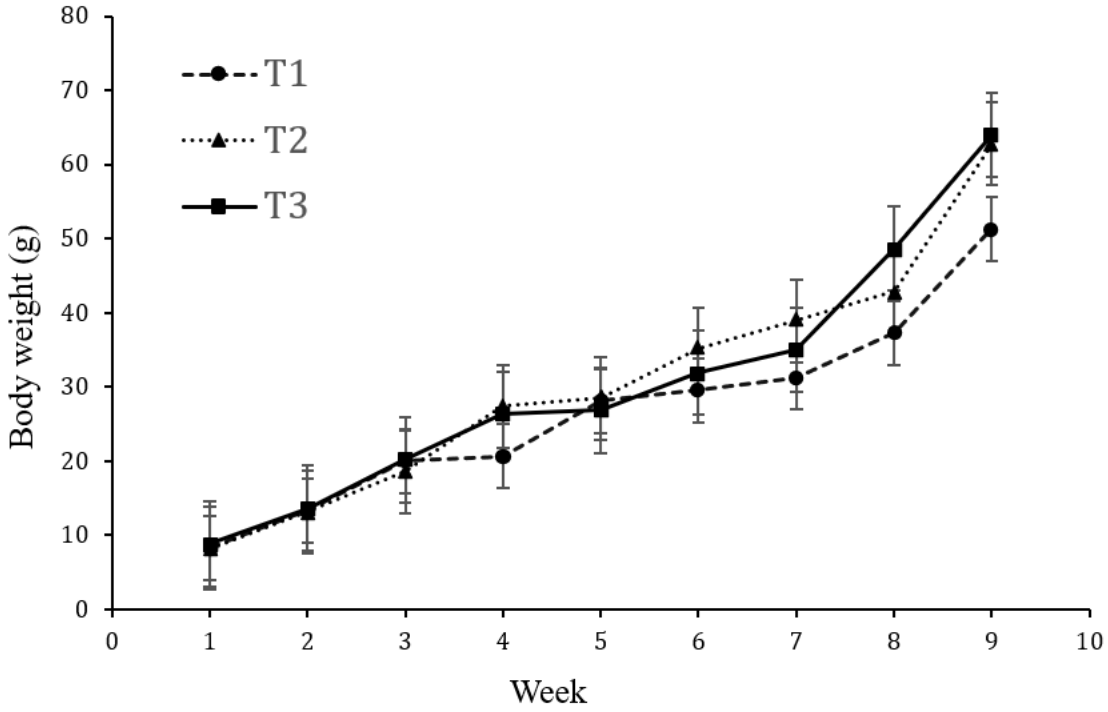

Figure 1

Evolution of body weight of Nile tilapia in ponds with three different waste stream mixtures used as substrates (2 ponds per treatment).

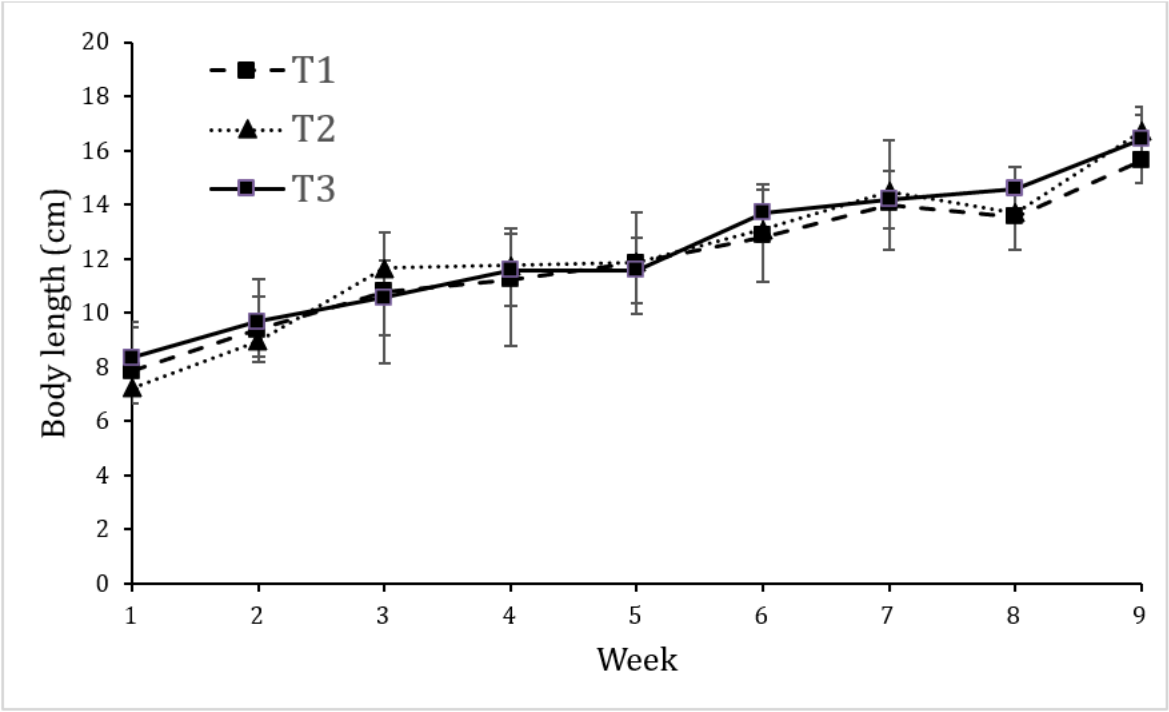

Figure 2

Evolution of body of Nile tilapia in ponds with three different waste stream mixtures used as substrates (2 ponds per treatment).

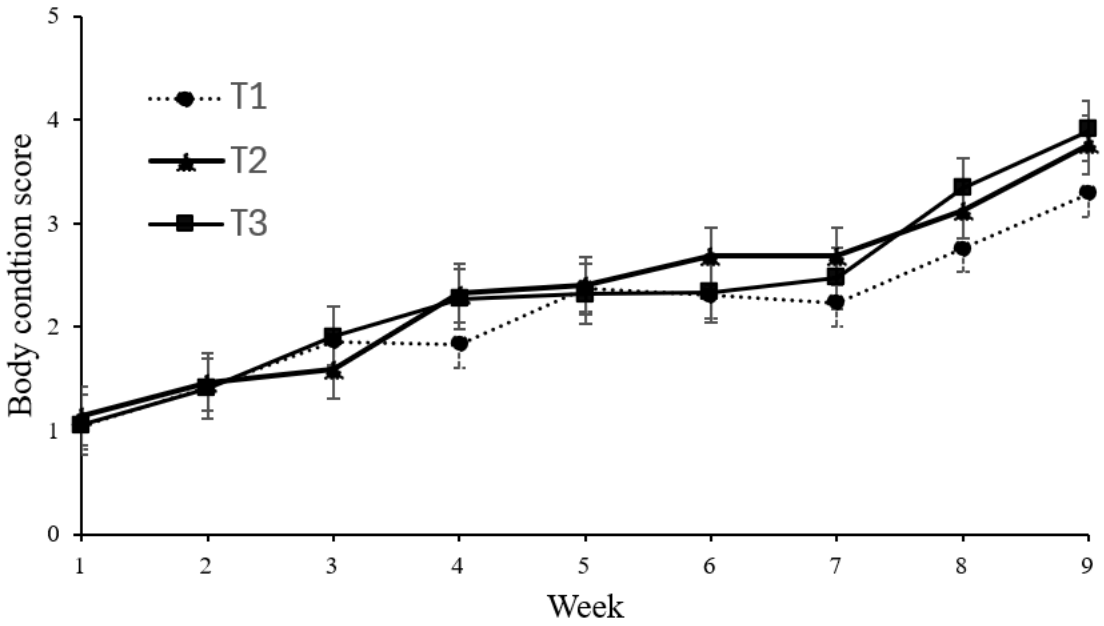

Figure 3

Evolution body condition score of Nile tilapia in ponds with three different waste stream mixtures used as substrates (2 ponds per treatment).