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Research Article

Chemical composition *of Tithonia diversifolia* (Hemsl.) and its effect on growth performance, feed efficiency and metabolic biochemistry of juvenile hybrid tilapia *Oreochromis mossambicus* × *Oreochromis niloticus*

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Abstract

The aquaculture due to the high cost and continued shortage of animal protein sources, the development of alternative plant protein sources has been one of the main challenges to establish sustainable aquaculture as economically viable. Objective of this investigation was to determine the chemical composition and effect of *Tithonia diversifolia* on the growth performance, feed efficiency and metabolic biochemistry of juveniles of *Oreochromis mossambicus* × *Oreochromis niloticus*. Five treatments were used: 0% (control), 4, 8, 12 and 16% levels of inclusion of *T. diversifolia* flour in diet to feed juvenile fish cultured in 15 plastic aquariums at a density of 15 fish/aquarium. The assay lasted eight weeks. The *T. diversifolia* flour was characterized for showing adequate levels of crude protein (21%), with low crude fat (4.5%) levels, neutral detergent fiber levels: 43.03% and acid detergent fiber: 24.40%. The growth performance presented significant differences *($p<0.05$)*, for weight gain, day average weight gain (DAW), length gain, day average length gain (DAL), specific growth rate (SGR), feed efficiency (FE), protein efficiency ratio (PER) and feed conversion ratio (FCR). In treatment with 16% of inclusion of *T. diversifolia* flour, values obtained for weight gain, DAW, SGR, FE and PER (15.88 g, 0.28 g. day^{-1}, 2.03 %, 0.34 $g.g^{-1}$ and 0.98, respectively), which did not differ with the control treatment, highlighting the benefits of *T diversifolia* flour. The metabolic biochemistry parameters experienced a decrease with the increase of *T. diversifolia* in diet, where the highest values of triglycerides, cholesterol, and glucose were for the control treatment *($p<0.05$)*, while the protein increased to 4% of the flour. *T. diversifolia* flour presents an adequate balance of nutrients for its chemical composition and amino acids, without causing negative effects on the growth performance, weight-length relationship, feed efficiency, survival and metabolic biochemistry.

Keywords: amino acid; glucose; growth; red tilapia; triglycerides

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Introduction

Aquaculture has grown exponentially in the last 50 years, from a production of less than 1 million tons (t) in 1950 to more than 90 million tons worldwide in recent years (FAO, 2023). Latin American and Caribbean (LAC) region produced a total of 3.75 million tons of food products (excluding algae production) (FAO, 2023). Aquaculture worldwide in recent years has shown exponential growth (FAO, 2022). The demand for conventional raw materials for the formulation of concentrates for commercially grown fish feed has also increased, especially those used as a protein source. In this regard, fishmeal and fish oil are the main ingredients of animal origin in commercial diets while soy and corn meals are those of vegetal origin. However, their high need for human food and livestock production systems causes an over demand for these products (Méndez-Martínez *et al*., 2021a).

One of the main factors that affects the success of this agroindustry is the selection of species with high productive potential, which are rich in protein and a variety of unsaturated fatty acids (Jim *et al*., 2017), such is the case of red tilapia, of which there are hybrids such as *Oreochromis mossambicus* × *O. niloticus* (Mastoi *et al.,* 2012; Méndez-Martínez *et al*., 2021b). Currently, aquaculture in LAC presents a deficit of quality and affordable food; as well as adequate methodologies to achieve efficiency in tilapia feeding and nutrition to meet their nutritional needs (Al-Sagheer *et al*., 2018). The high costs of concentrates constitute one of the fundamental factors for aquaculture activity to become less profitable every day (Herawati *et al.,* 2020) with this situation, one of the technological demands is to acquire knowledge about the use of raw materials alternatives, for the formulation of new commercial diets and unconventional ingredients in order to reduce the high production costs demanded by this activity (Sirakov and Velichkova, 2018).

In this sense, there are trees and shrubs with high potency forage biomass production with an adequate balance of nutrients, and we can call unconventional foods, because they are not of daily use in aquaculture production. However, with the correct form of presentation, they can become a vitally important food substitute due to their high nutritional content and low production costs, which will generate opportunities for new aquaculture development markets (Pineda-Santis *et al*., 2023).

Shrub sunflower (*Tithonia diversifolia*) is such kind of an unconventional feedstuff that is abundantly available. Its leaves and full-bloom flowers are good sources of protein, mineral matters, and other nutrients (Buragohain and Rajkhowa, 2019), has a high biomass production and more than chemical composition compared to most tree species in tropical conditions. *T. diversifolia* leaves have been documented to contain 18 to 23.5% crude protein (CP), 15 to 18% crude fibre (CF), 11 to 15% ash, 2.81 to 5.6% crude lipid (CL) and 44.38 to 52% nitrogen free extract (NFE) by various workers (Fasuyi *et al*., 2011; Ekeocha, 2012, Herrera *et al*., 2020). This perennial shrub contains low values of acid detergent fiber (ADF) and neutral detergent fiber (NDF), high content of nitrogen and calcium, as well as acceptable percentages of degradation and content of non-structural carbohydrates (Ekeocha, 2012; Rivera *et al*., 2018). Although, some secondary metabolites responsible for biological activity such as saponins, tannins, essential oils, flavonoids that act as antioxidants, repellent activity, anticancer, antiparasitic and reproductive stimulants are known (Ejelonu *et al*., 2017; Mabou *et al*., 2018; Verdecia *et al*., 2020ab; Akeumbiwo *et al*., 2023).

Studies of use *T. diversifolia* in fish and other monogastrics is limited. In this sense, Castro and Asencio (2012) found that when 15% of the shrub sunflower meal in diets for *Piaractus brachypomus* is included, there is low weight gain, feed conversion and feed efficiency, respectively. However, Puerta-Rico *et al*. (2017), evaluated the apparent digestibility coefficients of dry matter, crude protein and digestible energy in *P. brachypomus* and obtained percentages higher than 67% in these indicators, respectively. Hahn-von-Hessberg *et al*. (2016) reported values of apparent digestibility coefficients of shrub sunflower of crude protein (98.66%), ethereal extract (98.79%), neutral detergent fiber (87.73%), calcium (95.41%), phosphorous (93.75%); Nitrogen free extract (96.57%) and gross energy (78.47%) in *O. niloticus*, aspects that demonstrate the efficient use from this alternative source in some fish species.

However, studies are insufficient to elucidate the impact of the concentration of metabolites on the biological and metabolic response of fish. Due to the above, our objective was to determine the chemical composition and effect of *T. diversifolia* flour on productive performance, feed efficiency and metabolic biochemistry of juveniles of *O. mossambicus* × *O. niloticus.*

Materials and Methods

Location and growing conditions of T. diversifolia

The research was carried out in the period from May to December 2022, in Santo Domingo of the Tsachilas km 12 to road the Carmen, Quevedo, Ecuador. The climate belongs to the zone called Tropical Monsoon, whose ecological classification corresponds to Tropical Humid Forest (Holdridge, 1978) during the experimental period, climatic variables behaved as follows: rainfall of 110.87 mm, with an average, maximum and minimum temperature (21, 32 and 12 °C, respectively) and relative humidity of 85%.

Soil present in the area is dystrandeprt (Soil Survey Staff, 2014). Soil samples were taken at two depths (0-30; 30-60 cm), to determine the physical-chemical characteristics. The texture was determined by the hydrometer method (Murillo *et al*., 2014). Nitrogen, using the Kjeldhal technique (USDA, 2022); phosphorus (P) was determined according to Pérez *et al.* (2019), and potassium (K), calcium (Ca) and magnesium (Mg) were determined according to Molero *et al.* (2008), respectively (Table 1).

Table 1. Soil characteristics of the area of *T. diversifolia* cultivation

Indicator	Valor, as mean ± SD
pH	5.34 ± 0.02
N, $cmol_c.kg^{-1}$	1.44 ± 0.01
P, $cmol_c.kg^{-1}$	5.33 ± 0.04
K, $cmol_c.kg^{-1}$	0.55 ± 0.03
Ca, $cmol_c.kg^{-1}$	1.53 ± 0.02
Mg, $cmol_c.kg^{-1}$	0.78± 0.08
Sand, %	22.88 ± 2.06
Silt, %	54.00 ± 1.88
Clay, %	23.00 ± 2.76

Determination of chemical composition on flour and diets

For the elaboration of shrub sunflower forage flour was harvested at an age of 70 days and the leaves were manually separated from the stems, the leaves were taken and dried at 80 °C for 24 hours (Botello *et al*., 2023), in an air flow oven (Hafo 1600 Series, Sheldon Manufacturing Cornelius, OR), then ground in a hammer mill with a sieve (250 μm).

All chemical analyses were repeated three times (Tables 2 and 3). Dry matter (DM); crude protein (CP) by Kjeldhal method; crude fiber (CF) Weende's method, nitrogen-free extract (NFE) by difference were determined, phosphorus (P) was obtained by Covenin method, from ash of sample, calcium (Ca) by digestion of the sample with HNO_3 and H_3PO_4, and silica (Si) infrared absorption spectrophotometry, according to the AOAC (2005). While neutral detergent fiber (NDF), acid detergent fiber (ADF), acid detergent lignin (ADL), cellulose (Cel), hemicellulose (Hcel), cell content (CC) according to Goering and Van Soest (1970). The digestible energy (DE) was theoretically estimated according to Ramanathan *et al*. (2015) from the conversion factors of 4.25 animal protein, 8.0 for lipids, 2.0 (legume), 3.8 vegetable protein, and 3.0 kcal g^{-1} for carbohydrates (non-legume).

Essential amino acid profile (Table 3) in the foliage of *T. diversifolia* was quantified by high performance liquid chromatography (HPLC) (Agilent 1100- HPLC, Pleasanton, CA, USA), equipped with a flow-through vacuum degasser G1379A, a four-channel pump of the low-pressure gradient G13111A, an automatic injector G1313A, a column thermostat G13116A, and a diode array detector G1316A. Samples were analyzed using a column length ZORBAX-XDB-C18- 50 mm, inner diameter - 4.6 mm, and the diameter of octadecylsilyl sorbent grain 1.8 µm (Reverter *et al.*, 1997).

Formulation and elaboration of experimental diets

In the present study, all the diets (Table 2) were formulated (LINDO Systems, Inc. IL, USA), and the inclusion of different levels of *T. diversifolia* flour: 0 (control), 4, 8, 12 and 16%, for a total of five treatments. All ingredients were sieved with a 250 µm mesh sieve and weighed with a digital scale (0.01 g; PE 3600 Mettler-Toledo, OH, USA). For each diet, all the macro ingredients were mixed in an industrial mixer (Hobart 20 Qt-A200, OH, USA) until a uniform mixture was obtained. The micro-ingredients were mixed before adding to the macronutrient mix. The soy and fish oil were mixed until a homogeneous mixture was obtained. Water was added with an equivalent of 30% by weight of the ingredients in diet. The food was pelleted with a meat grinder (Tor-Rey MJ22 JR, NL, MX) to obtain 2 mm granules, which were then dried for 8 h at 45 °C in an air flow oven (Hafo 1600 Series, Sheldon Manufacturing Cornelius, OR), then the dry pellets were packed in plastic bags and kept at -4 °C until use (Méndez-Martínez *et al.*, 2017, 2021b).

Table 2. Formulation and chemical composition (%, wet basis) of experimental diets with *T. diversifolia* flour inclusion

Ingredients	***T. diversifolia flour*** **levels, %**				
	0	**4**	**8**	**12**	**16**
T. diversifolia flour	0.00	4.00	8.00	12.00	16.00
Fish meal[1]	33.00	31.00	29.00	27.00	25.00
Soybean meal[4]	16.00	18.20	20.30	22.50	24.60
Wheat meal[9]	22.40	19.50	16.50	13.60	10.70
Corn meal[3]	21.00	19.70	18.60	17.30	16,10
Mineral premix[9*]	1.00	1.00	1.00	1.00	1.00
Vitamin premix[8**]	2.00	2.00	2.00	2.00	2.00
Grenetine[5]	2.00	2.00	2.00	2.00	2.00
Vitamin C[9]	0.10	0.10	0.10	0.10	0.10
Vegetable oil[3]	1.00	1.00	1.00	1.00	1.00
Fish oil[7]	1.50	1.50	1.50	1.50	1.50
Proximal composition, %					
Dry mater	92.54	92.54	92.53	92.52	92.52
Crude protein	33.64	33.60	33.52	33.48	33.41
Crude lipid	5.94	6.12	6.16	6.19	6.22
Crude fiber	1.49	2.16	2.83	3.50	4.17
Ash	9.07	9.53	9.65	9.77	9.89
Nitrogen free extract (NFE)	42.40	41.12	40.37	39.58	38.83
NDF	2.95	4.75	6.56	8.36	10.16
ADF	1.29	2.33	3.37	4.41	5.45
DE ($MJ.Kg^{-1}$ of food) [10]	12.59	12.43	12.28	12.12	11.97
CP DE^{-1} ($mg.PC.MJ^{-1}$) [11]	26.72	27.04	27.31	27.62	27.90
Essential amino acids, $g.100g^{-1}$ MS [***]					
Lysine	2.04	2.00	1.96	1.92	1.88

Valine	1.81	1.77	1.73	1.69	1.65
Leucine	2.45	2.44	2.44	2.43	2.43
Histidine	0.89	0.87	0.85	0.84	0.82
Arginine	1.42	1.41	1.40	1.39	1.38
Threonine	1.52	1.51	1.50	1.50	1.49
Isoleucine	1.35	1.35	1.35	1.34	1.34
Methionine	0.77	0.76	0.75	0.74	0.73
Phenylalanine	1.26	1.24	1.23	1.21	1.20

[1] Commercial "El Gordillo" - Santo Domingo of The Tsáchilas, Ecuador; [2] Ullón Poultry –Valencia, Ecuador; [3, 4, 6] Supermaxi – Quevedo, Ecuador; [7] Fortidex S.A - Santa Elena; [5] Sigma Aldrich, USA; [8] Supplies AZ, La Paz, BCS, Mexico; [9] Super Success, Quevedo, Ecuador.
*mg.kg^{-1}: Magnesium sulfate 5.1 ; Sodium chloride 2.4; Potassium chloride 2; Ferrous sulfate 1; Zinc sulfate 0,2; Cupric sulfate 0.0314; Manganous sulfate 0.1015; Cobalt sulfate 0.0191; Calcium iodate 0,0118; Chlorine chloride 0.051.
**mg.kg^{-1}: Thiamine 60; Rivoflavin 25; Niacin 40; Vitamin B6 50; Pantothenic acid 75; Biotin 1; Folate 10; Vitamin B12 0,2; Hill 600; Myoinositol 400; Vitamin C 200; Vitamin A 5000 UI; Vitamin E 100; Vitamin D 0,1; Vitamin K 5. ***Essential amino acid requirements of tilapia according to NRC (1993).
Calculated value: [10]MJ.Kg^{-1} of feed: digestible energy; [11]mg.PC.MJ^{-1}: protein ratio for each MJ of energy in the diet.

Fish culture conditions

Red tilapia juveniles (6.74 ± 0.50 g) were obtained from private company "Piscicola Hidalgo", km 3 highway Valencia-Quevedo and transferred to the Aquaculture Laboratory of the Faculty of Livestock and Biological Sciences (01°03'18"S, 79°25′24"W) of the Universidad Técnica Estatal de Quevedo (UTEQ). They were acclimatized for a week and dewormed by applying 2.35 ml (API Stress Zyme, OH, USA)/L of water, every 48 hours, according to the manufacturer's instructions. The specimens were randomly placed in 15 plastic aquariums (27.6 cm × 16.5 cm × 12.5 cm at a density of 15 fish/ aquariums, for a total of 270 fish. They were fed the five experimental diets ad libitum for eight weeks.

The control of the physical-chemical parameters of the water was carried out, the temperature was measured with a mercury thermometer (0 to 50 °C), the dissolved oxygen with a digital oximeter (55-DO, YSI Incorporated, Yellow Springs, OH, USA), and the pH, NH_4, NO_2 and NO_3 with the colorimetric kit (Saltwater Master Test, OH, USA), with average values of 27.4 °C, 4.52 mg L^{-1}, 7.3, 0.52 mg L^{-1}, 0.71 mg L^{-1}, 0.68 mg L^{-1}, respectively. All tanks were siphoned daily in the morning before feeding, to remove the feces and excess food and the water (approximately 30 %) that was extracted during the siphoning was replaced.

Fish growth performance

For the biometrics the juveniles were anesthetized with 4-Allyl-2-methoxyphenol (clove oil): alcohol solution (ratio 1:3 mL in 5 L of water), for 1 min, to reduce stress in the fishs and have better handling. The fish were individually weighed on a digital balance (± 0.01 g; PE 3600 Mettler Toledo, Columbus, OH, USA), and the total length was determined with a digital vernier caliper (± 0.001 mm, GT-MA15 Gester, Xiamen, CHN). All calculations were obtained from average of three replicates. Feed intake was determined by assessing apparent satiation (Méndez-Martínez *et al*., 2021b). Surplus or not consumed pellets, which could be easily identified by its swollen shape, were removed every morning and quantified using a vacuum pump with Whatman No. 1 filter paper (Gast Manufacturing, Benton Harbor, MI) those were dry at 50 °C during 18 hours in an air flux oven (Hafo Series 1600, Sheldon Manufacturing, Cornelius, OR). Considering surplus food, daily rations were adjusted every week in order to minimize the amount of surplus food (Méndez-Martínez *et al*., 2017; 2021b). The following formulae were applied to obtain production and nutritional parameters:

Weight gain (WG, g) = Wx - Wi (1)

Lenght gain (LG, g) = Lx - Li (2)

Day average weight gain (DAW, g) DAW = (Wx - Wi) /t (3)

Day average lenght gain (DAL, g) DAL = (Lx - Li) /t (4)

Specific growth rate (SGR) = [(lnWx - lnWi)] / t × 100 (5)

Protein efficiency ratio (PER) = weight gain g, wet weight)/protein intake (g, dry weight) (6)

Food Efficiency (FE) = total weight gain (g, wet weight)/total feed consumed (g, dry weight) (7)

Feed conversion ratio (FCR) = total feed consumed (g) / total weight gain (g, wet weight) (8)

Survival rate (SR, %) = (final number of fishes / initial number of fishes) × 100 (9)

Where Wx is the final body weight (g), Wi is the initial body weight (g), and t is the duration of the experiment (days), Lx is the final body length (cm), Li is the initial body length (cm). Wd is the weight of diet consumed by the fish.

Metabolic-biochemical analysis

At the end of the culture period, they were fasted for 14 h before taking the samples. Blood samples were obtained as indicated by Azizoglu and Cengizler (1996), by caudal venipuncture with 1 mL disposable syringes and deposited in vacutenier tubes (Vacuette, Laborgeräte GmbH, Eschau, DE) and they were kept refrigerated at 4 °C for 24 h. They were then centrifuged (Gemmy, PLC-05, Taipei, TW) at 1200 rpm for 10 min to separate the plasma from the formed elements (Méndez-Martínez *et al*., 2022).

The evaluation in plasma was with kit reagents (Liquicolor, Wiesbaden, DE) for each variable respectively. The colorimetric reactions changed according to the variable, for cholesterol and triglycerides they were tested with lipid lightening factor and for glucose it was through the deproteinization method. Then they were incubated at a temperature of 37 °C for 25 min in a water bath. For protein it was through the Biuret method, samples were incubated 10 min at 37 °C for proteins. Readings were made in a spectrophotometer (SunostIk, SBA-733 Plus, Kunshan Road, CHN) at ABS: 510 nm for glucose, 456 nm for total proteins, 500 nm for cholesterol and triglycerides, respectively. The analyzes were carried out in triplicate.

Statistical analysis

One-way analysis of variance (ANOVA) was applied to the results of productive performance, feed efficiency and metabolic biochemistry. The difference between the means was quantified using the Duncan with a significance level of $p < 0.05$. The Kolmogorov-Smirnov ($p < 0.05$) test was used for the normal distribution of the data, and the Bartlett ($p < 0.05$) was used for the variances, respectively. For the weight-length relationship, an exponential correlation analysis was carried. Minitab 18 software (Minitab Inc., Philadelphia, PA, USA) was applied. The results are presented as means ± SD (standard deviation).

Results

The *T. diversifolia* leaf flour (Table 3) was characterized by presenting an adequate balance of nutrients in the chemical composition with high percentages of protein (CP) and cell content (CC) with 21.00 and 56.97%, respectively, while the constituents of the cell wall (NDF) were 43.03%. On the other hand, for the highest values of amino acids were for the phenylalanine, leucine, Lysine, Threonine, Valine, Arginine, Isoleucine and Methionine of 13.21, 19.08, 21.05, 12.43, 23.95, 9.02, 8.03 and 6.40 g.Kg^{-1} of protein, respectively.

Table 3. Chemical composition (%, as-dry basis) of *T. diversifolia* flour

***T. diversifolia* flour content, as mean ± SD**	
Proximal composition, %	
DM	88.67 ± 0.26
CP	21.00 ± 0.05
NDF	43.03 ± 0.39
ADF	24.40 ± 0.31
ADL	6.44 ± 0.19
Cel	12.34 ± 0.12
Hcel	16.72 ± 0.14
CC	56.97 ± 0.42
Ca	4.15 ± 0.021
P	0.29 ± 0.002
Si	3.01 ± 0.019
Essential amino acids, g.Kg^{-1} of protein	
Methionine (Met)	6.49 ± 0.015
Phenylalanine (Phe)	13.21 ± 0.432
Isoleucine, (Ile)	8.03 ± 0.221
Leucine (Leu)	19.88 ± 0.456
Lysine (Lys)	21.05 ± 0.34
Threonine (Thr)	12.43 ± 0.242
Valine (Val)	23.95 ± 0.44
Arginine (Arg)	9.02 ± 0.219
Histidine (His)	2.78 ± 0.02

The productive parameters (Table 4) showed significant differences (Duncan test, $p < 0.05$) for weight gain, day average weight gain (DAW), length gain, day average length gain (DAL), specific growth rate (SGR), feed efficiency (FE), protein efficiency ratio (PER) and feed conversion ratio (FCR), being highest for the control treatment (17.66 g, 0.32 cm, 2.23 %, 0.40 g.g^{-1} and 1.13) in weight gain, DAW, SGR, FE and PER, respectively. These indicators did not differ with 16% of inclusion of *T. diversifolia* flour. While, for length gain, DAL and FCR were higher than 16 and 12% of *T. diversifolia* flour with 4.35 cm, 0.78 cm.day^{-1} and 2.12, respectively. There were no differences in initial and final weight and length.

Table 4. Productive parameters (mean ± SD) of juvenile *O. mossambicus* × *O. niloticus* feed with different levels of *T. diversifolia* flour

Productive parameters		*T. diversifolia* flour levels, %					*p*
		0	4	8	12	16	
Weight, g	initial	6.99 ± 0.19	7.1 ± 0.11	6.98 ± 0.32	7.11 ± 0.41	6.98 ± 0.24	0.090
	final	23.88 ± 0.73	21.76 ± 0.73	21.82 ± 0.73	21.57 ± 0.73	22.45 ± 0.73	0.230
Length, cm	initial	6.90 ± 0.07	6.96 ± 0.08	6.86 ± 0.07	6.99 ± 0.07	6.87± 0.04	0.167
	final	10.85 ± 0.09	10.93 ± 0.15	10.84 ± 0.43	10.83 ± 0.13	11.22 ± 0.89	0.271
Weight gain, g		17.66 ± 0.88 [a]	14.66 ± 0.77[b]	15.17 ± 0.65 [ab]	14.43 ± 0.94 [b]	15.88 ± 0.66 [ab]	0.030
DAW, $g.day^{-1}$		0.32 ± 0.06 [a]	0.26 ± 0.03 [c]	0.27 ± 0.02 [bc]	0.26 ± 0.01 [c]	0.28 ± 0.02 [b]	0.032
Length gain, cm		3.95 ± 0.03 [b]	3.97 ± 0.02 [b]	3.98 ± 0.02 [b]	3.84 ± 0.02[c]	4.35 ± 0.03[a]	0.026
DAL, $cm.day^{-1}$		0.071 ± 0.02	0.070 ± 0.01	0.0711 ± 0.01	0.069 ± 0.01	0.078 ± 0.01	0.0761
SGR, %		2.23 ± 0.06 [a]	1.87 ± 0.03 [c]	1,98 ± 0.05 [bc]	1.84 ± 0.05 [c]	2.03 ± 0.04 [ab]	0.006
FE, $g.g^{-1}$		0.4 ± 0.01 [a]	0.33 ± 0.01 [b]	0.34 ± 0.04 [b]	0.32 ± 0.02 [b]	0.34 ± 0.05 [b]	0.036
PER		1.13 ± 0.07 [a]	0.95 ± 0.03 [b]	0.97 ± 0.03 [b]	0.9 ± 0.04 [c]	0.98 ± 0.02 [ab]	0.0381
FCR		1.88 ± 0.06 [c]	2.04 ± 0.44 [b]	2.06 ± 0.07 [ab]	2.12 ± 0.48 [a]	2.05 ± 0.34 [b]	0.019

[abc] Different letters between *T. diversifolia flour levels* denote significant differences (Duncan test, $p < 0.05$)

DAW: Day average weight gain; DAL: Day average length gain; SGR: Specific growth rate; FE: Feed Efficiency; PER: Protein efficiency ratio; FCR: Feed conversion ratio

The length-weight relationship (Figure 1) was adjusted to an exponential equation with all R^2 coefficient of 0.91-0.97, where a tendency to increase weight was shown with the increase in the length of juvenile red tilapia (*O. mossambicus* × *O. niloticus*). Survival (Figure 2) did not present significant differences, although the control (0 %) and 16% *T. diversifolia* flour presented 98.6%, and the rest of the treatments presented 100%.

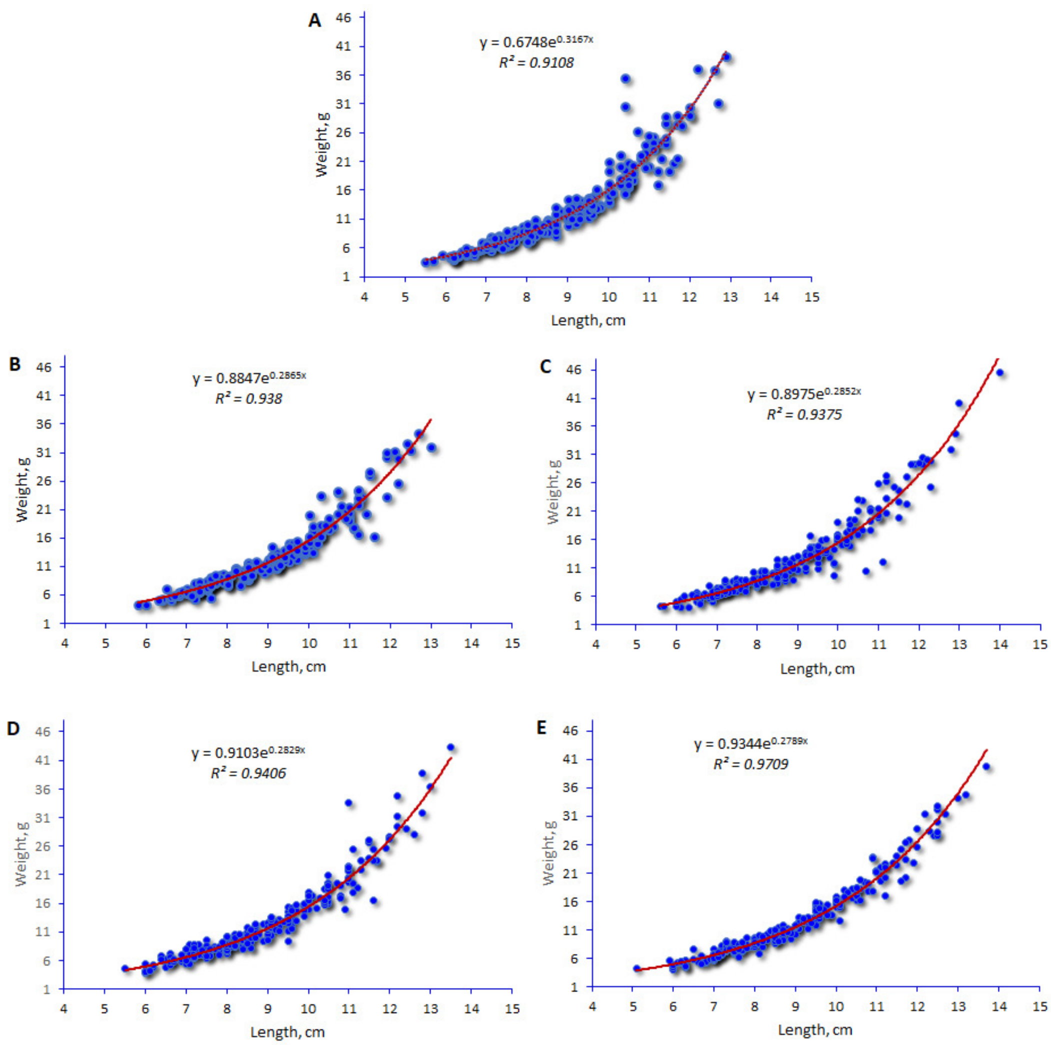

Figure 1. Length-weight relationship of juvenile *O. mossambicus* × *O. niloticus* feed with different levels [A) 0% -Control, B) 4%, C) 8%, D) 12% and 16%] of *T. diversifolia* flour

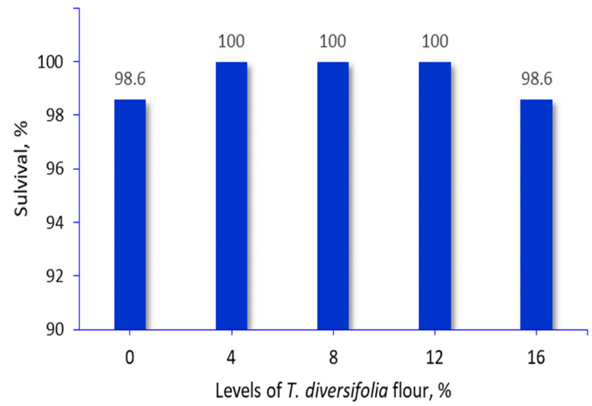

Figure 2. Survival of juvenile *O. mossambicus* × *O. niloticus* feed with different levels of *T. diversifolia* flour

The metabolic biochemistry parameters (Table 5) decreased with the increase of the inclusion levels of *T. diversifolia* flour. The highest values for cholesterol, glucose and triglycerides were for the control treatment (132.50, 139.50 and 120 mg.dL^{-1}, respectively), except for the proteins which were higher for the 4% flour of *T. diversifolia* with 21.55 g.dL^{-1}.

Table 5. Metabolic biochemistry (means ± SE) of juvenile *O. mossambicus* × *O. niloticus* feed with different levels of *T. diversifolia* flour

Biochemistry indicators	*T. diversifolia* flour levels, %					*p*
	0	4	8	12	16	
Protein, g.dL^{-1}	19.70 ± 0.46^{ab}	21.55 ± 0.44^{a}	19.00 ± 0.43^{b}	16.50± 0.38^{c}	19.00± 0.33^{b}	0.0002
Cholesterol, mg.dL^{-1}	132.50 ±1.24^{a}	79.00 ± 1.31^{bc}	81.50± 1.52^{b}	80.70 ± 1.44^{b}	78.50 ± 1.33^{c}	0.032
Glucose, mg.dL^{-1}	139.50 ±4.51^{a}	112.50 ±4.66^{c}	119.50 ± 4.37^{bc}	115.00 ±4.82^{c}	136.00 ± 4.12^{a}	0.0052
Triglycerides, mg.dL^{-1}	120.00 ±2.42^{a}	112.50 ±1.89^{b}	111.00 ± 2.32^{b}	110.00 ±2.05^{b}	82.50 ± 2.51^{c}	0.0001

^{abc} Different letters between *T. diversifolia flour levels* denote significant differences (Duncan test, *p < 0.05)*

Discussion

The chemical composition values reported in *T. diversifolia* flour (Table 3) coincide with those reported by Olmo-Gonzalez *et al.* (2022) for the flour of this plant cut at 70 days of regrowth (22.23% in CP, 2.62% Ca, 0.013% P, 45.7% NDF, 29.8% ADF). Authors stated that in tropical countries there is a great diversity of plants with these characteristics, among them *T. diversifolia*, which can be used in more environmentally friendly environments, with better responses in animal welfare and better feed conversion.

For their part, Rivera *et al.* (2018) found that *T. diversifolia* has low FDA content (12%) and high CP content (>20%), which are outlined as the main nutritional characteristics to be recommended for feeding non-ruminant species. In this sense, there is evidence that species of non-legume plants such as *T. diversifolia* accumulate similar nitrogen in their leaves as legumes, have high levels of phosphorus, high number of roots, good ability to take up the few nutrients from the soil, wide range of adaptation, tolerate conditions of acidity and low fertility in the soil; it can withstand pruning at ground level and has fast growth and low demand for inputs as well as for the management in its cultivation. In addition, information is available on its use in the feeding of non-ruminant species, such as birds in the form of flour (Parra-Ortiz *et al.,* 2019; Cabrera-Nunez *et al.,* 2019). The nutritional potential and phytochemical quality of shrub sunflower for its use as a supplement in pig feed were verified by Scull *et al*. (2022).

The protein values coincide with those reported by Verdecia *et al.* (2018; 2021) who reported that *T. diversifolia* has high protein values of 22.6%. Thus, due to the protein content (21%), its low cell wall content (43.03; 24.40; 6.44, 12.24; 16.72 %; FDN, ADF, ADL, Cel and Hcel, respectively) and the adequate cell content (59.97%), this shrub sunflower forage is a good candidate as raw material for feed due to its excellent ratio of nutrients in its composition.

In studies by Cabanilla-Campos *et al.* (2021), when evaluating the effect of the cutting system (30 to 75 days) on the nutritional value of *T. diversifolia*, the CP ranged between 12 and 24%, NDF between 41 and 47% and ADF between 29 and 35%, which denoted the possibility of considering this species as promising of interest for animal production. While Lodono *et al*. (2019); Navas and Montana (2019), reported similar values in CP, NDF, ADF and ADF (18- 25; 40-64; 17-48 and 6-22%). This shows that the cutting age, selected plant material, management and soil and climatic conditions can directly influence the chemical composition. Authors Guatusmal-Gelpud *et al.* (2020); Arias-Gamboa *et al.* (2023) stated that *T. diversifolia* is a plant that is characterized by excellent quality, by its adequate ratio of nutrients in its foliage and reported values of NDF (43.79 ± 2.96) and ADF (27.81 ± 3.60) respectively. The NDF levels in the investigated plant (45.7%) are higher than those reported by Conrado-Palma *et al*. (2022); Villarreal-Rivas *et al*. (2022), who found values of 35.3%, while for FDA (29.8-30.4%) the results were similar, these differences may be due to the material collected, analytical technique and quality of the reagents used.

Regarding the mineral content, this species has an appreciable amount of phosphorus reaching levels between 0.17 and 0.38% in the leaves. These values are even higher than those found in some legumes used in agroforestry systems that vary between 0.15 and 0.20% phosphorus. The calcium content is around 2.86% in the complete plant at 56 days (Lodono *et al*., 2019). On the other hand, Verdecia *et al.* (2018); Herrera *et al.* (2020); Olmo-González *et al.* (2022) report 2.62, 0.013 and 3.44% of calcium, phosphorus and silica, respectively, results in that denote that the values reached in the current investigation are in the range of studies carried out in different tropical zones.

The results for amino acid (Table 3) coincided with those reported by Fuentes-Martínez *et al.* (2019) and Olmo-Gonzalez *et al.* (2022) which reported low concentrations of essential amino acids, when compared to other plant sources such as *Phaseolus lunatus*, *Vigna unguiculata*, *Cajanus cajan* and *Vigna radiata* with amounts of 42-122 g/kg of protein. These differences are due to the intrinsic characteristics of each species, management, age, part of the plant and geographic area (Miquilena and Higuera-Moros, 2012; Marrugo-Ligardo *et al.,* 2016).

In this regard, Mejias-Diaz *et al.* (2017) obtained similar results, when carrying out a comparative study of *T. diversifolia* with several forage species (*Medicago sativa, Morus alba, Cenchrus clandestinus*) and soybean meal where, they found no differences in amino acid content between forages, although it was lower than soybean meal, where on average the forages presented 19% of protein notably below the 47% of soybean meal. Hence, the nutritional potential depends to a great extent on its protein quality, on the type and quantity of amino acids it contains, which is a determining factor in its nutritional assessment, since protein quality establishes the contributions of nitrogen and essential amino acids for the animal organism. From a nutritional point of view, protein is a macronutrient present in feed. The importance of the protein present in the diet is due to its ability to provide amino acids to attend to the maintenance of body protein and the increase of this during growth, thus, a better amino acid profile will be able to predict the impact of a new food on productivity and edible production (Basyuni and Wati, 2017; Zhou *et al*., 2017; Feng *et al*., 2020).

When including *T. diversifolia* flour in the diet of tilapia juveniles, it was found that the weights and length did not present differences ($p > 0.05$) (Table 4), The main criterion is the similar contribution of nutrients in the diets, which contributed to meet the nutritional requirements according to the NRC (1993, 2011), although the fiber levels in the ration with shrub sunflower flour, gradually increased and up to 16% did not significantly affect the productive indicators mentioned above, reaching a total neutral detergent fiber of the diet of 10.16%, respectively.

If we take into account the previously described acceptable nutrient concentrations found in *T. diversifolia* flour and the current trends in aquaculture worldwide in response to the growing need to reduce the use of conventional meals, plant proteins have been widely adopted as solution (Michael *et al*., 2016; Daniel, 2018). Although there is a need to reduce the use of conventional meals, some are necessary in the diet, such as fishmeal, due to its contribution of essential amino acids (FAO, 2016; Daniel, 2017). Increasing levels of aquaculture farming will continue to demand a simultaneous increase in the availability of high-quality, yet cost-effective dietary protein sources, where vegetable flour such as *T diversifolia* can be considered as an option for diets in omnivorous species such as tilapia (El Hack *et al.*, 2022).

Chirinos-Ochoa *et al.* (2022) and Pineda-Santis *et al.* (2023) when using alternative vegetable ingredients such as *T. diversifolia* flour in diet for juveniles of *P. brachypomus* and *O. niloticus*, they found a positive effect on food efficiency and growth rate of organisms when fed with 15 and 20% of shrub sunflower*,* respectively. In this sense, *T. diversifolia* contributes to correct balance of nutrients in diets, supplying the nutritional needs in juvenile stages of fish species. Botello *et al.* (2022) when including up to 10% palm kernel flour (*Elaeis guineensis*) in the diet of tilapia fingerlings (*O. niloticus*), did not observe significant differences ($p > 0.05$) in the nutrient digestibility indicators, growth and feed efficiency. The previous authors mentioned that up to 10% inclusion of the palm kernel in the diet, the contribution of the neutral detergent fiber was 10.19%, that higher levels caused an affectation in the productive indicators, similar results to the current investigation. It is known that in fish, the contribution of insoluble fiber in the diet is essential for the proper functioning of the digestive tract, intestinal health, participation in the immune response, reduction of intestinal pH, adjuvant in the production of volatile fatty acids, among others (NRC, 2011; Maas *et al.,* 2020).

The rest of the growth indicators presented significant differences in weight gain, DAW, SGR, FE and PER, these indicators did not differ with 16% of inclusion of *T. diversifolia* flour. While, for length gain, DAL and FCR were better for 12 and 16% of the flour. This response to the effect of unconventional raw materials in red tilapia juveniles is similar to that reported by Bowyer *et al*. (2020), when using lupine flours varieties in more than 15%, and Ghosal *et al*. (2021) when using different extracts from different medicinal plants found that the variations found in daily weight gain, protein efficient ratio; feed conversion ratio and weight gain, associating this behavior with the content of secondary metabolites and their beneficial effects as antioxidants and phytobiotics, which suggest that the presence of phenols and steroidal saponins are responsible for growth induction of fish in the study. Polysaccharides in medicinal herbal extracts have been reported to possess prebiotic properties, and thus improve gastrointestinal morphology or digestive systems, and increase nutrient digestibility, absorption and assimilation capacity of fish (Gabriel, 2019). Herbs have also been observed to positively influence eating patterns, secretion of digestive fluids and total feed intake. In addition, *T. diversifolia* has several compounds, such as α-pinene, β-pinene, germacrene D, and β-caryophyllene, these natural compounds can have diverse effects against different bacteria, depending on their biochemical characteristics (Nafiqoh *et al.,* 2020).

While, Khieokhajonkhet *et al*. (2021), reported increases in weight gain, SGR, FCR and PER by including up to 110 g of sacha inchi (*Plukenetia volubilis*) flour in red tilapia hybrid diets, found that inclusions of up to 420 g presented a decrease in indicators of the growth evaluated with the increase of the levels of the *Sacha inchi* flour. Regarding the weight-length relationship (Figure 1), exponential equations were adjusted (R^2 0.91-0.97), when analyzing this indicator Ghosal *et al*. (2021) found in red tilapia juveniles when using medicinal plant extracts in all the treatment groups showed a strong ($R^2 = 0.99$) correlation between length and weight in the power equation curve. While Pineda-Santis *et al*. (2023) when supplementing red tilapia fingerlings with *T. diversifolia* (15-20%) found values of linear correlation weight-height with 0.95 for 15% and 0.88 for 20%, reported that this behavior suggests a change in the transition between the two stages, from reversion, an open and random environment, to another from pre-levant with controlled variables, results based on the maintenance of culture conditions and appropriate sanitary management, accompanied by a balanced diet during the productive stages.

The forage of plant species such as *T. diversifolia* there is the presence of phytoconstituents, such as saponins, alkaloids and tannins have been reported to act as feed intake deterrents and digestive enzyme inhibitors in fish (Glencross *et al*., 2020). However, these antinutrients have varying effects on fish survival and growth depending on several factors. Tilapia species have been speculated to be more tolerant than the carp to the presence of antinutrients in their diets. Ghosal *et al*. (2021) when using extracts of medicinal plants found significantly higher ($p < 0.05$) growth values compared with control and high survival percentage (>90%) in all treatment groups indicated that the inclusion of plant extracts in diet might have no adverse effect on fish food consumption and survival, in the present study although they were not determined, there are reports in the literature (Fuentes-Martínez *et al*., 2019; Olmo *et al*., 2022) the secondary metabolites present in the foliage are in low proportions aspects that are not influenced survival.

In metabolic biochemical indicators, there were a decrease in glucose, triglycerides and cholesterol, in comparison with control treatment, while proteins increased up to 4% inclusion of *T. diversifolia* flour and then decreased. Therefore, it can be assumed that *T. diversifolia* contributed to the regulation of the metabolism of fats and fatty acids, and this could be attributed to the effect it exerts on the efficient use and metabolism of nutrients of diet (Ferreira *et al.,* 2011). The production of plasma glucose often indicates energy stress in fish (Li *et al.,* 2018; Khieokhajonkh *et al*., 2021). Therefore, our results suggest that the energy levels of the fish were within the recommended amounts for tilapia. Low plasma glucose levels to the extent that inclusion levels increase may indicate low stress and better metabolic efficiency, without causing liver damage or toxicity in tilapia (Li *et al.,* 2018; Méndez-Martínez *et al*., 2021b).

Triglycerides, cholesterol and glucose are in the range of studies carried out by Metwally (2009) when evaluating the effect of *Allium sativum* in species *O. niloticus*. Mastoi *et al*. (2011) when evaluating the effect of algae in diet of red tilapia fingerlings (*O. mossambicus x O. niloticus*), metabolic indicators were also improved. While, Khieokhajonkhet *et al*. (2021) in the metabolic biochemical characteristics of red hybrid tilapia fed with graded levels of *Sacha inchi* flour only the total proteins were higher in the alternative sacha inchi diets, cholesterol and triglycerides decreased linearly with increasing levels. As can be seen (Table 4), the values obtained coincide with the reports in the literature for tilapia species and hybrids (Hrubec *et al*., 2000; Metwally, 2009; Méndez-Martínez *et al*., 2021b). Blood biochemical indices have been used as an indicator for evaluating the metabolic response of fish to various factors such as season, temperature, and nutritional status. Reduction of triglycerides and cholesterol is associated with polyphenol contents, especially flavonoids that in low concentrations they have an antioxidant effect, regulate the metabolism of fats, fatty acids and improves the absorption of nutrients (Liland *et al.,* 2013; Khieokhajonkhet *et al*., 2021).

Food of vegetable origin such as *T. diversifolia* contain substances called phytosterols with their hypocholesterolemic effect, since they contribute to reduction of total cholesterol and that linked to low-density lipoproteins (Mabou *et al*., 2018; Ejelonu *et al*., 2017; Urbizo-Reyes *et al.,* 2021; Verdecia *et al*., 2021). In *O. niloticus*, it was found that organisms fed with flour of vegetable origin presented lower levels of total cholesterol, low-density lipoprotein (LDL), very low-density lipoprotein (VLDL) and high-density lipoproteins (HDL) compared to treatments with fish meal (Ferreira *et al.,* 2011; García-Caballero *et al.,* 2022).

Structurally, phytosterols are similar to cholesterol, causing competition for solubilization of mixed micelles at the intestinal level, which contributes to reduce the esterification of cholesterol in enterocyte cells by inhibiting the enzyme acyl-CoA: cholesterol acyltransferase (ACAT), and this prevents than cholesterol and triglycerides absorbed by enterocytes from being incorporated into the chylomicrons, allowing cholesterol that is not esterified to be sent back to the intestine (Urbizo-Reyes *et al.,* 2021). However, in studies carried out with atlantic salmon (*Salmo salar*), they found when replacing fishmeal with vegetable protein at levels of 80%, and replacing fish oil with vegetable oils at levels of 35% and 70%, the hypocholesterolemic properties were not activated in *S. salar* (García-Caballero *et al.,* 2022). This could be because, compared to freshwater fish such as tilapia, salmon is not an herbivorous animal, and its fatty acid requirements are different.

Vegetable flours such as shrub sunflower, contain various nutrients, and compounds such as: polyphenols, soluble fibers, alpha-galactosides and isoflavones, which give it functional food properties with a hypocholesterolemic character (Mabou *et al*., 2018; Verdecia *et al.,* 2020b; Akeumbiwo *et al*., 2023). *T. diversifolia* contributes from the diet to the growth development and health of fish, since favorably influence the digestion and metabolism of organisms. More research is required on using of *T. diversifolia* in diet of tilapia, and prepared by different procedures.

Conclusions

T. diversifolia flour presents an adequate balance of nutrients for its chemical composition and amino acids, without causing negative effects on the productive parameters, weight-length relationship, and survival, which may include up to 16%. While, for the biochemical indicators in fish, protein levels increased up to 4% inclusion, while glucose, triglycerides and cholesterol decreased when *T. diversifolia* was included in diets.

Authors' Contributions

The authors are responsible for any claims arising from the content of this article and will be held liable for any damages. Y.M.M.: Conceptualized the research, evaluated the data and produced the manuscript. R.I.N.N. and Y.G.T.N.: Collected the date of researched. C.A., E.C.J., A.B.L. and D.V.A.: Review and editing original drafts. All authors read and approved the final manuscript.

Ethical approval (for researches involving animals or humans)

This study was carried out without sacrificing organisms unnecessarily, to reduce and perfect the use of animals used for scientific purposes. We follow the principles of the EU Directive 2010/63/EU for experiments on animals. The study was carried out strictly following the Standard Operating Procedures (SOP) of the Bioethics Code and the Animal Welfare Regulations, approved by OCAS-UTEQ (Universidad Técnica Estatal de Quevedo) in ordinary section No. 02, developed on March 2, 2017.

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Conflict of Interests

The authors declare that there are no conflicts of interest related to this article.

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