

Effect of cannabis oil on growth performance, haematology and metabolism of Nile Tilapia *Oreochromis niloticus*

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Abstract

Cannabis sativa is a plant that produces an oil with psychoactive and stress reduction effects and thus illegal in many nations. Lately, the beneficial properties of the plant extract are becoming better understood and perceptions are changing. As the aquaculture industry matures from a primitive extensive pond system to an industrialized intensive system, fish stress and disease incidence are increasing, with negative economic results. A nutritional ingredient that could reduce stress and disease incidence in aquacultured fish would thus be opportune. In the present work we investigated whether ether extracted cannabis oil would relieve stress, improve growth and feed conversion, and/or improve haematological indicators of disease resistance. Three diets were made to contain either soy oil, industrial hemp oil or cannabis oil and offered to tilapia for 8 weeks. At termination, survival, growth, feed conversion and blood parameters were assessed. Fish were returned to their tanks, offered the same feeds as during the experiment and respiration assessed. Cannabis extract was found to increase metabolism and thus increase feed conversion. On the other hand, cannabis had no effect on blood cell counts, total plasma protein, haematocrit or lysozyme activity. Results thus suggest that cannabis does not improve immune response of tilapia or body composition but does reduce growth rate by increasing metabolic rate.

KEYWORDS

cannabis, haematology, metabolism, respirometry, THC, tilapia

1 | INTRODUCTION

Aquaculture is one of the fastest-growing sectors of the world food economy and has greatly contributed to global fish production (FAO 2016). With continued growth of the industry, we observe an increase in intensive aquaculture and fish over-crowdedness, which tend to increase stress by increasing intraspecific interactions and by causing deterioration in water quality. This in turn tends to increase fish stress, increase feed conversion ratio, increase disease incidence and reduce economic benefits to farmers. Any feed additive that could mitigate the negative effects of intensification would be welcome.

Indian hemp, marijuana, pot, hashish, *Cannabis sativa*, is an annual flowering plant species belonging to the family Cannabaceae, originating from Central Asia (Adams & Martin, 1996; Zuardi, 2006).

Chemicals produced by the plant have a variety of beneficial uses for little understood ailments including chronic pain, spasticity, seizure disorders, cancer, etc. (Adams & Martin, 1996; Russo, 2007). The plant was used as early as 10,000 BC (Abel, 1979) for various uses including its seed (Deferne & Pate, 1996), and its oil content (Oomah, Busson, Godfrey & Drover, 2002; Werf, Mathussen & Haverkort, 1996) which is often used for medicinal purposes.

Cannabis sativa comprises more than 400 chemical compounds, more than 60 of which are terpeno-chemical compounds known as cannabinoids, found in a resin secreted by the flowering tops and leaves (Adams & Martin, 1996; Atakan, 2012). The most notable of these cannabinoids is the Δ^9 -tetrahydrocannabinol (THC) which is the primary psychoactive constituent of the plant (Adams & Martin, 1996).

A considerable amount of research has been performed on the effect of THC on food intake and weight gain in both animal and human subjects (Le Foll, Trigo, Sharkey & Le Strat, 2013). Investigations into the acute effects of THC have documented no cases of fatality so far, an observation corroborated by the fact that THC has a very low toxicity. Extremely high doses of THC are needed to cause 50% mortality in rodents (Hall & Solowij, 1998), and as such it can be considered safe in animal feeds. In humans, increased appetite and weight gain were reported following acute and chronic exposure to marijuana (Greenberg, Kuehne, Mendelson & Bernstein, 1976; Williams, Himmelsbach, Wikler, Ruble & Lloyd, 1946). In a study intended to hinder weight loss in cancer patients, Regelson et al. (1976) reported an increase in food ingestion and a subsequent weight gain in patients administered with THC. In animals, hyperphagic effects were also observed following acute THC administration, but contradicting results were reported following chronic THC administration. THC has also been shown to relax some vertebrates. Santos, Sampaio, Fernandes and Carlini (1966) showed that acute administration of cannabis extract suppressed aggressiveness in mice. If THC exerts a sedative effect on aquacultured fish while helping in weight gain, then it could be very useful in intensive and super intensive aquaculture where stress could be a major concern.

Cannabinoids also appear to affect vertebrate immunity. The THC component of marijuana could have an immunosuppressive effect, impairing cell-mediated and humoral immunity by directly altering the functions of various immunocytes such as lymphocytes, macrophages, and natural killer cells (Cabral, 2001; Klein, Friedman & Specter, 1998), thus decreasing resistance to infections. Nevertheless, unsubstantiated rumours abound that marijuana is good for human immunity. The effects of THC on fish immune response have not been reported and need to be elucidated.

Nile tilapia *Oreochromis niloticus* is the second most widely aquacultured fish in the world after carps (Abdelhadi, 2011; Azaza, Dhraief & Kraiem, 2008). It is increasingly being aquacultured at stress inducing high densities in tanks and cages. The present study was designed to test whether cannabis extract could be incorporated in the diet of Nile Tilapia, and whether the potential sedative effect of this psychoactive component would exert a positive impact on the overall health and performance of the fish. Accordingly, the effects of THC on growth, haematological and biochemical parameters and oxygen consumption rate of juvenile *O. niloticus* were investigated.

2 | MATERIALS AND METHODS

2.1 | Fish acquisition and holding

All studies were performed at the aquaculture research laboratory of the American University of Beirut (AUB), Lebanon. Nile tilapia *Oreochromis niloticus* broodstock were maintained in outdoor 1 m³ circular holding tanks connected to a biological filter and a sump, and were offered a 40% crude protein, 8% lipid commercial feed (Rangen Inc., Buhl, Idaho, USA) twice daily to apparent satiation. Juvenile

tilapia were collected from these tanks, size-sorted by hand and transferred to an indoor environmentally controlled recirculating system. They were offered the commercial diet twice daily to apparent satiation and were allowed to acclimate for 2 weeks prior to the start of the feeding experiment.

2.2 | Experimental diet preparation

Preliminary data showed that although THC in the diet affected fish physiology, the actual quantity of THC in the feed did not seem to have a significant effect. Accordingly, only one diet containing cannabis extract was prepared in the present study to assess the effect of THC on Nile tilapia. Three iso-nitrogenous and iso-energetic (14 MJ/Kg⁻¹) diets were formulated (Diets 1, 2 and 3) (Table 1). The diets differed in oil type with diet 1 being the control and containing soy oil, diet 2 industrial hemp oil (no THC) produced and supplied by Dr. M. Farran at AUB, and diet 3 a 1:1 mix of soy oil and ether extracted cannabis oil which contains the psychoactive component THC. The diets contained 41% crude protein and 5.5% crude lipid. The values 33.5, 16.7, and 16.7 MJ/Kg⁻¹ were used to calculate digestible energy values for lipid, protein, and nitrogen-free extract, respectively (Bureau, Kaushik & Cho, 2002). Diets were prepared by mixing the ingredients with hot water using a dough mixer and then extruding in a meat grinder (2.5 mm die). Pellets were dried in a forced-air oven at 40°C to a moisture content of 8% and stored at -20°C.

2.3 | Growth experiment

The research setup consisted of nine 52-L (58 × 30 × 30 cm) indoor glass aquaria connected to a biological filter and a settling tank. Water in the system was aerated using a regenerative blower and submerged air diffusers, and temperature was maintained at 26°C. Salinity, temperature and dissolved oxygen were measured daily using a YSI Model 85 oxygen meter (Yellow Springs Inc., OH, USA). Total Ammonia Nitrogen and Nitrite Nitrogen were measured weekly using a HACH Aquaculture Test Kit, Model FF-3. pH was measured daily using a commercial hand-held pH meter. Photoperiod was maintained at 14:10 hr (light: dark).

At the start of the feeding experiment, 34 fish were randomly taken for individual weight and length measurements to calculate initial Fulton's condition index (K) (Table 2). Groups of eleven tilapia juveniles (10.81 ± 0.04 g; mean ± SE) were then weighed and stocked into each of the nine aquaria. Each of the diets was then randomly assigned to three tanks. Fish were offered prepared and commercial diets three times daily for 8 weeks divided as follows: prepared diet at 1% total average body weight (BW) at 7:00 a.m., and commercial feed at 4% of BW of the largest fish, divided into two equal rations at 12:00 p.m. and at 7:00 p.m. Fish were group-weighed biweekly after a day of fasting and ration adjusted accordingly. Leftover feed was removed within a half-hour of feeding. Dead fish were removed from tanks upon discovery.

TABLE 1 Ingredients and chemical composition of the three diets offered to juvenile *O. niloticus* over 8 weeks. Ingredients are reported on a dry weight basis

	Diet 1 Control	Diet 2 Industrial hemp (g/Kg diet)	Diet 3 Cannabis extract
Menhaden Fishmeal ^a	300.0	300.0	300.0
Soybean meal solvent extracted ^b	418.0	418.0	418.0
Soy oil	21.3	0.0	10.7
Industrial hemp ^c	0.0	21.3	0.0
Cannabis extract ^d	0.0	0.0	10.7
Wheat flour	224.7	224.7	224.7
Vitamin & Mineral premix ^e	20.0	20.0	20.0
Choline chloride	5.0	5.0	5.0
Stay C 250 ^f	1.0	1.0	1.0
Gelatin ^g	10.0	10.0	10.0
Chemical composition ^h (g 100 g ⁻¹ in dry matter)			
Crude protein	40.97	40.97	40.97
Crude lipid	5.50	5.51	5.51
Digestible energy	404.48	404.52	404.52
Phosphorous	1.23	1.23	1.23

^aFF Skagen Denmark. Havneavgvej 12.9990 Skagen.

^bDe-hulled solvent extracted soybean meal, Southern Sates Cooperative Inc., Richmond VA, USA.

^cIndustrial Hemp planted under license by Dr. M. Farran and extracted by cold press of seeds.

^dEther extracted from police confiscated cannabis.

^eThe vitamin and mineral premix provided the following per kg of experimental diet: vitamin A retinyl acetate 1 million IU, vitamin D3 cholecalciferol 0.1 million IU, vitamin E alpha-tocoph acet 7 g, vitamin K 0.5 g, folic acid niacin 0.1 g, niacin 4 g, calcium pantothenate 2.5 g, riboflavin (B2) 0.6 g, vitamin B12 0.001 g, thiamine (B1 nitrate) 0.5 g, pyridoxine (B6 HCl) 0.5 g, biotin 0.0125 g, vitamin C (ascorbic acid) 0.25 g, inositol 5 g, selenium (as sodium selenite) 0.0045 g, iodine (as calcium iodate) 0.25 g, iron (as sulphate monohydrate) 2 g, zinc (as oxide) 5 g, copper (as sulphate pentahydrate) 0.25 g, manganese (as sulphate monohydrate) 3.5 g, chlorine chloride 75, phosphorus (as monocalcium phosphate) 2.5, sodium chloride (salt) 225 g, and cellulose 75 g. Calcium carbonate carrier to balance.

^f250 mg/kg active vit C supplied by Stay C[®], (L-ascorbyl-2-polyphosphate 25% Active C), Roche Vitamins Inc., Parsippany, New Jersey, USA.

^gHimedia laboratories Pvt. Ltd., 23, Vadhani Ind. Est., LBS Marg, Mumbai, India.

^hBased on a calculated value.

2.4 | Sample collection and hematology

All fish were fasted for 24 hr prior to harvest. At experiment termination, fish were anaesthetized with a solution of Tricaine-S (Tricaine Methanesulfonate, MS 222, Western Chemical Inc., Ferndale, WA, USA), group-weighed and their individual weights and lengths measured to calculate final condition indices. At least three fish from each tank were randomly taken and used for hematology. If individual fish were too small to collect sufficient blood, an extra fish was

TABLE 2 Survival (S), initial body weight (IBW), final body weight (FBW), total length (TL) at harvest, Fulton's condition index (K), and feed conversion ratio (FCR) of juvenile *Oreochromis niloticus* offered various diets

Diet	S (%)	IBW ± SE (g)	FBW (g)	TL (cm)	K	FCR
Control	100 ^a	10.89 ± 0.19	49.96 ^a	13.80 ^a	1.88 ^a	1.8 ^a
IndHemp	100 ^a	10.76 ± 0.10	43.19 ^b	13.32 ^b	1.81 ^a	2.1 ^b
THC	100 ^a	10.79 ± 0.08	40.26 ^b	13.02 ^b	1.81 ^a	2.3 ^b
PSE			1.61	0.16	0.04	0.08

Values with different superscripts in the same column are significantly different from each other ($p < .05$).

PSE, Pooled Standard Error.

taken from the same tank for blood collection and samples were pooled. Three blood samples from each tank were ultimately tested. Blood samples were collected from the caudal arch and hematological and biochemical parameters were assessed within 24 hr of sample collection. Total red blood cells (RBC) and white blood cells (WBC) were counted using a modified Neubauer hemocytometer after staining with Natt-Herrick's vital diluent/stain (Natt & Herrick, 1952). For differential WBC counts, blood smears were stained with Wright and Giemsa and a total of 800 white blood cells per slide were counted as described by Ellis (1976) and Ainsworth (1992). Each type of blood cell was expressed as percentage of the total number of cells counted. Hematocrit (Hct) expressed as %, was determined using the indirect method for haematocrit measurement (Klontz, 1994). Total plasma protein (TPP) (g/dl) was quantified using a veterinary refractometer (RHC-200ATC, Westover Scientific, Inc., Mill Creek, WA, USA). Lysozyme activity was assayed using the turbidimetric method (Parry, Chandan & Shahani, 1965).

2.5 | Proximate analysis

The fish used for haematology were also used for analysis of proximate composition. Whole fish were completely macerated in a food processor, weighed, dried in a forced air oven at 95°C until constant weight and moisture content calculated. Dried samples were then ground with a mortar and pestle and stored at -20°C for later analysis. Lipid content was estimated using solvent extraction in a 9:1 mixture of petroleum ether/diethyl ether in an ANKOM XT20 Fat Analyzer and ANKOMXT Recovery System, (ANKOM Technology Corporation, Macedon, NY, USA). Protein content was determined using a modified Kjeldahl method (HACH Digesdahl). Nitrogen content values were multiplied by a correction factor (1.0739) based on glycine as a standard (5.665% N, 35.4% CP), then by 6.25 to estimate protein content (Alavanese & Orto, 1963). To determine ash content, 0.5 g of ground and dried sample were combusted in a muffle furnace at 500°C for 8 hr and final weight recorded.

2.6 | Respirometry

A Strathkelvin 929 6-Channel Dissolved Oxygen System was used for respiration measurements. A flow-through respirometry setup as

described by Babikian, Nasser and Saoud (2017) was used. The apparatus consisted of a glass water container (80 × 80 × 80 cm) equipped with a 4,000 ml Erlenmeyer flask, a submersible electric heater to maintain water temperature at 27°C, a thermometer to monitor temperature, and a submersible water pump to maintain a constant water level in the Erlenmeyer flask. Before each experimental run, the container was filled with freshwater that was chlorinated for 24 hr then de-chlorinated with sodium thiosulfate. Two air diffuser stones connected to an air pump were placed in the container to ensure continuous aeration and mixing. Water temperature was maintained at 26°C and pH between 8.0 and 8.2 in all experiments performed. The Strathkelvin RC400 respiration chambers (730 ml, 102 mm in diameter) were equipped with a magnetic stir bar under a perforated false bottom to slowly mix water in the chambers. All water tubes and respiration chambers were washed with 10% HCl prior to the start of each experimental run in order to eliminate bacterial growth that might affect oxygen uptake measurements.

Fish were fasted for 24 hr prior to the start of each experiment. Each experimental run consisted of three respirometry chambers operating simultaneously, with one chamber kept empty and used to calculate a correction factor for O₂ uptake. After setting the respirometer and calibrating the electrodes, fish were placed in the corresponding chambers and water was allowed to flow through the chambers. Flow rate was regulated to ensure that dissolved oxygen inside each chamber was kept above 4 mg/L. Fish were allowed to acclimate to the respirometry chamber for 6–7 hr until respiration rate was stable for at least one hour. Oxygen concentration in inflowing and outflowing water was recorded and flow rate measured. All chambers were covered with black plastic sheets to avoid any disturbance that might stress the fish.

2.7 | Oxygen measurements

At least six respirometry runs were performed for each of the various diets with one fish per chamber. Average total weight of fish in each chamber was 39.7 g ± 2.06 (mean ± SE). Mass-specific oxygen consumption rate measurements were obtained using the 929 Strathkelvin software. The calculations were based on the formula used by Saoud and Anderson (2004):

$$VO_2 = FR \cdot ([O_2]_i - [O_2]_f) / BW$$

where VO₂ is the oxygen consumption rate or respiration rate (mg O₂ g⁻¹ hr⁻¹), FR is the water flow rate (L/h), [O₂]_i is the oxygen content in inflowing water (mg O₂ L⁻¹), [O₂]_f is the final oxygen content of water in chamber (mg O₂ L⁻¹), and BW is the body weight of experimental animal in grams. Average oxygen consumption rate of all experimental runs per treatment was calculated as an estimation of standard metabolic rate of juvenile *O. niloticus*.

2.8 | Statistical analysis

Survival per tank was calculated as S (%) = (number of fish at the end of experiment/number of fish at the start of the experiment) × 100. Fulton's condition index was calculated as

$K = 10^5 \times W/L^3$ where W is weight (g) and L is length (mm). Feed Conversion Ratio was calculated as FCR = [weight of feed offered (g)]/[weight gain of fish (g)].

Statistical analysis of the data was performed using SAS (V.9.2, SAS Institute Inc., Cary, North Carolina, USA). All data were expressed as mean values of all replicates and compared using one-way ANOVA. Significant differences among treatment means were analysed using Student Newman-Keuls mean separation test. Differences were considered significant at $p < .05$.

3 | RESULTS

3.1 | Growth experiment

Juvenile *O. niloticus* in all treatments survived and grew in weight and length, with significant differences in growth among treatments (Table 2, Figure 1). Average weight of fish offered diet 1 (49.96 ± 1.88 g; mean ± SE) was significantly greater than weight of fish offered diets 2 and 3, with diet 3 yielding the lowest average fish body weight among the treatments (40.26 ± 1.08 g). No significant differences were observed in final body weights between diets 2 and 3. Similarly, total length of fish offered diet 1 (13.80 ± 0.19 cm) was significantly greater than length of fish offered diets 2 and 3, with diet 3 yielding the smallest fish body length (13.02 ± 0.11 cm). No significant differences were observed in total body lengths between diets 2 and 3 (Table 2).

Fulton's condition indices (K) did not vary significantly among the treatments, but was greatest in fish offered diet 1 (1.88 ± 0.06). Significant differences were observed in feed conversion ratio (FCR) among the treatments. Diet 1 yielded the best FCR among treatments with a value of 1.8 ± 0.06, whereas diet 3 gave an FCR of 2.3 ± 0.09. No significant differences were observed in FCR among diets 2 and 3 (Table 2).

3.2 | Hematological and biochemical parameters

Total RBC (cells/μl) and WBC (cells/mm³) counts of juvenile *O. niloticus* did not vary significantly among treatments. Differential blood counts of fish did not differ significantly among treatments; there

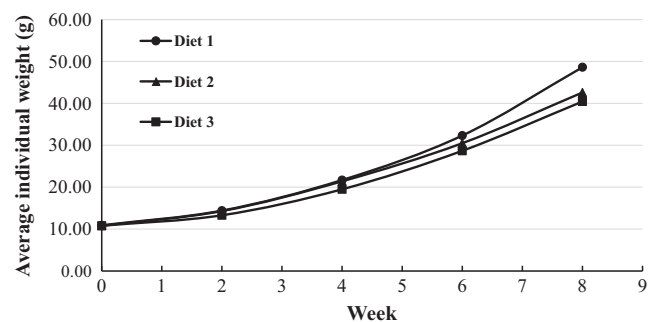


FIGURE 1 Growth in average individual body weight (g) over 8 weeks of juvenile *O. niloticus*, offered various diets

TABLE 3 Total counts of red blood cells (RBC), total white blood cells (WBC), differential blood cell counts of thrombocytes, lymphocytes, monocytes and neutrophils in juvenile *Oreochromis niloticus* offered various diets

Treatment	Parameter	RBC (*10 ⁶ cells μl ⁻¹)	WBC (*10 ⁴ cells mm ³ ⁻¹)	Thrombocytes (%)	Lymphocytes (%)	Monocytes (%)	Neutrophils (%)
Control	Mean ± SD	2.29 ^a ± 0.46	9.22 ^a ± 4.68	89.22 ^a ± 5.91	7.00 ^a ± 5.20	1.44 ^a ± 1.01	2.67 ^a ± 2.00
	Range	1.65–3.03	4.13–16.8	81–97	1–15	0–3	1–6
	N	9	9	9	9	9	9
Industrial hemp	Mean ± SD	2.20 ^a ± 0.58	8.31 ^a ± 2.90	91.67 ^a ± 3.14	6.17 ^a ± 2.79	1.17 ^a ± 0.41	1.33 ^a ± 0.82
	Range	1.42–3.15	3.73–11.6	88–96	3–10	1–2	0–2
	N	9	9	6	6	6	6
Cannabis extract	Mean ± SD	2.35 ^a ± 0.66	6.66 ^a ± 2.23	90.71 ^a ± 3.50	6.14 ^a ± 2.61	1.29 ^a ± 0.76	2.00 ^a ± 1.00
	Range	1.46–3.78	4.5–10.6	84–95	3–11	0–2	1–4
	N	9	9	7	7	7	7
	PSE	0.19	1.14	1.53	1.31	0.27	0.49

Values for eosinophils and basophils are not shown as none were observed. PSE: Pooled Standard Error.

were no significant differences in proportions of thrombocytes, lymphocytes, monocytes and neutrophils among the treatments. Basophils and eosinophils were not observed in fish from all treatments (Table 3). No significant differences were observed in haematocrit (Hct), total plasma protein (TPP) and lysozyme activity values among tilapia in the various treatments (Table 4).

3.3 | Proximate analysis

Whole body proximate compositions as a proportion of dry weight of fish are presented in Table 5. There were no significant differences in moisture, lipid content, protein content and body ash among fish in the various treatments.

TABLE 4 Hematocrit (Hct), total plasma protein (TPP) and lysozyme activity of juvenile *Oreochromis niloticus* offered various diets

Diet	Hct (%)	TPP (g/dl)	Lysozyme (μg/ml)
Control	23.44 ^a	5.57 ^a	3.05 ^a
IndHemp	22.00 ^a	5.29 ^a	3.51 ^a
THC	23.67 ^a	5.68 ^a	3.69 ^a
PSE	1.16	0.19	0.36

Values with different superscripts in the same row are significantly different based on SNK mean separation test ($p < .05$). PSE, Pooled Standard Error.

TABLE 5 Moisture, protein, lipid and ash content as percent of dry matter of juvenile *Oreochromis niloticus* offered various diets

Diet	Moisture (%)	Protein (%)	Lipid (%)	Ash (%)
Control	68.40 ^a	48.64 ^a	29.52 ^a	12.18 ^a
IndHemp	69.43 ^a	49.45 ^a	27.24 ^a	12.56 ^a
THC	69.06 ^a	51.73 ^a	28.63 ^a	12.61 ^a
PSE	0.40	1.99	0.83	0.26

Values with different superscripts in the same column are significantly different based on SNK mean separation test ($p < .05$). PSE, Pooled Standard Error.

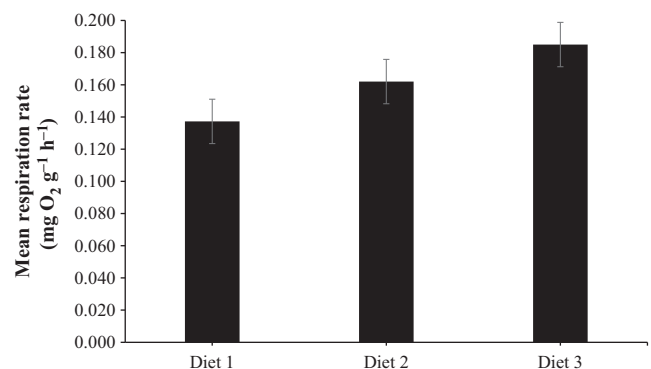
3.4 | Respirometry

Oxygen consumption rate of juvenile tilapia did not vary significantly among fish offered industrial hemp and those offered cannabis extract, with rates of 0.162 ± 0.01 and 0.185 ± 0.01 mg O₂ g⁻¹ hr⁻¹ (mean ± SE), respectively. However, oxygen consumption rate of fish offered cannabis was significantly greater than that of fish offered the control diet (0.137 ± 0.01 mg O₂ g⁻¹ hr⁻¹) (Table 6; Figure 2).

TABLE 6 Mean respiration rate of *Oreochromis niloticus* juveniles offered three various diets

Diet	N	Mean respiration rate (mg O ₂ g ⁻¹ hr ⁻¹)
1	6	0.137 ^a
2	8	0.162 ^{ab}
3	8	0.185 ^b
PSE		0.008

Values with different superscripts in the same column are significantly different from each other. PSE, Pooled Standard Error.

**FIGURE 2** Mean respiration rates (mg O₂ g⁻¹ hr⁻¹) of juvenile Nile Tilapia, *O. niloticus*, offered various diets, at 26°C

4 | DISCUSSION

4.1 | Effect of cannabis on growth and metabolism

Results of the present study suggest that chronic consumption of THC does not affect survival of tilapia but negatively affects metabolism, growth rate and feed conversion. The hemp extract had no observable effects on blood parameters, proximate composition or condition index.

The effects of marijuana seem to vary depending on whether application is acute or chronic. Whilst human studies have shown that acute and chronic exposure to marijuana leads to increased appetite and weight gain (Greenberg et al., 1976; Williams et al., 1946), contrasting effects were observed in laboratory animals. Hyperphagic effects and growth were observed in rats, mice and dogs following intermittent THC administration whereas weight loss was reported in the same animals subjected to chronic THC treatment (Le Foll et al., 2013). In the present study, tilapia were offered equal amounts of the various experimental diets daily for 2 months and results showed that dietary THC caused growth reduction in the fish as compared to fish not offered THC in their diet. Although such results corroborate studies reporting a decrease in growth rate as a result of chronic THC exposure performed on rats (National Toxicology Program, 1996), they give no clue on effects of acute administration of the drug.

The effect of cannabis on metabolism was studied by measuring oxygen consumption rates of fish offered the various diets. Generally, studies have shown that THC exerts a sedative effect on organisms. Gonzalez, Matsudo and Carlini (1971) showed that cannabis extract and THC acted as strong suppressors of aggressive behavior in Siamese fighting fish *Betta splendens*, although after prolonged administration (8–10 exposures) accustomization to the drug was observed. In the present study, we hypothesized that THC might have a sedative effect on tilapia, relieving stress which might in turn increase growth efficiency. However, our results did not validate the hypothesis as *O. niloticus* offered cannabis extract exhibited faster metabolism than those offered normal diet. Fish offered cannabis extract showed an increase in oxygen consumption rate which was translated into reduced growth and a high FCR, as opposed to fish that were not offered THC in their diet and appeared to allocate more energy for growth rather than for metabolism. The fact that we were not able to observe a sedative effect in tilapia could be attributed to the fact that fish were offered cannabis extract for 2 weeks i.e. chronic administration of cannabis, and might have developed tolerance to the drug, as was the case with the Siamese fighting fish.

4.2 | Effect of Cannabis on haematological and biochemical parameters

No significant differences were observed in any of the studied haematological parameters among treatments although total WBC counts, lymphocyte, monocyte and neutrophil counts were marginally lower in fish offered THC than those offered the control diet. Accordingly, THC in cannabis extract does not seem to perpetrate

any conspicuous haematological effect on fish. Oseni, Togun and Taiwo (2006) reported a similar lack of effect of marijuana uptake on haematological values in humans, with total leukocyte and lymphocyte counts being marginally lower in marijuana smokers than non-smokers. Nevertheless, Oseni et al. (2006) warn that the marginal decrease in lymphocyte counts in most cannabis smokers, attributed to the fact that cannabinoids inhibit T and B lymphocytes thus reducing humoral and cell mediated immune responses, can predispose organisms to infections (Hall & Solowij, 1998). This hypothesis was not tested in the present work.

4.3 | THC or another cannabinoid?

Results of the present study showed no significant differences in growth or oxygen consumption rates between fish offered industrial hemp and those offered cannabis extract, suggesting that there might be a chemical present in both industrial hemp and cannabis extract other than the psychoactive THC that caused the effects observed. Industrial hemp oil and cannabis extract comprise over 60 cannabinoids including cannabidiol (CBD). Studies involving the simultaneous administration of THC and CBD yielded contradictory results in animals and humans (Atakan, 2012). Whilst several studies showed an antagonistic interaction between these two compounds, others have shown that THC and CBD have similar effects (Atakan, 2012). For instance, the presence of CBD with THC was reported to potentiate the reduction in body weight of adolescent rats caused by chronic THC (Klein et al., 2011). Moreover, a significant decrease in body weight was observed in rats subjected to repeated CBD administration (Ignatowska-Jankowska, Jankowski & Swiergiel, 2011). In the present study, even though no significant differences were observed in growth between diets 2 and 3, the diet containing THC showed marginally lower body weights than the diet containing industrial hemp. Consequently, it might be safe to speculate that a cannabinoid present in both industrial hemp and cannabis extract might be responsible for the negative growth results observed, and that the combination of CBD with THC further retarded growth of the fish.

Fish in the present experiment were offered feeds with or without THC only once daily. The other two daily feedings were of the same feed and in equal amounts. If THC offered in the morning feeding was responsible for an increase in metabolic rate, then it is possible that if fish were offered feed ad libitum, those treated with marijuana extract might have consumed more and thus grown faster. There is anecdotal information that marijuana increases the appetite of human consumers (the munchies) and results in weight gain. However, even if the fish had grown faster, FCR would have been much higher, resulting in economic loss. Until further research yields different results, we do not believe fish should be given reefer.

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