JJBS

Volume 15, Number 4,December 2022
ISSN 1995-6673
Pages 611 – 619
https://doi.org/10.54319/jjbs/150409

Jordan Journal of Biological Sciences

Microbial Biomass Integrated with Sugarcane Wastes is a Proper Nutritive Supply for Nile Tilapia

Sayeda M. Ali[1,*], Ahmad M. Aboseif [2], Alkhateib Y. Gaafar[3], Ahamed D. El-Gamal[4] and Ahmed K. El-hammady[2]

[1] *Hydrobiology Department, Microbiology Laboratory, National Institute of Oceanography and Fisheries, NIOF, Aswan Research Station, Egypt ;*[2]*Fish nutrition Department, National Institute of Oceanography and Fisheries, NIOF, El-Qanater Research Station, Egypt;*[3]*Hydrobiology Department, Veterinary Research Division, National Research Center, Egypt ;*[4]*Botany Dept., Faculty of Science (boys), Al-Azhar University, Cairo, Nasr City, Egypt.*

Received: July 21, 2021; Revised: January 22, 2022; Accepted: February 9, 2022

Abstract

Single-cell proteins have a potential source for partial replacement of protein and lipids in animal feed. Also, using microorganisms in fish feed enhanced feed efficiency, growth performance, and disease resistance. The purpose of this study is to see if combining several microorganisms (*Azotobacter chroococcum*, *Chlamydomonas reinhardtii*, and baker's yeast) with sugarcane by-products may produce a low-cost fish feed formulation. A total of twelve treatments were completed (consisting of various microbial biomass combined with sugarcane by-products, and integrated with commercial fish feed in different levels, 0, 25, 50, 75, and 100 % $w.w^{-1}$). Nile tilapia (*Oreochromis niloticus*) were evaluated for growth performance, proximate composition, and histopathological examination. Results showed that using low amounts of the experimented formulations (25% and not more than 50% $w.w^{-1}$) increased fish productivity (weight gain and specific growth rate) and proximate compositions of fish without putting fish at risk. On the other hand, using higher levels from the combined diet (75, 100%) caused fish mortality. Although all the fish experimental treatments showed normal histological structure, the mortality of fish may be due to a lack of nutrients. In conclusion, this study is important for both the environment and the economy. More research is needed to extend the safe application of this study in aquaculture through the evaluation of fish in the field and for prolonged viability.

Keywords: Fish fodder, Sugarcane by-products, *Oreochromis niloticus*, *Azotobacter chroococcum*, *Chlamydomonas reinhardtii*, and baker's yeast.

1. Introduction

Aquaculture has become an important economic activity, particularly in developing countries (such as Egypt), to manage the shortage of protein food supplies (Gutierrez-Wing and Malone, 2006). Many factors limit aquaculture's expansion, including rising artificial feed prices due to the use of artificial substrates (organic and inorganic) and expensive protein sources (Delgado *et al.,* 2003). Therefore, many researchers have used alternative and complementary ingredients in feed formulations to reduce feed costs such as mango residue meal (Lima *et al.,* 2011), cassava sweep (Boscolo *et al.,* 2002), Pizzeria by-product (De Sousa *et al.,* 2019), and fenugreek seeds to improve the growth and immunity parameters in the *O. niloticus* (Abbas *et al*., 2019).

In a world where sugarcane industries remain one of the most popular agricultural industries, reusing sugarcane industrial by-products in aquaculture is critical. In Egypt, Egyptian sugar and integrated industries are considered the oldest and widespread industries. Actually, there are eight sugar factories distributed throughout the country from north to south, such as Abu Kerkas at Minya governorate, Gerga at Sohag governorate, Naga Hammadi, Deshna, Kous and Arment at Qena governorate, Edfu and Kom Ombo at Aswan governorate. The main product of these companies is sugar, and there are other related products such as ethanol, vinegar, glacial acetic acid, fodder yeast, solvents, animal feed, paper, and wood. In Egypt, Sugar factories operate intensively around six months from December to June in juicing, refining and sugar crystallization. During the other six months, the plants convert bagasse into wooden boards. The main by-products are bagasse, press mud (filter mud or clay industry), molasses, fly ash (produced during the burning of bagasse and causing air pollution), and wastewater effluent (discharged through the drainage pipe into a channel that finally reaches the Nile). Estimated amounts of by-products from sugarcane are 31% bagasse, 3.5% pressed clay (mud) and 4.5% molasses. According to Egyptian cultivated sugarcane, there are three million tons of bagasse, 316 thousand tons of filter mud and 370 thousand tons of molasses are generated annually (Nakhla and El Haggar, 2014). In fact, these by-products are used for different purposes; molasses is used in fermentation processes to produce fodder yeast, carbon dioxide, alcohol, vinegar, perfumes and medical solutions (Ryoheiet *al.,* 2003; Fadel *et al.,* 2013). In addition molasses, press mud and bagasse are used as a fertilizer in agriculture (Bento *et al.,* 2019) and aquaculture (Keshavanath and Shivanna, 2006; Raul *et al.,* 2020).

In addition, beneficial microorganisms serve a variety of roles in aquaculture productivity. They can be consumed directly or indirectly as food (Duncan and Moriarty, 1997), and they may help fish with their enzymatic digestion (Burford *et al.,* 2008). Microbes may also break down organic materials and transform nitrogenous compounds into microbial protein (Mishra *et al.,* 2008). Furthermore, some microorganisms can enhance the immune response of fish, such as *Azotobacter chroococcum* (Ali *et al.,* 2011) and *Saccharomyces cerevisiae* (Tukmechi *et al.,* 2011; Abdel-Tawwab *et al.,* 2020).

This study looks into the possibilities of making low-cost fish fodders by combining sugarcane industrial by-products with microbes, and the possibility of substituting a portion of the commercial diet. Consequently, this study aims to reduce both the environmental and economic problems of sugarcane and aquaculture industries.

2. Materials and Methods

2.1. Collection and chemical analysis of the sugarcane industry by-products

The Egyptian Sugar and Integrated Industries Company, Kom Ombo, Aswan, Egypt, provided the industrial waste, where all samples were obtained from inside the factory except wastewater effluent collected from the drainage tube. Pith was kept at room temperature, and industrial clay (press mud) was air-dried for 2-3 days before being packed and maintained at room temperature; molasses was kept in the refrigerator, and wastewater effluent was kept frozen. Chemical analyses for pith and press mud are presented in Table (1), where electrical conductivity (EC), pH, and total dissolved solids (TDS) were measured using CRISON multimeter (MM40+). Organic Carbon was determined using the method of Walkley-Black (Walkley, 1982). Nitrogen and ash content were determined according to the Association of Official Analytical Chemists, AOAC, (1995). Colorimetric methods were used to determine phosphorus (APHA, 1998). Heavy metals were determined using atomic absorption spectrometry (Perkin-Elmer 3110, USA) (APHA, 1998).

Table 1. Chemical composition of sugarcane solid wastes (pith and press mud)

Parameters	Pith	Press mud
pH	5.52	5.63
EC (dSm^{-1})	390	2910
Total dissolved solids (mgl^{-1})	250	1867
Moisture (%)	5.5	73.6
Total solids (%)	94.5	26.4
Ash (%)	6.4	4.9
Organic carbon (%)	20.7	9.5
Nitrogen (%)	3.3	4.2
Phosphorus (%)	1.0	1.4
Mn (ppm)	18.6	139.2
Zn (ppm)	34.5	65.4
Cu (ppm)	0.8	30.3
Fe (ppm)	460.5	627.3

2.2. Tested microorganisms

Three microorganisms were used: *Azotobacter chroococcum* isolated from the beach of Lake Nasser, microalgae *Chlamydomonas reinhardtii* isolated from soil sample collected from industrial zones of 6th October City and Baker's yeast obtained from an Egyptian market an accessible source of *Saccharomyces cervaicae*.

2.3. Fish experimental diets (preparation, composition and treatments)

Experimental diets (treatments) consisted of microbial biomass integrated with sugarcane by-products and mixed with commercial diet. Table (2) shows the experimental diets. The highest microbial biomass was obtained using batch culture technique. And culture media were prepared using sugarcane industrial wastewater effluent amendment with molasses and/or press mud as described in Ali *et al.* (2022). Briefly, *C. reinhardtii* was grown in the sugarcane wastewater effluent amendment with 1% molasses and 0.5% clay factor, *C. reinhardtii* batch culture were incubated at room temperature (30-32ºC) under continuous fluorescent light for 96 hours. *A. chroococcum* was grown in sugarcane wastewater effluent amendment with 1% molasses, 1% press mud and $0.5gl^{-1}$ $CaCO_3$, *A. chroococcum* batch culture were incubated in a rotary shaker of 100 rpm at 35 ± 2°C for 72 hours. Baker's yeast was grown in sugarcane wastewater effluent amendment with 5% molasses, baker's yeast batch culture were incubated in a rotary shaker of 100 rpm at 32 ± 2°C for 48 hours (Fig. 1A). After that, microbial biomass was mixed with sugarcane solid wastes (equal proportion of pith and dehydrated press mud was mixed well (Fig. 1B)); microbial cultures were added as 5 ml to each gram of the mixed sugarcane solid wastes, and mixed well (Fig.1C), to produce three basic mixtures. Mixture (1): containing only *A. chroococcum* integrated with sugarcane by-products (Azotobacter-integrated); mixture (2): containing microbial mix (*A. chroococcum*, *C. reinhardtii* and baker's yeast) integrated with sugarcane by-products (microbial mix integrated), and mixture (3): containing only sugarcane byproducts (only sugarcane byproducts). Microorganisms are estimated at about 10^7*Azotobacter* cells/g, 0.003 mg algal mass/g and 0.005 mg yeast mass/g. Then, each of the three previous mixtures was dried at room temperature (30-35°C) for 48 hours. Following that,

each of the three dried previous mixtures was combined with a commercial diet in different percentage (0, 25, 50, 75 and 100 % w.w^{-1}) as shown in Table (2). Proximate composition of the different experimental treatments is shown in Table (3).

Table 2. Treatment description for experimental diets.

Treatments	Treatment description
Control	100% commercial diet
Diets containing *A.chroococcum* integrated with sugarcane by-products (Azotobacter-integrated)	
25 % Azotobacter-integrated	Diet containing 25 % Azotobacter-integrated and 75% commercial diet.
50 % Azotobacter-integrated	Diet containing 50 % Azotobacter-integrated and 50 % commercial diet.
75 % Azotobacter-integrated	Diet containing 75 % Azotobacter-integrated and 25% commercial diet.
100 % Azotobacter-integrated	Diet containing 100 % Azotobacter-integrated.
Diets containing microbial mix integrated with sugarcane by-products (microbial mix-integrated)	
25 % microbial mix-integrated	Diet containing 25 % microbial mix-integrated and 75% commercial diet.
50 % microbial mix-integrated	Diet containing 50 % microbial mix-integrated and 50% commercial diet.
75 % microbial mix-integrated	Diet containing 75 % microbial mix-integrated and 25% commercial diet.
100 % microbial mix-integrated	Diet containing 100 % microbial mix-integrated
Diets containing only sugarcane byproducts (only sugarcane byproducts)	
25 % only sugarcane by-products	Diet containing 25 % only sugarcane by-products and 75% commercial diet.
50 % only sugarcane by-products	Diet containing 50 % only sugarcane by-products and 50% commercial diet.
75 % only sugarcane by-products	Diet containing 75 % only sugarcane by-products and 25% commercial diet.
100 % only sugarcane by-products	Diet containing 100 % only sugarcane by-products.

Table 3. Proximate composition for different experimental treatments

Treatments	CP%	EE%	Ash %
Control	24.85	11.95	7.97
25 % Azotobacter-integrated	21.42	10.90	11.74
50 % Azotobacter-integrated	17.99	9.85	15.50
75 % Azotobacter-integrated	14.56	8.80	19.27
100 % Azotobacter-integrated	11.13	7.75	23.03
25 % microbial mix-integrated	21.55	10.81	11.64
50 % microbial mix-integrated	18.25	9.67	15.31
75 % microbial mix-integrated	14.95	8.52	18.97
100 % microbial mix-integrated	11.65	7.38	22.64
25 % only sugarcane by-products	21.26	10.70	11.20
50 % only sugarcane by-products	17.66	9.46	14.43
75 % only sugarcane by-products	14.07	8.21	17.65
100 % only sugarcane by-products	10.47	6.96	20.88

CP%, crude protein % and EE%, ether extract %

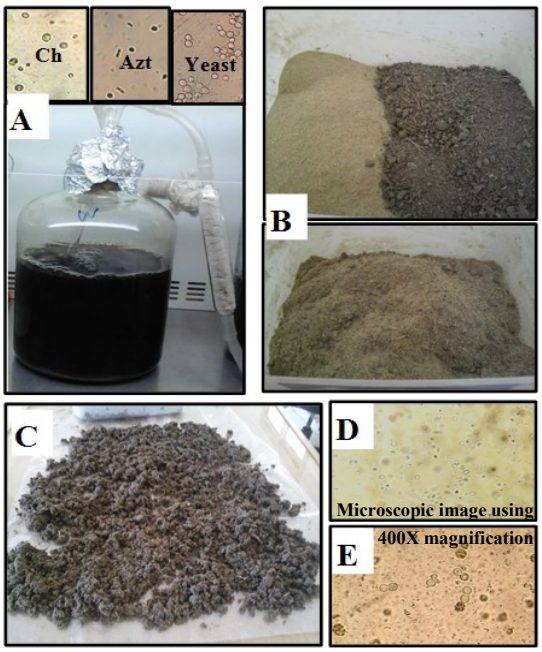

Figure 1.Preparation of experimental diets; (A) microbial biomass production (*Chlamydomonas reinhardtii* (Ch), *Azotobacter chroococcum* (Azt) and yeast) was obtained by using batch culture technique and sugarcane industrial wastewater effluent amendment with molasses and/or press mud, (B) equal ratio of pith and dehydrated press mud was mixed well, (C) microbial cultures were added as 5 ml to each gram of the mixed sugarcane solid wastes and mixed well. Three basic mixtures were produced; (D) diets containing *A. chroococcum* integrated with sugarcane by-products (Azotobacter-integrated), (E) diets containing microbial mix (Ch, Azt and yeast) integrated with sugarcane by-products (microbial mix-integrated) and diets containing only sugarcane byproducts (only sugarcane byproducts). After that, each of the previous three mixtures (after air drying) was mixed with a commercial diet in different percentage (0, 25, 50, 75 and 100 % $w.w^{-1}$) as shown in Table (2).

2.4. Fish feeding

One thousand Nile tilapia (*Oreochromis niloticus*) fingerlings with an average body weight of 0.5 ± 0.4 g (mean ± SE) were obtained from the General Authority for Fish Resources Development, Aswan, Egypt, and transferred to Aswan Research Station, National Institute of Oceanography and Fisheries, Aswan, Egypt. Fish were acclimated in glass aquaria (80 x 60 x 50 cm) for seven days, with two daily feedings of a basic diet.The aquaria were filled with clean, dechlorinated water with continuous aeration. The water temperature ranged from 28 to 30 °C, pH 7, and the dissolved oxygen was 7±1 mg/l. During the experimental period, the water was changed by 10 % daily. Fish were fed at 5% of their body weight daily in the first week, then 3 % of their body weight daily until the experiment ended. The experiment was conducted for 45 days, and fish samples were collected every two weeks.

2.4.1. Ethics Statement

The dealing with the experimental fish followed the National Institute of Oceanography and Fisheries institutional ethical guidelines of humane dealing with experimental animals. With no more than the least number of fish per group used, fish were anesthetized with eugenol either before sample collection or euthanized.

2.4.2. Fish analysis

2.4.2.1. Fish growth performance

Fish growth parameters (fish length and weight) were recorded for each treatment and control every 15 days. Total length was measured from the head to the end of the tail. Also, weight was measured in grams on a digital scale. Growth indices (weight gain (WG), specific growth rate (SGR), feed conversion efficiencies (FCE), the condition factor (CF), and the percentage of survival were calculated according to the following formula (Priestley *et al.*, 2006):

$$WG = \text{Final weight} - \text{Initial weight}$$

$$SGR = 100 \times \frac{(\ln \text{Final weight} - \ln \text{Initial weight})}{\text{Days of feeding}}$$

$$FCE = 100 \times \frac{(\text{Final weight} - \text{Initial weight})}{\text{Dry feed intake}}$$

$$CF = 100 \times \frac{\text{Total weight}}{\text{Total length}^3}$$

$$\text{Survival (\%)} = 100 \times \frac{\text{Final number of fish}}{\text{initial number of fish}}$$

2.4.2.2. Proximate composition

Diets and fish carcass samples were analyzed for dry matter (DM) and ash content, and crude protein (N x 6.25) using a Kjeltech auto-analyzer in accordance with AOAC guidelines (1995). Crude fat was measured according to Bligh and Dyer (1959). Nitrogen-free extract (soluble carbohydrate) was calculated by subtracting the difference.

2.4.2.3. Histopathological examination

Five fish were fixed whole for 24 hours in 10% phosphate-buffered formalin, then dehydrated with increasing concentrations of ethanol (70 %, 80 %, 90 %, 95 %, and 100 %), embedded in paraffin, and finally sectioned at 5 µm thick. Tissue sections were stained with Hematoxylin and Eosin (Presnell *et al.*, 1997) and examined by light microscopy (CX 41, Olympus, Japan), according to Roberts (2012).

2.5. Statistical Analysis:

Data were statistically analyzed using analysis of variance (ANOVA) using the STATISTICA computer programs.

3. Results

3.1. Fish growth performance

Figure (2) shows the effects of experimental diets on *O. niloticus* weight gain (WG), specific growth rate (SGR), feed conversion efficiencies (FCE), and condition factor. After 15 days of fish feeding, the treatment fed with 25% microbial mix–integrated diets had a higher WG (0.29 g) than the control (0.25 g), and after 30 days of fish feeding, the treatment fed with 25% Azotobacter–integrated diets had a higher WG (0.52 g) than the control (0.46 g). Moreover, after 45 days of fish feeding, several treatments recorded significantly higher WG values compared to control. In details, the treatment fed with 25, 50, 75% Azotobacter-integrated diets recorded 0.99, 1.18, 0.99 g WG respectively; the treatment fed with 25, 50% microbial mix-integrated diets recorded 0.99, 1.15g WG

respectively, and the treatment fed with 25% only sugarcane byproducts diets recorded 0.86 g WG, while control recorded 0.79 g WG (Fig. 2).

Statistically, the best SGR was recorded for 25 % microbial mix-integrated diets (2.95 SGR %), compared to control (2.58 SGR %) after 15 days of feeding, and 25 % Azotobacter-integrated diets (2.28 SGR %) and 25 % microbial mix-integrated diets (2.19 SGR %), compared to control (2.09 SGR %) after 30 days of feeding. After 45 days of feeding, the highest values were recorded in 25, 50, 75 % Azotobacter-integrated diets (2.36, 2.62, 2.69 SGR %, respectively); 25, 50 % microbial mix-integrateddiets (2.16, 2.58 SGR %, respectively) and 25 % only sugarcane byproducts mixed diet (2.36 SGR %) compared to control (2.04 SGR %).

Feed conversion efficiencies (FCE) values of fishes were improved, where after 15 days of fish feeding, the treatment fed with 25 % microbial mix-integrated diet recorded higher FCE (5.3 %) compared to control (4.5 %). In addition, after 30 days of fish feeding, the treatment fed with 25 % Azotobacter-integrated diet, and the treatment fed with 25 % microbial mix-integrated diet recorded higher FCE (12.9 and 12.2 % respectively) compared to control (11.4 %). Also after 45 days of fish feeding, significant increases ($P \leq 0.05$) in FCE in many treatments were recorded compared to control (19.8 %), such as FCE in 25, 50, 75 % Azotobacter-integrated diets (24.9, 29.5, 24.7 %, respectively); 25, 50 % microbial mix-integrated diets (24.8, 28.8 %, respectively).

Furthermore, Figure (2) showed that using experimental diets improved condition factor, particularly after 45 days of feeding, a phenomenon seen in all treatments except the highly concentrated one (100 % treatments), where 25, 50, 75% Azotobacter-integrated diets recorded 2.1, 3.1, 2.4 gm.cm^{-3} respectively; 25, 50, 75% microbial mix-integrated diets treatments recorded 2.4, 2.1, 2.1 gm.cm^{-3}respectively, and 25, 50, 75% only sugarcane byproducts diets recorded 2.1, 1.7, 2.4 gm.cm^{-3}respectively, compared to control (1.1 gm.cm^{-3}).

Lower concentrations of experimental diets (25, 50%) had considerably higher values in both WG and SGR, regardless of time, but higher concentrations (75, 100%) were deemed unsuitable for fish feeding (Table 4). Also, *A. chroococcum*- integrated and microbial mix-integrated diets had a significant effect on weight gain and specific growth rate compared to the only sugarcane byproducts mixed diets. In addition, all treatments except highly concentrated ones (100% treatments) recorded higher condition factor values compared to control (Table 4).

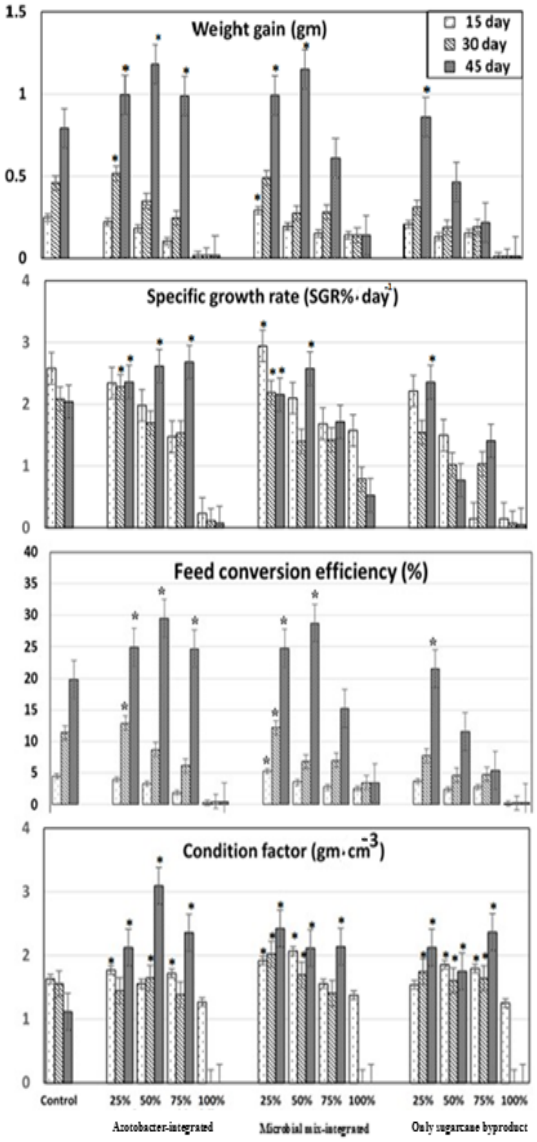

Figure 2. Growth performance (weight gain, specific growth rate, feed conversion efficiency and condition factor) for *Oreochromis niloticus* fingerlings feeding on *A. chroococcum* integrated with sugarcane by-products (Azotobacter-integrated) or microbial mix integrated with sugarcane by products (microbial mix-integrated) or only sugarcane byproducts after 15, 30 and 45 days of fish feeding.

Significant increases ($P \leq 0.05$), more than the control, at the same time are indicated by asterisks (*).

Table 4. Combined statistical analysis (regardless of sampling time) of weight gain, specific growth rate, feed conversion efficiencies and condition factor for different experimental treatments.

Treatments	WG (gm)	SGR (%)	FCE (%)	CF(gm. cm^{-3})
Control	0.50 ^{e}	2.24 ^{c}	11.91 ^{e}	1.43 ^{e}
25 % Azotobacter-integrated	0.58 ^{b}	2.33 ^{b}	13.92 ^{b}	1.78 ^{cd}
50 % Azotobacter-integrated	0.57 ^{c}	2.10 ^{d}	13.86 ^{c}	2.10 ^{a}
75 % Azotobacter-integrated	0.45 ^{g}	1.90 ^{f}	10.91 ^{g}	1.82 ^{c}
100 % Azotobacter-integrated	0.02 ^{l}	0.14 ^{k}	0.42 ^{l}	0.42 ^{f}
25 % microbial mix-integrated	0.59 ^{a}	2.43 ^{a}	14.10 ^{a}	2.12 ^{a}
50 % microbial mix-integrated	0.54 ^{d}	2.03 ^{e}	13.05 ^{d}	1.96 ^{b}
75 % microbial mix-integrated	0.35 ^{h}	1.61 ^{g}	8.34 ^{h}	1.70 ^{d}
100 % microbial mix-integrated	0.14 ^{k}	0.96 ^{i}	3.18 ^{k}	0.46 ^{f}
25 % only sugarcane by-products	0.46 ^{f}	2.04 ^{e}	10.98 ^{f}	1.80 ^{c}
50 % only sugarcane by-products	0.26 ^{i}	1.10 ^{h}	6.23 ^{i}	1.74 ^{d}
75 % only sugarcane by-products	0.19 ^{j}	0.86 ^{j}	4.34 ^{j}	1.93 ^{b}
100 % only sugarcane by-products	0.01 ^{m}	0.09 ^{l}	0.27 ^{m}	0.42 ^{f}

(WG) weight gain, (SGR) specific growth rate, (FCE) feed conversion efficiencies and (CF) condition factor

At the same column, means followed by the differ letter are significantly different ($P \leq 0.05$).

3.2. Proximate analysis of fish

Final proximate compositions of fish significantly affected by the treatments (Figure 3). Crude protein recorded significantly higher values in fish fed with 25, 50, 75% Azotobacter-integrated diets (65.7, 63.6, 67.3 % respectively), and 25, 50% microbial mix-integrated diets (65.1, 66.5% respectively), and 50, 75% only sugarcane byproducts diets (65.4, 65.2% respectively), compared with control (62.4%), while crude lipid concentrations in all treatments were lower than control (22.5%). Fish fed with 25% microbial mix-integrated diet and fish fed with 25% only sugarcane byproducts diet (21.4 and 20.7% respectively) did not significantly differ from control. The maximum nitrogen-free extract (soluble carbohydrate) was recorded in fish fed with 25% only sugarcane byproducts diet (3.7%), 50% microbial mix-integrated diet (2.9 %), 50% only sugarcane byproducts diet (2.7 %) and 50% Azotobacter-integrated diets (2.6 %), comparing to control (2.4%). Fortunately, lower ash content was recorded in fish fed with 25% microbial mix-integrated diet (11.4 %) compared to control (12.8 %), while fish fed with 100 % integrated diets recorded higher ash content (Fig. 3).

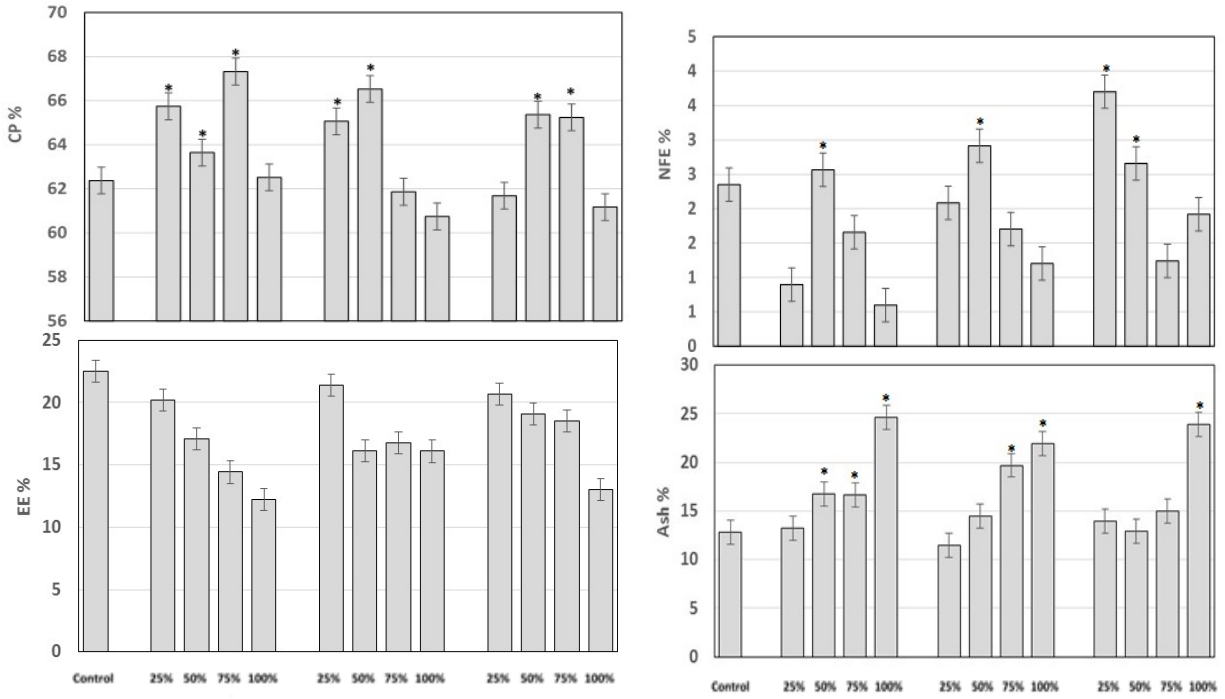

Figure 3. Final proximate compositions for *Oreochromisniloticus* fingerlings feeding on *A. chroococcum* integrated with sugarcane by-products (Azotobacter-integrated) or microbial mix integrated with sugarcane by-products (microbial mix-integrated) or only sugarcane byproducts after 45 days of fish feeding.

CP, crude protein; EE, ether extract; NFE, nitrogen free extract. Significant increases ($P \leq 0.05$), more than the control, are indicated by asterisks (*).

3.3. Histopathological examination

There were no significant differences in the histopathological picture of the different treatments as compared to the control. The majority of the tissues evaluated had normal histological structure (Fig. 4).

Although all experiment diets had no visible negative effects on fish health, there is a discernible effect on fish color (Fig. 5). As the amount of the experimental diet was increased, the blackness of the fish increased. The fishes that received 100 % of the experimental diet showed more darkness than those that received 75 % of the experimental diet. Moreover, fishes receiving 25 % of the experimental diets were more white and similar to the control (Fig. 5A, and 5B). This is related to the color of water (Fig. 5C and 5D), where the darkness of water increased with increasing the amount of the experimental diet (Fig. 5E).

Furthermore, the percentage of fish that survived was inversely proportional to the increase in the content of the experimental diets. It was higher in individuals who were given a 25% or 50% experimental diet, which is similar to a control diet (100 % survival rate). On the contrary, fishes receiving a 100 % experimental diet recorded more mortality than the others receiving 75 % experimental diet, and the mortality began in the 11th day of feeding. The lowest mortality was recorded in fishes fed with Azotobacter-integrated diets (Fig. 6).

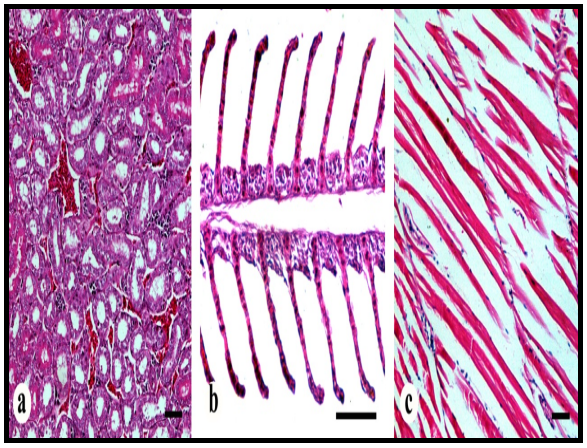

Figure 4. An example histopathological picture of *Oreochromis niloticus* fingerlings showing: (a) posterior kidney showing normal renal structure with moderate congestion, (b) Primary gill lamella showing normal structure, (c) musculature showing normal myofilamentos structure.

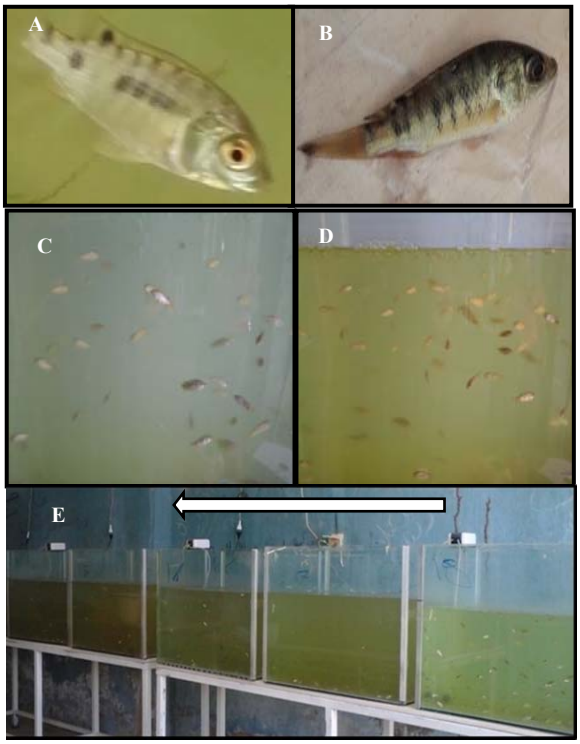

Figure 5. Photo image showing external color for *Oreochromis niloticus* fingerlings and water color for fish aquaculture. A, shows light and normal color of fish; B, shows dark color of fish, C, shows normal color of water aquaculture; D, shows dark color of water aquaculture which received high experimental diet; E, shows darkness of water aquaculture increased with increasing the amount of the experimental diet (with the direction of arrow).

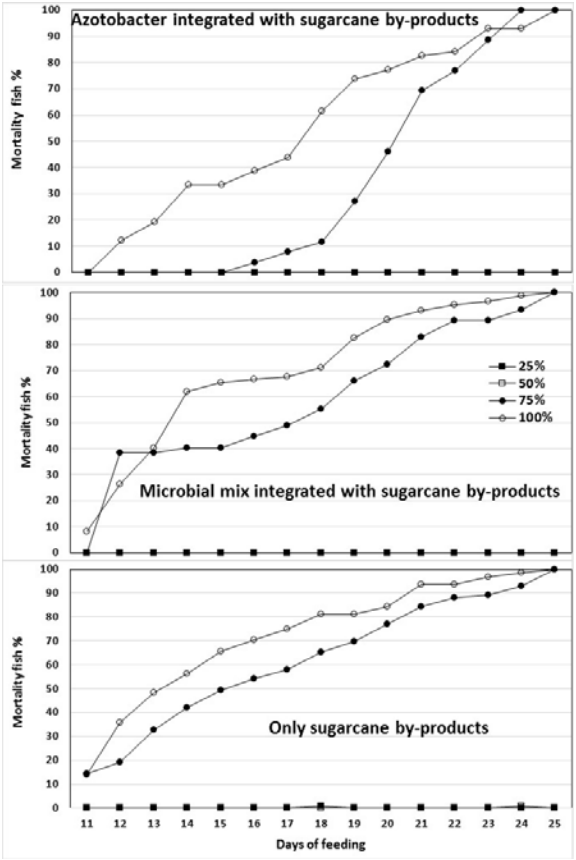

Figure 6. *Oreochromis niloticus* fingerlings mortality (%) from the eleventh day until the twenty-fifth day of feeding.

4. Discussion

Fish farming is an important industry, especially with the growing number of population and increasing food shortages. Using low-cost fish diets either in their ingredients or in their preparation is important to reduce the production cost and consequently increase fish productivity. In aquaculture, microorganisms play an important and useful role as nitrogen-fixing bacteria can raise net primary productivity, increase plankton production, and thereby increase fish biomass (Tripathy and Ayyappan, 2005; Ali *et al.,* 2015). Furthermore, nitrogen-fixing bacteria are used in controlling pathogens and improving the fish immunity system (Decamp *et al.,* 2008; Ali *et al.,* 2011).

The effect of sugarcane by-products on aquacultures have been studied by a number of researchers, such as Aderolu *et al*. (2013) who used molasses in the feed of catfish, and Gangadhar and Keshavanath (2012) who demonstrated that sugarcane bagasse can be applied in tank bottom as a substrate to increase rohu fish production.

The objective of this study is to provide low-cost, high-effective fish diets to lower fish production costs. The use of minor additions from the combination diet (25, 50%) considerably improved fish growing parameters, indicating that microbial biomass integrated with sugarcane wastes can replace a portion of the commercial fish diet (Table 4). On the other hand, using large amounts of combination diet (75, 100%) in fish feeding reduced fish growth performance, which could be due to a lack of accessible nutrients, as assessed by the approximation analysis (Table 3).

Furthermore, using microbial biomass integrated with sugarcane wastes increased fish growth performance more than using sugarcane by-products without microorganisms (only sugarcane byproducts), which indicates that microorganisms provided essential nutrients, such as essential amino acids, vitamins, and un-identified growth factors, increased digestibility of the raw materials, and catabolized anti-nutritional factors by the action of the produced enzymes. These results are compatible with the results of Keshavanath and Shivanna, 2006; Gangadhar and Keshavanath, 2012.

Moreover, the highest values for condition factor (used as an index to evaluate the aquatic ecosystem in which fish live) were found in fishes fed with 50% Azotobacter-integrated diets, which confirms that *Azotobacter* improved the water quality of aquaculture (Ali *et al.,* 2012). Additionally, the fish that were fed with 25% of microbial mix-integrated diets recorded higher carcass amounts of crude protein and lipid, which could be related to the high lipid content of *C. reinhardtii*cells (Yang *et al.,* 2018).

Despite the fact that there were no visible histological changes in fish tissues, the proportion of fish mortality increased with increasing amount of experimental diets. Fishes fed with 100 % integrated diets recorded higher mortality than those fed with 75 % integrated diet (Fig. 6). Furthermore, fish fed Azotobacter-integrated diets had a lower mortality rate than fish fed alternative treatments, although using higher amounts of the integrated diet (75 and 100 %) caused fish mortality and increased fish dark color. All of the fish samples from all of the experimental treatments lacked histological abnormalities in their tissues, indicating that nutrient deficiency was the cause of death.

5. Conclusion

It can be concluded that microbial mix–integrated sugarcane byproducts can be applied in Nile tilapia feeding after mixing with a commercial diet in between 25-50 %. Also, fodder factories must be complementary with sugarcane factories to reduce pollution and increase economic value. Further research is needed to confirm the safety and applicability of using such integrated diets in fish feeding and also to reduce feed cost through investigating the proper supplementary additions, especially with higher concentrations of integrated diets.

6. Declarations

Ethics approval and consent to participate: All applicable international, national, and/or institutional guidelines for the care and use of animals were followed by the authors. All the contributing authors consent to participate in this study.

Competing interests: The authors have no conflicts of interest to declare.

Authors Contributions SMA and ADE contributed to the study design and material preparation for microbial growth experiments. SMA and AKE contributed to the study design and material preparation for aquaculture experiments. AMA performed the proximate composition. AYG performed the histopathological examination.

SMA collected, analyzed and interpreted data and was a major contributor in writing the manuscript. All authors read and approved the final manuscript.

Funding

Not applicable.

Consent to participate

Not applicable.

Consent for publication

Not applicable.

Code availability

Not applicable.

Data availability

All data generated or analyzed during this study are included in this published article.

Acknowledgments

We would like to thank the General Authority for Fish Resources Development, Aswan, Egypt for providing the required fish. Thanks are also due to Mr. Ahmad El-Badry and Mr. Hassan El-Badry for their sincere efforts during the experiment.

References

Abbas WT, Abumourad IMK, Mohamed LA, Abbas HH, Authman MMN, Soliman WSE and Elgendy MY. 2019. The Role of the Dietary Supplementation of Fenugreek Seeds in Growth and Immunity in Nile Tilapia with or without Cadmium Contamination. *Jordan J Biol Sci*., **12 (5)**: 649 – 656.

Abdel-Tawwab M, Adeshina I and Issa ZA. 2020. Antioxidants and immune responses, resistance to *Aspergilus flavus* infection, and growth performance of Nile tilapia, *Oreochromis niloticus*, fed diets supplemented with yeast, *Saccharomyces serevisiae*. *Anim. Feed Sci. Technol*., **263**:114484.

Aderolu AZ, Aarode OO and Bello RA. 2013. Inclusion effect of graded levels of molases in the diet of *Clarias gariepinus* juvenile. *Int. J. Fish. Aquac.*, **5 (7)**: 172-176.

Ali SM, Aboseif AM, El-Gamal AD and El-hammady A. 2022. Microbial biomass production using sugarcane industrial by-products and their application to Nile tilapia aquaculture. *Res. J. Biotechnol*., 17 (5): 130 -142.

Ali SM, Nasr HS and Abbas MT. 2015. Using diazotrophic bacteria for biomass production of microalgae. *EJER,* **3**: 41-52.

Ali SM, Nasr HS and Abbas WT. 2012. Enhancement of *Chlorella vulgaris* growth and bioremediation ability of aquarium wastewater using diazotrophs. *Pakistan J Biol Sci*, **15 (16)**: 775-782.

Ali SM, Wafa MIA and Abbas WT. 2011. Evaluation of *Azotobacter* and *Azospirillum* biofertilizers as a probiotics in *Oreochromis niloticus* aquaculture. *J Fish Aquat Sci*, **6 (5)**: 535-544.

AOAC, Association of Official Analytical Chemists. 1995. **Official Methods of Analysis**. 16th Edition, Washington DC.

APHA, American Public Health Association. 1998. **Standard methods for the examination of water and wastewater,** 20th edition. American Public Health Association, Washington, DC.

Bento LR, Castro AJR, Moreira AB, Ferreira OP, Bisinoti MC and Melo CA. 2019. Release of nutrients and organic carbon in different soil types from hydrochar obtained using sugarcane bagasse and vinasse. *Geoderma*, **334**: 24–32.

Bligh EG and Dyer WJ. 1959. A rapid method for total lipid extraction and purification. *Can. J. Biochem. Physiol.* **37**: 911-917.

Boscolo WR, Hayashi C and Meure F. 2002. Farinha de Varredura de Mandioca (*Manihot esculenta*) na Alimentacao de Alevinos de Tilapia do Nilo (*Oreochromis niloticus* L.). *R. Bras. Zootec*. **31**: 546–551.

Burford MA, Alongi DM, McKinnon AD and Trott LA. 2008. Primary productivity in a tropical macrotidal estuary, Darwin Harbour, Australia. *Estuar. Coast. Shelf Sci.* **79**: 440-448.

De Sousa AA, Pinho SM, Rombenso AN, de Mello GL and Emerenciano MGC. 2019. Pizzeria by-product: A complementary feed source for Nile tilapia (*Oreochromis niloticus*) raised in biofloc technology?. *Aquaculture* **501**: 359–367.

Decamp O, Moriarty DJW and Lavens P. 2008. Probiotics for shrimp larviculture: review of field data from Asia and Latin America. *Aquac. Res.* **39**: 334–338.

Delgado LC, Wada N, Rosegrant WM, Meijer Sand Ahmed M. 2003. Fish to 2020: Supply and Demand in Changing Global Markets. *IFPRI*, Washington, D.C. pp. 237

Duncan T and Moriarty S. 1997. **Driving Brand Value: Using Integrated Marketing to Drive Stakeholder Relationships**. New York: McGraw-Hill.

Fadel M, Keera AA, Mouafi FE and Kahil T. 2013. High level ethanol from sugar cane molasses by a new thermotolerant *Saccharomyces cerevisiae* strain in industrial scale. *Biotechnol. Res. Int.*, Article ID 253286, 6 pages.http://dx.doi.org/10.1155/2013/253286

Gangadhar B and Keshavanath P (2012). Growth performance of rohu, Labeo rohita (Ham.) in tanks provided with different levels of sugarcane bagasse as periphyton substrate. *Indian J. Fish.* **59 (3)**: 77-82.

Gutierrez-Wing MT and Malone R. 2006. Biological filters in aquaculture: Trends and research directions for freshwater and marine applications. *Aquac. eng.* **34 (3)**:163-171.

Keshavanath P and Shivanna GB. 2006. Evaluation of sugarcane by-product pressmud as a manure in carp culture. *Bioresour. Technol.* **97**: 628–634.

Lima MR, Ludke MCMM, Neto FFP, Pinto BWC, Torres TR and Souza EJO. 2011. Farelo de residuo de manga para tilapia do Nilo. *Anim. Sci*. **33**: 65–71.

Mishra VK, Upadhyay AR, Pandey SK and Tripathi BD. 2008. Concentrations of heavy metals and aquatic macrophytes of Govind Ballabh Pant Sagar an anthropogenic lake affected by coal mining effluent. *Environ. Monit. Assess.* **141**: 49–58.

Nakhla DA and El Haggar S. 2014. Environmentally balanced sugarcane industry in Egypt. http://uest.ntua.gr/conference2014/pdf/nakhla.pdf

Presnell JK, Schreibman MP and Humason GL. 1997. **Humason's animal tissue techniques**. Johns Hopkins University Press.

Priestley SM, Stevenson AE and Alexander LG. 2006. Growth rate and body condition in relation to group size in black widow tetras (*Gymnocorymbus ternetzi*) and common goldfish (*Carassiusauratus*). *J. Nutr*. **136**: 2078S-2080S.

Raul C, Bharti VS, Dar Jaffer Y, Lenka S and Krishna G. 2020. Sugarcane bagasse biochar: Suitable amendment for inland aquaculture soils. *Aquac. Res.* https://doi.org/10.1111/are.14922

Roberts RJ. 2012. Fish pathology. John Wiley & Sons

Ryohei UENO, Naoko HS and Naoto U. 2003. Fermentation of molasses by several yeasts from hot spring drain and phylogeny of the unique isolate producing ethanol at 55°C. *J. Tokyo Univ. Fish.* **90**: 23-30.

Tripathy PP and Ayyappan S. 2005. Evaluation of *Azotobacter* and *Azospirillum* as biofertilizers in aquaculture. *World J. Microbial. Biotechnol*. **21**: 1339-1343.

Tukmechi A, Andani HRR, Manaffar R and Sheikhzadeh N. 2011. Dietary administration of beta-mercapto-ethanol treated *Saccharomyces cerevisiae* enhanced the growth, innate immune response and disease resistance of the rainbow trout, *Oncorhynchus mykiss*. *Fish Shellfish Immunol* **30 (3)**: 923-928.

Walkley A. 1982. Walkley-Black method. In: Page AL (Ed.) **Methods of soil analysis. Part 2. Chemical and microbiological properties.** Madison: American Society of Agronomy Inc., Soil Science Society of America Inc.; 1982. p. 570–1.

Yang LCJ, Qin SZM, Jiang Y, Hu L, Xiao P, Hao W, Hu Z, Lei A and Wang J. 2018. Growth and lipid accumulation by different nutrients in the microalga *Chlamydomonas reinhardtii*. *Biotechnol. Biofuels* **11**: 40.