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ORIGINAL ARTICLE

Replacing fish meal with rapeseed meal: potential impact on the growth performance, profitability measures, serum biomarkers, antioxidant status, intestinal morphometric analysis, and water quality of *Oreochromis niloticus* and *Sarotherodon galilaeus* fingerlings

Eman A. Sallam[1] · Aya F. Matter[2] · Liza S. Mohammed[3] · Aya E. Azam[4] · Ahmed Shehab[5] · Mohamed Mohamed Soliman[6]

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Abstract

The aim of this study was to assess the impact of using rapeseed meal as a partial replacement for fish meal in the diet of farmed tilapia. We evaluated the effect of this replacement on growth performance, profitability, serum biomarkers, antioxidant status, gut morphology, and water quality. A total of 960 apparently healthy *Oreochromis niloticus* (*O. niloticus*) and *Sarotherodon galilaeus* (*S. galilaeus*) fingerlings were randomly distributed into four dietary treatment groups for each tilapia species (triplicate design, 120 fish/group, and 40 fish/replicate). The diets consumed by these groups were formulated to replace fish meal (FM) with rapeseed meal (RM) at 0%, 10%, 20%, and 30%, respectively, for 12 consecutive weeks. Results indicated that replacing RM in the diet of *S. galilaeus* (up to 20%) and *O. niloticus* (up to 10%) resulted in increased growth performance parameters, including final weight, weight gain, length, length gain, weight gain rate, and specific growth rate (SGR), and return parameters such as a total return and relative return compared to the control group. Moreover, an increase in RM up to 30% improved net profit and increased the mucosal length, intestinal villi length, and the number of goblet cells compared with results in its relative control groups. Additionally, we observed a significant increase in serum and liver AST and ALT with increased RM replacement. With respect to water parameters, we observed a significant difference in the ammonia levels, turbidity, and conductivity with the changes to the percentage of RM in the diets. As for the effect on each species, *O. niloticus* showed a more significant increase in all examined parameters compared to results in *S. galilaeus*. In summary, up to 10% RM can be used to replace FM without any adverse effects on the growth performance, profitability measures, intestinal morphometric analysis, or water quality.

Keywords Rapeseed meal · Supplementation · Growth performance · Antioxidant activities · Comparative impacts · Tilapia

✉ Eman A. Sallam
Eman.salam@fvtm.bu.edu.eg

✉ Mohamed Mohamed Soliman
mmsoliman@tu.edu.sa

Aya F. Matter
ayamatter29@yahoo.com

Liza S. Mohammed
liza.reda@fvtm.bu.edu.eg

Aya E. Azam
aya.azzam@fvtm.bu.edu.eg

Ahmed Shehab
ahmed.shehab@fvtm.bu.edu.eg

[1] Animal and Poultry Production, Department of Animal Wealth Development, Faculty of Veterinary Medicine, Benha University, Benha 13736, Egypt

[2] Department of Aquatic Animals Diseases and Management, Faculty of Veterinary Medicine, Benha University, Benha 13736, Egypt

[3] Veterinary Economics and Farm Management, Department of Animal Wealth Development, Faculty of Veterinary Medicine, Benha University, Benha 13736, Egypt

[4] Animal Hygiene and Veterinary Management Department, Faculty of Veterinary Medicine, Benha University, Benha 13736, Egypt

[5] Department of Nutrition and Clinical Nutrition, Faculty of Veterinary Medicine, Benha University, Benha, Egypt

[6] Clinical Laboratory Sciences Department, Turabah University College, Taif University, P.O. Box 11099, Taif 21944, Saudi Arabia

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Introduction

For the last few years, aquaculture has been the fastest growing animal food industry, accounting for 80 million tons of total fish production worldwide (FAO 2018). Purchasing feed contributes to 65–70% of the total operating cost (Collins et al. 2013; FAO 2016); in Egypt, the cost is at the higher end of the range, representing ~ 70% of the total cost of aquaculture production (Dawood et al. 2020). Although fish meal is expensive and no longer a sustainable product, habit and a lack of alternatives means that production of aquafeeds rely heavily on its use (Hardy 2010; Oliva-Teles et al. 2015). In the future, the demand for fish meal is expected to rise due to expansion of the aquaculture industry. As a result, the cost of fish meal has risen by more than twofold in the last few years (Dalsgaard et al. 2009). In addition, expanded fish meal production has put extra pressure on aquatic ecosystems (Haghbayan and Shamsaie Mehrgan 2015). Therefore, finding ways to decrease the dependence on fish meals in aquaculture diets is now considered a priority in efforts to reduce feed cost (Luo et al. 2011). Many research studies have been conducted in an attempt to find an alternative good quality feed ingredient, which can be used efficiently in fish feeds and improve growth performance (Acar et al. 2018; Fazio et al. 2019). Since the fingerlings of tilapia, a popular farmed fish, require a good quality protein supplement such as fish meal in their feed, especially as they grow larger (Jatta and Sigurgeirsson 2013), several plant protein supplements have been examined for potential use in fish feed (El-Sayed 1999). These include alfalfa (Belal 1999), soybeans (Koumi et al. 2009), cottonseed (El-Sayed 1999), and rapeseed meal (Pettersson et al. 2009; Tanemura et al. 2016).

The production of rapeseed meal (RM) has increased steadily over the last few years, making it the second most commonly traded protein component after soybean meal (USDA 2017). Coupled with their good biological value, rapeseed proteins have fascinating functional characteristics that open up additional applications in the industry, including the eventual replacement of animal proteins (Gerzhova et al. 2015; Ghodsvali et al. 2005). Interestingly, RM is rich in protein content (32–45%, dry matter) and has a relatively well-balanced amino acid profile. Rapeseed protein is lower in lysine than soybean protein but richer in sulfur-containing amino acids (Drew et al. 2007). Unfortunately, rapeseed meal also contains a variety of antinutritional factors (ANF) such as glucosinolates, erucic acid, tannins, sinnapine, phytic acid, and indigestible carbohydrates. These antinutritional constituents may have a negative impact on fish growth and health (Adem et al. 2014; Ciska and Kozlowska 1998; Olukosi et al. 2017). Numerous previous studies have demonstrated that the ANF present in rapeseed meal limits its inclusion levels as a substitute for fish meal (FM) (Ciska and Kozlowska 1998; Nagel et al. 2012; Ngo et al. 2016). The main negative effects of the ANF present in rapeseed meal are low palatability (Rao et al. 2013), decreased feed intake, and low digestibility (Wu and Muir 2008). For these reasons, it has been proposed that RM be included in fish diets at limited levels ranging from 10 to 20% (Hertrampf and Piedad-Pascual 2000). Several studies have assessed the effectiveness of RM as a protein source for fish diets, but the efficiency with which RM is utilized differs considerably among fish species (Davies et al. 1990; Luo et al. 2012b). They reported that the practical inclusion limit was ~ 15% when substituting RM for FM, since it was observed that the feed intake and growth performance of tilapia declined as the inclusion level of RM in the feed increased (Nemati 2014). Recently, (Zhang et al. 2020) found that RM can be included in the diet of Asian red-tailed catfish at a level of 11.2% without any adverse effect on growth performance.

Four major species account for ~ 85% of total inland water catches. Tilapia and other cichlids are listed as the second-largest group after carp and other cyprinids in land water catches (FAO 2020). Nile tilapia, *Oreochromis niloticus* (*O. niloticus*), and Mango tilapia, *Sarotherodon galilaeus (S. galilaeus)*, are the two most dominant cultured tilapia species (Mensah et al. 2014). The main advantages of using tilapia species in the aquaculture industry are rapid growth, high environmental tolerance, fast spawning, disease resistance, and a high capacity to withstand a wide range of environmental stresses and the presence of antinutrient compounds in the diet, making it an ideal target species for upgrading low-quality ingredients to high quality-food (Nemati 2014). It is the preferred aquaculture fish in Egypt and has been enthusiastically embraced by customers (El-Saidy and Gaber 2005).

Based on previous reports, RM is less expensive than other protein sources, widely available all over the world, and of high biological value. Therefore, this study was conducted to evaluate the use of RM as a partial replacement for FM in the diet consumed by aquaculture fish. We examined growth performance, profitability measures, antioxidant status, serum biomarkers, gut morphology, and water quality in two such species, *O. niloticus* and *S. galilaeus*.

Materials and methods

Fish and experimental diet design

All experimental procedures, management conditions, handling, and sampling were approved by the Institutional

Animal Care and Use Committee Research Ethics Board, Faculty of Veterinary Medicine, Benha University, under ethical number BUFVTM 03-12-2020, and ethical approval of Taif University (42-0081). Fingerlings were obtained from a private fish farm in Kafr El-Sheikh Governorate, Egypt, and transported to the laboratory in double polyethylene bags supplied with oxygen (Amlacher et al. 1970). Fish were kept in aerated fiberglass tanks (750 L) for 2 weeks allow acclimatization to aquarium conditions and fed a commercial tilapia diet (30% CF). The water parameters were adjusted as follows: water temperature, 28 °C; dissolved oxygen, 6 mg/L; ammonia concentration, 0.53 mg/L; and pH, 7. The water was changed twice weekly at rate of 30%. After the 2 weeks of acclimatization to aquarium conditions, 960 healthy *O. niloticus* and *S. galilaeus* fingerlings (480 fingerlings of each species) with no previous history of disease underwent external inspection before being assigned to experimental groups (Klemm et al. 1993). Fingerlings with an average weight of 10.02 g and a length that ranged from 6.00 to 6.02 cm (for *O. niloticus* and *S. galilaeus*, respectively) were randomly distributed into four dietary treatment groups in 750 L tanks (triplicate design, 120 fingerlings/group, and 40 fingerlings/replicate). Aerators (RS Electrical, China) and multifunction internal filters were included in each tank and ~ 30% of the water in the tanks was replaced with fresh dechlorinated water (water stored overnight) 2–3 times a week. A dissolved oxygen meter placed at a distance of 10 cm below the water surface was used to measure the dissolved oxygen (DO) and temperature in each tank (El-Kassas et al. 2020).

The diets were formulated to meet the nutrient requirements of tilapia according to the National Research Council (Council 1993); the composition of diets used in the feeding trial is displayed in Table 1. The RM was obtained from a local company in Al Qalyubiyah Governate, Egypt; its chemical analysis has been described in a previous study (Jahan et al. 2013). In our study, RM was evaluated at four inclusion levels of 0%, 7%, 14%, and 21% used to replace 0%, 10%, 20%, and 30% FM, respectively.

The fish feeds were prepared by thoroughly mixing all components for 15 min, and then adding oil and water to form a moist, doughy mass. The dough mass was then pelleted without using steam to produce sinking pellets 2 mm in diameter. The pellets were finally dried at room temperature according to the methods of (He et al. 2016) and stored in clean, sterile plastic bags at -20 °C until use.

Table 1 Ingredients and proximate composition of the experimental diets

Ingredients	Basal diet	10%	20%	30%
Yellow corn	44.225	40.225	36.225	32.185
Soy bean meal 46%	18.40	18.40	18.40	18.40
Rapeseed meal	0	7	14	21
Fish meal 60% protein	30	27	24	21
Vegetable oil	2.60	2.60	2.60	2.60
Fish oil	2.20	2.20	2.20	2.20
Molasses	2	2	2	2
Vitamin and mineral premix	0.5	0.5	0.5	0.5
Choline chloride	0.05	0.05	0.05	0.05
Vitamin C	0.025	0.025	0.025	0.025
D L methionine	00,000	0.02	0.03	0.04
Total	100	100	100	100
Nutrient specification		Chemical analysis		
Dry matter	88.8	88.86	89.1	89.12
Crud protein	29.82	29.78	29.77	29.76
Crude fat	9.36	9.69	10	10.4
Crude fiber	2.23	2.85	3.2	4
Ash	8.22	8.09	7.91	7.74
Gross energy kcal/kg diet	4325	4326	4328	4326
Lysine	1.96	1.95	1.95	1.95
Methionine	0.7	0.7	0.7	0.7

Vitamin premix supplied each kg of feed with Vitamin A = 7000 IU; Vitamin D = 1400 IU; Vitamin E = 10 mg; vitamin K3 3 mg; vitamin B1 1 mg; vitamin B2 4 mg; Vitamin B12 0.01 mg; Folic Acid 1 mg; Niacin 20 mg; Pantothenic acid 8 mg; Biotin 0.025; vitamin B6 1 mg; Copper 10 mg; Cobalt 0.01 mg; Iron 15 mg; Zinc 40 mg; Selenium 0.01 mg; Manganese 40 mg; Iodine 0.05 mg

Proximate composition

The composition of the RM and diets were measured according to standard AOAC methods (AOAC 2000). Dry matter was estimated using a hot air oven; crude protein (N × 6.25) using the Kjeldahl method: ether extract using the Soxhlet method with ether extraction. Ash was measured by incineration at 550 °C for 16 h in a muffle Furance 6000 (Thermolyne, USA). The crude fiber (CF) was measured using Fibretherm (Gerhardt, Germany). The glucosinolate level in RM was measured as explained previously (Gardrat and Prevot 1987). The chemical composition of RM is shown in Table 2.

Determination of growth performance

Fish were counted and weighed biweekly for 12 weeks using a digital balance. The fish were fasted for 6 h before being weighed, and the fish in each tank were dried with clean, sterile filter paper to eliminate excess water before being weighed. Daily feed intake was tracked by collecting the remaining food, allowing it to air dry, and then subtracting the amount collected from the amount of feed provided. Fish were given the feed manually at the rate of 5% of body weight twice daily (8:00 am and 4:00 pm).

The growth performance was determined, including initial body weight, final body weight, weight gain (WG) = Average final body weight − Average of initial weight, and weight gain rate (%) = (Average body weight − Average initial body weight)\Average of initial body weight according to a method described previously (El-Kassas et al. 2020). The specific growth rate (SGR) = (Ln Final weight − Ln Initial weight)/(No of days in trial) × 100, initial length, final length of fish (as measured from the tip of the mouth to the tip of the caudal fin using a graduated ruler), length gain = Average final body legth – Average initial body length, feed conversion ratio (FCR) = Feed given (dry wt)\Weight gain (wet weight) as described previously (Elabd et al. 2020). The intestine somatic index (ISI) = (Wet weight of intestine(g))/(Wet body weight(g)) × 100 and hepatosomatic index (HSI) = (liver weight(g))/(body weight(g)) × 100 were calculated according to a standard method (Ramos et al. 2017).

Table 2 Chemical composition of rapeseed meal

Item	Percentage
Dry matter	89
Moisture	11
Crude protein	31
Ether extract	12.1
Crude fiber	11.2
Ash	7
Glucosinolates (µmol g–1)	115

Determination of the profitability measures

Profitability measures for each 100 fish per dollar (each dollar = 15.70 Egyptian pound (LE)) include different costs and return parameters. Different costs include total fixed cost (TFC), total variable cost (TVC), and total cost (TC). Different costs were measured according to the equations: Total cost (TC) = total fixed cost (TFC) + total variable cost (TVC).

TFC equals the value of the equipment/age of the equipment, which was then divided by the number of fish in each year to determine the depreciation value of the equipment for each fish. For this experiment, TFC equaled US$ 0.38 for each 100 fish.

TVC includes the price of the fish (about US$ 0.64) (100 fish multiplied by the value of each fingerling), feed cost (feed consumption per gram multiplied by the price of 1 g feed of each experimental diet), and the management costs, which include water, electricity, labor, and disinfectant (US$ 0.57).

Return parameters include total returns (TR), net profit (NP), the benefit–cost ratio (BCR), TR/TVC, and the return on investment (NP/TC) and NP/TVC where TR = weight of 100 fish per gram × the market price of each gram (US$ 0.00203) NP = TR-TC (Phiri and Yuan 2018), and the Benefit–Cost Ratio (BCR) = TR/TC (Adeniyi et al. 2015) where TR = Total Return and TC = Total Cost. The return on investment = net profit/TC (NP/TC) and NP/TVC = NP/TVC.

Relative efficiency measures include relative return = (TR of treated group ÷ TR of control group) × 100; relative net profit = (NP of treated group ÷ NP of control group) × 100; relative feed cost = (feed cost of treated group ÷ feed cost of control group) × 100; cost of each kg fish from feed (total feed cost ÷ body weight per kilogram), and the cost of each kg fish gain from feed = (feed cost ÷ body weight gain per kilogram) (Soltan et al. 2008).

Sample collection for serum biochemical analysis and oxidative stress

At the end of the 12-week feeding trial, the tilapia were sacrificed by addition of an overdose of anesthetic solution (MS 222; $NaHCO_3$ and tricaine methane sulfonate purchased from Sigma Aldrich Chemicals, CO, USA) in a dilution of 1:4000 in dechlorinated water for 2 min. Tilapia were exsanguinated by caudal vein and heart puncture using 3 mL capacity syringes. The blood was allowed to clot for 2 h at 4 °C to separate the serum. The serum was further separated

by centrifugation at 3500 × *g* for 25 min at 4 °C. The samples were stored at -80 °C until used in subsequent analysis.

The fish were dissected and liver samples collected in clean, sterile microcentrifuge tubes containing phosphate buffer saline (PBS). Next, the liver samples were homogenized using cool PBS with (pH 7.4) at ratio of 1:10 (w/v) using an electrical homogenizer (Heidolph, Germany). This procedure was performed on ice, and then the homogenates were centrifuged at 4 °C at 4000 × *g* for 15 min. The supernatants were stored at -20 °C.

Determination of digestive enzymes and oxidative stress biomarkers

The antioxidant enzyme activities of superoxide dismutase (SOD), glutathione peroxidase (GPx), and malondialdehyde (MDA) were determined and calculated as previously described (Fossati et al. 1980) (Satoh 1978). All enzyme activities were measured using Bio-diagnostic kits from Bio-diagnostic Company (Dokki, Giza, Egypt). Serum amylase and lipase digestive enzymes were estimated using ELISA kits (Cusabio Biotech Co. Ltd., China). Serum and liver biomarkers were measured spectrophotometrically with an automated analyzer (SPOTCHEM TM EZ model SP-4430, Arkray, Inc. Kyoto, Japan).

Histological examination and morphometric assessment of intestinal villi

Samples of intestinal villi ~ 2.5 cm long were collected from the middle segment of the intestine (the segment between the proximal and distal segments) from 6 fish per group. The tissues were fixed in phosphate-buffered formalin for at least 24 h. The samples were then dehydrated and rinsed several times in absolute alcohol and embedded in paraffin. Serial 5-µm longitudinal sections were cut using a Leica Rotary Microtome (RM 2145, Leica Microsystems, Wetzlar, Germany) and mounted on glass slides. The slides were routinely stained with hematoxylin and eosin (H&E) (Bancroft and Stevens 1996). Image J software for image processing and analysis, version 1.93 (National Institutes of Health, USA) was used to perform histomorphometry. The length and width of intestinal villi, as well as the whole mucosal length, were used as the intestinal morphometric variables. The villus length was measured from the villus tip to the villus-crypt junction, while the villus width was measured at the midpoint of the villus length. The whole mucosal length was considered the distance from the top of the villus to the muscularis mucosa. The number of goblet cells per unit of surface area (mm^2) was used to measure the density of goblet cells (Al-Deriny et al. 2020).

Water quality analysis

Water quality analysis was carried out every 2 weeks. Each tank was supplied with an aerator. About 30% of the water in the tanks was replaced by new water twice per week (El-Kassas et al. 2020). Samples were collected in the morning in triplicate over 3 separate days. Parameters were assayed based on the methods of Lloret et al. (Lloret et al. 2005; Riche and Garling 2003) using a photometer, pH meter, and ammonia analyzers (Nessler's method).

Statistical analysis

The effects of the experimental diets were tested using a two-way analysis of variance (ANOVA) to determine the effect of species, RM levels, and the interaction between species and RM levels using SPSS statistical software (version 16 for Windows). Orthogonal polynomial contrasts were done to determine whether the effect of the RM level was linear and/or quadratic (Rosales et al. 2017). Tukey's multiple comparison test was used to determine the significance of different treatment groups for both tilapia species (MSTAT program). Differences were considered statistically significant when $p \leq 0.05$ and highly significant when $p \leq 0.01$ (Spss 2007). In addition, to determine the dose–response curve, polynomial regression was done between the RM level and different parameters. The results are presented as mean ± SD (residual standard deviation).

Results

Growth performance

The results shown in Table 3 demonstrate that dietary replacement of FM with RM at a level of 20% resulted in a significant increase in weight gain, length gain, weight gain rate, and specific growth rate compared to replacement of FM with 30% RM in *S. galilaeus*. On the other hand, *O. niloticus* fed a diet replaced with different levels (0%, 10%, 20%, and 30%) of RM showed a significant difference in the final weight, weight gain, final length, length gain, weight gain rate, FCR, and SGR. The significantly higher values were recorded for *O. niloticus* fish that received the diet replaced with 10% RM. In the *S. galilaeus* groups, no significant difference was observed in the feed intake among the groups fed diets replaced at all four levels (0%, 10%, 20%, and 30%) of RM. However, in the *O. niloticus* groups, there was a significant difference among the different groups that received RM at different levels. The significant highest feed intake was consumed by the group that received the diet replaced with 10% RM.

Table 3 Effect of tilapia species and\ or rapeseed meal level on Growth performance among *S. galilaeus and O. niloticus*

Items	*S. galilaeus*				*O. niloticus*				SD	p value			
	Rapeseed level				Rapeseed level					Species	Rapeseed level		species × rapeseed level
	0%	10/%	20%	30%	0%	10/%	20%	30%			linear	quadratic	
Initial weight (gm)	10.02	10.01	10.04	10.00	10.00	10.03	10.01	10.02	1.14	NS	NS	NS	NS
Final weight	34.13^{c}	36.94^{bc}	38.48^{bc}	32.72^{c}	41.19^{b}	52.22^{a}	35.7^{bc}	37.72^{bc}	3.51	**	*	**	**
Weight gain (gm)	24.11^{cd}	26.93^{bcd}	28.44^{bc}	22.72^{d}	31.19^{b}	42.19^{a}	25.71^{cd}	27.70^{bc}	2.78	**	**	**	**
initial length (cm)	6.03	6.00	6.01	6.02	5.94	6.01	6.02	6.03	0.34	NS	NS	NS	NS
Final length (cm)	11.74^{c}	12.62^{bc}	13.49^{b}	12.09^{bc}	12.91^{bc}	15.38^{a}	12.26^{bc}	13.10^{bc}	0.90	*	NS	*	*
Length gain (cm)	5.71^{d}	6.62^{bcd}	7.48^{b}	6.07^{cd}	6.97^{bc}	9.37^{a}	6.24^{cd}	7.07^{bc}	0.65	**	NS	**	**
Feed amount	58.67^{c}	63.33^{bc}	66.24^{bc}	55.73^{c}	74.90^{b}	110.90^{a}	63.66^{bc}	65.31^{bc}	8.11	**	**	**	**
FCR	2.42^{b}	2.35^{b}	2.33^{b}	2.45^{ab}	2.41^{b}	2.62^{a}	2.47^{ab}	2.36^{b}	0.11	NS	NS	NS	NS
Weight Gain Rate	240.6^{cd}	269.51^{bcd}	288.39^{bc}	227.54^{d}	315.55^{b}	423.42^{a}	256.61^{c}	276.04^{bcd}	0.09	**	*	**	**
SGR	1.46^{cd}	1.56^{bcd}	1.61^{bc}	1.41^{d}	1.69^{b}	1.97^{a}	1.51^{cd}	1.57^{bcd}	0.33	**	*	**	**
ISI	9.24^{bc}	6.16^{d}	7.51^{cd}	7.55^{cd}	10.52^{ab}	10.54^{ab}	12.22^{a}	9.26^{bc}	1.62	*	NS	NS	*
HIS	2.07^{ab}	1.72^{b}	1.37^{b}	2.11^{ab}	2.44^{ab}	3.04^{a}	2.22^{ab}	1.98^{ab}	0.70	**	NS	NS	NS

Means carrying a-b-c within the same row are significantly different ($P \leq 0.05$) between different rapeseed replacements among different species. FCR: Feed conversion ratio; SGR: Specific growth rate; ISI: Intestinal Somatic Index; HIS: Hepato Somatic Index. NS, not significant ($p > 0.05$); * $p \leq 0.05$; ** $p \leq 0.01$

Increasing replacement of FM with RM by more than 20% in *S. galilaeus* and to more than 10% in *O. niloticus* diets resulted in a significant decrease in weight gain, final length, length gain, total feed, weight gain rate, and SGR. *S. galilaeus* fish that received the control diet (0% RM) had a higher value for the intestine somatic index (ISI) than fish that received the diets replaced with 10%, 20%, or 30% RM. The lowest value of ISI was recorded in the group fed the diet replaced with 10% RM. However, in *O. niloticus*, the group fed a diet replaced with 30% RM showed the lowest ISI value.

The hepatosomatic index (HSI) of *S. galilaeus* fish that received a control diet showed a non-significant higher value compared with those fed diets replaced with 10% and 20% RM. In *O. niloticus*, the higher value of HSI was recorded in fish fed the diet replaced with 10% RM. Taking into account the species difference, there was a highly significant difference ($p \leq 0.01$) between *O. niloticus* and *S. galilaeus* in final weight, weight gain, feed intake, SGR, weight gain rate, HIS, and ISI.

Profitability measures

The results shown in Table 4 represent the price of a kilogram of feed and different cost parameters including the total fixed cost (TFC), total variable cost (TVC) (which includes feed cost, fish price, and management cost) and total cost (TC), and different return parameters including the total return (TR), net profit (NP), benefit–cost ratio (TR/TC), total return/total variable cost (TR/TVC), net profit/total cost (NP/TC), and net profit/total variable cost (NP/TVC).

The cost of a kilogram of feed decreased as the value of the RM replacement increased, as the highest cost of a kilogram of feed was found in the control group (US$ 0.72) and the lowest was in the group fed the diet replaced with 30% RM (US$ 0.60). With respect to the cost of feed, the TVC and TC in both tilapia species decreased in the diets in which FM was replaced by RM by 20% and 30%. Moreover, the lowest values in both species were recorded for the diet replaced with 30% RM (the feed cost values were US$ 3.34 and 3.91, the TVC values were US$ 4.55 and 5.12, and the TC values were US$ 4.93 and 5.50 for *S. galilaeus* and *O. niloticus*, respectively), followed by the diet replaced with 20% RM of *O. niloticus*. The difference in species did have an effect; there was a highly significant difference ($p \leq 0.01$) in feed cost, TVC, and TC between *S. galilaeus* and *O. niloticus.*

The TR varies among different RM replacement levels for *S. galilaeus.* The TR increases with RM replacement between 10 and 20%; the highest value was recorded in 20% RM replacement (US$ 7.84) followed by 10% (US$ 7.53). For *O. niloticus*, the replacement of fish meal with RM by 10% resulted in increased TR (US$ 10.64), which attained the best return among different RM replacement for both

Table 4 Effect of tilapia species and\ or rapeseed meal level on profitability measures among *S. galilaeus and O. niloticus*

Items	*S. galilaeus*				*O. niloticus*				*SD*	p value			
										Species	Rapeseed level		species × rapeseed level
	Rapeseed level				Rapeseed level						linear	linear	
	0%	10/%	20%	30%	0%	10/%	20%	30%					
Feed price (US$ per Kg)	0.72	0.68	0.64	0.60	0.72	0.68	0.64	0.60					
TFC (US$)	0.38	0.38	0.38	0.38	0.38	0.38	0.38	0.38					
Management cost (US$)	0.57	0.57	0.57	0.57	0.57	0.57	0.57	0.57					
Fish price (US$)	0.64	0.64	0.64	0.64	0.64	0.64	0.64	0.64					
Feed cost (US$)	4.25^{cd}	4.32^{c}	4.24^{cd}	3.34^{d}	5.42^{b}	7.57^{a}	4.08^{cd}	3.91^{cd}	0.55	**	**	**	**
TVC (US$)	5.46^{cd}	5.53^{c}	5.45^{cd}	4.55^{d}	6.63^{b}	8.78^{a}	5.29^{cd}	5.12^{cd}	0.55	**	**	**	**
TC (US$)	5.84^{cd}	5.92^{c}	5.84^{cd}	4.93^{d}	7.02^{b}	9.16^{a}	5.67^{cd}	5.50^{cd}	0.55	**	**	**	**
TR (US$)	6.96^{c}	7.53^{bc}	7.84^{bc}	6.67^{c}	8.40^{b}	10.64^{a}	7.28^{bc}	7.69^{bc}	0.71	**	*	**	**
Net profit (US$)	1.11^{d}	1.61^{bc}	2.01^{ab}	1.74^{abc}	1.38^{cd}	1.48^{cd}	1.61^{bc}	2.19^{a}	0.28	NS	**	NS	NS
TR/ TC	1.19^{e}	1.27^{cd}	1.34^{abc}	1.35^{ab}	1.20^{de}	1.16^{e}	1.29^{bc}	1.40^{a}	0.04	NS	**	NS	*
TR / TVC	1.27^{d}	1.36^{c}	1.44^{ab}	1.47^{a}	1.27^{d}	1.21^{d}	1.38^{bc}	1.50^{a}	0.04	*	**	*	*
Net profit / TC	0.19^{e}	0.27^{cd}	0.34^{abc}	0.35^{ab}	0.20^{de}	0.16^{e}	0.29^{bc}	0.40^{a}	0.04	NS	**	NS	*
Net profit / TVC	0.20^{d}	0.29^{c}	0.37^{ab}	0.38^{ab}	0.21^{d}	0.17^{d}	0.31^{bc}	0.43^{a}	0.04	NS	**	NS	*

Means carrying a-b-c within the same row are significantly different ($P \leq 0.05$) between different rapeseed replacements among different species. TFC (total fixed cost), Management cost (water, electricity, labor and disinfection); TVC: total variable cost; TC: total cost; TR: total return. NS, not significant ($p > 0.05$); * $p \leq 0.05$; ** $p \leq 0.01$

species; the difference among 20%, 30%, and 0% RM was not significant. Taking into account the species difference, there was a highly significant difference ($p \leq 0.01$) between *O. niloticus* and *S. galilaeus* for TR.

In both tilapia species, the different economic efficiency measures such as TR/TC, TR/TVC, NP/TC, and NP/TVC increased with RM replacement between 20 and 30% compared to 0% replacement (control). The difference was not significant between 0 and 10% RM diet replacement in *O. niloticus*. Among the different RM replacement groups, the groups with 30% RM replacement for both species recorded the highest TR/TC, TR/TVC, NP/TC, and NP/ TVC, followed by 20%, then 10% RM replacement. The species effect showed a non-significant difference ($p > 0.05$) in the economic efficiency represented in TR/TC, NP/TC, and NP/ TVC.

Regarding the relative economic efficiency (Table 5) represented in relative feed cost, relative total return, and relative net profit, we found that the relative feed cost showed a significant decrease in the groups fed diets with 20% and 30% RM replacement. In contrast, the relative return showed a significant increase in the *O. niloticus* group fed the diet replaced with 10% RM, and a significant increase in the relative net profit for all 3 different replacement levels in S. *galilaeus* and for the diets replaced with 20% and 30% in *O. niloticus* compared to the control group. Moreover, the feed cost for each kg body weight gain showed a significant decrease in diets with RM replacement between 20 and 30% and the lowest value in the groups fed the diet replaced with 30% RM. From these data, we conclude that RM replacement of FM up to 30% improves economic efficiency parameters.

Determination of digestive enzymes and oxidative stress biomarkers

The ALT and ADT levels in serum and liver of *O. niloticus* and *S. galilaeus* are shown in Tables 6 and 7, while serum glucose, amylase, and lipase enzymes are shown in Table 6. The groups fed diets replaced with 20% and 30% RM had increased AST and ALT levels ($p \leq 0.01$) relative to those fed diets replaced with 10% RM and the control diet. In contrast, serum glucose concentrations showed a significant increase ($p \leq 0.05$) in the group fed the diet replaced with 10% RM compared to the control and other treated groups of *S. galilaeus*. However, the difference between groups fed the diet replaced with 10% and 20% RM and the control groups of *O. niloticus* was not significant. Serum amylase and lipase showed a significant reduction in the groups fed with the diet replaced with 20% and 30% RM in *O. niloticus* and *S. galilaeus* compared to the diet replaced with 10% RM and the control diets. A highly significant ($p \leq 0.01$) change

Table 5 Effect of tilapia species and\ or rapeseed meal level on relative profitability measures among *S. galilaeus and O. niloticus*

Items	*S. galilaeus*				*O. niloticus*				*SD*	P value			
	Rapeseed level				Rapeseed level								
	0%	10/%	20%	30%	0%	10/%	20%	30%		Species	Rapeseed level		species × rapeseed level
											linear	quadratic	
Relative TR (%)	100.00^{bcd}	108.19^{bc}	112.68^{ab}	95.82^{cd}	100.00^{bcd}	126.71^{a}	86.66^{d}	91.53^{d}	9.46	NS	*	**	**
Relative Net Profit (%)	100.00^{d}	145.32^{abc}	180.68^{a}	156.77^{ab}	100.00^{d}	107.17^{cd}	116.55^{bcd}	158.44^{a}	23.97	*	**	NS	NS
Relative feed cost (%)	100.00^{b}	101.75^{b}	99.87^{b}	78.51^{c}	100.00^{b}	139.71^{a}	75.26^{c}	72.14^{c}	11.24	NS	**	**	**
Feed cost/BW (US$)	1.24^{bc}	1.17^{cd}	1.10^{de}	1.02^{e}	1.32^{b}	1.45^{a}	1.14^{d}	1.03^{e}	0.05	**	**	**	**
Feed cost/BWG (US$)	1.76^{a}	1.61^{b}	1.49^{bcd}	1.47^{cd}	1.74^{a}	1.79^{a}	1.58^{bc}	1.41^{d}	0.07	NS	**	NS	*

Means carrying a-b-c within the same row are significantly different ($P \leq 0.05$) between different rapeseed replacements among different species. TR: total return; BW: body weight per kg; BWG: body weight gain per kg. NS, not significant ($p > 0.05$);* $p \leq 0.05$; ** $p \leq 0.01$

Table 6 Effect of tilapia species and\ or rapeseed meal level on serum biochemical parameters among *S. galilaeus and O. niloticus*

Items	*S. galilaeus*				*O. niloticus*				SD	P value			
	Rapeseed level				Rapeseed level								
	0%	10%	20%	30%	0%	10%	20%	30%		Species	Rapeseed level		species × rapeseed level
											linear	quadratic	
AST (U/L)	156.32^{e}	181.32^{de}	346.67^{ab}	381.95^{a}	167.75^{e}	173.69^{e}	242.82 ^{cd}	303.13^{bc}	35.66	**	**	NS	*
ALT (U/L)	75.31^{c}	74.36^{c}	107.92^{b}	130.43^{a}	58.15^{d}	57.40^{d}	83.32^{c}	99.18^{b}	8.14	**	**	**	NS
Glucose (mg/dl)	86.84^{bc}	117.56^{a}	71.27^{c}	71.72^{c}	88.63^{bc}	112.02^{ab}	84.67^{bc}	75.94^{c}	16.38	NS	*	*	NS
Amylase (U/L)	10.33^{a}	9.89^{a}	6.66^{b}	3.71^{c}	6.42^{b}	6.35^{b}	2.04^{c}	1.40^{c}	1.39	**	**	NS	NS
Lipase (U/L)	2.42^{ab}	2.78^{a}	1.26 ^{cd}	0.72 ^{cd}	3.34^{a}	3.32^{a}	1.65^{bc}	0.61^{d}	0.56	NS	**	*	NS

Means carrying a-b-c within the same row are significantly different ($P \leq 0.05$) between different rapeseed replacements among different species. NS, not significant ($p > 0.05$);* $p \leq 0.05$; ** $p \leq 0.01$

Table 7 Effect of tilapia species and\ or rapeseed meal level on liver biochemical parameters among *S. galilaeus and O. niloticus*

Items	*S. galilaeus*				*O. niloticus*				SD	P value			
	Rapeseed level				Rapeseed level								
	0%	10%	20%	30%	0%	10%	20%	30%		Species	Rapeseed level		species × rapeseed level
											linear	quadratic	
liver AST (U/L)	18.76^{c}	19.67^{c} ± 0.91	26.93^{c}	38.27^{b}	21.57^{c}	22.77^{c}	45.97^{b}	65.72^{a}	4.84	**	**	**	**
Liver ALT (N Mol/L)	15.99^{e}	16.47^{e} ± 2.11	32.23^{c}	42.57^{b}	15.63^{e}	15.55^{e}	25.61^{d}	53.74^{a}	2.96	NS	**	**	**
GPX (Ng/gm)	110.72^{a}	110.54^{ab}	58.45^{de}	48.53^{e}	96.51^{c}	97.83^{bc}	66.44^{d}	46.68^{e}	7.42	NS	**	*	NS
SOD (U/g)	75.10^{a}	76.87^{a}	35.36^{b}	11.62^{c}	69.19^{a}	68.54^{a}	43.87^{b}	18.53^{c}	6.89	NS	**	**	NS
MDA (N Mol/L)	28.21^{d}	27.92^{d}	45.17^{bc}	55.76^{a}	24.97^{d}	27.11^{d}	40.75^{c}	52.18^{ab}	4.56	NS	**	*	NS

Means carrying a-b-c within the same row are significantly different ($P \leq 0.05$) between different rapeseed replacements among different species. NS, not significant ($p > 0.05$);* $p \leq 0.05$; ** $p \leq 0.01$

was detected for AST, ALT, and amylase levels between *S. galilaeus and O. niloticus.*

The antioxidant activities, liver SOD, and GPx enzymes for *O. niloticus* and *S. galilaeus* are shown in Table 7. The groups fed diets replaced with 20% and 30% RM showed a significant reduction in SOD and GPx ($p < 0.05$) when compared to groups fed the diet replaced with 10% RM and the control diets. The groups fed diets replaced with 20% and 30% RM showed a significant increase ($p < 0.05$) in MDA compared with those fed diets replaced with 10% RM and the control diets.

Histopathology and morphometrical assessment of intestinal villi absorptive capacity

As shown in Table 8 and Fig. 1, plates 1 and 2, mucosal length and intestinal villi length were significantly increased in all groups fed on diets replaced with different RM levels compared their corresponding control groups. Dietary replacement of a portion of the FM by RM increased the number of goblet cells in all experimental groups compared with their relative control groups. The mucosal length, villus width, and villus length were significantly different ($p \leq 0.05$) in *S. galilaeus* compared to *O. niloticus* and the goblet cells were highly significantly different ($p \leq 0.01$) between the two species.

Water quality

For the duration of the study, the water temperature ranged from 30.59 °C in the first weeks to 22.18 °C at the end of the experimental period. No significant difference was observed between the two species fed either control meal (FM) or replacement RM (10%, 20%, and 30%) meals except in the sixth week (Table 9). The pH values (shown in Table 9) were monitored throughout the duration of the study and ranged from 6.83 to 8.65 without any significant difference among the groups fed diets replaced with 0%, 10%, 20%, and 30% RM.

The water ammonia levels ranged from 0.06 to 0.74 mg/L and showed no significant difference among the species fed diets replaced with 0%, 10%, 20%, and 30% RM (Table 10). Water turbidity ranged from 35.00 to 80.00 cm and showed a significant difference between the two tilapia species fed diets replaced with 0%, 10%, 20%, and 30% RM. The turbidity levels were relatively constant until the eighth week at which point they gradually increased (Table 10).

The measured conductivity reported in Table 11 ranged from 25.10 to 49.90 ms/cm; the values showed a significant difference between the groups fed diets replaced with 0%, 10%, 20%, and 30% RM. The average dissolved oxygen (DO) levels ranged from 4.15 to 6.53 mg/L during the entire 12-week study period (Table 11). The values for dissolved

Table 8 Effect of tilapia species and\ or rapeseed meal level on intestinal health among *S. galilaeus and O. niloticus*

Items	*S. galilaeus*				*O. niloticus*				*SD*	*P value*			
	Rapeseed level				Rapeseed level								
	0%	10%	20%	30%	0%	10/%	20%	30%		Species	Rapeseed level		species × rapeseed level
											Linear	quadratic	
Mucosal length	296.86^{e}	403.98^{d}	544.63^{b}	483.47^{c}	383.75^{d}	460.98^{c}	604.44^{a}	506.55^{bc}	52.33	*	**	**	*
villi length	225.90^{e}	357.91^{d}	507.56^{ab}	434.43^{c}	340.06^{d}	408.24^{cd}	568.44^{a}	470.78^{bc}	63	*	**	**	*
villi width	71.85^{ab}	61.96^{bc}	64.10^{bc}	55.44^{c}	82.32^{a}	77.17^{ab}	75.70^{ab}	66.58^{abc}	13.67	*	NS	NS	*
Goblet cells	23.00^{d}	26.67^{c}	39.67^{a}	33.00^{b}	12.67^{f}	16.33^{e}	26.00^{cd}	25.00^{cd}	3.13	**	**	**	*

Means carrying a-b-c within the same row are significantly different ($P \leq 0.05$) between different rapeseed replacements among different species. NS, not significant ($p > 0.05$);* $p \leq 0.05$; ** $p \leq 0.01$

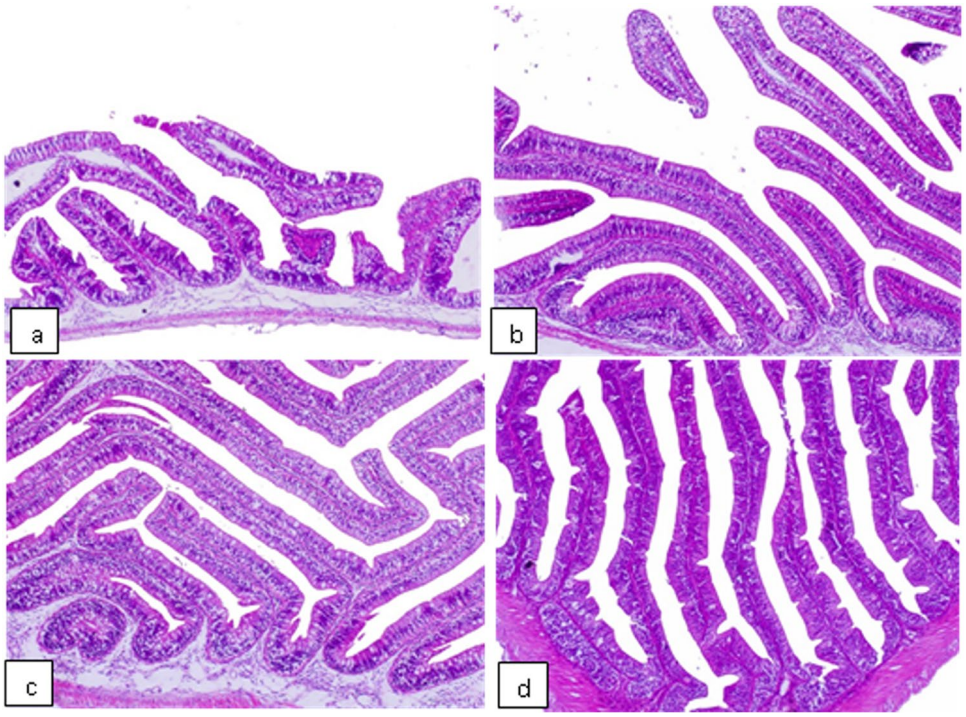

Fig. 1 plate 1. Effect of the replacement of a portion of the FM in fish feed by RM on histopathology and morphometrical assessment of the small intestine among *S. galilaeus* and *O. niloticus*. Plate 1(b): length, H&E, × 100, scale bar = 100 µm; **(c)**, intestine (jejunum) from the 20% RM group of *S. galilaeus* showing a marked increase in the length of the intestinal villi, H&E, × 100, scale bar = 100 µm; **(d)**, intestine (jejunum) from the 30% RM group of *S. galilaeus* showing a marked increase in the length of the intestinal villi, H&E, × 100,scale bar = 100 µm. **plate 2.** Effect of the replacement of a portion of the FM in fish feed by RM on histopathology and morphometrical assessment of the small intestine among *S. galilaeus* and *O. niloticus*. **(a)**, intestine (jejunum section) from the control group of *O. niloticus* showing normal villi, H&E, × 100, scale bar = 100 µm; **(b)**, intestine (jejunum section) from the 10% RM group of *O. niloticus* showing an increase in the length of the intestinal villi, H&E, × 100, scale bar = 100 µm; **(c)**, intestine (jejunum) from the 20% RM group of *O. niloticus* showing an increase in the length of the intestinal villi, H&E, × 100, scale bar = 100 µm; **(d)**, intestine (jejunum) from the 30% RM group of *O. niloticus* showing a marked increase in the length of the intestinal villi, H&E, × 100, scale bar = 100 µm

oxygen showed no significant difference between the two tilapia species fed diets replaced with 0%, 10%, 20%, and 30% RM during the growth period. The results in Table 12 indicate that the average coefficient of regression was 0.686 + ,-0.911, 0.659 + , 0.596 + and -0.523 for net profit, SOD, villi length, goblet cell, and ammonia, respectively.

Discussion

Compared with other plant proteins, RM is one of the most successful protein sources used in aquaculture feeds. RM has a relatively higher protein content, varying from 320 to 450 g/kg of dry matter (Burel et al. 2000a, b), with a favorable amino acid profile, and is rich in minerals, vitamins, and other microelements (Luo et al. 2012a). Despite these favorable attributes, RM has many anti-nutritional factors (ANF), such as various protease inhibitors, tannins, phytic acid, and glucosinolates (GLS), that limit its utility in aquaculture feeds. Because its nutritional composition clearly complements that of FM, with a comparatively balanced amino acid profile, availability, and fair price, soybean meal (SBM) has been recognized as one of the most suitable alternative protein sources for use as a replacement for a portion of FM in aquafeed (Wang et al. 2017).

In our study, replacing the FM in the diets consumed by both *O. niloticus* and *S. galilaeus* with 10% and 20% RM, respectively, resulted in increasing in the final weight, weight gain, length, length gain, weight gain rate, and SGR compared to the control groups (which received 0% RM). The increasing was not statistically significant in the case of *S. galilaeus* but was in *O. niloticus*. Increasing the replacement of FM with RM more than 10% or 20% in the diets of *O. niloticus* and *S. galilaeus*, respectively, resulted in a significant decrease in weight gain, length, length gain, total feed, SGR, and weight gain rate. Our results agree with those of others (Bu et al. 2018), who noted that fish fed diets in which the FM was replaced by 30% or more RM experienced a significant decrease in final body weight, weight gain, and SGR ($p < 0.05$). In agreement with this, (Davies et al. 1990)

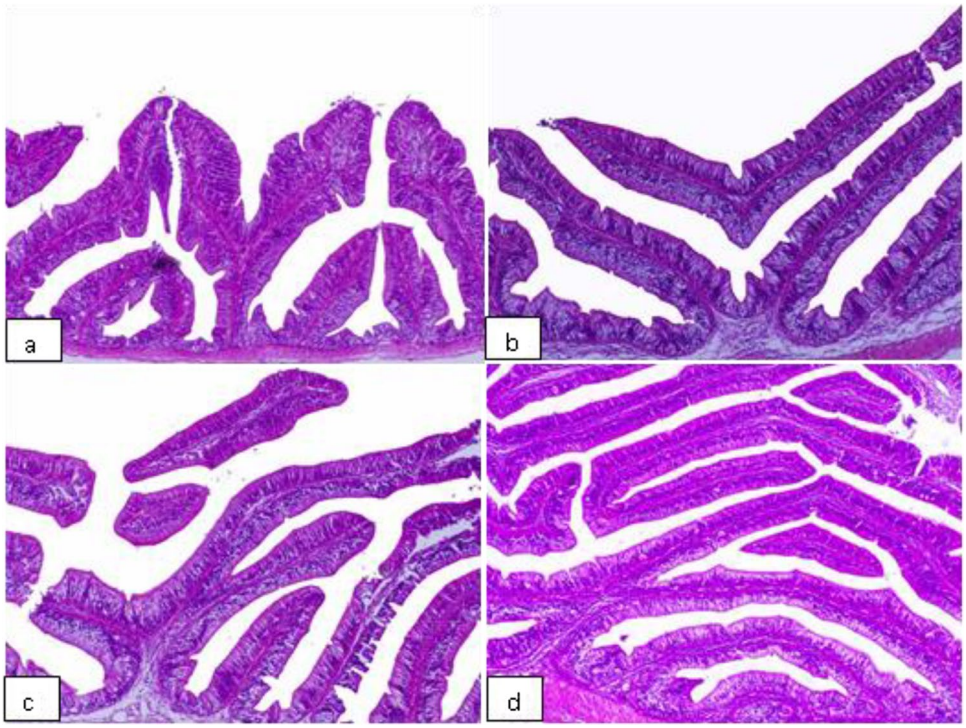

Fig. 1 (continued)

reported that limiting the inclusion of RM to ~ 15% when using it as a substitute for FM was good for growth performance and profitability measures. Others have confirmed a decline in feed intake and growth in proportion with the increasing levels of rapeseed cake added to the diet (Nemati 2014).

In reviewing our results, we found a significant decrease in the price of kg feed, total feed cost, total cost, total variable cost, and cost of kg feed per kg body weight and per kg body weight gain and relative feed cost in fish fed diets replacing 20% and 30% RM compared to the control groups. These results were due to the replacement of FM with inexpensive RM (Hardy 2010). Others found that partial or complete replacement of the FM in fish feed with a plant protein resulted in a reduction in production costs (Salze et al. 2010; Zhou and Yue 2010). Additionally, replacement of FM with RM resulted in improved returns parameters, as the total return and net profit and other economic efficiency measures, such as relative return, improved up to 20% in *S. galilaeus* and up to 10% in *O. niloticus.* Relative profits also improved with RM replacement due to increased body weight and a reduction in the cost of feed. These observations were in agreement with those of (Bu et al. 2018), who found no significant difference between the control group and groups fed a diet replaced with 10% and 20% RM, but significant decreases in the group fed a diet replaced with 30% RM. Similar findings were reported by (SLAWSKI et al. 2011; Tan et al. 2013) who reported no difference in body weight between the control group and fish fed diets with 25% reduced FM content achieved by inclusion of rapeseed protein concentrate.

AST and ALT are considered critical markers for protein utilization and/or ammonia excretion (Li et al. 2020; Solomon 2014; Zhang et al. 2019). In our study, groups fed diets replaced with 20% and 30% RM showed an increase ($p < 0.05$) in AST and ALT levels compared to the group fed a diet replaced with 10% RM and the control group. This is in agreement with (Zhang et al. 2020), who reported that both AST and ALT activities were enhanced following an increase in dietary RM inclusion level compared with groups fed diets supplemented with low amounts of RM. They concluded that high RM inclusion is associated with decreased and degraded AST and ALT activities in the blood and liver.

Serum amylase and lipase levels were significantly reduced in *O. niloticus* and *S. galilaeus* fed diets replaced with 20% and 30% RM compared to the groups fed diets replaced with 10% RM and the control diets. These results coincide with those of previous studies, which reported that the levels of amylase and lipase declined when the RM inclusion levels increased (Bu et al. 2018; Cheng et al. 2010; Zhang et al. 2020). The RM used in this study is a common, untreated commercial ingredient that contains a large number of anti-nutritional factors (ANF), such as glucosinolates (0.60%), isothiocyanate (0.53 g/kg), oxazolidinone (0.05 g/

Table 9 Effect of tilapia species and\ or rapeseed meal level on water temperature(c°) and water PH among *S. galilaeus and O. niloticus*

Items	*S. galilaeus*				*O. niloticus*				SD	P value			
	0%	10%	20%	30%	0%	10%	20%	30%		Species	Rapeseed level		species × rapeseed level
											linear	Quadratic	
water temperature (c°)													
2nd week	30.53	30.50	30.53	30.57	30.60	30.60	30.57	30.60	0.17	NS	NS	NS	NS
4th week	28.23	27.80	28.40	27.90	28.17	27.13	27.17	27.67	0.80	NS	NS	NS	NS
6th week	26.00^{abc}	25.00^{c}	25.30^{bc}	26.17^{ab}	25.00^{c}	26.00^{abc}	27.03^{a}	25.07^{c}	0.60	NS	NS	NS	**
8th week	23.90	24.00	24.00	23.80	24.00	24.00	24.03	24.00	0.51	NS	NS	NS	NS
10th week	24.00	24.00	24.20	24.00	24.00	24.00	24.00	23.77	0.84	NS	NS	NS	NS
12th week	22.00	22.00	22.33	22.33	22.27	22.00	22.40	22.07	0.63	NS	NS	NS	NS
PH													
2nd week	6.83	7.10	7.07	7.37	7.03	7.04	7.13	7.23	0.57	NS	NS	NS	NS
4th week	6.41	6.83	6.96	7.26	6.54	6.61	6.78	6.94	1.24	NS	NS	NS	NS
6th week	7.65	7.76	7.57	7.65	7.70	7.75	7.75	7.88	0.50	NS	NS	NS	NS
8th week	7.70	7.76	7.57	7.69	7.55	7.75	7.75	7.65	0.46	NS	NS	NS	NS
10th week	7.61	7.97	7.65	7.70	7.98	7.84	7.94	7.82	0.45	NS	NS	NS	NS
12th week	8.22	8.64	8.32	8.32	8.55	8.49	8.37	8.65	0.37	NS	NS	NS	NS

Means carrying a-b-c within the same row are significantly different ($P \leq 0.05$) between different rapeseed replacements among different species. NS, not significant ($p > 0.05$);* $p \leq 0.05$; ** $p \leq 0.01$

kg), tannin (0.41%), phytic acid (0.85%), sinapine (2.55 g/kg), and sinapic acid (0.60 g/kg). It has been reported that the nutritional quality of RM largely depends on its ANF contents, and that the presence of ANF in RM has a negatively effect on the activities of digestive enzymes, digestibility of nutrients, and growth rate of fish (Wang et al. 2018).

In our study, liver SOD and GPx activities decreased with increasing dietary RM levels. Our findings are close agreement with those of other recent studies (Bu et al. 2018; Cheng et al. 2010; Zhang et al. 2020), as SOD and GPx can reduce the excessive free radicals in different livestock. MDA is the end product of lipid peroxidation, which has strong biotoxicity and can cause bodily damage (Buege and Aust 1978). Excessive free radicals cause lipid peroxidation, which could explain the significant increase in liver MDA content in the two tilapia species in the groups fed diets replaced with 20% and 30% RM.

Absorption of nutrients occurs through the intestinal villi, and the epithelial cells of the intestinal villi play a pivotal role in the digestion and absorption of nutrients (Hu et al. 2016). Both the length and thickness of the intestinal villi are essential indicators for assessing the absorbability of the small intestine (Montagne et al. 2004), which is important since dietary protein sources influence the intestinal integrity of fish (Wang et al. 2017). Our findings were differ from those of (Figueiredo et al. 2003) and (Chiang et al. 2010), who observed no differences in villus length in response to different levels of canola meal in the diets of chicks. Mucin produced by goblet cells and epithelial cells acts as a mechanical barrier, which is considered an innate defense system (Pearson and Brownlee 2005). An increase in the number of goblet cells in the small intestine may indicate an increase in mucin secretion (Walk et al. 2011), which is important since it could affect absorption.

The maintenance of water quality is essential for the optimum growth of cultured (farmed) fish. Choosing the optimal diet has a direct impact on water quality (Ali et al. 2010). Successful fish aquaculture management requires a complete

Table 10 Effect of tilapia species and\ or rapeseed meal level on ammonia (mg\L) and turbidity (cm) of water among *S. galilaeus and O. niloticus*

Variables	*S. galilaeus*				*O. niloticus*				SD	P value			
	Rapeseed level				Rapeseed level								
	0%	10%	20%	30%	0%	10%	20%	30%		Species	Rapeseed level		species × rapeseed level
											linear	Quadratic	
Ammonia (mg/L)													
2nd week	0.12	0.12	0.09	0.11	0.12	0.07	0.12	0.10	0.08	NS	NS	NS	NS
4th week	0.13	0.18	0.11	0.13	0.11	0.09	0.11	0.10	0.09	NS	NS	NS	NS
6th week	0.21	0.18	0.50	0.12	0.17	0.30	0.43	0.22	0.22	NS	NS	NS	NS
8th week	0.30	0.15	0.24	0.47	0.30	0.36	0.18	0.18	0.21	NS	NS	NS	NS
10th week	0.40^{ab}	0.28^{ab}	0.07^{b}	0.06^{b}	0.64^{a}	0.51^{ab}	0.28^{ab}	0.26^{ab}	0.27	NS	*	NS	NS
12th week	0.38^{ab}	0.30^{ab}	0.10^{b}	0.06^{b}	0.74^{a}	0.49^{ab}	0.27^{ab}	0.25^{ab}	0.28	NS	*	NS	NS
Turbidity cm													
2nd week	50.00^{bc}	55.00^{b}	55.00^{b}	65.00^{a}	40.00^{de}	45.00^{cd}	35.00^{e}	45.00^{cd}	3.12	**	**	NS	*
4th week	50.00^{bc}	55.00^{b}	55.00^{b}	65.00^{a}	40.00^{de}	45.00^{cd}	35.00^{e}	45.00^{cd}	3.18	**	**	NS	*
6th week	50.00^{b}	65.00^{a}	65.00^{a}	65.00^{a}	40.00^{bc}	45.00^{bc}	35.00^{c}	45.00^{bc}	7.08	**	NS	NS	*
8th week	50.00^{ab}	65.00^{a}	65.00^{a}	65.00^{a}	40.00^{b}	45.00^{b}	35.00^{b}	45.00^{b}	10.13	**	NS	NS	NS
10th week	65.00^{ab}	70.00^{ab}	70.00^{ab}	75.00^{a}	80.00^{a}	55.00^{b}	80.00^{a}	75.00^{a}	10.96	NS	NS	NS	NS
12th week	65.00^{bc}	70.00^{ab}	70.00^{ab}	75.00^{ab}	80.00^{a}	55.00^{c}	80.00^{a}	75.00^{ab}	8.10	NS	NS	NS	*

Means carrying a-b-c within the same row are significantly different ($P \leq 0.05$) between different rapeseed replacements among different species. NS, not significant ($p > 0.05$);* $p \leq 0.05$; ** $p \leq 0.01$

understanding of how water quality is determined by abiotic factors such as temperature, dissolved oxygen (DO), turbidity, pH, and ammonia levels (Bhatnagar and Devi 2013). Water temperature is among the parameters that affect the growth rate, feed intake, and biological activities of aquatic life. In this study, the water temperature fluctuated from 30.59 to 22.18 °C, a range that the fish tolerated well and had no detectable effect on fish feed intake. This fluctuation may be due to seasonal changes, brightness of the sun, time of day, and the length of the day. The highest water temperature in our study did not reach a temperature harmful for Nile tilapia (salah et al. 2008).

The recorded pH value for all groups was approximately neutral and within the permissible limits (Ekubo and Abowei 2011). Ammonia is a normal parameter that reflects fish metabolism, protein utilization, and organic matter decay (El-Kassas et al. 2020). The recorded value showed no significant difference between the species fed different diets. All of our findings had no stressful effect on the cultured fish and were in agreement with (Asaduzzaman et al. 2006; Santhosh and Singh 2017), who recorded ammonia values ranging from 0.203 to 0.569 mg/L. We reported increases in the ammonia levels during the 6th week in the *S. galilaeus* fed the diet replaced with 20% RM, and in the 8th week in the *S. galilaeus* fed the diet replaced with 30% RM, of 0.50 and 0.47 mg/L, respectively. Similar increases in ammonia levels were recorded for the *O. niloticus* fed the control diet and the diet replaced with 10% RM in the 10th week (0.64 and 0.51 mg/L, respectively), and the 12th week (0.74 and 0.49 mg/L, respectively).

The higher levels of ammonia may be due to an increase in the production of fecal matter. Diet waste means deteriorated water quality and economic losses (Ali et al. 2010). Moreover, the significant differences in the turbidity of the water among the fish fed the different diets could be due to different feed particles, differences in food intake by the fish, differences in the fecal organic matter produced after being fed the various diets (replaced with 0%, 10%, 20%, and 30% RM) and decomposition of organic matter; these findings are in agreement with those of (Bhatnagar and Devi 2013; Boyd 1990). Also, the findings did not exceed the permissible limits mentioned by (Bhatnagar et al. 2004).

Conductivity is an index of the total ionic content of water and its freshness, which depends on its ionic concentration and the presence of dissolved solids. All of the values were within the permissible limits and were in agreement with those of (Stone and Thomforde 2004). The significant difference between water conductivity among the groups of fish fed different

Table 11 Effect of tilapia species and\ or rapeseed meal level on electric conductivity (ms/cm) and Dissolved oxygen (mg\L) of water among *S. galilaeus and O. niloticus*

Items	*S. galilaeus*				*O. niloticus*				SD	P value			
	Rapeseed level				Rapeseed level								
	0%	10%	20%	30%	0%	10%	20%	30%		Species	Rapeseed level		species×rapeseed level
											Linear	Quadratic	
Electric conductivity (ms/cm)													
2nd week	43.50^{bc}	45.10^{ab}	38.10^{d}	25.10^{e}	41.00 ^{cd}	37.70^{d}	46.40^{ab}	48.00^{a}	2.21	**	**	*	**
4th week	49.50^{a}	40.90^{bc}	47.00^{abc}	47.80^{ab}	49.50^{a}	40.20^{c}	47.50^{abc}	47.90^{ab}	4.28	NS	NS	*	*
6th week	41.50 ^{cd}	37.20^{d}	40.50 ^{cd}	42.40^{bc}	51.10^{a}	43.50^{bc}	43.90^{bc}	45.90^{b}	2.52	**	NS	**	**
8th week	41.50^{bcd}	38.20^{d}	40.50 ^{cd}	42.40^{bcd}	51.10^{a}	43.50^{bc}	43.90^{bc}	45.90^{b}	2.69	**	NS	**	*
10th week	57.70^{a}	40.00^{bc}	42.00^{bc}	47.00^{abc}	54.70^{a}	36.60^{c}	48.10^{ab}	49.70^{ab}	6.39	NS	NS	**	**
12th week	57.90^{a}	41.20^{bc}	42.30^{bc}	47.40^{abc}	54.90^{a}	36.80^{c}	48.30^{abc}	49.90^{ab}	7.15	NS	NS	**	**
Dissolved oxygen (mg\L)													
2nd week	6.00	6.50	6.70	6.90	6.00	6.30	6.30	6.50	0.69	NS	NS	NS	NS
4th week	5.00	5.60	5.70	6.00	5.10	5.20	5.40	5.60	0.70	NS	NS	NS	NS
6th week	5.00	5.50	5.70	5.90	5.10	5.40	5.40	5.60	0.70	NS	NS	NS	NS
8th week	4.67	5.17	5.03	5.47	5.00	4.97	5.13	5.50	0.56	NS	NS	NS	NS
10th week	4.50	4.40	4.40	4.50	4.50	4.50	4.70	4.70	1.10	NS	NS	NS	NS
12th week	4.00	4.27	4.40	4.40	4.10	4.10	4.10	4.30	0.72	NS	NS	NS	NS

Means carrying a-b-c within the same row are significantly different ($P \leq 0.05$) between different rapeseed replacements among different species. NS, not significant ($p > 0.05$);* $p \leq 0.05$; ** $p \leq 0.01$

diets with different RM replacements (0%, 10%, 20%, and 30%) could be due to the different contents of the diets combined with minerals and dissolved solids (Konovalenko et al. 2016). Any significant difference in conductivity between *S. galilaeus and O. niloticus* fed different diets may be because the fish differ in their ability to maintain osmotic pressure (Bhatnagar and Devi 2013). The higher conductivity could be attributed to the increased levels of uneaten feed dissolved in the water (Kosemani et al. 2017).

Maintaining healthy levels of DO in water directly influences feed intake, disease resistance, and metabolism. A sub-optimal level is highly stressful for fish. The difference in recorded values for the levels of DO in this study among the fish fed different diets was not statistically significant. The highest value (6.90 mg/L) was recorded

Table 12 Regression analysis of rapeseed level against net profit, villi length, goblet cells, ammonia, Electric conductivity and Superoxide dismutase

X	Y	Model	R^2	*P* value
Rapeseed level	Net Profit	Y = 1.27 + .686X	.470	.000
	Superoxide dismutase	Y = 80.531-.911X	.829	.000
	Villi length	Y = 314.593 + .659X	.434	.000
	Goblet cell	Y = 18.567 + .596X	.355	.002
	Ammonia	Y = -.014-.523X	.273	.009
	Electric Conductivity	$Y = 55.067–2.186X + 2.057X^2$	.391	.005

in the 2nd week in *S. galilaeus* fed the diet replaced with 20% RM and the lowest (4.00 mg/L) in the 12th week in same species fed the control diet. The findings of (Riche and Garling 2003) are in agreement with our results, where a decrease in the levels of DO may be due to water pollution and increased bacterial loads. Similarly, feed affects the productivity of water body (Leung et al. 2015). The water quality parameters measured for the duration of the experiment for both species of cultured fish fed the control fish meal, and the diets replaced with 10%, 20%, and 30% RM, were within the acceptable range, with no significant difference among the different diets; this provides evidence that replacement of a portion of high-cost FM with less expensive RM has no deleterious effect on water quality.

During our statistical analysis of the data, polynomial regression indicated that an increase in the level of RM by about 10% resulted in increases in net profit (6.86%), intestinal villi length (6.59%), numbers of goblet cells (5.96%), and reductions in SOD (9.11%) and ammonia (5.23%).

Conclusion

In conclusion, increasing replacement of fish meal with rapeseed meal over 20% in the diet of *S. galilaeus* and 10% in the diet of *O. niloticus* resulted in a significant decrease in weight gain, length gain, SGR, and weight gain rate. However, the addition of RM up to 30% RM demonstrated economic efficiency and kept the water parameters safe without any stressful effect on *O. niloticus*. Partial replacement of fish meal with rapeseed meal increased intestinal villi length and the number of goblet cells. Therefore, the replacement of fish meal with up to 10% RM is beneficial for tilapia species, whereas use of a higher concentration resulted in an oxidative stress effect.

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Data availability Data are available up on request.

Declarations

Ethical approval The procedures and protocols used in this study were designed in accordance with the guidelines for animal welfare and the use of animals prepared by the Institutional Animal Care and Ethical Committee of Taif University, Saudi Arabia for project TURSP-2020-09.

Consent to participate Not applicable.

Consent to publish All authors agree to the content of paper for publication.

Conflict of interest The authors declare no conflict of interest.

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