

Evaluation of Date Fiber as a Feed Ingredient For Nile Tilapia *Oreochromis Niloticus* Fingerlings

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Abstract:-This study was carried out to evaluate the use of date fiber (DF) as a feed ingredient for tilapia fingerlings in terms of growth parameters, body composition, anatomical alterations of the intestinal villi. In addition to, pellet strength and bacterial type and population in the test diets. Four isonitrogenous isocaloric diets containing 0, 100, 200 and 300 g kg⁻¹ DF as replacement of wheat bran were fed to triplicate groups of ten *O. niloticus* fingerlings (0.65 g) in a recirculating water system for 70 days. Fish fed diets contain up to 200 g kg⁻¹ DF had similar growth parameters. Further increase in dietary DF to 300 g kg⁻¹ resulted in significant retardation in all parameters. Body fat was reduced while protein, ash and moisture were increased by DF level. Increasing dietary DF level caused changes in tilapia's intestinal villi, reduced dietary microbial activity and bacterial population of selected species, and produced stronger pellets.

Keywords: Date fiber; fish; growth, bacteria; feed; electron microscopy.

1. INTRODUCTION

Tilapia culture has been growing at an outstanding rate during the past decade, in most of the tropical, subtropical and temperate regions. As a result, the production of farmed tilapia has jumped from 308,234 mt in 1988 to 2.8 million mt in 2008 [1]. In addition, tilapia culture has been gradually shifted from the traditional semi-intensive systems to the more intensive systems, which rely exclusively on artificial feeds. Therefore, formulating economic tilapia feeds has become a necessity.

Nutrition represents over 50% of total culture inputs in tilapia culture [2]. In addition, the prices of major feed ingredients, including fish meal (FM), soybean meal (SBM), corn, bran and oils have been sharply increasing during the past few years. This has been attributed mainly to one or more of the following reasons: 1) declining production, 2) increasing demands and competition among users, 3) increasing production cost, particularly fuel and fertilizer prices, and 4) conversion of some plant ingredients to biofuel (e.g. corn to ethanol) [2]. For example, the price of corn has jumped from US\$ 95 in 2006 to US\$ 230 in 2008. Similarly, the prices of soybean meal increased from about US\$200 to \$450 during the same period [2]. Therefore, the major challenge facing

tilapia culture industry is the production of cost effective and environmentally performing feeds for farmed tilapia, using inexpensive, locally available ingredients. Several studies have been conducted to evaluate the incorporation of different unconventional animal and plant proteins and energy sources for farmed tilapia with varying results [3].

Date palm tree is one of the most important cultivated trees in arid and semi-arid regions, especially in North Africa and Arabian Gulf countries. Date fruits play an important role in the economies of these countries, as a major source of nutrition. Egypt, Iraq, Iran, Saudi Arabia, UAE, Pakistan, Algeria, Sudan, Oman, Libya, China and Tunisia are the major date fruit producers. Over 6,700,000 mt of date fruits were produced in 2004 [4].

Date wastes include date pits and DF is produced annually. These by-products may have high potential as energy sources for farm animals and farmed fishes.

Date Fiber (DF), a by-product of date syrup production, is an insoluble, powder-like date by-product, connected with non-nutritive portion of the date flesh [5]. It is composed mainly of cellulose, hemicelluloses, lignin, ligno-cellulose, and insoluble proteins. This fiber is naturally broken down, by enzymes, during the ripening process, to more soluble compounds (glucose, sucrose, mannose and soluble pectin and galacto-mannan) to render the fruit more tender and soft. Date fiber represents 20-100 g kg⁻¹ of the date flesh, depending on the type and quality of the dates [5]. This means that a substantial amount of DF is produced annually, especially in tropical and subtropical regions, where dates are a major agricultural crop.

Only few studies have been carried out to investigate the use of this by-product as a feed ingredient in rats [6] and in human food fortification [7] They reported that patty formula replaced with up to 150 g kg⁻¹ DF produced healthier and better quality beef patties by possessing hypolipidemic effects. Dietary fibers, particularly water soluble, might influence lipid metabolism in rats [6] as it was found to possess hypolipidemic in rats fed 2 g kg⁻¹ cholesterol. Additionally, DF concentrates showed a high water and oil -holding capacity [8]. This suggests that DF concentrates may not only be an excellent source of dietary fiber but an ingredient for improving fish pellet quality.

The present study was conducted to investigate the use of DF as a replacement of dietary wheat bran in the diets of *O. niloticus* fingerlings growth and proximate body composition. Additionally, the effect of DF on pellet quality in terms of pellet strength, total and specific bacterial count, and anatomical alterations of the intestinal villi were investigated.

2.METHODS

(Culture Condition)

Nile Tilapia *Oreochromis niloticus* fingerlings (0.65 g average initial weight) were produced from tilapia brood stock kept in captivity at the Aquaculture Unit, College of Food and Agriculture, United Arab Emirates University, Al Ain, United Arab Emirates. Ten fish were stocked into 20 L fiberglass tanks in a closed, recirculating indoor system. The tanks were provided with central drainage pipes surrounded by outer pipes, perforated at the bottom, to facilitate self-cleaning and waste removal. The culture system was provided with a biological filter, aeration through an air blower, and heaters to maintain water temperature at 27 °C. Approximately 10% of the water volume was replaced by new freshwater daily. Lighting in the culture unit was set at 12:12 L:D cycle. Water quality parameters, including dissolved oxygen (DO) (Oxygen meter, YSI, model 58), ammonia (NH₄-N), Nitrates (NO₃-N), and nitrites (NO₂-N) (Orion Aquafast, Germany) and pH (pH meter, Jenway, UK) were monitored weekly

(Dietary formulations)

Four isonitrogenous (320 g kg⁻¹ CP), isocaloric (18.84 kJ g⁻¹) test diets with varying levels of date fiber as a replacement of wheat bran at 0, 100, 200 and 300 g kg⁻¹ were formulated. The diets were prepared as follows: all feed ingredients were ground in a commercial blender and then mixed in a kitchen mixer. Vitamin and mineral mixes were gradually added with continuous mixing. Distilled water (60°C) was slowly added while mixing until the mixture began to clump. Then, the diet passed through a kitchen meat grinder and was dried for 24 hours at 60°C in a vacuum drying oven. The dried diet was then chopped into pellets in a blender and then passed through laboratory test sieves (mesh 2 and 0.88 mm) to ensure homogenous particle size of sinking pellets and stored at -8°C until used. The amount of waste (powder form) as a result of the pelleting process for every test feed was calculated separately as a percentage of the total amount of every feed. This was used as an indicator of weak (high percentage) or strong (low percentage) pellets. The chemical composition of the DF and all test diets were determined according to [9] methods (Table 1 & 2).

Each diet was fed to triplicate groups of 10 fish each (0.65 g average initial weight) to satiation level, twice a day (09:00 and 16:00 h) for 70 days. Fish were weighed collectively at 10-day intervals, their average weights recorded.

(Feed efficiency performance)

Feed efficiency performance including fish weight gain (WG), Specific Growth Rate (SGR), feed conversion ratio

(FCR), Protein Efficiency Ratio (PER), were calculated with the following equations:

WG= $W_2 - W_1$, where WG is the mean of weight, W_2 is the Mean final Weight, W_1 is the Mean Initial Weight,

SGR = $(\ln W_2 - \ln W_1) / \text{time in days} \times 100$

FCR = feed (dry) intake (g) / wet weight gain (g)

PER= average weight gain (g)/average weight of protein fed

(Investigating intestinal wall under the scanning electron microscopy)

Two fish from the control 0 g kg⁻¹ DF, 100 g kg⁻¹ DF, and 200 g kg⁻¹ DF treatments were used for this study. All fish were killed and the ventral body wall was opened. The entire gastrointestinal tract of the six fish was excised and fixed in 30 g kg⁻¹ glutaraldehyde in phosphate buffered saline. Cross sections of the gastrointestinal tract were performed at several levels and processed for scanning electron microscopy. Selected gut fragments were taken from different levels of the intestine, fixed, dehydrated to critical dried point, further dissected if necessary, mounted, sputter coated with gold and viewed on a JEOL JSM 5500 LV SEM. The scanning electron microscopy was done in the Central Laboratory Unit of United Arab Emirates University.

(Microbial analyses enumeration of microbial populations)

The microbial populations of the test diet samples containing were estimated using the soil dilution plate method [10]. Three 10 g replicates, of each sample were dispensed into 100 mL of sterile 0.1 % (w/v) agar (Gibco Brl, Paisley, Scotland) solution in deionized water containing 20 g glass beads (3 mm diameter). The suspension was shaken 50 times and then placed in an ultra-sonic cleaner at a frequency of 55,000 cycles sec⁻¹ for 20 sec (Model: B- 221, 185 Warr, Branson Cleaning Equipment Company, USA). Ten-fold dilutions were made in sterile deionized water and 0.2 mL aliquots of what were considered appropriate dilutions were spread on the surface of the different media in sterile plastic Petri dishes (90 mm diameter) with a sterile glass rod. Nine plates were used per dilution. The plates were dried in a laminar flow cabinet for 1 h and then incubated at 25°C (\pm 2°C) and colony counts were carried out from day 2 onwards. The groups of organisms selected for enumeration and the media used were as follows: (i) total aerobic bacteria on 1/5 M32 medium [11], incubated for 2-4 days; (ii) fluorescent pseudomonads on 1/10 tryptic-soy agar (Difco laboratories, Michigan, USA) (TSA) containing ampicillin 50 mg mL⁻¹, (Sodium salt, Instituto Biochimico Italiano, Milano, Italy), cycloheximide 75 mg mL⁻¹ (Sigma) and chloramphenicol 12.5 mg mL⁻¹ (Sigma) (TSA + ACC), incubated for 2-4 days [12], (iii) Gram-negative bacteria on 1/10 TSA containing crystal violet (Sigma) at a concentrations of 2 μ g mL⁻¹ (TSA + CV), incubated for 2-4 [13]; (iv) filamentous fungi and yeasts on Martin's medium containing rose bengal 33 μ g mL⁻¹ (Sigma) and streptomycin 30 μ g mL⁻¹ (Sigma) incubated for 4-6 days [14]. Bacterial and fungal colonies

were counted from each medium and were expressed as colony forming units (cfu) g dry-1 sample

(Estimation of the total microbial activity)

The microbial activity of all test diet samples were measured by fluorescein diacetate hydrolysis and by arginine ammonification. The hydrolysis of fluorescein diacetate (FDA) (Sigma Chemical Co., St Louis, Mo., USA) was measured by the method of [15]. Briefly, 5 g of each sample were added to 20mL of sterile 60mM potassium phosphate buffer (8.7 g K₂HPO₄ and 1.3 g KH₂PO₄ in 1 L distilled water, pH 7.6) in 250mL flasks. FDA was dissolved in acetone and stored as a stock solution (2 mg mL⁻¹) at -20°C. The reaction was started by adding 0.2mL of FDA (400 µg) from the stock solution to a buffer-sample mix. Each treatment consisted of eight replicates and one blank to which no FDA was added. The reaction flasks were shaken (90 rpm) at 25°C for 20 min on a rotary shaker (Model G76, New Brunswick Scientific, Edison, NJ, USA). The reaction was then stopped by adding 20mL acetone to all samples. Sample residues were removed from the mixture by centrifugation at 500 rpm for 10 min and filtered through a No. 1 Whatman filter paper (Whatman, Maidstone, England). The filtrate was collected in a test tube, covered with parafilm and placed into an ice bath to reduce volatilisation of the acetone. The concentration of fluorescein was determined by reading the optical density at 490 nm, using a Shimadzu UV-2101/3101 PC scanning spectrophotometer (Shimadzu Corporation Analytical Instruments Division, Kyoto, Japan). This permitted the rapid handling of many samples, the concentrations of which were compared against a standard curve. The background absorbance was corrected for each treatment with the blank sample run under identical conditions but without the addition of FDA. Standard curves were prepared as described by [16]. The results were converted to µg hydrolysed FDA g dry-1 sample.

(Analyses Body composition analysis)

At the termination of the study, all fish in each tank were netted, weighed and frozen for body composition analyses at -20 °C. Initial body analyses were performed on a sample of fish, which were weighed and frozen prior to the study. Proximate analyses of body water, protein, lipid, and ash were performed according to standard [9] methods. *Statistical analyses* Fish growth rates, feed utilization efficiency and body composition results were subjected to a one-way analysis of variance (ANOVA) to test the effects of DF inclusion level on fish performance. Orthogonal polynomial procedure [17] were used to compare means at P = 0.05. Least significant difference (LSD) was used to test for the differences among treatment means when F-values from the ANOVA were significant. Statistical analysis for the date pit effect on microbial population

3.RESULTS

The average values of water quality parameters throughout the study were; DO = 6.4 ±13 mg-l, NH₄-N = 0.06 ± 0.002 mg-l, NO₃-N = 8.4±1.72 mg-l, NO₂ = 0.00 mg-l and pH = 8.0±0.09. Good binding properties were noted with increasing levels of DF in the experimental diet. The level of fines during the pelleting process decreased (277, 234, 183, 121 g kg⁻¹) for diets containing 0, 100, 200 and 300 g kg⁻¹ DF, respectively, with very high correlation ($r^2 = 0.97$, P < 0.05).

Table 1 Proximate analyses of DF on dry weight bases

Nutrient	DF g kg ⁻¹
Moisture	69
Crude protein	24
Crude fat	7
Crude fiber	515
Total ash	25
NFE	429

The proximate composition of the experimental diets (Table 2) showed little variation in nutrient levels of various diets and agreed with estimated values. The dietary DF significantly affected the growth performance of *O. niloticus* fingerlings (P<0.05).

The growth rates and feed conversion ratios of fish fed DF-based diets up to 200 g kg⁻¹ inclusion level were similar to that of fish fed the control (date fiber-free) diet (Table 3). Further increase in dietary DF to 300 g kg⁻¹ resulted in significant retardation in fish performance

Proximate body composition, namely moisture, crude protein, and total ash, of *O. niloticus* fingerlings fed test diets with DF up to a level of 200 in g kg⁻¹ were not affected (P < 0.05) by replacing dietary wheat bran while body fat was reduced at the 200 in g kg⁻¹. As the level of the DF incorporation increased to 300 in g kg⁻¹ DF in the test diets, body moisture, body protein and total ash were increased while body fat decreased significantly. (Table 4)

The results of total microbial activity and microbial populations in the test diets showed that samples with 0 g kg⁻¹ DF had a significantly (P<0.05) highest total microbial activity as compared to all samples with 100, 200, 300 g kg⁻¹ DF. There was significant (P < 0.05) gradual reduction of total microbial activity and microbial populations as the level of DF increased in the test diet (table 5). The estimated total populations of aerobic bacteria, fluorescent pseudomonads, Gram-negative bacteria, filamentous fungi and yeasts were significantly (P < 0.05) higher in the samples without DF than samples with DF. The population was gradually and significantly reduced as the level of DF was increased.

Table 2 Composition and proximate analyses of the test diets (g kg⁻¹ dry matter). Values represent the means of three replicates. Means in each row followed by a different letter are significantly different (P > 0.05).

Ingredients (g kg ⁻¹)	0g kg ⁻¹	100g kg ⁻¹	200g kg ⁻¹	300g kg ⁻¹
F M (700g kg ⁻¹ CP)	380	390	410	430
Wheat bran	530	420	290	160
DF	0	100	200	300
Sunflower oil	50	50	60	70
Vitamin and mineral mixes ¹	20	20	20	20
Binder (CMC)	20	20	20	20
Total	1000	1000	1000	1000
Proximate analysis				
Crude protein	327.8	324.3	328.9	320.2
Crude lipids	100.3	121.4	134.4	148.2
Total ash	120.1	122.1	123.2	121.1
Crude Fiber	54.2	96.7	120.1	174.5
NFE ³	399.6	335.5	293.4	286
GE ³ (kJ g ⁻¹)	185.3	182.7	181.7	183.9

¹Similar to [18], ²Nitrogen-free extract, calculated by difference. ³Gross energy, calculated based on 23.67, 17.17 and 39.79 kJ g⁻¹ for protein, carbohydrate and lipids, respectively

Table 3 Performance of *O. niloticus* fingerlings fed DF-based diets. Values represent the means of three replicates. Means in each column followed by a different letter are significantly different (P > 0.05).

g kg ⁻¹ DF	IW ¹	FW ²	WG ³	SGR ⁴	FCR ⁵	PER ⁶	Survival
0	0.65	6.5±0.17 a	9.14±0.42a	2.77±0.06 a	1.86±0.02 a	1.64±0.06	97±0.77 a
100	0.65	6.0±0.36 a	8.82±0.23 a	2.65±0.11 a	1.84±0.07 a	1.78±0.16	98±0.29 a
200	0.67	6.9±0.59 a	9.60±0.53 a	2.84±0.08 a	1.99±0.21 a	1.53±0.11	97±0.85a
300	0.66	4.5±0.05b	4.16±0.15b	2.27±0.04b	3.32±0.12b	0.97±0.21	97±0.87a

1 Mean Initial Weight

2 Mean Final Weights

3 Weight Gain = FW - IW

4 SGR, Specific Growth Rate = (ln FW - ln IW) / time in days / 100

5 FCR, Food Conversion Ratios = feed (dry) intake (g) / wet weight gain (g)

6 PER, Protein Efficiency Ratio = average weight gain (g) / average weight of protein fed (g).

Table 4 Proximate body composition of *O. niloticus* fingerlings fed test diets with different percentages of DF. Values represent the means of three replicates. Means in each column followed by a different letter are significantly different (P > 0.05).

DF g kg ⁻¹	Moisture g kg ⁻¹	Crude protein g kg ⁻¹	Lipid g kg ⁻¹	Total Ash g kg ⁻¹
0 (control)	700.6c	147.9a	73c	58a
100	716.5bc	146.1a	68.7b	58a
200	723ab	138.1a	55.3ab	74a
300	739a	138.5a	46.8a	71a

Table 5 Enumeration of microbial populations (colony forming units. g-1 dry sample) and estimation of the total microbial activity (Bg hydrolyzed FDA. g-1 dry sample) of sample amended with or without DF. Values are means of nine replicates and the values in parentheses are the standard error of the mean.

Microorganisms	Diet without DF	Diet with 200 g kg ⁻¹ DF
Total aerobic bacteria	41.22 x 105 ± (2.47)	1.85 x 103 ± (1.71)
Fluorescent pseudomonads	21.87 x 103 ± (2.02)	18.45 x 101 ± (1.87)
Gram-negative bacteria	53.67 x 104 ± (2.68)	22.87 x 102 ± (1.14)
Filamentous fungi and yeasts	12.98 x 103 ± (1.08)	9.54 x 101 ± (2.57)
Microbial activity	71.12 ± (2.81)	19.76 ± (1.91)

The scanning electron microscopy (SEM) of *Oreochromis niloticus* intestine It is important to indicate that the entire samples of fish intestine which were fed 100 g kg⁻¹ DF were lost during the analyses and could not recovered. The intestinal villi from fish fed 0 g kg⁻¹ DF (control group) were the smallest of all and the walls were the thinnest (Average width 793nm) as compared to those from fed 200 and 300 g kg⁻¹ DF (average width 1.16 μm and 2.28

μm respectfully). In other words there were gradual increases in size, height and thickness of the intestinal villi of *O. niloticus* fingerlings as the level of DF in the test diets fed increased. (Figures 1, 2, and 3) Unfortunately the intestinal samples of fish fed 100 g kg⁻¹ DF was lost, however, we can still get a clear idea of the effect of DF on the fish intestinal villi.

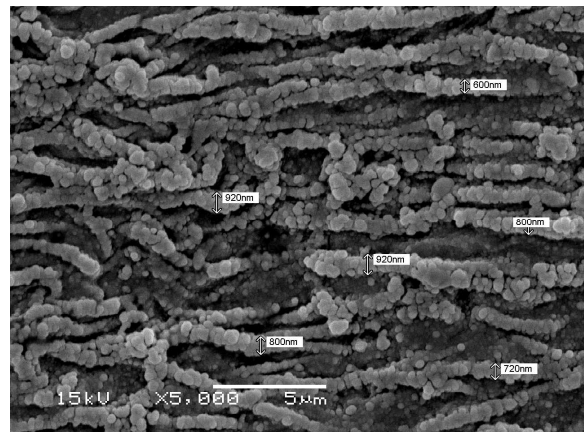
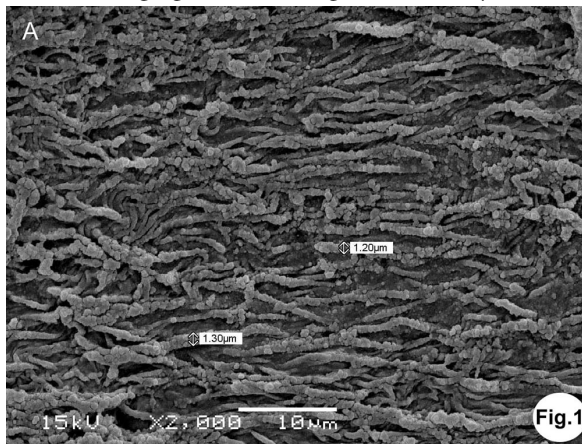


Fig. 1 Scanning electron micrograph of tilapia intestinal villi of that fed control diet (0 g kg⁻¹ DF). R: low magnification and L: high magnification

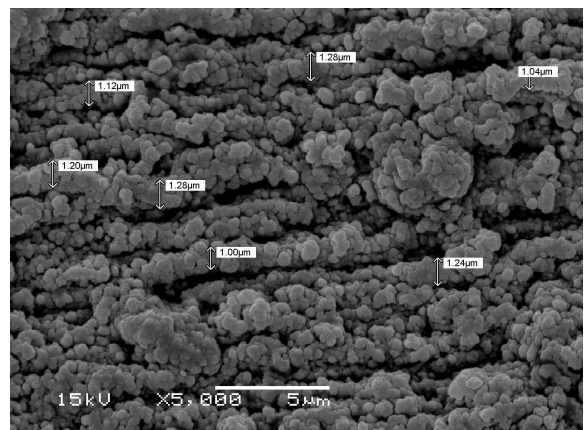
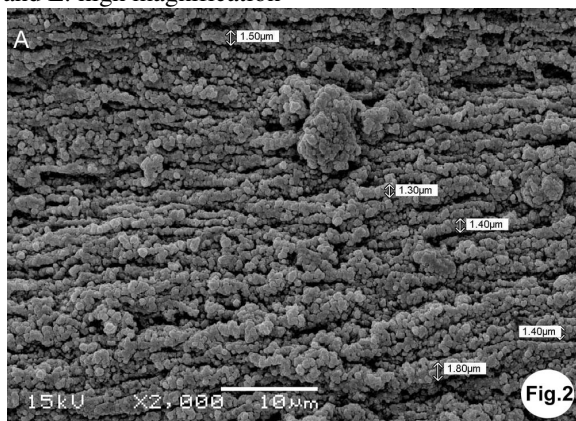


Fig. 1 Scanning electron micrograph tilapia intestine of that fed with 200 g kg⁻¹ DF. R: low magnification and L: high magnification

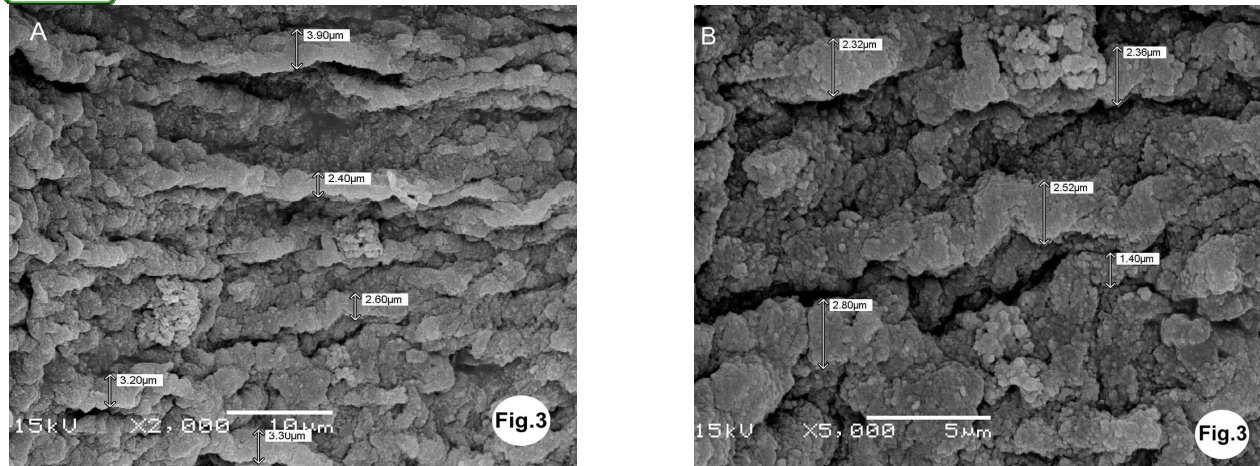


Fig. 3 Scanning electron micrograph of the intestine of tilapia fed diet with 300 g kg⁻¹ DF. R: low magnification and L: high magnification

4. DISCUSSION

Overall the closed recirculating culture system used in the experiment was capable of maintaining suitable water quality parameters for experimental fish [19]. DF inclusion in the test diets produced stronger pellets which were indicated by that reduction of powder after grinding. Up to my knowledge, there is no study on evaluating date fiber as a feed ingredient for fish. There is only one trail on feeding date fiber in starter ration for broiler with negative results which was due to inability of broiler to handle high fiber in their diet [20]. Few studies have been conducted on the use of dates and dates byproducts (date fiber not included) as feed ingredients in fish diets. For example, studies on *O. niloticus* [21], [22], [23], [24]. They revealed that dates and date by-products could be used as a nutritional source for these fish. Similarly, it was found [25] that date pits can replace wheat bran-barley mixture in common carp feed at up to 750 g kg⁻¹ inclusion level, without any significant retardation in fish growth and feed utilization efficiency. The present study indicated that even though, nutrient content of wheat bran is better than DF, no significant differences ($P < 0.05$) were detected in tilapia fed diets with DF at 0, 100 and 200 g kg⁻¹ in terms of growth parameters and feed utilization efficiency parameters (feed conversion ratios, specific growth rates, protein efficiency ratios). That was probably due to a combination of the following: first, the increase of digestible carbohydrates (oligo and monosaccharides). Tilapia gut microflora plays an important part in fiber digestion [26] Date fiber contains simple sugars (glucose and fructose) and polysaccharides (glucan, xylan, galactan, mannan, arabinan, and acid soluble and insoluble lignin) [27]; Secondly, it may also be due to a free sugar such as mannose which is a part of partly digested mannan which worked as growth promoters. Mannose and oligomannan are good growth promoters for chicken, turkey [28] and fish [24]; Thirdly,

the increase in intestinal villi in number, size, and thickness in fish fed test diet with DF 200 g kg⁻¹ (Figures 1, 2, and 3) could have improved nutrients absorption and make up for nutrients deficiency in DF composition. A researcher [29] has described that increased villus height suggests an increased surface area capable of greater absorption of available nutrients. Additionally, [30] showed that some fiber constituents (methoxylated pectin) causes changes in jejunal villus length and width and number in rates which villi function in digested feed absorption. It is understood that greater villus height and numerous cell mitoses in the intestine indicate that the function of the intestinal villi is activated [31], [32], and [33] Fourthly, the reducing effect of DF on microbial population, activity, total aerobic bacteria, fluorescent pseudomonads, filamentous fungi and yeasts may have played a role that cause a probiotic like effect to enhance tilapia growth. (Table 5) On the other hand, the present study indicates that growth and growth parameters were negatively affected when the level of DF increased to a level of 300 g kg⁻¹. This may be due to the significant reduction in feed intake (Table 3). Feed intake reduction may have been due to the increased levels of fibers in DF in the feed while the ability of tilapia to utilize them is limited, as has been reported by [34] Additionally feeds with high fiber intake increases the passage rate which reduces digestion and absorption, [35], [36], [37] and [38] and increases fecal fat content in rates [39]. Approximate body composition of *Oreochromis niloticus* fingerlings fed the test diets with 0, 100 and 200 g kg⁻¹DF were similar. This indicates that fish digestive system was able to adapt itself with the DF at those levels as shown in the scanning electron microscope pictures in figure 1, 2, and 3. However when DF level reached 300 g kg⁻¹DF fish body moisture was significantly increases while body fat was decreased when compared to fish fed at lower levels of DF. This could be due to lower feed intake of fish test diet with 300 g kg⁻¹DF (table 4) as compared to those fed diets with lower DF levels.



5. CONCLUSION

DF improved fish pellet quality; DF had significant effect on fish diet in reducing microbial counts of namely total aerobic bacteria, Fluorescent pseudomonads, Gram-negative bacteria, Filamentous fungi and yeasts. DF increased the intestinal mucosa surface area of tilapia which might play a role in dietary absorption.. The present study suggests 20 g kg⁻¹ of dietary wheat bran in tilapia feeds can be replaced with DF. This replacement can lead to a significant reduction in feed costs.

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