ISSN: 2642-9020

Research Article

Journal of Marine Science Research and Oceanography

Effect of Probiotics and Dietary Levels of Protein on Growth, Muscle Quality and Expression of Some Immune Genes in the Head Kidney of Nile Tilapia (*Oreochromis Niloticus*)

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Submitted: 2023, July 14; **Accepted**: 2023, Aug 05; **Published**: 2023, Sep 20

Citation: Ridha, M. T., Azad, I. S., Hossain, M. A., El-Dakour, S. (2023). Effect of Probiotics and Dietary Levels of Protein on Growth, Muscle Quality and Expression of Some Immune Genes in the Head Kidney of Nile Tilapia (*Oreochromis Niloticus*). *J Mari Scie Res Ocean, 6*(5), 174-187.

Abstract

This study evaluated the effect of dietary probiotics (P) to enhance the feed quality, growth parameters, muscle fatty acid (FA) profile, immunity, and the stimulation of immune genes in head-kidney of Nile tilapia. Healthy mixed-sex Nile tilapia (47.0 ± 2.07 g mean weight), were fed three tilapia commercial feeds; S, A, and a local feed K having 33.3, 32.4, and 13.4% protein, respectively. Dietary supplementation of Bacillus probiotic (P) into the commercial feeds resulting in six experimental feeds (S, SP, A, AP, K, and KP) were used. The non-probiotic feeds served as controls and each treatment had three replicates. Results showed that fish fed the SP feed had the highest (P<0.05) final mean weight (582.7 g), daily growth rate (2.52 g/fish/day), gross fish yield (44.0 kg/m3), and best feed conversion ratio (1.9), followed by S (570.4 g, 2.46 g/fish/day, 41.1 kg/m3, and 2.09, respectively). However, these values were the lowest (P<0.05) in tilapia fed KP and K (362.8 and 366.7 g, 1.48 and 1.49 g/fish/day, 27.4 and 26.8, kg/m3, 2.16 and 2.18, respectively). The feed A and AP had intermediate values. Fish fed the SP feed had the highest (P<0.05) muscle ∑n-3 fatty acid (10.5) and ∑n-3/n-6 ratio (0.75). In general, gut bacterial counts, lysozyme activity, phagocytic activity, and hemagglutination titer were the highest (P<0.05) in tilapia fed the SP, followed by tilapia fed AP, but it was the lowest for tilapia fed K and the KP feeds. Probiotics significantly up regulated β-actin, IgM, IL-β1, Mx, and TNF-α immune gene in the head kidney with better response in fish fed the SP feed. It can be concluded that improvement in tilapia growth, bacterial colonization, muscle quality, immune parameters, and the up regulation of immune genes in the head kidney due to probiotic supplementation would depend on the protein level of the feed used.

Keywords: Nile Tilapia, Protein Ratio, Probiotics, Growth, Fatty Acids, Immune Response, Immune Genes.

1. Introduction

Feed quality and protein level have significant effect on fish growth, production rate, feed conversion ratio (FCR), and flesh quality. However, feed represent over than 50% of the operating expenses, and any improvement in FCR will reduce the production cost [1]. Therefore, much research focused on replacing the expensive fishmeal and fish oil as sources of protein and fat with cheaper sources, leading in some cases to the production of low quality feeds [2-5]. Sargent stated that polyunsaturated fatty acids are essential for spawning, growth, survival, and immunity of fish larvae, and imbalanced ratio of fatty acids can be damaging to the cell membrane of the fish [6]. Glencross, Hawkins and Curnow observed considerable changes in the tissue fatty acid profile with changes in the fatty acid composition of dietary oils [7]. Fish fed with low quality diet that lacks sufficient energy and nutrition for maintaining homeostasis and for keeping the non-specific (innate) and specific (acquired) immune functionality ready for combating any disease-causing agent become much more vulnerable to diseases [8]. Moreover, the feed quality and protein levels might affect the expression of immune genes in the head kidney responsible for the regulation of inflammatory responses, antibody production, and cytoskeletal functions [9,10]. Tilapia farmers use probiotics supplementation extensively as feed additive to improve the feed efficiency and to enhance growth, immunity, and disease resistance against bacterial pathogens that can cause substantial mortality [11,12]. The overall objective of this study was to determine if the efficiency of a low protein feed improves through probiotic addition. Therefore, three tilapia commercial feeds with or without probiotic bacteria supplementation (two feeds with normal protein levels of 33.3% and 32.4%, and one with lower protein

level of 13.4%) were fed to Nile tilapia to compare growth parameters, feed utilization, proximate composition and fatty acid profile of fish muscles. The study also aimed at determining the effect of the different feeds on the colonization of the probiotic bacteria in the gut, immunological parameters, and the expression of selected immune genes in the head-kidney.

2. Materials and Methods

2.1. Experimental Set Up and Feeding

Healthy mixed-sex Nile tilapia, *Oreochromis niloticus* (Chitralada strain, Thailand) juveniles (47 g mean body weight), showing normal feeding and swimming behaviors, normal color, and free from morphological blemishes were stocked at a density of 83 fish m^{-3} in 0.43m^{-3} tanks in a recirculating water system arranged in four independent rows. Each row consisted of six tanks attached with a 0.5-m^3 submerged up-flow biological filter tank. Two rows were assigned for the probiotic treatments, and the other two rows were allocated for the probiotic-free feeds (control). Three commercial tilapia feeds namely local feed manufactured in Kuwait (L), Skretting feed of European origin but manufactured and imported from Egypt (S), and Arasco feed manufactured and imported from Kingdom of Saudi Arabia (A). The proximate composition of the feeds is shown in Table 1. Feeds S and A had 33.3% and 32.4% crude protein, respectively, while feed L had low protein level (13.4%). Feeds S and A had similar gross energy levels (18.2 KJ/g) while the Local feed had slightly lower energy level (16.9KJ/g). Each feed was fortified with probiotics (P) thus giving six experimental feeds; S, S+P, A, A+P, L and L+P. The feeds without probiotics served as respective controls. The feed pellets were coated with a mixed suspension of an equal proportion of *Bacillus subtilis* isolated from Nile tilapia gut cultured in Kuwait (Gene Bank NCBI 1701438), and the commercial probiotic Biostim® (Advanced Aqua Biotechnologies, India) used for aquaculture. The tilapia isolate was cultured in Brain Heart Infusion Agar/Broth, purified, maintained on agar (BHIA) slants, and identified using 16s rDNA sequencing [12]. The commercial Biostim® probiotic consisted of a mixture of Lactobacillus and Bacillus bacteria, yeast, and digestive enzymes [12]. The final bacterial load was 108 cfu g-1 as determined by spread plate bacterial enumeration of the feed. The probiotic properties of the supplemented bacteria were tested using competitive exclusion growth properties on BHI agar using Proteus vulgaris (hemorrhagic septicemia causative previously isolated from mullet and tested to produce similar effects in tilapia) and *Aeromonas spp* [12,13]. The probiotic diets were coated with a mixture of probiotic suspended in 20 ml sterile phosphate buffered saline (BS, pH 7.2) and 10 ml of vegetable oil. A similar mixture but without probiotic was used to coat the control diets. Oil addition to the feeds did not cause significant change in the lipid content of the test diets. The probiotic diets were prepared every 10 to 14 days to maintain the viability of the bacteria. Each diet represented a treatment, and each treatment was in triplicates using 18 experimental tanks.

Fish in all tanks were fed daily at a rate of 6% of their body weight and decreased subsequently to 2.5% using 3-mm and 5-mm pellets, respectively. The daily ration was divided into three equal portions. Tanks water temperature was controlled at 29.0 ± 2 oC using titanium heaters (AREA Inc, USA) and tanks water volume was kept at 300 l. About 20% of the water in the recirculating system was replaced daily with new freshwater. Total ammonia nitrogen (TAN), nitrite-nitrogen (NO_2-N) and nitrate-nitrogen (NO_3-N), pH, and dissolved oxygen (DO) were monitored every two weeks using HACH test kit (HACH Company, USA) and YSI-DO meter Model 55 (YSI Inc, USA), respectively. Water quality parameters monitored during the study period remained within the acceptable ranges for tilapia growth and averaged 0.48 mg l^{-1} for TAN (range of 0.1 to 6.0 mg l^{-1}), 0.026 mg l^{-1} for NH_3-N (range of 0.003 to 0.028 mg l^{-1}), 0.13 mg l^{-1} for NO_2-N (range of 0.02-2.0 mg l^{-1}) , 5.7 mg l-1 for NO_3-N (range of 0.5-11 mg l^{-1}), 7.6 for pH (range of 7-8.2), 6.1 mg l^{-1} for DO (range of 5.1-7.0 mg l-1) and 30.7 oC for temperature (range of 28 °C-32 °C). On a biweekly basis, all fish in each tank were weighed and counted to monitor growth, survival, and to adjust the feed quantity. At the beginning of the experiment, five fish from the stock were collected as initial sample and at the end of experiment, three fish from each replicate tank were collected as final sample for proximate and fatty acid composition analysis. On termination (213 days), mean body weight (MWT), daily growth rate (DGR), specific growth rate (SGR), feed conversion ratio (FCR), survival, gross fish yield (GY), and condition factor (K) were determined using the formulas reported by Ridha and Azad [12]. The protein efficiency ratio (PER) was calculated using the formula PER= Live weight gain ÷ crude protein fed. The apparent net protein utilization (ANPU %) was calculated using the formula ANPU% = (Final fish body protein - initial body protein) ÷ (total protein fed) x100.

2.2. Chemical Analysis and Organoleptic Test

The proximate composition of the experimental diets were analyzed in triplicate according to the standard procedure [14]. Proximate and fatty composition analysis was carried out on three fish $tank^{-1}$. The fat content of pooled ground and freeze–dried flesh was extracted by the Bligh and Dyer method [15]. The fatty acid composition was determined by preparing methyl esters and analyzing them by gas chromatography using an HP 6890 gas chromatograph (Hewlett-Packard, Palo Alto, California, USA) equipped with a chromapack column (CP-Sil 88 50 meter, ID 0.25mm, Varian Inc, Palo Alto, CA, USA) [16]. Fatty acid methyl ester standard mixture comprising of 25 different fatty acids (ranging from C8 to C22:6) were obtained from Altech Associates, Deerfield, USA. The fatty acids were identified by the comparison of their retention times with a mixture of standards containing all of the fatty acids identified in this study. Each fatty acid was quantified by calculating its peak area relative to the total peak area. These values are referred to as fatty acid content (%). An estimated amount of each fatty acid was calculated from the lipid content and fatty acid content, which approximately corresponds to a gram of fatty acid per 100 g of lipid.

3.3 Immunological Parameters and Immune Genes

Caudal vein blood was collected from three fish of each replicate in Eppendorf tubes using 1.0-ml syringe and centrifuged at 3000 x g/min for 10 min. The collected serum was pooled for each replicate of a treatment.

Lysozyme activity in the serum was determined based on the lysis of the lysozyme sensitive Gram-positive bacterium, *Micrococcus lysodeikticus* (Sigma, USA). The undiluted serum sample (50 µl) was placed into wells of a 96-well plate in triplicate. A 175 µl of *M. lysodeikticus* suspension (75 mg ml^{-1}) prepared in the same buffer was then added to each well. After rapid mixing, the change in turbidity was measured every minute for 5 min at 450 nm at approximately 20° C using a microplate reader. Reduction in the optical density by 0.001 min^{-1} is considered as one unit of lysozyme.

For hemagglutination titer (HAT), triplicate serum was serially diluted to yield double dilution (log_2) on a U-bottom microtiter plate. The double dilution was reacted with 50 µl of sheep red blood cells (SRBC) at density of 10^7 cells ml^{-1}. The highest dilution of the serum showing a clear agglutination with the blood was taken as a titer (log_2). Bacterial agglutinin titers (BAT) was carried out as mentioned above, by reacting a formalin-inactivated *Proteus vulgaris* suspensions (10^7cells ml^{-1}) with doubling dilutions (in sterile PBS) of the serum from three fish per replicate of the probiotic and control fish. The serum dilution showing clear agglutination of the bacterial suspension was considered as the titer and expressed as (log_2) titers.

The phagocytic activity (phagocytosis) of the head kidney leucocytes was determined by coating the wells of ELISA plate with 50-µl of the leucocytes suspension and incubating at 23°C for 1 h, followed by overnight incubation at 4°C. The unbound phagocytes were washed, and the adherent phagocytes were reacted for 1 h at 23°C with 50-µl tissue culture medium (L-15, Sigma), containing fluorescent latex beads (2-µm; Sigma) at 10^7 beads ml^{-1}. The wells were read using a fluorimeter (Mikrotek FLX-800). The luminescence (fluorescence) readings (LR) reflect the number of beads engulfed.

Gene expression related to fish immunoglobulin (IgM), interleukin for cell inflammatory response (IL-β1, 6 and 10), tumor necrosis factor protein (TNF-α), antiviral protein (Mx), stress response genes such as super oxide dismutase (SOD), heat shock protein (HSP), alkaline phosphatase (ALP) and skeletal muscle protein (β-actin) were evaluated. Head-kidney from three fish of a replicate were excised and 2-3 mg head kidney tissue was immediately lysed, and RNA was extracted using a commercial RNA extraction kit (SV total RNA isolation, Promega). Genomic DNA contamination was removed by digesting the extracted nucleic acid using DNAse treatment. The extracted RNA was used to obtain the cDNA (i-Script cDNA kit, BioRad, USA). Quality of RNA and cDNA was determined using Nanodrop 2000 (Thermo Scientific, USA). Primers (based on published literature) optimized for tilapia samples, for the amplification of selected genes, were used to obtain the PCR amplification on a Real time PCR (Stratagene MX-3005P, Agilent). Primers were evaluated for amplification in Nile tilapia, using melt curve analysis (Khansari et al., 2018 *Hernández-Cruz et al., 2015, **Leiva-Rebello et al., 2019). The details of the primers used are given in table 1.

Ingredient	S	A	K
Dry Matter	91.16	93.43	88.47
Crude Protein (%)	33.3	32.4	13.4
Crude Fat (%)	2.48	3.99	4.66
Ash (%)	8.43	9.74	4.58
NFE1	53.75	51.83	77.40
Gross Energy (KJ/g)[2]	18.20	18.28	16.92

[1]Nitrogen free extract calculated as 100 - % (moisture+ crude protein + crude fat + ash).
[2]Estimated according to NRC (1993) using the values of 23.6, 39.5, and 17.2 kJ/g for protein, fat, and total carbohydrate, respectively.

Table 1: Proximate composition of the tilapia feeds used in the study.

2.4 Statistical Analysis

Parameters means were subjected to one-way analysis of variance (ANOVA) at α=0.05 followed by Duncan's Multiple Range Test. Two-way ANOVA was carried out to study the interaction between diets and probiotics using the SPSS 14 statistical package.

3. Results

During the experimental period, fish in all treatments were actively feeding and consumed all of the administered feed. A significant enhancement in feed conversion and gross fish yield was obtained by feeding Nile tilapia with probiotic supplemented feed. Tilapia fed SP feed had the highest values for mean body weight (MWT) of 582.7 g, daily growth rate (DGR) of 2.25 g/fish/day, and 2.23 g/fish/day, respectively, and specific growth rate (SGR) of 1.18 %/day followed by the fish fed S (570.4 g, 2.46 g/fish/day, and 1.17%/day, respectively). Both A and AP showed almost similar values for MWT (529.8 g and 523.1 g, respectively), DGR (2.26 g/fish/day and 2.23 g/fish/day, respectively) and SGR (1.15 %/day, and 1.12%/day, respectively). On the other hand, tilapia fed the Local feed with low protein ratio and its respective probiotic (Local+P)

had significantly lower values for MWT, DGR, and SGR (Table 2). In all treatments, MWT increased linearly with time (Figure 1). However, biweekly sampling showed that at day 100 of the experiment, tilapia fed the Arasco feed had higher MWT than tilapia fed the Arasco+P. On termination (day 213 of the experiment), MWT of tilapia fed the Arasco+P recovered and had almost similar values to tilapia fed the control Arasco feed (Figure 2). The highest FCR ($P<0.05$) was obtained in fish fed the Local and the Local+P feeds. Fish fed the Skretting feed fortified with probiotics (Skretting+P) had the lowest ($P<0.05$) FCR of 1.9 followed by tilapia fed the control Skretting and the Arasco feed with probiotic (2.09 and 2.06, respectively). Tilapia fed the control Arasco and both of the Local feeds had the highest ($P<0.05$) FCR (Table 2). The highest ($P<0.05$) gross tilapia yield (GY) was recorded in tilapia group fed the Skretting+P feed (44.0 kg/m3), followed by its probiotic-free control (41.1 kg/m^3). Tilapia fed the Arasco+P feed had numerically higher GY value (39.5 kg/m^3) than its corresponding non-probiotic control (37.0 kg/m3), however, the difference was not statistically significant. The lowest ($P<0.05$) GY was recorded in tilapia received the low protein Local feed and those received the probiotic version (Local+P) (Table 2). Two-way ANOVA irrespective of probiotics showed Skretting feed to give the highest ($P<0.05$) MWT, DGR, SGR, GY, and the lowest (best) FCR, followed by the Arasco feed. The low protein Local feed resulted in the lowest values ($P<0.05$). However, data analyzed irrespective of feed type showed significant improvement in FCR only due to probiotic enrichment (Table 2). No significant differences were observed among fish fed the six feed types in survival and in condition factor (K). Fish fed the Local and Local+P feeds had significantly higher protein efficiency ratio (PER) and apparent net protein utilization (ANPU) values than tilapia fed the other feeds (Table 2). Two-way ANOVA irrespective of probiotics or feed type showed no significant difference in PER and ANPU among tilapia fed the different feeds (Table 2). No significant interaction between probiotic and feed type was obtained for MWT, DGR, SGR, FCR, GY, PER, and ANPU.

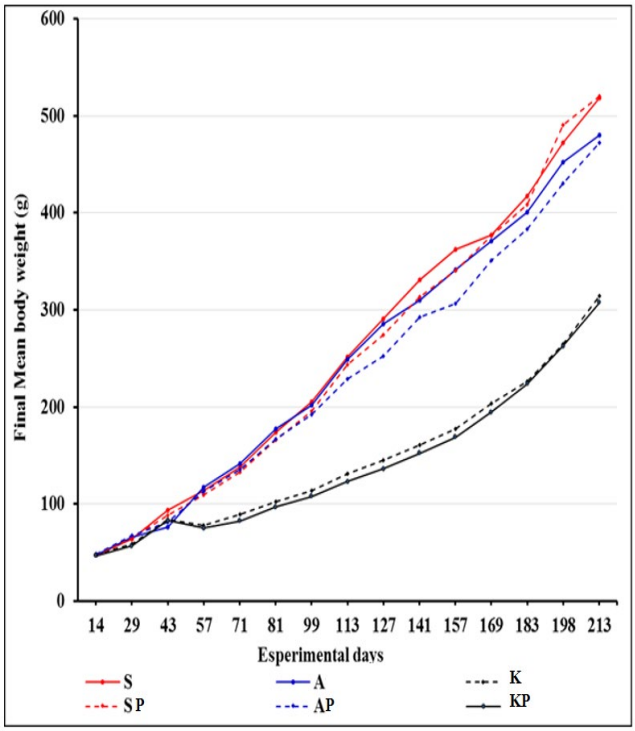


Figure 1: Mean body weight in tilapia fed different feeds for 214 days

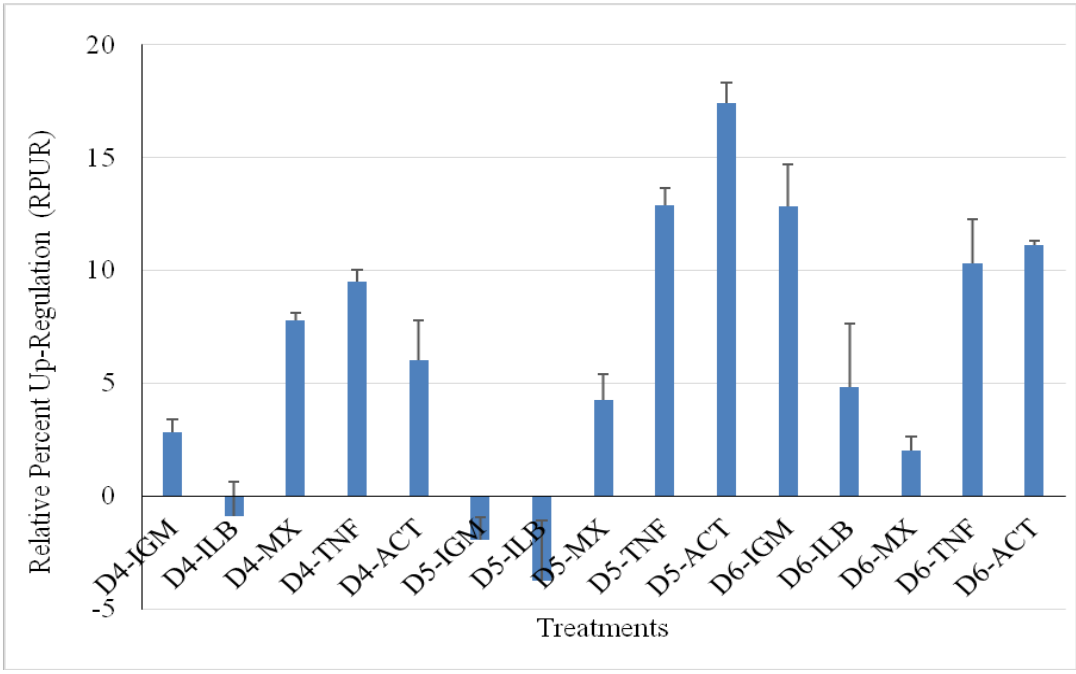


Figure 2: Immune gene regulation patterns in tilapia fed different feeds supplemented with probiotics.

Fatty Acids	KP	AP	SP	K	A	S
C14	3.41	4.19	4.01	3.36	3.92	3.92
C15	0.09	0.10	0.24	0.08	0.09	0.21
C16	16.12	21.95	17.20	15.57	19.52	16.99
C17	0.11	0.28	0.62	0.10	0.32	0.55
C18	3.04	3.34	11.17	2.83	3.16	9.91
C20	0.47	0.31	0.39	0.44	0.32	0.35
C21	0.13	0.17	0.47	0.12	0.16	0.49
C22	0.10	0.12	0.07	0.10	0.12	0.09
C24	0.10	1.16	0.37	0.11	0.98	0.41
C16:1	2.63	2.09	2.63	2.51	2.74	2.64
C17:1	ND	ND	0.24	ND	ND	0.21
C18:1n-9	30.89	26.95	24.56	31.48	27.87	25.71
C20:1n-9	0.44	0.45	0.37	0.38	0.32	0.36
C22:1n-9	0.09	0.29	0.21	0.06	0.30	0.26
C24:1n-9	0.09	0.22	ND	0.08	0.30	ND
C18:2n-6	34.18	28.31	32.34	35.09	30.86	32.91
C18:3n-3	2.21	1.80	1.87	1.84	1.62	1.59
C20:3n-3	0.11	0.29	ND	0.13	0.28	ND
C20:5n-3	1.22	1.15	1.17	1.22	1.16	1.15
C22:6n-3	2.79	3.25	1.88	2.85	3.29	1.79
∑SFA	23.57	31.62	34.54	22.71	28.59	32.92
∑MUFA	34.14	30.00	28.01	34.51	31.53	29.18
∑PUFA	40.51	34.80	37.26	41.13	37.21	37.44

∑n-3	6.33	6.49	4.92	6.04	6.35	4.53
∑n-6	34.18	28.31	32.34	35.09	30.86	32.91
n-3/n-6	0.19	0.23	0.15	0.17	0.21	0.14

SFA=Saturated fatty acid; MUFA= Monounsaturated fatty acid; PUFA= Polyunsaturated fatty acids, ND= Peak not detected.

Table 2: Fatty acid composition (% total fatty acids) of the experimental feeds.

The analyzed fatty acid (FA) profile of the experimental feeds is shown in Table 3. Among saturated FA (SFA), palmitic acid (C16) was the most dominant FA, while C18:1n-9 was the most dominant monounsaturated FA (MUFA) that accounted for about 25% to 31% of the total FA. The ∑n-3 FA in all the diets were comparatively low while the ∑n-6 FA values were exceptionally high in all the feeds, which resulted in the very low n-3/n-6 ratios. The initial and final muscle proximate composition of tilapia is shown in Table 4. Among feed, fish fed the low protein Local feed (K) fortified with probiotic (Local+P) had the highest (P<0.05) muscle moisture content (78.40%), whereas tilapia fed the Arasco feed with probiotic (Arasco+P) had the lowest value (77.19%). There were no significant differences between the muscle protein contents of tilapia fed Skretting, Arasco, Skretting+P, and Arasco+P. However, these values were significantly higher than fish fed the Local and the Local+P feeds with low protein level (13.4%). The highest (P<0.05) muscle lipid content (1.68%) was observed in tilapia fed the Local feed. Fish fed the Arasco and the Arasco+P feeds had the lowest values (P< 0.05). Data analyzed regardless of probiotic showed that feeding tilapia with the Local feed resulted in significantly higher dry matter and lipid content in the muscle than feeding tilapia with the Skretting or Arasco feeds. However, feeding tilapia with the Skretting and the Arasco feeds resulted in a significantly higher protein content in the muscle. Data analyzed regardless of feed type showed no significant effect of probiotic addition on the final muscle proximate composition. No significant interaction between probiotic and feed type was obtained for the proximate muscle composition.

Sl.No.	Gene	Primers	Amplicon (bp)	Citation
1.	LYS	F -5' TCATCGCTGCCATCATCTCC-3' R -5' TGTTCCTCACTGTCCCATGC-3'	211	Khansari et al., 2018; *Hernández-Cruz et al., 2015; **Leiva-Rebello et al., 2019; ***Khansari et al., 2018
2.	IL-1β	F -5' TCAGCACCGCAGAAGAAAAC-3' R -5' TAACACTCTCCACCCTCCAC-3'	148	
3.	IL-6	F -5' ATCCCCTCACTTCCAGCAGA-3' R -5' GCTCTTCGGCTCCTCTTTCT-3'	129	
4.	IL-10	F -5' GAGCGTGGAGGAATCTTTCAA-3' R -5' GATCTGCTGGATGGACTGC-3'	154	
5.	Mx**	F -5' AGGAGACGGTGGTTGACATC-3' R -5' TCTCTTTCCGTTGCCTCAGT-3'	127	
6.	HSP	F -5' AGGTTGGGTCTGAAAGGAAC-3' R -5' TGAACTCTGCGATGAAGTGG-3'	140	
7.	TNF-α	F -5' TCGTTCAGAGTCTCCTGCAG-3' R -5' AAGAATTCTTAAAGTGCAAACACACCAAA-3'	320	
8.	TGF-β1	F -5' AGACCCTTCAGAACTGGCTC-3' R -5' ACTGCTTTGTCTCCCCTACC-3'	145	
9.	GLP*	F -5' AGTTAATCCGGAATTCGTGAGA-3' R -5' TGAGTGTAGTCCCTGGTTGTTG-3'	168	
10.	ALP*	F -5' AGAACGCCCTGACGCTGCAA-3' R -5' TTCAGTATACGAGCAGCCGTCAC-3'	109	
11.	SOD*	F -5' GTTGGAGACCTGGGAGATGT-3' R -5' CTCCTCATTGCCTCCTTTTC-3'	159	
12.	ACT*	F -5' TCTGTCTGGATCGGAGGCTC-3' R -5' AAGCATTTGCGGTGGACG-3'	113	
13.	IgM***	F -5'- GATCGTGACATCGTCTGAGG-3' R -5'- TGTTGGGTTGTGGTTGTAGG-3'	187	

Table 3: Genes and Primers Used in the Gene Expression Procedures

Parameter	S	A	K	SP	AP	KP	Feed Effect			Probiotic effect	
							S	A	K	Control	Probiotic
MWT	570.4±24.0^{ab}	525.9±8.4^{ab}	366.7±9.5^{c}	582.7±22.2^{a}	523.1±25.7^{b}	362.8±11.1^{c}	576.5±14.8^{a}	524.5±12.1^{b}	364.8±5.2^{c}	487.7±31.9^{a}	489.5±34.3^{a}
DGR	2.46±0.19^{ab}	2.26±0.08^{ab}	1.49±0.04^{c}	2.52±0.11^{a}	2.23± 0.12^{b}	1.48± 0.03^{c}	2.49±0.07^{a}	2.24±0.06^{b}	1.49±0.02^{c}	2.07±0.15^{a}	2.08±0.16^{a}
SGR	1.17±0.03^{ab}	1.15±0.02^{ab}	0.95±0.01^{c}	1.18±0.02^{a}	1.12±0.02^{b}	0.96±0.01^{c}	1.18±0.01^{a}	1.14±0.01^{b}	0.96±0.0^{c}	1.09±0.04^{a}	1.09±0.03^{a}
FCR	2.09 ± 0.05^{ab}	2.20±0.04^{a}	2.18±0.03^{a}	1.9±0.08^{b}	2.06±0.05^{ab}	2.16±0.05^{a}	2.02±0.05^{c}	2.13±0.04^{ab}	2.17±0.02^{b}	2.16±0.03^{a}	2.05±0.04^{b}
GY	41.1±0.53^{ab}	37.0±0.62^{b}	26.8±0.83^{c}	44.0±2.07^{a}	39.5± 1.34^{b}	27.4±0.05^{c}	42.6±1.16^{a}	38.7±0.74^{b}	27.1±0.44^{c}	35.3±2.19^{a}	37.0±2.58^{a}
Survival	94.7 ± 2.67^{a}	97.3 ± 1.33^{a}	96.0 ± 4.0^{a}	98.7 ± 1.33^{a}	100± 0.0^{a}	98.7 ± 1.33^{a}	88.7±1.61^{a}	88.7±1.23^{a}	89.3±1.98^{a}	87.1±1.46^{a}	90.7±0.67^{a}
K	1.96±0.04^{a}	1.99±0.06^{a}	1.96±0.07^{a}	1.93±0.02^{a}	1.86±0.03^{a}	1.83±0.03^{a}	1.94±0.02^{a}	1.92±0.04^{a}	1.89±0.04^{a}	1.97±0.03^{a}	1.87±0.02^{a}
PER	1.62±0.07^{b}	1.64±0.04^{b}	3.65±0.1^{a}	1.85±0.03^{b}	1.69±0.01^{b}	3.60±0.19^{a}	1.74±0.07^{b}	1.67±0.01^{b}	3.63±0.85^{a}	2.30±0.61^{a}	2.38±0.67^{a}
ANPU	31.0±0.1^{a}	31.4±0.6b^{c}	66.3±2.7^{a}	36.0±0.6^{b}	32.9±0.3^{bc}	68.9±1.7^{a}	33.5±1.44^{b}	32.2±0.43^{b}	67.6±0.75^{a}	42.9±11.7^{a}	45.9±11.5^{a}

Table 4: Growth performance, feed conversion, gross yield, condition factor, protein efficiency ratio and apparent net protein utilization in *O. niloticus* fed different feeds for 213 days

Fatty acids of tilapia muscle lipid expressed as percentages of the total FA are given in Table 5. Palmitic acid (C16:0) was the dominant SFA amounting 23.5% to 25.3% of the total FA. The low protein Local feed resulted in the highest ($P < 0.05$) C16:0. Oleic FA (C18:1n-9) was the most dominant MUFA, ranging from about 26 to 32. Tilapia fed the Local and the Local+P feeds had significantly higher C18:1n-9 than those fed the other feeds. Fish fed Skretting and Skretting+P had significantly higher C18:2n-6 and eicosapentaenoic acid (EPA) than the other feeds. The lowest docosahexaenoic acid (DHA) content was reported in tilapia fed Skretting, Local, Skretting+P and Local+P. The ∑SFA, ∑MUFA and ∑PUFA values accounted for 36.5% to 37.8%, 33.6% to 41.1%, and 17.4% to 24.5%, respectively (Table 6). Fish fed Skretting and Arasco feeds supplemented with probiotic had significantly higher muscle ∑n-3 FA than their respective non-probiotic control feeds. However, no significant differences were observed in muscle ∑n-3 FA content of fish fed the Local feed and its probiotic version. Tilapia fed Skretting and Skretting+P had significantly higher ∑n-6 FA than the other diets. In general, the n-3/n-6 ratios were low, ranging from 0.57 to 0.99. Feeding Nile tilapia with the probiotics fortified Skretting and Arasco feeds resulted in a significantly higher muscle ∑n-3/n-6 ratio compared to their respective non-fortified controls. No significant differences was detected between the mean scores of different organoleptic attributes of raw and cooked tilapia muscle. The color of the raw muscles were pinkish white. Raw tilapia muscle from all treatments showed higher scores (4.7 to 5.0) out of 5.0. However, fish fed the Skretting impregnated with probiotics had slightly higher overall acceptance than those fed other feeds. Two-way ANOVA showed no effect of probiotic addition to the feeds on the muscle fatty acids profile. However, feed type had significant effect on the muscle fatty acids. Tilapia fed the Skretting and Arasco feeds had significantly higher ∑PUFA, ∑n-3 FA, ∑n-6 FA. Tilapia fed the Skretting feed had the highest EPA (C20:5n-3), whereas tilapia fed the Arasco feed had the highest ($P<0.05$) DHA (C22:6n-3), ∑SFA and ∑n-3/n-6. Feeding tilapia with the Local feed resulted in the highest ∑MUFA. No significant interaction between probiotic and feed type was obtained for the different fatty acids.

Parameter	S	A	K	SP	AP	KP	Effect of feed			Effect of Probiotic	
							S	A	K	Control	Probiotic
Dry matter	77.4±0.6^{bc}	77.6±0.5^{bc}	77.9±0.2^{ab}	77.5±0.1^{bc}	77.2±0.3^{c}	78.4±0.3^{a}	77.4±0.16^{b}	77.4±0.17^{b}	78.2±0.13^{a}	77.6±0.15^{a}	77.7± 0.19^{a}
Protein	19.1±0.1^{a}	19.1±0.3^{a}	18.2±0.1^{b}	19.2±0.1^{a}	19.3±0.3^{a}	18.0±0.1^{b}	19.2±0.05^{a}	19.2±0.12^{a}	18.1±0.06^{b}	18.8±0.16^{a}	18.9±0.21^{a}
Lipid	1.5±0.2^{ab}	1.3±0.1^{c}	1.7±0.2^{a}	1.5±0.1^{bc}	1.3±0.1^{c}	1.6^{ab}±0.1	1.49±0.04^{b}	1.30±0.02^{c}	1.65±0.5^{a}	1.50±0.07^{a}	1.46±0.05^{a}
Ash	1.3±0.06^{a}	1.2±0.12^{b}	1.1±0.21^{b}	1.3±0.04^{a}	1.1^{b}±0.06	1.0±0.02^{c}	1.29±0.01^{a}	1.17±0.02^{b}	1.08±0.03^{c}	1.21±0.04^{a}	1.15±0.04^{a}

Initial values for muscle proximate composition were 78.12% moisture, 18.05% protein, 1.33% lipid, and 1.78% ash.
Mean ± SEM in a column with different superscripts are significantly different ($P < 0.05$).

Table 5: Muscle proximate composition (% fresh matter basis) of in *O. niloticus* fed different feeds for 213 days

Fatty acid	S	A	K	SP	AP	KP
C14	1.74±0.06^{c}	1.91±0.01^{b}	2.50±0.02^{a}	1.58±0.03^{d}	1.78±0.04^{c}	2.45±0.02^{a}
C15	0.16±0.0b	0.18±0.0^{a}	0.16±0.0^{b}	0.13±0.01^{c}	0.17±0.0^{ab}	0.18±0.01^{a}
C16	23.5±0.09^{c}	24.4±0.1^{b}	25.3 ±0.08^{a}	23.6 ±0.18^{c}	24.3±0.03^{b}	24.4 ±0.27^{b}
C17	0.30±0.01^{b}	0.31±0.01^{b}	0.32±0.01^{ab}	0.23±0.0^{d}	0.26±0.0^{c}	0.33±0.0^{a}
C18	7.17±0.13^{b}	8.09±0.08^{a}	6.39±0.21^{c}	7.10±0.06^{b}	8.11±0.17^{a}	6.57±0.31^{bc}
C20	1.31±0.05^{a}	0.76±0.01^{d}	0.91±0.01^{c}	1.14±0.03^{b}	0.83±0.01^{cd}	0.83±0.02cd
C21	0.28±0.03^{ab}	0.21±0.01^{c}	0.32±0.01^{a}	0.24±0.02^{bc}	0.24±0.0^{bc}	0.28±0.02^{ab}

C22	2.02±0.09^{a}	1.79±0.03^{b}	1.41±0.03^{c}	2.13±0.06^{a}	1.74±0.01^{b}	1.37±0.09^{c}
C24	0.16±0.03^{cd}	0.25±0.01^{b}	0.12±0.0^{d}	0.18±0.01^{c}	0.30±0.02^{a}	0.17±0.01^{cd}
C16:1	3.11±0.08^{bc}	3.09±0.07^{bc}	5.23±0.21^{a}	2.79±0.11^{c}	3.40±0.06^{b}	4.75±0.26^{a}
C17:1	0.14±0.01^{b}	0.08±0.0^{c}	0.24±0.0^{a}	0.09±0.0^{c}	0.10±0.0^{c}	0.25±0.02^{a}
C18:1n-9	28.3±0.44^{b}	26.4±0.09^{c}	31.7 ±0.25^{a}	26.2 ±0.04^{c}	26.3±0.09^{c}	32.2 ±0.64^{a}
C20:1n-9	1.38±0.01^{b}	1.26±0.01^{c}	1.67±0.05^{a}	1.34±0.01^{b}	1.24±0.03^{c}	1.65±0.0^{a}
C22:1n-9	0.0±0.0^{a}	0.09±0.08^{a}	0.05±0.04^{a}	0.0±0.0^{a}	0.11±0.01^{a}	0.06±0.06^{a}
C24:1n-9	3.63±0.26^{b}	2.62±0.02^{cd}	2.24±0.06^{cd}	4.81±0.12^{a}	2.72±0.03^{c}	2.12±0.22^{d}
C18:2n-6	13.9±0.14^{a}	12.9±0.03^{b}	10.6 ±0.04^{d}	14.0 ±0.03^{a}	12.3±0.04^{b}	11.7 ±0.29^{c}
C18:3n-3	0.49±0.05^{bc}	0.57±0.01^{a}	0.46±0.01^{c}	0.42±0.02^{c}	0.55±0.01^{ab}	0.47±0.01^{c}
C20:3n-3	4.79±0.47^{b}	4.86±0.04^{b}	2.82±0.07^{c}	6.05±0.34^{a}	5.14±0.04^{b}	2.66±0.22^{c}
C20:5n-3	1.97±0.14^{ab}	1.44±0.04^{b}	1.67±0.03^{b}	2.19±0.04^{a}	1.57±0.02^{b}	1.49±0.12^{b}
C22:6n-3	1.88±0.01^{c}	4.45±0.05^{b}	1.92±0.02^{c}	1.85±0.10^{c}	4.84±0.14^{a}	2.09±0.17^{c}
∑SFA	36.6±0.02^{bc}	37.8±0.06^{a}	37.4 ±0.34^{a}	36.5 ±0.32^{c}	37.3±0.29^{ab}	36.6 ±0.10^{bc}
∑MUFA	36.6±0.36^{b}	33.6±0.08^{d}	41.1 ±0.41^{a}	35.2±0.01^{c}	33.8±0.19^{d}	41.1 ±0.75^{a}
∑PUFA	23.1±0.44^{b}	24.3±0.09^{ab}	17.4 ±0.14^{c}	24.5±0.16^{a}	24.4±0.12^{ab}	18.4 ±0.79^{c}
∑n-3	9.1±0.57^{c}	11.3±0.12^{ab}	6.9±0.11^{d}	10.5±0.19^{b}	12.1±0.16^{a}	6.7±0.50^{d}
∑n-6	13.9±0.19^{a}	12.9±0.03^{b}	10.6±0.03^{e}	14.0±0.03^{a}	12.3±0.04^{c}	11.7±0.29^{d}
n3/n6	0.66±0.45^{d}	0.88±0.02^{b}	0.65±0.01^{d}	0.75±0.01^{c}	0.99±0.02^{a}	0.57±0.03^{d}

[1]Data are percentage of total fatty acids

Means in each row having different superscripts are significantly different ($P < 0.05$).

SFA=Saturated fatty acid; MUFA= Monounsaturated fatty acid; PUFA= Polyunsaturated fatty acids

Table 6: Muscle fatty acid composition of *O. niloticus* fed different feeds for 213 days

The highest (P<0.05) gut bacterial count was observed in fish fed the probiotic versions of the Skretting and the Arasco feeds. Tilapia fed the Skretting+P feed had the highest (P< 0.05) serum lysozyme activity (LA) value, followed by tilapia fed the Arasco+P and those fed the low protein Local+P. Tilapia fed Skretting+P feed had the highest (P<0.05) phagocytic activity (PA) followed by those fed the Arasco+P feed. Feeding tilapia with the low protein Local and the Local+P feeds resulted in significantly lower phagocytic activity. The Skretting+P feed resulted in significantly higher hemagglutination titer (HAT) value in tilapia serum than fish in the other treatments. The non-probiotic feeds had significantly lower HAT values (Table 7). No significant difference was observed among treatments in the bacterial hemagglutination titer (BAT) against the pathogenic bacteria Proteus vulgaris. However, the probiotic fortified diets showed higher values than the non-probiotic control diets. Except for BAT, two-way ANOVA irrespective of feed type showed significant improvement in the bacterial count, LA, PA, and HAT due to supplementation of probiotics to the commercial feeds (Table 7). However, data analyzed irrespective of probiotic showed significant improvement in bacterial count and phagocytosis due to feeding tilapia with the higher protein feeds (Skretting and Arasco). A significant interaction between probiotic and feed type was evident only for the bacterial count.

Fatty acid	Effect of feed			Effect of probiotic	
	S	A	K	Control	Probiotic
C14	1.66±0.05^{c}	1.85±0.04^{b}	2.48±0.02^{a}	2.05±0.15^{a}	1.94±0.17^{a}
C15	0.143±0.01^{b}	0.168±0.0^{a}	0.175±0.05^{a}	0.17±0.01^{a}	0.16±0.03a
C16	23.6±0.09^{b}	24.3±0.05^{a}	24.9±0.27^{a}	24.4±0.33^{a}	24.1±0.17^{a}
C17	0.27±0.02^{b}	0.28±0.01^{ab}	0.32±0.0^{a}	0.31±0.00^{a}	0.27±0.02^{a}
C18	7.13±0.06^{b}	8.1±0.08^{a}	6.48±0.16^{c}	7.21±0.32^{a}	7.26±0.30^{a}
C20	1.22±0.05^{a}	0.79±0.02^{b}	0.87±0.03^{b}	0.99±0.10^{a}	0.93±0.07^{a}
C21	0.26±0.02^{ab}	0.23±0.01^{ab}	0.30±0.01^{a}	0.27±0.02^{a}	0.25±0.01^{a}

C22	2.07±0.05[a]	1.76±0.02[b]	1.39±0.04[c]	1.73±0.11[a]	1.74±0.14[a]
C24	0.17±0.01[b]	0.27±0.02[a]	0.14±0.01[b]	0.17±0.02[a]	0.22±0.03[a]
C16:1	2.95±0.11[b]	3.25±0.10[b]	4.99±0.19[a]	3.81±0.45[a]	3.64±0.37[a]
C17:1	0.11±0.01[b]	0.09±0.01[b]	0.24±0.01[a]	0.15±0.03a	0.15±0.03[a]
C18:1n-9	27.3±0.64[b]	26.4±0.07[b]	31.9±0.32a	28.8±0.97[a]	28.2±1.28[a]
C20:1n-9	1.36±0.01[b]	1.25±0.01[c]	1.66±0.01[a]	1.44±0.08[a]	1.41±0.08[a]
C22:1n-9	0.0±0.0[b]	0.1±0.04[a]	0.05±0.03[ab]	0.04±0.03[a]	0.06±0.02[a]
C24:1n-9	4.22±0.36[a]	2.67±0.03[b]	2.18±0.10[b]	2.83±0.27[a]	3.21±0.52[a]
C18:2n-6	13.99±0.06[a]	12.62±0.19[b]	11.13±0.35[c]	12.50±0.64[a]	12.67±0.44[a]
C18:3n-3	0.45±0.03[b]	0.56±0.01[a]	0.46±0.0[b]	0.50±0.02[a]	0.48±0.03[a]
C20:3n-3	5.42±0.43[a]	5.0±0.08[a]	2.74±0.10[b]	4.16±0.44[a]	4.62±0.65[a]
C20:5n-3	2.08±0.09[a]	1.50±0.04b	1.58±0.07[b]	1.69±0.10[a]	1.75±0.14[a]
C22:6n-3	1.87±0.04[b]	4.65±0.13[a]	2.0±0.09[b]	2.75±0.54[a]	2.93±0.61[a]
∑SFA	36.6±0.13[b]	37.6±0.19[a]	37.0±0.28[b]	37.28±0.25[a]	36.79±0.20[a]
∑MUFA	35.9±0.42[b]	33.7±0.11[c]	41.1±0.35[a]	37.08±1.38[a]	36.68±1.42[a]
∑PUFA	23.8±0.45[a]	24.3±0.07[a]	17.9±0.43[b]	21.59±1.34[a]	24.44±1.29[a]
∑n-3	9.8±0.47[b]	11.7±0.24[a]	6.8±0.22[c]	9.09±0.83[a]	9.77±1.02[a]
∑n-6	13.9±0.06a	12.6±0.19[b]	11.1±0.35[c]	12.49±0.64[a]	12.67±0.44[a]
n-3/n-6	0.70±0.33[b]	0.93±0.03[a]	0.61±0.26[b]	0.73±0.05[a]	0.77±0.08[a]

[1]Data are percentage of total fatty acids

Means in each row having different superscripts are significantly different (P < 0.05.

SFA=Saturated fatty acid; MUFA= Monounsaturated fatty acid; PUFA= Polyunsaturated fatty acids what was the sample number used in these analyses

Table 7: Effect of feed type and probiotic on muscle fatty acid composition of *O. niloticus* fed different feeds for 213 days

Immune genes showed significant up regulation due to supplementation of the feed with the probiotic preparations. The regulation pattern of the different genes in the head-kidney is presented in Table 8. In general, feeding tilapia with Skretting feed fortified with probiotic resulted in the lowest cycle threshold (Ct) value for β-actin, Mx, cytokines IL-1β, and IL-10. On the other hand, feeding tilapia with Arasco feed enriched with probiotic resulted in the lowest (P<0.05) cycle threshold for cytokines IL-6 and TNF-α. Both Skretting+P and Arasco+P stimulated the production of IgM. Two-way ANOVA regardless of feed type showed immune genes to be significantly up-regulated with probiotic addition. Data analyzed irrespective of probiotic showed Skretting to cause the best (P<0.05) stimulation for β-actin, IgM, Mx, cytokines IL-1β, and IL-10 genes, whereas Arasco feed resulted in the lowest (P<0.05) Ct for cytokines IL-6 and TNF-α genes (Table 8). A significant interaction between probiotic and feed type was evident for the tested genes.

	S	A	K	SP	AP	KP	Effect of feed			Effect of probiotic	
							S	A	K	Control	Probiotic
BC	37.3±6.3[b]	42.7±8.5[b]	24.3±4.9[b]	533.3±147.7[a]	516.7±112.6[a]	31.7±7.8[b]	285.3±129.1[a]	279.7±117.4[a]	28.0±4.4[b]	34.8±4.3[b]	360.5±98.2[a]
LA	11.3±3.4[bc]	8.3±2.8[c]	7.0±1.0[c]	25.7± 4.1[a]	21.0± 3.8[ab]	16.3±3.3[abc]	18.5±3.99[a]	14.7±3.5[a]	11.7±2.6[a]	8.88±1.5[b]	21.0±2.3[a]
PA	45.7±4.8[c]	46.2±0.21[c]	19.4±1.14[d]	115.3±9.4[a]	69.4±3.9[b]	20.9±1.4[d]	80.5±15.9[a]	57.8±5.3[b]	20.2±2.1[c]	37.1±4.6[b]	68.5±13.8[a]
HAT	3.67± 0.33[b]	2.67±0.33[b]	2.67±0.67[b]	6.67± 1.45[a]	4.67±0.33[ab]	5.0± 1.0[ab]	5.17±0.95[a]	3.67±0.49[a]	3.83±0.7[a]	3.00±0.3[b]	5.4±0.6[a]
BAT	1.67± 0.33[a]	2.33±0.88[a]	2.67±0.67[a]	4.67± 0.67[a]	4.33± 1.45[a]	3.0± 0.58[a]	3.17±0.75[a]	3.33±0.88[a]	2.83±0.4[a]	2.22±0.4[b]	4.0±0.6[a]
IgM	20.2±0.14[d]	28.3 ±0.07[a]	24.7± 0.26[b]	19.9± 0.12[d]	19.6±0.07[d]	21.5± 0.33[c]	19.9±0.14[c]	23.9±1.93[a]	23.1±0.73[b]	24.4±1.17[a]	20.2±0.33[b]
Mx	26.3± 0.05[e]	26.8 ± 0.09[d]	29.2 ± 0.07[a]	24.3 ± 0.07[f]	27.2 ±0.08[c]	28.6± 0.05[b]	25.3±0.46[c]	27.0±0.11[b]	28.9±0.14[a]	27.4±0.45[a]	26.7±0.64[b]
TNF-α	30.1 ± 06[c]	28.4 ± 0.05[d]	34.5 ± 0.14[a]	27.3 ± 0.08[e]	26.5± 0.23[f]	30.9 ± 0.41[b]	28.7±0.64[b]	27.5±0.44[c]	32.8±0.82[a]	31.0±0.91[a]	28.2±0.70[b]
IL-β1	26.4±0.19[c]	29.9± 0.15[b]	28.3± 0.07[b]	26.7 ±0.15[c]	29.9 ±0.14[a]	26.9 ±0.49[c]	26.5±0.12[c]	29.4±0.22[a]	27.6±0.37[b]	27.9±0.38[a]	27.8±0.54[a]
IL-6	19.3±1.36[b]	12.6± 0.02[d]	40.0± 0.0[a]	15.0 ±0.67[c]	13.7 ±0.44[cd]	40.0 ±0.0[a]	17.2±1.31[b]	13.2±0.36[c]	40.0 ±0.0[a]	23.9±5.22[a]	22.9±5.41[a]
IL-10	15.8±0.73[b]	12.9±0.15[c]	40.0± 0.0[a]	12.6 ±0.12[c]	40.0 ±0.0[a]	40.0 ±0.0[a]	14.2±0.99[c]	26.5±7.82[b]	40.0 ±0.0[a]	22.9±5.43[b]	30.9±5.79[a]
HSP	13.8±0.52[b]	13.0± 0.23[b]	40.0± 0.0[a]	12.9 ±0.14[b]	40.0 ±0.0[a]	13.6 ±0.95[b]	13.4±0.33[c]	26.5±7.80[b]	40.0 ±0.0[a]	22.3±5.61[a]	22.2±5.64[a]

LYS	25.4±3.94[a]	26.5± 2.92[a]	24.9±4.59[a]	30.1 ±1.57[a]	26.5±3.45[a]	26.3 ±3.39[a]	27.7±2.20[a]	26.5±1.84[a]	25.6 ±2.36[a]	25.6±1.76[a]	27.6±1.52[a]
SOD	24.89±1.34[a]	27.6± 2.57[a]	26.0±3.46[a]	31.46 ±0.49[a]	25.6±0.28[a]	27.1 ±4.31[a]	28.2±1.98[a]	26.6±1.20[a]	26.6 ±2.27[a]	26.2±1.27[a]	28.1±1.58[a]
GLP	34.09±3.83[a]	35.2± 3.24[a]	34.4±4.13[a]	39.4 ±0.61[a]	33.7±0.94[a]	35.5 ±4.54[a]	36.7±2.20[a]	34.4±1.44[a]	34.9 ±2.52[a]	34.6±1.69[a]	36.17±1.61[a]
AKP	27.3±2.75[a]	35.89± 3.75[a]	25.8±4.99[a]	30.0 ±0.72[a]	27.6±3.29[a]	27.5 ±2.98[a]	28.6±1.41[a]	31.7±3.15[a]	26.6 ±2.42[a]	29.6±2.67[a]	28.3±1.27[a]
βACT	21.9±0.30[a]	23.1±0.33[a]	22.6±0.18[a]	23.2±0.01[a]	22.4±0.07[a]	22.8±0.18[a]	22.5±0.40[a]	22.8±0.26[a]	22.7 ±0.37[a]	22.5±0.26[a]	22.8±0.27[a]

In each row, means ± SEM having different superscripts are significantly different (P <0.05).

BC: bacterial count (x10^{4}cfu/cm), LA: lysozyme activity (units/ml), PA: phagocytic activity, HAT: hemagglutination titer (log_2), BAT: bacterial agglutination titer (log_2).

Table 8: Gut bacterial count, immunological parameters Ct values of immune genes in head kidney of *O. niloticus* fed different feeds for 213 days

4. Discussion

Results of this study showed a trend of significant enhancement of growth parameters, feed conversion, gross fish yield, and some immune parameters and stimulation of some immune genes by feeding tilapia with probiotic-supplemented feed. The better growth parameters obtained in this study with the commercial tilapia feed Skretting makes this feed more favorable for tilapia growers over the Arasco feed. On the other hand, the significantly lower growth parameters and production rate of tilapia fed the Local feed with low protein ratio (13.4%) indicate that this commercial tilapia feed is not suitable for tilapia farming and should not be used by tilapia growers. The probiotic supplement, containing primarily Bacillus and lactobacillus spp., was responsible for the growth promotion in tilapia fed the fortified diet Skretting+P as evident by the total bacterial count observed in the gut of the Skretting+P fish. These probiotic bacteria bring about prominent growth enhancement in Nile tilapia [11,12,17,18]. The reason behind the failure of the probiotic-supplemented Arasco feed to improve tilapia growth could be only speculated. However, the nutritional factors could be responsible for this failure. Welker and Lim and Cha at al. stated that variables including nutrition would result in variable effects of the added probiotics on fish growth, making it difficult to arrive at definite recommendations on the actual effect of probiotics on growth enhancement [19,20]. Ridha and Azad obtained higher growth performance than controls in 19 g O. niloticus only after 40 to 61 days of withdrawing Arasco feed (44% protein) enriched with Bacillus. amyloliquefaciens probiotic bacteria [12]. Therefore, it is possible that the positive effect of the probiotics on growth would be more evident sometimes after termination, since the biweekly sampling showed that at on day 213 mean body weight of tilapia fed the Arasco+P recovered and was similar to those fed the control Arasco feed. The failure of probiotics to enhance growth in tilapia fed the Local+P to the low protein content (13.4%) and lower nutrients digestibility. Moreover, it is possible that this low quality feed did not help the probiotic bacteria to bind with it. Ghazalah et al. demonstrated that Nile tilapia fry (1.2 g) fed with a relatively low crude protein level of 25% without or with commercial probiotic bacteria for 120 days had the lowest growth performance and worst FCR than those fed with higher crude protein (27.5% and 30%) [21]. Lara- Flores et al., (2003) found that the Nile tilapia fry (0.15 g) fed diets containing 40% protein for 63 days showed higher growth than lower protein diet of 27%.

In fish farming, commercial feed represents more than 50% of the operational cost (El-Sayed, 2006). Therefore, any enhancement in FCR would be positively reflected on the cost of fish production. The lower FCR obtained in fish fed the control Skretting feed indicates that fish were able to convert the feed components into fish muscle more efficiently than Arasco. The fact that Local feed resulted in the worst FCR values support the conclusion that the Local feed with the low protein level is not suitable for tilapia culture. Moreover, results suggest that supplementing with feeds containing optimum protein level such as Skretting and Arasco fortified with probiotic bacteria had a beneficial effect in enhancing FCR. These results are in line with those reported by Ridha and Azad [11,12]. FCR improvement in fish fed probiotic diets Skretting+P and Arasco+P is correlated with the significantly higher bacterial counts and colonization observed in the gut of tilapia fed these diets. Such colonization enhances fish appetite, digestion of the feed ingredients, extraction of nutrients, and the absorption rate of nutrients due to increased villous heights in the intestine [22,23]. On the other hand, the failure of probiotics to improve FCR when tilapia fed the probiotic diet Local+P could be related to the low protein content of the diet (13.37%) and to the significantly lower bacterial counts in the gut. The ingredients of this feed probably required lower pH (higher acidic medium) to be digested compared with Skretting and Arasco, thus negatively affecting survival, growth, and adhesion ability of the added probiotic bacteria to the inner mucosa of the intestinal epithelium. Therefore, results of this study may suggest that colonization of the added probiotic bacteria in the host gut is dependent on the quality of the used feed. The improvement in growth and FCR is probably caused by the increased absorption rate of nutrients because of increased villous heights and surface area, and to the improved digestion due to the digestive enzymes added to the commercial probiotic [22,23].

Although Local and Local+P showed the worst FCR values among all diets, the PER and ANPU values in these two diets were significantly higher than the other diets. This could be due to the lower dietary protein levels (13.4%) in the Local and Local+P feeds. It is generally observed in fish that protein retention efficiency increases with low protein intake [24]. Therefore, less of the dietary protein is either excreted or used as energy substrate. In this study, the PER range of 1.62% to 1.85% and the ANPU range of 31% to 36% obtained in fish fed Skretting, Arasco, Skretting+P, and

Arasco+P are similar to the range of 1.29% to 1.93% reported by Younis, Al-Quffail, Al-Asgah, Abdel-Warith and Al-Hafedh, and by Hossain, Tarafder, Kader and Becker, for Nile tilapia, respectively [25,26]. However, in this study, fortification of commercial diets with probiotics did not improved the PER or APNU values significantly.

Condition factor (K) reflects the fish condition in the culture unit. Crab, Kochva; Verstraete and Avnimelech reported that K above 1.8 indicates good condition. In the present study, K was above 1.8 indicating the well-being of the fish despite the slow growth in tilapia fed Local and Local+P [27].

The proximate composition of fish is affected by the composition of their food and a range of other factors such as species, genetics, size, reproductive stage, season, and environmental factors [28]. The present study showed changes in the chemical composition of tilapia flesh which appear to be related to the variation in the nutrients of the diets. Tilapia fed Skretting, Arasco, Skretting+P, and Arasco+P had significantly higher muscle protein levels than fish fed Local and Local+P. However, these levels are higher than the values reported by Vieira, Hilsdorf and Moreira for the Red-Stirling tilapia (18.6%) and hybrid tilapia (18.5%), but similar to those reported for the Chitralada stain of the Nile tilapia (19.4%) fed a commercial feed containing 32% protein diets [29]. Ahmed and Abdel-Tawwab also reported slightly lower levels of whole body protein content (16.7% to 17.2%) in Nile tilapia fed caraway seed meal as additive in a commercial diet containing 20% crude protein [30]. In the present study, muscle lipid levels were generally low (1.30% to 1.68%), but higher than the range of 0.58% to 0.72% reported by Thongprajukaew et al. for sex-reversed Nile tilapia fed diets containing 8.43% to 9.21% lipid [31]. Ahmed and Abdel Tawwab used diets with higher lipid levels (7.3% to 7.4%) than those used in this study and reported higher levels for the whole body lipids (3.5% to 4.5%) [30].

It is well known that fatty acid composition of tissues is determined mainly by their dietary lipids. In the present study, the muscle fatty acid composition reflected the dietary fatty acid content particularly DHA, which was significantly higher in fish fed Arasco and Arasco+P (4.45% and 4.84%, respectively) than those fed other diets. However, these DHA levels were higher than the range of 2.85% to 3.35% obtained in the GIFT strain of the Nile tilapia fed diets containing different levels of perilla oils, but lower than the ranges of 7.54% to 7.93% reported by Vieira et al. for the Red-Stirling tilapia hybrid and the Chitralada strain [29,32]. It is surprising that although fish fed Skretting+P resulted in highest growth parameters, the muscle DHA content was significantly lower than those fed Arasco and Arasco+P. However, the muscle ∑PUFA levels in fish fed Arasco, Skretting+P, and Arasco+P were not statistically different.

The n-3/n-6 ratio is a better index for comparing the relative nutritional value of different species of fish [28]. However, this index is of limited value without considering which fatty acids are present. Generally, C20, C22 fatty acids are more valuable than C18 fatty acid [3]. Owning to their predominating quantity, DHA and EPA are responsible to the greatest extent for changes in the n-3/n-6 ratios. According to Sargent, the optimum ratio of n-3/n-6 PUFA should be 1:5 (0.2) [6]. Therefore, the higher the n-3/n-6 ratios, the higher the ability of the body to utilize n-3 fatty acids. In the present study, the n-3/n-6 ratio varied between 0.57 to 0.99 with fish fed Arasco+P showing significantly higher n-3/n-6 ratio compared to fish fed the other diets. Young stated that in farmed tilapia, the ratio of n-3/n-6 did not exceed 1.0, which corroborated the n-3/n-6 ratios obtained in the present study [34]. However, this low n-3/n-6 ratio could be attributed to the higher proportion of n-6 fatty acids present in the diets used, in particular the C18:2n-6 fatty acid.

The pinkish white color of the raw muscles from all treatments with high scores of 4.7 to 5.0 out of 5.0 indicates better quality of the muscles in terms of color. In general, consumers prefer more or less firm and elastic fish muscle. It seems that fat-rich tissues usually taste smooth and succulent. In the present study, the fat content in tilapia muscles from all treatments were comparatively low. However, the scores of 'taste' of cooked muscle from fish fed Arasco and Local were comparatively higher than those fed the remaining diets.

Aquatic environment exposes the aquatic animals to pathogens to a higher degree than the animals in the terrestrial environment with typically a million bacteria and 10 million viruses per milliliter of seawater (Fuhurman, 1989). Husbandry-related stress in aquaculture makes the fish much more vulnerable to diseases if the diet provided to the fish lacks sufficient energy and nutrition for maintaining homeostasis [8]. The immune functionality in fish, hence, requires a considerable support through diet for keeping the non-specific and specific components of immunity ready for any challenge by a disease-causing agent. The diets used in the present study showed a clear influence on the immune functions of tilapia. The non-specific factors such as lysozyme, phagocytic cellular response, and agglutinins were significantly activated due to the probiotic supplementation, irrespective of the diet type. These results are supported by the better nutritional quality of Skretting, D2, Skretting+P, and Arasco+P. The better non-specific immune response in the probiotic-supplemented diets obtained in this study is in conformity to our previous studies on Nile tilapia and to those reported in the literature [11,12,35,36]. However, the failure of the probiotic- supplemented diet Local+P to improve the phagocytosis further indicate that this feed is not suitable for use.

Teleost head kidney, analogous to the mammalian adrenal gland, is an active site of lymphoid cell generation and endocrine functionalities (Uribe et al, 2011) [9]. The β-actin, apart from being an important gene responsible for the cytoskeletal functions, plays an important role in regulating the immune gene expression. β-actin specifically controls cell growth, migration, and the G-actin pool [37]. Probiotic-supplemented diets in the present study showed

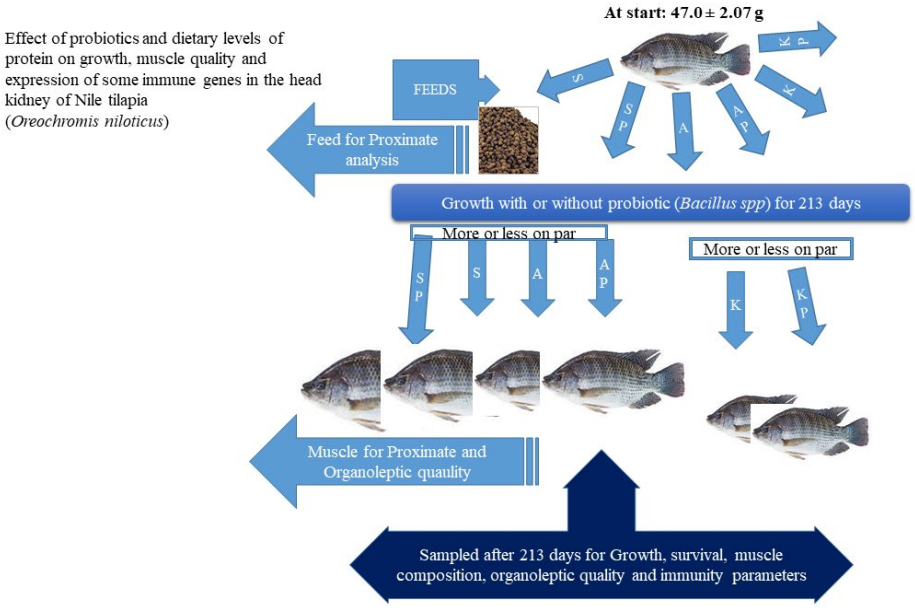
significantly up regulated β-actin gene, which was reflected in the better growth performances of the probiotic fed tilapia. The IL-1 β is one of the cytokines that is involved in the regulation of inflammatory responses in fish. This interleukin enhances antibody production when administered with bacterial vaccines [38,39]. In the present study the live bacterial supplement used as a probiotic, probably acted as a triggering factor mimicking live vaccines and hence, resulting in up regulation of IL-1 β genes. This is also reflected in the up regulation of immunoglobulin gene (IgM) in two of the three probiotic-supplemented diets (Skretting+P and Arasco+P). In this investigation, an up regulation of anti-viral cytokine Mx gene in the probiotic-supplemented diets was recorded. Mladineo et al. reported a partial up regulation of Mx and other genes following probiotic administration in European seabass [40]. Zou and Secombes indicated that Mx role in fish immunity is broadened by its ability to interact with bacterial and viral agents, most likely through triggering the production of IFN-γ [41]. The present study also showed the evidence of up regulated pro-inflammatory cytokine (TNF-α) which is consistent with Grayfer and Belosevic wherein TNF-α, IFN-γ, IL-1β were inferred to have induced antimicrobial functions of immune cells [42-45].

5. Conclusion

Results of the present study showed that probiotics fortified diet Skretting+P resulted in the highest growth and feed utilization in tilapia. Probiotics supplemented diets improved muscle n-3 fatty acids levels, positively influenced the non-specific immune factors, and significantly up regulated β-actin gene which was reflected in the better growth performance and up regulated the pro-inflammatory cytokine (TNF-α) which induces the antimicrobial functions of the immune cells. On the other hand, adding probiotics to low quality feed such as Local does not help in bringing significant improvements in tilapia growth and immune responses.

Acknowledgements

The authors gratefully acknowledge the financial grant provided by KISR to carry out the research (FM100K). Thanks are due to Ms. S. B. Yassen and T. Al-Sharrah for their help with the proximate and fatty acid analysis of the samples.



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