Results

THE FIRST EXPERIMENTAL STUDY (PART I)

WATER QUALITY

The water quality parameters of concrete ponds measured during the eight weeks feeding study were not affected by feeding various experimental diets containing probiotics to tilapia (Table 3). The mean water temperature in all ponds was 28.2 ±0.30 °C. Average pH ranged from 6.90 ±0.14 to 7.45± 0.13 and total ammonia levels were 0.065 ± 0.002 mg/l to 0.082 ± 0.01 mg/l. The dissolved oxygen levels in all ponds were above 6 mg/l and these values ranged from 6.05±0.14 to 6.62±0.44 mg/l in tanks used to test the five experimental diets (Figure 1).

GROWTH PERFORMANCE

The growth parameters of fish including initial body weight (IBW), final body weight (FBW), specific growth rate (SGR), feed intake (FI), feed conversion ratio (FCR) and survival rate of Nile tilapia fed various experimental diets containing probiotics are summarized in Table 4 and Figures (2 and 3). The four experimental diets containing different probiotics, *Bacillus subtilis* NIOFSD017, *Lactobacillus plantarum* NIOFSD018, a mixture containing bacterial isolates (*B. subtilis* NIOFSD017 and *L. plantarum* NIOFSD018) and a yeast, *Saccharomyces cerevisiae* NIOFSD019 are referred to as D1, D2, D3 and D4 respectively. No probiotics were added to the control diet.

At the beginning of the experiment, no significant differences were observed in the IBW among the treatment groups and the control group (Figure 2). However, at the end of the experiment, the feeds containing different probiotics (D1, D2, D3 and D4) exhibited higher FBW and SGR

than the control diet (probiotics free diet). Diet containing *L. plantarum* NIOFSD018 (D2) showed the highest FBW (56.05±2.18 g) and SGR (1.378±0.07% day^{-1}) as compared to FBW (40.63±1.51 g) and SGR (0.837±0.07% day^{-1}) recorded for fish fed the control diet. No significant differences were observed in the average FBW and SGR among the three diets; D2, D3 and D4 (Figure 2).

The total feed intake (FI) ranged from 49.24±0.03g (for fish fed the control diet) to 58.13g±1.96 (for fish fed D2). Feed intake by fish in all dietary treatments was statistically ($P<0.05$) higher than the control group.

Results of FCR show that fish fed diets containing different probiotic groups had significantly lower FCR (better) than those fed the control diet. The lowest value (1.84±0.07) of FCR was recorded for fish feed D2 and the highest value (3.08±0.30) was found for fish fed the probiotic free diet. No significant differences in FCR were observed among the four dietary treatments, D1, D2, D3 and D4 (Figure 3).

No mortality was recorded in any of the experimental groups throughout the rearing period. It is obvious from Table 4 that the addition of probiotics to Nile tilapia diets have significantly improved their growth performance, and the diet containing *L. plantarum* NIOFSD018 (D2) showed the highest FBW, SGR, FI and the lowest FCR.

DAILY WEIGHT GAIN (DWG)

Nile tilapia daily weight gain fed diets containing various probiotic groups is shown in Table 5 and also illustrated in Figure 4. The mean values of DWG for fish fed on different probiotic diets were found to be significantly higher than that of the control group. But no significant differences were detected among the DWG values for fish fed diets D2, D3 and D4.

Table 4

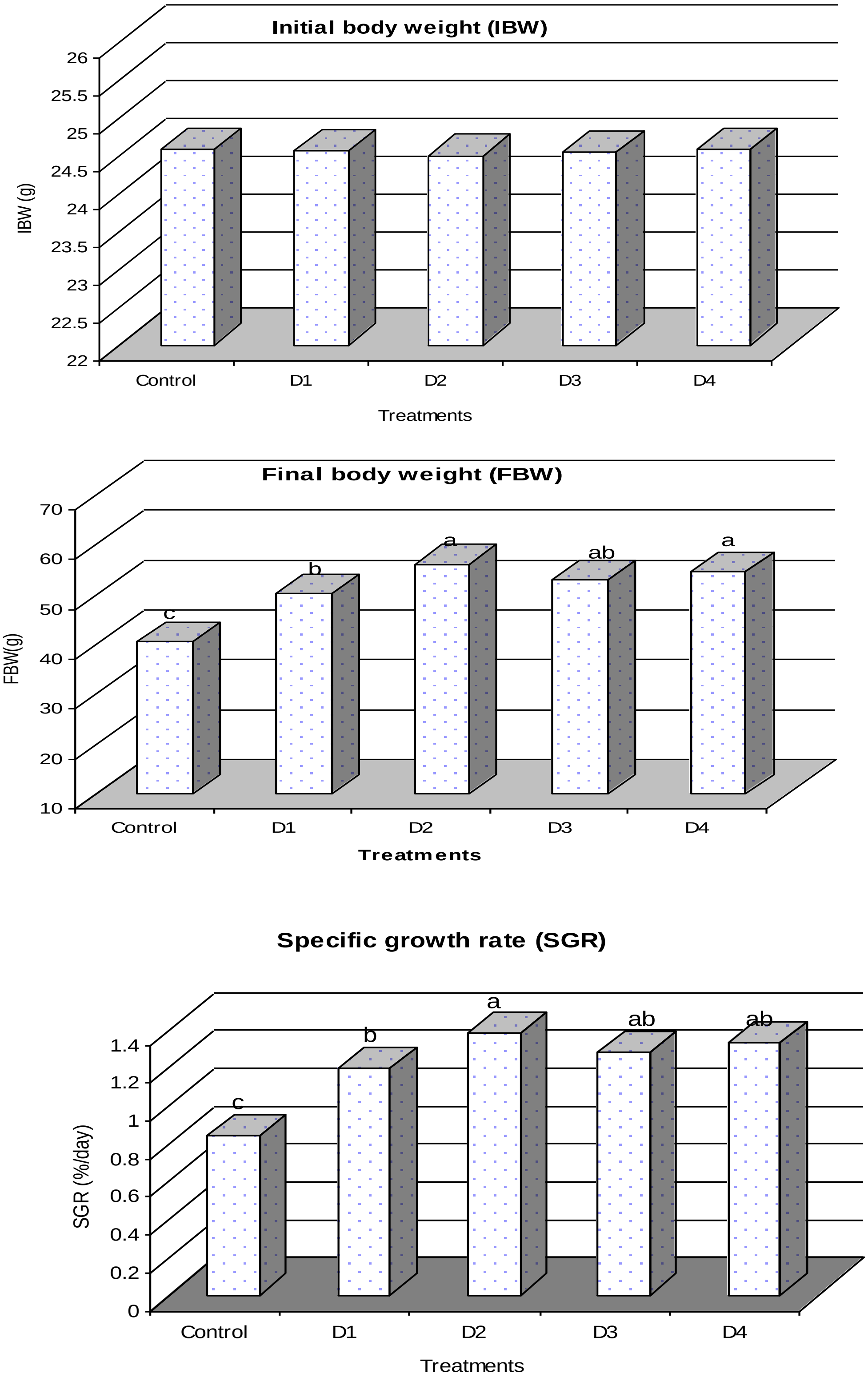
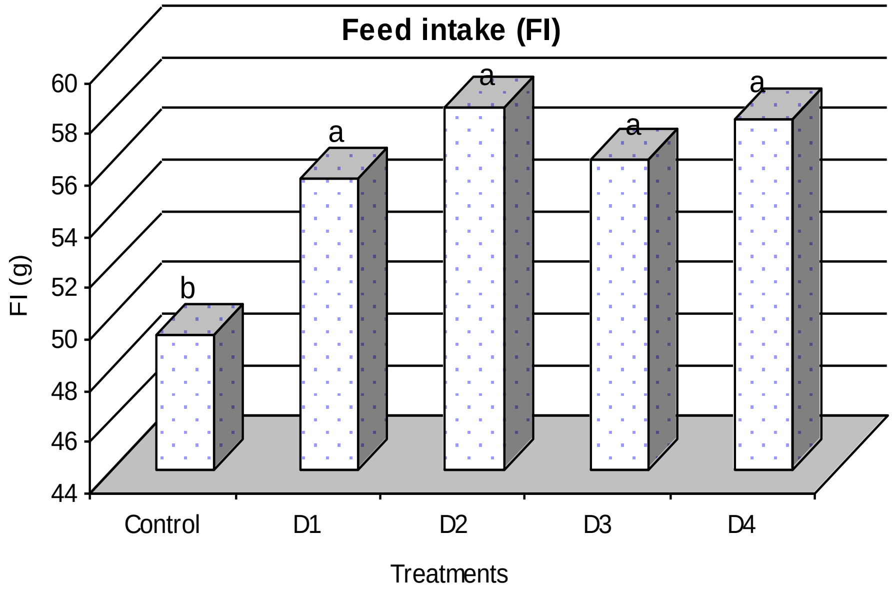


Figure 2 Initial body weight, final body weight and specific growth rate of Nile tilapia fed various probiotic groups.

Values sharing the same superscript are not significantly different (P<0.05)

D1: *B. subtilis* NIOFSD017, D2: *L. plantarum* NIOFSD018, D3: mixture of both bacterial isolates; *B. subtilis* NIOFSD017 and *L. plantarum* NIOFSD018, D4: *S. cerevisiae* NIOFSD019 and control: probiotic free diet



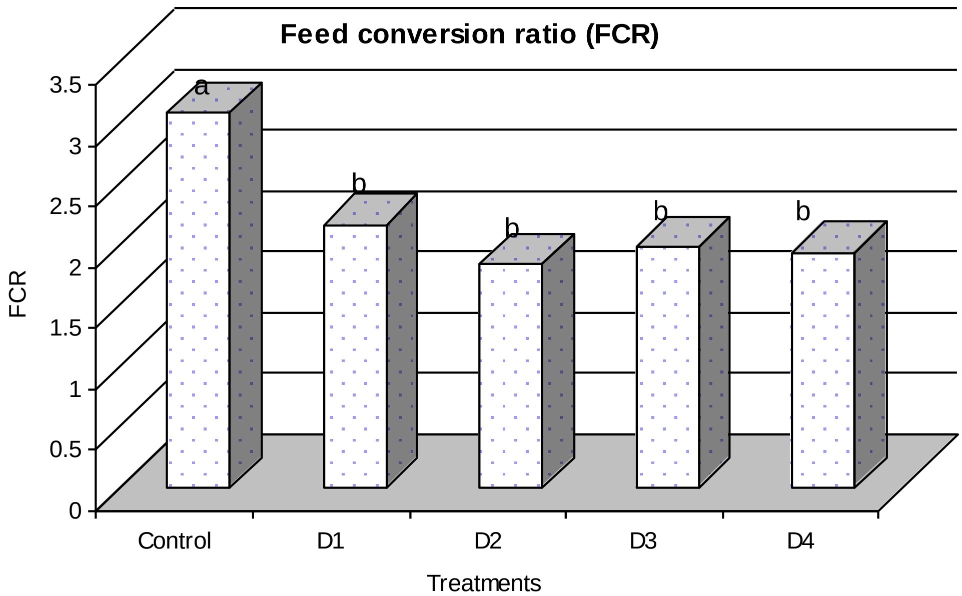


Figure 3 Feed intake and feed conversion ratio of Nile tilapia fed various probiotic groups.

Values sharing the same superscript are not significantly different (P<0.05)
D1: *B. subtilis* NIOFSD017, D2: *L. plantarum* NIOFSD018, D3: mixture of both bacterial isolates; *B. subtilis* NIOFSD017 and *L. plantarum* NIOFSD018, D4: *S. cerevisiae* NIOFSD019 and control: probiotic free diet

Table 5

FEED UTILIZATION

Feed utilization including protein efficiency ratio (PER), protein productive value (PPV) and energy retention (ER) are outlined in Table 6 and Figure 5. The average PER, PPV and ER significantly increased with the administration of various probiotics in the experimental diets compared to the control group. The highest PER (1.81±0.06), PPV (29.55±1.11%) and ER (18.48±0.78%) were recorded with fish fed with D2. These values were not significantly different from those fed diets D3 and D4.

CARCASS CHEMICAL COMPOSITION

Carcass chemical composition including moisture, crude protein (CP), ether extract (EE), gross energy (GE), ash and nitrogen free extract (NFE) content of Nile tilapia fed diets containing probiotics, are shown in Table 7 and graphically illustrated in Figure 6. No significant differences were observed in the moisture content of fish fed different probiotics supplemented diets and the control. The carcass moisture content ranged from 74.05±0.01 to 74.47±0.15%.

The two highest values (61.65±0.15 and 61.36±0.20%) of crude protein (CP) were achieved with fish fed diets D1 and D2, and no significant difference between these two diets was detected. Average crude protein content of fish fed diets D3, D4 and control were 60.85±0.004, 60.90±0.04 and 60.79±0.09% respectively. After eight weeks of feeding the experimental diets, there was a significant increase in the protein content of the fish in all the dietary groups compared to the initial protein content.

Average fat content (ether extract, EE) of fish fed diets containing different probiotic groups D1, D2, D3, D4 and the control were 22.16±0.01, 21.82±0.11, 21.77±0.20, 21.85±0.004 and 22.11±0.17% respectively (Figure 6).

Table 6

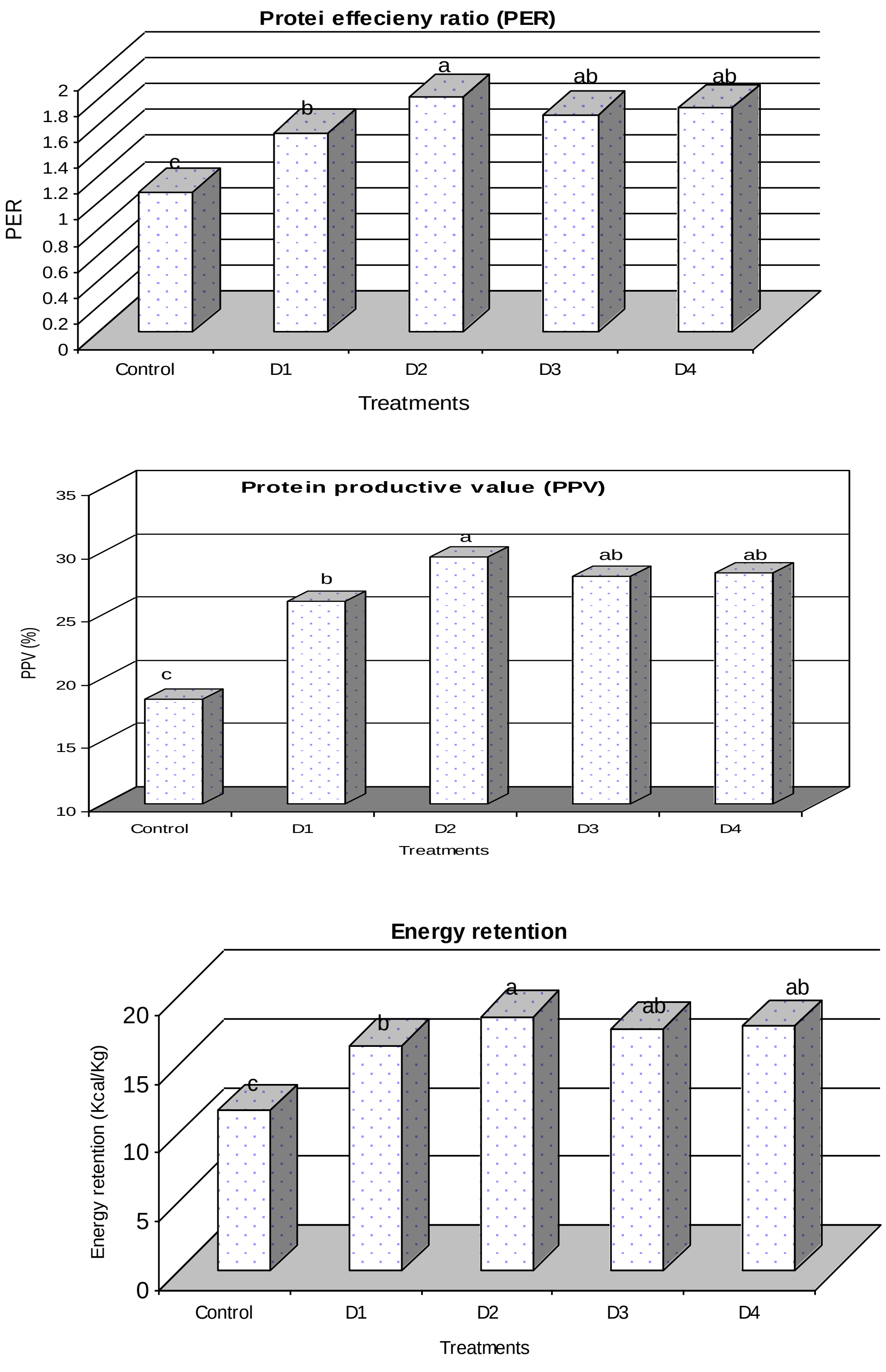


Figure 5 Feed utilization values of Nile tilapia fed various probiotic groups**.**

Values sharing the same superscript are not significantly different (P<0.05)
D1: *B. subtilis* NIOFSD017, D2: *L. plantarum* NIOFSD018, D3: mixture of both bacterial isolates; *B. subtilis* NIOFSD017 and *L. plantarum* NIOFSD018, D4: *S. cerevisiae* NIOFSD019 and control: probiotic free diet

Table 7

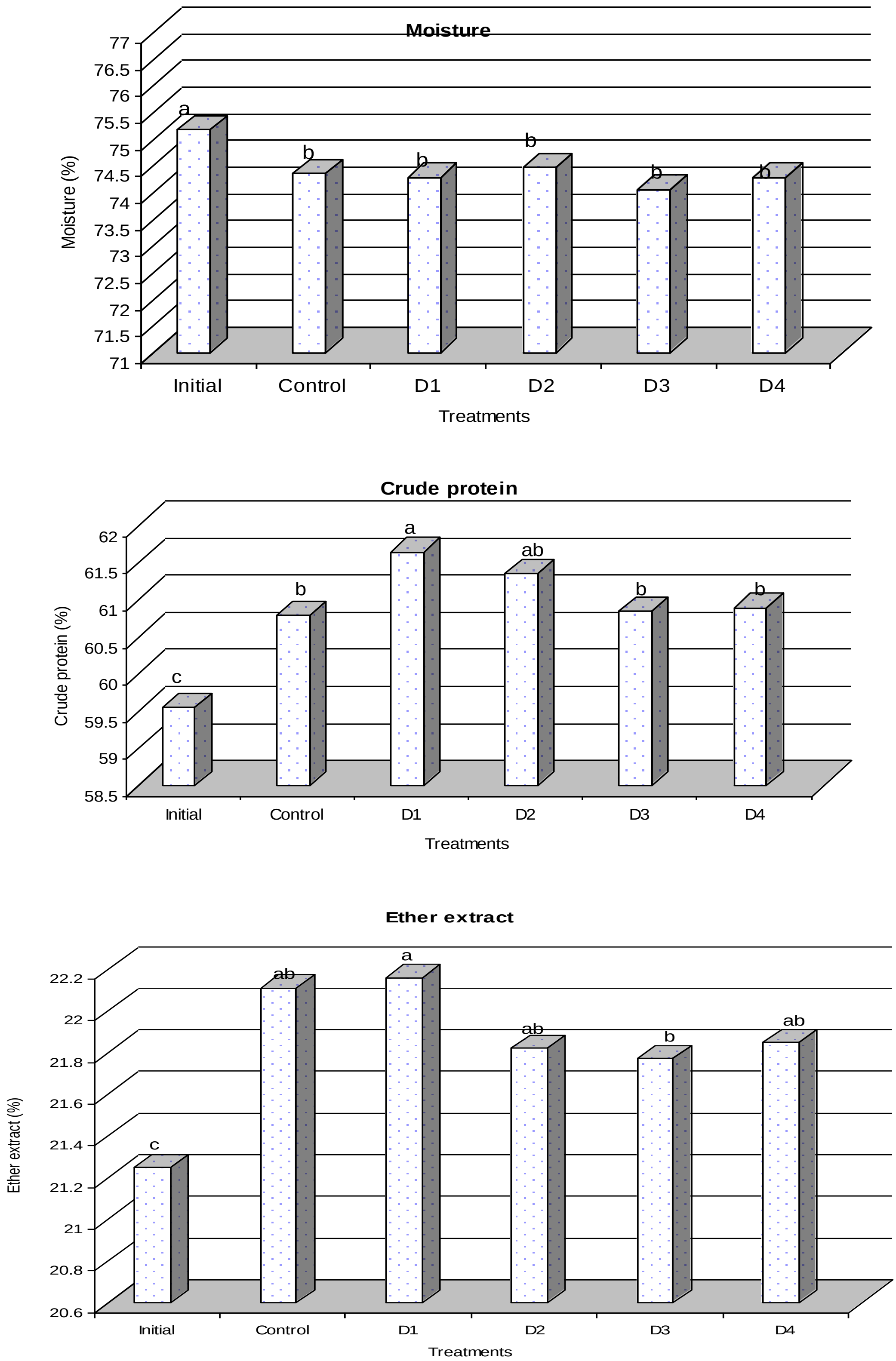


Figure 6 Moisture, crude protein and ether extract of Nile tilapia (carcass) fed various probiotic groups.

Values sharing the same superscript are not significantly different (P<0.05)
D1: *B. subtilis* NIOFSD017, D2: *L. plantarum* NIOFSD018, D3: mixture of both bacterial isolates; *B. subtilis* NIOFSD017 and *L. plantarum* NIOFSD018, D4: *S. cerevisiae* NIOFSD019 and control: probiotic free diet

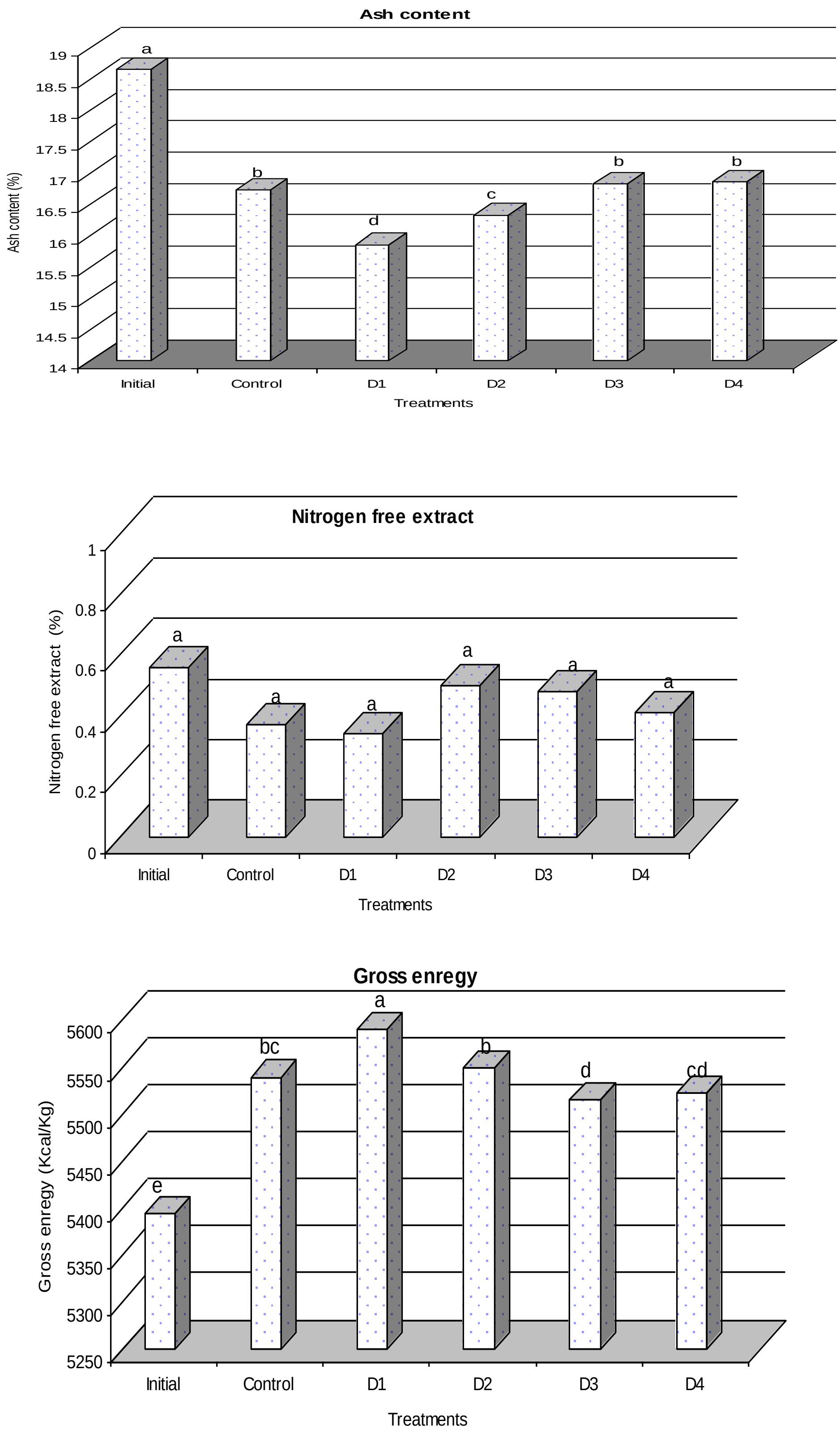


Figure 7 Ash content, nitrogen free extract and gross energy of Nile tilapia (carcass) fed various probiotic groups.

Values sharing the same superscript are not significantly different (P<0.05)
D1: *B. subtilis* NIOFSD017, D2: *L. plantarum* NIOFSD018, D3: mixture of both bacterial isolates; *B. subtilis* NIOFSD017 and *L. plantarum* NIOFSD018, D4: *S. cerevisiae* NIOFSD019 and control: probiotic free diet

Whole body ash content ranged from 15.85±0.18 to 16.85±0.08 % for fish fed the diets D1 and D4. The mean NFE were not significantly different among the fish fed various experimental diets and they ranged from 0.34±0.04 to 0.50±0.03 % (Figure 7).

The highest GE content (5591.2±8.93 kcal/kg) of carcass was recorded for fish fed D1 and fish fed D3 showed significant lower GE content (5515.1±12.80 kcal/kg) (Figure 7).

FLESH CHEMICAL COMPOSITION

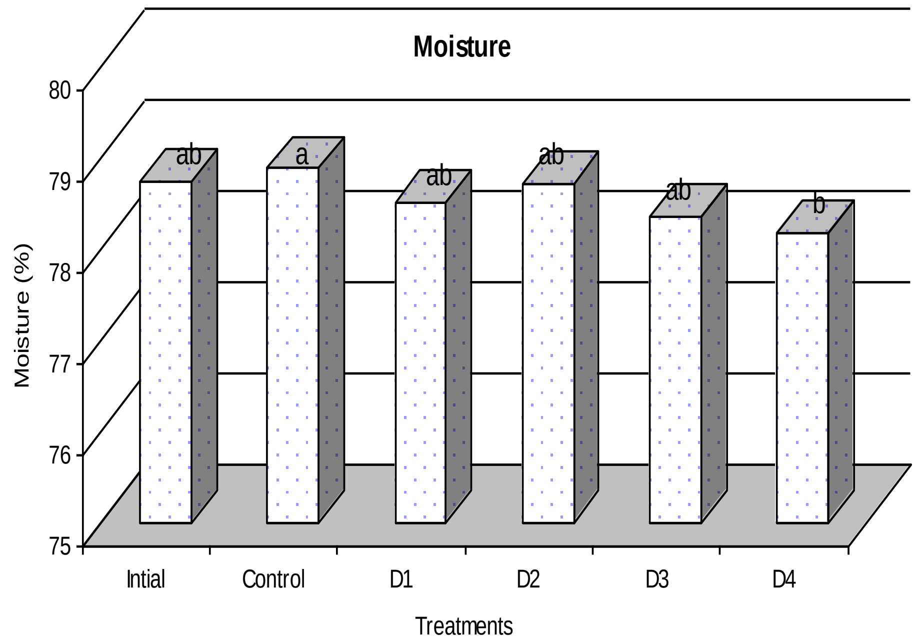
Chemical composition of Nile tilapia flesh (without bones) including moisture, crude protein (CP), fat (ether extract, EE) and ash content are presented in Table 8 and Figures 8 and 9. Highest moisture content (78.87±0.49%) was recorded in fish fed the control diet; this value was found to be statistically similar to the moisture averages that were recorded for D1, D2 and D3 and also was similar to the initial value (78.73±0.20%).

Crude protein content of the fish fed the five experimental diets did not exhibit significant differences among the dietary groups as compared to the initial level. Flesh CP content was 84.97±0.48% for fish fed the control diet and 86.15±0.48% for fish fed D1 (Figure 8).

The highest EE content was recorded for fish fed D2 (7.75±0.07) and D3 (7.49±0.01) and these values were significantly higher than the EE values of 6.85±0.04% and 6.76±0.21% observed for fish fed D4 and D1 respectively.

Average flesh ash content significantly decreased by the addition of different probiotics to the experimental diet. Hence the highest average (6.56±0.37%) of ash content was recorded for fish fed the control diet; this value was significantly higher than the values of 5.66±0.08, 5.56±0.12 and 5.39±0.36% for fish fed diets D1, D2 and D3 respectively (Figure 9).

Table 8



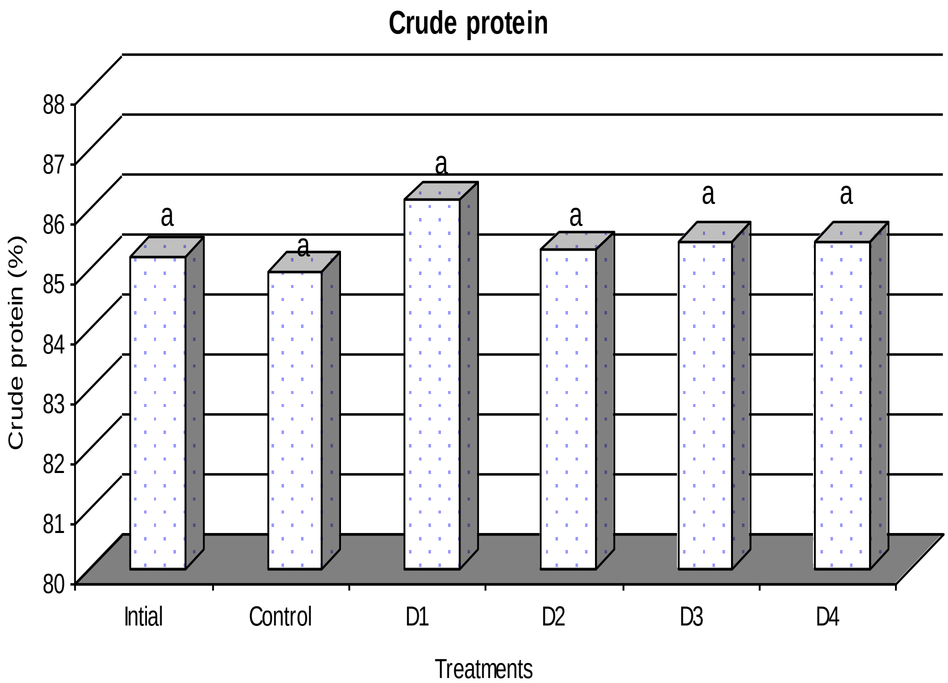
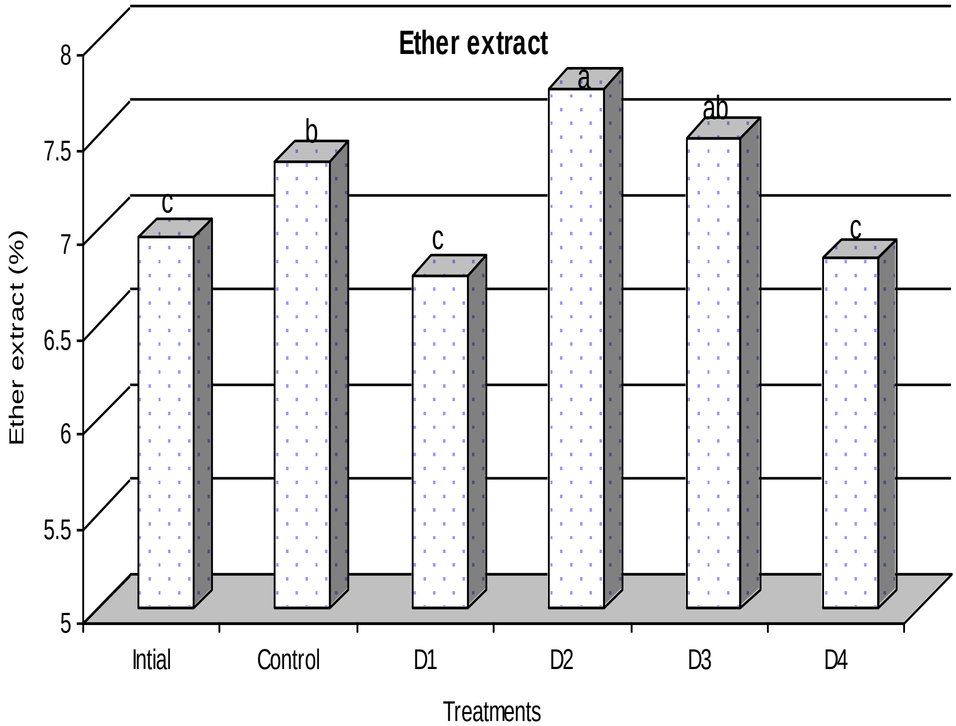


Figure 8 Moisture content and crude protein of Nile tilapia (flesh) fed various probiotic groups.

Values sharing the same superscript are not significantly different (P<0.05)
D1: *B. subtilis* NIOFSD017, D2: *L. plantarum* NIOFSD018, D3: mixture of both bacterial isolates; *B. subtilis* NIOFSD017 and *L. plantarum* NIOFSD018, D4: *S. cerevisiae* NIOFSD019 and control: probiotic free diet



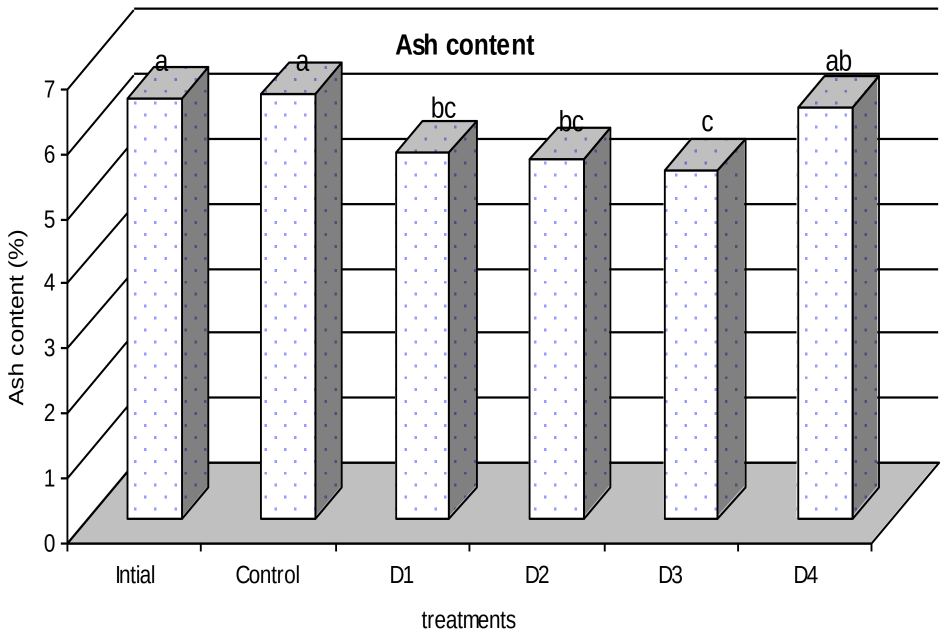


Figure 9 Ether extract and ash content of Nile tilapia (flesh) fed various probiotic groups.

Values sharing the same superscript are not significantly different ($P<0.05$)
D1: *B. subtilis* NIOFSD017, D2: *L. plantarum* NIOFSD018, D3: mixture of both bacterial isolates; *B. subtilis* NIOFSD017 and *L. plantarum* NIOFSD018, D4: *S. cerevisiae* NIOFSD019 and control: probiotic free diet

DIGESTIVE ENZYMES

Amylase enzyme

The total and specific amylase activities of Nile tilapia fed diets containing various probiotic groups are shown in Table 9. Total and specific amylase activities for all the probiotic groups were significantly higher than the control group. The highest total amylase activities were 41.88±2.92, 40.87±2.97 Uml^{-1} for fish fed D2 and D4 respectively. This was followed by the value; 39.58±3.85 Uml^{-1} for fish fed D3. There were no significant differences in the total amylase activities of fish fed diets D2 and D4 but they are still significantly higher than 34.28±3.95 Uml^{-1} and 29.20±1.61 Uml^{-1} recorded in fish fed D1 and control diet respectively (Figure 10).

Similarly for amylase total activity the specific activity was of similar trend, the highest recorded averages of specific activities were 3.496±0.31, 3.335±0.28 unit (mg protein) $^{-1}$ for fish fed D2 and D4 respectively without any significant difference. Whereas, these specific activities values were significantly different than those for fish fed control diet (2.4274±0.13 unit (mg protein) $^{-1}$ and D1 (2.8254±0.28 unit (mg protein) $^{-1}$ (Figure 10).

Protease enzyme

Incorporation of probiotic into the experimental diets has significantly increased the total and specific protease activities in all treatments over the control, except D4 which was not significantly different from the control group, Table 10 and Figure 11. The highest protease activity was found for fish fed diets D2 and D1 having averages of 5.47±0.69 and 5.41±0.59 Uml^{-1} respectively with no significant difference. These were followed by the value; 4.45±0.50 Uml^{-1} for D3. The lowest values of total protease activity were recorded for fish fed D4 (3.17±0.72 Uml^{-1}) and control (3.16±0.58 Uml^{-1}) diets.

Table 9 Total and specific amylase enzyme activity of Nile tilapia fed various probiotic groups[1]

Treatments [2]	Protein content mg ml^{-1}	Enzyme activities[3]	
		Total Unit ml^{-1}	Specific Unit (mg protein)$^{-1}$
Control	12.04 ±0.59	29.20^{d} ±1.61	2.427^{d} ±0.13
D1	12.13 ±0.59	34.28^{c} ±3.95	2.825^{c} ±0.28
D2	12.01 ±0.55	41.88^{a} ±2.92	3.496^{a} ±0.31
D3	12.15 ±0.69	39.58^{b} ±3.85	3.266^{b} ±0.36
D4	12.28 ± 0.52	40.87^{ab} ±2.97	3.335^{ab} ±0.28

[1] Values (Mean ± Standard Deviation) in the same column sharing the same superscript are not significantly different (P<0.05)
[2] D1: *B. subtilis* NIOFSD017, D2: *L. plantarum* NIOFSD018, D3: mixture of both bacterial isolates; *B. subtilis* NIOFSD017 and *L. plantarum* NIOFSD018, D4: *S. cerevisiae* NIOFSD019 and control: probiotic free diet
[3] Amylase unit is defined as the amount of enzyme that hydrolyze 1 mg of starch in 30 minutes
Starch hydrolyzed (mg) = [D (control) – D (digest) / D (control)] x 60, where D is the optical density

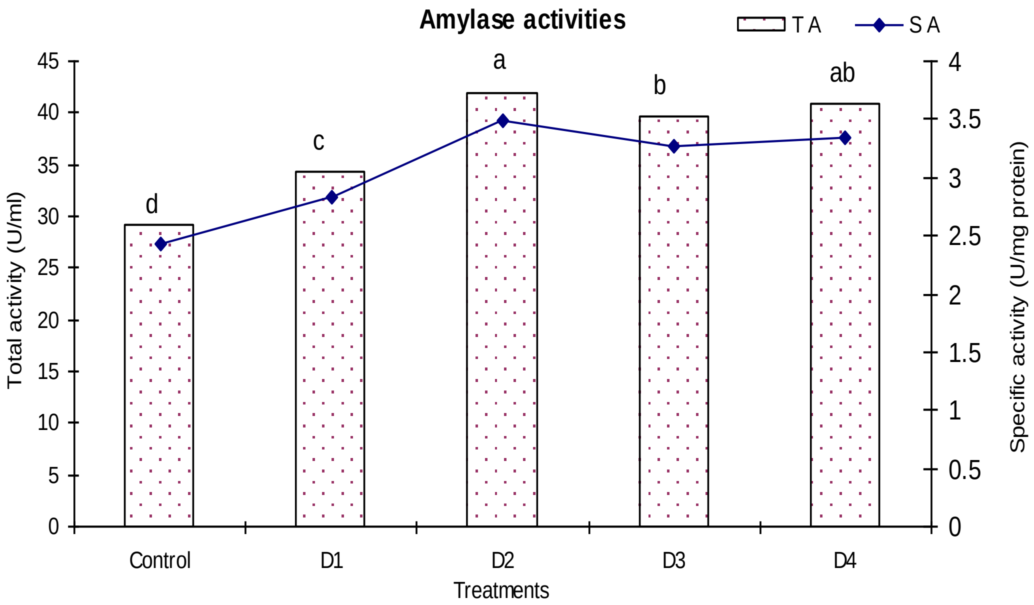


Figure 10 Total activity (TA) and specific activity (SA) of amylase enzyme of Nile tilapia fed various probiotic groups.

Values sharing the same superscript are not significantly different (P<0.05)
D1: *B. subtilis* NIOFSD017, D2: *L. plantarum* NIOFSD018, D3: mixture of both bacterial isolates; *B. subtilis* NIOFSD017 and *L. plantarum* NIOFSD018, D4: *S. cerevisiae* NIOFSD019 and control: probiotic free diet

Specific protease activity also followed the previous trend, the highest specific protease activity were noticed in fish fed D2 (0.456±0.06 Unit (mg protein)$^{-1}$ and D1 (0.448±0.06 Unit (mg protein)$^{-1}$) and the differences between these two dietary treatments were not statistically significant. However, the lowest two averages (0.259±0.06 and 0.262±0.04 Unit (mg protein)$^{-1}$) of specific protease activity were recorded for fish fed D4 and the control diets respectively (Figure 11). This increase in the total and specific protease activity in fish fed the probiotic diets were observed as a result of the significant increase in the amount of µmol tyrosine liberated by the action of protease enzyme (Table 10). Hence, the amount of µmol tyrosine was significantly higher in fish fed the diets D1, D2, D3 as compared to that obtained from fish fed D4 or the control diet.

Lipase enzyme

Total and specific lipase activities are illustrated in Table 11. It is obvious that the supplementation of different probiotic isolates to the diet of Nile tilapia improved the total and specific activities of lipase compared to the control diet and D4. The highest total (4.12±0.34 Uml^{-1}) and specific lipase activities (0.344±0.034 Unit (mg protein)$^{-1}$ were observed in fish fed D2 However, the lowest two averages; 2.47±0.31 and 2.26±0.34 Uml^{-1} of total lipase activity were detected in fish fed D4 and the control diets respectively. There was no significant difference in the total lipase activity of fish fed these two diets. Similarly, the lowest two values 0.203±0.029 and 0.189±0.033 Unit (mg protein)$^{-1}$of specific lipase activity were recorded for the same dietary treatments. Again, there was no significant difference in specific enzyme activities between D4 and the control (Figure 12).

Table 10 Total and specific protease enzyme activities of Nile tilapia fed various probiotic groups[1]

Treatments [2]	Protein content mg ml^{-1}	µ mol tyrosine	Enzyme activities	
			Total Unit ml $^{-1}$	Specific Unit (mg protein) $^{-1}$
Control	12.04 ±0.59	0.0566^{c} ±0.0091	3.16^{c} ±0.58	0.2624^{c} ±0.04
D1	12.13 ±0.59	0.0983^{a} ±0.0107	5.41^{a} ±0.59	0.4476^{a} ±0.06
D2	12.01 ±0.55	0.0995^{a} ±0.0125	5.47^{a} ±0.69	0.4559^{a} ±0.06
D3	12.15 ±0.69	0.0811^{b} ±0.0091	4.45^{b} ±0.50	0.3673^{b} ±0.04
D4	12.28 ± 0.52	0.0577^{c} ±0.0130	3.17^{c} ±0.72	0.2592^{c} ±0.06

[1] Values (Mean ± Standard Deviation) in the same column sharing the same superscript are not significantly different (P<0.05)
[2] D1: *B. subtilis* NIOFSD017, D2: *L. plantarum* NIOFSD018, D3: mixture of both bacterial isolates; *B. subtilis* NIOFSD017 and *L. plantarum* NIOFSD018, D4: *S. cerevisiae* NIOFSD019 and control: probiotic free diet

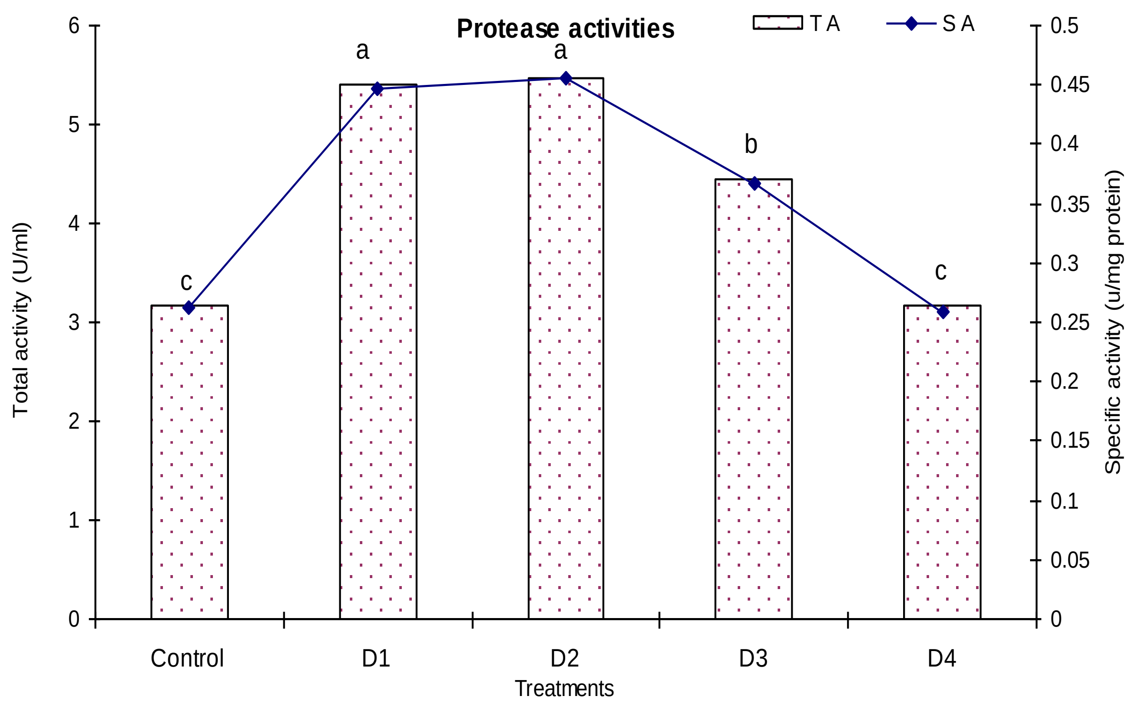


Figure 11 Total activity (TA) and specific activity (SA) of protease enzyme of Nile tilapia fed various probiotic groups.

Values sharing the same superscript are not significantly different (P<0.05)
D1: *B. subtilis* NIOFSD017, D2: *L. plantarum* NIOFSD018, D3: mixture of both bacterial isolates; *B. subtilis* NIOFSD017 and *L. plantarum* NIOFSD018, D4: *S. cerevisiae* NIOFSD019 and control: probiotic free diet

Table 11 Total and specific Lipase enzyme activities of Nile tilapia fed various probiotic groups[1]

Treatments [2]	Protein content mg ml^{-1}	Enzyme activities[3]	
		Total Unit ml $^{-1}$	Specific Unit (mg protein) $^{-1}$
Control	12.04 ±0.59	2.26^{c} ±0.34	0.1888^{c} ±0.033
D1	12.13 ±0.59	3.19^{b} ±0.55	0.2642^{b} ±0.051
D2	12.01 ±0.55	4.12^{a} ±0.34	0.3438^{a} ±0.034
D3	12.15 ±0.69	3.12^{b} ±0.46	0.2584^{b} ±0.046
D4	12.28 ± 0.52	2.47^{c} ±0.31	0.2019^{c} ±0.029

[1] Values (Mean ± Standard Deviation) in the same column sharing the same superscript are not significantly different (P<0.05)
[2] D1: *B. subtilis* NIOFSD017, D2: *L. plantarum* NIOFSD018, D3: mixture of both bacterial isolates; *B. subtilis* NIOFSD017 and *L. plantarum* NIOFSD018, D4: *S. cerevisiae* NIOFSD019 and control: probiotic free diet
[3] Lipase activity was expressed as the volume of 0.05 N NaOH required to neutralize the fatty acids released during the incubation period

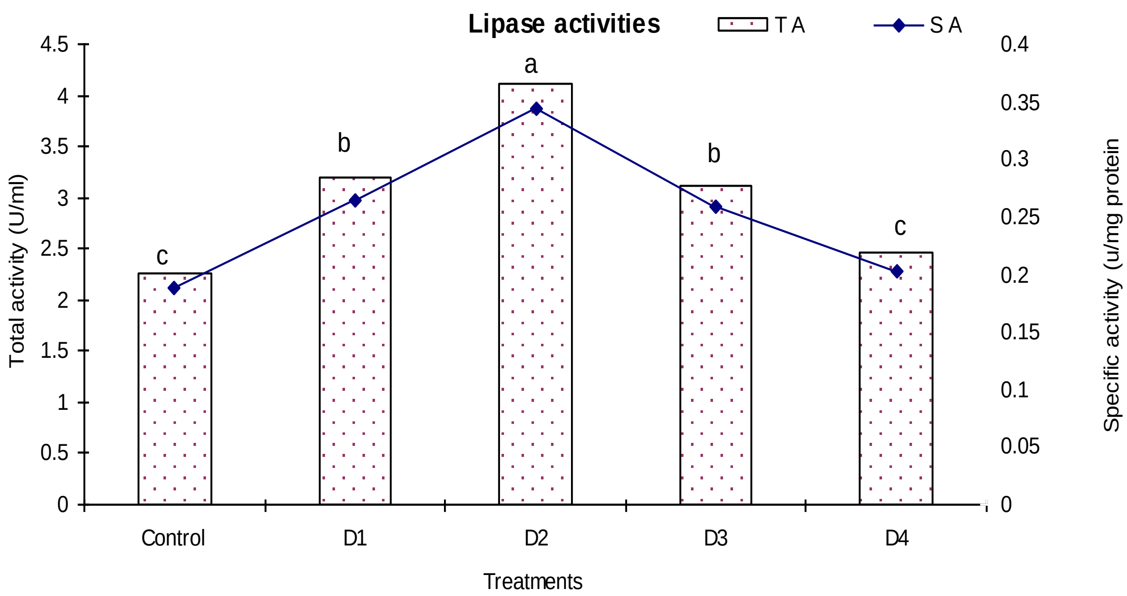


Figure 12 Total activity (TA) and specific activity (SA) of lipase enzyme of Nile tilapia fed various probiotic groups.

Values sharing the same superscript are not significantly different (P<0.05)
D1: *B. subtilis* NIOFSD017, D2: *L. plantarum* NIOFSD018, D3: mixture of both bacterial isolates; *B. subtilis* NIOFSD017 and *L. plantarum* NIOFSD018, D4: *S. cerevisiae* NIOFSD019 and control: probiotic free diet

IMMUNE RESPONSE

Phagocytic activities

The effect of four experimental diets (D1, D2, D3 and D4) containing four different probiotic groups on the immune response of Nile tilapia, are summarized in Table 12 and Figures 13. It is clear that the probiotic diets had a significant influence on the fish immunity; the phagocytic activities of fish fed diets D1, D2, D3 and D4 were significantly higher than the control group. The maximum average (79.31±1.58%) phagocytic activity was measured in fish fed the diet D4 followed by the values; 79.0±1.32%, 78.50 ±1.21 % and 77.56±1.55 % for fish fed the diets D2 and D3 and D1 respectively. However, phagocytic activity of fish fed diets D2, D3 and D4 did not show significant differences among each others, the lowest average (74.50± 1.50%) of phagocytic activity was obtained for fish fed the control diet.

Acid phosphatase activities

Serum acid phosphatase activity significantly increased in fish fed D1, D2 and D3 as compared to the control group. The highest acid phosphatase activity (8.14±0.36, 8.11±0.17 and 8.27±0.28 Uml^{-1}) were recorded for fish fed the diets D1, D2 and D3, however, no significant differences were found among these treatments. Meanwhile, the lowest acid phosphatase activity (6.76±0.0.22 Uml^{-1}) was observed in fish fed the probiotic free diet (control), Table 12 and Figures 13.

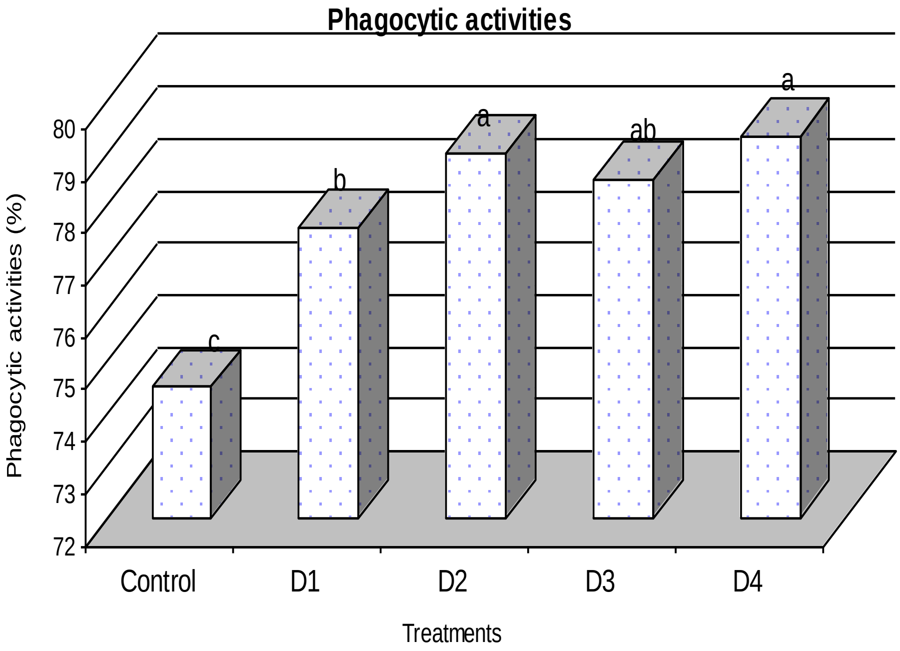
Lysozyme activities

Lysozyme activities in fish fed diets supplemented with the above mentioned four probiotic groups were significantly different than fish fed the control diet. Although the highest average lysozyme activity (342.35± 20.44 Uml^{-1}) was recorded for fish fed the diet D3, it was not significantly different when compared to the lysozyme activity found in fish fed the diet D1 and D2 (331.18±19.45 and 330.61±14.996 Uml^{-1}). Significantly lower lysozyme activity (288.60±13.23 Uml^{-1}) was observed for fish fed the control diet (Table 12 and Figures 14).

Total serum immunoglobulin

According to Table 12 and Figure 14, results of total serum immunoglobulin level of Nile tilapia was found to be significantly higher in diets supplemented with various probiotics than the control group. The highest results of total immunoglobulin (2.115±0.238 g/dl and 2.055±0.107 g/dl) were observed for fish fed the diets D3 and D1 respectively and there was no significant difference between these two groups. Results revealed also that, the lowest average total immunoglobulin (0.998±0.038 g/dl) was recorded in fish fed the control diet.

Table 12



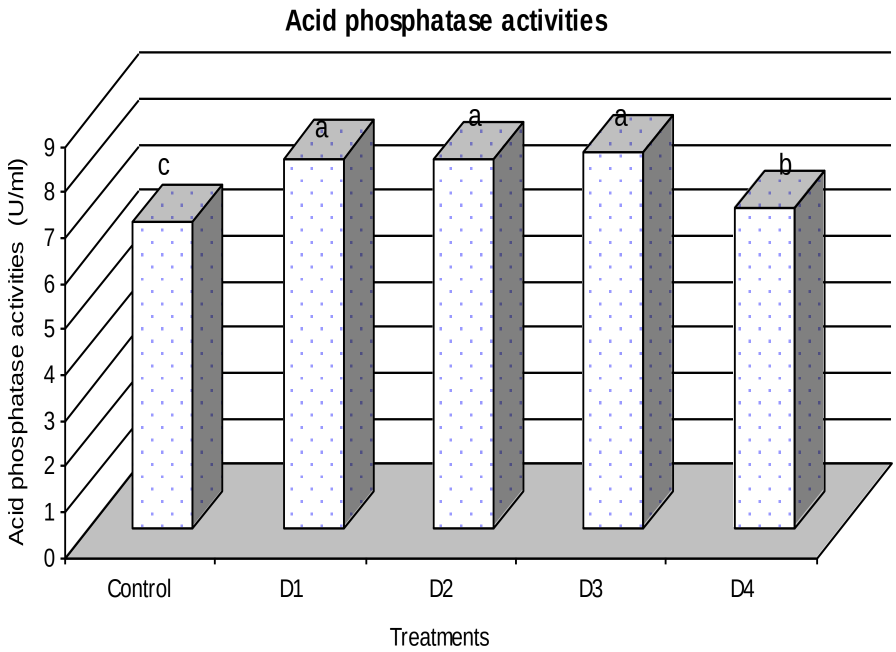
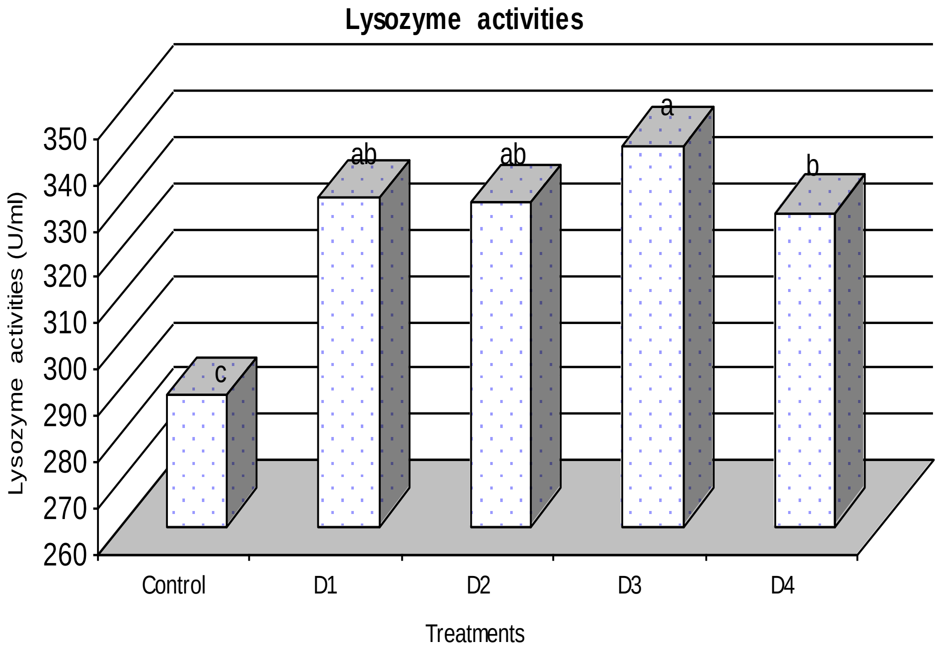


Figure 13 Phagocytic and acid phosphatase activities of Nile tilapia fed various probiotic groups

Values sharing the same superscript are not significantly different (P<0.05)
D1: *B. subtilis* NIOFSD017, D2: *L. plantarum* NIOFSD018, D3: mixture of both bacterial isolates; *B. subtilis* NIOFSD017 and *L. plantarum* NIOFSD018, D4: *S. cerevisiae* NIOFSD019 and control: probiotic free diet



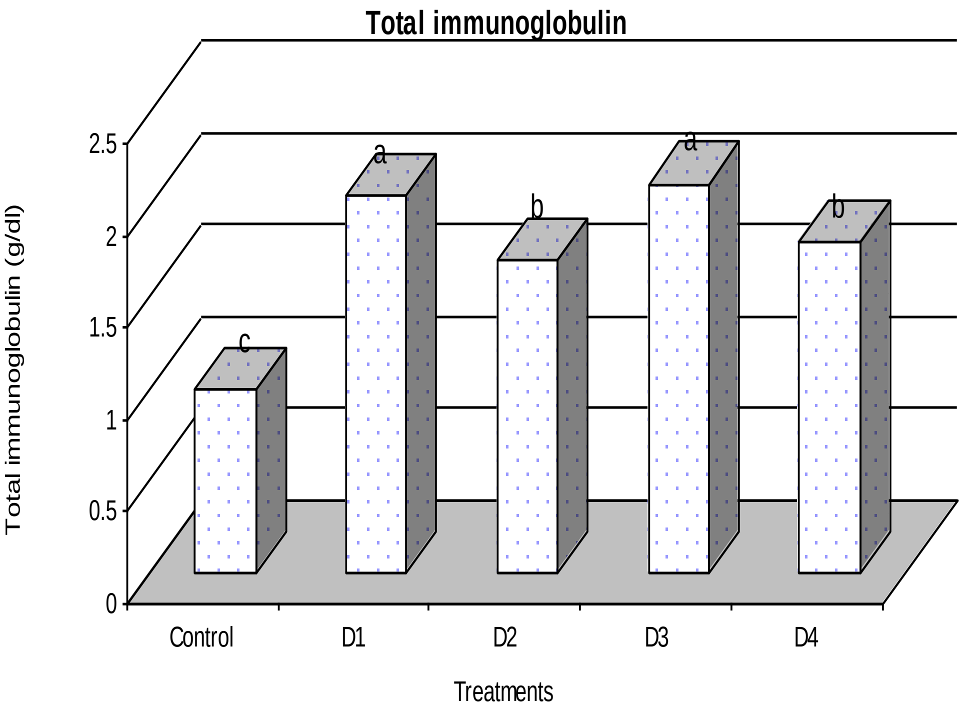


Figure 14 Lysozyme activities and total immunoglobuline of Nile tilapia fed various probiotic groups.

Values sharing the same superscript are not significantly different (P<0.05)
D1: *B. subtilis* NIOFSD017, D2: *L. plantarum* NIOFSD018, D3: mixture of both bacterial isolates; *B. subtilis* NIOFSD017 and *L. plantarum* NIOFSD018, D4: *S. cerevisiae* NIOFSD019 and control: probiotic free diet

THE SECOND EXPERIMENTAL STUDY (PART II)

WATER QUALITY

Water quality of the rearing water during the experimental period of the second experiment is presented in Table 13 and graphically illustrated in Figure 15. Water temperature was 28.36 ±0.10 °C in all ponds, and there was no apparent effect of the probiotic on the water quality. However, pH ranged from 7.19± 0.11- 7.39±0.15 for the different treatments and these values were found to be slightly lower than the control treatment (7.52±0.02). Total ammonia varied from 0.058 ± 0.004 mg/l - 0.069 ± 0.004 mg/ l. The table also shows that mean values of dissolved oxygen ranged from 6.92±0.19 mg/l to 7.21±0.16 mg/l. All measured water quality parameters were stable and within the acceptable ranges.

GROWTH PERFORMANCE

The growth performance including initial body weight (IBW), final body weight (FBW), specific growth rate (SGR), feed intake (FI), feed conversion ratio (FCR) and survival rate of Nile tilapia fed diets containing varying levels of *L. plantarum* NIOFSD018 is illustrated in Table 14 and Figure 16. There were no significant differences among the IBW of fish used to test the different *L. plantarum* NIOFSD018 levels. At the end of the feeding trial a significant increase in both FBW and SGR were recorded by increasing the probiotic doses from 10^5 to 10^7 and 10^9 CFUg^{-1}.

The highest averages FBW (73.45±3.61 g and 72.94±4.01 g) of fish were recorded for fish fed diets D2 and D3 ($10^7$and 10^9 CFU g^{-1} *L. plantarum* NIOFSD018 g^{-1}) respectively, these were significantly different from values for fish fed the control diet (probiotic free diet) and D1 (10^5 CFU $g^{-1)}$. The lowest mean FBW values (60.39 ±0.72 g and 61.84±0.44 g) were recorded for fish fed control diet and D1 respectively without showing any significant difference (Table 14).

Table 13 Water quality of Nile tilapia aquaria during the second experimental period[1]

Items[2]	Diets[3]			
	Control	D1	D2	D3
Temperature (^oC)	28.36±0.10	28.36±0.10	28.36±0.10	28.36±0.10
pH	7.52±0.02	7.39±0.15	7.20±0.19	7.19±0.11
Ammonia (mg/l)	0.067±0.002	0.058±0.004	0.067±0.005	0.069±0.004
Dissolved Oxygen (mg/l)	7.08±0.18	6.92±0.19	7.21±0.16	7.01±0.19

[1]Values (Mean ± Standard Deviation) of pH, ammonia and dissolved oxygen were measured at morning while temperature was measured at one o'clock after mid-day.
[2] pH, ammonia and dissolved oxygen were measured at morning while, temperature was measured at one o'clock after mid-day
[3] D1: diet containing 10^5, D2: 10^7 , D3: 10^9 CFU/g of *L. plantarum* NIOFSD018 and Control: probiotic free diet.

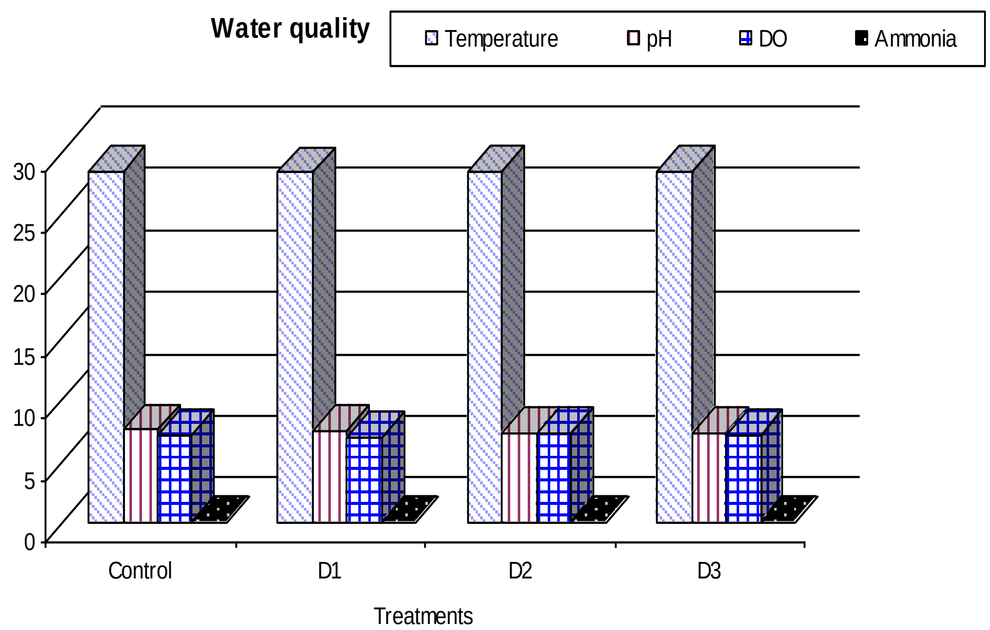


Figure 15 Water quality in the aquaria during the second experimental period.

pH, ammonia and dissolved oxygen were measured at morning while, temperature was measured at one o'clock after mid-day
D1: diet containing 10^5, D2: 10^7 , D3: 10^9 CFU/g of *L. plantarum* NIOFSD018 and Control: probiotic free diet, Whereas, Temperature (^oC), Ammonia ($mg\ l^{-1}$) and Dissolved Oxygen (mgl^{-1})

Dietary supplementation of three different doses (10^5, 10^7 and 10^9 CFU g^{-1}) of *L. plantarum* NIOFSD018 significantly improved SGR from 0.907 ± 0.02 % day^{-1} and 0.946 ±0.01 % day^{-1} for fish fed the control diet and 10^5 CFU/g respectively to 1.231 ±0.083 and 1.220 ±0.09 for those fish that received D2 and D3. SGR for fish fed the control diet and D1 were not significantly different. Fish fed D2 and D3 also did not exhibit any significant changes in their SGR values.

Table 14 outlines that feed intake for fish fed control, D1, D2 and D3 diets, which were 70.28 ±0.82 g, 71.08 ±0.81 g, 79.20 ±4.03 g and 78.30 ±4.85 g respectively. Growth performance data also showed that the best FCR (lowest) was observed for fish fed D2 and D3 (2.07 ±0.09 and 2.07 ±0.10), without showing a significant difference between D2 and D3. However, the highest FCR (2.77±0.06 and 2.65±0.05) were recorded for fish fed the control and D1 diets respectively and no significant difference was observed between FCR of fish fed the control and D1 (Figure 17). The survival rate, in all treatments and during the whole fed trial period was 100%.

DAILY WEIGHT GAIN

Daily weight gain (DWG) is summarized by Table 15 and Figure 18. DWG of 0.64 ±0.06 g and 0.63±0.07 g for fish fed D2 and D3 were found to be significantly higher than (0.42± 0.01 g and 0.45±0.01 g) values recorded for fish fed the control and D1 diets respectively. On the other hand, there were no significant differences between D1and D2 or between D2 and D3. It was shown that administration of the doses $10^7$and 10^9 CFU/g probiotic; *L. plantarum* NIOFSD018 significantly increased the growth performances and decreased the feed conversion ratio (FCR).

Table 14

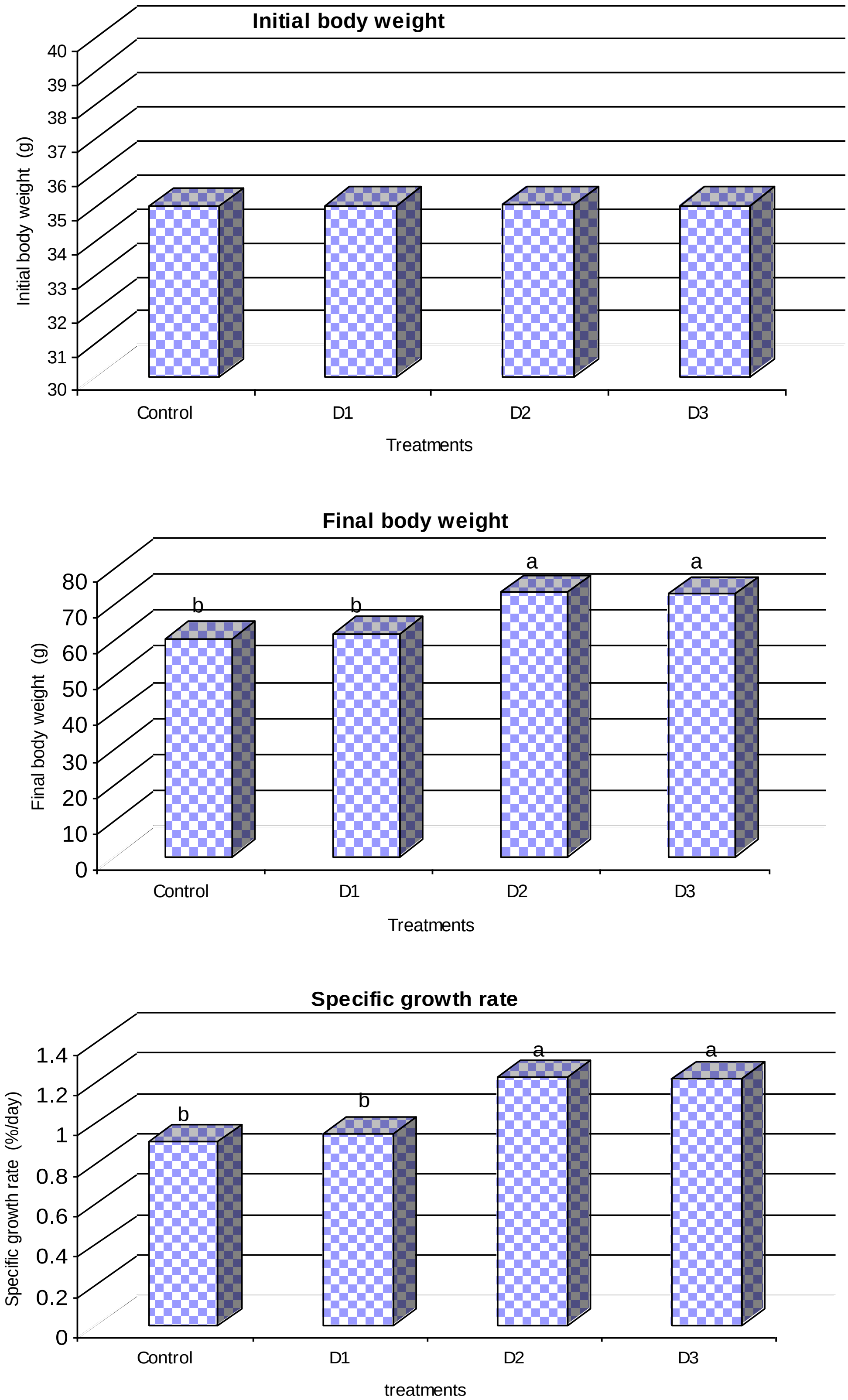
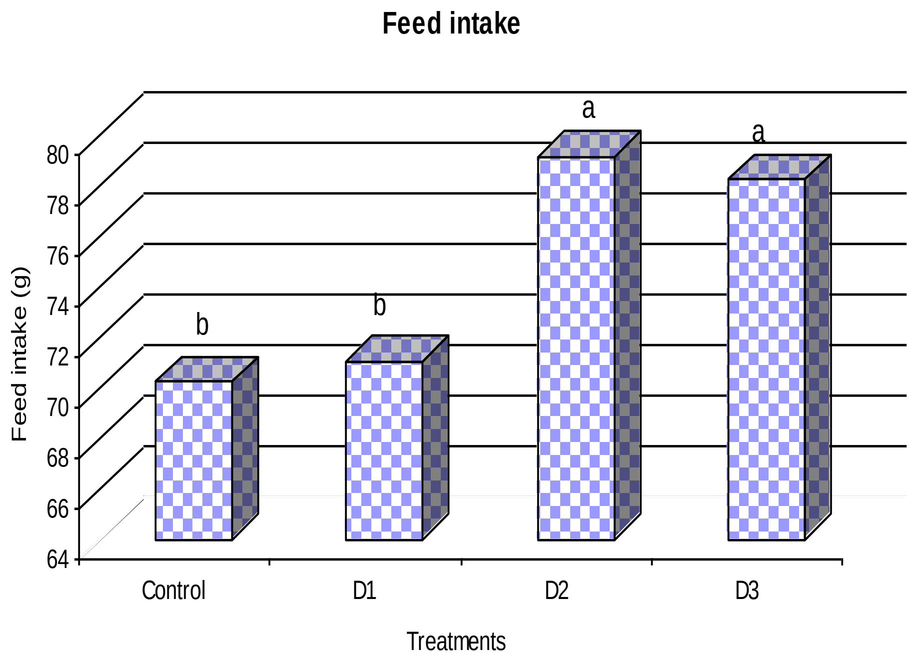


Figure 16 Initial body weight, final body weight and specific growth rate of Nile tilapia fed varying levels of *L. plantarum* NIOFSD018**.**

Values sharing the same superscript are not significantly different (P<0.05)
D1: diet containing 10^5, D2: 10^7 , D3: 10^9 CFU/g of *L. plantarum* NIOFSD018 and Control: probiotic free diet.



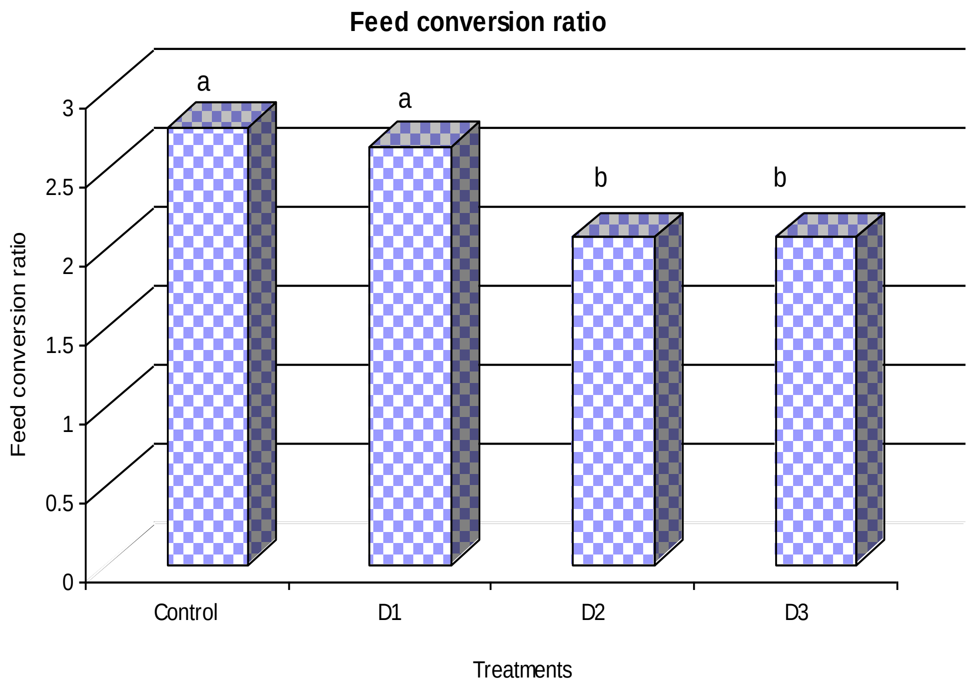


Figure 17 Feed intake and feed conversion ratio of Nile tilapia fed varying levels of *L. plantarum* NIOFSD018.

Values sharing the same superscript are not significantly different (P<0.05)
D1: diet containing 10^5, D2: 10^7 , D3: 10^9 CFU/g of *L. plantarum* NIOFSD018 and Control: probiotic free diet.

Table 15 Daily weight gain of Nile tilapia fed varying levels of *L. plantarum* NIOFSD018[1]

Weeks	Diets[2]			
	Control	D1	D2	D3
Two weeks	0.35	0.36	0.62	0.61
Four weeks	0.38	0.42	0.62	0.60
Six weeks	0.52	0.54	0.69	0.63
Eight weeks	0.44	0.47	0.62	0.69
Mean[3]	$0.42^b \pm 0.01$	$0.45^b \pm 0.01$	$0.64^a \pm 0.06$	$0.63^a \pm 0.07$

[1] Values (mean ± standard deviation) in the same column sharing the same superscript are not significantly different (P<0.05)
[2] D1: diet containing 10^5, D2: 10^7 , D3: 10^9 CFU/g of *L. plantarum* NIOFSD018 and Control: probiotic free diet.
[3] The means ± standard error were recorded for three tanks within the same treatment

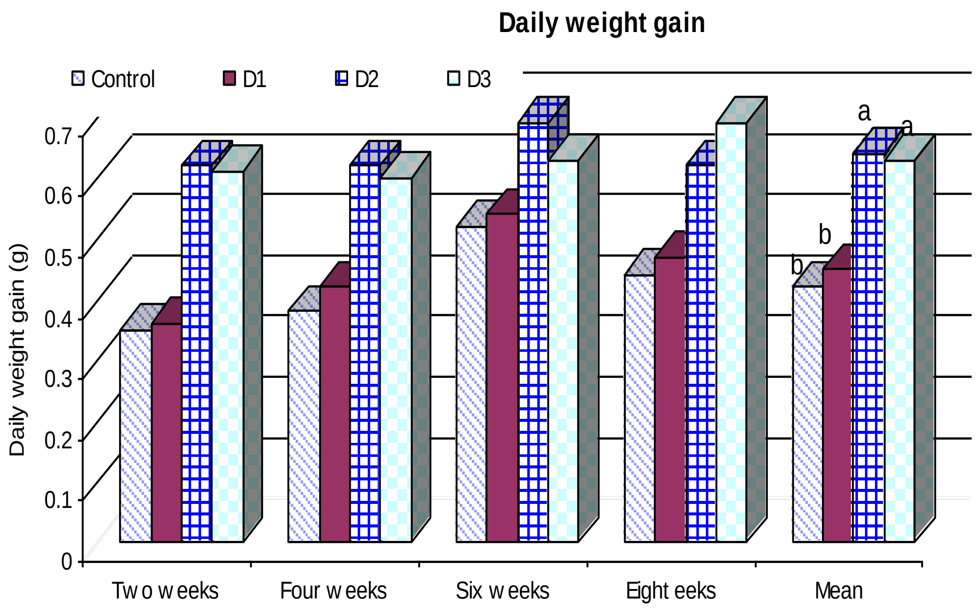


Figure 18 Daily weight gain (g) of Nile tilapia fed varying levels of *L. plantarum* NIOFSD018.

Values sharing the same superscript are not significantly different (P<0.05)
D1: diet containing 10^5, D2: 10^7 , D3: 10^9 CFU/g of *L. plantarum* NIOFSD018 and Control: probiotic free diet.

FEED UTILIZATION

Data presented in Table 16 and Figure 19 indicates that the administration of different concentrations of *L. plantarum* NIOFSD018 in the diet had a noticeable effect on tilapia feed utilization. Nutrient utilization data including protein PER, PPV and energy retention ER showed that PER (1.59±0.07 and 1.59±0.072) recorded for fish fed the diets D2 and D3 respectively were significantly higher than the values (1.18±0.03 and 1.24±0.02) recorded for fish fed the control and D1 diets respectively. Similar to PER, the results of PPV showed that, the means (26.31±1.71% and 27.33±0.43%) for fish fed diets D2 and D3 respectively were significantly higher than those 19.09±0.06 7% and 20.57±0.80 observed for fish fed the control and D1diets respectively. No significant differences were observed in PPV between fish fed D1and D2 or between D2 and D3. The highest average ER (18.60±0.30%) occurred in fish fed D3; this value was found not to be significantly different from the ER (17.95±0.93%) of fish fed D2 and was significantly different from the average ER (13.21±0.42% and 12.03±0.24%) for D1 and the control group respectively. The lowest overall GE value was recorded for the control group.

WHOLE BODY COMPOSITION

The varying levels of *L. plantarum* NIOFSD018 supplemented to the Nile tilapia diets did not show any significant differences in moisture content (Table 17 and Figures 20 and 21). The moisture content ranged from 72.73±0.32 % for fish fed D2 to 73.19±0.55 % for the control group. The moisture content for all the probiotic doses were significantly lower than the initial one. Average crud protein (CP) was statistically enhanced by increasing the dose of *L. plantarum* NIOFSD018 in the diet to 10^7 $CFUg^{-1}$. The highest average CP content (59.31±0.43 %) was observed for fish fed D3, which was significantly higher than the protein contents of all the other treatments or the initial group.

Table 16 Feed utilization of Nile tilapia fed varying levels of *L. plantarum* NIOFSD018[1]

Items	Diets[2]			
	Control	D1	D2	D3
Protein efficiency ratio[3]	1.18[b] ±0.03	1.24[b] ±0.02	1.59[a] ±0.07	1.59[a] ±0.072
Protein productive value (%)[4]	19.09[b] ±0.67	20.57[b] ±0.80	26.31[a] ±1.71	27.33[a] ±0.43
Energy retention (%)[5]	12.03[c] ±0.24	13.21[b] ±0.42	17.95[a] ±0.93	18.60[a] ±0.30

[1] Values (mean ± standard deviation) in the same row sharing the same superscript are not significantly different ($P<0.05$)
[2] D1: diet containing 10^5, D2: 10^7 , D3: 10^9 CFU/g of *L. plantarum* NIOFSD018 and Control: probiotic free diet.
[3] Protein efficiency ratio = Weight gain /protein intake
[4] Protein productive value (%)=[final body weight×(CP/100)-initial body weight (CP/100)/ protein intake]x 100
[5] Energy retention (%) = [final body weight × (CE/1000) - initial body weight (CE/1000)/ energy intake]x100

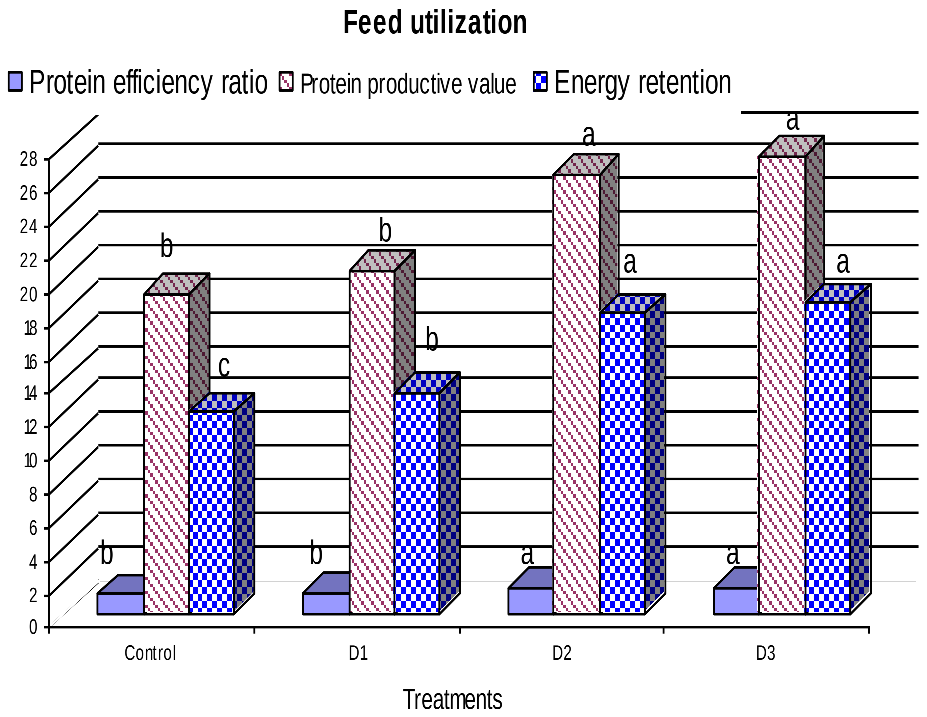


Figure 19 Feed utilization of Nile tilapia fed varying levels of *L. plantarum* NIOFSD018.

Values sharing the same superscript are not significantly different ($P<0.05$)
D1: diet containing 10^5, D2: 10^7 , D3: 10^9 CFU/g of *L. plantarum* NIOFSD018 and Control: probiotic free diet.

Fat content (ether extract; EE) was proportionally increased by increasing *L. plantarum* NIOFSD018 doses in the diet. No significant difference was observed between the EE of D3 (23.84±0.17%) and that measured in fish fed D2 (23.14 ±0.32%). On the other hand, EE of fish fed with D2 and D3 were significantly different from those fed D1 (21.46±0.94) or the control group (20.73±0.89%).

The highest average ash content (21.18±0.63%) was recorded for fish fed the control diet while, the lowest (16.28±0.32%) was obtained for fish fed D3. Both the highest and the lowest average ash content values were significantly different than all other groups.

No significant differences in nitrogen free extract (NFE) were apparent among the fish fed varying doses of *L. plantarum* NIOFSD018. The average NFE ranged from 0.58±0.06% to 0.86±0.06 %.

Gross energy (GE) content of the body was significantly influenced by the addition of different doses of *L. plantarum* NIOFSD018 to the diet, where GE averages were significantly increased by the each increasing dose of probiotic in the diet. Fish fed with diet D3 (10^9 $CFUg^{-1}$) of *L. plantarum* NIOFSD018 had the highest GE; 5627.22±11.27 kcal kg^{-1}while the lowest GE; 5228.42±71.40 kcl kg^{-1} was recorded for fish fed the control group.

FLESH CHEMICAL COMPOSITION

Chemical composition of flesh including moisture, crude protein, EE and ash content of Nile tilapia, fed on diets containing *L. plantarum* NIOFSD018 in varying levels are showed in Table 18. The moisture contents were 79.06±0.12%, 79.08±0.18% and 78.99±0.17% for fish the diets D1, D2 and D3 respectively. These moisture values (Figure 22) were significantly higher than the control moisture (78.61±0.13%). Crude protein content did show a consistent pattern in terms of differences among the various treatments, CP ranged from 84.39±0.38% to 85.62 ±0.59%.

Table 17

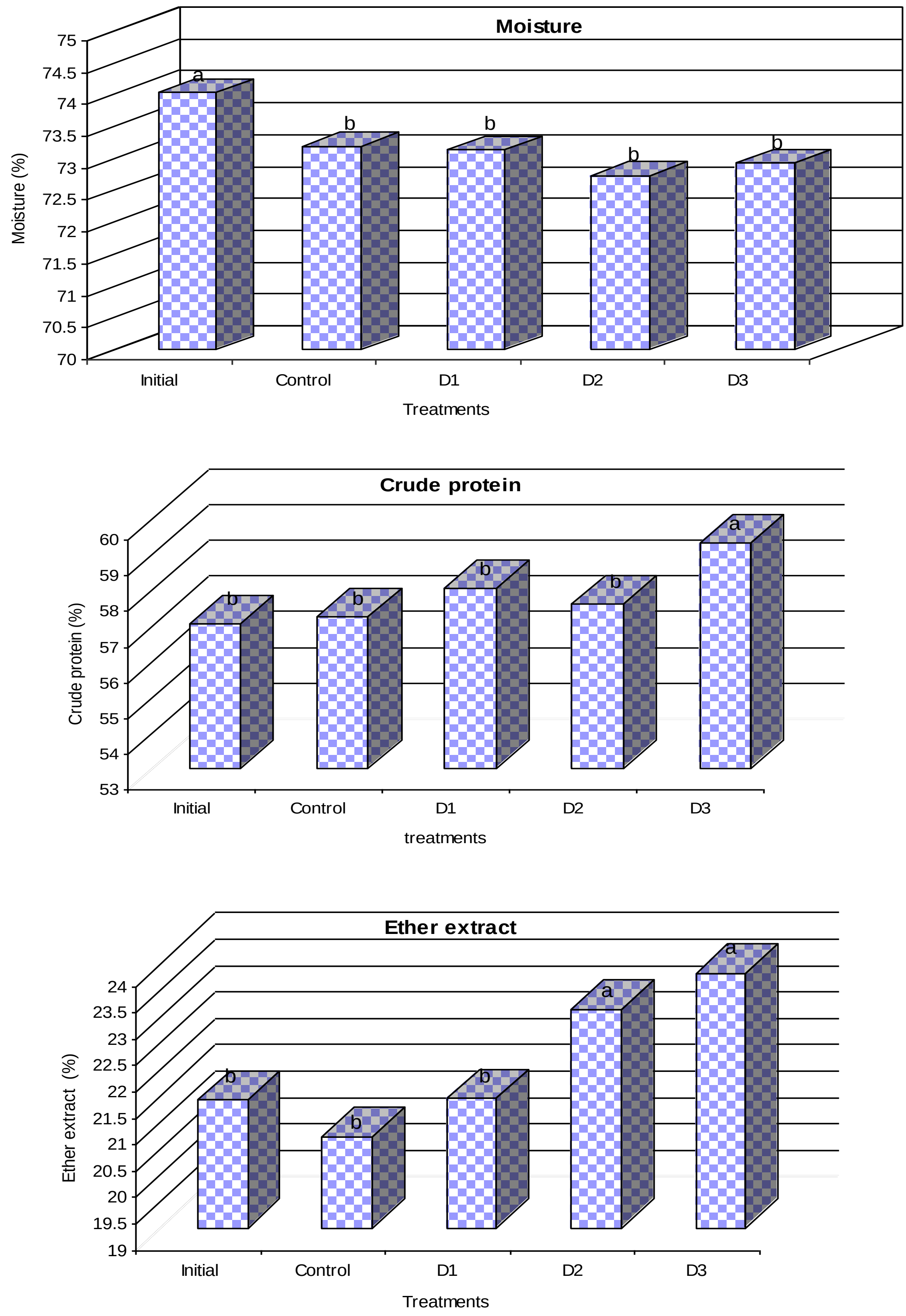
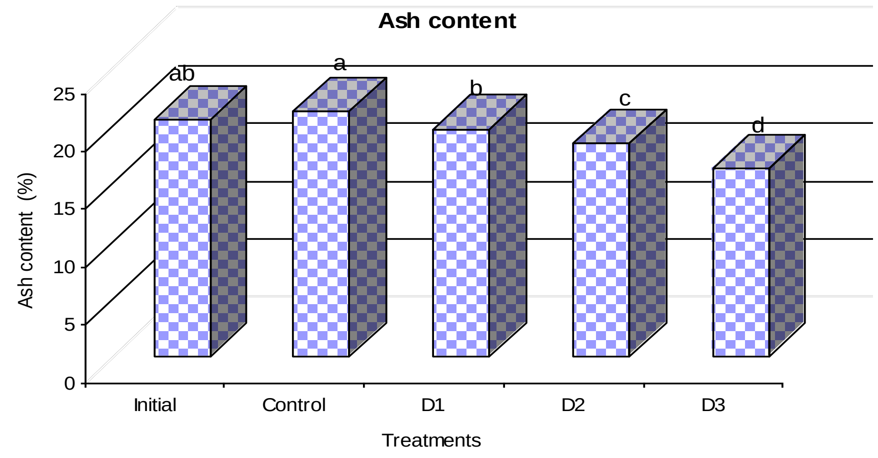
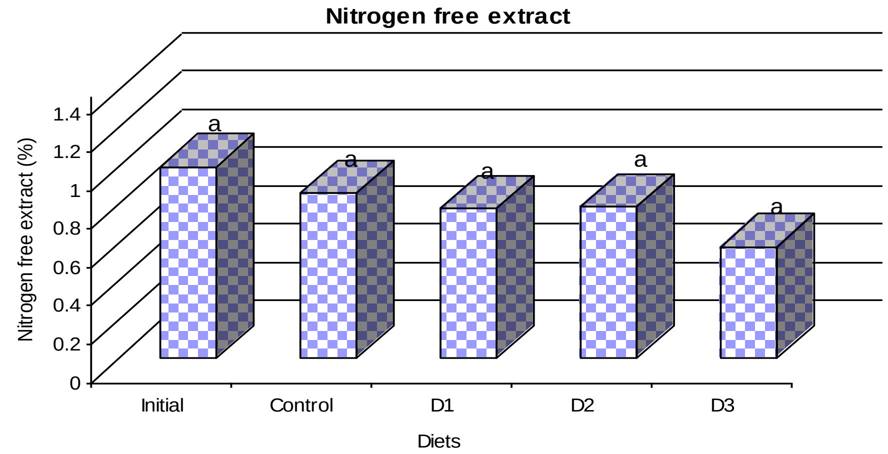


Figure 20 Moisture content, crude protein and ether extract of Nile tilapia (carcass) fed varying levels of *L. plantarum* NIOFSD018.

Values sharing the same superscript are not significantly different (P<0.05)
D1: diet containing 10^5, D2: 10^7 , D3: 10^9 CFU/g of *L. plantarum* NIOFSD018 and Control: probiotic free diet.





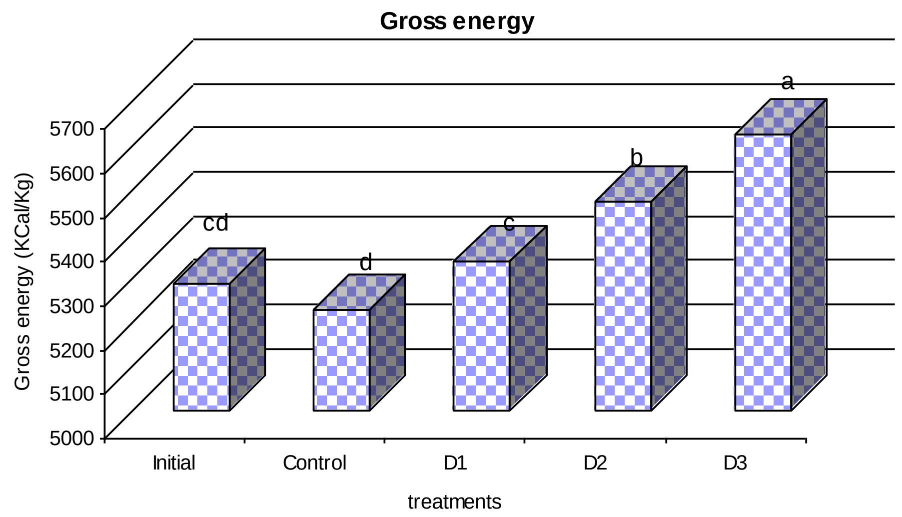


Figure 21 Ash content, nitrogen free extract and gross energy of Nile tilapia (carcass) fed varying levels of *L. plantarum* NIOFSD018.

Values sharing the same superscript are not significantly different (P<0.05)
D1: diet containing 10^5, D2: 10^7 , D3: 10^9 CFU/g of *L. plantarum* NIOFSD018 and Control: probiotic free diet.

Whereas, no significant differences were observed among the protein content of fish fed on D1, D3 and the initial fish, also there were no significant differences among the treatments D1, D2 and the control group (Figure 22)

The highest averages fat content (7.40±0.19% and 7.31±0.05%) were observed in fish fed the diets D3 and D2 respectively, these two values were significantly different as compared to the values; 6.77±0.29 % or 6.81±0.15 % for fish fed D1 and the control group respectively (Figure 23).

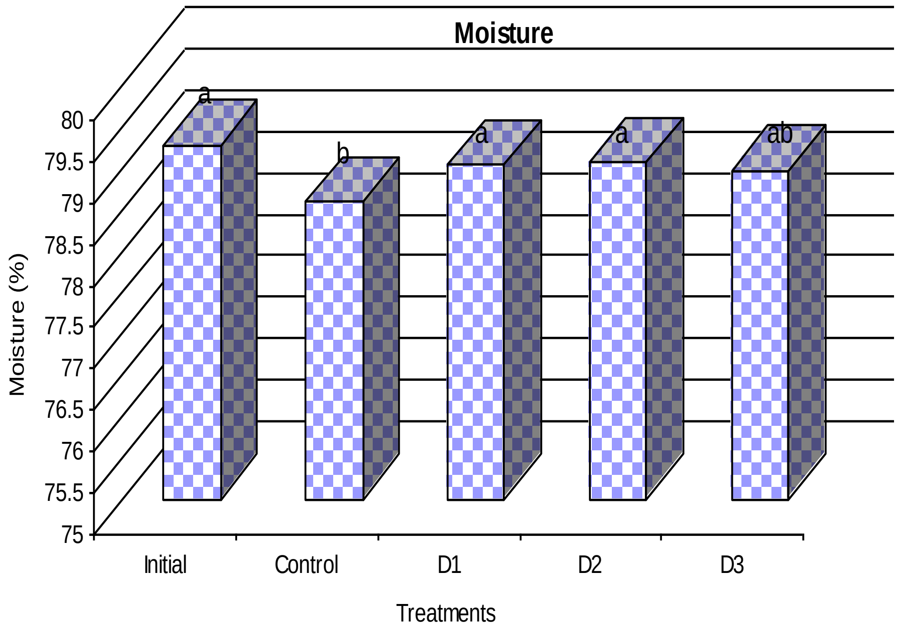
Average ash content were similar among all the treatments and were similar to the initial group with the exception of the treatment D3 which exhibited the average; 5.87±0.12% and found to be significantly lower than all other fish in various groups (Figure 23).

DIGESTIVE ENZYME ACTIVITIES

Amylase activities

Amylase activities data including both total and specific activity of Nile tilapia, fed on different *L. plantarum* NIOFSD018 doses are presented in Table 19 and Figure 24. It is obvious from these results that both of total and specific amylase activities were significantly increased by the addition of *L. plantarum* NIOFSD018 to the diet at 10^5 and 10^7 CFU/g^{-1}. Amylase activities were not significantly improved by increasing *L. plantarum* NIOFSD018 dose from 10^7 to 10^9 (D2 and D3 respectively). However, the total amylase activity (36.61±4.90 Uml^{-1} and 36.70±3.91 Uml^{-1}) for fish fed D2 (10^7 CFU/g) and D3 (10^9 CFU/g) respectively, were found to be significantly different than the amylase activity; 28.52±2.72 Uml^{-1} and 23.89±3.16 Uml^{-1} of D1 and the control group respectively. Amylase specific activities for fish fed the diets D2 and D3 were significantly higher than those of control or D1 diets. The specific activities of amylase for sampling at the day 60 (end of the experiment) were 2.75±0.32, 3.44±0.57,

Table 18



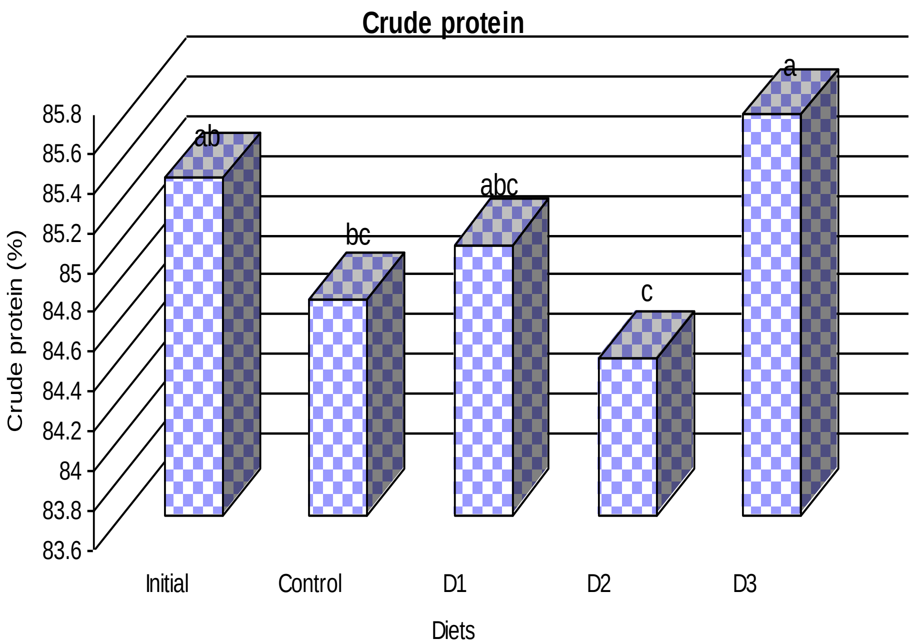
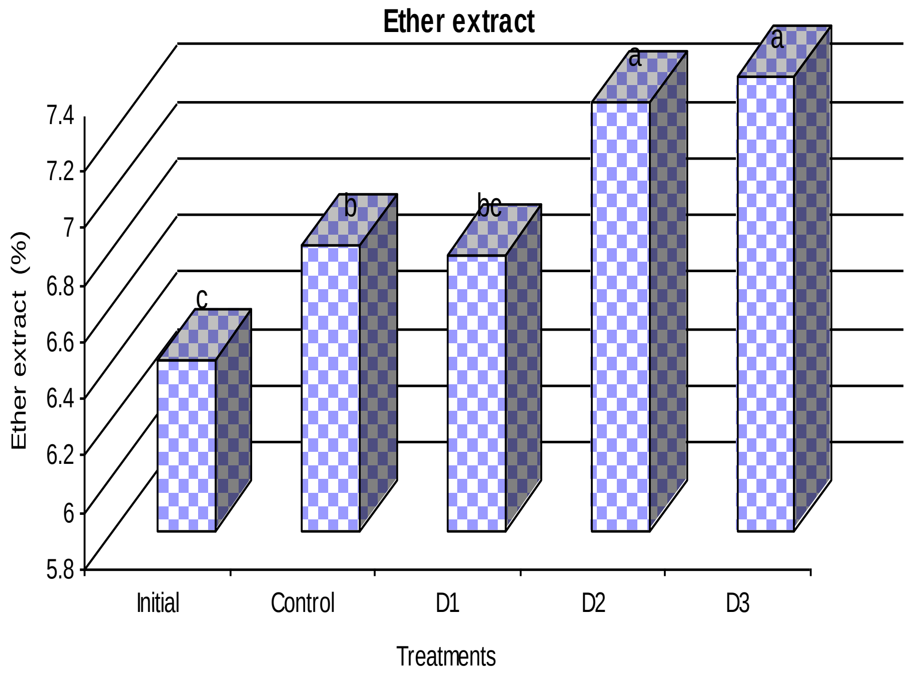


Figure 22 Moisture content and crude protein of Nile tilapia (flesh) fed varying levels of *L. plantarum* NIOFSD018.

Values sharing the same superscript are not significantly different (P<0.05)
D1: diet containing 10^5, D2: 10^7 , D3: 10^9 CFU/g of *L. plantarum* NIOFSD018 and Control: probiotic free diet.



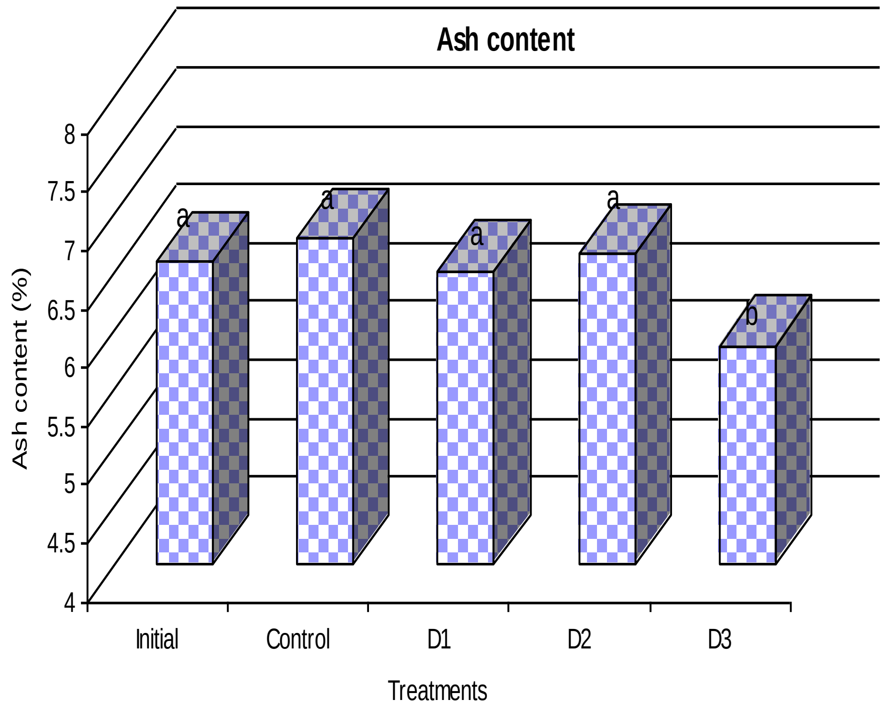


Figure 23 Ether extract and ash content of Nile tilapia (flesh) fed varying levels of *L. plantarum* NIOFSD018.

Values sharing the same superscript are not significantly different (P<0.05)
D1: diet containing 10^5, D2: 10^7 , D3: 10^9 CFU/g of *L. plantarum* NIOFSD018 and Control: probiotic free diet.

Table 19 Amylase enzyme activity of Nile tilapia fed varying levels of *L. plantarum* NIOFSD018[1]

Treatments [2]	**Protein content mg ml^{-1}**	**Amylase activities**[1]	
		Total (Unit ml^{-1})	**Specific (Unit mg protein)$^{-1}$**
Control	10.62 ± 0.66	23.89^{c} ±3.16	2.26^{c} ±0.34
D1	10.40 ±0.62	28.52^{b} ±2.72	2.76^{b} ±0.32
D2	10.72 ±0.58	36.61^{a} ±4.90	3.44^{a} ±0.57
D3	10.44 ±0.57	36.70^{a} ±3.91	3.53^{a} ±0.41

[1] Values (Mean ± Standard Deviation) in the same column sharing the same superscript are not significantly different (P<0.05)
[2] D1: diet containing 10^5, D2: 10^7 , D3: 10^9 CFU/g of *L. plantarum* NIOFSD018 and Control: probiotic free diet.

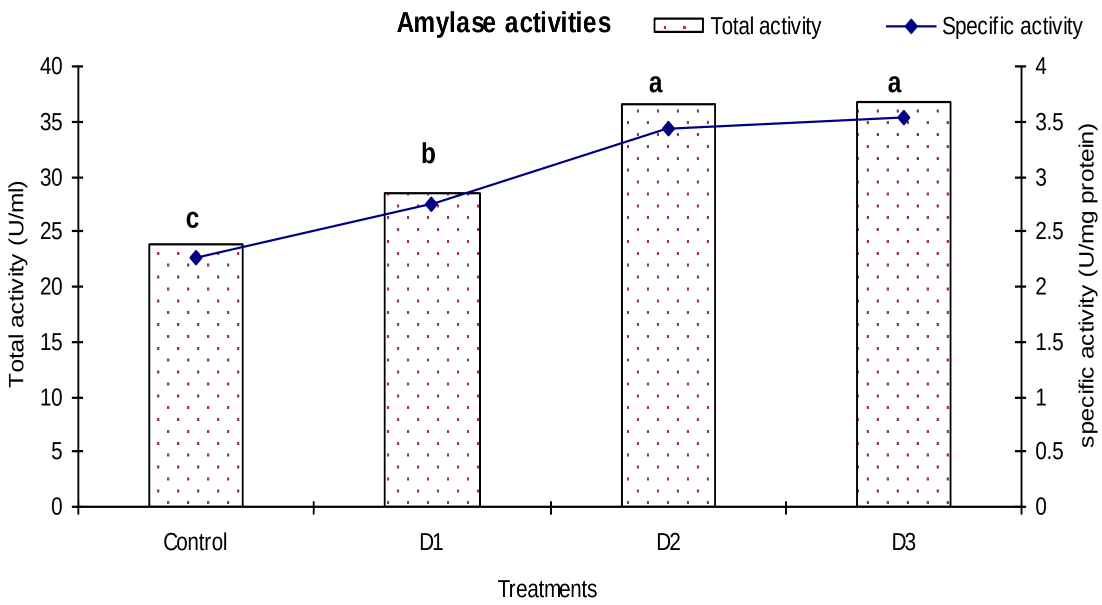


Figure 24 Amylase enzyme activities of Nile tilapia, fed varying levels of *L. plantarum* NIOFSD018.

Values sharing the same superscript are not significantly different (P<0.05)
D1: diet containing 10^5, D2: 10^7 , D3: 10^9 CFU/g of *L. plantarum* NIOFSD018 and Control: probiotic free diet.

3.53±0.41 and 2.26±0.34 U (mg protein) $^{-1}$ for fish fed D1, D2, D3 and the control group in the order mentioned.

Protease activities

Addition of *L. plantarum* NIOFSD018 to the diet at different doses has improved the total and specific protease activities (Table 20 and Figure 25). The highest amount (0.103±0.0111 µmol) of tyrosine was recorded for fish fed D2, while the lowest average (0.066±0.006 µmol) of tyrosine was noticed for fish fed the control diet. Table 20 outlines also that fish fed diet with 10^7 CFU/g (D2) had significantly higher total protease activity (5.655±0.61 unit ml^{-1}), which was followed by the protease activities of 4.992±0.44, 4.08±0.54 and 3.613±0.35 unit ml^{-1} in fish fed diets D3, D1 and the control respectively. There were significant differences among all of these treatments. As in the case of the protease total activities, protease specific activities recorded in fish fed D2 exhibited significantly higher (0.529±0.057 U (mg protein) $^{-1}$ protease specific activity as compared fish fed D1 and D3 or as compared to the fish of the control group.

Lipase activities

It is obvious from the data represented in Table 21 that both of total and specific lipase activities were increased by the increasing of probiotic doses (Figure 26). The highest total lipase activity (4.346±0.48 U ml^{-1}) was recorded in fish fed D3 (10^9 CFU/g) and the activity of this enzyme was found to be significantly different from the activities of 3.336±0.45, 2.467±0.68 and 2.358±0.52 U ml^{-1} found in fish fed D2, D1 and the control diets respectively. The recorded values of specific lipase activities were 0.239±0.070, 0.313±0.045, 0.417± 0.049 and 0.222 ± 0.048 U (mg proteins) $^{-1}$ in fish fed D1, D2, D3 and the control diets respectively. Fish fed D3 exhibited significantly higher lipase specific activity than those received D1, D2, and control diets; however fish fed D1 did not show significant difference compared to the control.

Table 20

Table 21 Total and specific lipase enzyme activities of Nile tilapia fed varying levels of *L. plantarum* NIOFSD018[1]

Treatments[2]	Protein (mg ml^{-1})	Lipase activities[3]	
		Total (Unit ml^{-1})	Specific (Unit mg protein)$^{-1}$
Control	10.62 ± 0.66	2.358^{c} ±0.52	0.222^{c} ±0.048
D1	10.40 ±0.62	2.467^{c} ±0.68	0.239^{c} ±0.070
D2	10.72 ±0.58	3.336^{b} ±0.45	0.313^{b} ±0.045
D3	10.44 ±0.57	4.346^{a} ±0.48	0.417^{a} ±0.049

[1] Values (mean ± standard deviation) in the same column sharing the same superscript are not significantly different (P<0.05)
[2] D1: diet containing 10^5, D2: 10^7 , D3: 10^9 CFU/g of *L. plantarum* NIOFSD018 and Control: probiotic free diet.
[3] Lipase activity was expressed as the volume of 0.05 N NaOH required to neutralize the fatty acids released during the incubation period

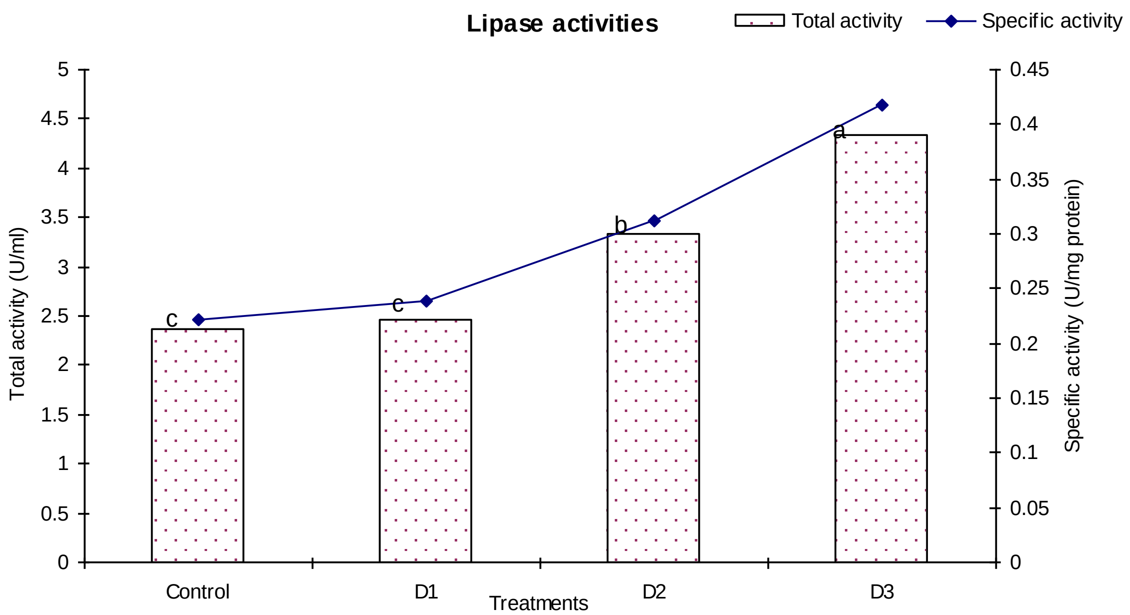


Figure 26 Lipase enzyme activities of Nile tilapia fed varying levels of *L. plantarum* NIOFSD018.

Values sharing the same superscript are not significantly different (P<0.05)
D1: diet containing 10^5, D2: 10^7 , D3: 10^9 CFU/g of *L. plantarum* NIOFSD018 and Control: probiotic free diet.

IMMUNE RESPONSE

The effect of *L. plantarum* NIOFSD018 (three different levels) on the immune response including phagocytic activity, seum acid phosphatase activity, serum lysozyme activity, serum phenoloxidase activity and total immunoglobulin of Nile tilapia, are reported in Table 22 and Figures 27 and 28 . It is obvious that the different tested immunological parameters have responded to the varying probiotic doses in different manners. The phagocytic activity was significantly increased by increasing the probiotic dose from 10^5 to 10^7 $CFUg^{-1}$. However, by increasing the dose of probiotic to 10^9 $CFUg^{-1}$ the phagocytic activity has decreased again but still significantly higher than the control. The average (78.5±2.20%) of phagocytic activity for fish fed D2 was found to be significantly higher than the values; 76.67±2.45%, 73.39±2.12% and 72.72±1.36% which were recorded in fish fed D3, D1 and the control group respectively. No significant differences of the phagocytic activity were observed between fish fed the D1 and the control diets.

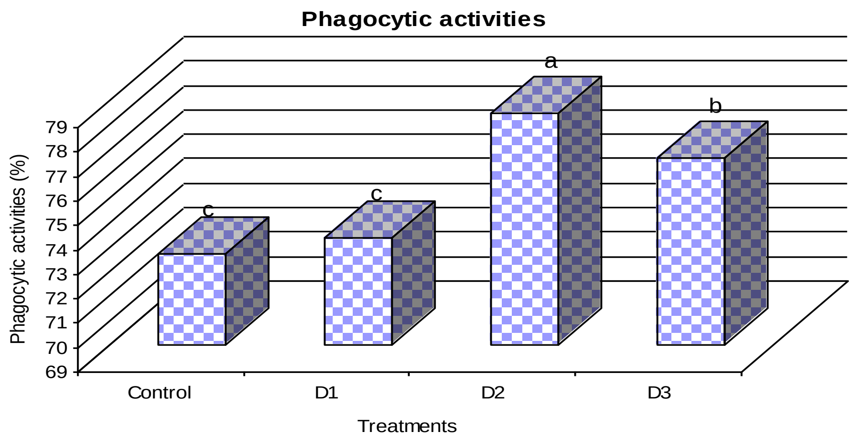
Acid phosphatase activity was significantly enhanced only at the dose; 10^9 CFU g^{-1} of *L. plantarum* NIOFSD018 by the average; 9.25 ±2.90 Uml^{-1}. However, the other acid phosphatase activities (5.50 ±0.31 and 6.28 ±0.07) detected in fish fed D1 and D2 respectively were found to be not significantly different as compared to the control (3.47 ±0.91 Uml^{-1}).

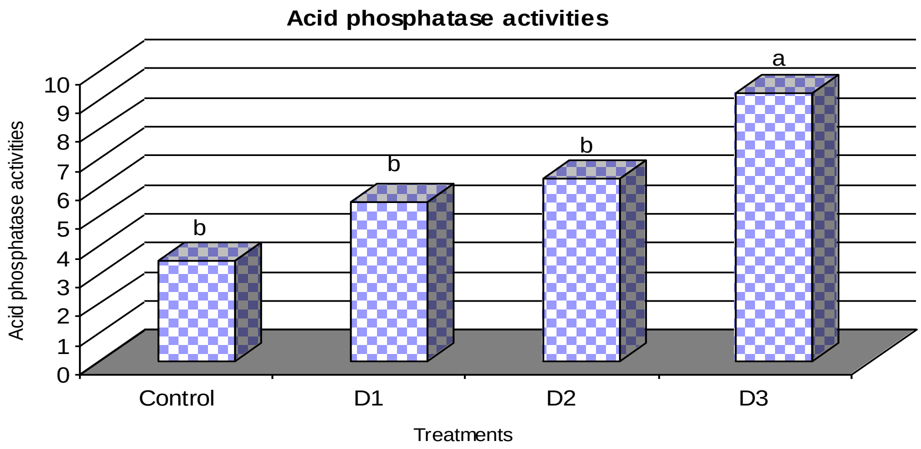
No significant differences appeared in lysozyme activity between fish fed 10^5 $CFUg^{-1}$ of *L. plantarum* NIOFSD018 (128.30 ±49.06) and the control (80.0±42.40 Uml^{-1}). On the other hand, Nile tilapia received the diets containing 10^7 and 10^9 $CFUg^{-1}$ of probiotic exhibited the highest averages lysozyme activities of 889.78 ±89.74 and 913.78±93.71 Uml^{-1} respectively. Also no significant difference in lysozyme activity was found between fish fed D2 and D3.

Phenoloxidase activities were slightly (insignificantly) increased with each increase in *L. plantarum* NIOFSD018 dose. Phenoloxidase activities have been ranged from 7.93 ±5.06 to 10.67±2.47.

Total immunoglobulin for fish fed different doses of *L. plantarum* NIOFSD018 were 1.66±0.072 g/dl, 2.044±0.121 g/dl, 2.21±0.077 g/dl and 2.054±0.154 g/dl for the control, D1, D2 and D3 diet respectively. No significant difference was observed between D1 and D3. From the previous immunological data, it is clear that Nile tilapia, fed D2 (10^7 CFU/g) had significantly the highest values of phagocytic activity and total immunoglobulin. However, no significant differences were observed in lysozyme activity or phenoloxidase activities between D2 and D3 (10^7 CFU/g^{-1}). Only in the case of acid phosphatase activity, it is noticed that fish fed D3 exhibited significantly higher activity than fish fed D2.

Table (22





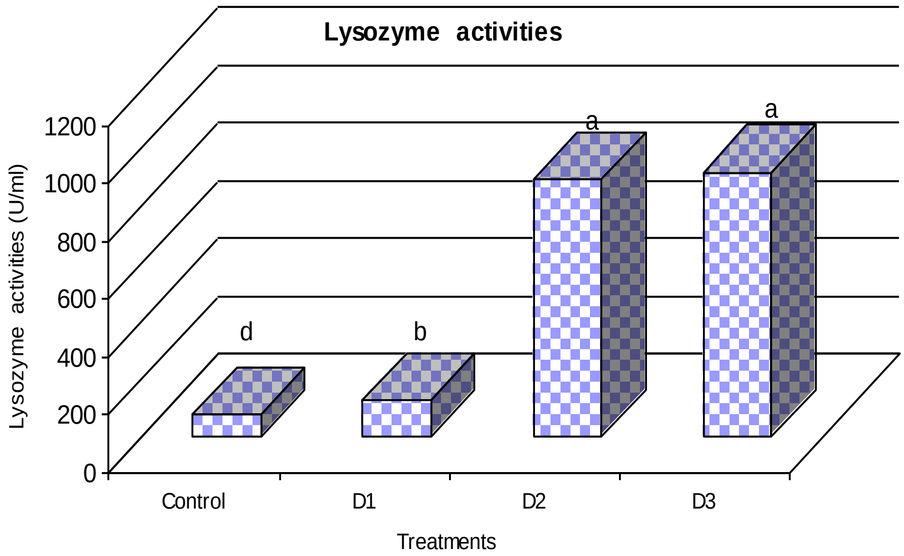
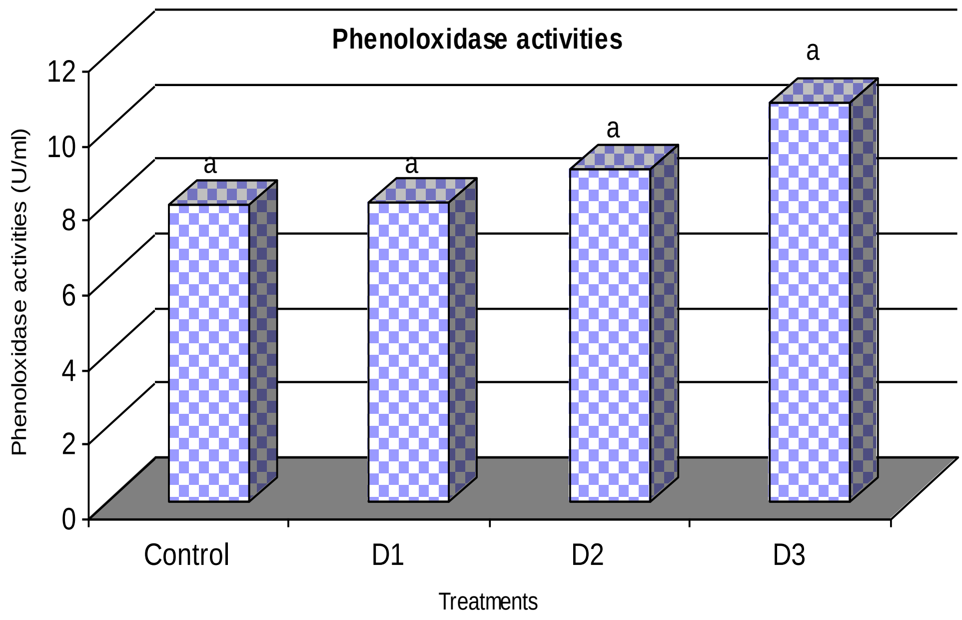


Figure 27 Phagocytic, acid phosphtase and lysozyme activities of Nile tilapia fed varying levels of *L. plantarum* NIOFSD018.

Values sharing the same superscript are not significantly different (P<0.05)
D1: diet containing 10^5, D2: 10^7, D3: 10^9 CFU/g of *L. plantarum* NIOFSD018 and Control: probiotic free diet.



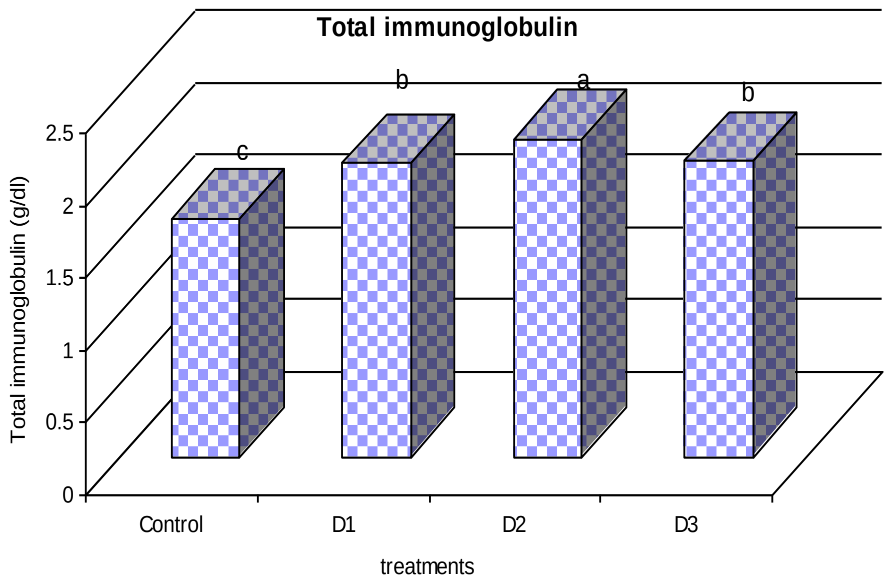


Figure 28 Phenoloxidase activities and total immunoglobulin of Nile tilapia fed varying levels of *L. plantarum* NIOFSD018.

Values sharing the same superscript are not significantly different (P<0.05)
D1: diet containing 10^5, D2: 10^7 , D3: 10^9 CFU/g of *L. plantarum* NIOFSD018 and Control: probiotic free diet.