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Original Research Article

IMPACT OF ALTERING DIETARY OMEG 6 TO OMEGA 3 FATTY ACIDS RATIO ON GROWTH PERFORMANCE, CARCASS COMPOSITION, HEMATO-BIOCHEMICAL PARAMETERS AND ABSORPTIVE CAPACITY OF THE INTESTINE OF NILE TILAPIA

Eldsokey Nassef[1]*, Elsayed Hegazi[1], Ahmed Sherif[2], Mohamed Elshamy[2], Abdelnasser Bakr[1], Tarek k. Abouzed[3]

[1]Nutrition and Clinical Nutrition Department, Faculty of Veterinary Medicine, Kafrelsheikh University, Kafrelsheikh, Egypt, [2]Agriculture Research Center, Animal Health research Institute, Kafrelsheikh, Egypt, [3]Biochemistry Department, Faculty of Veterinary Medicine, Kafrelsheikh University, Kafrelsheikh, Egypt.

*Corresponding author, E-mail: dsokeynassef@yahoo.com

Abstract: The present study investigated the influence of altering omega 6 to omega 3 fatty acids (FA) ratio on growth, hemato- biochemical parameters and absorptive capacity of Nile tilapia. One hundred and fifty fingerlings (25.1±0.3 g), were assigned into 3 groups (5 replicates each) and fed on iso-caloric and iso-nitrogenous diets. Diets were supplemented with fish and soy oil to create 3 different ratio of omega 6 to omega 3 FA, namely, 0.91, 2.85 and 9.3 parts of omega 6 to 1 part of omega 3 (R1, R3 and R9, respectively). Increasing the ratio from R1 to R9 significantly decreased average daily gain and increased feed conversion ratio. Also this alteration decreased ash content in the carcass on the expense of ether extract. Number of erythrocytes, packed cell volume, hemoglobin concentration were significantly increased in fish fed R1. Feeding R9 increased heterophils and decreased lymphocytes as compared with R1 and R3. Fish fed R1 significantly had high serum protein and serum antibacterial activity (47.6%) than R9 (43.5%) and R3 (44.9%). Feeding R1 significantly decreased serum triglycerides, cholesterol and creatinine. Feeding R3 and R1 enhanced absorptive capacity of jejunum villi more than the group fed R9. Increasing the ratio of omega 6 to omega 3 FA from R1 to R9 decreased growth performance through decreasing absorptive capacity of jejunum villi. However, Feeding R3 had a positive effect on growth performance and absorptive capacity of the intestine of Nile tilapia.

Key words: fish oil; growth performance; intestinal villi; Nile tilapia; soybean oil

Introduction

Fish oil is the main lipid source used in the formulation of commercial aqua feeds. The continuous little global production of fish oil into 2030 (1), the highly variable cost have forced intensive research activities to evaluate alternative lipid sources (2). Vegetable oils are important lipid sources with low cost and wide availability. To our knowledge, there was scarce information on the assessment impact of altering omega 6 to omega 3 fatty acids (FA)

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ratio on antibacterial activity and absorptive capacity of the intestine of Nile tilapia.

Tilapia is the most common type of fish used in aquaculture where its production reaches to 66.8% of total aquaculture production (3). Fish meals and oils are essential part of diet composition in tilapia as a source of protein and lipid (2). Dietary lipids are important for regular growth and modulating immune responses in mammals, fish, sea cucumber, and shrimps (4). Because the expansion of aquaculture, it is expected that the requirements of fish meals and oils by the aquaculture sector will increase, but there are troubles of the high cost and limiting availability of fish meals and fish oils (1; 4).

We hypothesized that vegetable oils could replace fish oil due to its low cost and wide availability. However, the difference in the ratio of omega 6 to omega 3 FA can impact fish health and disease resistance. Lipids affect on the immune system by influencing the phospholipids of immune cell membranes, membrane-associated signaling molecules (eicosanoids) and receptor sites (5). Many previous studies used fish meal as a protein supplement when evaluating vegetable oils which supplied a certain amount of long chain poly unsaturated fatty acids. This study aimed to investigate the influence of altering omega 6 to omega 3 FA on growth performance, hematological and biochemical parameters, carcass composition and antibacterial activity of Nile tilapia and assessment of the absorptive capacity of duodenum, jejunum and ilium.

Materials and methods

Fish and experimental design

One hundred and eighty fingerlings of Nile Tilapia (25.1±0.3 g) were purchased from commercial farm in Kafrelsheikh governorate and were acclimatized to tank conditions for 2 weeks. One hundred and fifty apparently healthy fingerlings of homogenous size were selected and randomly distributed into 3 groups (5 replicates each). Each replicate contained 10 fish per tank of 50-L which was equipped with continuous aeriation, inlet and outlet. The procedures have been approved by the Institutional Aquatic Animal Care and Use Committee, Faculty of Aquatic and Fisheries Sciences, Kafrelsheikh University.

Three experimental diets were supplemented with fish and soy oils to create 3 different ratio of omega 6 to omega 3 FA. The first ratio (R1) was of 0.91 parts of omega 6 to 1 part of omega 3 FA. The second ratio (R3) was of 2.85 parts of omega 6 to 1 part of omega 3 FA. The final ratio (R9) was of 9.3 parts of omega 6 to 1 part of omega 3 FA. The diets were nearly similar in protein and energy (Table 1). The ingredients were ground to pass through a sieve of 1 mm and mixed for 20 min. the oil was added with the continuous mixing. Distilled water was added to the diets till forming soft dough. The diets were pelleted in a laboratory pellet mill through 2 mm diameter die. Pellets were dried in an oven at 60 °C for 2 h and freshly used. The experimental period lasted for 8 weeks. The fish were fed twice daily at 8.00 and 15.00 h at a level of 4% of body weight for 2 weeks; 3.5% from 3 to 4 weeks then 3% from 5-8 weeks. Feed refusal was recovered after feeding and dried using oven at 60 °C for 2 h then subtracted from the offered feed.

Growth trial

Growth parameters were determined according to the following equations:

Body weight gain (BWG, g $fish^{-1}$) = Final BWT - Initial BWT;

Average daily gain (ADG, g) = BWG/duration of the experiment (56 days);

Feed intake (FI, g $fish^{-1}$, 56 days) = (offered feed–feed refusal recovered and dried)/no of fish

Feed conversion ratio (FCR) = FI (g)/ BWG (g);

Protein efficiency ratio (PER) = BWG (g)/dry protein intake (g);

Protein retention (PR, %) = (protein gain, g/protein intake, g) ×100

Energy retention (ER, %) = (energy gain, kcal)/energy intake, kcal) ×100

Chemical analysis of feed and fish

Representative feed samples were ground through 1mm screen (Cyclotec, Foss Sweden). The ground samples were analyzed for crude

protein based on Kjeldahl method and ether extract (Ankom Technology method), according to (6). NFE was calculated according to the following formula: NFE= 100- (moisture + crude protein + ether extract + ash + crude fiber). Lysine, methionine, calcium and available phosphorus were calculated based on feed composition tables in (2).

At the end of the experiment, 3 fish were randomly collected from each replicate; dried in oven at 60 °C for 48 h. Dry matter and moisture were determined. The dried samples were analyzed for crude protein, ash and ether extract according to (6).

Blood analysis and immune response assay

At the end of the experiment, pooled blood sample was collected from 5 fish per tank via the caudal vein into heparinized disposal syringe for complete blood count (CBC). Further blood sample without anticoagulant was collected, centrifuged at 3000 rpm for 15 min to obtain the serum and stored at -20 °C for the biochemical analysis. Blood film was prepared according to (7). Differential leukocyte count was calculated according to (8). Hematocrit, erythrocytes, white blood cell (WBCs) and hemoglobin (HB) were examined according to (9). Serum protein and albumin were calorimetrically measure based on (10, 11) respectively. Serum globulin was determined by subtracting the concentration of albumin from protein. The activity of alanine amino transaminase (ALT) and aspartate amino transaminase (AST) was determined as previously described (12).

Histopathological analysis

Intestinal tissue specimen were obtained at the end of experiment to examine absorptive capacity of villi. The samples were immersed in formalin 10%, embedded in paraffin, stained with haematoxylin and eosin (H & E) and documented photographically with a digital camera (DCM 130E/1.3 megapixels, CMOS Software Scopephoto, China) connected to a light microscope (Leica).

Serum antibacterial test

Serum bactericidal activity was done following the procedure of (13). Equal volumes (100µl) of serum and A. hydrophila bacterial suspension $2x10^8$ (CFU) were mixed and incubated at 25 ^oC for 1 h. Blank control was made by replacing serum with sterile phosphate buffer saline (PBS). The mixture was then diluted with sterile PBS at a ratio of 1:10. The diluted mixture (100µl) was plated on blood agar and incubated at 37 ^oC for 24 h. The number of viable bacteria was determined by counting the colonies grown on the agar plates.

Statistical analysis

Analysis of variance (ANOVA) was done for all data using the SPSS program (14). Duncan's multiple range was used to determine the significant difference among means at $P<0.05$.

Results

Growth performance

As shown in Table 2, the final weight, BWG and ADG were significantly higher in fish fed R1 than those fed R9. But, fish fed R3 had nearly similar growth parameters to fish fed R1. In sum, ADG of fish fed R1 was the greatest followed by R3 then R9, There was no significant difference in feed intake of fish fed either R1 or R3 or R9. But, FCR was significantly improved in fish fed R1 followed by R3 then R9. PER and PR were greater in fish fed R1 than those fed the other diets. In contrast, ER was significantly greater in fish fed R9 followed by R3 then R1.

Body chemical composition

As illustrated in Table 3, there is no significant differences between different groups in dry matter, moisture and crude protein. Feeding omega 3 FA (R1) significantly increased mineral density in the skeleton as compared to R9. In contrast, feeding R1 decreased fat deposition in the whole body of fish. Whereas, Feeding R3 still had the same effect of R1 on ash and ether extract concentrations.

Hematological parameters

As illustrated in Table 4, feeding R1 increased RBCs count, Hb concentration and PCV as compared to R3 or R9. Feeding R1, R3 increased WBCs than R9. However feeding R9 increased heterophil but decreased lymphocyte when compared to the other groups, while monocytes, eosinophil and basophil still unchanged among the dietary treatments.

Biochemical analysis

As shown in Table 5, although the ALT and AST were significantly lower in fish fed R1. The values of further groups were within the acceptable range. The same trend was recorded in creatinine analysis. Serum globulin was markedly high in fish fed R1. Subsequently, it elevated serum protein because the serum albumin was nearly similar among all treatment groups. Serum triglyceride and cholesterol were significantly lesser in fish fed R1 as compared to R9 or R3 groups. No significant difference in cholesterol concentration between R9 and R3 groups was detected. Feeding R1 increased HDL and decreased LDL than the other two groups. On the other hand, fish fed R3 had a higher HDL and a lower LDL than R9 group.

Antibacterial activity

As shown in Table 6, the result indicated a significant enhancement of the immune status of group fed R1 but differences in other groups were in acceptable range which showed no threat on fish life.

Table 1: Physical and chemical composition (%) of the diets for Nile tilapia.

Items	Dietary treatments [a]		
	R1	R3	R9
Corn gluten meal	10	10	10
Soybean oil	0	2.5	5
Fish oil	5	2.5	0
Corn grain	30.0	30.0	30.0
Dehulled soybean meal	51.6	51.6	51.6
Monocalcium phosphate	1.57	1.57	1.57
Limestone	0.68	0.68	0.68
Salt	0.35	0.35	0.35
Methionine, DL	0.2	0.2	0.2
Premix b	0.1	0.1	0.1
Antimycotoxin	0.2	0.2	0.2
Vitamin C	0.1	0.1	0.1
Binder	0.2	0.2	0.2
Chemical composition of dried pelleted diets			
Digestible energy, Kcal/kg c	3325	3313	3302
Crude protein %	32.23	32.23	32.23
Lysine %	1.73	1.73	1.73
Methionine %	0.75	0.75	0.75
Crude fat %	6.93	6.93	6.93
Calcium %	0.7	0.7	0.7
Available phosphorus %	0.45	0.45	0.45

[a] Treatments represent the ratio between omega 6 to omega 3 FA in the diet. R1 is a ratio of 0.91 parts of omega 6 to 1 part of omega 3; R3 is a ratio of 2.85 parts of omega 6 to 1 part of omega 3; R9 is a ratio of 9.3 parts of omega 6 to 1 part of omega 3. [b] Premix: Magnesium 40mg ; Manganese 10mg ; Zinc150mg ; Iron 30mg ; Copper 5mg ; Iodine 5mg ; Cobalt 0.005mg ;Selenium 0.1mg; vit A 5500 UI; vit D3 1000UI; vit E 50mg ; vit B1 20mg ; vit B2 20mg ; vit B6 20mg ; vit B12 0.02 mg ; niacin 100mg; vit K3 10mg ; biotin 0.1mg ;folic acid 5mg. [c] Calculated according to (2).

Table 2: Impact of altering omega 6 to omega 3 FA ratio on growth performance and feed utilization

	Dietary treatments [1]		
Items	R1	R3	R9
Initial weight	25.23±0.18	25.17±0.03	25.40±0.10
Final weight	54.96±0.6 [a]	51.12±2.84 [ab]	50.27±3.1 [b]
Body weight gain	29.3±0.90 [a]	25.96±2.87 [ab]	24.87±1.1 [b]
Average daily gain	0.52±0.02 [a]	0.47±0.05 [ab]	0.44±0.06 [b]
Feed intake	57.16±0.72	55.2±1.41	54.4±1.10
Feed conversion ratio	1.95±0.04 [a]	2.1±0.17 [ab]	2.24±0.21[b]
Protein efficiency ratio	1.59± 0.01[a]	1.45± 0.12 [b]	1.42b± 0.13
Protein retention	25.8± 1.5 [a]	23.55± 0.1 [b]	23.19± 1.05 [b]
Energy retention	45.6± 2.28 [c]	48.78± 0.16 [b]	50.05± 0.6 [a]

Means ± SE with different letter within the same raw are significantly different at P<0.05. [1] Treatments represent the ratio between omega 6 to omega 3 FA in the diet. R1 is a ratio of 0.91 parts of omega 6 to 1 part of omega 3; R3 is a ratio of 2.85 parts of omega 6 to 1 part of omega 3; R9 is a ratio of 9.3 parts of omega 6 to 1 part of omega 3.

Table 3: Impact of altering omega 6 to omega 3 FA ratio on body chemical composition (on dry matter basis)

	Dietary treatments [1]		
Items	R1	R3	R9
Dry matter	28.37±0.56	29.01±0.29	28.97±0.88
Moisture	71.63±0.58	71.0±0.29	71.03±0.88
Crude protein	55.73±0.15	55.7±1.7	55.9±0.11
Ether extract	21.8±1.25 [b]	23.1±1.1 [a]	23.8±0.4 [a]
Ash	19.6±1.98 [a]	17.98±1.9 [b]	16.46±0.78 [c]

Means ± SE with different letter within the same raw are significantly different at P<0.05. [1] Treatments represent the ratio between omega 6 to omega 3 FA in the diet. R1 is a ratio of 0.91 parts of omega 6 to 1 part of omega 3; R3 is a ratio of 2.85 parts of omega 6 to 1 part of omega 3; R9 is a ratio of 9.3 parts of omega 6 to 1 part of omega 3.

Table 4: Impact of altering omega 6 to omega 3 FA ratio on hematological parameters

	Dietary treatments [1]		
Items	R1	R3	R9
RBCS	2.57±0.09 [a]	2.01±0.08 [b]	1.88±0.1 [b]
WBCS	121.9±0.3 [a]	119±0.7 [a]	116.3±1.4 [b]
Heterophil	25±0.6 [b]	26.3±0.6 [b]	30±0.6 [a]
Monocyte	4.7±0.3	3.7±0.9	5.3±0.7
Lymphocyte	66.7±0.3 [a]	65.7±1.2 [a]	60.7±0.7 [b]
Eosinophil	3.7±0.3	4.3±0.6	4±0.6
Basophil	0.3±0.3	0.3±0.3	0.7±0.3
Hb	8.7±0.26 [a]	7.14±0.14 [b]	6.66±0.1 [b]
PCV	26.1±0.8 [a]	22.1±0.4 [b]	21±0.03 [b]

Means ± SE with different letter within the same raw are significantly different at P<0.05. [1] Treatments represent the ratio between omega 6 to omega 3 FA in the diet. R1 is a ratio of 0.91 parts of omega 6 to 1 part of omega 3; R3 is a ratio of 2.85 parts of omega 6 to 1 part of omega 3; R9 is a ratio of 9.3 parts of omega 6 to 1 part of omega 3.

Table 5: Impact of altering omega 6 to omega 3 FA ratio on biochemical parameters and antibacterial activity

	Dietary treatments 1		
Items	R1	R3	R9
ALT (µ/l)	34.3±1.3 [c]	41.2±0.8 [b]	47.3±0.9 [a]
AST (µ/l)	106.3± 2.96 [b]	112.5±1.3 [b]	130±6.4 [a]
Creatinine (mg/dl)	0.89±0.05 [c]	1.47±0.09 [b]	1.86±0.14 [a]
Total protein (g/dl)	6.6±0.15 [a]	5.7±0.2 [b]	5.3±0.09 [b]
Albumin (g/dl)	4.1±0.06	4.07±0.02	4±0.03
Globulin (g/dl)	2.5±0.15 [a]	1.6±0.3 [b]	1.2±0.1 [b]
Triglycerides (mg/dl)	149.5±1.9 [c]	295.5±1.45 [b]	303.3±2 [a]
Cholesterol (mg/dl)	98.5±1.6 [b]	118.2±0.7 [a]	123.5±2.4 [a]
HDL (mg/dl)	56.2±0.7 [a]	51.3±0.9 [b]	48.6±0.6 [c]
LDL (mg/dl)	2.87±0.3 [c]	11.5±3.3 [b]	20±0.8 [a]
Serum antibacterial (%)	47.6 ±0.6 [a]	44.9 ±0.4 [b]	43.5 ±0.8 [b]

Means ± SE with different letter within the same raw are significantly different at P<0.05. [1]Treatments represent the ratio between omega 6 to omega 3 FA in the diet. R1 is a ratio of 0.91 parts of omega 6 to 1 part of omega 3; R3 is a ratio of 2.85 parts of omega 6 to 1 part of omega 3; R9 is a ratio of 9.3 parts of omega 6 to 1 part of omega 3

Table 6: Impact of altering omega 6 to omega 3 FA ratio on mucosal and villi parameters of the intestine

Dietary treatments	Duodenum		Jejunum		Ilium	
1	length	width	length	width	length	width
R1	541±33	141±13	938±35 [a]	115±4 [b]	570±37 [a]	149±23
R3	510±30	139±13	769±27 [b]	113±6 [b]	187±23 [b]	133±12
R9	539±31	150±10	901±37 [a]	142±10 [a]	405±23 [c]	140±10

Means ± SE with different letter within the same column are significantly different at P<0.05. [1]Treatments represent the ratio between omega 6 to omega 3 FA in the diet. R1 is a ratio of 0.91 parts of omega 6 to 1 part of omega 3; R3 is a ratio of 2.85 parts of omega 6 to 1 part of omega 3; R9 is a ratio of 9.3 parts of omega 6 to 1 part of omega 3

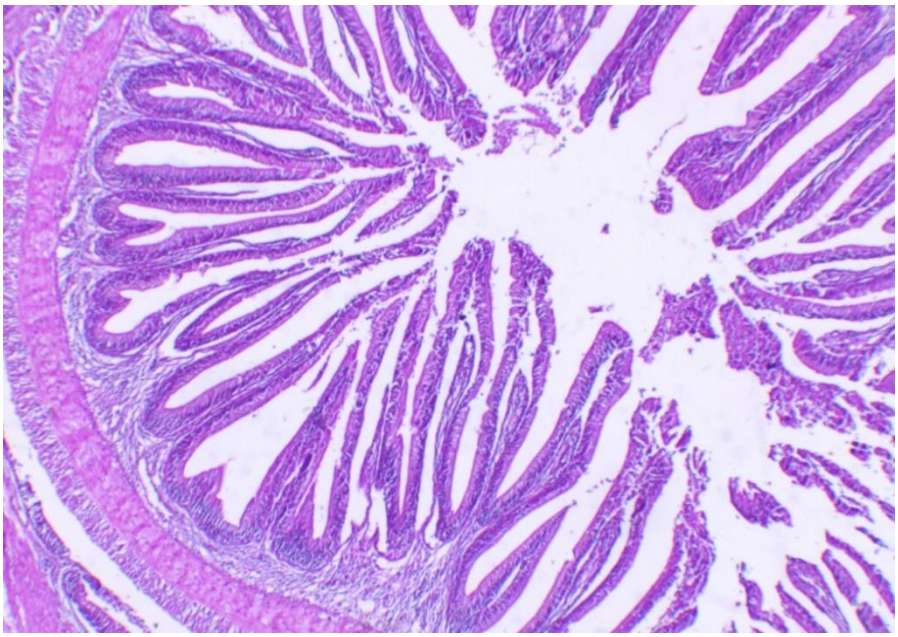

Figure 1: Jejunum of fish supplemented with diet containing fish oil showing normal long villi lined with normal epithelium, (H&E, X200)

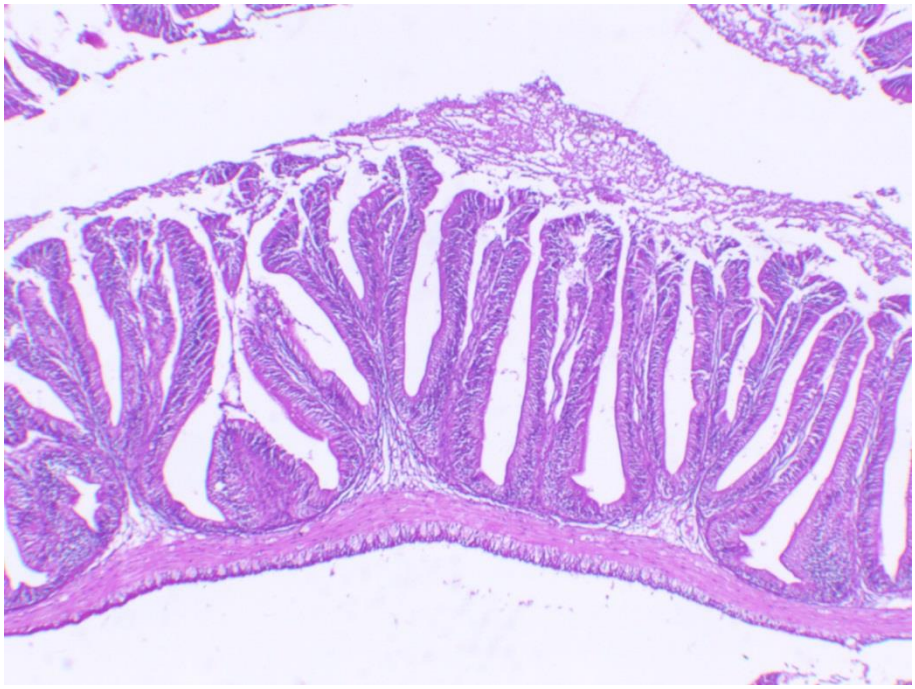

Figure 2: Jejunum of fish supplemented with diet containing vegetable oil showing normal villi, (H&E, X200)

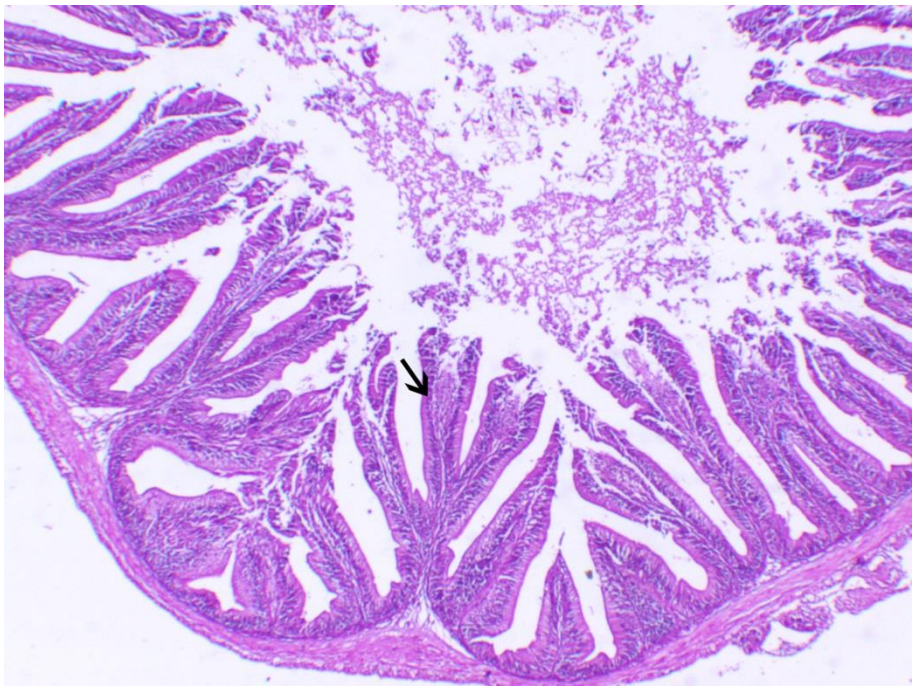

Figure 3: Jejunum of fish supplemented with diet containing a mix of fish and soybean oils showing most of villi length was similar to group 1 with increase branches (arrow), (H&E,X200)

Histopathological analysis

Increasing ratio of omega 6 to omega 3 FA from R1 to R9 decreased length of jejunum and ilium villi. Feeding R3 improved the absorptive capacity of intestinal villi as similar to the group fed R1. Jejunum of fish fed R1 showing normal long villi lined with normal epithelium (Fig. 1). Jejunum of fish fed R9 showing normal villi but

was shorter than the other groups (Fig. 2). Jejunum of fish fed R3 showing most of villi length was similar to fish fed R1 with increase branches and width (Fig. 3). The effect of dietary treatments on duodenum histopathology was not significant (Table 6).

Discussion

The result of the present study revealed that the fish fed a diet containing a blend of omega 6 and omega 3 FA (R3) had similar BWG to the group fed more omega 3 FA (R1) but higher than that fed omega 6 FA (R9). Similarly, (15) found that the final weight was lower when increasing dietary omega 6 FA by using 6% soy oil in fish diet for 6 weeks. Also, a recent study denoted that increasing omega 6 to omega 3 FA ratio for 8 weeks depressed growth of tilapia (16). (17) who reported that a moderate increase of omega 6 FA by using 4.5% SO in replacement of FO in hybrid tilapia diets for 10 weeks, found that all groups had similar final weight. Also, (18) investigated a moderate omega 6 to omega 3 ratio in red hybrid tilapia diets for 5 months and detected no significant difference in either the growth performance or feed efficiency. Some studies have designated that both omega-3 and omega-6 FAs are indispensable for tilapia (2). These results implied that the presence of moderate dietary levels of omega 6 FA may spare a part of the requirement for omega 3 FA series.

This study revealed that feeding R1 reduced body fat content. This might be due to the high phospholipid content of fish oil than the soy oil. Lipid rich in omega 3 PUFA is reported to impede the fat synthesis and to reduce the deposition of these FAs in the liver and whole body of fish (19). With regard to phospholipids, (20) found that it decreased total lipids of whole body of rainbow trout as compared to a mixture of olive and linseed oils, also, (21) stated that omega 3 FA decreased lipid concentration in rainbow trout than the omega 6 FA. On the other hand, feeding R1 increased mineral deposition in the skeleton. The relation between type of FA and mineral metabolism in fish is less investigated. In some studies on European sea bass, (22) demonstrated that EPA and DHA of marine origin supported vertebral and cephalic growth with less deformities. In terrestrial animals, phospholipid facilitated cartilage mineralization and stimulated insulin like growth factor production which activate bone formation and matrix production. On the other hand, prostaglandins E2 derived from arachidonic acid is a powerful stimulator of bone resorption (23).

These results clarify that feeding R1 improved general health condition of Nile tilapia as indicated by increasing HB concentration, PCV and RBCs count which positively affected the growth performance. Blood cells reflect dietary changes due to their fast renovation (24). Feeding omega 3 FA increases DHA incorporation in cell membrane of erythrocytes which maintains osmotic pressure and nutrient transport across the cell membrane. On the other hand, feeding omega 6 FA to channel catfish increased C18 PUFA and decreased LC-PUFA which appeared to increase erythrocyte fragility (25). In another study on salmon, vegetable oils (blend of rapeseed and linseed oils), which are rich in omega 6 FA, decreased erythrocyte counts and hematocrit (26).

Fish fed more omega 3 FA or a mixture of omega 6 and omega 3 FA (R3) had high lymphocyte and low heterophil count than those fed omega 6 FA (R9). However, total count of WBCs was high in fish fed R1 than the other groups. These results differed from the previous reports which documented that omega 3 PUFA inhibit lymphocyte proliferation (27).

The current results revealed that feeding R1 or R3 enhanced liver function as indicated by lowering serum ALT and AST levels than fish fed R9. This may be due to the associative influence of dietary lipid types on the histological structure of the liver (28). Increasing omega 6 FA in gilt head sea bream diets increased fat accumulation in hepatocyte (29) which subsequently reduced hepatocyte activity leading to metabolic imbalance. Omega 6 FA had a lipogenic effect and lowered oxidation capacity of fish (30). More certainly, feeding omega 6 FA to Atlantic salmon increased molecular expression of adipophilin in the liver (31) which is a marker for lipid accumulation.

Altering the ratio of omega 6 to omega 3 FA modified immune status (32). Our result of serum antibacterial test indicated that the group fed R1 had significant enhancement of antibacterial activity of fish over the other groups. The use of a moderate omega 6 to omega 3 FA ratio did not alter the health conditions (33).

Results of the histopathology confirmed the beneficial effect of partial replacement of omega 3 with omega 6 FA on absorptive capacity of jejunum villi. Increasing ratio of omega 6 to omega 3 FA from R1 to R9 had a harmful effect on the absorptive capacity of the intestine, subsequently it decreased the growth performance of Nile tilapia fish. To our knowledge, there was scarce information on the effect of altering omega 6 to omega 3 FA ratio on the absorptive capacity of the intestine. Fish oil is rich in omega 3 FA which maintain integrity of cell membrane of the enterocytes which might show the higher length of the jejunum villi than soy oil.

Conclusion

Increasing the ratio of omega 6 to omega 3 FA from R1 to R9 decreased growth performance and feed utilization of the Nile tilapia. Also, it cleared that feeding more omega 3 and less omega 6 FA was very essential for blood and liver cells. Feeding R9 weakened immune response against bacterial infection and decreased absorptive capacity of jejunum villi. However, it could feed R3 without adverse effect on growth performance, feed utilization and absorptive capacity of the intestine.

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Conflict of interest

There is no conflict of interest.

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