EFFECT OF DIFFERENT LEVELS OF DIGESTAMIN SUPPLEMENTATION ON PERFORMANCE OF NILE TILAPIA (*OREOCHROMIS NILOTICUS*) FINGERLINGES.

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(Received 30/4/2011, Accepted 18/5/2011)

SUMMARY

The present study was performed to evaluate the response of Nile tilapia, *Oreochromis niloticus* to supplemental dietary digestamin with respect to growth performance, feed utilization, body composition and some blood parameters. Digestamin was supplemented at different levels (0.0, 0.05, 0.10, 0.15 and 0.20 % on DM basis) and experimental diets were isonitrogenous and isocaloric (29.38 % CP and 4.55 kcal/GE/g in average). A total number of 150 Nile tilapia fingerlings with average initial body weight 5.93g/fish were randomly divided to five experimental groups and stocked into 15 glass aquaria (70 liter each). Potential groups were disturbed into triplicate aquaria and fed a rate of 5, 4 and 3% of the body weight per a day through 12 weeks (1-4, 5-8 and 9-12 weeks, respectively) as experimental periods depending on the increase in BW gain. Results indicated that, Growth performance parameters included the final body weight, weight gain, daily gain, specific growth rate and feed utilization criteria increased significantly ($P<0.05$) by digestamin supplementation, compared with the control one. In addition, the same trend of significant effect ($P<0.05$) was detected for the food conversion ratio, protein efficiency ratio in fish groups receiving supplemented digestamin diets compared with those fed the control diet. Concerning the influence of digestamin on the proximate composition of carcass, no significant differences ($p>0.05$) were observed among different fish groups in carcass moisture, crude protein, lipid, ash and energy content. Results showed that, no significant differences ($p>0.05$) were noticed among different experimental fish groups in plasma glucose, total lipids, Aspartate aminotransferase and alanine aminotransferase. Plasma total protein was increased significantly ($P<0.05$) by digestamin supplementation. In conclusion, the addition of digestamin in the diets, at levels 0.10, 0.15 and 0.20%, improves and enhances the growth performance of Nile tilapia, *Oreochromis niloticus*, fingerlings

***Keywords**: feed additives; Nile Tilapia; Oreochromis niloticus; Digestamin, growth parameters.*

INTRODUCTION

More than half of world's population depends on fish as a principal source of animal protein (Corpei, 2001). In Egypt, production from cultured tilapia had increased from 9000 ton in1980 to 1000000 ton in 2009 (FAO, 2009). To sustain the high rates of increase in aquaculture production, there should be a matching increase in the levels of production of fish feed. Feed additives have gained remarkable public interest and importance by improving the immunity, productivity and economic efficiency of fish via its improvement in body weight of the fish (Carnevali et al., 2006), weight gain (Venkat, 2004), feed conversion ratio and efficiency (Abdel Hamid and Mohamed, 2008).

In recent years, there has been great interest in the use of lactic acid bacteria and their metabolic products as potential probiotics in aquaculture (Ringb and Gatesoupe, 1998 and Gatesoupe, 1999), to improve population growth in rotifer cultures (Planas et al., 2003), their nutritional value for turbot larvae (Gatesoupe, 1991). They have also been used in the disinfection treatment of *Artemia auplii* (Gatesoupe, 2002), as growth promoters of *Oreochromis niloticus* (Lara-Flores et al., 2003) and in the increase of the immunological response of turbot (Villamil et al., 2003a,b) or of rainbow trout (Nikoskelainen et al., 2003).

Digestamin one of natural feed additives which consists of 1. lactic acid fermented soybean, 2. lactic acid fermented, dried grass from natural pastures rich in herbs, 3. dried horse radish roots, 4. lactic acid

fermented oak bark shavings. Digestamin is produced by controlled lactic acid fermentation with natural lactic acid bacteria by using highly sophisticated drying-techniques. The high quality dietary fiber from the fermented grass contained in digestamin is very important for the digestive hygiene of the animals and very helpful for young animals to build up an optimal working digestive system. Soya, used as a fermentation substrate, is converted by the fermentation and additionally different metabolites, like short chain peptides, which are known to enhance mucosal immunity and thereby the general health, are produced. The horseradish also contained in digestamin has a positive influence on the immune- and digestive system, and it is also a very effective agent against respiratory diseases (Piro, 2000) of animals.

As far as we are aware, there are no studies relating digestamin and fish nutrition. Therefore, it was the purpose of the present research to investigate the influences of digestamin supplementation on fish growth and performance. Specifically, we were interested in determining if the consumption of a digestamin-supplemented diet at different levels (0.0, 0.05, 0.10, 0.15 and 0.20 % on DM basis) would affect growth performance, feed utilization, body composition and some blood parameters of Nile tilapia (*Oreochromis niloticus*) fingerlings .

MATERIALS AND METHODS

This study has been carried out at the Wet Fish Laboratory, Department of Animal Production, Faculty of Agriculture, Kafrelsheikh University, Egypt.

Experimental fish:

Nile tilapia, *Oreochromis niloticus* fingerlings were brought from a fresh water commercial farm in Motobas, Kafr El-Sheikh governorate, Egypt. Prior to the start of the experiment, fingerlings were placed in a fiberglass tank and randomly distributed into glass aquaria to be adapted to the experimental condition until starting the experiment. Fish were fed on the unsupplemented (control) diet for two weeks, during this period healthy fish at the same weight were replaced by died ones. All the experimental supplementations were conduced under an artificial photo period equal to natural light/darkness period (12h light: 12h darkness).

Experimental Diets:

Five diets were formulated to contain digestamin for tilapia fingerlings: one a control diet without supplements; a second to fifth supplemented with digestamin at different levels (0.05, 0.10, 0.15 and 0.20 % on DM basis), respectively. The basal and the other diets were formulated from the commercial feed ingredients. The dry ingredients were grounded through a feed grinder to very small size (0.15 mm). Digestamin was obtained from Produktionsgemeinschaft F.U.H. Egger GmbH Company, Austria. Experimental diets were formulated (Table, 1) to be isonitrogenous and isocaloric (about 29.38% crude protein and about 454.38 kcal GE/100g diet. The ingredients were weighted and mixed by a dough mixer for 20 minutes to homogeneity of the ingredients. The estimated amount of oil components (sunflower oil) was gradually added (few drops gradually) and the mixing operation was continued for 20 minutes. The diets were pelleted through fodder machine and the pellets were dried under room temperature. The diets were collected, and stored in plastic bags in refrigerator at 4 °C during the experimental period to avoid the deterioration of nutrients.

Experimental design of rearing fish:

A total of 150 Nile tilapia, *Oreochromis niloticus* fingerlings with an average initial body weight about 5.93g were randomly divided into five supplemental groups and stocked into 15 glass aquaria (70 liter each). Three aquaria were assigned for each supplement.

Fresh tap water was stored in fiberglass tanks for 24h under aeration for dechlorination. One third of all aquaria were replaced daily. Five air stones were used for aerating the aquaria water. Fish feces and feed residues were removed daily by siphoning. Fish from each replicate were weighted at the start of each experiment and counted and weighted every two weeks throughout the experimental period (12 weeks).

Potential groups were disturbed into triplicate aquaria and fed a rate of 5, 4 and 3% of the body weight per a day through 12 weeks (1-4, 5-8 and 9-12 weeks, respectively) as experimental periods depending on the increase in BW gain. The feed amount was given three times daily (900, 1200 and 1500 h) in equal proportions, six days a week for 12 weeks. Fish were weighed biweekly and feed amounts were adjusted on the basis of the new weight.

Table (1): Composition and proximate analysis of the experimental diets.

Item	Diet[5] No (On DM basis, %)				
	D1 Control	D2	D3	D4	D5
Feed ingredients					
Herring fish meal, 72% CP	12	12	12	12	12
Soybean meal, 44% CP	32	32	32	32	32
Yellow corn	36	35.95	35.90	35.85	35.80
Wheat bran	15	15	15	15	15
Sunflower oil	3	3	3	3	3
Vitamins and minerals premix[1]	2	2	2	2	2
Digestamin[2,3]	0	0.05	0.10	0.15	0.20
Total	100	100	100	100	100
Chemical composition %					
Dry matter	91.02	91.11	91.13	91.14	91.14
Crude protein	29.34	29.36	29.37	29.40	29.44
Ether extract	5.21	5.20	5.22	5.24	5.22
Crude fiber	4.21	4.22	4.20	4.21	4.24
Total ash	4.54	4.52	4.51	4.48	4.50
Nitrogen free extract	56.70	56.70	56.70	56.67	56.60
Calculated energy value					
GE (kcal/kg)[4]	4543	4543	4545	4548	4545
DE (kcal/kg)[5]	3407	3407	3409	3411	3409
P/E,mg/kcal[6]	86.12	86.18	86.15	86.19	86.36

[1]Vitamins and minerals premix at 2 % of the diet supplies the following per kg of the diet: 75000 IU Vit.A; 9000 IU Vit. D3 ; 150 mg Vit. E ; 30 mg Vit. K3 ; 26.7 mg Vit. B1; 30 mg Vit. B2; 24.7 mg Vit. B 6 ; 75 mg Vit.B12; 225 mg Nicotinic acid ; 69 mg Pantothenic acid ; 7.5 mg Folic acid; 150 mg vit. C; 150 mg Biotien; 500 mg Choline chlorid 300 mg DL-methionine; 93 mg Fe; 11.25 mg Cu; 210 mg Zn; 204 mg Mn; 5 mg Se and Co 5 mg (Local market).

[2]Digestamin supplemental diets: D1 (control): 0%, D2: 0.05% ,D3: 0.10%, D4: 0.15% and D5: 0.2% .

[3]Chemical composition of Digestamin: 88% dry matter, 33% crude protein, 2% ether extract, 12% crude fibre, 7% total ash, 44 % nitrogen free extract , 0.50% methionine, and 2% lysine.

[4]GE (Gross energy) was calculated according to NRC (1993) by using factors of 5.65, 9.45 and 4.22 K cal per gram of protein, lipid and carbohydrate, respectively .

[5]DE (Digestible energy) was calculated by applying the coefficient of 0.75 to convert gross energy to digestible energy according to Hepher et al., (1983).

[6]P/E (protein energy ratio) = crude protein x 10000 / digestible energy, according to Hepher et al., (1983).

Chemical analysis:

Proximate chemical analyses were made for diet ingredients and a sample of fish at the beginning and end of the experiment according to standard methods (A.O.A.C., 1992) for dry matter, crude protein, ether extract, crude fiber and ash. Gross energy (GE) contents of the experimental diets and fish samples were calculated by using factors of 5.65, 9.45 and 4.22 kcal/g of protein, lipid and carbohydrates, respectively (NRC, 1993) .

Measurements of water parameters:

Water samples were taken each two days for ammonia and pH analysis. Analytical methods were done according to the American Public Health Association (APHA, 1985). The pH values were determined by (A digital pH-meter). Water temperature and oxygen level were measured daily at 8 o'clock by (Oxygen meter model 9070). In all treatments water quality parameters for water temperature ranged between 27 to 28.50°C, pH (7.3 to 8.2); dissolved oxygen (5.80 to 6.32 mg/L) and water ammonia (0.07 to 0.11 mg/L). All the water quality parameters were within the acceptable ranges for fish growth (Boyd, 1984).

Blood parameters:

Blood samples were collected at the end of experiment, fish in each aquarium were weighted and 5 fish were taken randomly for blood sampling. The blood was collected using heparinized syringes from the caudal vein. Blood samples were centrifuged at 4000 rpm for 20 minutes to allow separation of

plasma which was subjected to determine plasma total protein (Tietz, 1990). Blood plasma total lipids were determined according to the method of McGowan *et al.* (1983). Glucose concentration was determined according to Trinder (1969). Alanine aminotransferase (ALT) and activity of aspartate aminotransferase (AST) were determined by the methods of Young (1990).

Statistical analysis:

The obtained numerical data were statistically analyzed using SPSS (1997) for one-way analysis of variance. When F-test was significant, least significant difference was calculated according to Duncan (1955).

RESULTS AND DISCUSSION

Chemical composition of diets:

Experimental diets (Table, 1) contained nearly similar levels of DM, CP, EE, CF, Ash, NFE, GE, DE and P/E ratio. The CP and GE content of experimental diets were around 29.38 % and 4.55 kcal/g, respectively. These values were within the range recommended for tilapia by Jauncey and Ross (1982) and NRC (1993).

Growth performance and survival rate:

Data in Table (2) show the growth performance and nutrient efficiencies of Nile tilapia fingerlings fed diets containing digestamin. Results showed that, average initial live body weight of tilapia among the different experimental treatments ranged between 5.80 and 5.93 g without significant differences among the different experimental groups, referred to the accuracy of randomization process between the experimental groups.

Table (2): Growth performance parameters of Nile tilapia (*O. niloticus*) fed on the experimental diets.

Item	Diets No (On DM basis, %)					SE*
	D_1,Control	D_2	D_3	D_4	D_5	
Initial weight, g/fish	5.90	5.93	5.85	5.80	5.90	0.06
Final weight, g/fish	30.00^b	32.05^b	37.54^a	38.35^a	38.62^a	0.68
Average total gain[1], g/fish	24.10^b	26.12^b	31.69^a	32.55^a	32.72^a	1.23
Average daily gain[2], g/fish/day	0.29^b	0.31^b	0.38^a	0.39^a	0.39^a	0.02
Specific growth rate[3] (SGR % /day)	1.94^b	2.01^b	2.21^a	2.25^a	2.24^a	0.09
Survival rate[4], %	90	95	95	100	100	2.22
Feed intake (FI), g/fish	39.62	41.10	42.21	42.82	43.17	1.97
Feed conversion ratio[5] (FCR)	1.64^a	1.57^a	1.33^b	1.32^b	1.32^b	0.13
Protein efficiency ratio[6] (PER)	2.07^b	2.16^b	2.56^a	2.59^a	2.58^a	0.19
Protein productive value[7] (PPV, %)	31.83^b	34.51^b	41.71^a	43.30^a	43.67^a	2.14
Energy retention[8] (ER, %)	20.10^b	21.37^b	25.07^a	25.57^a	25.58^a	0.87

**Means in the same rows having different superscript letters were significantly different at 0.05 level.*

** Standard error of the mean derived from the analysis of variance.*

1. ATG (g/fish) =Average final weight (g) – Average initial weight (g).

2. ADG (g/fish/day) = [ATG (g)/experimental period (d)].

3. SGR (%/day) = 100(Ln final weight–Ln initial weight)/experimental period (d).

4. SR =100[Total No of fish at the end of the experimental/Total No of fish at the start of the experiment].

5. FCR = DM Feed Intake (g)/Live weight gain (g).

6. PER = Live weight gain (g)/ Protein intake (g).

7. PPV (%) =100 [Final fish body protein (g)–Initial fish body protein (g)]/crude protein intake (g).

8. ER % = 100 [gross energy gain / gross energy intake]

Although, the pervious results of initial live body weight of tilapia, significant differences (P<0.05) were noticed between examined fish groups for all the estimated growth parameters (gain, ADG and SGR). Fingerlings fed supplemented diets with digestamin had higher increases of gain, ADG, and SGR compared to unsupplemented one specifically, with 0.20% digestamin supplementation. Fish fed control diet (D_1) exhibited the lowest final body weight (30.00 g/fish) while, the highest weight detected with D5 group (38.62 g/fish).

Results explain that, feeding supplemental diets with digestamin to fish groups (D_3 to D_5) had significant higher values (P< 0.05) of feed conversion ratio compared with, the control group. The best conversion ratio was achieved by 0.10 to 0.20% digestamin additions (1.32-1.33 feed intake/live weight gain).

Generally, the better feeding efficiency was detected by fish fed supplemental diets with digestamin than those fed the control diet. Digestamin supplementation increased the protein efficiency ratio (PER), protein productive value (PPV %) and energy retention (ER %) significantly (P<0.05) especially, with 0.15 and 0.20% supplementations. The lowest values of PER, PPV and ER were recorded for the control fish (2.07 Live weight gain (g)/ Protein intake (g), 31.83%, and 20.10 % respectively).

Growth performance parameters estimated in present study indicated that, digestamin has a positive effect on growth rates and feed utilization of Nile tilapia fingerlings especially; fish fed diets with 0.10 - 0.20% digestamin (D5). Growth performance of fish could be improved by digestamin fermented materials like soybean meal which agree with Yamamoto et al. (2010) who found that, diet containing fermented soybean meal at 47.6% level attained similar growth performance and nutrient digestibility in rainbow trout to fish fed the fish meal-based diet their diet showed the highest increase (P < 0.05) in growth rate and feed utilization.

A similar trend was found when lactic acid bacteria were used as dietary supplements which have been widely employed to improve the immune response and protect fish from various infections (Verschuere et al., 2000 and Nikoskelainen et al., 2001). On the other hand, Fagbenro and Jauncey (1994) explained that, feeding lactic acid fermented fish silage with soybean meal as a protein source had no adverse effects on the consistency of the diet, diet acceptance, nutrient and energy digestibility, or on the growth, quality and health of juvenile catfish, *Clarias gariepinus*. While, the addition of 0.1% probiotics (bacteria *Streptococcus faecium* and *Lactobacillus acidophilus* and the yeast *Saccharomyces cerevisiae*) in tilapia diets improves animal growth, and mitigates the effects of stress factors but, the yeast was effective in stimulating fish performance (Lara-Flores *et al.,* 2003).

Significantly worst feed conversion (P<0.05) appeared in control fish group fed on control diet (1.64) compared with the other experimental groups, D3 (1.33), D4 (1.32), and D5 (1.32). However, supplementation with digestamin (0.20%) provides the fish culturist to save 0.32 kg feed per kg body weight gain, which gives about 19.51% less than the feed required for the control group. This aspect is of practical importance for the fish culturist both from an ecological and economic point of view. The significant differences (P<0.05) of FCR in the experiment may suggest that, the improved growth was related to feed consumption and better feed utilization efficiency. Therefore, the improved performance of Nile tilapia in the experiment with digestamin supplementation is presumably due to improve moderately the physiological conditions such as billiard conjugated bile salt composition and morphologies of the distal intestine and liver as it was explained by Yamamoto et al. (2010) when rainbow trout fed on fermented soybean meal compared to fish fed the unfermented one.

Body composition:

The influence of different dietary digestamin levels on chemical a proximate analysis of carcass is shown in Table (3). Digestamin had no significant effect (*P*>0.05) on Nile tilapia body composition (dry matter, crude protein, ether extract, ash and energy). However, control fish had lower dry matter, crude protein, and ash contents but, fish fed diet supplemented with 0.20% digestamin had lower ether extract and energy contents.

Enhancing feed intake in supplemented diets with digestamin may have been due to increase fish appetite resulting in a higher feed intake and therefore improved growth.

Biochemical blood parameters:

Results in Table (4) showed that, blood plasma glucose and total lipids, were not significantly affected (*P*>0.05) by the different levels of digestamin. On contrast, total protein was increased significantly (P < 0.05) by digestamin supplementation specifically, by 0.10 - 0.20% level. Although there is a slight decrease in plasma glucose and slight decrease in plasma total lipid. These results

suggested that, fish health was improved when fed digestamin supplemented diets. Moreover, digestamin supplementation had no significant effect ($P>0.05$) on Aspartate aminotransferase (AST) and alanine aminotransferase (ALT) concentrations compared with unsupplemented group. Biochemical analyses often provide vital information for health assessment and management of cultured fish (Cnaani *et al.*, 2004).

Table (3): Effect of digestamin on Nile tilapia body composition (%, on DM basis).

Item	Initial fish	Diets No (On DM basis, %)					SE*
		D_1,Control	D_2	D_3	D_4	D_5	
Dry matter, %	21.42	26.33	26.68	26.88	27.12	27.22	0.21
Crude protein, %	54.10	55.44	56.78	57.99	58.86	59.27	2.12
Ether extract, %	16.35	18.24	18.27	17.68	17.43	16.87	1.11
Ash, %	11.47	12.47	12.57	12.66	12.74	12.58	1.02
Energy, Kcal/100g	537	544	546	544	544	542	4.12

Insignificant differences between means per each item were observed at 0.05 level.

* *Means of the standard error derived from the analysis of variance.*

Table (4): Blood plasma parameters of Nile tilapia fed on the experimental diets.

Item	Diets No (On DM basis, %)					SE*
	D_1,Control	D_2	D_3	D_4	D_5	
Plasma glucose, mg/dl	57.75	57.33	56.68	56.42	55.87	0.36
Plasma total protein, g/dl	4.24^b	4.37^b	4.98^a	5.27^a	5.80^a	0.09
Plasma total lipid, g/dl	4.21	4.09	3.78	3.68	3.61	0.04
AST, U/dl	125	122	121	119	120	2.54
ALT, U/dl	55	50	52	53	57	1.10

**Means in the same rows having different superscript letters were significantly different at 0.05 level.*

* *Standard error of the mean derived from the analysis of variance*

This result agrees with Abdel-Tawwab *et al.*, (2008) who investigated the use of commercial probiotic as a growth and immunity promoter for Nile tilapia, *Oreochromis niloticus;* they found that, biochemical parameters were improved in fish fed probiotic.

CONCLUSION

It can be concluded that, the addition of digestamin in tilapia fingerlings diets improves fish growth and feed utilization. Based on these results, use of 0.10 - 0.20% of digestamin in tilapia fingerlings feeds is recommended as the best level to stimulate growth performance. On the other hand, using digestamin as a promising natural feed additive in fish nutrition need more advanced studies especially, with different conditions of fish husbandry.

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تأثير اضافة مستويات مختلفة من الديجستمين على أداء اصبعيات البلطي النيلي

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أجرى هذا البحث بمعمل بحوث الأسماك بقسم الإنتاج الحيواني بكلية الزراعة – جامعة كفر الشيخ وذلك لبحث تأثير أضافه خمسة مستويات مختلفة من الديجستمين (0 و 0.05 و 0.10 و 15، 0 و 20، 0%) على أداء النمو والاستفادة من الغذاء والعناصر الغذائية والتركيب الكيماوي لجسم الأسماك في نهاية التجربة وكذلك بعض مكونات الدم لأسماك البلطي النيلي . حيث تم تركيب خمس علائق تجريبية متشابهه في نسبه البروتين (29.38%) والطاقة (4.55 ك كالورى/جم مادة جافة) . تم استخدام عدد 150 من أصبعيات البلطي النيلي (بمتوسط وزن ابتدائي 5.93جم/سمكه) وزعت عشوائيا على أربع معاملات في خمسة عشر حوض زجاجي سعة كل منها 70 لتر بواقع ثلاثة أحواض لكل معامله وتم تخزين عشرة اسماك في كل حوض . وكانت معدلات التغذية اليومية تتم بنسبة 5 و 4 و 3 % من الوزن الحي خلال الفترات من الأسبوع الأول إلى الرابع والخامس إلى الثامن والتاسع إلى الثاني عشر على الترتيب تبعا لزيادة الأسماك في الوزن وذلك خلال فترة التجربة وهى أثنى عشر أسبوعا .

أظهرت نتائج التجربة أن أصبعيات البلطي النيلي التي تغذت على العلائق المضاف لها الديجستمين بنسب من 0,1 – 0,2 % من العليقه أعطت أحسن النتائج في مقاييس النمو وكفاءة الاستفادة من الغذاء مقارنة بمجموعة المقارنة. بالإضافة إلى ذلك فإن كفاءة التحويل الغذائي ، كفاءة استخدام البروتين قد تحسنت معنويا في المعاملات المضاف إليها الديجستمين مقارنة بمجموعة الكنترول. بالنسبة لتأثير الديجستمين على التركيب الكيميائي لجسم الأسماك في نهاية التجربة فلم تكن هناك اختلافات معنوية بين المعاملات المختلفة في محتوى الجسم من الرطوبة والبروتين الخام والدهون والرماد وكذلك محتوى الطاقة . كما أظهرت النتائج عدم وجود اختلافات معنوية بين المعاملات المختلفة المضاف إليها الديجستمين ومجموعة المقارنة . ولم يكن هناك اختلافات معنوية بين المعاملات بالنسبة لمحتوى بلازما الدم من الجلوكوز والليبيدات بينما زاد محتوى بلازما الدم من البروتين اضافة الديجستمين. كما ان أضافة الديجستمين لم تؤثر سلبيا على وظائف الكبد لاصبعيات البلطى النيلى .

من النتائج السابقة فإن أضافه الديجستمين في العلائق بمستويات من10، 0 و 20، 0 % تعمل على تحسين أداء النمو والاستفادة من الغذاء لأصبعيات البلطي النيلي تحت الظروف التجريبية الحالية .