J. Agr. Sci. Tech. (2011) Vol. 13: 1045-1051

Growth Performance of Nile Tilapia (*Oreochromis niloticus*) Fed with Diets Containing Different Levels of Hydrolysable and Condensed Tannin

H. M. Buyukcapar[1], A. İ. Atalay[2], and A. Kamalak[2*]

ABSTRACT

This study was undertaken to evaluate the effect of hydrolysable tannin (HT) and condensed tannin (CT) on growth performance and body composition of Nile tilapia. Hydrolysable tannin and CT were included into diets at four levels (0, 5, 15, and 25 g kg^{-1} diet). The diet with zero tannin level acted as control and the response of fish fed diets containing tannin was compared to that of the control diet. All the diets were iso-nitrogenous and iso-energetic. Hydrolysable and condensed tannin had a significant (P<0.05) effect on body weight gain (WG), feed intake (FI), feed conversion ratio (FCR), specific growth rate (SGR) and protein efficiency ratio (PER). Weight gain, SGR and PER of fish fed on the diets containing 15 and 25 g HT/ kg diet were significantly (P< 0.05) lower than those fed on the other diets. Feed conversion ratio of fish fed diets containing 15 and 25 g kg^{-1} HT were significantly (P<0.05) higher than those fed on the other diets. Feed intake of fish fed diets containing 15 and 25 g HT/kg diet were significantly (P< 0.05) lower than those fed on the other diets, except for diet containing 15 g kg^{-1}condensed tannin (CT2). It is concluded that adverse effect of HT is higher on tilapia compared to that of CT and that protein sources of plant origin containing high amounts of tannins, in particular HT, should be used with caution as fish meal substitutes in tilapia diets.

Keywords: Condensed tannin, Growth performance, Hydrolysable tannin, Tilapia.

INTRODUCTION

Research on the inclusion of plant-based ingredients in practical diets for salmonids and omnivorous fish such as channel catfish, tilapia, and carp has shown that partial replacement of fish meal is feasible (Davies *et al*., 1990). However, they have the disadvantage of containing a number of anti-nutritional factors, of which tannins are known to be the most potent (Liener, 1989). Protein digestibility appears to be directly dependent on the tannin content (Makkar, 1993). Tannins may cause a number of different anti-nutritional effects such as reduced feed intake, decreased nutrient digestibility, and reduced growth performance (Jansman, 1993). Tannins usually occur in two groups: hydrolysable tannins (HT) and condensed tannins (CT). Hydrolysable tannins are esters of gallic acid and ellagic acid with core molecules consisting of polyols such as sugars and phenolics as in catechin (Reed, 1995) Hydrolysable tannins are more susceptible to enzymatic and non-enzymatic hydrolysis and, usually, more soluble in water than CT, which is a polymer of flavan-3-ols linked

[1]Department of Fisheries, Faculty of Agriculture, University of Kahramanmaras Sutcu Imam, 46100 Kahramanmaras, Turkey.
[2]Department of Animal Sciences, Faculty of Agriculture, University of Kahramanmaras Sutcu Imam, 46100 Kahramanmaras, Turkey.
*Corresponding author; e-mail: a_kamalak@hotmail.com

through an interfalavan carbon bond that is not susceptible to hydrolysis (Reed, 1995). The effect of tannin on growth of some species of animals has been reported (Makkar and Becker, 1998), but little is known about the effects of tannins on growth of fish (Becker and Makkar, 1999; Pinto *et al*., 2001).

This study was conducted to evaluate growth performance of Nile Tilapia (*O. niloticus*) fed diets with different levels of hydrolysable and condensed tannin.

MATERIALS AND METHODS

Nile tilapia (*O. niloticu*s) fingerlings derived from brood stock held at the University of Cukurova (Adana, Turkey) were transferred to the Department of Fisheries, Faculty of Agriculture, University of Kahramanmaras Sutcu, Imam and were maintained in a 250-L fiberglass tank at 26±0.8^{o}C with a diet containing approximately 350 g kg^{-1} protein, 80 g kg^{-1} lipid, 7 g kg^{-1} ash and energy content of 13.54 MJ (3.249 Kcal) kg^{-1} dry matter until they reached individual mean weight of approximately 16.18±0.03 g. From this population, groups of 10 tilapia fingerlings with similar body weight were selected and randomly allocated to each aquarium with three aquaria for each experimental diet. After 5 days feed acclimatization to the experimental diets, the fingerlings in each aquarium were fed twice daily at a fixed feeding rate of 3% body weight per day for eighty days with seven isonitrogenous and isoenergetic experimental diets. The diets were formulated from fish meal, soyabean meal, maize flour with hydrolysable and condensed tannin at four levels (0, 5, 15, and 25 g kg^{-1}, air-dry basis). Proximate composition and estimated gross energy content of the ingredients that were used to formulate the experimental diets is given in Table 1. The experimental diets were incorporated into the daily diets in powder form. The composition of the experimental diets is given in Table 2. All diets were supplemented with 2 g kg^{-1} methionine and lysine (Yanar *et a*l. 2008).

All ingredients were finely grounded to a fine powder using a laboratory hammer mill and were passed through a 0.25 mm sieve. The sieved ingredients were mixed in a Hobart mixer for 20 minutes and moistened with double distilled water. After passing the moist mixture through a meat mincer to form strands of about 2 mm in diameter, the strands were air-dried in an oven at 45^{o}C. The dry strands were broken into suitable sizes before being fed to the fish.

Water quality was monitored daily throughout the experimental period. Temperature, and dissolved oxygen were recorded using oxythermometer (Hach HQ10, Hach, Loveland, CO, USA) while pH was measured using a pH meter (Hach sensION1, Hach, Loveland, CO, USA).

Table 1. Proximate compositions and estimated gross energy content of the ingredients used in the experimental diets.

Constituents (g kg^{-1})	Fish meal	Soybean meal	Maize flour
Dry matter	900.6	890.0	881.3
Crude protein	701.6	470.0	78.0
Crude fat	113.3	10.0	35.2
Crude ash	135.0	60.0	55.2
Crude fiber	5.0	60.0	26.2
Crude lipid	113.3	10.0	35.0
NFE	0	290.0	729.0
Methionine	21.2	4.5	1.2
Lysine	67.5	27.8	2.8
Gross energy [a], MJ kg^{-1}	21.3	17.5	16.21

[a] Gross energy was estimated using the following coefficients: 23.6 KJ g^{-1} for crude protein, 39.5 KJ g^{-1} for crude fat and 17.2 KJ g^{-1} for carbohydrates (NRC, 1993).

Table 2. Ingredients and compositions of the experimental diets for *O. niloticus* fingerlings (as fed).

	Experimental diets						
		Hydrolysable tannin			Condensed tannin		
Ingredients (g kg^{-1})	Control	HT1	HT2	HT3	CT1	CT2	CT3
Fish meal	250	250	250	250	250	250	250
Soya bean meal	400	400	400	400	400	400	400
Maize flour	250	245	235	225	245	235	225
Sunflower oil	89	89	89	89	89	89	89
Tannin	0	5	15	25	5	15	25
DCP [a]	1	1	1	1	1	1	1
Vit-Min [b]	5	5	5	5	5	5	5
Methionine	2	2	2	2	2	2	2
Lysine	2	2	2	2	2	2	2
Salt	1	1	1	1	1	1	1
Total	1000	1000	1000	1000	1000	1000	1000
Gross energy [c] (MJ kg^{-1})	19.9	19.9	19.7	19.5	19.9	19.7	19.5
Crude protein (g kg^{-1})	382.9	382.5	381.7	381.0	383.1	382.5	381.9
Crude lipid(g kg^{-1})	130.0	129.9	129.5	129.2	129.9	129.5	129.2
NFE(g kg^{-1})[3]	298.3	294.6	287.3	280.0	294.6	287.3	280.0

[a] Di calcium phosphate

[b] Per 5 kg vitamin-mineral premix: 20.000.000 IU vitamin A; 2.00.000 IU vitamin D3, 200.000 mg vitamin E; 12.000 mg vitamin K_3; 20.000 mg vitamin B_1; 30.000 mg vitamin B_2; 200.000 mg niacin; 50.000 mg Ca-panthothenate; 20.000 mg vitamin B_6; 50 mg vitamin B_{12}; 500 mg D-biotin; 1.200 mg folic acid; 200.000 mg vitamin C; 300.000 mg inositol; 1.200.000 mg cholin chloride; 40.000 mg manganese; 30.000 mg Zinc; 800 mg Copper; 1.000 mg iodine; 150 mg Selenium, 40.000 mg magnesium.

[c] Gross energy was estimated using the following coefficients: 23.6 KJ g^{-1} for crude protein, 39.5 KJ g^{-1} for crude fat and 17.2 KJ g^{-1} for carbohydrates (NRC, 1993).

During the experimental period, mean temperature, pH, and dissolved oxygen were 28±0.4°C, 7.03±0.16, and 7.1±0.36 mg l^{-1}, respectively. Water was supplied to the aquaria through a re-circulated system, which comprised a biological filter and an electronic heater. Aeration was provided by an aerator (Resun LP-40 Low Noise Air Pump) and water was passed through the filter at a rate of 5 l min^{-1}.

The fish were individually weighed at the start of the experiment and, thereafter, at 20-day intervals (20, 40, 60 and 80 days). Phenoxyethanol was used to immobilize the fish before handling to reduce stress. At the end of the experiment, five fish per aquarium were minced in a blender and homogenized to determine the whole body proximate composition. The proximate composition of experimental diets and fish samples were analyzed according to the procedures described in AOAC (1990).

Fish performance was evaluated by body weight gain (WG; g), feed conversion ratio (FCR), feed intake per fish (FI; g), specific growth rate (SGR; %) and protein efficiency ratio (PER). These parameters were determined using the following equations:

Body weight gain (WG; g): $WG= [W_F-W_I]$

Where W_F represents final body weight and W_I represents initial body weight;

Feed conversion ratio: FCR= [dry feed consumed (g)/Live body weight gain (g)];

Specific growth rate: SGR= $[(\ln W_F - \ln W_I)/\text{Days}]\times 100$;

Feed intake: F1= [Total feed consumption per aquarium/Number of fish per aquarium];

Protein efficiency ratio: PER= [body weight gain/Protein fed]

One-way analysis of variance (ANOVA) was applied to determine the effects of the diets on growth and whole body composition parameters, using SPSS 10.0 for windows (SPSS, 1999). Significant differences between individual means were evaluated using the Duncan's multiple comparison tests at P< 0.05 (Duncan, 1955).

RESULTS

The effects of hydrolysable and condensed tannins on the growth performance of *O. niloticus* are presented in Table 3. Hydrolysable tannin had a significant effect on W_F, *WG*, FI, and *PER* with fish fed on the diets containing 15 and 25 g kg^{-1} of HT having significantly (P< 0.05) lower values than the control diet (Table 3). The FCR of fish fed diets containing 15 and 25 g kg^{-1} *HT* were significantly (P< 0.05) higher than those fed the control diet. Feed intake with diets containing 15 and 25 g kg^{-1} HT was also significantly (P< 0.05) lower than those fed all other diets, except for diet containing 15 g kg^{-1}condensed tannin (CT2).

The effect of hydrolysable and condensed tannin on the whole body proximate composition of Nile tilapia is presented in Table 4. Both hydrolysable and condensed tannins had no significant effect on whole body dry matter, crude protein, and ash contents of Nile tilapia. But, the body fat of fish fed diets containing 15 and 25 g kg^{-1} HT was significantly lower than that of fish fed on the other diets.

DISCUSSION

Hydrolysable tannin significantly influenced the growth performance of *O. niloticus*. As can be seen in Table 3, inclusion rate of hydrolysable tannin at levels of 5 g kg^{-1} and below had no significant influence on the growth performance of *O. niloticus,* whereas inclusion rates of condensed tannin in diets up to 15 g kg^{-1} had no significant (P< 0.05) effect on the growth performance when compared with the control diet. There was significant reduction in growth performance of fish with increasing dietary tannin levels. Levels of 15 g kg^{-1} inclusion of hydrolysable tannin depressed growth while no growth reductions were observed with condensed tannin until inclusion levels of 25 g kg^{-1}. Although inclusion rate of hydrolysable tannin at levels of 5 g kg^{-1} had no significant influence on the growth performance of *O. niloticus,* Al-Owafeir (1999) observed a significant growth reduction when *O. niloticus* were fed with diets containing 0.27% tannic acid. Mukhopadhyay and Ray (1996) found that incorporation of defatted sal (*Shorea robusta*) seed meal, which contains high amounts of tannins, into diets of *Labeo ruhita* fingerlings reduced growth performance. Becker and Makkar (1999) found that 2 % hydrolysable tannin in the diet reduced the growth of carp. However,

Table 3. The effect of hydrolysable and condensed tannin on the body weight, feed intake, feed conversion ratio, specific growth rate and protein efficiency rate of *O. niloticus* fingerlings (Mean±SE, n= 30).

	Experimental diets						
Parameters	Control	HT1	HT2	HT3	CT1	CT2	CT3
W_I (g)	16.3^a±0.1	16.2^a± 0.1	16.1^a± 0.1	16.3^a±0.1	16.1^a± 0.1	16.1^a± 0.1	16.2^a± 0.1
W_F (g)	36.9^{ab}±0.1	35.6^{ab}±0.5	27.6^c±0.4	27.4^c±0.4	37.0^a±0.2	34.3^{ab}±0.1	34.0^b±0.1
WG (g)	20.7^a± 0.2	19.3^{ab}±0.4	11.6^c±0.4	11.1^c±0.5	20.9^a±0.2	18.2^{ab}±0.3	17.8^{ab}±0.1
FI (g)	37.9^a±0.2	37.2^a±0.3	34.3^b±0.5	35.2^b±0.2	37.7^a±0.5	36.1^{ab}±0.5	37.3^a±0.3
FCR	2.0^a±0.2	2.1^a±0.2	3.1^{cb} ±0.3	3.5^c±0.5	1.9^a±0.2	2.3^{ab}±0.5	2.2^{ab}±0.1
SGR %	1.3^a ±0.16	1.3^a±0.16	0.9^c±0.10	0.9^c±0.19	1.4^a±0.15	1.3^{ab}±0.23	1.2^b±0.10
PER	1.4^a±0.18	1.3^{ab}±0.19	0.9^c±0.15	0.8^c ±0.20	1.4^a±0.17	1.3^b±0.25	1.2^b±0.10

Row means with common superscript do not differ (P> 0.05); W_I: Initial body weight (g); W_F: Final body weight; WG: Body weight gain; *FCR*: Feed conversion ratio; *FI*: Feed intake (g $fish^{-1}$); *SGR*: Specific growth rate, *PER*: Protein efficiency ratio.

Table 4. The effect of hydrolysable and condensed tannin on the body composition of *O. niloticus* fingerlings (% wet weight basis) (Mea ±SE, n= 15).

	Experimental diets						
%	Control	HT1	HT2	HT3	CT1	CT2	CT3
DM[a]	28.9[a]±0.8	29.1[a]±0.8	30.6[a]±0.4	30.1[a]±0.4	28.8[a]±0.4	28.0[a]±0.2	28.8[a]±0.6
Protein	15.2[a]±0.6	14. 9[a]±0.1	14.8[a]±0.2	14.6[a]±0.3	15.5[a]±0.3	15.2[a]±0.2	14.8[a]±0.2
Fat	7.5[a]±0.3	7.7[a]±0.1	5.3[b]±0.2	5.3[b]±0.3	8.0[a]±0.2	7.8[a]±0.1	7.9[a]±0.4
Ash	2.8[a]±0.08	2.8[a]±0.07	2.9[a]±0.03	2.9[a]±0.02	2.8[a]±0.06	2.8[a]±0.04	2.8[a]±0.07

Row means with common superscript do not differ significantly (P> 0.05).
[a] Dry matter.

the condensed tannins were found to be less toxic to common carp, which has been shown to tolerate a 2% additional pure condensed tannin without any adverse effect on growth. Reduction in growth performance could be the result of several factors including reduced nutrient and energy digestibility or energy utilization efficiency (Makkar *et al*., 1987).

The effect of tannin of *Dinorphandra mollis* Benth on the apparent digestibility of diet for Nile tilapia (*O. niloticus*) was evaluated by Pinto *et al.* (2000). The result indicated that tannin levels equal to or more than 0.63 % affected significantly the digestibility of dry matter, crude protein, and ether extract. Moreau *et al*. (2003) also showed that growth performance of Nile tilapia decreased when fish were fed with a diet containing fresh or ensiled coffee pulp due to high tannin content. The anti-nutritional effects of tannins include interference with the digestive process, either by binding the enzymes or by binding to feed components such as protein or minerals (Francis *et al.*, 2001). As can be seen in Table 3, *FI* and *PER* also decreased while *FCR* increased with increasing inclusion levels of hydrolysable and condensed tannin.

It has also reported that tannins create palatability problems due to their astringent taste (Joslyn and Goldstein, 1964). Generally, carp are known to be quite sensitive to adverse taste (Becker and Makkar, 1999), based on visual observation of the behavior of *O. niloticus* fingerlings during feeding time. The adverse effects of tannins and their toxicity have generally been attributed to binding of tannins to nutrients and enzymes, thereby decreasing the availability of nutrients and reducing the capacity of the enzymes for digestion. (Becker and Makkar, 1999). Hydrolysable tannins are easily degraded in the biological systems by non-specific esterases, with the hydrolyzed products entering into blood and causing organ toxicity. (Garg *et al*., 1992; Muller-Harvey and McAllan, 1992). Condensed tannins are complex and the larger molecules that are not easily hydrolyzed in biological systems (Becker and Makkar, 1999; Terril *et al*., 1994). Condensed tannin does not appear to bind to proteins or other nutrients to decrease their availability in the tilapia as is evidenced from the similarity in feed intake and growth parameters between condensed tannin diets and the control diet (Table 3).

Diets containing higher level of HT (HT2, HT3) produced the significantly lowest body fat values (Table 4). This finding is in agreement with Yurkowski *et al*. (1978) and Hossain and Jauncey (1989) who observed similar reductions in body fat of rainbow trout and carp, respectively, when these fish fed diets containing rapeseed meal and mustards oil cake that contained tannin and glucosinolates. The low body fat content may be attributed to decrease in digestibility coefficient of the diet lipid fraction due to enzymatic decrease by tannin.

It is concluded from this study that adverse effect of HT is higher in tilapia than that of CT and that plant protein sources containing tannins, and in particular HT, should be used with caution as fish meal substitutes in diets for *O. niloticus*.

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تاثیر رژیم خوراکی دارای مقادیر مختلف تانن محلول و تانن متراکم بر رشد ماهی تیلاپیا(*Oreochromis niloticus*)

ح. م. بیوک کاپار، ا. ی. آتالای و ا. کمالک

چکیده

این تحقیق به منظور ارزیابی تاثیر تانن محلول(HT) و متراکم(CT) روی رشد ماهی تیلاپیا (*Oreochromis niloticus*) و ترکیب مواد دراندام آن اجرا شد. این دو نوع تانن در مقادیر مختلف (صفر، ۵، ۱۰، ۱۵، و ۲۵ گرم در هر کیلو گرم خوراک ماهی) به خوراک معمول آنها اضافه شد و تاثیر تانن بر رشد ماهی مزبور با ماهی های شاهد(تیمار بدون تانن) مقایسه شد. همه تیمار های خوراکی از لحاظ نیتروژن و انرژی همسان بودند. بر اساس نتایج تحقیق، تاثیر هر دو نوع تانن بر افزایش وزن بدن ماهی (WG)، مقدار خورده شده خوراک (FI)، نسبت تبدیل خوراک (FCR)، نرخ رشد ویژه (SGR)، و نسبت کارآیی پروتئین، در سطح($P< 0.05$) معنی دار بود. افزایش وزن SGR ، و PER ماهی هایی که رژیم خوراکی آنها دارای **۱۵** و 25 g kg^{-1}تانن بود به طور معنی داری($P< 0.05$) کمتر از ماهی هایی بود که رژیم خوراکی دیگری داشتند. نسبت تبدیل خوراک ماهی هایی که خوراک آنها ۱۵ یا ۲۵ g kg^{-1} تانن محلول داشت به طور معنی داری($P< 0.05$) بیشتر از آنهایی بود که رژیم خوراکی دیگری داشتند. خوراک خورده شده ماهی هایی با رژیم **۱۵** و25 g kg^{-1} تانن محلول به طور معنی داری($P< 0.05$) کمتر از دیگر ماهی ها بود ، به استثنای ماهی هایی که رژیم خوراکی آنهاg kg^{-1} ۱۵ تانن متراکم داشت. بنا براین نتیجه گیری می شود که اثرهای منفی تانن محلول روی **تیلاپیا** (*Oreochromis niloticus*)از تانن متراکم بیشتر است. نیز باید در استفاده از منابع پروتئینی گیاهی که دارای مقادیر زیادی تانن، به ویژه تانن محلول، هستند به عنوان جایگزین خوراک ماهی برای ماهی های مزبور احتیاط کرد.