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IMPROVEMENT OF GROWTH PERFORMANCE IN TILAPIA *(OREOCHROMIS AUREUS* LINNAEUS) BY SUPPLEMENTATION OF RED CLOVER *(TRIFOLIUM PRATENSE)* IN DIETS

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Key words: growth, *Oreochromis aureus*, red clover, tilapia, *Trifolium pratense*

Abstract

This study was conducted to investigate the effects of red clover on growth, body composition, and survival of tilapia, *Oreochromis aureus*. Three concentrations of dietary red clover (50, 100, 200 mg/kg) were tested for 90 days. The growth rate was significantly better in fish fed 100 mg red clover/kg feed than in the control that contained no red clover ($p<0.01$). The specific growth rate ranged from 3.79±0.10 in the 200 mg/kg red clover treatment to 4.41±0.05 in the 100 mg/kg treatment. The food conversion ratio, protein efficiency ratio, and apparent net protein utilization were significantly highest in groups fed the 100 mg red clover/kg diet ($p<0.01$). Survival in groups fed red clover did not differ from that of the control. The highest protein content (19.74%) was obtained in the 100 mg/kg group and it was significantly higher than in all other groups ($p<0.05$). Moisture and ash contents did not differ among treatments. Lipid content was not significantly affected by red clover dosage. The findings of the present study suggest that 100 mg red clover/kg feed improves the growth rate and feed utilization of tilapia.

Introduction

The increasing world-wide importance of cultured tilapia as a food fish has prompted considerable research on improvement of growth performance in tilapia. Most studies have concentrated on the development of sex control techniques. For example, all-male populations have been established by using hormonal sex reversal and manual sexing or species hybridization. Fish growth can also be stimulated by hormones. Steroid hormones as well as growth hormones encourage growth (Donaldson et al., 1979; Higgs et al., 1982;

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Turan and Akyurt, 2003). Steroids are eliminated from the fish and have no harmful effects (Tave, 1992). Although hormone treatments on fish have no negative effects, they sometimes generate marketing problems. Hence, investigators are searching for alternative applications.

Phytoestrogens are plant substances with varying degrees of estrogenic activity and may or may not be structurally similar to gonadal estradiol-17β. There are three main groups of phytoestrogens: isoflavones and lignans, coumestans, and resorcylic acid lactones. Studies generally deal with isoflavones and lignans (Aldercreutz et al., 1986; Lotke, 1998). Natural isoflavones are widely distributed in the Leguminosae family and are plant chemicals with estrogenic activity.

Red clover (*Trifolium pratense* L.) is a legume and an important forage plant grown around the world. Red clover is a rich source of isoflavones, containing high levels of isoflavone compounds such as genistein, which have estrogenic properties (Lotke, 1998). Red clover was used as a medicinal herb by indigenous natives of North America to treat coughs, asthma, bronchitis, and cancer (Leung and Foster, 1996; Rijke et al., 2001). Extracts of red clover are commercially available as isoflavone enriched dietary supplements in the USA and Europe for women suffering menopausal complaints. Red clover has also been used to promote growth in sheep. Moorby et al. (2004) reported that lambs fed red clover had significantly increased plasma concentrations of growth hormone and the insulin-like growth factor (IGH)-I. Turan and Akyurt (2005a) found that administration of red clover significantly increased growth and improved protein and lipid levels in African catfish (*Clarias gariepinus*).

The positive effects of isoflavone-rich plants in humans and some animals led us to seek possible benefits to other important cultured fish species. The main objective of this study was to investigate the effects of red clover on growth, body composition, and survival in tilapia (*Oreochromis aureus*).

Materials and Methods

Fish with an average body weight of 0.31±0.02 g were obtained from the Mustafa Kemal University Aquaculture Research Unit. Fish were randomly stocked into 50-l aquaria at a density of 15 fish per aquarium.

A commercial carp diet (28% protein, 12% lipid, 4% cellulose, 16% ash, wet basis; Akuamak, Turkey) was used to prepare the experimental diets. Red clover powder (Menoflavon®) was supplied by Melbrosin (Vienna, Austria). The powdered red clover extract was mixed with the pulverized carp diet, water (450 ml/kg) was added, and the mixture was extruded through a food grinder (Lee et al., 2004). Three dosages of red clover were tested (50, 100, and 200 mg/kg feed). The control diet was mixed with 450 ml water but contained no red clover powder. The extrusions were broken into small pieces and stored in a freezer until feeding. Each diet was randomly assigned to three groups of fish. Each group was fed their respective diet twice daily, at a rate of 4% of their body weight per day, for 90 days. Aquaria were aerated, supplied with continuously flowing water (2 l/min), and kept at 26±1°C. The photoperiod was maintained at 12 h light:12 h dark.

Fish in each aquarium were counted and individually weighed twice a month after anesthetizing the fish with 300 mg/l lidocaine-HCL/1000 mg/l $NaHCO_3$ (Park et al., 1988). Weight gain (Watanabe et al., 1990), food conversion ratio (Steffens, 1989), specific growth rate (Clark et al., 1990), protein efficiency ratio (Steffens, 1989), apparent net protein utilization (Bender and Miller, 1953), and survival rate (Watanabe et al., 1990) were calculated.

In the beginning of the experiment, five fish from each treatment (n = 20 fish) were killed by an overdose of lidocaine-HCL/1000 mg/l $NaHCO_3$ solution and stored at -20°C for determination of proximate body composition. At the end of the feeding trial, five fish from each triplicate (n = 60 fish) were analyzed for final whole body proximate composition. Standard methods (AOAC, 1990) were used to determine the initial and final whole body proximate composition.

All data were subjected to a one-way analysis of variance to determine significant differences in weight gain and body composition among treatments. Duncan test was used to compare treatment means when significant differences were found (Norusis, 1993).

Results

The effects of dietary red clover concentration on growth and survival of tilapia for 90 days are shown in Table 1. Survival ranged from 95.55% in the control to 97.78% in the 100 and 200 mg/kg treatments and did not significantly differ among treatments. The growth rate was significantly highest in fish fed 100 mg/kg (p<0.01). The specific growth rate significantly differed among all treatments (p<0.01) and was highest in the 100 mg/kg group. The food conversion ratio and protein efficiency ratio were also significantly highest in this treatment (p<0.01), as was the apparent net protein utilization (p<0.001).

The body composition of the fish is given in Table 2. Moisture and ash contents did not differ among treatments. The highest protein content (19.74%) was obtained in the 100 mg/kg treatment and was significantly higher than that of the other treatments (p<0.05). The lipid contents were not significantly affected by the red clover dosage and, on the contrary, dramatically decreased after the 100 mg/kg supplementation level.

Discussion

To our knowledge, this is the first report regarding the potential of red clover as a growth-promoting agent in tilapia culture. The present findings indicate that supplementation of red clover at a level of 100 mg/kg feed increases the growth rate of tilapia. The 100 mg/kg treatment resulted in a significantly higher growth rate than the 50 and 200 mg/kg supplementation levels. The growth rate dramatically decreased beyond 100 mg/kg, indi-

Table 1. Effects of dietary red clover concentration on growth performance, feed utilization efficiencies, and survival in tilapia (*Oreochromis aureus*) after 90 days (means of triplicates±SE).

	Red clover (mg/kg feed)			
	0	*50*	*100*	*200*
Initial body wt (g)	0.30±0.01^{a}	0.30±0.01^{a}	0.30±0.01^{a}	0.30±0.01^{a}
Final body wt (g)	12.37±0.63^{b}	11.97±0.55^{b}	15.74±1.03^{c}	9.35±0.69^{a}
Wt gain (g)	12.07±0.63^{b}	11.67±0.55^{b}	15.44±1.03^{c}	9.05±0.69^{a}
SGR[1]	4.14±0.05^{b}	4.08±0.07^{b}	4.41±0.05^{c}	3.79±0.10^{a}
FCR[2]	1.52±0.08^{b}	1.58±0.07^{b}	1.19±0.05^{a}	2.05±0.16^{c}
PER[3]	2.35±0.12^{b}	2.27±0.11^{b}	3.0±0.12^{c}	1.76±0.13^{a}
ANPU (%)[4]	41.49±1.10^{b}	40.68±0.52^{b}	60.51±3.43^{c}	32.38±2.12^{a}
Survival (%)	95.55±2.22^{a}	97.77±2.21^{a}	97.78±2.22^{a}	97.78±2.21^{a}

Values in a row with different superscripts indicate significant differences at a level of p<0.01 (p<0.001 for ANPU).

1 Specific growth rate = [(lnW^{2}-lnW^{1})\(T^{2}-T^{1})] x 100, where W^{1} and W^{2} are mean body weights at times T^{1} and T^{2} when samples were taken.

2 Food conversion ratio = dry feed intake in g/wet wt gain in g.

3 Protein efficiency ratio = live body wt gain in g/protein intake in g.

4 Apparent net protein utilization = (protein retained/protein intake) x 100.

Table 2. The effects of feeding supplementary dietary red clover on the chemical body composition of tilapia, *Oreochromis aureus*, after 90 days.

Chemical composition (% wet wt)	*Initial*	*Red clover (mg/kg feed)*			
		0	*50*	*100*	*200*
Moisture	74.03±0.30	75.15±0.40[a]	74.50±0.31[a]	73.73±0.27[a]	75.33±0.79[a]
Crude protein	16.23±0.46	17.30±0.62[a]	17.52±0.56[a]	19.74±0.41[b]	17.85±0.59[a]
Crude lipid	7.11±0.61	7.71±0.37[b]	7.68±0.49[b]	8.94±0.30[b]	6.12±0.39[a]
Ash	0.99±0.01	0.99±0.01[a]	0.98±0.01[a]	0.99±0.01[a]	0.98±0.01[a]

Values in a row with different superscripts indicate significant differences at a level of $p<0.05$.

cating that the higher concentration (200 mg red clover/kg feed) had a negative effect on tilapia growth. This study also showed that red clover treatment generally enhances nutrient utilization, as reflected by the improvements in weight gain, food conversion ratio, specific growth rate, protein efficiency ratio, and apparent net protein utilization.

Turan and Akyurt (2005a) used pelleted diets to assess the relative growth promoting efficiency of feed supplemented with red clover on African catfish, *Clarias gariepinus*. In their study, red clover increased the growth rate to a significant level over the control. It may be that the presence of phytoestrogen (isoflavones) in red clover stimulates growth hormones in fish. Isoflavones are plant chemicals with estrogenic activity. Studies have shown that exogenous administration of estrogen promotes the growth in yellow perch *Perca flavescens* (Malison et al., 1988), goldfish (Trudeau et al., 1992), and African catfish (Turan and Akyurt, 2005b). The results of the current study suggest that phytoestrogens have a similar effect on tilapia growth. Further, Moorby et al., 2004 reported that lambs fed red clover had significantly increased plasma concentrations of growth hormones and the insulin-like growth factor (IGH)-I, suggesting a physiological mechanism for the increased growth rates of these animals.

The survival rate, though not significantly different, was higher in groups fed red clover than in the control. Similarly, a higher survival rate among red clover treated fish was previously reported by Turan and Akyurt (2005a) who found that mortality did not occur to catfish treated with dietary red clover for 120 days.

From a chemical composition point of view, the 100 mg/kg dose of red clover increased the level of crude protein in tilapia but did not significantly affect the lipid content. A similar finding was reported in catfish where 75 mg/kg increased the protein level (Turan and Akyurt, 2005a). Moorby et al. (2004) reported that a high protein content and possible growth promoting properties made red clover highly attractive as a natural means of increasing the growth rate and crude protein level in lamb production.

In conclusion, the present findings suggest that red clover inclusion at 100 mg/kg feed can improve feed utilization in fish, resulting in a higher growth rate. Since the dietary red clover treatment significantly increased the protein level in tilapia, it can be used as a natural alternative to synthetic hormones. Further studies are required to investigate plasma metabolite and hormone concentrations in fish and determine the effects of red clover on other culturable fish species.

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