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Dietary Red Clover (*Trifolium pratense*) on Growth Performance of Common Carp (*Cyprinus carpio*)

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Abstract: The effects of dietary red clover on growth and body composition were investigated in common carp *Cyprinus carpio*. The fish (mean body weight 2.01±0.03 g) were fed with diets supplemented with three concentrations (50, 100 and 200 mg kg^{-1}) of red clover and a control diet without red clover, for 12 weeks. The fish fed with diet supplemented with 100 mg kg^{-1} red clover exhibited significantly higher growth rates than fish fed with diets supplemented with 50 and 200 mg kg^{-1} red clover ($p<0.001$). The highest values of specific growth rate and food conversion ratio were 2.35±0.07 and 1.23±0.01, respectively at 100 mg kg^{-1} red clover diet group. Protein efficiency ratio and apparent net protein utilization were significantly higher in 100 mg kg^{-1} red clover group than that with other groups ($p<0.001$). However, significant differences were not observed in carcass moisture and proximate protein, lipid and ash contents among the all diet groups ($p>0.05$). Survival rate was higher (88%) in all dietary groups. The findings of the present study suggest that 100 mg kg^{-1} red clover inclusion improves growth rate, feed utilization and survival of common carp.

Key words: *Trifolium pratense*, red clover, common carp, *Cyprinus carpio*, growth

INTRODUCTION

Common carp *Cyprinus carpio* L. is undoubtedly one of the most widely cultured and domesticated fish in the world, especially in Asia and Central and Eastern Europe. This species is mostly reared in ponds under extensive or semi-intensive management. Most of the studies on carp culture have concentrated on the development of sex control techniques with varied applied objectives. The potential advantages of these applications can include elimination of reproduction, reduction of sexual or territorial behavior and risk of environmental impact resulting from escapes of exotic species, but mainly achievement of higher average growth rate and reduction of variation in harvest size (Cherfas *et al*., 1996; Beardmore *et al*., 2001). Steroid hormones as well as growth hormones encourage increased growth (Donaldson *et al*., 1979; Higgs *et al*., 1982; Turan and Akyurt, 2003). The steroid hormones are eliminated from the fish and have no harmful effects (Tave, 1992). Although the hormone treatments on fish have no negative affect it sometimes generates problems in marketing. Furthermore, synthetic hormones have been reported to have the potential to accumulate in the sediment water and aquatic biota (Contreras-Sanchez *et al*., 2001), which lead investigators to find possible alternative applications.

In the last decade, some studies show the positive effects of dietary medicinal herbs on growth and the immune response of different fishes (Kwon *et al*., 1999; Lee *et al*., 2004). Red clover (*Trifolium pratense* L.) was originally used as a medicinal herb by native indigenous people of North America in the treatment of cough, asthma, bronchitis, coughs, cancer (Leung and Foster, 1996; Rijke *et al*., 2001). Extracts of red clover are commercially available as isoflavone enriched dietary supplements on the US and European markets for women suffering under menopausal complaints. There have been growing bodies of literature on the use of isoflavone in various subjects (Gambacciani *et al*., 1994; Lampe *et al*., 1994). Dornstaudera *et al*. (2001) reported that an average isoflovane content of Red Clover (RC) was found to be approximately 9% (dry weight) determined by HPLS. Phytoestrogens has also been used to promote growth in poultry (Jurani *et al*., 1987). Turan and Akyurt (2005) found that administration of red clover significantly increased the growth and improved the level of protein and lipid in African catfish (*Clarias*

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gariepinus). Moreover, Turan (2006) found that administration of Red clover extract in tilapia (*Oreochromis aureus*) improved growth rate and feed utilization. Therefore, we studied the possibility of using red clover in carp culture in terms of growth performance, carcass proximate and survival rate.

MATERIALS AND METHODS

Herb and test diets: Red clover powder (Menoflavon®) was obtained from Melbrosin (Vienna, Austria). Carp diets (Aquamak, Turkey) were used to prepare experimental diets. The crude protein, lipid content of the control diet was 28 and 12% on wet weight basis, respectively. Proximate composition of the experimental diets is determined by analysis (AOAC, 1990). The red clover was supplemented into this diet at the levels of 0, 50, 100 and 200 mg to 1 kg of basal diet. In the preparation of experimental diet, powder red clover extract were mixed with a pulverized carp diet in which, water (450 mL kg^{-1}) were added and extruded through a food grinder with a 2-mm diameter die plate (Lee *et al*., 2004). The control diet was also mixed with 450 mL water. The extrusions were broken into small pieces and stored in freezer until feeding.

Fish and feeding trial: Common carps with mean body weight 2.01±0.03 were obtained from the Water Management Institute, Adana, Turkey. Groups of 15 fish were transferred into 60 L aquaria after acclimating to rearing conditions using the control diet for two weeks. The rearing trial was performed in triplicate for each diet. The fish were fed the diets twice a day (10:00 and 15:00 h) until ca. 4% body weight for 12 weeks. A photoperiod of 12 h light and 12 h dark (06:00-18:00 h) was used. The aquaria were equipped with aeration and supplied with continuously flowing water (2 L min^{-1}) and controlled temperature (26±1°C).

Assays: At the end of the rearing trial, body weights were measured in each aquarium. Growth performance parameters including weight gain (Watanabe *et al*., 1990), Specific growth rate (Clark *et al*., 1990), Food conversion ratio (Steffens, 1989), Protein efficiency ratio (Steffens, 1989), Apparent net protein utilization (Bender and Miller, 1953) and Survival rate (Watanabe *et al*., 1990) were analyzed. These indicators were calculated as:

WG (Weight Gain) (g) = Final weight (g) -Initial weight (g).
SGRW (Specific Growth Rate Weight) (%) = $[(\ln W_2-\ln W_1)/(T_2-T_1)] \times 100$

Where W_1 and W_2 are mean body weight at times when the first and second samples were taken (T_1 and T_2).
FCR (Food Conversion Ratio) = Dry feed intake (g)/wet weight gain (g).
PER (Protein Efficiency Ratio) = Live body weight gained (g)/protein intake (g).
Survival (%) = 100(initial fish number - dead fish number)/ initial fish number.
ANPU (Apparent Net Protein Utilization) (%) = [protein retained/unit of protein intake]× 100.

At the start of experiment, 5 fish from each group (N = 20 fish) were treated with an overdose of lidocaine- HCL\1000 mg\L $NaHCO_3$ solution and stored at -20°C for the determination of body proximate composition. At the end of the feeding trial, 5 fish from each dose group (n = 15 fish\per dose) were analyzed for final whole body proximate composition. Standard methods (AOAC, 1990) as described for the experimental diets were used to determine the initial and final whole body proximate composition.

Statistical analysis: In the experiment, all data were subjected to a one-way analysis of variance to determine if there is a difference in weight gain and body composition among treatments. Duncan test was used to compare the means of the treatments when differences occurred (Norusis, 1993).

RESULTS

Growth performance and survival rate of common carp fed different concentrations of dietary red clover for 12 weeks are shown in Table 1. Survival rate was higher than 88% in all dietary groups. Growth rate significantly increased in fish fed with red clover-supplemented diets in comparison with the control groups ($p<0.001$). Weight gain and Specific Growth Rate (SGR) of 100 mg kg^{-1} red clover diet supplemented group was significantly higher than for control and other groups (Table 1). These results

Table 1: Growth performance, feed utilization efficiencies and survival rate of common carp *C.carpio* fed diets containing different concentrations red clover for 12 weeks*

	Red clover (mg kg^{-1})			
	0	50	100	200
Weight gain (g)	12.08±0.10 ^{ab}	12.33±0.36^{b}	14.60±0.20^{c}	11.33±0.27^{a}
SGR	2.17±0.05^{ab}	2.19±0.03^{b}	2.35±0.07^{c}	2.10±0.02^{a}
FCR	1.49±0.01^{b}	1.46±0.04^{b}	1.23±0.01^{a}	1.59±0.03^{c}
PER	2.40±0.02^{ab}	2.45±0.07^{b}	2.90±0.03^{c}	2.25±0.05^{a}
ANPU (%)	52.06±3.95^{ab}	52.03±4.03^{ab}	63.08±3.33^{b}	50.30±2.03^{a}
Survival (%)	88.89±2.22^{a}	93.33±2.10^{a}	93.33±3.85^{a}	88.90±2.22^{a}

*Values (mean±S.E. of triplicate) with different superscripts in each line indicate significant differences ($p<0.001$) (Water temperature 26±1°C)

Table 2: The chemical composition of the whole-body common carp *C.carpio* fed diets containing different concentrations red clover for 12 weeks*

Chemical composition		Red clover (mg kg^{-1})			
(%)	Initial	0	50	100	200
Moisture	74.74±0.30	73.19±0.42^a	73.41±0.35^a	71.75±0.21^a	73.11±0.41^a
Crude protein	17.85±1.31	18.62±1,29^a	18.25±1.01^a	19.16±0.81^a	19.01±0.81^a
Crude lipid	6.42±0.60	7.21±0.75^a	7.36±0.84^a	8.10±0.32^a	6.90±0.53^a
Ash	0.99±0.01	0.98±0.01^a	0.98±0.01^a	0.99±0.01^a	0.98±0.01^a

*Values (mean±S.E. of triplicate) with different superscripts in each line indicate significant differences ($p<0.05$). Body composition data presented on a wet basis

indicated that the increase in the Red Clover (RC) dosage to some extent in diets enhance weight gains in common carp.

Food Conversion Ratio (FCR), Protein Efficiency Ratio (PER) and Apparent Net Protein Utilization (ANPU) were significantly higher in 100 mg kg^{-1} RC diet group than that with control group ($p<0.001$, Table 1). Also, 100 mg kg^{-1} RC diet supplemented group exhibited significantly higher feed utilization efficiency than those supplemented with 50 and 200 mg kg^{-1} RC ($p<0.001$).

The chemical composition of the whole-body Common carp *C. carpio* fed diets containing different concentrations red clover for 12 weeks are shown in Table 2. Among the diet groups, significant differences were not observed in carcass moisture and proximate protein, lipid and ash contents (Table 2).

DISCUSSION

The results of the present work have clearly demonstrated that dietary red clover promoted the growth performance of common carp, *Cyprinus carpio*. This is a first report to our knowledge regarding the potential of red clover as a growth-promoting agent in carp culture. The 100 mg RC^{-1} kg diets induced better effects on the fish than the control and other diets. Furthermore, these results showed that red clover treatment generally enhances nutrient utilization which is reflected by improvement in weight gain, FCR, PER and SGR. Red clover extract in diets promoted growth and feed efficiency in African catfish, *Clarias gariepinus* (Turan and Akyurt, 2005) and Tilapia, *Oreochromis aureus* (Turan, 2006). A similar optimum level for a commercially available medical herb 'Gokshura' *Tribulus terrestris* has been applied to Guppy, *Poecilia reticulata,* (Çek *et al*., 2007a), Convict Cichlid, *Cichlasoma nigrofasciatum* (Çek *et al*., 2007b) and African catfish, *Clarias gariepinus* (Turan and Çek, 2007). Kim *et al*. (1998) suggested that unknown factors in various medicinal herbs led to favorable results in fish trials. Plants can contain substances that mimic estrogenic effects in vertebrates. Phytoestrogens are defined by their hormonal activity in vertebrates, whereas their actual function in the plant is quite different, like herbivore defense and protection against radicals or reactive oxygen forms. The major phytoestrogens are diphenolic compounds with structural similarities to natural or synthetic estrogens or antiestrogens. They have been categorized according to their chemical structures in isoflavones, lignans and coumestans (Ibarreta *et al*., 2001). One of the first reported signs of the estrogenic effects of phytoestrogens, the ''clover disease'', was produced by isoflavones. Sheep in Australia during the 40's had fertility problems that were finally linked to the clover in their diet, which was a source of high concentrations of isoflavones (Bennetts *et al*., 1946). Moreover, Jurani *et al*. (1987) found that phytoestrogens has also been used to promote growth in poultry. These findings may indicate that the presence of phytoestrogen (isoflavones) in the red clover stimulate growth in fish. In addition, isoflavones are plant chemicals with estrogenic activity and studies have shown that estrogen promotes the growth in common carp (Kocour *et al*., 2005). Therefore, the red clover that could promote growth performance in common carp may become a valuable and effective agent to induce effective technical and economical propagations in fish. The survival rate was found to be high (88%) in all dietary groups. The high survival rate at red clover treatment in Tilapia and African catfish was also previously reported (Turan, 2006; Turan and Akyurt, 2005).

CONCLUSION

In conclusion, dietary red clover, which fortify growth performance and feed utilization of cultured carp, are useful and reliable method for propagating seedling production and rearing strategy. Therefore, future research should focus on the improvement of rearing technology for different fish by red clover.

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