Kufa Journal for Veterinary Medical Sciences Vol.(8). No.(1) 2017

Kufa Journal for Veterinary Medical Sciences

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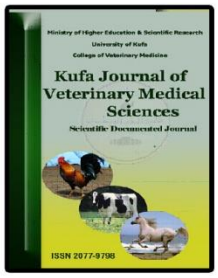

Efficiency of Dietary Turmeric on Growth Performance, Hematology and Survival Rate in Common Carp *Cyprinus carpio* Challenged with *Flexibacter columnaris*

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Abstract

The present study was carried out to assess the effect of turmeric (*curcoma longa*) supplemented diet on growth performance, hematology and survival rate of *Cyprinus carpio* L. against the pathogenic bacteria *Flexibacter columnaris*. For this purpose, 180 common carp were used, 60 fish were used to determine LD_{50} and 120 fish were weighed and randomly distributed into six treatments (two replications for each treatment). Fish groups were fed six dissimilar diets up to 45 days, the first group was fed with 0.25% turmeric, the second group was fed supplemented with 0.5% turmeric, the third group was supplemented with 0.75% turmeric, the forth group was fed with 1% turmeric, the fifth group was supplemented with 1.25 % turmeric and the sixth group was served as the control group fed basal diet without supplementing with turmeric. After 45 days of feeding trail all fish in experimental groups were challenged intramuscularly with 0.2 ml *F. columnaris* at a concentration of 120 × 10^6 CFU/ml. after 45 day of feeding trail and post challenge the different parameters were determined including: growth performance, hematological parameters (WBC and RBC count, Hb content and PCV value) and survival rate were also determined. The obtained results showed that T5 was the best treatment followed by T4, T3, T2 and T1 respectively, which revealed a significant increase ($P \leq 0.05$) in comparison with the control group in all growth performance parameters (final weight, daily gain, SGR(%), relative growth rate, FCR and FCE%). RBC, HB and PCV% after 45 and 60 days (pre and post challenged with *F. columnaris*) revealed significant differences ($P \leq 0.05$) in all experimental groups relative to the control group. In conclusion, our findings indicate that turmeric powder has improved the growth rate in *C. carpio* when challenged with *F. columnaris.* Also, addition of turmeric t into commercial feeds increased survival rate of carp fish challenged with a bacterial pathogen. This will be worth and useful for application in fish aquaculture.

Keywords: turmeric; *Flexibacter columnaris*, Cyprinus carpio, survival rate, hematology

1. Introduction

Fish farming is the sub-set of aquaculture that focuses on rearing of fish under controlled or semi-controlled conditions for economic and social benefits (Kinsey and Darin, 2006). In recent years, fish are protected from infectious diseases using vaccination or antimicrobial agent. But, due to widespread use of the these agents, the increase of antimicrobial resistance among pathogens and the related of environmental threats have been well documented. Hence, several substitutes strategies such as using immune-stimulants have been recommended. The use of immune-stimulants and feed additives could suppress fish pathogens and several harmful environmental conditions (Sakai, 1999). Feed additives are substances that included in diet in trace amounts in order to supply a pathway by which such deficiencies of diet can be addressed which advantage not only the nutrition and the growth of the animal enhanced (Kiron, 2012). In this regard, turmeric (*Curuma longa*) is a perennial herb which is a member of the ginger family. It is a native of South East India and cultivated throughout tropical Asia, India and China. The plant grows to a height of 0.9 to 1.5 m, bears large oblong leaves and funnel –shaped, dull yellow flowers. It is a thick rhizome, which is yellowish on the outside and deep orange or reddish brown yellow coloring than the bulb. The dried primary bulb and secondary lateral rhizomes are collected, cleaned, boiled and dried for use in medicinal and food preparations (Soni *et al.,* 1997).

Turmeric is used as additives due to its active ingredient *Curcumin* (Louis, 2009). The curcuminoids, yellowish pigments present in turmeric powder have shown protective effects against Agri-Food and Sciences Institutes (AFBI) (Soni *et al,* 1997. The significant biological properties of turmeric powder make it a potential feed additive in livestock diets. A number of studies have been conducted to evaluate its effects on the performance of broiler chickens, laying hens and fishes (Emadi and Kermanshahi 2008; Sahu *et al*., 2008 and Mahmoud *et al*., 2014), however, the results have not been consistent. Hence, this study was designed to evaluate the effects of turmeric powder on growth performance, hematology and survival rate of common carp challenged with *F. columnaris*.

Materials And Methods

Preparation of diet

The raw materials were grinded by food grinder and weighed . Then mixed well after that *Curcumin* was added with different doses (0.25% , 0.5%, 0.75%, 1%, 1.25%) and mixed well and converted into paste. These paste was extruded through food mixer and dried at 40^{o}C, pellets were stored at -20^{o}C.

2.1 Experimental design

Healthy fish of *C. carpio* weight ranged between 96.25- 96.75 g used in this experiment were obtained from a commercial farm (Al-Mahaweel, Babylon). Fish were acclimated for two weeks before starting the experiment and they were fed on commercial diet at 3% body mass. After that, fish were stocked in two bath trough (150 × 80 × 50 cm). Then 100 fish were randomly selected and divided into 12 tanks trough at rate of 10 fish for each tank (two replicates /treatment) Fish groups were fed six dissimilar diets up to 45 days, the first group was fed with 0.25% turmeric , the second group was fed supplemented with 0.5% turmeric, the third group was supplemented with 0.75% turmeric, the forth group was

fed with 1% turmeric, the fifth group was supplemented with 1.25 % turmeric and the sixth group was served as the control group fed basal diet without supplementing with turmeric. Tanks filled with chlorine free tap water. Chemo-physical parameters of water such as temperature during the experiment, ranged between 24 -26 ˚C, pH of the water in the tanks ranged between 6.72- 7.13 and the concentration of DO was 6.25 -6.49 mg/l for the duration of the experiment. Temperature of water was measured using a thermometer, pH and dissolved oxygen (DO) were measured using pH meter and digital DO respectively, feeding rate about 3% body weight twice daily for 45 days. Every day tanks was partially cleaned and water was partially changed. Fish weighed every two weeks intervals to determine growth performance and survival rate.

2.2 Bacterial Strain and Challenge Study

Isolation of *F. columnaris* bacteria were taken from College of Veterinary Medicine/Baghdad University. Experimental fish were challenged with *A. hydrophila* by injection fish intramuscular with 0.2ml 120×10^6 CFU/ml after 45 days of the experimental period. Mortality was recorded daily up to 14 days and relative percentage survival were calculated.

2.3 Growth performances

Growth weight: It was calculated every two weak throughout the experimental trail

Body weight gain: Weigh gain = Fish weigh at first day of experiment - weigh at 45 days of experiment (Schmalhusen, 1926).

Daily gain (D.G): It was calculated according to Schmalhusen (1926) via the following equation**:**

$$D.G = \frac{WT-Wt}{T-t}$$

WT = final body weight Wt= initial body weight (T-t) = time

Relative growth ratio (RGR): was calculated via the following equation (Uten, 1978)**:**

$$RGR = \frac{\text{Final body weight} - \text{Initial body weight})}{\text{Initial body weight}} \times 100$$

Specific growth rate SGR (%): It was calculated via the following equation (Brown, 1957).

$$SGR(\%) = \frac{Ln\,W2 - Ln\,W1)}{T} \times 100$$

Ln W1 = Ln of Initial weight of fish
Ln W2 = Ln of final weight of fish
T = Days of experiment (45 days).

Feed Conversion Ratio (FCR): It was estimated via the following equation (Uten, 1978).

$$FCR = \frac{\text{Total feed consumed by fish (g)}}{\text{Total weight gain by fish (g)}}$$

Food Conversion Efficiency (FCE): It was estimated via the following equation (Uten, 1978)**.**

$$FCE = \frac{\text{Total weight gain by fish (g)}}{\text{Total feed consumed by fish (g)}} \times 100$$

Relative Percentage Survival

RPS calculated using mortality number according to Amend (1981).

RPS=1- [(The percentage of mortality in treated group) x 100]

(The percentage of mortality in control group)

2.4 Hematological Parameters

Four individual blood samples were collected from the caudal vessels from each group pre 45 days of the experiment and post challenge test. Blood samples were collected to determine the RBC count, Hematocrit and Hemoglobin content. All the techniques that have been used previously explained by Al-Dohail *et al*. (2009)

2.6 Statistical Analysis

The Statistical Analysis System- SAS (2012) program was used to show the effect of different treatments in study

parameters. Least significant difference –LSD test and ANOVA were used to show the significant differences and compare between means in this study.

Results and Discussion

3.1 Growth parameters and nutritional performance

The mean body weight data on of *C. carpio* are presented in table (1). The body weight of all groups at one day of experimental ranged 100.21-100.75 g and there were no statistical differences between them ($P > 0.05$). The valuable result of turmeric diet was detected throughout the first 15 day. After 15, 30 and 45 days all treatment groups showed significant differences ($P \leq 0.05$) between them. However, at 45 day of the trial time, the growth rate revealed the practicality of a clear growth for all treatments comparison with the control group. T5 showed the greatest value (160.63 g) followed by T4, T3, T2 and T1 (155.29, 151.11, 144.73 and 140.66 g) respectively.

Table (1) Effect of turmeric on body weight in different groups of *C. carpio.*

Groups	Mean ± SE of weight (g)			
	1 Day	15 Day	30 Day	45 Day
Control	100.21 ±0.17 A	105.11 ±0.06 D	115.10 ±0.05 D	130.50 ±0.28 E
T1	100.37 ±0.18 A	105.15 ±0.08 C	118.50 ±0.28 D	140.66 ±0.38 E
T2	100.75 ±0.43 A	107.50 ±0.29 B	120.75 ±0.43 C	144.73 ±0.42 D
T3	100.43 ±0.25 A	110.40 ±0.23 B	120.85 ±0.49 C	151.11 ±0.06 C
T4	100.50 ±0.29 A	110.55 ±0.32 B	125.50 ±0.29 B	155.29 ±0.17 B
T5	100.35 ±0.20 A	112.30 ±0.17 A	130.10 ±0.05 A	160.63 ±0.37 A
LSD value	0.833 NS	0.664 *	0.975 *	0.953 *

* ($P<0.05$).

Data are mean ± SE groups with the different alphabetic letters within the same column indicate significant different at $P<0.05$; (n=4).

Table (2). Effect of turmeric on growth performance (DGR, SGR%, RGR% and BWG) in treatment groups of *C. carpio* fed different concentrations of turmeric for 45 days

Groups	Mean ± SE			
	DGR (g)	SGR%	RGR%	BWG (g)
Control	0.67 ± 0.04 b	0.60 ± 0.04 b	30.17 ± 1.74 d	30.25 ± 1.57 e
T1	0.89 ± 0.06 ab	0.75 ± 0.04 ab	40.16 ± 2.57 cd	40.31 ± 2.63 d

T2	0.97 ± 0.06 ab	0.80 ± 0.06 ab	43.65 ± 2.52 bc	43.98 ± 2.07 cd
T3	1.12 ± 0.08 ab	0.91 ± 0.08 ab	50.46 ± 2.94 abc	50.68 ± 2.55 bc
T4	1.21 ± 0.08 a	0.95 ± 0.09 ab	54.50 ± 2.47 ab	54.79 ± 2.74 ab
T5	1.33 ± 0.11 a	1.06 ± 0.09 a	60.04 ± 3.07 a	60.26 ± 2.81 a
LSD value	0.402 *	0.369 *	11.72 *	9.86 *

* (P<0.05).
Data are mean ± SE groups with the different alphabetic letters within the same column indicate significant different at P<0.05 ; (n=4).

Table (3) Effect of turmeric on growth performance (FCR, FCE and TFI) in treatment groups of *C. carpio* fed different concentrations of turmeric for 45 days.

Groups	Mean ± SE		
	FCR	FCE	TFI
Control	3.17 ± 0.16 a	31.51 ± 1.67 e	96.00 ± 3.68 a
T1	3.40 ± 0.39 a	41.53 ± 1.94 d	97.05 ± 3.82 a
T2	2.24 ± 0.18 ab	44.57 ± 1.83 cd	98.55 ± 4.26 a
T3	1.96 ± 0.16 b	50.96 ± 2.14 bc	99.45 ± 4.63 a
T4	1.84 ± 0.11 b	54.35 ± 2.52 ab	100.80 ± 5.15 a
T5	1.70 ± 0.09 b	58.64 ± 2.33 a	102.75 ± 5.67 a
LSD value	0.882 *	7.803 *	8.519 NS

* (P<0.05).
Data are mean ± SE groups with the different alphabetic letters within the same column indicate significant different at P<0.05 ; (n=4).

There were greater improvements in the feed conversion ratio (FCR) of fish fed on dietary turmeric than the control fish. Also, the highest value of FCE was observed in T5 followed by T4, T3, T2 and T1 respectively, and all these groups showed significant differences (P<0.05) compared to control (that means all treatment group supplemented turmeric diets improved feed utilization of common carp compared with control group) (Table 3).

The enhanced growth response indicated by turmeric supplementation in this study may be due to improved feed consumption, improved feed utilization, which is an indication of increased nutrient digestibility and antioxidant activity of turmeric (Osawa *et al.,* 1995) that stimulates protein synthesis by enzymatic system. This is in agreement with Pransin,(2006) who

reported that goldfish fed turmeric supplemented diets, had highest acid protease, alkaline protease and lipase activity, enhanced growth rate and yellow pigmentation. Prasad and Aggarwal (2011) reported that turmeric is a digestive stimulant and it favorably enhanced the activities of digestive enzymes such as lipase, chymotrypsin, and amylase. The obtained results are in line with those obtained by Sahu *et al*. (2008), who reported that TP-supplemented diets significantly improved growth performance of *rohu*, *Labeo rohit*a, and their optimal growth was obtained at 1.0 g TP/kg diet. Mahmoud *et al*. (2014) concluded that 0.5% turmeric supplementation improved growth performance of Nile tilapia.

In fact, the present results are in agreement with (Kumar,2005) who observed asignificant ($P< 0.05$) increase in weight gain in broiler fed turmeric (1%) over those of control group. On the contrary, Namagirilakshmi (2005) stated that broiler fed with turmeric (0.25,0.50,0.75 and 1%) levels did not significantly affect body weight gain. The result of the weight gain is in line with the result reported by Lawhavinit *et al*. (2011) who observed that ethanolic turmeric extracts could improve weight gain when supplemented in white shrimp diet at 15 g/kg. On the other hand , Emadi and Kermanshahi (2008) showed that was no enhancement in growth performance of broiler after feeding 0.25% ,0.50% and 0.75% of dietary turmeric. But in other poultry studies 0.5% dietary turmeric can improved growth performance (Singh *et al*., 2010). While Mahmoud *et al*. (2014) was disapproved with these result because he noticed Turmeric supplementation non-significantly improved the measured growth and feed utilization parameters in tilapia especially in the higher supplementation level (0.50%). The result of the FCR is in contrast with that of Lawhavinit *et al*. (2011) who reported that there was a significant difference ($P<0.05$) in feed conversion ratio in shrimp fed ethanolic extract of turmeric.

Table (4) Survival rate and mortality rate in treatment groups of *C. carpio* fed different concentrations of turmeric for 45 days and challenged with *F. columanris*

Treatment groups	**Total number of fish**	**Survival rate %**		**Mortality rate %**	
		45 day	**60 day**	45 day	60 day
C	20	70%	60%	30%	40%
T1	20	75%	65%	25%	35%
T2	20	80%	75%	20%	25%

T3	20	80%	75%	20%	25%
T4	20	85%	80%	15%	20%
T5	20	90%	85%	10%	15%
LSD value	---	8.92 *	10.37 *	7.62 *	7.09 *

The results of survival rate were in agreement with Amany Diab *et al.*, (2014) who recorded that the mortality rate in *Oreochromis niloticus* curcumin-treated groups (with 1% and 2% curcumin in diet) after challenging with *Pseudomonas fluorescens* were decreased than in control group. They concluded that curcumin in diets of *Oreochromis niloticus* improve growth performance and immunity which increases the resistance of challenged fish *to Pseudomonas fluorescens*. Also, Sahu *et al.*, (2008) detected 100% and 89% survivability in *Labeo rohita* groups fed with 5.0 and 1.0 g tumeric/kg feed for 60 days, respectively after challenge with *Aeromonas hydrophila*

3.2 Haematological parameters

The results of hematological parameters are illustrated in table 5 and table 6. RBCs count showed a significant improvement ($P \leq 0.05$) in turmeric fed group of common carp (T1, T2 , T3 ,T4 and T5) in comparison with the control group at 45 days (before challenging) and 60 days (after challenging). The highest mean value of RBC was observed in T5 on 45th day ($2.75\times 10^6 mm^3$) compared with T1, T2, T3 and T4 ($2.05\times 10^6 mm^3$, $2.15\times 10^6 mm^3$, $2.53\times 10^6 mm^3$, $2.68 \times 10^6 mm^3$ respectively) and the control group ($1.89\times 10^6 mm^3$) RBC numbers showed a significantly increase ($P\leq 0.05$) in treated group post challenge (day 60) than the pre-challenge, the maximum increase was observed in T5 ($2.27\times 10^6 mm^3$), followed by treatment T1,T2,T3 and T4 ($1.79\times 10^6 mm^3$, $1.86\times 10^6 mm^3$, $2.05\times 10^6 mm^3$, $2.17 \times 10^6 mm^3$ respectively), while in the control group ($1.86\times 10^6 mm^3$).

Table (5) The Effect of turmeric on RBC count in treatment groups *C. carpio* fed different concentration of turmeric for 45 days and challenged with *F. columanris.*

Groups	Mean ± SE of RBC × $10^6 mm^3$	
	45 Day	60 day
Control	1.89 ± 0.03 e	1.68 ± 0.01 f
T1	2.05 ± 0.03 d	1.79 ± 0.01 e
T2	2.15 ± 0.03 c	1.86 ± 0.02 d

T3	2.53 ± 0.02 b	2.05 ± 0.03 c
T4	2.68 ± 0.01 a	2.17 ± 0.01 b
T5	2.75 ± 0.03 a	2.27 ± 0.01 a
LSD value	0.077 *	0.053 *

* (P<0.05).
Data are mean ± SE groups with the different alphabetic letters within the same column indicate significant different at P<0.05 ; (n=4).

The results showed a significant increase (P ≤0.05) in hemoglobin content and packed cell volume in all treated groups of fish (T1, T2 , T3 ,T4 and T5) that were (8.13, 8.31 , 8.82 . 9.22 and 9.42 dl^{-1}) respectively and (25.46 , 25.73 , 26.13 , 26.62 , 26.91%) respectively compared with the control group 7.52 g dl^{-1} and 22.92 % at 45 days (pre challenge). After 60 day (post challenge), the results showed a significantly increase (P ≤0.05) in hemoglobin and packed cell volume in all treated groups (T1, T2 , T3 ,T4 and T5) that were (7.22 , 7.42 , 7.71 , 8.15 and 8.30 dl^{-1}) respectively and (13.4 , 14.23 , 16.62 , 16.91 , and 17.19 %) respectively as compared with the control group 6.71 dl^{-1} and 11.83 %.

Table (6) The Effect of turmeric on hemoglobin content and PCV % in treatment groups C. carpio fed different concentration of turmeric for 45 days and challenged with *F. columanris*.

Groups	Hb content and PCV%			
	Hb g/dl		PCV%	
	45 Day	60 Day	45 Day	60 Day
Control	7.52 ±0.01 f	6.71 ±0.01 f	22.92 ±0.01 f	11.83 ±0.01 f
T1	8.13 ±0.02 e	7.22 ±0.01 e	25.46 ±0.01 e	13.40 ±0.01 e
T2	8.31 ±0.01 d	7.42 ±0.01 d	25.73 ±0.001 d	14.23 ±0.01 d
T3	8.82 ±0.01 c	7.71 ±0.01 c	26.13 ±0.01 c	16.62 ±0.01 c
T4	9.22 ±0.01 b	8.15 ±0.02 b	26.62 ±0.01 b	16.91 ±0.01 b
T5	9.42 ±0.01 a	8.30 ±0.01 a	26.91 ±0.01 a	17.19 ±0.01 a
LSD value	0.037 *	0.042 *	0.017 *	0.018 *

* (P<0.05).
Data are mean ± SE groups with the different alphabetic letters within the same column indicat significant different at p<0.05 ; (n=4).

The RBC count increased with the administration of turmeric, which might indicate that it acts as an immunostimulatory agent (Sahu,

2004). Also, this finding supported by the earlier work of Sahu *et al*. (2007a) in which the number of erythrocytes was found to be significantly increased in *rohu* fed with a diet containing garlic for 60 days. These results are in agreement with Sahu *et al*. (2008) who used turmeric in *Labeo rohita*, they noticed the RBC increase in turmeric treated group The increase of Hb content may be attributed either to increasing the synthesis of enzymes needed for biosynthesis of hem (El-Tahir *et al.*, 1993) or increasing the size of red blood cells (El-Fek *et al.*, 1993). Similarly, Ashok and Meenakshi (2004) found that rats treated with aqueous and alcoholic extract of turmeric for two months produced insignificant effect in Hb and PCV values. Also, Duncan and Klesius (1996) reported that the number of erythrocytes in channel cat fish fed with a diet containing b-glucan was significantly ($P \leq 0.05$) greater. While Sodamola *et al*. (2016) disapproved with these results, who noticed that different doses of dietary turmeric did not effect on RBC count, PCV% and Hb content.

Taken all together our findings indicate that dried turmeric powder has improved the growth rate in *C. carpio* when challenged with *F*. columnaris. Also, addition of turmeric t into commercial feeds increased survival rate of carp fish challenged with a bacterial pathogen. This will be worth and useful for application in fish aquaculture.

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