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Study some immunological parameters of dietary supplementation turmeric powderinhealthy common carp *Cyprinus carpio L.*

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Abstract
The research was conducted at a pathology laboratory, College of Veterinary Medicine, University of Diyala, Iraq, to determine the effect of turmeric powder Curcuma longa L. as a feed additive on immune response in common carp (Cyprinus carpio L.).All in all, 100 C. In the sample carpio weighing around 180±5 g. Fish were held in parallel (10 fish / tank) in five treatment groups, fish were fed a separate concentration of turmeric as follows: T1 (0.5 percent), T2 (1.0 percent), T3 (1.5 percent) and T4 (2 percent) as well as the control group (C) were fed basal diets without any addition of turmeric. Six fish from each treatment group T1, T2, T3, T4 and control (C) obtained blood samples from 6 fish chosen from each treatment group for the study of differentiated white blood cells and white blood cell counts (WBCs) after 60 days of the feeding cycle. Considerable improvements that have been made in WBC criteria fall in very categories. The test showed a significant improvement ($p<0.05$) in T4, T3 relative to T1, T2 and in WBC control group. The lymphocyte ratio and neutrophil reported phagocytic activity increased significantly in T4 and T3 as opposed to control groups (C).

Keywords:*Cyprinus carpio* L., *Curcuma longa L.*, White blood cellcount, Differential leukocytes count.
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INTRODUCTION

Turmeric (a specified source of the Curcuma longa L. rhizomes) is widely used as a dietary spice. Curcuminoids, composed of curcumin, demethoxycurcumin and bisdemethoxycurcumin (1), were called active biologically complexes.In fact, curcumin's providing a preventive agent for cancers, autoimmune, coronary, neoplastic, respiratory, neurodegenerative, metabolic diseases, inflammation, oxidative stress, and immunity (2) is cheap, healthy at actual high doses of pharmacological products.Therefore, pathogens are the main stumbling blocks in aquaculture production causing heavy industrial losses (3,4). While antibiotics and chemotherapeutics used in aquaculture therapy, their negative impact has been widely criticized (5).Up to now, the use of conventional herbal medicines as immunostimulants is restricted although such medicines tend to be a rich source of active immunotherapy substances (6). Curcumin used as medical regimens to improve recovery capacity decline with enhanced efficacy of synthetic drugs (7).The current research explored immunological parameters of dietary supplement Curcuma longa L. Common carp (*Cyprinus carpio L*) used in healthy fish.

MATERIAL AND METHODS

The study conducted in the Fish Disease Laboratory at the Pathology Department, College of Veterinary Medicine, Diyala University, Iraq during the period extended between 2/1/2019 to 1/6/2019.A total of 100 *C. carpio* in the studywas obtained from a commercial farm (Al–Mahaweel, Babylon), weighing approximately 180±5g. Five separate treatment groups (10 fish / tank) processed in two paths with 80 L chlorine-free tap water, fish were fed a different turmeric concentration as follows:T1 (0.5 %), T2 (1.0 %), T3 (1.5 %) and T4 (2%) as well as the control group (C) was fed basal diet without any addition of turmeric. All the treatment groups and control fed twice a day at a rate of 2% body weight.During the experimental time, chemical parameters of the water reported daily, such as water temperature (ranged between 24-26ºC), pH of water in the aquaria ranged between 7.2-7.6 and the concentration of dissolved oxygen (DO) (ranged between 6.5- 7 mg/l). Six individual blood samples of fishes collected randomly from each group. Blood taken from the caudal vein by puncture using a plastic syringe of 3 ml (G 23× 1). The blood samples collected to determine the hematological and immunological parameters like WBCs and the count of differential leukocytes.

Total White Blood Cells (WBCs) Count

WBCs count existedas described by (8), the formula was applied to calculate the WBCs:

$$\text{Total WBCs count} = \frac{N}{4} \times 20 \times \frac{1}{10} \quad \text{cell/ mm}^3$$

N: number of the cells in 4 large squares.
20: diluting factor

10: depth factor
0.1: volume factor = (width x length x height).

Differential leucocyte count

Differential leukocyte counts with the use of Giemsa staining method and blood films detected under a light microscope (9).

Statistical Analysis

Statistical Analysis System, Users Guide. Statistical. Version 9.1th ed. SAS. Inst. Inc. Cary. N.C. USA (10). Used to regulate significant differences among variables. The differences between means were determined and compared using LSD test with a significance level of $P < 0.05$.

RESULTS ANDDISCUSSION

Total White Blood Cells (WBCs) Count

WBCs count showed a significant increase ($P< 0.05$) in fish treated with curcumin supplemented diets (Table 1). The highest values were recorded in T4 (39.2 ×103 cell/mm3) followed by T3, T2 and T1 (37.2 × 103/mm3, 33.2× 103/mm3, 32.2 ×103cell /mm3, respectively) compared to the control (C) group (23.5 ± 1.07 cell /mm3).

Table 1. WBCs count of *C. carpio* fed different concentrations of diet *Curcuma longa* L.for 60 days.

Treatment	Mean ± SE of WBC (103/mm3)	T-Test
Control (C)	23.5 ± 1.07 E	-
T1	32.2 ± 2.42 Cd	3.27 NS
T2	33.2 ± 1.94 bc	3.09 NS
T3	37.2 ± 2.35 ab	2.71 NS
T4	39.2 ± 3.17 a	2.50 NS
Level of Sig.	5.163 *	---
Means values indicated with different letters in the same column are significantly different (P<0.05). *		

Blood parameters calculated to give information about the common carp's food response and safety importance (11). Leukocytes are among the most cells that activate fish's immune responses and act as one of the first lines of body defense and increase sharply with infections. Furthermore, the leukocytes and their count were known as health status indicators (13). The results of total leukocyte count of *C. carpio* groups treated with curcumin showed a significant increase of WBCs count associated with control groups. These results were fully accordant with those reported by (14) who noticed that snakehead (*Channa striata*) fingerlings fed with supplemented diets curcumin, the number of WBC significantly increased in all groups related withcontrol groups. On the contrary, (15) reported that no effect on haematological parameters (WBC) of Channel catfish (*Ictalurus punctatus*).In addition, current results were in agreement with those obtained by (16) who found significant improvement in leukocyte of *C.carpio* treated withcurcumin for 60 days as compared with the control group**.**

Differential leukocyte count:

The lymphocyte and monocyte indicated a significant decrease ($P<0.05$) in the control group. In addition, lymphocytes (percentage) recorded significant differences among all treated withcurcumin supplemented diets groups (T1, T2, T3 and T4). While, neutrophils (%) recorded significant increases ($P<0.05$) in all treated curcumin supplemented diets relative to the control group(Table 2) with significant differences in all treated curcuminsupplemented diets groups (T1, T2, T3 and T4). Such, the neutrophils (percentage) was significantly increased in treated groups (T1, T2 and T3) in comparison to the Cgroup (Table 2).

Table 2. Differential leukocyte count of *C. carpio* treated with supplemented diets.*Curcuma longa* L. for 60 days.

Mean ± SE (%)					
Treatment Lymphocyte %		Monocyte %	Neutrophil %	Eosinophil %	Basophil %
C	74.3 ± 3.69a	8.0± 0.61ab	17.3±1.15c	0.33 ± 0.00	0 ± 0.00
T1	71.3 ± 3.72a	7.3 ± 0.52b	21.0±1.08bc	0.33 ± 0.00	0 ± 0.00
T2	69 ± 3.64ab	7.6 ± 0.66b	23.0±1.76ab	0.33 ± 0.00	0 ± 0.00
T3	68.3 ± 2.51ab	9.0 ±0.40ab	22.3±2.09ab	0.33 ± 0.00	0 ± 0.00
T4	65.0 ± 2.78b	9.3± 0.56ab	25.3 ± 2.58 a	0.33 ± 0.00	0 ± 0.00
Level of significant	*	*	*	NS	NS
Values indicated with different superscript letters in the same column are significantly different (P<0.05).					

The WBCs are the non-specific immune factors which may be associated with fish immune status to increase the level of these factors (17). Such results are in the contract with the previous study (18), which found that there were significant differences in leukocyte (lymphocyte, neutrophils and common carp monocyte). In addition, on the same line, findings of (19) indicated that administration of curcumin have effects on However, on the same side, findings of (19) suggested that curcumin administration had effects on sturgeon hematological parameters (*Acipenser persicus*) so that the amount of blood lymphocytes decreased while the granulocytes were both increased.Via differential leukocyte counting, these results showed differences between treatments as compared to control.In the present study, the elevation in the number of WBCs defined the immune-stimulant. It generally activates white blood cells, such as macrophages, granulocytes and monocytes) additionally able to multiply fishes granulocytes, lymphocytes and macrophages as same as those in higher vertebrates (20) resulted in a significant rise in the number of WBCs.

CONCLUSION

Results from this study showed that curcumin can significantly stimulate some of these (immune parameter)

differential white blood cell counts and white blood cell counts (WBCs) without any side effects, and work with low doses.

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ETHICAL APPROVAL

All applicable international, national, and/or institutional animal care and utilization guidelines have been followed. All procedures carried out in animal-related studies were in accordance with the institution's ethical standards or practice in which the studies were conducted.

Disclosure of potential conflicts of interest and current submission

This manuscript has not been previously published and is not under consideration in the same or substantially similar form in any other peer-reviewed media.

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