EFFICACY OF PROPOLIS AS FEED ADDITIVES ON GROWTH AND HEMATOLOGICAL PARAMETERS ON *CYPRINUS CARPIO* CHALLENGED WITH *AEROMONAS HYDROPHILA*

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ABSTRACT

The present study was conducted to investigate the effects of propolis [(crud propolis and Water Ethanol Extract Propolis (WEEP)] as feed additive on growth performance, hematology and survival rate in common carp, *Cyprinus carpio* challenged with *Aeromonas hydrophila.* A total of 60 *C. carpio,* (average weight 75±10g) were randomly distributed into five treated groups as follows: control group (C) fish were fed basal diet without any addition of propolis; T1 fish were fed basal diet supplemented with 10g/kg crud propolis; T2, T3 and T4 fish were fed WEEP at concentrations of 2g, 4g and 8 g/kg diet respectively for 42 days. Results indicated that the best Final weight, specific growth rate (SGR%) and feed conversion ratio (FCR) were observed in T2 followed by T1 compared to control and other treated groups. At the end of the feeding period, fish were intraperitoneally challenged by *A. hydrophila*. The survival rate recorded highest value (100%) in T2 compared to control positive group (42%). Hematological indices showed considerable changes in the mean values of RBCs, Hb, PCV and WBCs. This study suggests that growth performance and survival rate against any infection with *A. hydrophila* of common carp can be improved by dietary supplementation with crude propolis and WEEP propolis at concentration of 2 g/kg diet which is beneficial for fish culture.

Keywords: bees glue- Common carp –-Hematology- Specific growth rate

مجلة العلوم الزراعية العراقية- 2025 :56 (2):916-922 الجبوري ومصطفى

تأثير العكبر كإضافات علفية في اداء النمو والصفات الدمية في اسماك الكارب الشائع *Cyprinus carpio* المصابة ببكتريا *Aeromonas hydrophila*

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المستخلص

أجريت الدراسة الحالية لمعرفة تأثير العكبر الخام ومستخلص الإيثانول المائي كإضافات علفية الى علائق الاسماك في أداء النمو والصفات الدمية في اسماك الكارب الشائع *Cyprinus carpio* المصابة تجريبيا ببكتريا*Aeromonas hydrophila*. *تضمنت* التجربه استخدام 60 سمكه معدل وزنها 75 ± 10 غم. تم توزيعها عشوائياً على خمس معاملات علاجية مكررة ، تم تغذية الأسماك على النحو التالي:مجموعة السيطرة غذيت الاسماك على عليقة اساسية دون اضافة العكبر: المعاملة الاولى غذيت على عليقه اساسية مع اضافة العكبر الخام بمعدل 10غم/كغم علف: المعاملات الثانية والثالثة والرابعة غذيت على عليقة اساسية مع اضافة مستخلص الايثانول المائي للعكبر بمعدل 2 و4 و 8 غم/كغم علف على التوالي. بعد مرور 42 يومًا من التغذية لوحظ زيادة في الوزن النهائي ومعدل النمو النوعي ومعدل التحويل الغذائي في المعاملة الثانية تليها المعاملة الاولى (T1) مقارنة مع مجموعة السيطرة والمعاملات الاخرى بعد انتهاء فترة التغذية اجرى فحص التحدي *ببكتريا A. hydrophila* (1.57×10^6 CFU/ml) حيث سجل اعلى معدل بقاء(100%) في المعاملة (T2) مقارنة بمجموعة السيطرة الموجبة(42%) . اظهرت الصفات الدمية تغيرات ملحوظة في معدل الكريات الحمر والبيض والهيموكلوبين وحجم كريات الدم المضغوطة في المعاملات المعالجة. تشير النتائج ان معدل النمو ومعدل البقاء ضد الاصابة ببكتريا *A. hydrophila* في اسماك الكارب الشائع ممكن ان يعزز باضافة العكبر الخام ومستخلص الإيثانول المائي بتركيز 2 غم/كغم علف وبالتالي ممكن ان يكون مفيدا لتربية الاسماك.

الكلمات المفتاحية: صمغ النحل–الكارب الشائع –علم الدم– معدل النمو النوعي

Received:12/10/2022, Accepted:25/1/2023

INTRODUCTION

Propolis is bee glue which is a sticky resin material produce by honey bee *(Aspis millfera l*) (16). The bees collect it from tree, flowers and other plant mixes with saliva and enzymes to form propolis secretion (23). Propolis use for protection the bees from invaders and protect the honey from fungi and other microorganisms also protect their house from wind and heat (29). It has different biological and pharmacological properties like antifungal, antibacterial, antiviral, local anesthesia, anti-inflammatory, antiprotozoal, promotes growth and strengthens immunity (2). The most component of propolis is flavonoids, flavanones, flavones and flavanols, Enzymes, ketones, sterols and steroid hydrocarbons, sugar, vitamins and minerals (8). One of the most common fish diseases is bacterial hemorrhagic septicemia that caused by *A. hydrophila* which has a relatively high resistance to antibiotics (26). This disease can cause serious economic losses to fish. The motile *A. hydrophila* infects a wide range of freshwater fish and is associated with tail and fin rot, epidemic ulceration syndrome, hemorrhagic septicemia, ascites, intestinal infection and exophthalmic eyes (13), *C. carpio* is an important species for freshwater aquaculture and improving its culture and diseases resistance is one of the most important the challenge facing fish farmers. The aim of this study was designed to evaluate *C. carpio* to *A. hydrophila* resistance using crude propolis and water ethanolic extract as study its effect on growth performance, survival rate and hematological parameters

MATERIALS AND METHODS

Crude Propolis and its Water Ethanolic Extract (WEEP) preparation

The ethanolic extract of propolis was prepared according to Eraslan *et al*. (12), by using a dark sterile vial (200 ml), the propolis was cute into small pieces, frozen for 6 hours, mixed with an electric mixer to become a powder. Then, 40g of propolis was added to 50 ml of water for 24 hours and mixed by a magnetic stirrer and then added 150 ml of 99% ethanol for 48 hours using a magnetic stirrer. After that, filtrate the insoluble component with clothe tulle and evaporate at room temperature to obtained powder which was used for experimental feed preparation.

Diet preparation

Commercial basal diet (crude protein 35%) was crushed, and then divided into five parts. Control was fed basal diet without any addition of propolis, Diet for treatment 1 (T1) was mixed with 10g/kg curd propolis. Ethanolic extract of propolis was added to the feed at 2 g/kg, 4 g/kg, 1 and 8 g/kg. then, the diet was reformed into pellets, spread to dry and stored at room temperature for the feeding experiment.

Experimental setup : A total of *C. carpio* (average weight 75±10g; total length 15cm) were randomly collected from local ponds of fish farms, at Al-Mesiab/Babylon. fish were adapted for two weeks, and then divided into five equal groups in duplicate as follows: control group (C) fish were fed basal diet without any addition of propolis; T1 fish were fed basal diet supplemented with 10g/kg crud propolis; T2, T3 and T4 fish were fed WEEP at concentrations of 2g, 4g and 8 g/kg diet respectively for 42 days. Fish were fed twice a day during the 42 days experiment. Throughout the experiment, water quality was registered: temperature 23.2±0.1°C, pH 6.8-7.2, dissolved oxygen 6.82±0.2 mg/L. The water of the aquaria was changed daily. The fish were weighed at 0, 14, 28 and 42 days from the starting of the experiment.

Growth performance

Growth performance: Growth weight was calculated every two weeks for 42 dayaccording to Al-Hassani and Mustafa (5).

According to the following equations:

Body weight gain= final fish weight (g)-initial fish weight(g)

Daily gain (D.G) = WT-Wi/T-t

Specific growth rate SGR%= (In WT-In Wt)/ (T-t) ×100

Relative Growth Rate RGR= (Final fish weight (g)-initial fish weight (g) / Initial fish weight (g) ×100

Feed conversion efficiency FCE= Total weight gain by fish (g)/ Total food intake by fish (g) ×100

Feed Conversion Ratio FCR= Total food intake by fish (g)/ Total weight gain by fish (g).

Blood collection and hematological analysis

At the end of the feeding experiment, fish (n = 4of each treatment) were anaesthetized by clove powder at concentration of 0.25mg/L, Blood samples were collected from the caudal vein in plastic EDTA vials for determination of (Hb) hemoglobin using commercial colorimetric kits (Cyan-methaemoglobin (9). packed cell volume (PCV%) (3), Red blood cell and white blood cell (7).

Determination LD_{50} of *A. hydrophila* The bacterial strain was cultured in Brain Heart Infusion Agar at 28 ºC for 24 h, LD_{50} according to Suhail *et al*. (26) (exactly at a concentration of 0.1 ml 1.57×10^6 colony forming units CFU/ml fish, 1 ml 1.57×10^8 CFU/ ml which causes high mortality 100% and no mortality0% on 0.1ml $1,57\times10^4$ CFU /fish of approximately.

Challenge test

After 42 days of the feeding experiment, fish from each group were challenged intraperitoneally with 0.1 ml (1.57×10^6 CFU/ml). The challenged fish were kept under observation for 14 days. Dead fish were removed from the aquarium daily and mortality was reported daily for 14 days. Survival rate (%) =final number of fish survivor/initial number of stocked fish ×100.

Statistical analysis

The Statistical Analysis System-SAS (22) program was used to detect the effect of various factors on the study variables. Least significant difference - LSD test (Analysis of Variance-ANOVA) was used for significant comparison between means in this study. Probability level were assessed at ≤0.05 and ≤0.01.

RESULTS AND DISCUSSION

Growth performance: Following 42 days from the start of feeding, the highest growth rate was obtained in T2 using WEEP and in T1 (crude propolis), while the lowest growth was obtained in T4. The growth rate was significant increase in T2 and T1) compared to T4 after 7 ,14 and28 days from starting of feeding, when compared with control group (Table 1), The daily gains (DG), feed efficiency ratio (FER), total weight gain (TWG), specific growth rate (SGR), relative growth rate (RGR), feed conversion ratio (FCR) and feed conversion efficiency (FCE) were highly significant in T2 followed by T) and the lowest of these performance were observed in T3 followed by T4 (Table 2). Crud propolis is a very complex mixture of plant and raisins (50%), wax (30%), aromatic oil and essential oil (10%), pollen (5%) and of various other substances (5%) like vitamins and sugars (2). At the end of the 42 days, there was a significant increase in T2 compared to all treatments, and there was a significant increase in T1 and T4 compared to the T3 and control groups in Table (2). Growth is measured in units of weight and height, and the increase in daily gain and body weight are among the important factors for raising the value of the diet and the protein in it. Table (2) show the Water Ethanol Extract Propolis WEEP have the best TWG, DG, SGR, RGR, FCE and the survival rate 100% was in T2 due to increased total counts of psychrophilic and mesophilic bacteria in digestive tract, which have the ability to improve gut health, blood parameters, improve digestion and absorption and thus improve growth performance (27). Adding 10g of Crud Propolis in the diet of *C. carpio* was significantly enhance the daily growth performance of the body increase, (SGR) and (FCR), feed efficiency rate (FER) similar to Abd-El-Rhman (2) and to Meurer *et al.* (17). preferred use small amount of crud due to contain on wax. when use high amount of extract propolis (T3 and T4) due to side effect as in (28), Decrease in FCR at T1 and T2 (3.8 and 3.33) to get well fed and allow more fish to grow in the aquarium similar to Abbass *et al*. (1) and to Nur *et al.* (19). in other species, catfish receives ethanol Extract propolis (10g/kg) in their diet significantly improved feed efficiency and growth performance. Several studies focus on applying different concentrations of propolis and different extraction methods to modify the intestinal micro-flora. Thr results are in disagreement with Alishahi *et al.* (6) who indicated that using a different dose of water ethanol extract of propolis there were no significant differences in all growth parameters including: SGR, FCR, TWG and length.

Table 1. Body weight (g) of *C. carpio* post dietary supplementation with propolis during 42 days.

Growth Groups	zero-day weight	14day weight	28day weight	42day weight
Control	444.5±2 a	457.75±12.25 a	495.8±0.8 a	517.3±6.3 b
T1	446.5±16.5 a	458.25±17.25 a	527.75±19.25 a	545.75±18.25 ab
T2	450.5±2.5 a	462.75±2.25 a	524.2±12.8 a	564±5 a
T3	449.5±2 a	461.75±2.25 a	509.55±1.45 a	527.55±1.15 ab
T4	419.25±13.75 a	456.75±9.75 a	486.2±11.8 a	509.3±12.6 b
P value	0.2721	0.9904	0.186	0.068
LSD	35.454	38.225	42.282	38.399

Means with the same letter in the same column are not significantly different*(P≤0.05

Table 2. Growth performance of *C. carpio* post dietary supplementation with propolis for 42 days

Growth performance Groups	Total Weight Gain	Daily Weight Gain (g)	Specific Growth Rate (SGR%)	Relative Growth Rate (RGR)%	Feed Conversion Efficiency (FCE)%	Feed Conversion Ratio (FCR)
Control	72.8 ± 4.3 d	1.73 ± 0.102 d	0.36 ± 0.018 c	16.37 ± 0.89 c	19.259 ± 1.14 d	5.21 ± 0.3 a
T1	99.25 ± 1.75 b	2.36 ± 0.04 b	0.478 ± 0.0083 b	22.24 ± 0.43 b	26.3 ± 0.46 b	3.8 ± 0.08 bc
T2	113.5 ±2.5 a	2.70 ± 0.059 a	0.53 ± 0.008 a	25.19 ± 0.42 a	30.026 ±0.66 a	3.33 ± 0.073 c
T3	78.05 ±0.85 d	1.858 ± 0.02 d	0.38 ± 0.0054 c	17.36 ± 0.266 c	20.65 ± 0.22 d	4.84 ± 0.05 a
T4	90.05±1.15 c	2.144±0.027 c	0.4788 ±0.004 b	22.27 ± 0.21 b	23.82±0.304 c	4.198±0.053 b

Means with the same letter in the same column are not significantly different (P≤0.05).

Survival rate

The fish mortalities kept increasing in time until the 12 days, after 14 days of challenged with *A. hydrophila*, the highest survival rate was observed at T2 (100%) followed by T3 (75%) and T1 and T4 (67%) as shown compared with the C+ group (42%) as presented in Table (3). shows the ability of propolis to increase the survival rate in T2 due it contains on antibacterial activity compounds like flavonoids, phenolic acid, artepillin C which has bacteriostatic activity (25 and 30), the Ethanol Extract propolis EEP and crud propolis have effect against *Aeromonas hydrophila* (20).

Table 3. Results of survival rate of *C. carpio* post challenge with *A. hydrophila* for 14 days.

Day group	Total number	number dead fish	Mortality (%)	Survival rate%
C -	12	0	0	100
C+	12	7	58	42
T1	12	4	33	67
T2	12	0	0	100
T3	12	3	25	75
T4	12	4	33	67

Hematological parameters

The results of RBCs significant decrease (P≤0.01) in treated groups T1, T2, T3 and T4 compared to C- group. PCV % recorded significant decrease in T1 and T4 (P≤0.01) compared to C- and C+ groups. Hb content in all propolis dietary groups recorded significant decrease (P≤0.01) compared to C- and C+ groups, the best treatment in table (4) was in T2 than T3 in RBCs, PCV and Hb. WBCs was showed significantly increase (P≤0.01) T3 and T4 in compared to C- group. Hematological indicators for commonly health status of fish, In the current study, the decrease in RBC number in non-treated C+ group could be enterotoxin cytotoxin also known as 'aerolysin', which has cytotoxic, enterotoxin, and hemolytic activities, has been described as the strongest virulence agent associated with Aeromonase-mediated gastrointestinal disease (18). which have ability to lyse red blood cells (11). Decreased RBC, PCV and Hb at T1 and T4 could indicate that erythrocytes are damaged with increased leukocyte activity in carp experimentally infected with A. hydrophila. This result is consistent with (10), This decrease in the level may be due to the increased dose of propolis in the C. carpio and may be a symptom of anemia with inhibition of erythrocyte formation in the hematopoietic organ. Added crud propolis in diet T1 have benefit on growth performance only but not on blood performance. However, elevated leukocyte values depend on the stimulatory effects of cytotoxic agents on the immune system (14,15 and 24). Propolis supplementation with 2g/kg for 42 day can improve carp growth, body composition, biochemical parameters and hematology. Because propolis contains a variety of flavonoids, minerals, vitamins, and other compounds with diverse biochemical structures and biological activities, it functions in increasing erythropoiesis in fish hematopoiesis (4). WBC plays a role in immune processes and changes in the number of white blood cells after exposure to pathogens indicate a decrease in non-specific immunity of fish (21). Increase T2, T3 and T4 The increase in leukocyte counts at T3 and T4 may have enhanced non-specific defense mechanisms, since leukocytes are major components of the immune system and are the main effector cells affecting propolis.

Table 4. Hematological parameter of *C. carpio* after 14days post challenged with *A.hydrophila* (P≤0.01).

Group	Mean ± SE RBC×10^6 / mm^3	Hb (g/dl)	PCV (%)	WBC×10^3 /mm^3
C+	1.36 ±0.08 b	5.64 ±0.37 b	16.95 ±1.13 bc	34.36 ±1.65 a
C-	1.75 ±0.01 a	10.84 ±0.02 a	32.55 ±0.05 a	24.41 ±0.13 c
T1	1.17 ±0.09 bc	4.43 ±0.32 d	13.27 ±0.96 de	27.65 ±3.28 bc
T2	1.43 ±0.06 b	6.62 ±0.62 b	19.87 ±0.77 b	29.59 ±2.13 abc
T3	1.27 ±0.07 bc	5.43 ±042 cd	16.29 ±1.25 cd	31.84 ±1.08 ab
T4	1.01 ±0.15 c	4.16 ±0.59 e	12.49 ±1.79 e	32.31 ±0.67 ab
LSD value	0.269 **	1.117 **	3.347 **	5.378 **
P-value	0.0005	0.0001	0.0001	0.010

Means having with the different letters in same column differed significantly. **

CONCLUSION Administration of local propolis appears to have a positive effect on the growth performance, survival rate, enhances RBCs, WBC at dose 2g/kg, It could be concluded that the water ethanolic extract of propolis was more effective than the crude propolis in protecting fish against *A.hydrophila* infection.

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