

Effects of propolis, a honeybee product, on growth performance and immune responses of *Barbus barbulus*

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Barbus barbulus, Propolis, growth indices, immuneresponse, hematological parameters.

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Abstract:

BACKGROUND: Propolis, a honeybee product, has been used empirically for centuries and was always mentioned as an immunomodulatory. **OBJECTIVES:** In this study, the effects of propolis on growth indices, innate immune responses and haematological parameters of *Barbus barbulus* were investigated. **METHODS:** Three hundred and sixty juvenile *Barbus barbulus*, weighing 102 ± 8.2 g were randomly divided into four equal groups in triplicate. Groups 1 to 3 were fed basal food supplemented with 0.1, 0.5, 1% of Propolis-ethanolic -extract (PEE) in diet for 60 days. Control group received basal diet free of PEE. At the end of experiment, growth indices were measured in all groups. Various immunological parameters (serum lysozyme and bactericidal activity, complement activity, total serum protein and globulin) as well as hematological parameters (RBC, WBC, hemoglobin, hematocrit, MCV, MCH and MCHC) of *Barbus barbulus* were compared among the groups. Then the fish were challenged with *Aeromonas hydrophila*. **RESULTS:** Oral administration of different level of PEE induce no significant change, neither in growth indices nor in haematological parameters of *B. barbulus* ($p > 0.05$). Significant increase in serum Lysozyme and bactericidal activity, total serum protein and WBC were seen in G2 and G3 compared to the control group ($p < 0.05$). Meanwhile, mortality after challenge of fish fed on diet containing 0.5% PPE significantly decreased compared to control group. **CONCLUSIONS:** It was concluded that, although supplementation of food with 0.5 and 1% PEE enhanced some immune response indicators of *B. barbulus*, growth indices and hematological parameters were not affected by this supplementation.

Introduction

Immunostimulants, a heterogeneous group of compounds, are able to enhance the innate immune system, thereby preventing infectious diseases. In fish, several immunostimulants such as vitamins (Anderson, 1992), substances with microbial origin (Dalmo and Bogwald, 2008) extracts from animals and plants (Esteban et al., 2000; Bricknell and Dalmo 2005; Alishahi et al., 2010), synthetic compounds like levamisole (Sakai, 1999) and sub products of

other industries such as chitozan and propolis (Sforcin, 2007), that play a promising role in aquaculture by enhancing the disease resistance in fish species have been reported. By-product immunostimulants (such as propolis) have recently received increasing attention due to their lower costs and low impact on the environment (Sforcin, 2007).

Propolis (bee glue), a resinous sticky substance collected by bees from various plant sources and mixed with secreted beeswax, is a multifunctional material used by bees in the construction, maintenance, and protection of their hive. Propolis has been

used since ancient times as a medicine due to several biological and pharmacological properties such as antibacterial, antifungal, antiviral, anti-protozoa, anti-inflammatory and immune-stimulant (Kosalec et al., 2003; Cuesta et al., 2005). Egyptians, Greeks and Romans reported the use of propolis for general healing qualities and cure of some lesions of the skin (Sforzin, 2007). In general, it is composed of 50% resin and vegetable balsam, 30% wax, 10% essential and aromatic oils, 5% pollen and 5% various other substances, including organic debris (Burdock, 1998). It has been shown that propolis has both immunomodulatory and anti-inflammatory effects in mammals. Propolis was able to enhance macrophage functions, lymphocyte proliferation and the number of plaque-forming cells in the spleen as well as resistance to several pathogens and tumors in a number of mammalian species (Dimov et al., 1991; Dimov et al., 1992; Ansorge et al., 2003; Cuesta et al., 2005; Abd-El-Rhman., 2009; Kanbur et al., 2009).

Current developments in aquaculture have led to more effective production systems and introduction of new species. *Barbus barbulus*, a native fish species, has recently been artificially propagated and cultured under poly culture system in cyprinid ponds in Iran. In the last decade several studies have been carried out on propolis, with various medicinal effects (Orsolich and Basic, 2003; Hu et al., 2005; Kanbur et al., 2009), but few studies focused on the effect of propolis on immune system of fish species (Zhang et al., 2009; Abd-El-Rhman, 2009). Thus, this study aimed to evaluate the effect of different level of propolis on some haematological and non-specific immune parameters as well as growth factors of *Barbus barbulus*.

Materials and Methods

Fish: A total of 360 Juvenile *B.barbulus* ($102\pm 8.2\text{g}$) were obtained from the reproduction center of Iranian native fish (Dasht-e - Azadegan) in Ahvaz, Iran. Fish were kept in 300 L tanks, with running aerated and dechlorinated water at $25\pm 1^\circ\text{C}$ and kept 1 week to acclimate. Tanks were equipped with external biofilters and thermostatic heaters.

Fish were fed with commercial pellets (Behparvar Company, Iran) twice a day. Water quality factors were recorded during the experiment as: temperature,

$25\pm 1^\circ\text{C}$; Dissolved oxygen, 8-10 ppm; pH, 7.8 ± 0.2 ; $\text{NO}_2 < 0.01\text{ppm}$ and $\text{NH}_3 < 0.1\text{ppm}$. Water exchange rate was 20% of water volume daily.

Crude propolis and its ethanolic-extract: Propolis composition is highly variable, creating a problem in the medical use and standardization. In the present work, crude propolis sample was collected in summer from the north of Khuzestan province (Masjed-soleiman) using propolis traps and kept in a dark and dry place until used. Propolis-ethanolic-extract (PEE) was prepared by adding 30 mL of absolute ethanol to 3 g minced propolis in bottles which were sealed and shaken in darkness for 1 day at room temperature. The extract was then filtered twice and stored in sealed bottles at 4°C until used (Cuesta et al. 2005).

Experimental settings: Three hundred and sixty fish were divided into 4 equal groups, each group with three replicates (each replicate contained 30 fish) and fed for 60 days under the following treatments: the first group (G1) received 0.1% PEF/ kg diet, the second and third groups (G2 and G3) received 0.5% and 1% PEE/kg diet respectively and the control group (G4) received no PEF. Fish were hand-fed ad libitum twice a day.

Fish were anesthetized with MS222 and blood-samples were collected from the caudal peduncle vein of 6 fish from each Aquarium. Hematological parameters were assessed after sampling on the same day. Remained blood samples were centrifuged (3000 g for 15 min) and sera was separated and stored at -20°C until used (Schaperclaus 1991).

Feed preparation: Commercial barbus diet (Beiza Co, Iran) was used as a basal diet. For better homogenation of PEE with food, initially granulated food became paste by adding distilled water to it, then 0.1, 0.5 and 1% (w/w) PEE was added to food and homogenized with an electric mixer. Finally, food was pelleted by means of a special meat grinder. Control food was prepared in the same way without supplementation with PEE. Prepared experimental foods were packed in nylon bags, labeled and stored at 4°C until use (Cuesta et al., 2005).

Growth performance: The average weight gain (AWG), specific growth rate (SGR), feed conversion ratio (FCR) and feed efficiency ratio (FER) were calculated at day 60 according to the following equations:

AWG (g/fish) = Average final weight (g) - Average initial weight (g) / experimental period (day).

SGR (%/day) = 100(In final body weight (g) - In initial body weight (g) / experimental period (day).

FCR = Feed intake (g) / weight gain (g).

FER = Body weight gain (g) / Feed intake (g).

Lysozyme activity: Lysozyme activity was measured by the method of Parry et al., (1965) with minor modifications of Ellis (Ellis, 1990). In this turbidimetric assay, 0.3 mg mL⁻¹ lyophilized *Micrococcus lysodeikticus* in 0.05 mM sodium phosphate buffer (pH=6.2) was used as substrate. Ten microlitres of fish serum was added to 200 µL of bacterial suspension in triplicate wells of ELISA plate and the reduction in absorbance at 490 nm was determined after 0.5 and 4.5 min of incubation at 22°C using a microplate reader. One unit of lysozyme activity was defined as a reduction in absorbance of 0.001 per min.

Serum bactericidal activity: Serum bactericidal activity was measured according to Kajita et al. 1990 with slight modification. Sera samples from each group were diluted three times with 0.1% gelatin-veronal buffer (GVBC2) (pH 7.5, containing 0.5 mM mL⁻¹ Mg²⁺ and 0.15 mM mL⁻¹ Ca²⁺). *Aeromonas hydrophila* (live, washed cells) was suspended in the same buffer to make a concentration of 1 × 10⁵ cfu mL⁻¹. The diluted sera and bacteria were mixed at 1:1, incubated for 90 min at 25°C and shaken. The number of viable bacteria was then calculated by counting the colonies from the resultant incubated mixture on TSA plates in triplicate after 24 h incubation.

Alternative complement activity: Alternative complement activity was assayed following the procedure of Yano et al. (1992) by using rabbit red blood cells (RaRBC). Briefly, RaRBC were washed and adjusted to 2 × 10⁸ cell mL⁻¹ in ethylene glycol tetra acetic acid-magnesium-gelatin veronal buffer (0.01 M). Exactly 100 µL of the RaRBC suspension was lysed with 3.4 mL of distilled water and the absorbance of the haemolysate was measured at 414 nm against distilled water to obtain the 100% lysis value. The test serum was appropriately diluted and different volumes ranging from 0.1 to 0.25 mL were made up to 0.25 mL total volume before being allowed to react with 0.1 mL of RaRBC in test tubes. After incubation at 20°C for 90 min with occasional shaking, 3.15 mL of a saline solution was added to

each tube and centrifuged at 1600 g for 10 min at 4°C. The optical density of supernatant was measured using a spectrophotometer at 414 nm. A lysis curve was obtained by plotting the percentage of haemolysis against the volume of serum added. The volume yielding 50% haemolysis was determined and used for calculating the complement activity of the sample (ACH50) as follows:

ACH50 value (units mL⁻¹) = 1/K × (reciprocal of the serum dilution) × 0.5

where K is the amount of serum (mL) giving 50% lysis and 0.5 is the correction factor since this assay was performed on half scale of the original method.

Total serum protein and globulin: The total serum protein level was estimated by the method of Bradford (Bradford 1976) using the standard protein estimation kit (Zist Shimi Co., Iran). For globulin estimation 50 µL saturated ammonium sulphate solution was added drop wise to 50 mL serum followed by vortexing. Centrifugation was done at 10,000g for 5 min. Then 20 mL of this sample was dissolved with 80 mL carbonate-bicarbonate buffer (pH 9.3), and the protein content estimated through the method of Bradford using the standard protein estimation kit (Zist shimi co, Iran).

Hematological parameters: Blood samples were immediately analysed for the estimation of numbers of erythrocytes, hemoglobin (Hb), hematocrit (Hct), the mean corpuscular volume (MCV), the mean corpuscular hemoglobin (MCH) and the mean corpuscular hemoglobin concentration (MCHC). Numbers of erythrocytes counts were determined by the hemocytometer method (Ellis 1990); haematocrit was determined by the micro-hematocrit method (Fox et al. 1997), and hemoglobin measurement was determined by the cyanomet-haemoglobin method (Goldenfarb 1971). MCV, MCH and MCHC were calculated using the formulas as follow (Hu et al., 2005):

MCV (µm³ cell⁻¹) = (Packed cell volume as percentage/RBC in millions cell mm³) × 10

MCH (pg cell⁻¹) = (Hb in g 100 mL⁻¹ / RBC in millions cell mm³) × 10

MCHC (g 100 mL⁻¹ Hct) = (Hb in g 100 mL⁻¹ / packed cell volume as percentage) × 100

White blood cell count (WBC), Differential cell count: White blood cell count was made from 6 animals of each group in a Neubauer counting

chamber as described by Schaperclaus et al. (1991). For Differential count of leukocytes whole blood on glass microscope slides, dried in air, and stained with May-Grunwald/Giemsa. One hundred white blood cells from each smear were assessed and the percentage of different types of leucocytes was calculated following the method of Schaperclaus et al., (1991).

Challenge with bacterium: Twenty fish from each aquarium were injected intraperitoneally with 0.1 mL of LD₅₀ suspension of *A. hydrophila* (1.6×10^7 cfu per fish) in PBS. Mortality of challenged fish was recorded daily for 10 days. The cause of death was ascertained by re-isolating the infecting organism from kidney and liver of dead fish according to Misra et al. (2006).

Statistical analysis: All statistical analyses were performed using SPSS 16 software. Data were tested for normal distribution with Shapiro-Wilk's test and for homogeneous variance with Levene's test. Differences between means of data in groups were tested with one-way analysis of variance (ANOVA) and Tukey's comparison of means; significance level was defined as $p < 0.05$.

Results

Growth performance: The results of growth indices were shown in Figure 1. Supplementation of food by PEE did not induce any specific change in all growth indices including: AWG, SGR, FCR and FER ($p > 0.05$).

Lysozyme activity: The highest serum lysozyme activity was seen in G2 and G3 respectively. G1 showed no significant difference with control group ($p > 0.05$) (Figure 2).

Serum bactericidal activity: As shown in Table 1, the use of 0.5% and 1% of PEE significantly decreased the bacterial colonies in comparison to 0.1% PEE and control group ($p < 0.05$).

Alternative complement activity: As shown in Table 1, although there were no significant difference among the groups in alternative complement activity, the highest alternative complement activity was seen in G2.

Total serum protein and globulin: The highest serum protein and globulin were in G2 and G3 respectively, but just total protein increased in a

significant extent compared to control ($p > 0.05$) (Table 1).

Hematology: The results of hematological parameters have been shown in Table 2. Total leukocyte count increased significantly in G2 and G3 ($p < 0.05$). The red blood cells count, packed cell volume (PCV), MCV, MCH, and MCHC did not show any significant difference among the groups (Table 2).

Discussion

According to the results, food supplemented by different concentration of PEE for 60 days had no stimulating effect on the growth indices including: average weight-gain (AWG), specific growth rate (SGR), feed conversion ratio (FCR) and feed efficiency ratio (FER) in *B. barbatus*. Cuesta et al. (2005) found that the specific growth rate was not affected by the dietary intake of propolis in gilthead seabream, but Abd-El-Rhman (2009) reported significant increase in AWG, SGR and FCR in tilapia fed with propolis enriched diet. They used propolis with the origin of northern Egypt, whereas our propolis was originally from the southern region of Iran. The difference between the origins of propolis, which influence its quality, may be one of the main reasons for the incoherence among the different works. Silici and Kutluca (2005) reported that honeybee race has a great impact on the chemical composition and bioactivity of propolis yield. Difference between the physiology of fish species can be another reason.

The lysozyme activity is an important indicator of the immune defence in both invertebrates and vertebrates (Ellis, 1999). Lysozyme causes hydrolysis of the N-acetylmuramic acid and N-acetylglucosamine, which are constituents of the peptidoglycan layer of bacterial cell wall and activate the complement system and phagocytes by acting as an opsonin (Magnado ttir, 2006). In this study food supplemented with 0.5% and 1% PEE significantly increased the serum lysozyme activity and stimulated the immune response in *B. barbatus*. Increased lysozyme activity has been reported after supplementing the fish diet with propolis (Abd-El-Rhman, 2009), propolis and Chinese herbs (Zhang et al., 2009), herbal extracts (Alishahi et al., 2010), and

Table 1. Changes in the immunological parameters in *B. barbulus* fed with diet containing different level of PEE. Legends are the same as in Figure. 1. Significant differences ($p < 0.05$) are marked by different letters.

Parameters	G1	G2	G3	Control
Serum bactericidal activity*	198±12.63 ^b	171±15.1 ^a	186±15.9 ^b	200±18.2 ^b
ACH ₅₀ (unit mL ⁻¹)	428±18.1 ^a	451±17.7 ^a	448±18.1 ^a	435±14.7 ^a
Total serum protein (g dL ⁻¹)	3.16±0.23 ^a	3.71±0.2 ^b	3.56±0.26 ^b	3.15±0.27 ^a
Serum globulin (g dL ⁻¹)	1.93±0.2 ^a	2.15±0.23 ^a	2.17±0.22 ^a	2.03±0.19 ^a

Table 2. Changes in the hematological parameters in *B. barbulus* fed with diet containing different level of PEE. Legends are the same as in Table. 1. Significant differences ($p < 0.05$) are marked by different letters. (*) Number of live bacteria, counted in TSA media.

Parameters	G1	G2	G3	Control
RBC count ($\times 10^6$ cell/mm ³)	1.21±0.15 ^a	1.31±0.14 ^a	1.23±0.08 ^a	1.29±0.15 ^a
PCV (%)	31.73±5.84 ^a	34.87±5.68 ^a	33.07±4.92 ^a	31.47±2.19 ^a
Hb	8.06±0.40 ^a	8.18±0.47 ^a	8.57±0.42 ^a	8.15±0.32 ^a
MCV (fl)	275±23 ^a	291±10.7 ^a	286±20.5 ^a	269±16.7 ^a
MCH (pg)	64±4.5 ^a	65.4±3.4 ^a	66.1±4.5 ^a	61.8±4.1 ^a
MCHC (%)	24.9±3.5 ^a	21.8±2.4 ^a	21.6±2.3 ^a	25.3±2.1 ^a
Lymphocyte (%)	81.7±1.02 ^a	83.2±1.02 ^a	82.8±1.20 ^a	84.9±0.82 ^a
Neutrophil (%)	17.73±1.00 ^a	16.27±0.77 ^a	16.58±0.96 ^a	15.18±0.66 ^a
Eosinophil (%)	0.37±0.13 ^a	0.37±0.13 ^a	0.37±0.16 ^a	0.44±0.13 ^a
Monocyte (%)	0.71±0.17 ^a	0.67±0.18 ^a	0.67±0.15 ^a	0.63±0.13 ^a

vaccine injection (Alishahi and Kurt, 2006).

Results also showed that although supplementing diet with 0.5% PEE significantly increased the serum bactericidal activity against *A. Hydrophila* ($p < 0.05$), diet supplemented with 0.1% and 1% PEE had no stimulating effect on serum bactericidal activity compared to the control group. Abd-El-Rhman, 2009 reported significant increase in serum bactericidal activity following the administration of propolis in tilapia. Divyagnaneswari et al., (2007) in tilapia and Kajita et al., (1990) in rainbow trout reported an increase in serum bactericidal activity after administration of biological immunostimulants.

Complement, another component of the non-specific humoral immune response, was also studied in the present study. Diet supplemented with PEE had no inducing effect on any changes in the alternative pathways compared with control treatments ($p > 0.05$). Although complement activity increased following oral administration of propolis in gilthead sea bream (Cuesta et al., 2005) and immunostimulants (Sakai, 1999), other workers found that oral administration of some immunostimulants had no inducing effect on alternative complement pathway in fish (Alishahi et al., 2010; Selvaraj et al., 2005). These contradictory results may be due to differences in immune system

of fish species or immunostimulant characteristics.

Total serum proteins increased in G2 and G3 treatments ($p < 0.05$), but no significant differences were observed in serum immunoglobulins among the groups ($p = 0.067$). Probably stimulating immune related proteins such as, lysozyme, complement component, antibacterial peptides and so on, caused an increase in the total serum proteins of propolis treated fish.

Significant enhancement in the WBC count was observed in fish fed with 0.5% and 1% PEE supplemented food in comparison with the control ($p < 0.05$). The administration of 0.1% PEE had no stimulating effects on any change ($p > 0.05$) in WBC count. The present results concur with the reports of previous workers which describe increments in the intensity, mobility and activities of leukocytes, and in the production of IL-1, TNF and activating factors of mammalian leucocytes after in vitro or in vivo treatment with propolis (Dimov et al., 1992, Ivanovska et al., 1995; Murad et al., 2002; Cuesta et al., 2009). Probably, the increase in the leucocyte count might have resulted in the enhancement of the nonspecific defence, because leucocytes are the key elements in the immune system and are the major affecter and effector cells on which propolis exerts its

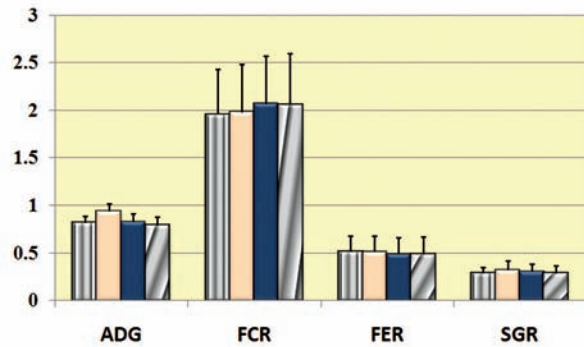


Figure 1. Effect of oral administration of different concentration of PEE on growth parameters: Average daily gain (ADG), food conversion ratio (FCR), feed efficiency ratio (FER) and specific growth rate (SGR) of *B. barbulus* fed for 60 days. G1: groups fed with commercial basal diet containing 0.1% PEE, G2: groups fed with commercial basal diet containing 0.5% PEE, G3: group fed with commercial basal diet containing 1% PEE and Control: groups fed with commercial basal diet free from PEE.

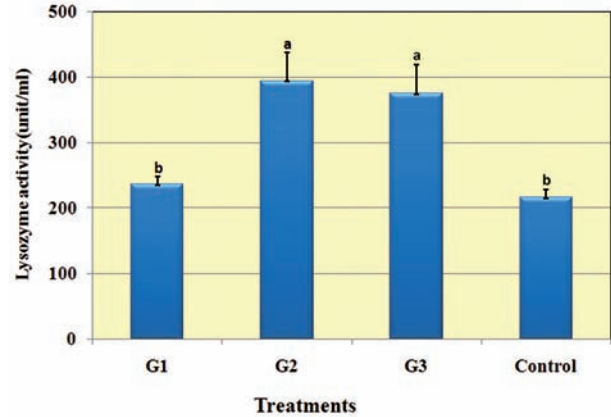


Figure 2. The effect of administration of food supplemented with different dose of PEE on Lysozyme activity in *B. barbulus*. Legends are the same as in Figure 1. Significant differences ($p < 0.05$) are marked by different letters.

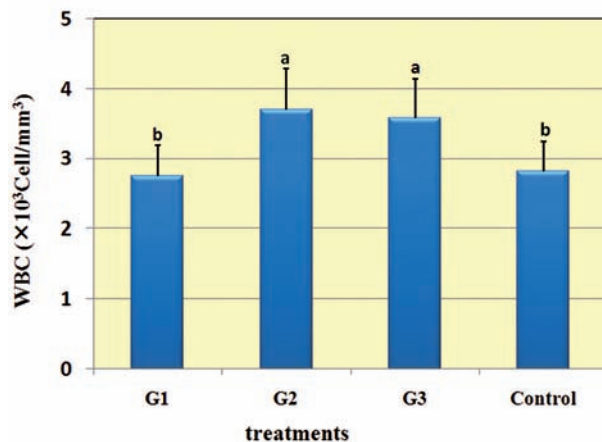


Figure 3. The effect of administration of food supplemented with different dose of PEE on WBC value in *B. barbulus*. Legends are the same as in Figure 1. Significant differences ($p < 0.05$) are marked by different letters.

activities.

The differential leucocytic-count is an indicator of health in fish (Fox et al., 1997). The current study showed no significant changes in the type of leucocytes (lymphocytes, neutrophils, monocytes and neutrophils) among the experimental groups ($p > 0.05$). Conversely, Abd-El-Rhman et al., (2009) reported a decrease in the neutrophils under oral administration of propolis in tilapia. Also, Cuesta et al., (2005) reported that water and ethanolic-extracts

of propolis increased the percentage of phagocytes in gilthead seabream. Besides, Talas and Gulhan (2009) reported deep changes in differential leucocytic-count following administration of toxic concentration of propolis via immersion route in rainbow trout. Propolis enhanced the macrophage functions and lymphocyte proliferation in several mammalian species (Manolova et al., 1987; Tatefuji et al., 1996; Murad et al., 2002; Ansoerge et al., 2003). The difference of fish species and their physiology may be the reason for these contradictory results.

It is known that exogen agents can change hematological parameters, such as erythrocyte number, Hb amount, hematocrit value and haemoglobin indexes (Selvaraj et al., 2005). In this study, there were no significant differences in PCV, RBC, Hb, MCV, MCH and MHCH in the control or PEE treated groups. PCV and RBC are general indicators for fish health and help to describe abnormalities caused by immunostimulants (Selvaraj et al., 2005). Contrary results were observed between previous studies: Abd-El-Rhman (2009) reported an increase in hematocrit value following oral administration of propolis in tilapia. Also, Talas et al., (2009) emphasized that toxic concentration of propolis (in water) induced changes in hematological parameters in rainbow trout. These contradictory results suggest that further studies using other sources of propolis and other fish species are necessary to confirm or refute these findings (Kleinrok et al., 1978).

It is important to estimate bacterial resistance of

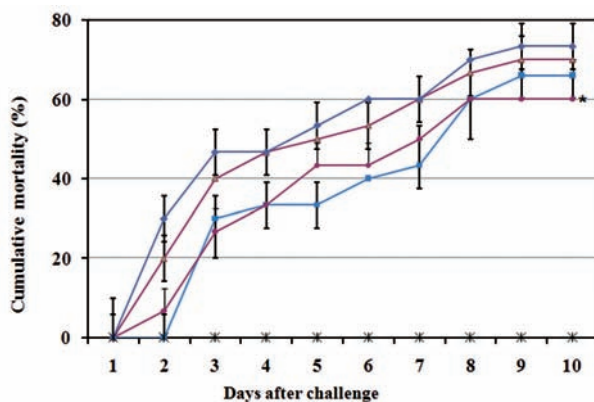


Figure 4. Cumulative mortality of *B. barbulus* in bacterial challenge after oral administration of different dose of PAA. "Shieh" stands for a control challenge without bacteria. The results represent Mean \pm SD (n=20) in each treatment. Statistical significance between treatments: (*) ($p < 0.05$). In cumulative mortality, the difference between G2 (fish fed with food supplemented with 0.5% PEE) and Control is statistically significant.

— G3 — G2 — G1 — Control ✕ Shieh

treated fish to determine the efficiency of an immunostimulant. The supplemented diet with 0.5% PEE significantly decreased the *A. hydrophila* induced mortality when compared to the control group ($p < 0.05$). These results indicated that the PEE activated the immune system of the *B. barbulus*. Decreased mortalities, after challenge with *A. hydrophila* were reported in tilapia fed on propolis extract (Abd-El-Rhman, 2009), vaccinated *Carassius auratus gibelio* injected with propolis water extract (Chu, 2006), Chinese sucker fed on propolis and Herbal extracts (Zhang et al., 2009), *C. carpio* fed on Aloe vera extract (Alishahi et al., 2010) and Chinese herbs (*Astragalus radix* and *Ganoderma lucidum*) (Yin et al., 2008).

To conclude, we have found that oral administration of propolis (0.5% in food) had the best stimulating effects on the *B. barbulus* innate immune system, but had no effect on growth-performance and hematological parameters. The use of propolis as a fish immunostimulant for fish farming purposes needs validation with further studies, using different propolis products and other fish species.

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بررسی اثر بره موم محصول زنبور عسل بر رشد و برخی فاکتورهای ایمنی و خونی ماهی برزم

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چکیده

زمینه مطالعه: بره موم، محصول جنبی زنبور عسل، برای قرن‌ها بصورت تجربی و به عنوان محرک ایمنی مورد توجه بوده است. **هدف:** در این تحقیق اثرات تجویز بره موم بر فاکتورهای رشد، پاسخ ایمنی ذاتی و پارامترهای خونی ماهی برزم مورد ارزیابی قرار گرفت. **روش کار:** ۳۶۰ قطعه ماهی با خوراک حاوی ۰، ۱/۰، ۰/۵ و ۱٪ عصاره الکلی بره موم (PEE) در خوراک به مدت ۶۰ روز تغذیه گردیدند. در انتهای تحقیق فاکتورهای ایمنی (میزان فعالیت لایزوزیم سرم، قدرت باکتری کشی سرم، فعالیت کمپلمان، پروتئین تام و میزان ایمونوگلوبولین سرم) و پارامترهای خونی (شامل تعداد گلبولهای قرمز، تعداد گلبولهای سفید، هموگلوبین، هماتوکریت و اندیس‌های گلبولی (MCH, MCHC) و MCV) بین تیمارها مقایسه گردید. بعلاوه فاکتورهای رشد نیز بین تیمارها مقایسه گردید. سپس ماهی‌ها با باکتری آئروموناس هیدروفیلا بصورت تزریق داخل صفاقی چالش داده شده و تلفات در مدت ۱۰ روز ثبت شد. **نتایج:** افزایش معنی دار فعالیت لایزوزیم سرم و قدرت باکتری کشی سرم، میزان پروتئین تام و تعداد گلبولهای سفید خونی در تیمارهای ۰/۵ و ۱٪ نسبت به تیمار کنترل بود ($p < 0.05$). در صورتیکه در فاکتورهای رشد (ضریب رشد ویژه، ضریب تبدیل غذایی و میزان بازده غذایی) و فاکتورهای خونی تغییر معنی داری ایجاد نگردید ($p > 0.05$). ضمناً تلفات بعد از چالش با باکتری زنده آئروموناس هیدروفیلا نیز تنها در تیمار ۰/۵٪ کاهش معنی داری در میزان تلفات نسبت به سایر تیمارها مشاهده شد. **نتیجه‌گیری نهایی:** اضافه نمودن ۰/۵٪ عصاره الکلی بره موم به خوراک ماهی برزم باعث تحریک برخی پاسخ‌های ایمنی و افزایش مقاومت در برابر عفونت باکتریایی می‌گردد، هرچند تجویز این ماده در خوراک تأثیری در شاخص‌های رشد و فاکتورهای خونی این ماهی ندارد.

واژه‌های کلیدی: ماهی برزم، بره موم، رشد، پاسخ ایمنی، فاکتورهای خونی.

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