Iraqi Journal of Veterinary Sciences, Vol. 27, No. 1, 2013 (57-60)

The effect of replacing fishmeal with *Spirulina* on microbial load in common carp *Cyprinus carpio* L

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(Received February 17, 2013; Accepted April 7, 2013)

Abstract

The use of blue green algae *Spirulina* in aquaculture has several potential advantages over the production of fish. This study was designed to investigate the effect of different replacement levels of fishmeal with *Spirulina* on microbial load of common carp *Cyprinus carpio* L., the trail was conducted for 105 days and for this purpose 200 fingerlings common carp. Mean initial weight was (32.7 g). The fish were acclimated to laboratory conditions and fed with control pellets (32% protein) prior to the feeding trials for 21 days. Five experimental diets were used and *Spirulina* replaced fishmeal protein from the standard diet at 0% (T1), 5% (T2), 10% (T3), 15% (T4) and 20% (T5) levels. Bacterial total account in rearing water and the bacterial total count in carp intestine for the third treatment was higher significantly as compared to other treatments, which is the conclusion of this study.

Keywords: *Spirulina*: Fishmeal; Microbial load; Intestine.
Available online at http://vetmedmosul.org/ijvs

تأثير استبدال مسحوق السمك بالطحلب الأخضر المزرق في الحمل الميكروبي لأسماك الكارب العادي

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الخلاصة

إن لاستخدام الطحلب الاخضر المزرق *Spirulina* عدة فوائد في زيادة إنتاجية الاسماك، تم تصميم هذه التجربة لتقييم تأثير استبدال مستويات مختلفة من مسحوق السمك بالطحلب *Spirulina* في الحمل الميكروبي لاسماك الكارب العادى *Cyprinus carpio* L.، استغرقت التجربة مدة 105 يوم ولهذا الغرض تم استخدام 200 اصبعية كارب عادى .كان متوسط الوزن الاولى (32.7غم)، تم اقلمة الاسماك للظروف المختبرية والعلف التجريبي باستخدام حبيبات نسبة البروتين فيها 32% قبل البدء بالتجربة الفعلية ولمدة 21 يوم .تم استخدام خمس علائق تجريبية واستبدل مسحوق السمك بالطحلب Spirulina من الاساس وبنسب (T1) 0% ، و (T2) 5% ، 10% (T3) ، 15% (T4) ، 20% (T5) سجلت المعاملة الثالثة اعلى القيم معنويا في كل من العد الكلي البكتيري لمياه التربية واعلى عد بكتيري لأمعاء الكارب المرباة.

Introduction

Diet supplementation is an important aspect in aquaculture management especially in intensive or in semi-intensive fish culture, and is promising for increasing fish production (1,2). However, protein is essential for normal tissue function, for the maintenance and renewal of fish body protein and for growth. Due to the cost of the protein, the feed will be more cost effective if all the protein is used for tissue repair and growth and little catabolized for energy (3).

Spirulina is a cyanobacterium that has been commercially cultivated for more than 10 years due to its high nutritional content; e.g. protein, amino acid, vitamin, minerals, essential fatty acid and β- carotene (4). Preclinical testing suggests *Spirulina* has hypocholesterolemic, immunological, antiviral, and antimutagenic properties (5).

Spirulina can be considered a nutritional supplement that has various health benefits for humans, and a feed supplement for animals having economic benefits, as an example, it can be a suitable food supplement when fed to trout, sea bass, fancy carp, red tilapia, shrimp and mollusk. It has been found that the alga can be used as an alternative source of protein and can be used to improve the color, flavor and quality of meat (6). Researchers have reported the therapeutic effects of *Spirulina* as a growth promoter, probiotic, and booster of the immune system in animals including fishes (7). *Spirulina* is used to promote the growth of livestock, poultry, prawn, carp, canaries and exotic birds (8).

Previous researchers demonstrated that *Spirulina* capable to breaking down indigestible feed components and due to that improves the intestinal flora in fish (9). The production and releasing of enzymes that transport fats for growth instead of storage in fish. In addition, β-carotene in *Spirulina* firmly maintains the mucous membrane and thereby prevents the entry of toxic elements into the body. Chlorophyll in *Spirulina* acts as a cleansing and detoxifying factor against toxic substances (10). The Aim of this study was to examine the effects of replacing fishmeal with different levels of *Spirulina* on microbial load of common carp water rearing and intestine.

Materials and methods

This study was conducted in the Fish Laboratory of the Department of Animal Production, Faculty of Agricultural Sciences, University of Sulaimaniya, Iraq.

Experimental system and design

Twenty plastic aquariums (100 L) were used in this trial. Each tank was provided with a proper continuous aeration. Each aquarium was stocked with seven fish and fed two times a day. The numbers of treatments in the trial were five with four replicates for each. The aquaria (replicates) were randomly allocated to minimize differences among treatments. The continuous water flow discharged non-consumed feed and feces particles from the aquaria. Also, a daily cleaning by siphon method was applied to remove remained particles from the system.

In T1 fish were fed a diet replacing fishmeal with 0% *Spirulina*, While in T2, fish were fed a diet replacing fishmeal with 5% *Spirulina*, T3 represents the third treatment, in which fish were fed on a diet replacing fishmeal with 10% *Spirulina*, While, in T4 fish were fed a diet replacing fishmeal with 15% *Spirulina*, and final treatment T5 replacing fishmeal with 20% *Spirulina*.

Diet formulation

Experimental diets were prepared with fishmeal, wheat bran, soybean, broken rice, vitamin and *Spirulina*, and the chemical composition of the different diet shown in Table (1). The ingredients were mixed with water to obtain dough. Then, the dough was passed through an electrical mincer for pelleting by using Kenwood Multi-processors. The pellets were dried at room temperature for a few days and crushed to yield fine particles. The fish were fed 2 times a day.

Table 1: The structure of experimental diet.

	Basis on 100 kg				
Spirulina	0%	5%	10%	15%	20%
Fishmeal	24.2	21.7	19.2	16.8	14.2
Wheatbran	35	35	35	35	35
Soybean	20	20	20	20	20
Broken Rice	20.3	17.8	15.3	12.7	10.3
Vitamin	0.5	0.5	0.5	0.5	0.5
Chemical composition					
Crude Protein %	32	32	32	32	32
Crude Fat %	6.7	6.4	6.0	5.7	5.4
Fiber %	7.6	7.6	7.5	7.5	7.5

Used *Spirulina*

500g of premium sinking *Spirulina* wafers, these top quality-sinking wafers are rich in *Spirulina* suitable for all herbivorous fish such as pleco's and catfish as well as shrimps and snails. Their chemical composition as labeled in the below table (2).

Table 2: Chemical composition of used *Spirulina* as labeled.

Composition	Ratio %
Crude Protein	34%
Crude Fat & Oils	6%
Fibre	5%
Ash	10%
Vitamin A(Per kg)	24000lU
Vitamin D	2600lU
Vitamin E	280lU
Vitamin C	550mg/kg

Microbial load

Water samples were collected at the end of experiment with separate sterile plastic containers, which were transferred to the laboratory for the microbial study.

Aseptically, 1 ± 0.1 g of the sample were weighed, transferred into a sterile blender jar, then 9 ml sterile phosphate buffer were added and blended at high speed for two minutes. This became the 1:10 dilution. The foam was permitted to settle, and then 10 ml of the blended 1:10 dilution were pipetted into a 90 ml dilution blank to make 1:100 dilutions. The procedure was repeated to prepare serial dilutions of 10^{-3}, 10^{-4}, etc. All dilutions were shaken 25 times in a one-foot arc.

The dilutions were prepared before use according to the procedure of APHA (11). About 1ml from the 10^{-1}, 10^{-2}, 10^{-3}, 10^{-4} etc., then the melted plate count agar was used (Biolife, Italy). After culturing, the plates were incubated at 35 ºC (Memmert, Germany) for 48 hrs. For calculation, the dilution reverse was multiplied by the average count of double plate. A colony counter (W.T.W.BZG30, Korea) was used to count colonies on the duplicate plates.

After fish dissection the intestine will be taken and washed with peptone water carefully, then make the microbial counts as the above procedure used for water samples.

Statistical analysis

Analysis of variance was conducted using the general linear models (GLM) procedure of XLSTAT. Pro. 7.5 one way (ANOVA). Fisher's L.S.D test's was used to compare between means of the control and experiment treatments. The modle of analysis was as follows:

$Yij = \mu + Ti + Eij$

μ = The overall mean.

Ti = The effect of treatment.

Eij= The random error.

Results

Table 3: Effect of replacing fishmeal with different levels of *Spirulina* on total bacterial counts in rearing aquarium water and carp intestine.

Treatment	Bacterial Total Counts	
	In water pond $*10^4$	In carp intestine $*10^4$
T1	50.667 c	421.333 b
T2	190.000 b	149.000 c
T3	231.667 a	683.333 a
T4	172.333 b	78.667 cd
T5	72.000 c	6.667 d

Mean values with different superscripts within a column differ significantly (P≤0.05).

In table (3) Bacterial total counts in rearing water showed 231.667, 190.000, 172.333, 72.000 and 50.667 for the (T3) 10% *Spirulina*, (T2) 5% *Spirulina*, (T4) 15% *Spirulina*, (T5) 20% *Spirulina* and (T1) 0% *Spirulina* respectively observed (T3) had higher significant (*P<0.05*) difference among other dietary groups. In the same table bacterial total counts in carp intestine data ranged 683.333, 421.333, 149.000, 78.667 and 6.667 for the (T3) 10% *Spirulina*, (T1) 0% *Spirulina*, (T2) 5% *Spirulina*, (T4) 15% *Spirulina* and (T5) 20% *Spirulina* respectively observed that (T3) had higher significant (P<0.05) difference among other dietary groups.

Discussion

In a study of (12), the results of bacteria challenge, bactericidal activity suggest the increase in phagocytosis in blood, which have an important role for prevention of infectious disease. Phagocytosis by these cells is a process of internalization, killing and digestion of invading microorganisms. (13-19).

Watanuki *et al*. (20) estimated the fluctuation in the number of bacterial cells in *Spirulina*-treated fish organs after an experimental challenge with *A. hydrophila*. They found that the bacterial numbers were lower in the liver and kidney of carp treated with *Spirulina* than the control group suggesting the increased resistance *A. hydrophila* infection.

There also established the absence of *E. coli*, *Salmonella*, and *Staphylococcus aure*us, total aerobic bacteria of less than 200,000 colony forming units per gram (cfu/g), and total coliforms of less than 10 cfu/g. the composition of *Spirulina*. As an example of *Spirulina* powder used is Pacifica™ which is a free-flowing green to bluish-green powder. It has a mild seaweed odor and is not soluble; it forms a suspension (21).

Antibacterial activity of various extracts of *Spirulina* was tested against different strains of fish and shellfish pathogens. The *Chlorella* spp., *Scenedesmus* spp., *Chlamydomonas* spp., *F. ambigua*, *Microcystis aeruginosa*, have been reported as the main groups of microalgae to produce antimicrobial substances. The ability to produce antimicrobial substances may be significant not only as a defensive mechanism for the algal strains but also as a good source of the new bioactive compounds from a pharmaceutical point of view (22).

The results concerning the biological antimicrobial activity of antimicrobial agents produced by *Spirulina* were recorded. It is clear from the above result that ethanolic extract of *Spirulina* is comparatively more effective against three strains of *A. hydrophila* (AH2, AH4, ATCC 49140), two species of *Vibrio* (VAN,VFS) and two strains of *E. coli* (O1, O115), the ethanol, acetone, diethyl ether and methanol extracts of *S. platensis* revealed antibacterial activity on *E. coli*, *S. aureus* and *P. aeruginosa.*

Screening efforts aimed to identify antimicrobial agents in microalgae have revealed several promising compounds. Significant differences in the antimicrobial activity were

found depending on the solvent used. In general, extracts obtained using ethanol were more active than those obtained with hexane, the functional properties of *Spirulina* have been attributed to different compounds such as, phycocyanins, carotenoids, phenolic acids and ω-3 andω-6 polyunsaturated fatty acids (23). *Spirulina* lipids were investigated as a natural source off functional bioactives because of its usefulness for human health.

Several authors (24) have attributed the cyanobacteria antimicrobial activity to different compounds. As only crude extracts of *Spirulina* examined, thus, the result of the present study supports the folkloric usage of the studied cyanobacteria and suggests that the *Spirulina* extract possesses certain constituents with antibacterial properties that can be used as antimicrobial agents for the therapy of microbial infectious diseases (25).

The extracts showed maximum activity against pathogenic microbes subjected to isolation of the therapeutic antimicrobials and hence the need to carry out further pharmacological evaluation (23).

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