

MICROBIAL LOAD IN THE LINE COMMON CARP REARING WATER FED DIFFERENT LEVELS OF DRY YEAST

(Received: 28.8.2012)

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ABSTRACT

This study was conducted at the Fish Laboratory, Animal Production Department, College of Agriculture, University of Sulaimani using commercial dry yeast at four levels (zero, 3, 5 and 7%) to study their effects on the microbial load in fish aquaria water, total weight gain (g), daily growth rate (g/day) and relative growth rate (%) as well as the pH of aquaria water. The experiment included four treatments, each in three replicates (plastic aquaria) in which 10 finger lings of common carp of the same weight (3.5 gram) were stocked in each aquarium. The actual duration of experimental feeding trial was three months. The results showed that 7% of dietary yeast gave the best result of total weight gain, daily growth rate, and relative growth rate. Moreover, it had the lowest microbial load which decreased from 6.2×10^5 to 3.15×10^4 at the end of the experiment as compared to the control without dry yeast that increased from 2.01×10^3 to 5.4×10^3 . No significant differences were observed among the treatments in total weight gain (g), daily weight gain (g/day) and relative growth rate (%). The pH value ranged from 6.8 in the control treatment to 7.4 in the second treatment (5% yeast). Accordingly, dry yeast could be used safely in feeding common carp up to 7% to attain the best microbial load.

Key words: carp, dry yeast, microbial load, relative growth weight, total daily growth rate, weight gain

1. INTRODUCTION

Probiotics can be defined as live microbial feed supplement that beneficially affect the host animal by improving its intestinal balance (Fuller, 1989). Most studies on the effects of probiotics on cultured aquatic animals have emphasized a reduction in mortality or the improved resistance against putative pathogens (Denev *et al.*, 2009; Irianto and Austin, 2002). However, the beneficial effects are sometimes temporal, depending on the time of exposure (Balcázar *et al.*, 2006; Verschuere *et al.*, 2000).

In the last decade, the scientific community carefully examined the roles and effects of probiotics in aquaculture as an alternative to antimicrobial drugs, demonstrating positive effects on fish survival (Denev *et al.*, 2009), growth (Burr *et al.*, 2005), stress resistance, immune system enhancement and finally general welfare (Balcázar *et al.*, 2006; Denev *et al.*, 2009).

Microbial quality of farmed fish is largely determined by the quality of the water in which they were cultivated (Buras, 1993; APHA, 1998; Balcázar *et al.*, 2006 and Fafioye, 2011). Fish dependency on water is crucial. Hence the source,

volume and the quality of physico-chemical parameters such as dissolved oxygen, total hardness, pH, alkalinity, carbonate, nitrate, and temperature are the major factors to consider in relation to fish health. This brings about revelation on the influence of bacterial load and types of genera in water bodies (Denev, 2008; Liu *et al.*, 1992). Similarly, aquafeeds fed to cultured fish have been reported to have a considerable impact on the load of bacteria (Guo *et al.*, 1988 and Azad *et al.*, 1997).

The relationship between bacteria and the environment has received the attention of researchers (Fang *et al.*, 1989; Fuller, 1989). However, the studies undertaken have been mostly limited to the relationship among aerobic heterotrophic bacteria and not all bacteria in the environment. Therefore, this study was designed to:

- (i) Estimate the level of bacteria abundance in each aquarium.
- (ii) Estimate pH variations among treatments.

2. MATERIALS AND METHODS

The experiment was conducted for 12 weeks,

using common carp (*Cyprinus carpio*, 3.5 g average weight) finger lings obtained from Dukan hatchery. The experimental system included 100 L plastic tanks, each represents a replicate. Water samples were collected twice per month with separate sterile plastic containers, which were transferred to the laboratory for the microbial study.

Aseptically, 25 ± 0.1 g of the sample were weighed, transferred into a sterile blender jar, then 225 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 (1998). 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 °C (Mermert, 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.

The tested commercial dry yeast probiotics (*Saccharomyces cerevisiae*) were used to study their effects on growth performance of common carp (*Cyprinus carpio* finger lings. The animals were allowed to adapt to the experimental system for a week and fed with a conventional diet, contained 30% crude protein and 2880 kilocalori energy. There after the different treatments (the control treatment without dry yeast, 3% dry yeast, 5% and 7% dry yeast) were randomly assigned to the tanks with three replicates per treatment. Feed was manually administered *adlibitum* 2 times a day for 12 weeks. A daily record was kept of feed offered. Bulk weight of the cultured fish in each aquarium was measured weekly to calculate growth in weight. The experimental diets used in the study represent the standard diet found in local markets.

All data were analyzed by a one-way ANOVA using a Statistical Analysis software Program of SPSS (XLSTAT-PRO 7.5). The Duncan's Multiple Range Test was used to determine the differences between the treatment means. The results were considered statistically significant at the level of $P < 0.05$ (Duncan, 1955).

The weight gains (%), RGR were evaluated based on the standard formula as follows as mentioned in (Jafaryan *et al.*, 2011):

$$WG = W_1 - W_0$$

Where: WG = Average weight gain (g)

W_1 = Average final weight (g)

W_0 = Average initial weight (g)

Daily weight gain = $(W_1 - W_0) \div T$

Relative growth rate (%) =

$$[(W_1 - W_0) \div W_0] \times 100.$$

Where, W_0 = Initial body weight (g), W_1 = Final bodyweight (g) and T= Time (day)

3. RESULTS AND DISCUSSION

The bacterial loads in water are shown in Table (1). The total bacterial load varied from 2.01×10^3 to 5.4×10^3 cfu ml⁻¹ in the control, and from 2.63×10^3 to 3.0×10^4 in the first treatment (3% dry yeast). With increasing yeast level, the total bacterial load changed from 4.8×10^4 to 3.65×10^3 , and from 6.2×10^5 to 3.15×10^4 in the second and third treatments, respectively. The results showed pronounced variations in total bacterial load of aquarium water over time.

The bacterial load in the samples was the highest in August. This may have resulted from the high water temperature (32°C). While, low temperature (15°C) may be the major factor in decreasing bacterial loads in the pond water. It was reported that bacterial load might be increased with an increase in water temperature (Al-Salim *et al.*, 2009; APHA, 1998).

Table (1): Microbial load in aquarium water

Sampling	Control	T1(3% DY)	T2(5% DY)	T3(7% DY)
Time 1	2.01×10^3	2.63×10^3	4.8×10^4	6.2×10^5
Time 2	5.65×10^2	1.505×10^4	9.6×10^5	4.15×10^3
Time 3	4.25×10^1	3.3×10^3	4.4×10^3	1.1×10^4
Time 4	5.4×10^3	3.0×10^4	3.65×10^3	3.15×10^4

DY: dry yeast; Time 1 at 17th august; Time 2 29th august; Time 3 at 6th September; Time 4 at 29th September.

The data illustrated in Table (2) show that 7% yeast treatment was more effective in enhancement of body weight of common carp *Cyprinus carpio*. These results are in agreement with the findings of Ozório *et al.* (2012) who found that 10% brewer's yeast were the optimal level, where more than this level (20 and 30%) performed worse as regards to body weight. Different workers (Allam, 2007; Mohapatra *et al.*, 2012; Pooramini *et al.*, 2009; and Tewary and Patra, 2011) reported similar positive effects of dietary Pronifer, algae, and yeast on the average body weight gain of tilapia fish.

Table (2): The effect of dry yeast levels on the growth performance of common carp fingerlings:

Dry yeast (%)	Characteristics		
	Weight gain (g)	Daily weight gain (g/day)	Relative growth rate (%)
Zero	12.32 ±0.0 a	0.15 ± 0.0 a	353.01 ± 0.0 a
3	9.37 ± 2.22 a	0.12 ± 0.03 a	286.73 ± 24.14 a
5	7.39 ± 2.59 a	0.09 ± 0.03 a	249.17 ± 79.27 a
7	11.20 ±1.06 a	0.13 ± 0.01 a	318.94 ± 40.39 a

All the probiotic-supplemented diets resulted in higher growth than that of the control diets, suggesting that the addition of the probiotics mitigated the effects of the stress factors. This resulted in better fish performance, with better growth results in the diets supplemented with the dry yeast (Iranshahi *et al.*, 2011; Lara-Flores *et al.*, 2003). Further more, it was reported that the inclusion of yeast in the diet improves feed efficiency, organic phosphorus (phytic acid) utilization, and fiber digestion (Al-Salim *et al.*, 2009, Jafaryan *et al.*, 2011; Tewary and Patra, 2011). In this respect, Noh *et al.* (1994) studied the effect of supplementing yeast (*S. cerevisiae*) and bacteria (*Streptococcus faecium*) in the diet of Israeli carp and reported the better growth response of fish fed probiotic-supplemented diets than that of the diet without probiotic supplementation.

Many published reports demonstrated positive effects of probiotics and prebiotics in feeds for various fish species, including common carp (*Cyprinus carpio*) (Denev, 2008; Randall, 1991; Staykov *et al.*, 2005a,b, and c, d; Staykov *et al.*, 2006a,b,c; Staykov *et al.*, 2007a,b; Yanbo and Zirong, 2006).

However, the obtained results could be explained by some factors as dietary yeast improved growth performance due to yeast is a source of protein (De Silva, 1989). Yeast acts as a source of enzymes, *i.e.* amylase, protease and lipase that may improve food digestion and consequently food utilization. Moreover, yeast is a very good source of vitamin B6 (39.8 mg/kg dry matter) as reported by Mc Dowell (1989). Vitamin B6 may act as a stimulator of growth hormone (Allam, 2007).

In fish culture, water pH is lowered by CO₂ produced from the respiration of fish and bacteria. Metabolism, and CO₂ production, increase as fish grow in the system (Attramadal, 2011). Abd El-Halim *et al.* (1989) found that the addition of living yeast in diet improved the performance of *O. niloticus*. Also, Scholz *et al.*

(1999) reported that *S. cerevisiae* improved the growth and survival of sea bass fry. They attributed this action to adherence of *S. cerevisiae* cells to the gut and the secretion of amylase enzymes, which share in increased digestibility of the diet. On the other hand, the increased growth performance of *O. niloticus* treated with commercial products (Megalo and Diamond-V yeast, containing living *S. cerevisiae* with *B. subtilis* and dead *S. cerevisiae*, respectively) could be also attributed to the inhibition of some intestinal bacterial flora and increasing the non-specific immunity of the treated *O. niloticus*. The adherence capacity of *S. cerevisiae* and *B. subtilis* to the intestinal mucosa inhibits the attachment of the other intestinal bacteria to these binding sites and so preventing the disease occurrence with its negative impact on fish growth (Marzouk *et al.*, 2008).

The values of pH are within the ranges recommended for warm water fish cultured (pH of 6.8±0.2) which falls within the 5-9 range given by (Staykov *et al.*, 2007 a & b) and with that of Abdulrahman and Muhammad, (2012), though the average pH taken from the aquaria gave 6.8; it was still desirable for fish production as shown in Table (3). The desirable range for pond pH is 7.97- 8.06 and the acceptable range is 5.5 - 10.0 (DWAMD, 1994). The present results are in agreement with (El-Nemaki *et al.*, 2008).

In conclusion, the adding of 7% dietary yeast gave the best result of the total weight gain, growth rate and it had the lowest microbial load. Therefore, we recommend adding 7% dietary yeast for obtaining the best microbial load in common carp water.

Table (3): The effect of yeast addition on aquaria water pH.

Dry yeast (%)	pH
Zero	6.55 a
3	6.85 a
5	7.2 a
7	6.9 a

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الحمل الميكروبي لمياه تربية اسماك الكارب العادي المغذاة على مستويات مختلفة من الخميرة الجافة

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ملخص

أجريت هذه الدراسة في مختبر الأسماك في قسم الإنتاج الحيواني، كلية الزراعة، جامعة السليمانية باستخدام الخميرة الجافة التجارية بارب مستويات (صفر، ٣٪، ٥٪، ٧٪) لدراسة تأثيرها على الحمل الميكروبي لمياه أحواض الأسماك، والزيادة الوزنية (غم)، ومتوسط النمو اليومي (غم/يوم) ومعدل النمو النسبي (٪) ودرجة حموضة مياه الأحواض. شملت التجربة في كل المعاملات الاربع ثلاث مكررات (أحواض بلاستيكية) خزن في كل منها عشرة إصبعيات من اسماك الكارب الشائع *Cyprinus carpio* من نفس الحجم والوزن (٣.٥ غرام) في كل حوض. إستمرت مدة التجربة الفعلية ثلاثة أشهر. اوضحت النتائج تفوق المعاملة الثالثة (٧٪ خميرة) حيث اعطت أفضل نتائج الزيادة الوزنية الكلية، معدل النمو اليومي، معدل النمو النسبي فضلا عن انخفاض الحمل الميكروبي الذي تغير من 6.2×10^4 الى 3.15×10^4 في نهاية التجربة مقارنة مع معاملة السيطرة الخالية من الخميرة والذي تراوح بين 20.1×10^2 الى 54×10^2 وذلك بدون أي فروقات معنوية بين المعاملات. وبوجه عام تراوحت درجة الحموضة في مياه الاحواض من ٦.٨ في معاملة السيطرة إلى ٧.٤ في المعاملة الثانية (٥٪ الخميرة). لذلك يمكن استخدام الخميرة الجافة في تغذية اسماك الكارب العادي حتى ٧٪ للوصول الى افضل حمل ميكروبي في المياه.

المجلة العلمية لكلية الزراعة - جامعة القاهرة - المجلد (٦٣) العدد الرابع (أكتوبر ٢٠١٢): ٤١٣-٤١٨.