



Evaluation the Role of Effective Microorganisms (EM) on the Growth Performance and Health Parameters on Common Carp (*Cyprinus carpio* L.)

Dh. M. Jwher* and M. R. Al-Sarhan

Department of Veterinary Public Health, College of Veterinary Medicine, University of Mosul, Iraq

*Corresponding Authors: Dh. M. Jwher, E-Mail: deea@gmx.us

ABSTRACT

The study aimed to evaluate the effects of Effective microorganisms (EM) on *C. carpio* by studying their effect on some growth and health Parameters. The study dealt with the calculation of weekly and total weight gain, food conversion and Somatic index. The Micromorphometric parameters, including Goblet cells, length of both villus of intestine and gills (primary and secondary) lamellae were also studied. Also estimation titer count of Lactic acid bacilli in the intestine. The results showed that there were significant differences at $P \leq 0.05$ between the control group and the EM treated group, and this difference was clear on the weekly and total weight gain, the effect was reflected on the organs weights represented by the liver, gills, anterior kidneys, posterior kidneys, intestines and spleen, micromorphometric study showed that there is a significant increase of villus lengths and primary and secondary lamellae for EM treated group, and this effect was withdrawn on the number of goblet cells present in the intestinal epithelium, a very significant increase in the mean number of lactic acid bacilli was recorded in the EM treated groups. The use of effective organisms is a useful strategy that has a clear impact on growth and health parameters in fish.

Keywords: Common carp fish, Effective microorganisms, gills lamellae, Villus lengths.

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INTRODUCTION

Fish cultures are essential to improve and promote the rapid growth and wide fish production. Such activity led to increase the quantities of consumed feed as well as food additives and supplements followed in feed system regimes due to initiate rapid growth and decrease production costs (Naylor *et al.*, 200). The use of therapeutic and chemical agents has led to the trial of an alternative approach to protection and disease prevention rather than the use of antibiotics that increase the survival chance (Ismail and Alhamdani 2019). However, prolonged administration of antibiotics may lead to the development of microbial resistance, with concerns about its unexpected consequences for public health (Lee and Ko 2016).

Effective Microorganisms (EM) is considered as food additive, immunostimulant, increase host resistance to diseases as well as it represented a good ecofriendly (Wuertz *et al.*, 2021). The results of several studies showed that the great EM on the hygienic status of the fish (Stephens *et al.*, 2015; Liu *et al.*, 2016). The improvement of the health is attributed to the modulation of EM on the causative agents as well as their improvement of water quality by

the adjustment of the various microbes of the water and sediment (Verschuere *et al.*, 2000). There are many works on the different uses of EM in multiple field of animal production as feed additives, However such studies lack the role of EM on the improvement of growth, productive parameters, gastro-intestinal and respiratory tracks of the fish. Therefore, the current throw light on the effect of EM addition to fish farms on some Growth and health parameters of the common carp fish.

MATERIALS AND METHODS

Fish and feeding:

Forty carp fish (*Cyprinus carpio* L.), their weight ranged between 28 to 36 gm were used in this experiment. Fish were kindly obtained from Yanki hatchery/Ministry of Agriculture of Erbil governorate/Iraq, Then transported to the animal house care in the Collage of Veterinary Medicine/ University of Mosul.

Fish were fed on carp feed produced by " GÜRDAL®"- Turkey Ltd, composed of 33% crude protein, 7.5% crude fat, 4.53% cellulose, 9.64% ash, 1.84% calcium, 1.40% phosphorous, 0.29% sodium,

0.62% methionine and 1.84% Lysine, with a granule size of 3 mm

Effective Microorganism (EM):

Liquid form of EM product (Maple Orgteeh™ - India Ltd under the supervision of EMRO Japanese institute - Okinawa-Japan) was used. EM stock solution is formed from Lactic acid bacilli: *Lactobacillus plantarum*; *L. acidophilus* *L. casei* *Streptococcus Lactis*; *B. subtilis*. Photosynthetic bacteria *Rhodospseudomonas palustris*; *Rhodobacter sphaeroides*, Yeast; *Saccharomyces cerevisiae*; *Candida utilis torula*, *Pichia jadinii*; Actinomycetes; *Streptomyces albus*; *S. griseus* and Fermenting fungi; *Aspergillus oryzae*; *Mucor hiemalis*.as described by the manufacturer

Fish groups:

After one week of expand acclimation period, fish were divided in to into two groups of two replicates for each group as follows:

The first group: Control group (C) (10 fish).

The second group: Treatment group (T). This group consists of ten fish treated with effective microorganism (EM). (Fish ration was treated with EM by spraying after their dilution in the rate 2ml of EM/1liter water. Later, the treated ration was put in the incubator at 37°C for five days).

Growth and health parameters:

According to (Shaima *et al.*, 2020), total and weekly increase in fish body were calculated as well as feed conversion rate (FCR) according to the following formulas :

Total body gain(gm/fish) = final weight (gm) - primary weight (gm).

$$FCR = \frac{\text{Total consumed Feed}}{\text{Total weight}}$$

Also, weights of liver, gills, anterior and posterior kidneys, intestine and spleen were calculated.

Micromorphometric parameters:

After the end of the experiment the fish were anesthetic (Al-koye, 2013) for collection the internal organs includes liver, Intestine, Spleen, Gills, Anterior kidney Posterior kidney were kept in the 10% formalin for traditional sectioning technique and stain with hematoxylin and Eosin (Al-Sabaawy *et al.*, 2021).

Histologic sections were proponed, Micromorphometric parameters including Length of the intestinal villi, Goblet cell count of the intestine, measurements of the length of primary and secondary lamellae of the gills were achieved by using the color USB 2.0 digital image camera (Scope Image 9.0-

China) which was provided with image processing software (Al-Haaik, 2019).

Lactic Acid Bacilli (LAB) count:

MRS agar was used for count of LAB present in the intestine of each group of the inspected fish. The procedure involved the emptying the intestinal contents in a sterile glass beaker. Deciliter serial dilutions were performed and were cultured on plates. The culture were incubated in anaerobic condition at 37°C for 48 hours. Bacteria counts were achieved according to (Kangawa, 2018).

Statistical analysis:

One-way ANOVA was followed for means and standard errors of the obtained data. The analysis was done using IBM SPSS V25 program UK at P≤0.05 (Handel, 2013).

RESULTS

The study revealed a significant difference at (P≤0.05) in the studied groups i.e. T and C groups. This variation started after the 1st week of the experiment. The significant difference was noted in weekly weight, mean increase of body weights, total increase of body weights, mean of feed conversion efficiency at P≤0.05 in the treated group (with EM) as compared with the control group as shown in table (1).

Table 1: The effects of EM on the growth performance in *C. carpio* (mean ± standard error).

Wks	Groups					
	C			T		
	Weight *	weight gain	FDR	Weight *	weight gain	FDR
1	34.21 ± 0.8 ^a	20	3.05	34.11 ± 0.8 ^a	20	3.16
2	44.58 ± 1.2 ^a	22	2.12	63.10 ± 0.9 ^b	32	1.10
3	50.05 ± 1.06 ^a	25	4.56	77.46 ± 5.4 ^b	35	2.44
4	53.95 ± 2.6 ^a	30	7.68	86.13 ± 1.7 ^b	37	4.26
Total		97	4.93		124	2.08

*Different letters mean a significant difference in the groups of experiment at (P≤0.05).

Gross examination of the fish showed a difference in the color, texture and consistency of the liver, intestine, anterior and posterior kidneys between the two groups. The color of the liver of treated groups of fish (T) with EM is reddish - brown as compared with those of control groups (C) which had brownish - pale pink color. The intestine of the treated group (T)

had whitish-pink color as compared with those of the control group (C) which had whitish- or whitish-pale color. As concerning to the kidneys (of both anterior and posterior lobes) of all the groups, there was no difference in their colors which was brownish-red. Dark red color was the color of the spleen of all groups of fish. Fig. (1).

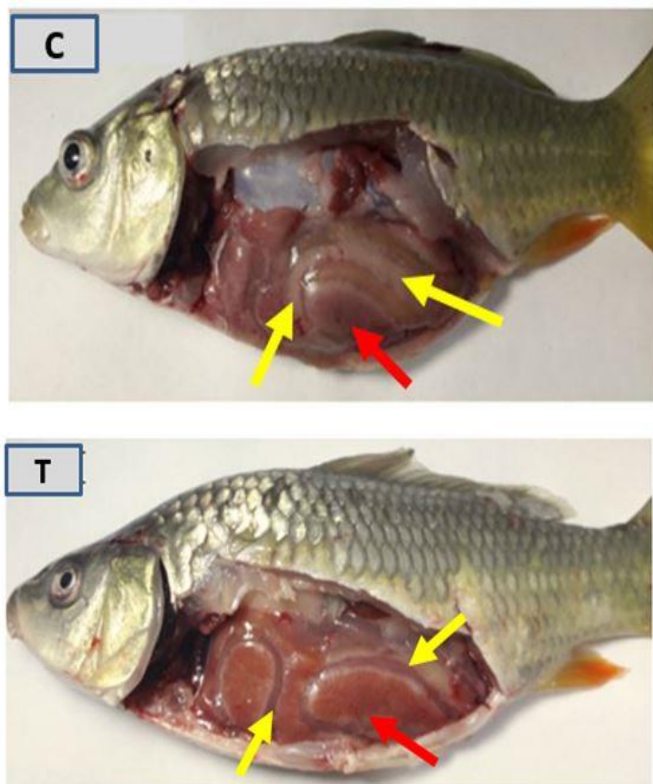


Fig. 1: Difference in the color of liver and intestine between controls group (C) and EM treated group (T) of fish. The liver of treated group had brownish-red color as compared with the brownish-pale pink color of the control groups (red arrow). The intestine of treated group had whitish pink color as compared with whitish pale color in the intestine of the control group (blue arrow).

There was no difference in the colors of the posterior kidney which was dark red in all groups of the fish the texture and consistency of the visceral organs (liver, intestine, anterior and posterior kidneys, spleen) of the treated groups with EM were more firm, dense and compact in their form structure as compared with similar organs of the control groups whose their organs are less stiff and more fragile.

The relative weights of the liver, gills, anterior and posterior kidneys, intestine and spleen of the treated groups with EM were more than those of the control groups with significant difference ($P \leq 0.05$) as explained in table (2).

Table 2: The application of EM on the weights of liver, gills, anterior and posterior kidneys, intestine and spleen of the different groups of common carp fish of the experiment (mean± standard error).

Organs	Groups *	
	C	T
liver	1.70 ± 0.6 ^a	3.62 ± 0.05 ^b
Gills	1.23± 0.04 ^a	2.56± 0.02 ^b
Anterior kidney	0.19± 0.004 ^a	0.40± 0.017 ^b
Posterior kidney	0.37± 0.003 ^a	0.56± 0.01 ^b
Intestine	1.29± 0.3 ^a	2.23± 0.03 ^b
Spleen	0.17± 0.004 ^a	0.31± 0.004 ^b

* Different letters mean a significant difference in the groups of experiment at ($P \leq 0.05$).

The findings of the histologic study concerning the measurements, dimensions and morphological structure referred a significant increase ($P \leq 0.05$) in the heights of intestinal villi with a clear significant increase in Goblet cell counts of the intestinal epithelium in the treated group (T) as compared with control group (C) as indicated in Figs.2,3 and table (3).

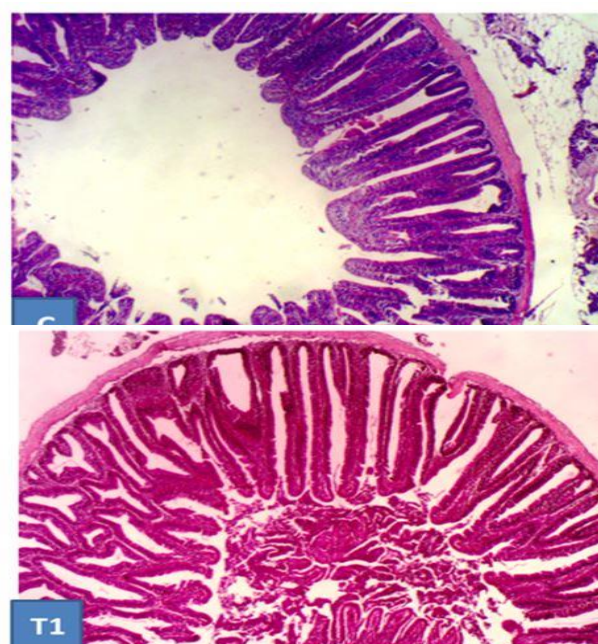


Fig. 2: Histological Section of the intestine of fish shows the height of intestinal villi of the control group of the experiment (H & E, 10X).

Table 3: Effects of EM on the on Goblet cell counts, length of both villi of intestine and gills lamellae of fish (mean± standard error).

Parameter	Group *	
	C	T
Goblet cell count	15.60 ± 0.6 ^a	34.00 ± 0.7 ^b
Villi lengths	798.52 ± 1.2 ^a	990.10 ± 3.3 ^b
lengths of primary lamellae off gills	2254.83 ± 7.9 ^a	2823.12 ± 137.4 ^b
lengths of secondary lamellae gills	15.60 ± 0.6 ^a	34.00 ± 0.7 ^b

*Different letters mean significant difference between group of experiment (P≤0.05).

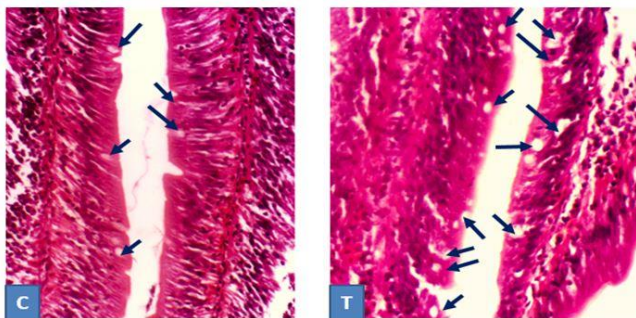


Fig. 3: Histological section of the intestine of fish shows Goblet cell counts in the epithelium of intestinal villi of experiment groups. (H & E, 40X).

Also, measurements and histological changes in the histology of the gills showed a clear significant difference in the lengths of primary and secondary lamellae in treated group (T) as compared with control group (C) as shown in Figure (4) and (5) and table (3).

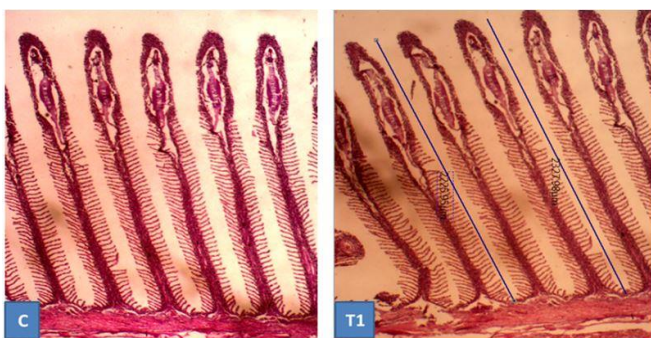


Fig. 4: Histological section in the gills of fish explain the lengths of the primary lamellae of the different groups of the experiment (H & E,10X).

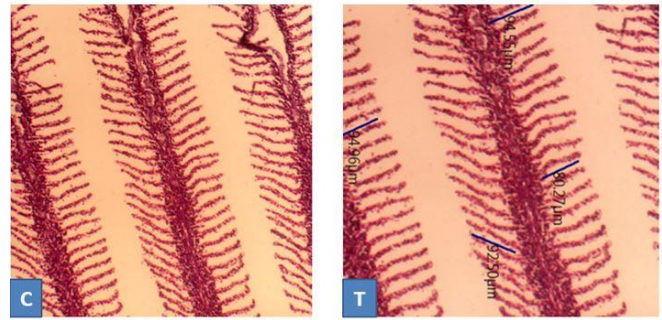


Fig. 5: Histological sections in the gills of fish explain the lengths of the secondary lamellar of the different groups of the experiment (H & E, 10X).

The results of LAB count of the intestinal contents of fish experiment informed a great difference in the means of LAB counts in the treated group (with EM), which reached (0.22 x 10⁴) compared with the control group (C) which reached (101x 10⁴) as explained in figure (6).

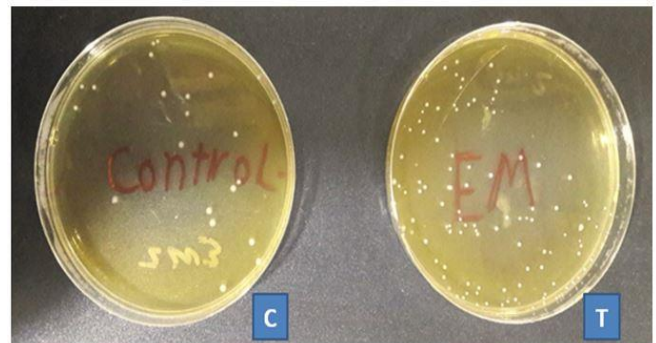


Fig. 6: Lactic acid bacilli count cultured on MSR agar of treated (T) and control (C) groups.

DISCUSSION

Undoubtedly, in the context of rapid increase in the aqua culture in the globe, researches were directed to explore alternative routes for improvement of fish production through rapid growth and best performance as well as a provision of multiple avenues of therapy, protection and environment biosecurity. Consequently, EM offer a good opportunity and successful approach for modulation of flora of the digestive system which is commonly known as "microbiome". Microbiome is a general term describes the genome of all living microorganisms normally inhabiting the alimentary tract of all vertebrates *i.e.* useful, beneficial and mutual organisms besides the pathogenic including bacteria, viruses and fungi. As a sequela, administration of such microorganisms is reflected by useful and beneficial and healthy outcome representing by increase feed conversion efficiency, increase growth as well as playing a role for resistance against diseases and stresses (Wuertz *et al.*, 2021).

Amazingly, the capability of beneficial microorganisms or activated and effective microorganisms for destruction and digestion of compounds in which the fish host is not able to do it alone, such as carbohydrate digestion and toxin removal from foodstuffs. These merits are the main base and corner stone in researches of fish culture (Stephens *et al.*, 2015). It is worthy to mention that "effective microorganism" is a natural blend of various microorganisms which has greater impact on the animal than one ingredient. The mechanism of action of effective organism is to modulate microbiome normally exist in the digestive system by improving the intestinal microbes in a mutual symbiotic relationship and production of receptors which stimulate immune responses and increase defense means against pathological agents.

The activity of several strains of lactobacilli constituting a huge proportion of "effective microorganism" is well-documented acting as an agent against several species of viruses. Certainly, the role of "effective microorganisms" will be greater if it suitably serves against viral diseases of the fish (Lee and Ko, 2016). Proteobacteria and bacteroides form the most predominant species of intestinal microbiome in several species of fish (Dulski *et al.*, 2020). However, herbivores normally inhabit flora of Clostridia, Leptotrichia and Citrobacter in their intestines for supporting of cellulose digestion derived from the plant, so they have the utmost variety of microbiome (Liu *et al.*, 2016). Owing to their wide variations and their histology of particular microscopic structures, the intestine equips favorable and multiple environments of various concentrations of oxygen and pH. Such status creates several ecological reactions to occur and interfere. Many studies indicated that the increased microbial variation is positively reflected on the health and physiology of intestine with subsequent increase in their functional efficiency due to a provision of broad surface for absorption (Bajinka *et al.*, 2020; Merrifield *et al.*, 2009).

Nevertheless, our study confirmed this proof concerning the histology of the intestinal structures of the treated group which showed an improvement in the villi lengths as well as in the Goblet cell counts. Such recent induced condition will provide a wider surface area for absorption with a better movement and peristalsis of the intestine for physiology of intestine. However, these findings were confirmed in earlier studies carried out on fish (Merrifield *et al.*, 2009). The same observations were recorded in poultry administered *Bacillus licheniformis*, *B. subtilis* and *Enterococcus faecium* (Jwher *et al.*, 2013). Contextually, these improvements were noticed in poultry rations supplemented with enzymes produced by the previous microorganism which create an

additional digestion of feed constituents and other organic substances (Merrifield *et al.*, 2009). Many works referred that administration of probiotics which their constituents are similar to "effective microorganisms" *i.e.* consisting of multiple good and useful living bacteria had led to an improvement in growth rate. Better growth rate was attained by increased appetite and regulation of growth by supporting and improving digestion processes with subsequent increase in conversion efficiency of these feed. Another probable improvement may be obtained through the activity of enzymes *viz.*, trypsin and chymotrypsin as well as the increased tolerance of the animal against stress and diseases. The mode of action in the former condition is due to the inhibition of living pathogenic microorganisms causing diseases by adhesion and competition with later improvement in the health status and general body condition. Such observations were typically found in our study which were also similar to those of (EL-Haroun *et al.*, 2006; Ghasempour *et al.*, 2016).

It is regarded that the presence of *B. subtilis*, yeast, fungi and molds within the constituents of "effective microorganisms" offers additional merits and advantages to the enzymatic digestion. Furthermore, the presence of *Lactobacillus acidophilus* provokes the gene of "hunger hormone" which is known as "Ghrelin" which will later stimulate feed intake as mentioned by (Giorgia *et al.*, 2018; Young and Liptak, 2018).

The results of the current study referred the plain impact of the "effective microorganisms" on the structure of primary and Secondary lamellae of the gills of the treated group of fish. However, according to our best information on the topic, there is no study explains the precise mechanism or the role of beneficial microorganisms on the respiration process in fish. Consequently, there are few studies which revealed to the role of useful microorganisms in protection of mucous epithelia which is considered as the major defense system. "Effective microorganisms" consist of beneficial mutual and symbiotic organisms serve as a protective coat embedding the mucous surfaces.

This formed cover acts as a biological promoter keeps these surfaces against different disease etiology and maintain the aqua ecology which involves a broad mass of elements and physical/chemical hazards (Lazado and Caipang 2014). The ability of beneficial organisms to stimulate animal immunity initiated a broad-spectrum revolution. The characters that promote immunity of animals achieved by microorganisms were found out in two fields. The first domain is the Complicated mechanism to identify the host (the ability to differentiate between factors of pathogenic and non-pathogenic agents that cause diseases) to protect the newly introduced

microorganisms to the body. It follows that a reverse mechanism may occur which means the ability of the beneficial microorganisms to identify the host. The second field is concerned with the immunity of the mucous surfaces which were mentioned by authors (Merrifield *et al.*, 2009). Other investigators started that these organisms are capable of diluting the necrotizing effects of insecticides on the gills (Lazado *et al.*, 2011). The findings of LAB count of the intestinal content of both groups indicated a large difference between the two groups. The increase of bacilli counts may be attributed to the prolonged survival of beneficial microorganisms in the digestive system with subsequent of higher multiplication rate then less elimination process, which leads to maximum benefit from its presence for a longer period.

CONCLUSION

From the findings of the present study, it can be concluded that EM play an important role in the growth and health performance so it can be used as feed promoter in fish aquaculture.

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Conflict of interest:

The Authors profess that no conflict interest .

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