Journal of Animal and Veterinary Advances 13 (11): 660-663, 2014
ISSN: 1680-5593

Locally Produced Probiotic and Their Effect on Total Counts, Proteolytic Bacteria and Lipolytic Bacteria of Common Carp Fed Two Level of Protein

[1]Nasreen M. Abdultrahman and [2]Saaid A. Al Shawi
[1]Department of Animal Production, Faulty of Agricultural Sciences,
University of Sulaimaniyah, Sulaimaniyah, Iraq
[2]Department of Animal Sources, College of Agriculture,
University of Baghdad, Baghdad, Iraq

Abstract: Two types of fish probiotics produced and extracted, single contained the genus of the bacteria *Lactobacillus* and the genus *Bifidobacterium* and mixed probiotics from the earlier bacterial genus mixed with the prepared yeast *Saccharomyces cerevisiae* that extracted from the first part of the intestine (foregut) which represents the gut in these omnivore's fish (represented by the common carp *Cyprinus carpio*) and the herbivores (represented by the grass carp *Ctenopharyngodon idella*). The probiotics added to the fish diet in two levels of protein 32 and 10% by conducting two experiments and for that purpose 300 fish fry weighted 0.2 g reared for 5 months included with acclimation period about 27 days whereas each experiment include seven treatments. The first treatment represented the control without using any addition to the probiotics, the second one was the single probiotics with the Lactobacillus bacteria. The third one was also the single probiotics but with Bifidobacterium whilst the fourth and fifth treatments were the mixed probiotics which of Lactobacillus and Bifidobacterium bacteria with the yeast *Saccharomyces cerevisiae* in ratio 1:1:1 one from common carp and the other from grass carp, respectively. The commercial probiotics was in the sixth treatments and the Iraqi probiotic was the seventh one.

Key words: Locally produced probiotic, total counts, proteolytic bacteria, lipolytic bacteria, common carp, two level of protein

INTRODUCTION

Probiotic consists either of one type or a mixture of live microorganisms that positively affect the health of the host and production through the improvement of the properties of the normal flora of the intestinal flora (intestinal micro flora) in which it is originally located in the gastrointestinal tract.

The colonies of the genus *Lactobacillus* are isolated rarely in farmed fish and it's appeared that when there are effects on the growth of intestinal flora it will changes along the gastrointestinal tract from the mouth to the internal director and across the cavity to the epithelial layer (Kraha and Sakata, 1997). Fish that do not contain the genus *Lactobacillus* such as farmed fish have the intestinal mucosa layer less developed and mild mucus may be devoid from the important mechanisms to sustain health (Korsnes *et al*., 2006).

The problem faced by most of the species are digesting the food industry pellets during the early stages of the development of larval and which may be due to non-completion of the growth of the digestive system and the lack of enzyme levels capable of digesting food (Kolkovski *et al*., 1997). So, the addition of probiotic will raise the levels of the important enzymes that help in the digestion of the food industry so will be reduced for feeding and then the cost will be reduced (Vine, 2004).

The intestinal flora reflects the microbial load of fish food and their environment (Gatesoupe, 1999), in general the bacterial genus that dominant in common carp were *Aeromonas hydrophila*, *Citro bacterfreundii*, *Escherichia coli*, *Enterobacter aerogenes*, *Pseudomonas*, *Bacillus* and *Staphylococcus* sp. (Kumar *et al*., 2006; Apun *et al*., 1999).

MATERIALS AND METHODS

The experiment was conducted for 5 months and for purpose 300 fingerlings common carp *C. carpio* L. were brought from a fish hatchery in Dukan belongs to the Ministry of Agriculture. Mean initial weight was 0.2 g. The fish were acclimated to laboratory conditions and fed with control pellets (31% protein) prior to the feeding trials for 27 days, fish were starving for 3 days and then feed were presented 2% of body weightat a rate of two meals, morning and night for 2 days sand then raised the

Corresponding Author: Nasreen M. Abdultrahman, Department of Animal Production, Faulty of Agricultural Sciences, University of Sulaimaniyah, Sulaimaniyah, Iraq

Table 1: Experimental diets

Total number of microorganisms/g of the products	Type of microorganisms
10^8	*Lactobacillus acidophilus*
10^9	*Lactobacillus* sp.
10^{10}	*Bacillus subtilis*
10^9	*Saccharomyces cerevisiae*

rate to 3% and by two meals for 2 months and after that when it was sure that the feed was consumed completely by the fish it raised the rate to 4% of the weight of the fish at a rate of two meals a day.

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 seven with two 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. In addition, a daily cleaning by Siphon Method was applied to remove remained particles from the system.

Diet formulation: Experimental diets were prepared as shown in Table 1. Fish were distributed randomly after the localization stage on 14 plastic basins by repeating each transaction where they put seven fish each repeater. The experiment was conducted for a period of 16 weeks and the fish fed on experimental diets containing probiotic level (0.5%) for the following treatments:

- T1: control treatment (without any additions)
- T2: singular probiotic Lactobacillus which contains the bacteria Lactobacillus
- T3: singular probiotic Lactobacillus which contains the bacteria Bifidobacterium
- T4: probiotic mixture consisting of Lactobacillus+Bifidobacterium+yeast

Saccharomyces cerevisiae (1:1:1) extracted from fish common carp:

- T5: probiotic mixture consisting of Lactobacillus+Bifidobacterium+yeast

Saccharomyces cerevisiae (1:1:1) extracted from grass carp fish:

- T6: foreign probiotic BIOSB-Gold*
- T7: local probiotic (Iraqi probiotic)**

Each gram of BIOSB-Gold consists of:

- *Saccharomyces cerevisiae* $>3\times10^{11}$ CFU g^{-1}
- *Bacillus subtilis* $>4\times10^{9}$ CFU g^{-1}

Table 2: The amount of food items, their amount in diet and the percent of protein within it

	Amount in diet (g)		Protein in diet (%)	
Food items	First experiment	Second experiment	First experiment	Second experiment
Soybean meal	35	3	15.40	1.32
Protein concentrate	35	3	14.00	1.20
Flour	14	15	1.79	1.92
Starch	14	77	1.07	5.85
Premix	2	2	0.00	0.00
Total	100	100	32.26	10.29

Table 3: Microbial load for the first experiment fish with different types of probiotics (with 32% protein)

Treatments	Bacterial total count (cfu)	Proteolytic bacteria	Lipolytic bacteria
T1	336.0 ± 21.21^{b}	15.5 ± 0.71^{cd}	25.5 ± 0.71^{b}
T2	374.5 ± 21.92^{ab}	15.0 ± 1.41^{cd}	25.0 ± 1.41^{bc}
T3	318.5 ± 41.72^{b}	16.0 ± 1.41^{bc}	29.5 ± 0.71^{a}
T4	325.5 ± 6.360^{b}	14.0 ± 1.41^{cd}	22.5 ± 0.71^{cd}
T5	445.0 ± 62.23^{a}	19.5 ± 0.71^{a}	21.5 ± 0.71^{d}
T6	392.5 ± 3.540^{ab}	13.0 ± 1.41^{d}	21.5 ± 2.12^{d}
T7	428.5 ± 31.82^{a}	18.5 ± 0.71^{ab}	28.5 ± 0.71^{a}

- Biomass metabolites (QS)
- Carrier (QS)

**Represent the Iraqi probiotic and consist of the following experimental diets which are shown in Table 1. Two types of diet were prepared of difference protein container (32 and 10%) but it is a fixed content of energy 3000 kilo calories/100 g. Moreover, Table 2 and 3 illustrate the contents of the diet.

First experiment: The 98 randomly selected fish weight srate of 0.2±0.01 g and distributed to the 14 plastic aquaria 100 L water, each aquarium contain seven fish where fed with 3% by weight for 2 months from the duration of the experiment and then increased to 4% after confirmation of entry into force of the amount of feed additives have since continued duration of the experiment for 5 months and the amount of feed adjusted relative to the weight of fish weekly. The aquaria were cleaned every 2 days.

Second experiment: The same as in the first experiment except the diet formula which differ accordingly.

Microbial counts: First part of intestine taken from each treatment at the end of the experiments in sterilizes conditions make dilution series by peptone water for estimated Lactobacillus count by Pour-Plate Method in which 15 mL of sterilized MRS used and the coliform count by using MacConkey agar.

RESULTS AND DISCUSSION

Significant differences appeared from data in Table 3 among experimental treatments in bacterial total count in

the first experiment, the highest observed in T2, T5, T6, and T7 with 374.50, 445.00, 392.50 and 428.50 cfu, respectively which differ significantly than other treatments.

Highest count of lipolytic bacteria found in T5 and T7 with 19.500 and 18.500 cfu, respectively while the lowest were in T1, T2, T4 and T6. In regarding lipolytic bacteria, T3 and T7 obtain the highest count 29.5 and 28.5, respectively. In the second experiment, the bacterial total count observed in T1, T3, T4, T5, T6 and T7 with 412.5, 370.0, 416.0, 403.0 and 372.5, respectively.

The using of single probiotic Lactobacillus in the first experiment a significant difference observed in which the bacterial total count was 374.5 cfu and the mixture produced from grass carp was better than that from common carp 445.0 and 325.5 cfu, respectively the Iraqi probiotic 428.5 cfu was better than the commercial 392.5 cfu in the first experiment.

In the second experiment when using single probiotic Bifidobacterium better than using of Lactobacillus with 370.00 cfu but when adding the mixture of probiotic from both sources improves the parameters but not significant, the same trend observed with the commercial and Iraqi probiotics.

No significant differences observed in proteolytic bacteria in the T2 and T3, the probiotic produced from grass carp differ significantly than that from common carp which obtain 19.50 cfu, T7 with Iraqi probiotic differ significantly than the commercial probiotic by 18.50 cfu. In the second experiment (Table 4) the T2 with Lactobacillus differ significantly by 16.50 cfu, T5 with probiotic produced from common carp was better significantly than probiotic produced from grass carp in which the proteolytic bacterial count was 18.50 cfu whereas no significant differences observed between Iraqi and commercial probiotic in proteolytic bacterial count.

Lipolytic bacterial counts were higher in all treatment than the proteolytic bacterial counts in both experiments, no significant differences observed between treatments with Bifidobacterium from both types whereas T7 differ significantly which contain Iraqi probiotic with 28.50 cfu. In general no significant differences observed between Iraqi and commercial probiotics in in lipolytic bacterial counts.

Total bacterial counts in intestine of first experiment fish (Table 5) show higher counts in T2 with 2466.50 cfu while the lowest was in T6 with 453.50 cfu. In the second experiment, the higher count was in T4 with 788.0 cfu.

Bagheri *et al.* (2008) found no significant differences in bacterial total count in in *O. mykiss* intestine when Bacillus added to the experimental diets. While Ghosh *et al.* (2005) fond an increase in total count when adding 0.1% as compared with other treatments. Different species of Lactobacteria recorded by Querioz and Boyd (1998) in healthy fish as normal flora.

Table 4: Microbial load for the second experiment fish with different types of probiotics (with 10% protein)

Treatments	Bacterial total count (cfu)	Proteolytic bacteria (cfu)	Lipolytic bacteria (cfu)
T1	412.50±12.02^{a}	15.50±0.707^{b}	22.00±0.7070^{d}
T2	342.50±13.44^{b}	16.50±0.707^{ab}	28.50±0.7070^{a}
T3	370.00±43.84^{ab}	15.50±0.707^{b}	23.00±1.4140^{d}
T4	416.00±7.070^{a}	13.00±1.414^{c}	25.50±0.7070^{bc}
T5	403.00±4.242^{a}	18.50±0.707^{a}	23.50±0.7070^{cd}
T6	372.50±20.51^{ab}	12.50±0.707^{c}	25.50±0.7070^{bc}
T7	390.50±2.121^{ab}	11.00±1.414^{c}	26.00±1.4140^{b}

Table 5: Total count of intestinal microbial load in both experiment fish (cfu)

Treatments	First experiment	Second experiment
T1	506.0±49.497	211.0±26.8710
T2	2466.5±71.418	41.5±13.4350
T3	768.5±13.435	670.5±62.9330
T4	1065.0±70.711	788.0±52.3260
T5	480.0±42.710	138.5±4.95000
T6	453.5±2.1210	169.5±64.3470
T7	997.5±33.234	102.0±12.7280

CONCLUSION

The ability of yeast to improve weight gain, food conversion ratio and decrease stress on fish was due to their production of some vitamins and unidentified growth factor which reduce stress and enhance growth (Cooney, 1980). Yeast also considered a source of protein in fish diets that acts as growth enhancer and producer of some amino acids so it considered as probiotics, the being of live yeast in the intestinal tract of fish and secreting some digested enzymes and their freely occurrence in the lumen will increase fatty acids assimilation and bioavailability of some minerals like calcium and potassium by secreting phytase (Abdul-Halim, 1991) while the being of died yeast with their solid cell wall could absorbed nutritive minerals which make it a suitable storage for nutritive items (Avelar *et al*., 1999).

REFERENCES

Abdul-Halim, A.M., 1991. Microbial protein in fish feeding. Ph.D. Thesis, Faculty of Agriculture, Alexandria University, Egypt.

Apun, K., A.M. Yusof and J. Kumbang, 1999. Distribution of bacteria in tropical freshwater fish and ponds. Int. J. Environ. Health Res., 9: 285-292.

Avelar, K.E.S., L.J.F. Pinto, L.C.M. Antunes, L.A. Lobo, M.C.F. Bastos, R.M.C.P. Domingues and M.C. De Souza Ferreira, 1999. Production of bacteriocin by *Bacteroides fragilis* and partial characterization. Lett. Applied Microbiol., 29: 264-268.

Bagheri, T., S.A. Hedayati, V. Yavari, M. Alizade and A. Farzanfar, 2008. Growth, survival and gut microbial load of rainbow trout (*Onchorhynchus mykiss*) fry given diet supplemented with probiotic during the two months of first feeding. Turk. J. Fish. Aquat. Sci., 8: 43-48.

Cooney, D.O., 1980. Activated Charcoal Antidotal and Other Medical Uses. Marcel Dekker Inc., New York, pp: 1425-1426.

Gatesoupe, F.J., 1999. The use of probiotics in aquaculture. Aquaculture, 180: 147-165.

Ghosh, K., S.K. Sen and A.K. Ray, 2005. Feed utilization efficiency and growth performance in rohu, *Labeo rohita* (Hamilton, 1822), fingerlings fed yeast extract powder supplemented diets. Acta Ichthyologica et Piscatoria, 35: 111-117.

Kolkovski, S., A. Tandler and M.S. Izquierdo, 1997. Effects of live food and dietary digestive enzymes on the efficiency of micro-diets for Sea bass (*Dicentrarchus labrax*) larvae. Aquaculture, 148: 313-322.

Korsnes, K., O. Nicolaisen, C.K. Skar, A.H. Nerland and O. Bergh, 2006. Bacteria in the gut of juvenile cod *Gadus morhua* fed live feed enriched with four different commercial diets. ICES J. Marine Sci., 63: 296-301.

Kraha, M. and T. Sakata, 1997. Fermentation of dietary carbohydrates to short-chain fatty acids by gut microbes and its influence on intestinal morphology of a detritivorous teleost tilapia (*Oreochromis niloticus*). Comp. Biochem. Physiol. A: Physiol., 118: 1201-1207.

Kumar, R., S.C. Mukherjee, K.P. Prasad and A.K. Pal, 2006. Evaluation of *Bacillus subtilis* as a probiotic to Indian major carp *Labeo rohita* (Ham.). Aquacult. Res., 37: 1215-1221.

Querioz, J.F. and C.E. Boyd, 1998. Effect of bacterial inoculums in channel Catfish ponds. J. World Aqua. Soci., 29: 67-73.

Vine, N.G., 2004. Toward the development of a protocol for the selection ofprobiotics in marine fish Larviculture. Ph.D. Thesis, Rhodes University, South Africa.