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ENHANCING THE BACTERIAL SYSTEM IN THE INTESTINES OF THE GRASS CARP *CTENOPHARYNGODON IDELLA* BY FEEDING IT ON DIETS OF DIFFERENT PROPORTIONS OF COW RUMEN CONTENTS ENRICHED WITH THEPAX YEAST

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Abstract
The study was conducted in the Fish Laboratory, College of Agricultural Engineering Sciences / University of Baghdad, with the aim of using cow rumen contents enriched with prebiotic as a ration for grass carp fish. 15 fingerlings, the fish were fed on four types of diets equal in protein after crushing and grinding the materials and adding the rumen contents at rates of 10%,15%,20%, supplemented with prebiotic 2 g/kg except for the control diet free of addition. The fish were weighed every 14 days. The grass carp fish were dissected, cut with scissors along the side line of the fish, and the intestines were extracted. We took the middle and last part of the intestine, and one gram of the fish intestines was transplanted into two activated media in petri dishes after sterilization, left in the incubator for 48 hours. Colonies for Bacillus nervosa were counted. The results of the research indicated that the second treatment was significantly ($p<0.01$) superior to all treatments in terms of body weight rates, weight gain, relative and qualitative weight rates, food conversion ratio, food conversion efficiency and apparent digestion coefficient, followed by the third treatment, then the fourth treatment and for all durations of the experiment. The results showed that the second treatment was significantly superior to the rest of the treatments in this trait for the preparation of Bacillus subtilis isolated from the intestines of grass carp fish.
Key words: prebiotics culture, grass carp. rumen content

Introduction:
Many beneficial bacterial species live in water bodies, such as Bacillus subtilis and others (Norris, 1981), and are active in aquatic environments depending on the temperature and depending on the decomposing food from the remains of plants and sediments at the bottom. The water body sticks together like lactic acid bacteria, and some remain in the cavity of the canal. The bacterial species work synergistically together and create conditions for each other depending on pH and oxygen (Wang, 2010.(

Certain types of microorganisms settle in the gut of fish at an early age, and the microorganisms develop inside the gut. In the beginning, a tube forms next to the yolk sac immediately after the fish larvae hatch. The gut develops depending on live food, and soon different types of germs settle. It was successful to isolate acidophilus bacteria Lactobacillus is one of the Atlantic cod fish, but the isolation process faces difficulties because the fish are still small and in their early stages, as well as the absence of glucose from the ocean, which is important in the growth of bacteria and germs. Ringo et al., 1996

The contents of the rumen promote the growth of large numbers of microorganisms, including bacteria and ciliates, containing grains and weeds, which ferment in the rumen (Mac Donald et al.,

1998). The biological precedents work to enhance the role of microorganisms in colonizing the gastrointestinal tract and competing with other organisms through their adhesion in the rumen. The mucous layer of the alimentary canal, modesty is affected by the diversity of food and temperature (Madigan and Brock.,1991). Al-Jubouri (2016) showed that the addition of nutrients from mannan sugar and beta-glucan fiber to carp fish diets works to perpetuate the survival of beneficial bacteria in the digestive system of the fish and increases the strength of their adhesion between the folds of the epithelial layer of the fish's intestines.

Materials and methods

The experiment

was conducted for a period of 1/20/2022 and lasted 96 days in the fish laboratory of the College of Agricultural Engineering Sciences / University of Baghdad, and 250 fish were selected out of 1000 fish from a private fish hatchery for the production of caviar, their weights ranged from 40-44 g. They were transported by a tank car at five in the morning to the laboratory and then transferred To a large basin equipped with oxygen made of stainless steel with a capacity of 1000 liters, after equalizing the temperature of the basin water and the water of the tank of the fish transport car, it was left in it for 7 days. Primary 40 g.

Experiment tanks

Tanks made of glass, with a capacity of 72 cm3, rectangular in shape, covered from the outside with plastic nets to prevent jumping of fish. They were supplied with an air pump for the purpose of maintaining oxygen concentration, and a heating heater within a temperature of 24 AH for the purpose of providing growth conditions. Measurements for each of oxygen, temperature and acidity of the water are measured twice a day in the morning. And at evening. The ponds were prepared after repairing the fractures and cracks with silicone material, the ponds were cleaned and sterilized with 5 g/l sodium chloride salt. The fish were fed gradually for 15 days depending on their ability to consume feed, down to 4% of body weight.

The fish were fed at the beginning with 1% gradually until they stabilized on 4% of the fish's mouth at the rate of three meals a day at 7 am, 12 pm, and 5 pm. The pH was measured by a portable Chinese-made PH meter. The temperature was recorded daily at 7:00 AM, 1:00 PM, and 5:00 PM, during the experiment period, using a portable mercury thermometer, to the nearest degree Celsius

Preparing the bush

The components of the diet were prepared from the local markets, and the contents of the rumen were collected from the slaughterhouse in the city of Kut, near the Al-Taqi Institute of the city, and dried in the sun for ten days, when the air temperature was more than 50 AH. water 37 H for ten minutes, and after mixing the ingredients and observing the consistency of the mixture, the process of pressing the ration was done in a Chinese-made SONIO meat grinder, and the diameter of the fodder grains was 3 mm

Table 1: Components of experimental diets

Components of experimental relationships	**T1**	**T2**	**T3**	**T4**
Fish protein	5	5	5	5
Protein concentrate	18.75	18.75	18.75	18.75
Soybean meal	35	35	35	35
yellow corn	8	7	3	1
barley	7	3	3	2
bran	5	3	2	1
Fish fat	1	1	1	1
salt	1	1	1	1
vitamins and minerals *	1	1	1	1
flour	10	7	2	1
cow rumen content	6.25	6.25	6.25	6.25
Thepax	2	2	2	2

Chemical analysis of cow rumen content	
dry matter	93%
Crude protein	10%
ether extract	3.5%
raw fiber	25%
starch	30%
Ash	10%

Table 2: Chemical analysis of grass carp diets

treatments	Moisture	protein	fiber	ash	energy	carbohydrate	Energy kcal/kg
T1	11%	31.3	3.2	7.2	5.2	53.1	2461
T2	10%	31,1	4	6.2	5	53.3	2448
T3	9%	31.73	4.3	6.3	6	53.7	2516
T4	9.7%	30.9	5.1	5.5	4.2	54.3	2550

Counting and diagnosis of Bacillus subtilis

Microbial counting was carried out in the laboratory of the College of Agriculture/University of Baghdad, and all experimental tools were sterilized, including tubes, lubes, petri dishes, beakers, and dilutions.

-1Prepare the two mediums, Nutrient Agr and Nutrient Broth, by dissolving 2.3 gm in 1 liter of distilled water for each.

-2Three samples of fish from each treatment were dissected using a scalpel. The fish were cut longitudinally from the alignment of the side line, and from the sides, the meat was cut. The inner viscera appeared. The intestines were isolated, then divided into three equal parts, leaving the front part. 1 gm of fish intestine was taken from the middle and last part and added to 9ml of medium water activated with Broth, from which I made eight dilutions.

-3The eight dilutions were placed in a water bath at a temperature of 85 °C for 15 minutes for the purpose of isolating Bacillus bacteria, as they can withstand these temperatures and are isolated by killing the bacteria that are with them in the tubes to remain only them.

-4The operations of weighing the media and pouring the dishes were carried out in a device (Hood: an oven-like device with a closed shape with a glass door inside which is a torch of flame. We work close to the flame to prevent contamination inside the device.(

Pour 1ml of each dilution on the medium used to harden the dishes, then turn the dishes and place them in the incubator for forty-eight hours at a temperature of 29°C.

-4Colonies are shown in yellow 3 Growth parameters

3- The results

3-1 Average weights of experimental fish

The results of the statistical analysis shown in Table (2) indicated that the second treatment with a content of 10% of cow rumen ($p<0.01$) was significantly superior in the characteristic of the average body weights of fish, as the final weight was 73 gm fish, and the superiority was on all treatments compared to the first control treatment. Final weight 58g. A fish and extended the experiment collect. It was followed by the third and fourth treatments 63.62 gm of fish (in which the rumen contents were 15, 20% per kg) respectively significantly superior to the control treatment and for all periods as well. The third treatment outperformed the fourth treatment for the periods mentioned in the experiment, but the weights of the fish became more distinguished, as they increased in recent periods. The alimentary canal may have developed and the area of contact between it and the food mass increased, which led to an improvement in digestion and absorption. Another factor was the incorporation of the seven-day digestion experiment within the period. (96 days.(

Table (1) Weight averages of grass carp for the periods and length of the experiment (mean ± standard error)

Periods/ Average weights of experimental fish								
Treatment	Initial Weight	14	28	42	**56**	**70**	**84**	**96**
T1	**40 ±0 a**	**43 0.40± d**	**45 0.46± d**	**. 46± 0.35 d**	**50 0.69± d**	**50 0.69 ± d**	**51 0.52± d**	**59 0.57± d**
T2	**40 0± a**	**44 0.41± ab**	**47 ±0,51 a**	**50 0.80 a**	**53 0.57± a**	**57 1.72± a**	**61 0.91± a**	**73 0.57± ab**
T3	**40 ±0 a**	**45 0.41± a**	**46± 1.05± ab**	**49± 0.32 ab**	**51 0.52± b**	**56 2.30± b**	**57 2.30± b**	**63.8 0.43± bc**
T4	**40 0± a**	**44.5 0.29± Ab c**	**44.9 0.31± abc**	**47.5± 0.88± abc**	**49.6 0.87± abc**	**53.6 1.68± abc**	**54 1.52± abc**	**62 1.35± c**
	n.c	*	*	**	*	*	**	**

weight gain

The results of the statistical analysis shown in Table (12) showed a significant superiority of the second treatment over all treatments and throughout the trial periods. 4.4 g/day) compared to the control treatment with a total increase (19 g/day) followed by each of the third and fourth treatments with a total weight increase (23.8, and 22 g/day) and it was superior to the control treatment in terms of weight gain, and so that the second, third and fourth treatments were Superior to the control treatment, but the best results were in favor of the second treatment, whose diet contained 10% of the rumen contents, supplemented with Thepax yeast 2 g / kg.

And it is noted from the mentioned table that this weight increase decreases for the third and fourth treatments with additives (15.20% g/kg of feed) content of cow rumen to the diets of this type of fish, but this increase in weight remains higher than what is found in the weight increase in the control treatment, which amounted to (19 g/fish.(

This weight gain has decreased in some periods, as it is in the period (56 days) due to turning off the heating devices during the high air temperature, and the weight gain has become significantly high during the period (96 days) due to conducting a digestion experiment within it, which is Seven days, digestion developed in the experimental fish, and the aquarium water heaters were restarted for the same period.

Table (3) Weight gain rates of grass carp fish fed on diets supplemented with rumen content treated with Thepax yeast by 2% (standard ± error

	Periods/day , weight gain							
Treatment	**14**	**28**	**42**	**56**	**70**	**84**	**96**	**Weight gain Total**
T1	**3.4 0.46± d**	**0.8 0.46± d**	**1.5 0.03± d**	**1.4 0.26± d**	**3.5 1.03± d**	**1.3 0.17± d**	**7.2 0.11± d**	**19.00± 0.5 d**
T2	**4.4 0.29± c**	**3.3 1.15± a**	**2.7 0.46± a**	**2.9 0.74± a**	**3.3 0.33± a**	**4.4 1.63± a**	**11.3 0.36± a**	**33.00± 0.57 a**
T3	**5.2 0.41± a**	**1.6 0.70± c**	**2.6 0.89± b**	**1.6 0.66± c**	**1.8 0.42± c**	**1.3 0.33± b**	**6.4 2.56± b**	**23.80 0.43 b**
T4	**4.5 0.29± b**	**4.0 0.05± b**	**2.6 0.56± c**	**2.0 0.26± b**	**2.3 0.32± b**	**1.03 0.54± c**	**6.00 1.15± c**	**22.00± 0.57 c**
	*	*	*	*	*	*	**	**

3-3Bacillus subtillus

The results shown in Table (8) showed that the second treatment, which contained 10% of the rumen contents enriched with the prebiotic Thepax 2 g/kg in counting Bacillus subtilis, was distinguished over all treatments, as the number of colonies was 119 x 108 per mm 3 compared to the control treatment of 11 x 108 colonies.

However, the number of colonies of Bacillus subtilis in the second treatment was the highest than the rest of the treatments, followed by the third and fourth treatment, respectively, compared to the control treatment (untreated diet with Thepax yeast.(

Table (4) Number of Bacillus subtilis bacteria in the intestines of grass carp fish for different treatments, average ± standard error

	Microbial and digestive characteristics		
treatment	**Bacillus Subtillus colony/mm^3**	**Feed Conversion Ratio**	**Apparent Digestiblity Coefficient**
T1	**11x10^8 57735026± d**	**55 0±**	**9.51 0.27± a**
T2	**119x10^8 470224533± a**	**75 0±**	**5.60 0.30± a**
T3	**30x10^8 66666666± b**	**60 0±**	**7.08 1.05± a**
T4	**15x10^8 88191710± c**	**49 0±**	**7.64 0.65± a**

4-Discussion

It is noticed from the review of the two tables (2,1) that the improvement in the productive qualities represented in the final weight, weight gain and food conversion factor of grass carp fish for the different treatments. The second treatment was characterized by 10% of rumen contents with the best growth characteristics mentioned, followed by the third and fourth treatments 15%,20%. respectively compared to the control treatment attributed to the following reasons:

1 - Yeast works by containing mannan sugar (a monosaccharide sugar with formula C6H12O6 which is an isotope of glucose, it gathers to form mannans which are found in yeasts and bacteria and is included in the composition of the cell coverings of the mentioned microorganisms) as it stimulates the growth of beneficial bacteria such as lactic acid bacteria to stick to In the epithelial layers of the intestinal wall and increases the thickness of the mucous layer of the intestine and increases bacterial colonization of the fish intestine. The earlier the colonization of microorganisms, the better the dominance of beneficial bacteria on the canal, and it has a positive effect on growth. glucans important in feeding the intestinal flora, enzymes secreted when they are broken down, nucleotides and vitamin B12 important in the digestive process

2- The addition of Thepax yeast would enhance muscle growth by increasing the readiness of essential and non-essential amino acids and improving digestion and absorption as a result of the

work of microorganisms and their analysis of the feed material represented by percentages of rumen contents, which contain amino acids such as lysine, arginine, alanine and thyronine (Gibson. 2006, El-Feki, Abdel-Aty, 2015.(

3- Restricted yeasts increase the absorption of glucose sugar after the breakdown of carbohydrate chains linked to other elements, and also increase their absorption and that their availability and supply to the blood freely are an important factor in many vital activities (Bongers Van den Heuvel, 2009).

It is noted from Table (3) that the second, third and fourth treatments were superior in the number of Bacillus subtilis bacteria, and that the best results were recorded for the second treatment. The productive and growth characteristics of the species mentioned in our study are attributed to the following reasons:

4 -The role of Thepax yeast has improved the presence of Bacillus bacteria in the intestines of grass carp fish in this study, as it is available in water bodies in low percentages, and that sugars and fibers (mannan sugar and beta-glucan fibers in the prebiotics promoted the growth of Bacillus Subtilius

5- The presence of this bacterial type in the central part of the fish intestine is appropriate because the conditions are relatively aerobic for Bacillus subtilius bacteria, which in turn lowers the oxygen rates towards the back of the gut and creates anaerobic conditions and good fermentation of sugars by lactic acid bacteria (Jiraphocakul, 1990).

REFERENCE

- **Elfaki, M.O.A.; Abdelatti, K.AElfaki, M.O.A.; Abdelatti, K.A. and Malik, H.E.E. (2015)**. Effect of dietary dried rumen he diets of starter chicks. Nig. J. Anim. Prod., 28(2): 153-157
- **Jiraphocakul , S., T. W. Sullivan, and K. M . Shahani, 1990.** Infiuencews Of a dried Bacillus subtilis Culture and antibiotics .
- **WU, Shangong,2012**. Composition, diversity, and origin of the bacterial community in grass carp intestine. *PloS one*, 2012, 7.2: e30440.
- **Ringø, E., Sperstad, S., Myklebust, R., Refstie, S., & Krogdahl, Å. (2006).** Characterisation of the microbiota associated with intestine of Atlantic cod (Gadus morhua L.): the effect of fish meal, standard soybean meal and a bioprocessed soybean meal. *Aquaculture*, *261*(3), 829-84
- **Brock, T. D., & Madigan, M. T. (1991**). Biology of Microorganisms. Prentice-Hall. *New Jersey*, *19862*.
- **Yousefian, M., & Amiri, M. S. (2009).** A review of the use of prebiotic in aquaculture for fish and shrimp. *African Journal of Biotechnology*, *8*(25).
- **Bongers, A., & van den Heuvel, E. G. (2003).** Prebiotics and the bioavailability of minerals and trace elements. *Food Reviews International*, *19*(4), 397-422.

- **Mac Donald , p.; Edwards ,R. A.; Greenhalg ,J. F. D. and Morgan, C. A.1998** .Animal Nutrtion .5TH ed.LONDON Longman GPR.LTD
- **Norris, J. R., et al. 1981. "**The genera Bacillus and Sporolactobacillus." *The prokaryotes* 2: 1711-1742.