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To cite this article: H. S. Abedalhammed *et al* 2021 *IOP Conf. Ser.: Earth Environ. Sci.* **761** 012088

Effect of Addition of *Lactobacillus reuteri*, Inulin and Jerusalem Artichoke (*Helianthus tuberosus*) Powder in Diets of Common carp (*Cyprinus carpio* L.) on Growth Performance and Feed Utilization

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Abstract. The experiment was conducted to demonstrate the effect of addition Lactobacillus reuteri with different levels of both inulin, and Jerusalem artichoke (Helianthus tuberosus) powder in common carp (Cyprinus carpio L.) fingerling diet, on growth performance and feed utilization: Weight gain (W.G), Daily weight gain (D.W.G), Relative growth rate (R.G.R), Specific growth rate (S.G.R), Feed conversion rate (F.C.R), Feed efficiency rate (F.E.R), and Protein efficiency rate (P.E.R). 60 fish were elected randomly distributed to five treatments, with three replications and 4 fish in each aquarium, per treatment, at a period of 70 days. Fish were fed at 3% of the weight of the biomass, feed prepared by protein ratio 28.95%, the control treatment without additives, 108 cfu/ml (colony formation unit)\ml were added to the meal provided for the first treatment, adding 2% inulin from the provided meal with 108 cfu/ml bacteria for the second treatment, for the third treatment, the meal containing Jerusalem artichoke powder was provided at 1% from the provided meal with 108 cfu/ml bacteria and the fourth treatment is by 2% Jerusalem artichoke powder from the provided meal, with 108 cfu/ml bacteria. These additions were provided with the nutrition program for research on Mondays and Thursdays of each week, with meals continuing to be given without additions for the rest of the days. The fourth treatment significantly ($P \leq 0.05$) exceeded for all growth parameters criteria, It did not differ significantly ($P \leq 0.05$) from the third treatment for weight gain criteria over all treatments, the feed utilization criteria, fourth treatment showed significantly ($P \leq 0.05$) exceeded over all treatments for all characteristics, it did not differ significantly ($P \leq 0.05$) from the third treatment for feed conversion rate and feed efficiency rate criteria.

1. Introduction

Common carp fish is the most important species among the aquatic organisms invested for breeding and commercial production that contribute globally to more than 72% of freshwater fish production[1]. The means of global fish farming have evolved greatly in recent years to become of economic importance within the agricultural sectors because their meat is of high nutritional value due to its protein, fat, mineral and salts[2].

The Inulin is found in nature in a wide range of plants that are used as a routine food such as fruits, vegetables and grains, as well as onions, garlic, Jerusalem artichoke (earth apples), tomatoes, and bananas [3]. Inulin is precisely vital (Prebiotic) specifically and has more health benefits and boosts immunity [4] it resists digestion and reaches the intestine to stimulate Bifidobacterium and Lactobacillus for beneficial species to the host [5]. Jerusalem artichoke tubers contain nutritional fibers and inulin [6] which are functional nutritional components that are able to play an important role in improving health and boosts immunity as they resist digestion and absorption in the digestive tract and reach the intestine intact to function as a prebiotics to improve nutrition and reproduce the beneficial microorganisms selectively which promotes health and improves growth performance excluding pathogenic in the intestine [7].

Jerusalem artichoke (Helianthus tuberosus) belongs to the sunflower family (Helianthus annuus) all Helianthus species are native to North America and Central America [8]. Most studies indicated the health and therapeutic benefit resulting from the use of this plant in the diet, as it contains inulin in addition to dietary fiber thus Jerusalem artichoke plant improves performance and quality attributes in fish diets [9], as inulin contains 67.6% of its nutritional components and protein 71.88% when it is fresh [10].

Lactobacillus bacteria is one of the most widely used types of probiotics to obtain many nutritional and curative benefits for the host [11]. the genus of lactobacilli consists of a large and heterogeneous group of anaerobic bacteria or with microaerophilic needs[12] because its nutritional requirements are complex, the intestinal cavity of humans and animals is its typical environment [13]. Many studies indicated to the role of lactobacilli in treating cases of digestive disorders and intestinal infections, in addition to its role in preventing colon and rectal cancer and colon syndrome [14]. The probiotic trait of bacteria lies through its ability to produce organic acids, ethanol and rutriene, and inhibit the growth of many pathogenic microorganisms in the host's body [15].

2. Materials and Methods

This study was completed in the Fish Laboratory of the College of Agriculture, University Of Anbar, for a period of ten weeks.

2.1. Fish and housing

We have used the fingerlings of common carp that are prepared for rearing by local farmers involved in fish farming, the fish were transported to the preset laboratory with the available means of transportation without anesthesia as they were transported in the early morning hours because of the proximity distance to the laboratory. Fish sterilized with 3% (1.5 kg/50L) NaCl solution for 5-10 minutes at once they arrived, and they were acclimatized before the 10-days of trial in the aquaria for study (30×30×60 cm.) equipped with a continuous air supply source with air pumps, 60 fish were randomly elected after the acclimation period an average weight of 17.98 ± 0.82 gm. per fish and a weight average of 72.16 ± 0.71 gm. for a living mass of 4 fish in each aquarium with three replicates per treatment, it also replaces 30% of aquaria water daily with new water stored for this purpose, after completing meals, waste and solid materials are removed to control the quality of the aquaria water. Water temperature was controlled for the duration of the experiment. The water quality measurements were taken in cooperation with the Anbar Environment Directorate every week. The growth measurements were taken in a laboratory every two weeks for a period of ten weeks.

2.2. Feeding

The diets were prepared in vitro from feedstuffs available in the local market (Table 1) after they were well ground, sifted and mixed until homogeneous with a content of 28.97% crude protein. The bush was divided into five parts. The control treatment was without addition, while the first treatment contained 108 cfu/ml of Lactobacillus reuteri only, the second treatment contained 2% inulin of the weight of the serving provided with 108 cfu/ml of bacteria, the third treatment consisted of 1% Jerusalem artichoke powder and 108 cfu/ml of bacteria and the fourth treatment contained 2% Jerusalem artichoke powder with 108 cfu/ml of bacteria. The fingerlings of common carp were fed on feed additives twice a week,

according to the process of addition to each treatment during the days of the week at 3% of the weight of the living mass (wet), and on two meals, morning at nine o'clock and evening at two o'clock (except for the weight day) and the amount of feed is adjusted according to the change in the weight of the biomass which counted every two weeks during the study period lasted 70 days. Jerusalem artichoke was obtained from the local market during the period from 15-November-2018 to 15-December-2018, the tubers were taken and washed well and their outer shell was removed to get rid of the fibers, then the tubers were cut into thin slices as possible and then the slices were directly immersed in boiling water for 5 minutes followed by submerging them in a cool solution of citric acid 1%, slides are dried at a temperature of 55-65 ° C using the Taiwan-YCO 01036L electric drying oven until its weight is fixed and then crushed and preserved as a powder with polyethylene bags and placed in the freezer at -18º C until use (16), Inulin was extracted from the dried tubers powder according to the method described by [8].

Table 1. The feed components used in feeding experimental fish. The values are based on the dry matter. [1 *7*]

The contribution of feedstuffs to various nutrients						
Energy Kcal / Kg	Ether extract	Raw fibers	Crude protein	Dry matter	Ratio%	Ingredients
484.61	1.15	0.51	9.20	21.37	23	Protein conc.
780.50	0.39	2.45	15.4	31.15	35	Soybean meal
670.00	0.72	0.44	1.70	17.80	20	yellow corn
264.00	0.19	0.55	1.10	8.90	10	Barley
130.00	0.40	1.10	1.57	8.90	10	bran
----	----	----	---	---	1	Vit. & minerals
----	----	----	---	---	1	salt
1329.11	2.85	5.05	28.97	88.12	100	Total

Lactobacillus reuteri was obtained from the laboratories of the Department of Food Sciences - College of Agriculture - University of Anbar, biological and microscopic tests were performed to ensure that they were alive and free from contamination. The bacteria were active through their development three consecutive times in the middle of the pre-sterilized MRS-broth using the preservative and at a temperature of 121°C/ 15 minutes, the culture incubated at a temperature of 37°C, after the completion of the third activation stage, 250 ml of skim milk was sterilized at 115°C for 10 minutes was inoculated with the bacteria, and this was considered as a mother culture will be adopted in the next stages of research [12]. In order to obtain a fixed number of bacteria, the method described by [1*8*] was followed, as the number 108 cfu\ml was adopted. The live number of bacteria was counted by following the total plat count method with MRS-Agar medium according to the method described by [12].

2.3. The parameters studied.

The studied parameters were calculated on growth performance and feed utilization as follows:

Weight gain, W.G (gm) = W2 (gm) - W1 (gm)
Daily weight gain, D.W.G (gm/day) = WG(gm)- Trial period (day)
Relative growth rate, R.G.R (%) = W2 (gm) - W1 (gm) / W1 (gm) × 100
Specific growth rate and, S.G.R (%) =ln W2 –ln W1/ Trial period (day) × 100
Feed conversion rate, FCR = dray food intake (gm)/ WG (gm)

Feed efficiency rate, F.E.R (%) = WG (gm) / dray food intake (gm) × 100
Protein efficiency rate, P.E.R (%) = dray protein intake (gm) / WG (gm) × 100

3. Results and Discussion

Water quality tests: The results of the water temperature checks for the current experiment showed suitable rates throughout the research period, as they recorded 24 ± 1.5º C, for the period from 10/ March/ 2019 until 19/ May/ 2019, and they are within the appropriate limits for breeding common carp fish [19]. The ranges of oxygen concentration ranged between 6.5-9 mg/ l, which exceeds the critical ranges (2 mg / l), and the pH scale ranged between 6.5-7.5 and it falls within the appropriate limits for breeding that lie between 6.0 and 8.5 [20]. The experiment was performed without any mortality.

3.1. Effect of feeding on bacteria, inulin and Jerusalem artichoke on growth performance and feed utilization criteria.

Results of the study showed the superiority of the fourth treatment, 2% Jerusalem artichoke powder with 108 cfu/ml Lactobacillus reuteri significantly (P≤0.05), on all treatments did not differ significantly (P≤0.05) from the third treatment 1% Jerusalem artichoke powder with 108 cfu/ml for the weight gain feature. Figure (1) clarify the weight increase in the fourth treatment was 52.88 grams over the initial weight 71.95 gm., while the increase amounted to 50.41 gm. over the primary weight of 71.82 gm. in the third treatment, which contained 1% of the powder of Jerusalem artichoke with 108 cfu/ml of bacteria, this was followed by the second treatment containing 2% anulin, with 108 cfu/ml of bacteria, where the weight increase in it reached 46.49 gm. over the initial weight 71.71 gm, then the first treatment containing the bacteria separately, recorded a weight increase of 35.30 gm. over the initial weight of 72.22 gm. final weight, the control treatment that recorded a weight gain of 29.09 gm. over the initial weight was 72.18gm.

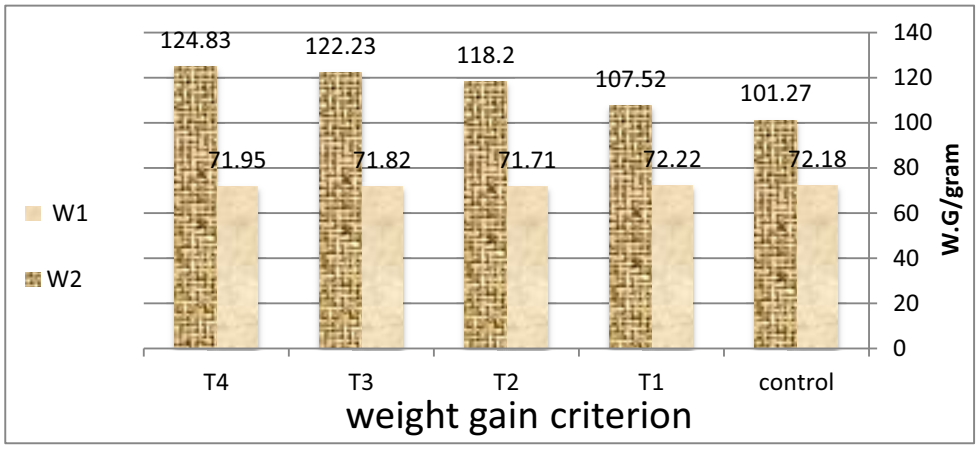

Figure 1. Effect of addition of inulin, bacteria and Jerusalem artichoke powder on the weight gain of fish (W1 initial fish weight . W2: final fish weight)

Results also indicate the positive role played by the bacteria L.reuteri in the weight gain of fish by improving their digestibility and increasing the diet metabolism in addition to the health and therapeutic benefits that bacteria cause in the intestinal cavity of the fish [21], which reflects positively on its weight, as the results demonstrated the role of inulin in increasing the activity of lactobacilli through the ability of these bacteria to divide this sugar into fructose, which is a carbon source that supports cell growth during the bosom period [22, 23], This is observed by comparison between the weight gain of fish in the first treatment that contained the bacteria individually and the second treatment that contained the bacteria in

addition to the prebiotic. The increase in fish weight when adding Jerusalem artichoke tubers powder, by 1% or 2%, was due to the fact that this Jerusalem artichoke powder contains inulin in addition to many other nutrients that play a positive role in supporting the activity of bacteria added to the diet, which was positively and significantly reflected in improve the weight gain of fish in the fourth treatment [16].

The first treatment showed a significant superiority over the control treatment, as it is one of the vital enhancers (prebiotics) that resist somewhat enzymatic digestion, after that it was reaching the intestine intact in proportions capable of causing a change in the social composition of its microorganisms in favor of the host, which improves growth performance [21], the second treatment is the addition of anulin and bacteria in a synergistic way, its significantly ($P \leq 0.05$) outperforming on the control treatments and the first one. This may be attributed to the addition of anulin with bacteria present, as inulin is a functional nutrient, which applies the definition of prebiotics, meaning that it is the specific substances that support the growth and/ or activity of beneficial bacteria endemic in the digestive system in order to promote health [24], Studies have agreed that the prebiotics improves the host's health and provides it with a beneficial physiological effect [25], it is the active ingredient for fermentation of beneficial bacteria in the intestine, harmful bacteria do not benefit from it which helps to multiply beneficial bacteria colonies Increase in number and size and exclude the presence of pathogenic bacteria because it narrows the space available inside the intestine [26, 27].

Jerusalem artichoke powder was added to the third treatment instead of inulin, which gave results that exceed the previous three treatments, and this may be due to the presence of dietary fiber in the Jerusalem artichoke powder by 16.6% (dry) which stimulates beneficial bacteria and boosts immunity [28] in addition to the presence of inulin by 67.6% when its fresh [10], which microorganisms use in the colon for fermentation and are represented and this results in few calories for the host [8].

Fermentation is one of the important mechanisms of dietary fiber in the colon Which changes the formation of the intestinal microflora in the large intestine, which is the location of microorganism activity and the processes of metabolism of microorganisms in the body, this makes the medium more acidic in the intestine, since the by-product of metabolism is short chain fatty acid [24].

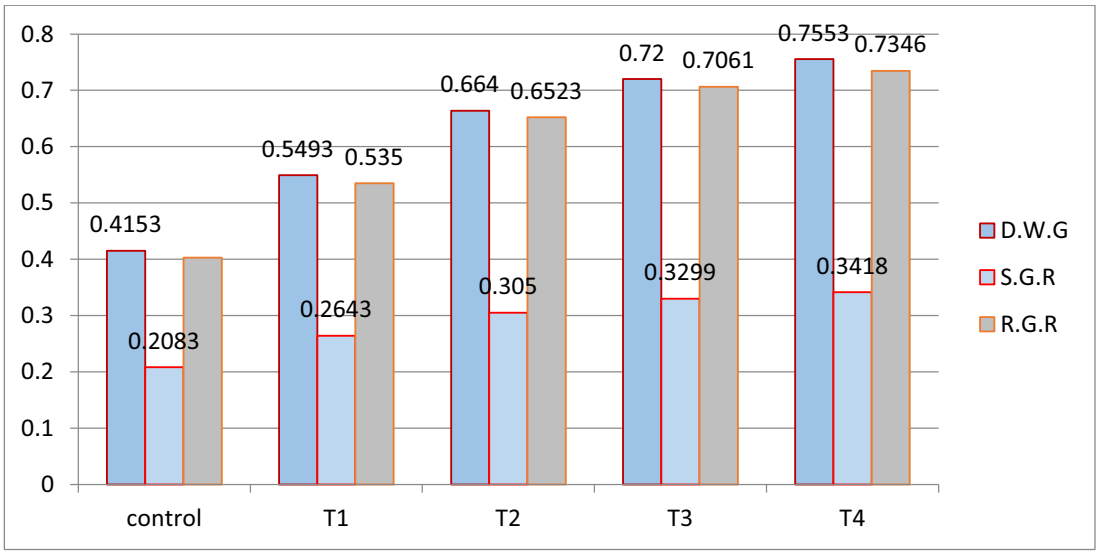

Figure 2. Effect of addition of inulin, bacteria and Jerusalem artichoke powder on fish growth.

As for the fourth treatment, the percentage used from Jerusalem artichoke powder was added twice as much as the third treatment which showed superior results in most of the criteria for growth and feed utilization, it may be attributed to the increase in the active and stimulating substance for bacterial activity, it positively affected, by increasing its efficacy on the intestinal microorganisms and making a greater impact on growth performance, which raised the values of these criteria (Figure 2).

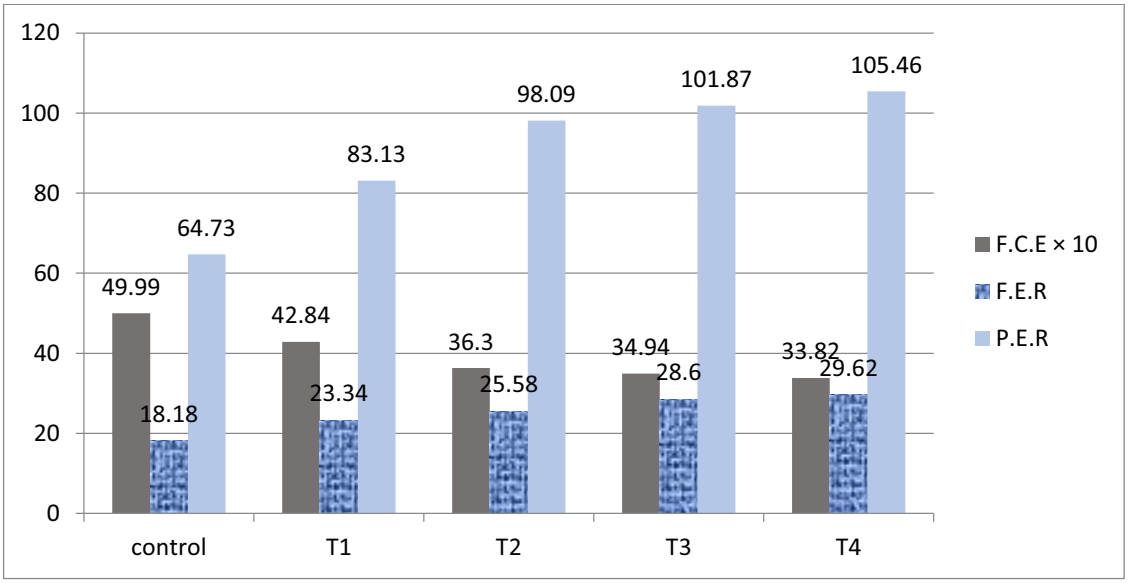

Figure 3. The effect of addition of inulin, bacteria and Jerusalem artichoke powder on feed utilization criteria

The fourth treatment indicates outperformed 2% Jerusalem artichoke tubers powder with 108 cfu/ml of L. reuteri significantly (P≤0.05) over all treatments. The daily weight increase was 0.7553 gm/fish/day, while the specific weight rate was 0.3418% day, and the relative growth rate was 0.7346%, and did not differ significantly (P≤0.05) from the third treatment 1% Jerusalem artichoke powder with 108 cfu/ml L reuteri for the daily weight gain feature, daily 0.72 gm/fish/day , while the specific weight rate was 0.32.99 % and the relative growth rate was 0.7061%.

The differences were significant between the previous two transactions and the first and second treatment, in which the average daily weight increase was 0.5493 gm/fish/day and 0.664 gm/fish/day, the specific weight rate was 0.2643 % day and 0.305 % day, and the relative growth rate was 0.535% and 0.6523%, respectively.

This is due to the ability of lactobacilli to ferment in the gut and produce lactic acid [29], the by-products of fermentation are short chain fatty acids that improve health and support immunity to the host [30], this would reduce the pH inside the gut, and this would determine the effectiveness of pathogenic bacteria and limit their activity [8] which supports and enhances the overall health of fish and pushes the improvement of growth indicators, and improves the coefficient of feed digestibility, and the role of prebiotic appears in the promotion and dominance of beneficial bacteria over the intestinal flora, as Lactobacillus sp. can secrete the digestive and degrading enzymes of the constituent elements of the diet [3]. This may be improves the value of feed use indicators and improves growth performance [32, 33] (Figure 3).

Conclusion:

This study aims to reach an improvement in fish diets through available food additives at reasonable costs, to obtain better production performance of common carp, enhance the immunity of the fry, which increases the quantity of the final product. The feeds were supplemented with various levels of Jerusalem artichoke (Helianthus tuberosus) powder and inulin with Lactobacillus reuteri to the meals of young common carp, 60 fish were distributed randomly into five treatments, including the control treatment. The fourth and third treatments outperformed all treatments in terms of growth and weight gain, as well as the criteria for feed use, feed conversion rate and feed efficiency. On the basis of the results obtained from this study, we can recommend using Jerusalem artichoke tuber powder 1-2% with 108 cfu/ml L. reuteri bacteria for the diets of young common carp fish.

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