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Efficiency of betaine and β-glucan as feed additives on the growth performance and survival rate of common carp (*Cyprinus carpio* L.) fingerlings

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Abstract
The present study was aimed to evaluate the efficiency of betaine and 0.2% β-glucan on growth performance and survival rate of common carp. 120 fingerlings of *Cyprinus carpio* weight ranged between 46-46.5g were randomly distributed into six treatment groups. Fish were fed different betaine concentration: 0.15% (T1), 0.2% (T2), 0.25% (T3) and 0.3% (T4). The fifth group (T5) was fed 0.2% β-glucan, control group was fed 0% of betaine and β-glucan in the diet. All treatments fed twice a day at rate 3% B.W for 56 days. The study was conducted at College of Veterinary Medicine/ University of Baghdad, Ichthyology laboratory, Baghdad, Iraq, from 27/4/ to 21/6/2016.
Results of feeding trail in treated groups with betaine supplementation showed significant increase ($P< 0.05$) in all growth parameters (i.e:final weight, DWG, RGR, SGR(%), FCR, FCE) compared with the control group, and the highest values were recorded in T4 supplemented with betaine 0.3%. Also, survival rate recorded highest value (100%) in treatment groups supplemented with betaine 0.3% and β-glucan 0.2% in comparison with control group (80%), suggesting that the high level of betaine (0.3%) improve growth rate and survival rate in common carp

Keywords: Common carp, betaine, β-glucan, growth performance, survival rate

1. Introduction
Fish production increased rapidly in the last years by several methods such as the establishment of fish farms and intensive rearing in cages, closed recirculating system and artificial breeding [1, 2], that encouraged researchers to develop fish production in different scientific ways to raise quality and quantity production and lower costs, which including special fish diets supplemented with growth promoters as a feed additives to improve and raise the production level without any negative effect on public health of the consumer [1]. Fishes are one of the important methods to provide food for human consumption in the world [2].
Chemicals and antibiotics are widely used to control or prevent infection by many diseases, but if use of high doses or long period will lead to side effects and sometime fish die, antibiotics have negative effect on immune system, in addition to accumulate residual in tissues [3], as well as development of pathogenic resistance to the drug [4]. Also, the use of chemicals and antibiotics lead to prevent growth of aquatic organisms which benefit as natural food for fish.
For these reasons scientists trended toward the use of alternative treatments such as feed additives which are added to the fish feed to improve the growth and production and to stimulate immune system and increase resistance to different diseases as well as lower side effect comparison with chemically synthesized drugs [5].
One of the new substances which affect positively body gain increasement and which decrease food consumption, include betaine, this substance share in the metabolic processes. Betaine is amino acid derivatives which is derive from amino acid glycin (is the trimethyl glycin), is a metabolite of plant and animal tissues [6]. Betaine is considered as a by- product of sugar beet processing [7], betaine has nutritional function commonly used as feed additive in animal, poultry and aquatic nutrition, betaine improve growth performance, health status, feed digestability, palatability, flesh quality and immune status of fish species [8, 9, 10, 11].
Another feed additives like β-glucans, β-glucans as a prebiotics are the most popular immuno-stimulants used in aquaculture, which promising for stimulating of non-specific immune response

in fish [12], β-glucans are sugars that are found in the cell wall of bacteria, fungi, yeast, mushroom and plants [13]. β-glucans used as drug (medicine) in human, while in livestock and aquatic farming used as feed supplements. In aquaculture practices used as immune stimulants, enhancing growth, survival and protection against infections pathogen [14, 15, 16, 17]. On the light of above information there are few studies to determine the efficient of betaine and β-glucan addition to the diet, on growth parameters and survival rate of common carp. Therefore, this study aimed to assess the efficiency of Betaine and β-glucan on growth performance and survival rate in common carp.

2. Materials and Methods

The present study was carried out at College of Veterinary Medicine/ University of Baghdad, Ichthyology laboratory, Baghdad, Iraq, from 27/4/ to 21/6/2016.

Fish and Experimental condition: A total of 120 fingerlings of *C. carpio* weight ranged between 46.0- 46.5 g were used in this experiment, were provided from a commercial hatchery (Al-Messayab, Babylon). Initially, fish were immersed in a salt bath of NaCl at concentration 2.5% for five minutes to get rid of external parasites and fungal infections, after that two weeks of acclimation for fish before starting the experiment and fed on commercial diet (at rate 1% of their body weight). During this time, they were stocked in three bath trough (150 × 40 × 20 cm). Then, 120 fish were randomly selected and treated with formalin at concentration 25ml/100L water for 30 minutes to eradicated of bacterial, fungal and parasitic infections, and then distributed into 12 glass aquaria filled with chlorine-free tap water and supplied with air pump at rate of 10 fish per aquarium (two replicates/ treatment) were maintained for each of the six treatments (T1, T2, T3, T4, T5 and control). Chemo-physical parameters of water were measured by Lovibond water testing apparatus. Water temperature was adjusted at 22-24 ºC and recorded daily; pH (7.2-7.6) and dissolved oxygen (6.5-7 mg/L) were noted every two days.

Fish were fed basic diet supplemented with different betaine concentration: 0.15% (T1), 0.2% (T2), 0.25% (T3) and 0.3% (T4). The fifth treatment (T5) was fed basic diet supplemented with β-glucan at ratio 0.2%, control group (C) was fed basic diet without any addition of betaine and β-glucan in the diet. All treatments fed twice a day at rate about 3% of body weight for 56 days.

One day intervals aquaria were cleaned and water was partially changed. Fish weighed every two weeks intervals to determine different growth performance (BWG, DWG, FCR, FCE, RGR%, SGR%). After 56 days from feeding trail final body weight, total feed consumption and survival rate are measured.

Experimental Diet: A floating diet was used as a basal diet in this study obtained from Faradaneh Co.(Iran), containing crude protein 36%, approximate analysis of a basal diet presented in Table 1, pellet size 5±0.4mm. Diet was prepared by weighed individually feed of basal diet for each replicate based on 3% of body weight. Different level of betaine (0.15%, 0.2%, 0.25% and 0.3%) and β-glucan (0.2%) were dissolved separately into flask with 100 ml pure water (completely dissolved), and then sprayed by sprayer on special amount of feed (with agitation) for each replicate. Diet was dried using air and sun heat with agitation; after thoroughly dryness, diet stored in a clean plastic container in dry place at room temperature. Only control group given basal diet without any supplementation, as shown in the Table 2.

Growth performance: Growth parameters were calculated every two weeks throughout the experimental period.

Body weight gain: Final fish weigh (g)- initial fish weight(g).

Daily gain (D.G): $\frac{WT-Wi}{T-t}$ were estimated [18].

Specific growth rate (SGR %): It was calculated according to the following equation SGR(%) = $\frac{\ln W_2 - \ln W_1}{T} \times 100$ [19].

Relative growth ratio (RGR)**,** Food Conversion Ratio (FCR), Food conversion efficiency (FCE) was calculated [20].

Survival Rate (survival%)

The fish were counted at the end of experimental treatment (at 56 days from the beginning of experiment) to determine the survival percentage, according to [21]. Survival%= No. of fish counted at 56 days / No. of stocked fish x 100.

Statistical analysis

All values were statically analysis using SAS (Statistical Analysis System –version 9.1). One -way ANOVA with Least significant differences (LSD). $P < 0.05$ was considered significant values [22].

Table 1: Proximate analysis of basal diet.

Analysis (%)	Pre grower
Crude protein (min)	36
Crude fat (min)	9
Crude fiber (max)	5
Charbohydrates (max)	29
Moisture (max)	10
Phosphrous (min)	1.1
Ash (max)	10

Table 2: Ingredients and composition of the experimental diet.

Treatment / Ingredients	Control	T1	T2	T3	T4	T5
Basal diet	Present	Present	Present	Present	Present	Present
Betaine	------	0.15%	0.2%	0.25%	0.3%	-----
β-glucan	------	-----	-----	-----	-----	0.2%

3. Results

Growth performance

Data of average body weight of experimental fish (*C. carpio)* are summarized in Table 3. The initial weight of all groups at first day of experimental period ranged between 46.0–46.5 g and there was no statistically differences among them ($P \geq 0.05$). The effect of betaine supplementation on growth performance was observed during the first 14 days. After 14, 28, 42 and 56 days all groups showed significantly differences ($P < 0.05$) among them. Moreover, at 56 days of the

experimental period, all treatments showed the existence of a clear increase in the growth rate compared with the control group. Results showed that the body weight increased significantly (P< 0.05) with advanced age in all groups (Table 3). The fourth group (T4) surpassed on the other groups. The superiority of this group was detected in the second period (at 14 day) (50.25 g) and continued till the end of experiment (89 g). However, similar results were found concerning the third group (T3=86 g). This could be and indicator that the two levels of betaine are more useful compared to other levels. Also, slight increase showed between T5 and T1. But, T2 showed a clear increase in body weight but not surpassed on T3 and T4.

Body weight gain (WG), Daily gain (DG), Relative growth rate (RGR), Food conversion ratio (FCR), Food conversion efficiency (FCE), Specific growth rate (SGR) are shown in Table 4. There were significant differences (P< 0.05) observed in body weight gain and daily gain in all group between them.

Our results showed there were no significant differences in FCR values between T1, T2 and T5. However, there were significant differences in FCE values (P< 0.05) among all groups. But decrease in the FCR values at level 2.0 and 1.89 and increase in FCE values at level 49.47 and 52.63 with T3 and T4 respectively, that means treatment groups supplemented with betaine at level (0.25% and 0.3 %) improved feed utilization and growth performance of common carp compared with other groups.

The results of present study showed that were significant differences at level (P< 0.05) between T1, T2, T3, T4, T5 and control group in RGR values. Also, there were significant differences at level (P< 0.05) between T1, T2, T5 and control group in SGR values. While, no significant difference between T3 and T4 in SGR which recorded highest values (1.13% and 1.15%) respectively from all other groups. Table 4 confirmed that the fourth group was the best group as it was differed significantly (P< 0.05) as compared with other groups in all parameters. Also, results of the third group were found similar with results of the fourth group.

Survival rate

At the end of experimental period (at 56 days) all treatment groups revealed significant decrease in mortality rate when compared with control group. The results in Table 5 showed significant differences concerning survival rate between treatments, the best survival rate was recorded in T4 and T5 that was 100%, followed by T3 and T2 was 95% while T1 and control treatment were 90% and 80% respectively.

Table 3: Average of body weight of *C. carpio* feeding different level of betaine, β-glucan and a control diet (± SE) during 56 days and fish weight each 14 days.

Weight (g) / Treatment	Initial weight at one day	At 14 day	28 day	42 day	56 day
Control	46.50±0.25 a	48.00±0.57 b	53.00±0.57 e	61.00±1.15 d	70.00±0.57 f
T1	46.00±0.57 a	48.25±0.28 b	54.75±0.15 d	62.45±0.26 d	75.00±0.58 e
T2	46.25±0.14 a	48.75±0.14 b	56.25±0.14 bc	64.35±0.20 c	79.00±0.59 c
T3	46.35±0.20 a	49.50±0.28ab	57.50±0.27 ab	67.75±0.43 b	86.00±1.16 b
T4	46.30±0.17 a	50.25±0.16 a	58.50±0.16 a	70.00±0.66 a	89.00±1.17 a
T5	46.25±0.14 a	48.45±0.25 b	55.25±0.13 cd	63.35±0.20 c	76.75±0.44 d
LSD	1.42				

Means with different small letter in the same column differ significantly (P< 0.05).

Table 4: Growth performance of *C. carpio* post treatment with betaine, β-glucan and a control diet during 56 days.

Parameters / Treatment	BWG (g)	DWG g/d/fish	FCR	RGR%	FCE	SGR%
Control	23.50±0.28 f	0.41±0.005 d	3.20±0.11 c	50.53±0.28 f	31.30±0.57 f	0.73±0.03 d
T1	29.00±0.43e	0.51±0.007 cd	2.62±0.07 b	63.04±0.58 e	38.05±0.60 e	0.87±0.02 c
T2	32.75±0.43c	0.57±0.01 bc	2.37±0.21 b	70.81±0.29 c	42.20±0.70 c	0.95±0.03 b
T3	39.65±0.29b	0.70±0.004 ab	2.00±0.07 a	85.54±0.57 b	49.47±0.49 b	1.13±0.02a
T4	42.70±0.40a	0.76±0.02 a	1.89±0.11 a	92.22±0.70 a	52.63±0.35 a	1.15±0.02 a
T5	30.50±0.28d	0.54±0.005 c	2.51±0.05 b	65.94±0.54 d	39.78±0.45 d	0.90±0.01 bc
LSD	1.11	0.156	0.356	1.60	1.66	0.08

Means with different small letters in the same column differ significantly (P< 0.05).

Table 5: Survival rate of experimental fish post feeding with betaine, β-glucan during 56 days.

Treatment	Total number	No. of dead fish	Survival rate%	Mortality rate%
Control	20	4	80	20
T1	20	2	90	10
T2	20	1	95	5
T3	20	1	95	5
T4	20	Zero	100	Zero
T5	20	Zero	100	Zero

4. Discussion

Growth performance and survival rate

Good nutrition is critical in promoting good health of all fish species, including those raised in aquaculture. A healthy condition is needed if the animals are surviving and growing rapidly, which is considered the most important goal in aquaculture [23].

Betaine is an attractant substance, and used in order to increase food intake and growth promoters for several fish species and crustacean [24, 25]. Betaine can improve feeds delicious flavor to promote ingestion, short ingestion time, increase feed utilization rate and reduce water pollution.

In the present study the results of growth parameters and survival rate especially with betaine treatments are in agreement with the results were obtained by [6] who studied the effect of 0.2% betaine addition to carp fry feed improved the growth rate, final body weight increases, food conversion

ratio (1.22) and the highest survival rate (91.6%) were obtained in this treatment. Also, [26]. reported that added betaine (0.5 and 1%) with the experimental diet of Caspian roach (*Rutilus rutiluscaspicus*) fingerlings, resulted an increasing in final weight, body weight gain and a decreasing effect on FCR, but not significantly. Food intake increased significantly as added betaine [27]. reported that body weight gain and daily weight gain are important parameters to evaluate kinds of diet and protein value in it [28]. reported that food conversion ratio means the capability of any fish species to convert diet to body weight gain with kept healthy. Also, [27] reminded that the evaluation of growth performance on fish accurate by use relative growth rate (RGR) and specific growth rate (SGR) criteria more than the body weight gain (BWG) and daily gain (DG) criteria, due to that the RGR and SGR criteria reduce the effects of variant in the initial weight between fish.

The effect of add betaine hydrochloride to the diet of common carp *C. carpio* was investigated by [29], who studied that treatment group with 0.25% betaine fed common carp showed highest growth in parameters of specific growth rate, survival, and food conversion rate and protein efficiency ratio compared with other treatment groups.

Our results in β-glucan treatment showed no significant increases in growth rate but showed highest survival rate. Several studies showed the effect of β-glucan on growth, survival and immune response of fish species against infectious pathogens alone or in combination with other stimulants or probiotics. For example, Nile tilapia (*Oreochromis niloticus*) were fed with different concentrations of β-glucan showed no effect on growth and survival rate after 10 weeks [30, 15] used β-glucan at a dose of 250 mg/kg diet was recommended for enhancing immunity, growth, and survival against opportunistic pathogens such as *Aeromonas hydrophila and Edwardsiella tarda* in rohu (*Labeo rohita*). Oral administration of three different quantity of β -glucan low (38 g/kg), average (52 g/kg), high (82 g/kg) in Oncorhynchus mykiss showed decrease in the growth rate but enhanced survival against IHNV(infectious hematopoietic necrosis virus) [17]. Pacific White shrimp (*Litopenaeus vannaemi*) fed with diet containing inactive yeast cell wall (β-glucan) 1 and 2 g/kg feed showed no significant differences in weight, survival, and growth rate, but showed better effects on immune parameters compared to control [31]. These differences in results due to different in types of β-glucan were utilized, and dosage, route and duration of administration as well as, fish species.

5. Conclusion

This study showed positive effects of betaine on growth performance when adding to the diet of common carp in all treatments. The largest value on growth rate was recorded in T4 intake diet supplemented 0.3% betaine, as well as, give protection by increasing survival rate. There was no positive effect of 0.2% β-glucan on growth performance when supplemented to diet of common carp but has a positive effect on survival rate. In conclusion, dietary betaine supplementation improves growth performance, survival rate in common carp. This information could be useful for fish aquaculture. More studies are required to detect the molecular and cellular pathways of the effect of betaine.

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