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Effect of adding blue-green algae, probiotic and antibiotic to the diet on the productive performance of common carp *Cyprinus carpio* L.

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Abstract

This current study aimed to study the effect of adding blue-green algae (Spirulina), symbiotic (poultry star®me) at a ratio of 1 to 2% and colistin antibiotic to the control diet of common carp fish reared in glass aquariums for 56 days terms of three replicates for each treatment. The growth results showed that the fish fed on the third diet was significantly superior to the fish fed on the other experimental diets in the rate of total and daily weight gain, feed conversion ratio, feeding efficiency ratio and protein efficiency ratio criteria over all other experimental diets except for the second diet, 1% spirulina. The fish fed on the second diet significantly outperformed in the precipitated protein criterion and the protein productive value of all the experimental diets under study. The value of crude protein in the edible portion increased significantly when fish were fed on spirulina, symbiotic and antibiotic diets, compared to the control diet, while the percentage of fats in the fish fed on diets containing the food additives under test decreased significantly compared to the control diet. No significant differences were recorded in the percentage of ash. It is evident from what was mentioned above that the best results of the studied criteria were when adding spirulina at had positive effects on growth criteria and food utilization.

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Introduction

Arthrospira has cylindrical, multicellular trachomas in an open left-hand helix and can be found in tropical and subtropical lakes with high pH and alkalinity, where other microorganisms struggle to survive (1). *A. platen*sis thrives in alkaline, saline water > 30 g L with a high pH 8.5-11.0. It's also in the soil, marshland, rivers, and thermal springs (2). *A. platensis* is found in Africa, Asia, and South America, whereas *A. maxima* is found only in Central America (1). In Central Africa, around Lakes Chad and Niger, and in East Africa, in the Great Rift Valley, the largest spirulina lakes may be found (3). The primary reason for the microalgae's promise is that it includes an excellent mix of protein, fat, and carbohydrates to protect the health of the fish. When compared to certain other additives like bacteria and yeast, microalgae are really a high source of protein and fat (4). *Spirulina platensis*, a type of microalgae, has a lot of promise and is a great feed ingredient in many aspects. According to studies, this alga includes a wide range of chemicals, including phycobiliproteins, carotenoids, phycocyanin, polysaccharides, unsaturated fatty acids, superoxide dismutase, various vitamins, and others, all of which may help fish improve their color, growth, and immunity (5). Probiotics are administered in a variety of ways, from the oral/water regimen to feed additives, the latter of which is extensively employed in aquaculture. Probiotics can be used in single or multiple strains, as well as in conjunction with prebiotics and immunostimulants like symbiotic. They can also be used in living or dead forms (6). Between 2008 and

2018, 67 antibiotic compounds were utilized in 11 out of 15 nations, according to Lulijwa *et al.* (7). 73% of these countries used oxytetracycline, sulphadiazine, and florfenicol. Antibiotics have been used by 15 countries, with Vietnam, China, and Bangladesh being the country's most commonly used (8-11).

The purpose of this study is to compare the effects of various food additives at various levels on growth and food utilization of common carp *Cyprinus carpio* L.

Materials and methods

Experimental diets

The common carp *Cyprinus carpio* L. were fed on sixth experimental diets contained 25.45% crude protein and 13.17 MJ/Kg. Control diet (diet 1) which supplemented with blue green algae (spirulina) by 1 and 2% (diet 2 and 3); symbiotic by 1 and 2% (4 and 5 diets) and antibiotic (Colistin) 3000 IU/Kg (diet 6) (Table 1). Experimental feeds were made after their components were milled and formed as pellets in a meat mincer machine. Twice a day, the fishes were fed 3-5% of their total body weight. The fishes were acclimatized for two weeks in a glass aquarium environment (72 L), containing 10 fish per aquaria. After the acclimatization period ended, the fish were fed on experimental diets for fifty-six days. The fish were randomly distributed at an average weight of 14.31 g/fish to the experimental treatments, with three replications for each treatment.

Table 1: Dietary ingredients and chemical composition of the experimental diets containing different supplementary food

Ingredients	Control	Spirulina 1%	Spirulina1 2%	Symbiotic 1%	Symbiotic 2%	Colistin
Spirulina)	-	1.0	-	-	-	-
Spirulina)	-	-	2.0	-	-	-
Symbiotic	-	-	-	1.0	-	-
Symbiotic	-	-	-	-	2.0	-
Colistine	-	-	-	-	-	3000 IU/gm.
Animal protein	10	10	10	10	10	10
Soybean meal	30	30	30	30	30	30
Local barley	20	20	20	20	20	20
Yellow corn	18.5	18.5	18.5	18.5	18.5	18.5
Wheat bran	19	19	19	19	19	19
Food salt	1	1	1	1	1	1
Vitamin + minerals	0.5	0.5	0.5	0.5	0.5	0.5
Lime stone	0.5	0.5	0.5	0.5	0.5	0.5
binder (Bentonite)	0.5	0.5	0.5	0.5	0.5	0.5

Chemical composition

Crude protein	Ether extract	Ash	Crude fiber	Nitrogen free extract	ME (MJ/Kg)
25.45	3.54	6.97	4.75	52.20	13.17

*Calculated according to Smith's equation: ME (MJ/Kg) = Protein x 18.8 + Fat x 33.5 + NFE X13.8. (11).

Water quality

Air conditioners maintain the water's temperature between 23 and 26 degrees Celsius. The pH is between 7.2 and 7.6 and the dissolved oxygen content ranges from 5.6 to 6.8 mg/L. Every two weeks, the amount of food given to the fish is raised in accordance to the increase in weight.

Chemical analysis

The major chemical components of the feed, as well as the edible portion components of fish fed on various experimental diets, were determined using the standard methodologies Association of Official Analytical Chemists (12).

Growth criteria

To determine growth rates, food consumption, protein intake, protein efficiency ratio, protein retention standards, and protein productive value, arithmetic formulae based on Hepher (13) are applied.

Statistical analysis

All of the data was analyzed and processed using SPSS (14). The mean standard error of three replicates of pooled samples is provided. The analysis was applied to a one-way analysis of variance. Duncan's (15) novel multiple-range test was utilized to find differences in the criteria's means. $P \leq 0.05$ was used to test for significant differences.

Results

Final weight, weight gain and daily weight gain

Final weight, weight gain and daily weight gain for the initial weight criterion, statistical analysis of the growth criteria assessed in Table 2 indicated no significant differences (P≤0.05) between all treatments. The fish fed on the third diet (2% Spirulina) significantly outperformed the total weight gain criterion, which amounted to 21.55 g/fish, then the fish fed on diets, control, fourth (1% Symbiotic), fifth (2% Symbiotic) and the sixth (Colistin), which amounted to 16.72 and 14.98 and 15.33g/fish, respectively, as well as the daily weight gain. The daily weight gain (gm/fish/day) characteristic followed the same trend as the total body weight characteristic, as fish fed the third diet, with a value of 0.385, were significantly superior to fish fed the first, fourth, fifth, and seventh diets, with values of 0.297, 0.298, 0.268, and 0.264 respectively.

Relative growth rate and specific growth rate

All treatment groups showed 100% survival rates throughout the trial, demonstrating that all fish had adapted to the study's conditions and test diets. Fish given spirulina at 1.0 and 2.0% (diet 2 and 3) exhibited the greatest significant (P≤0.05) relative growth rates when compared to the control diet and other treatments. The second and third diets exhibited the highest relative growth rates 138.21 and 151.01%, as well as the highest specific growth rates, according to table 3. (1.56 and 1.52). Between the control (diet 1) and the fish-fed probiotics (diets 4 and 5) groups, as well as between the antibiotic group and the antibiotic group, there were no significant differences (diet 6).

Table 2: Effect of different supplementary food on final weight, weight gain, and daily growth rate

Diets	Initial body weight (gm/fish)	Final body weight (gm/fish)	Total weight Gain (gm/fish)	Daily weight gain (gm./fish/day)
Control	14.13±0.13 a	30.74±0.46 a	16.61±0.59 bc	0.297±0.01 bc
Spirulina 1%	14.35 ±0.29 a	34.18±0.35 a	19.83±0.55 ab	0.354±0.01 ab
Spirulina 2%	14.29±0.14 a	33.34± 4.79 a	21.55±2.61 a	0.385±0.05 a
Symbiotic 1%	14.420±.15 a	30.14 ± 1.46 a	16.72±0.61 bc	0.298±0.01 bc
Symbiotic 2%	14.18±0.10 a	29.16±0.18 a	14.98±0.19 c	0.268± 01 c
Colistin	14.47± 0.17 a	29.80± 0.62 a	15.33±0.79 c	0.2740±0.01 c

* Means not sharing a common superscript letter are significantly different (P ≤ 0.05).

Table 3: Effect of different supplementary food on relative growth rate, specific growth rate and survival rate

Diets	Survival Rate (%)	Relative Growth Rate (%)	Specific Growth Rate (SGR)
Control	100	117.60 ± 5.26 b	1.39 ±0.88 b
Spirulina 1%	100	138.21± 2.41 a	1.56± 0.02 a
Spirulina 2%	100	151.01± 2.09 a	1.52± 0.07 a
Symbiotic 1%	100	115.98± 5.40 b	1.33± 0.11 b
Symbiotic 2%	100	105.65± 1.41 b	1.29± 0.01 b
Colistin	100	105.95± 6.63 b	1.30± 0.04 b

* Means not sharing a common superscript letter are significantly different (P ≤ 0.05).

Feed conversion ratio and feed efficiency ratio

Table 4 demonstrates that there was no significant difference in total food consumption (P≤0.05) between fish fed the control diet and fish fed other diets containing different supplemental foods at varying rates, with the exception of fish fed the second and fifth diets. The FCR of common carp fed 2.0% spirulina (second diet) decreased to 2.02, which was less significantly (P≤0.05) than the control group 2.61 and fish fed probiotic (diets 4 and 5) and antibiotic (diets 4 and 5) diets (diet 6) (Table 4).

Protein intake and protein efficiency ratio

Table 5 demonstrates that there were no significant differences in protein intake between the fish fed various additional materials and the other treatments, with the exception of the fifth diet (P≤0.05). Supplementing with spirulina at a rate of 2.0% (diet 2) raised the PER value to 1.97 (diet 4), 2.05 (diet 2), and 1.50 for the fourth, fifth, and sixth diets, which were respectively 1.53, 1.44, and 1.46. The amount of protein deposited by fish fed second diets was 3.95 gm/fish, which was significantly (P≤0.05) more than the control and other treatments.

Table 4: Effect of different supplementary food on food intake, food conversion ratio and feed efficiency ratio

Diets	Feed consumption (gm/fish)	Feed conversion ratio	Feed efficiency ratio (%)
Control	43.52±0.09 ab	2.61± 0.08 ab	38.17±1.28 b
Spirulina 1%	44.80± 0.80 a	2.26± 0.03 bc	44.05±0.44 a
Spirulina 2%	42.90±0.50 ab	2.02± 0.22 c	50.17±5.50 a
Symbiotic 1%	43.00±1.40 ab	2.57±0.01 ab	38.87±0.14b
Symbiotic 2%	41.00 ±1.40 b	2.74±0.13 a	36.60±1.71b
Colistin	44.20± 0.20 ab	2.88± 0.12 a	34.67±1.62 b

* Means not sharing a common superscript letter are significantly different (P≤0.05).

Table 5: Effect of different supplementary food on protein intake, protein efficiency ratio, protein retention and protein productive value

Diets	Protein Consumption (gm/fish)	Protein Efficiency Ratio (PER)	Protein Retention (gm / fish)	Protein Productive Value (%)
Control	11.08± 0.03 ab	1.50±0.22 b	2.84± 0.02b	25.60 ±0.26 bc
Spirulina 1%	11.41± 0.21a*	1.74±0.05 ab	3.95± 0.01 a	34.63±0.54 a
Spirulina 2%	10.92± 0.13 ab	1.97±0.03 a	2.90± 0.06 b	25.29±0.94 bc
Symbiotic 1%	10.95± 0.36 ab	1.53± 0.21b	3.12±0.41b	28.30±2.77 b
Symbiotic 2%	10.44± 0.36 b	1.44±0.01b	2.87±0.01b	27.50± 0.99 bc
Colistin	11.25± 0.05 ab	1.46±0.07 b	2.60±0.10 b	23.07±0.83 c

* Means not sharing a common superscript letter are significantly different (P≤0.05).

Protein productive value

The protein productive value criterion was significantly higher (P≤0.05) for fish fed a diet containing green blue algae (Spirulina) at 1.0% 34.63% than in fish fed diets 1, 3, 4, and 5, which were 25.26, 25.29, 28.30, 27.25%, and 23.07%, respectively.

Protein, fat and ash retention

At the ending of the feeding trial, Table 6 shows the biochemical composition of the common carp's edible portion. Moisture and dry weight of all fish groups fed diets containing various feed additives at various doses could not differ significantly from the control diet, according to biochemical analyses of the edible portion of the fish. All experimental treatments of the edible portion content had significantly lower percent ages of ether extract than the control treatment (P≤0.05). Spirulina (diets 2 and 3) and probiotic (diets 4 and 5) offered to fish showed a significant increase in crude protein (P≤0.05) when compared to the control and antibiotic diets. (Diets 1, 6, and 8). When compared to fish given the third and fourth diets (symbiotic 1 and 2%), the ash percentage of fish fed the second diet (1% spirulina) increased significantly (P≤0.05), reaching 3.61, 2.85, and 2.81, respectively. treatments.

Table 6: Effect of different supplementary food on chemical composition (%) of the edible portion of common carp

Diets	Moisture	Dry weight	Total protein	Ether extract	Ash
Control	73.58±0.60	26.43 ±0.34	16.42±0.19 b	9.22±0.20 a*	3.10±0.15 ab
Spirulina 1%	73.18±0.52	26.82±0.37	18.12±0.02 a	7.66±0.19 b	3.61±0.07 a
Spirulina 2%	73.32±1.64	26.68±1.16	17.63±0.31 a	7.11±0.10 b	2.85±0.05 b
Symbiotic 1%	74.66±0.74	25.35±0.53	17.82±0.43 a	7.42±0.11 b	2.81±0.05 b
Symbiotic 2%	73.08±1.32	26.93±0.94	17.44±0.10 a	7.50±0.22 b	2.95±0.10 ab
Colistine	73.48±0.38	26.52±0.34	16.22±0.01 b	7.47±0.13 b	3.39±0.44 ab

*Means not sharing a common superscript letter are significantly different (P≤0.05).

Discussion

The fish fed on the third diet 2% spirulina were significantly superior to the other experimental diets in the parameters of the total weight gain rate and the daily weight gain. This is due to the highly valuable nutritional properties of blue-green algae. These results are in agreement with Raji *et al.* (16) and El-Sheek *et al.* (17) found the fish fed the control diet had the least growth performance in terms of weight gain compared with fish fed on an algae diet.

The results of the current study showed that the fish fed on the second and third rations (1% and 2% Spirulina) were significantly superior in terms of relative growth rate and specific growth rate over all other experimental rations. This result agrees with El-Sheek *et al.* (17) Sarker *et al.* (18) found that the value of RGR and SGR for fish fed control diets decreased significantly when compared to fish fed algae diets (*S. platensis and C. vulgaris*). The high digestibility of nutrients, notably protein, lipid, amino acids, and fatty acids, which were attributed to 2% *S. platensis* in our study, was credited with the increase in growth (18,19). The addition of 3-5% *S. platensis* to fish diets has been demonstrated to enhance *C. gariepinus'* uptake of nutrients and, as a result, its growth (20,21). Ibrahem *et al.* (22) observed a significant increase in the growth performance parameters and survival rate of *Oreochromis niloticus* in Spirulina-supplemented groups at a concentration level of 10g/kg. Adding 0.5 g/kg of numerous types of probiotics to carp fish diets resulted in a substantial increase ($P \leq 0.05$) overall body weight gain, growth rate, relative growth rate, and especially poultry star®me compared to the control ration for common carp, according to Mohammad and Al-Safo (23). While Amer (24) reported that supplementing with *S. platensis* at a concentration of up to 1.5% had no deleterious effects on growth, there were no significant variations in SGR% between the two groups.

The best feed conversion rate and feed efficiency were achieved when feeding on the second and third diets. In contrast to plant components, the addition of *S. platensis* as a feed supplement may improve feed efficiency by boosting gut bacterial colonization, according to a trend for better growth rate at 2% *S. platensis* in the current study. Teimouri *et al.* (25) showed the Spirulina contains highly digestible protein 60-70% with all essential amino acids, polyunsaturated fatty acids (PUFA), vitamins, minerals, and a variety of photosynthetic pigments (26,27). This also increases the synthesis of enzymes that transfer lipids from the fish to the liver for metabolism rather than storage. These results are consistent with those of Amer (24), who found that adding 1% spirulina to the diet resulted in the best food conversion ratio, but no significant differences in total feed intake or antioxidant protective capabilities. Adding different types of probiotics (star®me) to carp fish diets in amounts of 0.5 g/kg resulted in significant differences ($P \leq 0.05$) in the feed conversion ratio and feed efficiency ratio of common carp fish, according to Mohammad and Al-Safo (23).

The findings revealed that while the protein efficiency ratio rose when feeding fish supplemented with blue-green algae, there were no significant differences in the amount of protein consumed overall amongst the various treatments. Raji *et al.* (16) agreed with the findings, indicating the importance of a fish-fed spirulina diet in protein intake and protein efficiency ratio criteria as compared to fish fed fish meal. The group supplemented with 1% outperformed the control and other groups *S. Platensis* had a considerable rise in PER (24).

The result reveals that the fish fed the second diet, that contained 1% blue-green algae, performed significantly superior than the fish fed the other experimental diets. The algal source influences the algal digestibility of nutrients in a fish species (18,28). Raji *et al.* (16) revealed our findings, finding that fish fed a spirulina diet had a significant effect on protein intake and protein efficiency ratio when compared to fish fed fish meal, and that all of the experimental diets had an apparent protein digestibility of > 90%, indicating that juvenile *C. Gariepinus* could absorb their proteins adequately. Teuling *et al.* (19) found 80.7% digestibility for Chlorella and 81.4% for Spirulina in catfish, and 85.4% protein digestibility for cell-ruptured Chlorella in juvenile Atlantic salmon *Salmo salar*. The protein retention and protein productive value of common carp fed a probiotic (star®me) diet were significantly higher ($P \leq 0.05$) than the control diet, according to Mohammad and Al-Safo (23).

The findings revealed that fish fed probiotic and blue-green algae diets retained a significant amount of crude protein compared to fish fed control and antibiotic rations, while the amount of fat in the edible portion increased in comparison to the control group rations. The highest protein concentration was found in the muscles of fish fed a 10% spirulina diet, according to Abdel Latif (29). El Sheek *et al.* (17) found that feeding Nile tilapia spirulina instead of fish meal significantly increased the amount of protein and decreased the amount of ether extract in their bodies. Qasab-bashi and Mohammad (30) mentioned the possibility of substituting spirulina in spite of 20% of the soybean meal, where common carp fish outperformed in growth and food utilization criteria. The addition of *B. subtilis* to diets significantly altered the activity of the digestive enzymes ADCP, EFU, FCR, PER, RGR, and SGR, according to results of Rachmawati (31).

Conclusion

The addition of blue green algae produced the best results for the growth criteria, which included the rate of daily and total weight gain, feed conversion ratio, feed efficiency ratio, protein efficiency ratio, protein retention, and protein productive value, while the percentage of fat significantly decreased in fish fed on diets.

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Conflict of interest

No conflict of interest.

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تأثير إضافة الطحالب الخضراء المزرقة والمعزز الحيوي إلى العلائق على أداء اسماك الكارب الشائع

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الخلاصة

هدفت هذه الدراسة الحالية الى بيان تأثير اضافة الطحالب الخضر المزرقة والمعزز الحيوي التآزري بنسبة ١ و ٢% و المضاد الحيوي كوليستين الى عليقة السيطرة لأسماك الكارب الشائع. المرباة في الاحواض الزجاجية لمدة ٥٦ يوما وبواقع ثلاث مكررات لكل معاملة. بينت نتائج النمو تفوق الاسماك المغذاة على العليقة الثالثة معنويا عن الاسماك المغذاة على العلائق التجريبية الاخرى في معيار معدل الزيادة الوزنية الكلية واليومية ومعامل التحويل الغذائي ونسبة كفاءة التغذية ونسبة كفاءة البروتين عن كافة العلائق التجريبية الأخرى (باستثناء العليقة الثانية، ١% سبايرولينا). تفوقت الاسماك المغذاة على هاتين العليقة الثانية معنويا في معيار البروتين المترسب والقيمة المنتجة للبروتين عن كافة العلائق التجريبية قيد الدراسة. ارتفعت قيمة البروتين الخام في الجزء المأكول عند تغذية الاسماك على علائق احتوت على السبايرولينا والمعزز الحيوي التآزري و المضاد الحيوي معنويا عن عليقة السيطرة. فيما انخفضت نسبة الدهون معنويا في الاسماك التي تم تغذيتها على علائق احتوت على الاضافات الغذائية قيد الاختبار مقارنة بعليقة السيطرة. لم يتم تسجيل اختلافات معنوية في نسبة الرماد. يتبين مما تم ذكره انفا ان افضل النتائج للمعايير المدروسة كانت عند اضافة السبايرولينا بنسبة ١ و ٢% لتأثيرات الايجابية في معايير النمو والاستفادة من الغذاء.