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The effect of algae meal (*Spirulina*) on the growth performance and carcass parameters of rainbow trout (*Oncorhynchus mykiss*)

ABSTRACT

The effect of algae meal (*Spirulina spp.*) on the growth performance and carcass parameters of rainbow trout (*Oncorhynchus mykiss*) were studied. Algae meal was used as supplementary feed. One experimental diet – consisting of 10% Spirulina's meal + basal diets (10% SD) – was used in the trial and compared to a control diet – a basal diet (BD) with 0% algae. The initial size of rainbow trout from the control group was 14.95 g, whereas the group fed with experimental feed was at 14.66 g. They were stocked in semi-closed RAS at a density of 26 pcs/m³. The fish were acclimatized in the RAS for two weeks. The duration of the trial was 35 days. The weight gain, condition factor and average daily growth of rainbow trout fed with 10% SD were higher than those from the group fed with feed without algae supplement, but the differences were not statistically proven (p>0.05). The fish fed with experimental feed showed better weight of eviscerated fish, consumable yield and carcass weight compared with the carcass parameters showed from fish fed with BD, but difference was statistically proven (p<0.05) just for consumable yield.

Key words: spirulina meal, rainbow trout, supplement, growth, carcass parameters

Introduction

The algae are a strongly appreciated source of protein, essential amino acids (Fabregas & Herrero, 1985; Becker, 1994) and vitamins (Becker, 2004). They could play a considerable role in the humans' nutrition as well as in the feeding of livestock –e.g. chicken (Marton et al., 1968) or pigs (Fevrier & Seve, 1976).

In aquaculture the feeding experiment conducted with algae are related with their use as general protein sources - due to the complete replacement of fish meal in fish feed - or as additive substances in the feed for cultivated hydrobionts.

Concerning the usage of algae meal for the replacement of fish meal in the feed for hydrobionts, experiments were conducted with the following species – fish from genera *Oreochromis* (Appler & Jauncey, 1983; Appler, 1985; Chow & Woo, 1996), *Pagrus major* (Kalla et al., 2008), *Dicentrarchus labrax* (Valente et al., 2006), *Siganus canaliculatus* (Tacon et al., 1990) *Penaeus monodon* (Briggs

& Funge-Smith, 1996).

Better growth and protein assimilation was proved for fish species *Paralichthys olivaceus* when the algae meal was used as an additive in their feeding. The positive effect of the used additive decrease the cholesterol and fat level and improved lipid metabolism in fish too.

Because of the great variety of biologically active agents found in *Spirulina spp*. they were object of numerous investigations for their use in biotechnology and medical science. *Spirulina* is a rich source of the pigment C-phycocyanin C-PC (Richmond, 1986), whose role as an antioxidant (Romay et al., 1998; Bhat & Madyastha, 2000; Pinero Estrada et al., 2001) as well as it's anticancer properties (Dasgupta et al., 2001) are well known. The studies of Nandeesha et al., 1998; Olvera-Novoa et al., 1998; Mu et al., 2000; Nandeesha et al., 2001 have shown that the blue-green algae *Spirulina platensis* holds potential for inclusion in diets of various fish species due to its attractive nutrient profile and digestibility.

The study conducted by Ayyappan et al. (1991) clearly demonstrated the advantages of spirulina meals in their quality as additives in the feed for carps. They established that Rohu and Mrigal carp showed a better growth rate in comparison to those received for the indian carp and common carp. The potential of *Spirulina* as nutrient source in diets for abalone such as for *Haliotis midae* (Britz et al., 1994; Britz, 1996) or *Haliotis asinina* (Bautista-Teruel et al., 2003) was reported too.

The aim of the current study was to investigate the effect of dietary spirulina meal in diets for rainbow trout on their growth rate, feed conversion efficiency and carcass parameters.

Materials and Methods

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The trial was conducted in the Experimental Aquaculture Base at the Trakia University (Stara Zagora, Bulgaria). The duration of the experiment was 35 days. For the aim of the trial a recirculation aquaculture system (RAS) was used. It consisted of a concrete tank connected to a common recirculation water supply (Figure 1). The total volume of each tank was 1 m³.

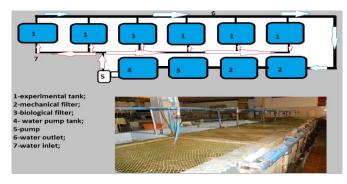


Figure 1. Used RAS in the trial.

The mechanical and biological filter was used in the RAS for the cleaning of the water from waste. The bottom of the experimental tank was syphoned daily. A compensation of water losses from the evaporation, the syphoning of the bottom and for the reduction of nitrate up to 20% pure water from total volume of RAS was added every day.

Hydrochemical parameters

The temperature of water was measured once a day at 9 o'clock. The measurement was made by a temperature probe. Dissolved oxygen and pH was measured with a portable

meter – HQ 40D (Hach Lange®) – with appropriate probes every day.

Experimental fish

The used experimental fish originated from the fish farm "Bukovetc" - Tvyrdica, where 120 rainbow trout in good health condition (Figure 2) and similar size were selected and transported to the Trakia University. The transportation of fish was made in 50 l. polyethylene bags with aerated water.



Figure 2. Experimental rainbow trout.

The average initial weight of the fish from 3 replications for two 2 experimental variants were as follows:

- -Control group (fed with control diet a basal diet with 0% algae) 14.95 ± 2.10 g;
- -Experimental group (fed with experimental diet -consisting of 10% Spirulina's meal + basal diets (10% SD) 14.66 ± 2.09 g.

Two experimental variants were conducted in three replications. The fish were acclimatized in the RAS for two weeks before starting the experiment. In this period the fish were fed with pellets with a size appropriate for their age. The fish were stocked in a tank under a stocking density of 26 pcs.m⁻³. For measuring the growth rate of the experimental rainbow trout they were measured in technical balance with 0.01g precision.

Experimental diet

Dried algae meal (*Spirulina spp.*) and commercial trout feed (39.6% protein) were milled using a laboratory mill. Commercial trout feed was mixed with 10% dried algae meals and commercial feed that contained no algae meal serving as the control diet. Milled trout feed and algae meals were mixed thoroughly using an electronic mixer device by

adding warm water and mixing until a homogeneous dough was obtained. The received dough was then passed through a mincer to produce feeds with pellets with appropriate size. After being dried in an oven at +40°C, the feeds were kept in a refrigerator (+4°C) until the use (Promya & Chitmanat, 2011).

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The chemical analysis of the basal and experimental diet was made by standard methods in the department "Animal nutrition and physiology" at the Faculty of Agriculture of the Trakia University. The results are shown in Table 1.

Table 1. Proximate composition (% dry weights) of experimental diets.

| | Crude protein (%) | Fat (%) | Moisture (%) | Ash (%) |
|-------------------|-------------------------|---------|-----------------|---------|
| Basal Diet | 39.67 | 20.09 | 5.64 | 5.47 |
| (0% algae) | | | | |
| Experimental Diet | 41.44 | 18.99 | 9.58 | 5.25 |
| (10% algae) | | | | |

Daily feed ratio was determined on the base of temperature and weight of the experimental fish. For this reason 10% of the fish were weighed each day and the amount of feed was recorded each day throughout the 35 days experimental period. The fish were fed three times a day by hand.

At the beginning of trial for all fish and at the end of trial for 50% of experimental rainbow trout weight (g) and standard length (cm) were measured. After the end of the experiment calculations were made to determine values of the condition factor, weight gain, specific growth rate (SGR), average daily growth, survival rate, feed conversion rate to the following formula:

-Condition factor is equal to:

$$K = \frac{(Weight in grams)}{(lenght in cm)^2} X100$$

$$(Weight\ gain,\%) = \frac{Final\ weight-Initial\ weight}{Initial\ weight} X100$$

-Specific growth rate (%/day) is equal to:

$$\frac{(\ln Fish\ weight\ at\ end\ of\ experiment\ -\ \ln Fish\ weight\ at\ the\ beginning\ of\ experiment\)}{Number\ of\ days\ during\ experiment} X100$$

-Average daily growth (g/fish/day) is equal to:

-Survival rate (%) is equal to:

$$\frac{\textit{Number of surviving fish}}{\textit{Number of initial fish}} \textit{X} 100$$

-Feed Conversion Rate (unit) is equal to:

Carcass parameters of rainbow trout have been measured at the end of trial:

- Weight of eviscerated fish (g) = weight of fish weight of viscera;
- Consumable yield (g) = (weight of fish (weight of gills + internal organs))
- Consumable weight (g) = (weight of trout (mass of head + internal organs))

Statistical analysis

The data from the measurements were analyzed by variance analysis (ANOVA) in order to determine the effects and differences of treatment using ANOVA single factor test at p<0.05. The SPSS program was used.

Results

Hydrochemical parameters

The water temperature varied between 11.9 and 14.3°C during the experiment. Statistically proven differences in temperature between both experimental variant were not found. Average value of dissolved oxygen was higher than 8 mg.L⁻¹, and its value didn't distinguish significantly between the experimental variants (Table 2). The values received for pH were slightly alkaline and they were similar for the tanks from the control and experimental groups (Table 2).

Growth parameters

At the start of the trial standard length and condition factor for rainbow trout from the control group were 11.06 ± 1.36 cm and 1.12 ± 0.13 respectively and for the fish from experimental variant 10.74 ± 0.69 cm and 1.18 ± 0.08 respectively (Table 3). At the end of the experiment weight and length of rainbow trout fed with 10% SD were higher, but the differences were not statistically proven.

Table 2. Water quality in RAS.

| Parameter | BD | 10% SD | |
|------------------------------|---------------|---------------|--|
| | $x\pm Sx$ | $x\pm Sx$ | |
| Temperature (°C) | 12.94±1.13 ns | 12.95±1.08 ns | |
| pН | 8.00±0.20 ns | 7.99±0.23 ns | |
| Oxygen (mg.L ⁻¹) | 8.38±0.36 ns | 8.37±0.38 ns | |

Table 3. Weight, standard length and condition factor of the rainbow trout during the trial.

| Parameter | BD | 10% SD |
|----------------------|--------------------|----------------------|
| | x±Sx | x±Sx |
| Start of trial | | |
| Weight (g) | 14.95±2.10 ns | 14.66±2.09 ns |
| Standart length (cm) | 11.06±1.36 ns | 10.74±0.69 ns |
| Condition factor | 1.12±0.13 ns | 1.18±0.08 ns |
| End of trial | | |
| Weight (g) | 22.53±1.49 ns | 22.54±1.70 ns |
| Standart length (cm) | 13.63±0.65 ns | 13.66±0.66 ns |
| Condition factor | 0.88 ± 0.01^{ns} | $0.88\pm0.02^{\ ns}$ |

The same tendency was observed for weight gain, specific growth rate (SGR) and average daily growth which were lower for the experimental variant in which fish were fed with the basal diet (BD), compared with the values of these parameters received for rainbow trout fed with the experimental diet with 10 % algae supplement, but these differences were not significant (p>0.05) (Table 4). The survival rate was the same for both experimental variants -96.8±2.74. No differences among the two treatments were observed for the feed conversion ratio (p>0.05) (Table 4).

Table 4. Average growth rates, survival rates and feed conversion ratio of rainbow trout for two used feed during 35 days experimental period.

| BD | 10% SD |
|----------------------------|---|
| $x\pm Sx$ | $x\pm Sx$ |
| 51.07 ± 6.48^{ns} | 54.08±1.61 ns |
| 1.21 ± 0.12^{ns} | 1.27±0.03 ns |
| 0.22 ± 0.01^{ns} | 0.23 ± 0.004 ns |
| | |
| 96.8±2.74 ns | 96.8±2.74 ns |
| $1.32\pm0.10^{\text{ ns}}$ | 1.26±0.02 ns |
| | x±Sx 51.07±6.48 ^{ns} 1.21±0.12 ^{ns} 0.22±0.01 ^{ns} 96.8±2.74 ^{ns} |

Carcass parameters of rainbow trout

The weight of the eviscerated fish and carcass weight (Table 5) were higher for rainbow trout fed with 10%SD, but differences were not significant (p>0.05). The consumable yield of rainbow trout receiving 10% SD was significantly

(p<0.05) higher than those gained from fish fed with BD (Table5).

Table 5. Weight of eviscerated fish, consumable yield and carcass weight of rainbow trout for two used feed during 35 days experimental period.

| Parameters | BD | 10%SD |
|----------------------------|--------------------------|--------------------------|
| | $x\pm Sx$ | $x\pm Sx$ |
| Weight of eviscerated fish | 80.31±8.29 ^{ns} | 84.01±1.87 ^{ns} |
| Consumable yield | 78.48 ± 1.53^{a} | 85.38 ± 1.79^{a} |
| Carcass weight | 64.86 ± 5.78^{ns} | 65.35 ± 7.08^{ns} |

Note: Different letters (a,b,c,d) show significant statistical differences (p<0.05), ns=no significant difference

Discussion

The measured temperature and oxygen were optimal for the fish species used in the trial, because the optimal temperature for rainbow trout must be in a range between 12 and 16°C and the oxygen level should not fall below 8 mg.l⁻¹ (Zajkov, 2006). The used experimental diet did not significantly change the pH of the water in the tanks, whose received results were in the contradiction of the results noted from Promya & Chitmanat (2011) which received higher values of pH in a pond with fish fed with a diet consisting algae. This received from us values of pH perhaps are the result from the daily syphoning of the tanks bottom, which did not allow a piling up of uneaten food on the tanks' bottoms.

The results received from the trial stated that rainbow trouts fed with the experimental diet consisting of algae meal (Spirulina spp.) showed higher growth (final weight, condition factor, weight gain, SGR, average daily growth) when compared to rainbow trouts fed with a standard trout diet, but the differences were not statistically proven. Our results were in confirmation of the data received from Pókniak (2010), who found out that 5% of the spirulina meal can be incorporated in the feed for rainbow trout fry without a significant affect to their productive performance (body weight, feed intake, feed conversion and specific growth rate) and the mortality of the fry. In recent research survival and feed conversion rate of experimental fish were not affected by the presence of algae meal in the diet. Similar data were received in a research conducted to investigate the replacement of fish meal in the feed for Pangasianodon gigas Chevy with spirulina meal at 0, 15, 30 and 100% (Ahmadzadenia et al., 2011a). It showed no significant

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differences in average daily gain, specific growth rate and feed conversion rate between the different experimental variants.

A study conducted with Red Tilapia demonstrated that a 10-15% replacement of crushed fish as a protein source with Spirulina algae led to an increase of average daily growth rate and specific growth (Promya & Chitmanat, 2011). Tilapia species are herbivorous species and because of this reason they can more effectively assimilate the plant protein in comparison to carnivorous species to which the rainbow trout belongs. We believe that in our study the differences in growth performance, feed conversion ratio and carcass parameters in favor of rainbow trout fed with feed with algae additives compared with those fish fed with a basal diet are not so highly expressed, because in our experimental diet in common with replacement of plant meal with algae also the fish meal was partly replaced. These ratiocinations are confirmed from a study conducted by Ahmadzadenia et al., (2011b), who received better body length, carcass weight, crude protein, crude fat and ash in trout's carcass for rainbow trout fed with a diet in which soybean meal is replaced with algae meal. The better consumable yield in rainbow trout fed with feed containing algae supplement (Spirulina) was confirmed in recent research too.

Conclusion

The results of the present study indicate that *Spirulina* could be incorporated in experimental feed for rainbow trout at 10% as a supplement to their feed. Not only did the standard length, final weight, average daily gain and specific growth rate increase, but the weights of eviscerated fish, carcass weight and consumable yield of the rainbow trout were higher for fish fed with the experimental diet with algae as a supplement and for the consumable yield this difference was statistically proven.

References

- AhmadzadeniaY, Nazeradl K, Ghaemmaghami S, Hejazi M, Zamanzad Ghavidel S, Hassanpour S, Chaichisemsari M. 2011. Effect of replacing fishmeal with spirulina on carcass composition of rainbow trout. ARPN Journal of Agricultural and Biological Science, 6(6): 66-71.
- Ahmadzade-Nia Y, Nazer Adl K, Ghaemmaghami S, Hejazi M, Hassanpour S, Chaichisemsari M, Riyazi S. 2011. Effect of replacing different levels of Soybean meal with *Spirulina* on

- performance in Rainbow Trout. Annals of Biological Research, 2(3): 374-379.
- Appler H, Jauncey K. 1983. The utilization of a filamentous green alga (Cladophoraglomerata (L) Kutzin) as a protein source in pelleted feeds for *Sarotherodon* (Tilapia) niloticus fingerlings. Aquaculture, 30: 21-30.
- Appler H. 1985. Evaluation *of Hydrodictyon reticulatum* as protein source in feeds for *Oreochromis* (Tilapia) niloticus and *Tilapia zillii*. Journal of Fish Biology, 27: 327-334.
- Ayyappan S, Pandey B, Sarkar, S, Saha D, Tripathy S. 1991. Potential of *Spirulina* as feed supplement for carp fry. pp. 86-88. In Proceedings of the National Symposium of Freshwater Aquaculture, CIFA, Bhubaneswar, India. Bhubaneswar, CIFA.
- Bautista-Teruel M, Fermin A, Koshio S. 2003. Diet development and evaluation for juvenile abalone, *Haliotis asinina*: animal and plant protein sources. Aquaculture, 219(1-4): 645-653.
- Becker E. 1994. Microalgae biotechnology and microbiology. Cambridge University Press, Cambridge, Great Britain, p. 293.
- Becker E. 2004. Microalgae in human and animal nutrition. In: Richmond A. (eds), Handbook of microalgal culture, Blackwell, Oxford, p. 312-351.
- Bhat V, Madyastha K. 2000. C-Phycocyanin: A potent peroxyl radical scavenger in vivo and in vitro. Biochem. Biophys. Res. Commun., 275: 20-25.
- Briggs M, Funge-Smith S. 1996. The potential use of *Gracilaria* sp. meal in diets for juvenile *Penaeus monodon* Fabricius. Aquaculture Research, 27: 345-354.
- Britz P. 1996. The suitability of selected protein sources for inclusion in formulated diets for the South African abalone *Haliotis midae*. Aquaculture, 140: 63-73.
- Britz P, Hecht T, Kanuer J, Dixon M. 1994. The development of an artificial feed for abalone Farming. South African Journal of Science, 90: 7-8.
- Chow C, Woo N. 1990. Bioenergetic studies on an omnivorous fish, *Oreochromis mossambicus*: evaluation of utilization of *Spirulina* algae in feed. In Hirano R, Hanyu I. (eds), The Second Asian Fisheries Forum. Manila, Asian Fisheries Society p. 291-294.
- Dasgupta T, Banejee S, Yadav P, Rao A. 2001. Chemomodulation of carcinogen metabolising enzymes, antioxidant profiles and skin and forestomach papillomagenesis by *Spirulina platensis*. Mol. Cell. Biochem., 226: 27-38.
- Fabregas J, Herrero C. 1985. Marine microalgae as a potential source of single cell protein (SCP). Appl. Microbiol. Biotechnol., 23: 110-113.
- Février C, Seve B. 1976. Essais d'incorporation de spiruline (*Spirulina maxima*) dans les aliments des porcins. Ann. Nutr. Alim., 29: 625–650.
- Kalla A, Yoshimatsu T, Araki T, Zhang D, Yamamoto T. Sakamoto S. 2008. Use of *Porphyra spheroplasts* as feed additive for red sea bream. Fisheries Science, 74: 104-108.
- Marton G, Peter L, Szajko J, Schmidt J. 1968. Mosonmagýaróvári Agrártud. Főiskola Közlemé., 11: 213-228.
- Mu Y, Lam T, Shim K. 2000. Protein digestibility and amino acid availability of several protein sources for juvenile Chinese hairy crab, *Eriocheir sinensis* H. Milne-Edwards (Decapoda, Grapsidae). Aquaculture Research, 31 (10): 757-765.
- Nandeesha M, Gangadhara B, Manissery J, Venkataraman L. 2001. Growth performance of two Indian major carps, catla

- (Catlacatla) and rohu (Labeorohita) fed diets containing different levels of *Spirulina platensis*, Bioresource Technology, 80: 117-120.
- Nandeesha M, Gangadhara B, Varghes T, Keshavannath P, 1998. Effect of feeding *Spirulina platensis* on the growth, proximate composition and organoleptic quality of common carp, *Cyprinus carpio* L. Aquaculture Research, 29(5): 305-312.
- Olvera-Novoa M, Dominguez-Cen L, Olvera-Castillo L, Martinez-Pacios C. 1998. Effect of the use of microalgae Spirulina maxima as fish meal replacement in diets for tilapia, Oreochromis mossambicus (Peters), fry. Aquaculture Research, 29(10): 709-715.
- Pinero Estrada J, Bermejo Bescos P, Villardel Fresno A. 2001. Antioxidant activity of different fractions of *Spirulina platensis* protean extract. Farmaco, 56: 497-500.
- Pókniak J. 2010. Incorporación de Espirulina (*Spirulina maxima*) en dietasparaalevines de truchasarco iris (*Oncorhynchus mykiss*) Avances en Ciencias Veterinarias. [Online] 22: 1-2.
- Promya J, Chitmanat C. 2011. The effects of *Spirulina platensis* and *Cladophora* algae on the growth performance meat quality and immunity stimulating capacity of the African sharptooth catfish (*Clarias gariepinus*). Int. J. Agr. Biol., 13(1): 77–82.

- Richmond A, 1986. Microalgae of economic potential. In: Richmond, A. (eds), Handbook of Microalgal Mass Culture. CRC Press Inc, Boca Raton, FL., p. 199-244.
- Romay C, Armesto J, Remirez D, Gonzalez R, Ledon N, Garcia I. 1998. Antioxidant and anti-Inflammatory properties of Cphytocyanin from blue-green algae. Inflamm. Res., 47(1): 36-41
- Tacon A, Rausin N, Kadari M, Cornelis P. 1990. The food and feeding ofmarine finfish in floating net cages at the National Seafarming Development Centre, Lampung, Indonesia: rabbitfish, Siganus canaliculatus (Park). Aquaculture and Fisheries Management, 21: 375-390.
- Valente L, Gouveia A, Rema P, Matos J, Gomes E. Pinto I. 2006. Evaluation of three seaweeds *Gracilaria bursa-pastoris*, *Ulva rigida* and *Gracilaria corneaas* dietary ingredients in European sea bass (*Dicentrarchus labrax*) juveniles. Aquaculture, 252: 85-91.
- Zajkov A. 2006. Aquaculture Principle and Technology. Kabry, Sofia, p. 376 (in Bulgarian).