ISSN 1313 - 8820
Volume 6, Number 4
December 2014

AGRICULTURAL SCIENCE AND TECHNOLOGY

2014

An International Journal Published by Faculty of Agriculture,
Trakia University, Stara Zagora, Bulgaria

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The journal is accepted to be indexed with the support of a project № BG051PO001-3.3.05-0001 “Science and business” financed by Operational Programme “Human Resources Development” of EU. The title has been suggested to be included in SCOPUS (Elsevier) and Electronic Journals Submission Form (Thomson Reuters).

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Agricultural Science and Technology
Faculty of Agriculture, Trakia University
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Bulgaria
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AGRICULTURAL SCIENCE AND TECHNOLOGY, VOL. 6, No 4, pp 431 - 436, 2014

Effect of linseed and sunflower oils in the diet on the growth parameters in rainbow trout (*Oncorhynchus mykiss* W.) cultivated in a recirculating system

G. Zheliazkov*

Department of Biology and Aquaculture, Faculty of Agriculture, Trakia University, 6000 Stara Zagora, Bulgaria

Abstract. *The aim of this study was to determine the influence of linseed and sunflower oils in the diet on the survival rate, weight gain and the feed conversion ratio in rainbow trout, reared in a recirculating system. The fish of the three groups were raised in concrete tanks with effective capacity of 0.8 m^3, which were part of the recirculating system. Rainbow trout received extruded feed (6 mm pellets), produced by "Aqua garant". The feed of the fish from the experimental group (EL) was supplemented with 5% linseed oil, while that of the trout of the group (ES) – 5% sunflower oil. The diet of the fish of the control group (C) did not contain any of the above mentioned oils. The duration of the trial was 60 days. In order to study the effect of linseed and sunflower oils in the diet on the weight gain of the fish, control catches were carried out at 15 days' interval. The live weight (g) and linear growth (mm) of the control catches were determined as the fish were individually weighed and measured. The final live weights of the trout in the experimental groups as well as that in the control were as follows: EL – 334.79±67.52g, ES – 329.39±70.31 g and C – 323.58±67.77 g. The values of this parameter in EL were higher than those of fish from ES and the control group by 1.64% and 3.47%, respectively, but the differences were not significant (P>0.05). This trend persisted also for the linear measurements - in the trout of EL they were the highest. The fish of EL displayed the lowest feed consumption. This trait had lower values than the ones of the trout of ES and the control group by 2.84% and 5%, respectively, though the differences were not significant (P>0.05). The survival rate of the fish from the groups did not differ substantially.*

Keywords: rainbow trout, linseed oil, sunflower oil, growth parameters, feed conversion ratio

Introduction

Rainbow trout is the most widely raised species among the freshwater cold-water fish. The feed production for trout is of great importance for the industry survival (Ng et al., 2007). The main feed for rainbow trout is extruded pellets. Considerable changes in the formulation and composition of the diets for rainbow trout have occurred in the past 15 to 20 years (Atanasov et al., 2010). Feeds used nowadays may contain up to 35-40 % fat, mainly fish oil (Geurden et al., 2007). The increase of its price in the global market (Barlow, 2000; Delgado, et al., 2003; Mráz, 2012) makes aquaculture feed producers search for the possibilities to replace it by more stable alternatives (Pickova and Morkore, 2007). These are oils obtained from unicellular algae, pelagic organisms or demersal invertebrates that are rich in n-3 PUFA and tested in the fish feeds (Hertrampf and Piedad-Pascual, 2000; Carter et al., 2003; Olsen et al., 2004). Their price at this point is still too high for them to be used largely in the fish feeds (Turchini et al., 2009). This is the reason why usually as replacement some vegetable fat sources are tested. The production of the latter has increased substantially during the last decades (IFFO, 2008). This makes them a good alternative of the fish oil due to their availability, price, efficiency and sustainability (Pettersson, 2010).

The inclusion of vegetable oils in the feed for some fish species might be a problem because of the presence of antinutritional factors. These compounds are natural toxins or antimetabolites acting as insecticides in the plants or as part of the survival mechanisms of the species (Hendricks, 2002). The vegetable oils utilized must guarantee the growth of the fish, must not have any negative effects on the feed consumption and last but not least, they must not deteriorate the qualities of the products (Karalazos, 2007).

The aim of this study was to determine the effect of linseed and sunflower oil supplementation of the diet on the survival rate, weight gain and feed conversion ratio in rainbow trout (*Oncorhynchus mykiss* W.), raised in a recirculating system.

Material and methods

The rainbow trout of the three groups was raised in concrete tanks with a capacity of 0.8m^3, which were part of the recirculating system. The fish were fed three times a day. They received 6 mm extruded pellets "Aqua UNI", produced by "Aqua garant". The feed of the fish from the experimental group (EL) was supplemented with 5 % linseed oil, while the feed of the trout of the group (ES) contained additionally 5% sunflower oil. The fish of the control group (C) did not receive any of the above mentioned vegetable oil with their diet. The nutrient content of the feed of the three groups is presented in Table 1. Here the data from the specification of the firm and as well as the obtained after our analysis according AOAC 2012 are presented. The trial continued 60 days.

Methods adapted for fish-farming (Bessonov and Privezentsev,1987; Todorov, 1992) were applied to determine the hydrochemical parameters in the recirculating system of the rainbow trout (*Oncorhynchus mykiss* W.) as follows:

- Quantity of the dissolved oxygen, mg/l – MultiLine P4;
- pH – MultiLine P4;
- Electrical conductivity – MultiLine P4 and BDS EN 27888;
- Quantity of nitrates, mg/l – BDS 17.1.4.12:1979;
- Quantity of nitrites, mg/l –BDS ISO 26777:1997;

In order to study the effect of the included sunflower and linseed oils on the weight gain of the fish, control catches were carried out at 15 days' interval. The live weight was determined within 0.1 g, and

* e-mail: georgi_ij@abv.bg

Table 1. Nutrient content in the feed of the rainbow trout (*Oncorhynchus mykiss* W.)

№	Item	According to the specification of "Aqua garant"	Groups		
			C	ES	EL
1	Crude protein, %	42.00	43.00	43.00	43.00
2	Fat, %	16.00	17.12	22.12	22.12
3	Fibre, %	2.50	3.31	3.31	3.31
4	Moisture, %	7.62	7.62	7.62	7.62
5	Lysine, %	1.68	1.68	1.68	1.68
6	Methionine +cysteine, %	2.84	2.84	2.84	2.84
7	Ca, %	1.42	1.42	1.42	1.42
8	P, %	1.40	2.50	2.50	2.50
9	Chlorides, %	1.84	1.84	1.84	1.84
10	ME, MJ/kg	18.20	18.64	20.54	20.54
11	ME, kcal/kg	4352	4455	4910	4910

* 1 kg feed contains: vitamin A – 10000 IE; vitamin D3 – 1500 IE; vitamin – E – 200 mg; vitamin K – 3 mg; thiamin – 10 mg; riboflavin – 15 mg; pyridoxine– 8 mg; vitamin B12 – 0.02 mg; nicotinic acid– 40 mg; folic acid– 3 mg; biotin– 0.3 mg. ** 1 kg feed contains: Fe – 145 mg; Mn – 67 mg; Cu – 16 mg; Zn – 68 mg; I – 1.5 mg; Co – 0.5 mg; Se – 0.6 mg

the linear measurements of the fish – total body length (longitudo totum corporis-L, mm), standard body length (longitudo corporis-l, mm), trunk length (T, mm), head length (longitudo capitis-C, mm), body height (altitudo corporis maxima-H, mm), body width (latitudo corporis-D, mm) and circumference (O, mm) – within 1 mm. The body measurements of the fish were carried out according to a scheme of Pravdin (1966), for measuring trout species. The linear measurements of the fish were done using a measuring board, triangle and tape, and the weights were recorded by electronic balance, as the fish were weighed and measured individually. At the end of the trial the weight gain (g), survival rate (%) and feed conversion ratio were determined.

The data were statistically evaluated using STATISTICA 6.0 software (StatSoft Inc., 2002).

Results

The hydrochemical parameters during the trial period are presented in Table 2. The water temperature of the different groups was 18.4 ºC. The content of the dissolved oxygen was within the range of 6.93 mg/l – 8.87 mg/l. The pH of the water in the recirculating system varied for the individual experimental designs and was 7.48 to 8.08. The quantity of nitrates was measured between 0.27 mg/l to 0.33 mg/l, while the nitrite content was within the range of 0.005 mg/l to 0.009 mg/l. The values of electrical conductivity of the water were 556.0 µS/cm^{-1} to 695.0 µS/cm^{-1}. The analysis of the data concerning the hydrochemical traits during the trial showed that they were optimal for the species, resulting in good survival rate, weight gain and feed conversion ratio in the rainbow trout of the experiment.

The survival of the rainbow trout during the trial is presented on Figure 1. The values of this parameter in the fish of the replicates of the experimental groups supplemented with linseed and sunflower oils were 100%. Such was the survival of the fish from the control group as well.

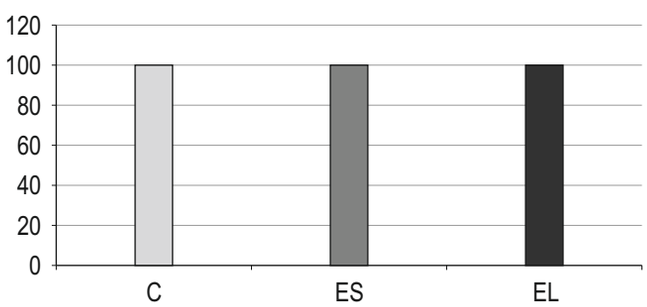

Figure 1. Survival rate of the rainbow trout

Table 2. Hydrochemical parameters in the recirculating system during the experiment with the rainbow trout

Parameter	n	Min.	Max.	Optimal values, (according to Zaykov and Staykov, 2013)
Temperature, °C	60	13.00	18.40	12.0-16.0
Dissolved oxygen O_2, mg/l	60	6.93	8.87	Not below 8
pH	60	7.84	8.08	7.0-8.0
Nitrates, mg/l	60	0.27	0.33	Up to 2.0
Nitrites, mg/l	60	0.005	0.009	Up to 0.01
Electrical conductivity, µS/cm^{-1}	60	556.00	695.00	-

Table 3. Mean linear measurements and weight of the rainbow trout at the beginning of the trial

Parameter	n	C	ES	EL
		Mean ± SD	Mean ± SD	Mean ± SD
Total body length, mm	40	229.38 ± 12.65	231.35 ± 15.25	232.03 ± 15.26
Standard body length, mm	40	203.75 ± 9.99	204.13 ± 11.19	204.25 ± 11.19
Head length, mm	40	40.73 ± 2.56	40.80 ± 2.52	40.98 ± 2.78
Trunk length, mm	40	106.25 ± 5.77	106.43 ± 6.13	105.95 ± 6.04
Body height , mm	40	48.55 ± 2.65	48.45 ± 2.48	48.50 ± 2.61
Body width, mm	40	26.53 ± 1.18	26.78 ± 1.33	26.83 ± 1.34
Body circumference, mm	40	116.75 ± 9.19	116.70 ± 8.83	116.80 ± 8.66
Live weight, g	40	122.10 ± 17.38	123.6 ± 18.14	123.20 ± 17.88

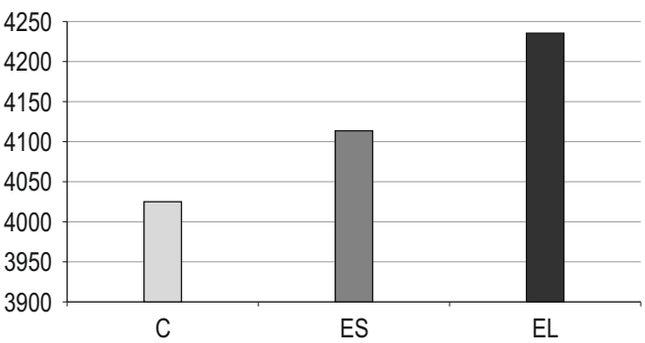

Figure 2. Total weight gain of the rainbow trout

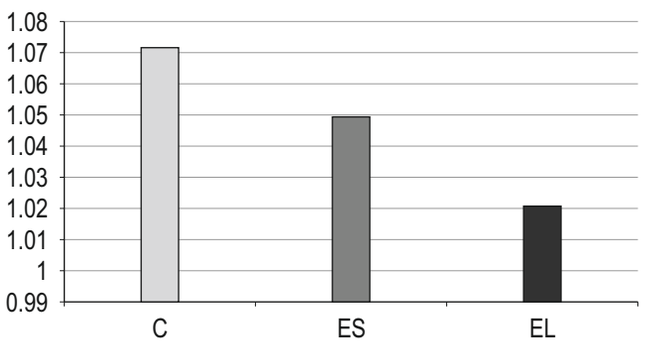

Figure 3. Feed conversion ratio

The linear measurements and the live weight of the trout of the experimental groups at the beginning of the trial are presented in Table 3. The values of the total body length of the fish of the control and experimental groups were respectively 229.38±12.65 mm, 231.35±15.25 mm and 332.03±15.26 mm, but the differences were not significant (P>0.05). The same trend was observed for the mean values of the rest of the linear measurements of the rainbow trout from the three groups, though they did not differ significantly (P>0.05).

The mean values for the total length of the replicates of both control and the two experimental groups at the end of the trial were 287.66±17.07 mm, 288.83±16.83 mm and 290.51±15.30 mm (P>0.05), respectively (Table 4). The same tendency persisted for the rest of the linear measurements with no significant differences (P>0.05). The final live weight of the fish of the experimental groups as well as of the trout of the control group was as follows: EL – 334.79±67.52 g, ES – 329.39±70.31 g and C – 323.58±67.77g.

At the end of the trial the weight gain of the fish from the control group was 4029.50 g, while those of the experiments with sunflower and linseed oils were 4115.75 g and 4232.25 g, respectively (Figure 2). During the trial the rainbow trout was fed three times a day. The feed consumed daily was analyzed in the control and the experimental groups. The values of the feed conversion ratio are presented on Figure 3 and for the fish of the control, ES and EL they are 1.072, 1.050 and 1.021, respectively.

Discussion

The analysis of the hydrochemical parameters (temperature, dissolved oxygen, pH and electrical conductivity) during the experimental period indicated that they were within the optimal range for this particular fish species. The same could be said for the

Table 4. Mean linear measurements and weight of the rainbow trout at the end of the trial

Parameter	n	C	ES	EL
		Mean ± SD	Mean ± SD	Mean ± SD
Total body length, mm	40	287.66 ± 17.07	288.83 ± 16.83	290.51 ± 15.30
Standard body length, mm	40	260.53 ± 16.08	261.89 ± 16.11	263.34 ± 14.84
Head length, mm	40	57.48 ± 3.88	57.80 ± 3.95	58.23 ± 3.74
Trunk length, mm	40	132.83 ± 10.82	133.76 ± 11.58	134.66 ± 11.47
Body height , mm	40	74.98 ± 4.76	75.20 ± 4.76	75.58 ± 4.45
Body width, mm	40	36.35 ± 2.82	36.60 ± 2.83	36.80 ± 2.64
Body circumference, mm	40	171.30 ± 11.52	172.24 ± 11.47	173.21 ± 10.75
Live weight, g	40	323.58 ± 67.77	329.39 ± 70.31	334.79 ± 67.52

maximum contents of nitrates and nitrites in water. The values of these parameters for the rainbow trout must be up to 2 mg/l and 0.01 mg/l respectively, as they are much higher than those maintained in the water during our experiment (Regulation № 4/ 20.10.2000; Zaykov and Staykov, 2013). The maintenance of these values in the water of all the groups is due to the fact that the rainbow trout was cultivated in optimal parameters of farming technology. The tanks were cleaned three times daily and fresh water was added in amount of 10% of the capacity of the recirculating system. The optimal hydrochemical characteristics in the recirculating system during the trial were maintained by filter and in particular by a biofilter.

The addition of 5% vegetable oils (linseed and sunflower) to the extruded feed for rainbow trout, cultivated in recirculating system did not affect the survival rate. The data obtained at the end of the trial, as mentioned above, showed that this parameter in the fish of the two experimental groups was 100%. Such was the survival of the rainbow trout of the control group (Figure 1). This is due to the maintenance of hydrochemical characteristics in the ranges required for the species at applied optimized technological parameters – stocking density, daily diet, feeding frequency. The analysis of the data for the total weight gain of the rainbow trout showed values of 4029.50 g in the control group and this was lower than the fish of the experimental groups ES and EL by 2.14% and 5.03%, respectively, but the differences were not statistically significant (P>0.05) (Figure 2).

At the end of the trial period the analyzed data for the feed consumption indicated that the feed conversion ratio in the rainbow trout, raised in recirculating system, receiving 5% linseed oil to the diet was 1.02 l. It was 2.84% lower than that of the fish fed 5% sunflower oil supplemented diet and 5.00% lower that the feed conversion ratio of the fish of the control group as the differences were not significant (P>0.05) (Figure 3). According to many authors the issue of the full replacement of the fish oil by various vegetable and animal fats is a problem and particularly important for the predatory fish (Bell et al., 1991; 1994; 2002; Martino et al., 2003; Seierstad et al., 2005). The studies of other authors prove that it could be partially (60 – 80%) replaced in the feed by vegetable fats (Ng et al., 2007; Drew et al, 2007; Rinchard et al., 2007; Turchini et al., 2009; Pettersson, 2010; Masiha et al. 2013 a, b; Turchini et al., 2013; Yıldız et al., 2013). Nevertheless in our study the results are due to the improved metabolism of nutrients in the fish from the groups supplemented with sunflower and linseed oils because the quantities of the two essential fatty acids – linoleic (LA) and α-linolenic (ALA) are substantially increased.

The improved feed consumption in the groups that received 5% sunflower and linseed oils affected positively the growth of the fish from these groups, cultivated in recirculating system. At the beginning of the experiment the rainbow trout had equal live weight and linear measurements (P>0.05). At the end of the trial higher growth in the fish of the experimental groups with vegetable oils was observed when compared to the control group. The mean live weight of the rainbow trout of EL was 334.79 g and it was higher than that of the fish of ES and C by 1.64% and 3.47% (P>0.05), respectively (Table 4). These results are confirmed by several authors who have conducted experiments with various kinds of vegetable oils in different fish species. Experiments with linseed, cottonseed and rapeseed oil as supplements in the diet of rainbow trout were carried out by Turchini et al. (2013), Yıldız et al. (2013), who obtained positive results for the growth of the fish. Similar data are obtained in experiments with hazelnut, linseed and soybean oils added to the feed for Balkan trout (Arslan et al., 2012) and zander (*Sander lucioperca)* (Kowalska et al., 2010) raised in recirculating systems. According to these authors, this was a result of the high level of linoleic acid in the rapeseed and soybean oil, as well as α-linolenic acid in the linseed oil. These two fatty acids are essential for the freshwater fish, and besides most of these species, including trout, have the abilities to convert these fatty acids into long–chain n-3 and n-6 PUFA (Sargent et al., 1995). Thus, the vegetable oils rich in LA and ALA are particularly important lipid sources for the freshwater fish.

During the last few years the researches in the area of aquaculture increase and they are mainly focused on the possibilities to use vegetable oils as replacements of fish oil in the extruded feed for trout (Rinchard et al., 2007; Torstensen et al., 2008; Pettersson, 2010; Twibell et al., 2012; Arslan et al., 2012; Turchini et al., 2013; Masiha et al., 2013a, b; Yıldız et al., 2013). The reason for this interest is on the one hand the constantly rising price of fish oil, and on the other hand – the search for the dietetic and healthy food products. Many farmers focus their efforts in this area and the search for alternatives of fish oil for the feed industry increases.

The main objective of our experiments was to study the possibility for using sunflower and linseed oils in extruded feeds for rainbow trout. The first results were promising and the researches in this area continue. In order to respond to the requirements of the growing aquaculture, more and more scientists focus their attention towards finding the replacements of the fish oil, which is becoming scarce on the market and its price is rising (Barlow, 2000; Delgado et al., 2003; Mráz, 2012).

Conclusions

The supplementation of 5% linseed or sunflower oils in extruded feed for rainbow trout, cultivated in recirculating system had positive effect on the following traits:

- Decreased the feed conversion ratio in the trout of EL and ES by 5.00% and 2.84%, respectively, compared to the values of this parameter in the fish of the control group;
- Increased the weight gain of the fish of EL and ES when compared with the rainbow trout of C, by 5.03% and 2.14%, respectively;
- Did not affect the survival rate of the fish from all the groups;
- Improved the metabolism of nutrients in rainbow trout.

The study showed that linseed as well as sunflower oils might be successfully used as a supplement to the extruded feed and to replace part of the fish oil in the diet of rainbow trout. Their inclusion in the diet did not influence negatively the growth and the survival rate, increased the weight gain and decreased the feed conversion ratio. The vegetable oils in the diet improved the nutrient metabolism increasing the content of the two essential fatty acids – linoleic (LA) and α-linolenic (ALA) which can make the lipid profile of the meat more beneficial for the human health.

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CONTENTS 1 / 2

Review

Genetics and Breeding

Nutrition and Physiology

CONTENTS 2 / 2

Instruction for authors

Preparation of papers

Papers shall be submitted at the editorial office typed on standard typing pages (A4, 30 lines per page, 62 characters per line). The editors recommend up to 15 pages for full research paper (including abstract references, tables, figures and other appendices)

The manuscript should be structured as follows: Title, Names of authors and affiliation address, Abstract, List of keywords, Introduction, Material and methods,Results, Discussion, Conclusion, Acknowledgements (if any), References, Tables, Figures.

The title needs to be as concise and informative about the nature of research. It should be written with small letter /bold, 14/ without any abbreviations.

Names and affiliation of authors The names of the authors should be presented from the initials of first names followed by the family names. The complete address and name of the institution should be stated next. The affiliation of authors are designated by different signs. For the author who is going to be corresponding by the editorial board and readers, an E-mail address and telephone number should be presented as footnote on the first page. Corresponding author is indicated with *.

Abstract should be not more than 350 words. It should be clearly stated what new findings have been made in the course of research. Abbreviations and references to authors are inadmissible in the summary. It should be understandable without having read the paper and should be in one paragraph.

Keywords: Up to maximum of 5 keywords should be selected not repeating the title but giving the essence of study.

The introduction must answer the following questions: What is known and what is new on the studied issue? What necessitated the research problem, described in the paper? What is your hypothesis and goal ?

Material and methods: The objects of research, organization of experiments, chemical analyses, statistical and other methods and conditions applied for the experiments should be described in detail. A criterion of sufficient information is to be possible for others to repeat the experiment in order to verify results.

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Discussion: The objective of this section is to indicate the scientific significance of the study. By comparing the results and conclusions of other scientists the contribution of the study for expanding or modifying existing knowledge is pointed out clearly and convincingly to the reader.

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Thesis:

Hristova D, 2013. Investigation on genetic diversity in local sheep breeds using DNA markers. Thesis for PhD, Trakia University, Stara Zagora, Bulgaria, (Bg).

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AGRICULTURAL SCIENCE AND TECHNOLOGY

Volume 6, Number 4
December 2014

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