

ТОВАРНАЯ АКВАКУЛЬТУРА И ИСКУССТВЕННОЕ ВОСПРОИЗВОДСТВО ГИДРОБИОНТОВ

COMMODITY AQUACULTURE AND ARTIFICIAL REPRODUCTION OF HYDROBIONTS

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The effectiveness of adding hot-pressed flaxseed oil and hydrothermal white lupine in compound feeds for rainbow trout (*Oncorhynchus mykiss*)

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Abstract. The results of experimental feeding of juvenile rainbow trout in a cage farm using mixed feeds with full and partial replacement of fish oil, incorporating an alternative protein source – barohydrothermally treated (BHTO) white lupine seeds are presented. Based on the results of the experiment, it was found that the use of the traditional source of lipids – fish oil in the compound feed for trout led to a significant increase in the final weight (582 g) of fish, as well as a decrease in the feed conversion ratio (1.21). The second experimental formulation also had a positive effect on the growth rate of rainbow trout, increasing the absolute growth rate by 20%. Clinical examination and control dissection showed that all experimental individuals exhibited no pathological disorders and had high marketable qualities. In both experimental groups, a significant increase in the indicators of protein metabolism in blood serum was revealed (total protein up to 54.7 g/L). In the first experimental group, a reliable decrease in the relative number of monocytes and basophils was recorded. Calculation of economic efficiency showed that the experimental formulation with the inclusion of protein and fatty acid sources in the form of white lupine and flaxseed oil is more cost-effective (at a cost of 161.32 rubles/kg) compared to feeds with fish oil only, as well as feeds using rapeseed oil and fish oil. The presented data indicate that the investigated feed formulations can be used in the industrial cultivation of rainbow trout without loss of nutrient characteristics of feed, fish-biological parameters, and meat quality.

Keywords: aquaculture, fish feeding, hot-pressed linseed oil, fatty acids, rainbow trout, barohydrothermal treatment

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Эффективность добавления масла льняного горячего отжима и белого люпина, прошедшего барогидротермическую обработку, в комбикорма для радужной форели (*Oncorhynchus mykiss*)

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Аннотация. Приведены результаты экспериментального кормления молоди радужной форели в условиях садкового хозяйства комбикормами с полной и частичной заменой рыбьего жира и введением альтернативного источника белка – барогидротермически обработанных (БГТО) семян белого люпина. По результатам опыта было установлено, что использование традиционного источника липидов – рыбьего жира – в составе комбикорма для форели приводит к существенному увеличению конечной массы (582 г) рыбы, а также снижению кормового коэффициента (1,21). Вторая опытная рецептура также оказывала положительное влияние на скорость роста радужной форели, увеличивая абсолютный прирост на 20 %. Клинический осмотр и контрольное вскрытие показали, что все экспериментальные особи не имели патологических нарушений и обладали высокими товарными качествами. В обеих опытных группах было выявлено достоверное увеличение показателей белкового обмена в сыворотке крови (общий белок до 54,7 г/л). В первой опытной группе было зафиксировано достоверное уменьшение относительного количества моноцитов и базофилов. Расчет экономической эффективности показал, что опытная рецептура с включением источников белка и жирных кислот в виде БГТО белого люпина и льняного масла представляется более выгодной (при стоимости 161,32 руб./кг) в сравнении с кормами только с рыбьим жиром, а также кормами с использованием рапсового масла и рыбьего жира. Приведенные данные показывают, что исследованные кормовые рецептуры могут применяться при промышленном выращивании радужной форели без потери нутриентных характеристик корма, рыбоводно-биологических показателей и качества мяса.

Ключевые слова: аквакультура, кормление рыб, льняное масло горячего отжима, жирные кислоты, радужная форель, барогидротермическая обработка

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Introduction

Rainbow trout farming in industrialized conditions has proven to be the most profitable branch of aquaculture. This type of fish can be cultivated both in natural water bodies (cage fish farming) and in recirculating aquaculture system (RAS). The greatest source of costs in fish production is quality feeds that ensure optimal fish growth and high-quality fish products [1]. Feed production is based on the use of fishmeal as the main protein component, which has a high cost and varies considerably in terms of quality and quantity [2]. The main task of modern aquaculture is to find ways to replace fishmeal with cheaper components, including plant-based ingredients.

White lupine (*Lupinus albus*) is a leguminous crop that is also grown in the Russian Federation. Its undemanding growing conditions and high protein content

in the fruits of the plant make it a viable alternative to fishmeal, meat and bone meal, and other protein components of feed [3]. However, the disadvantage of this type of raw material is the poor bioavailability of nutrients without pre-treatment.

Barohydrothermal treatment (BHTO) is a method of processing grain in which the raw material is loaded into a chamber, the chamber is sealed, and steam is pressurized into it. After a preset time has elapsed, the chamber is instantly depressurized, and the raw material is moved with excess pressure to the receiving hopper and bloated. This treatment leads to the destruction of the natural polymers that make up the grain under the action of thermal, mechanical, and chemical processes [4]. Since white lupine beans contain a large number of alkaloids [5], it is impossible to use them in pure form without a loss of feed quality. The majority

of alkaloids are contained in the shell, and this technological process allows the beans to be made available for use in compound feeds for aquaculture facilities.

The second important component of feeds for coldwater fish species is essential fatty acids and phospholipids [6]. Fish oil, like fishmeal, is a scarce product with a short shelf life and susceptibility to oxidation. Various vegetable oils, which have a similar fatty acid composition and relatively low cost, can serve as substitute sources of fatty acids of animal origin [7]. Linseed oil is particularly capable of replacing fish oil in complex formulations for aquaculture facilities in terms of fatty acids [8]. Linseed is used both for the production of plant fibers and for the production of high-quality oils. Along with the widely used rapeseed oil, imported feed companies also use linseed oil in their formulations due to its more complete fatty acid composition [9]. However, the volume of raw products of this oil in Europe cannot meet the needs of the feed industry. Since fish oil significantly increases the cost of feed [10], incorporating a vegetable fat component will improve the economic efficiency of cultivating commercial aquaculture products.

The goal of this study is to evaluate the effectiveness of the complex use of hot-pressed linseed oil with white lupine BHTO in the feed composition for juvenile rainbow trout in a cage cultivation environment.

Materials and methods

Studies were performed in the aquaculture enterprise Ladozhskaya LLC (Republic of Karelia). Yearlings of Donaldson's rainbow trout bred from Aquasearch Fresh (Denmark) eggs with an average weight of 250 g were used as an object in the studies.

Fish rearing was carried out in cages with a diameter of 12.7 m, installed in the water area of Lake Ladoga.

For the experiment, 45,000 rainbow trout (*Oncorhynchus mykiss*) individuals were selected and divided into 3 groups: 2 experiments and control. The fish were fed manually twice a day, with the ration equally divided into evening and morning feeding. Daily feeding was calculated depending on biomass and water temperature using special feeding tables. The measurements were carried out using the Milwaukee MW605 MAX oximeter and the TP700 electronic thermometer with a measurement error of 1 °C. The average weight was determined on platform scales VSP-5KS with a measurement error of ± 10 grams. The average weight was determined by hanging 100 individuals in 5 replicates. 2% was taken as the error for water and mucus. Efficiency indicators were evaluated by fish-biological indicators, economic feasibility of product introduction, biochemical indicators of serum and clinical blood picture.

Fish feeding was conducted using mixed feed of the Aquarex trade mark produced by Melcombinat JSC (Russia, Tver). Before the beginning of the experiment both groups of fish were fed with control feed. The mixed feeds of the experiment group were produced on the basis of feeds of the control group for maximum reliability of the obtained data. Biochemical parameters of feed for the groups of experience and control are given in Table 1.

Table 1

Biochemical composition of feeds for rainbow trout

Parameter	Control	Group 1	Group 2
Moisture	6.44	6.20	6.30
Crude Protein	44.00		44.20
Crude Fat	23.00		
Raw Fiber	1.29	1.34	0.99
Raw Ash	5.34	5.80	4.32

The use of lupin and fish oil in the production of BHTO formulations resulted in an insignificant change

in the amino acid composition of feed, the data on which are shown in Table 2.

Table 2

Amino acid composition of rainbow trout feeds

Parameter	Control	Group 1	Group 2
Lysine	3.23	3.29	3.38
Methionine	0.82	0.80	0.87
Threonine	1.80		1.62
Arginine	2.20	2.15	2.07
Isoleucine	1.50	1.42	1.61
Valin	2.29	2.28	1.89
Histidine	1.27	1.29	1.09

The design of the research is presented in Table 3.

Table 3

Research design

Parameter	Control	Group 1	Group 2
Lupin BHTO, %		0	5
Fish oil, % of dusting	50	100	35
Rapeseed oil, % of spraying	50	0	
Linseed oil, % of spraying		0	65

The control formulation included fish oil as a protein component, and rapeseed oil was used as a source of fatty acids. In the first experimental formulation only fish oil was used, and in the second a combination of lupin BHTO, fish oil and linseed oil was used.

Spraying percentage means spraying of liquid part

by vacuum sprayer during mixed fodder production. In this table the composition of liquid part of mixed fodder was taken as 100% spraying. Quality indicators of hot-pressed linseed oil, rapeseed oil and fish oil are given in Table 4.

Table 4

Fatty acid composition of lipids and quality characteristics of feed components

Parameter	Fish oil	Rapeseed oil	Flaxseed oil
Myristolein, % of LC amount	7.34	0	0.05
Myristolein, % of total LCs	0.17	0	
Pentadecane, % of LC amount	0.36	0	
Palmitic acid, % of LC amount	16.37	5.46	5.52
Palmitoleic acid, % of LC amount	11.45	0.45	0.07
Stearic acid, % of total LC	3.01	2.2	3.22
Elaidonic, % of the sum of LCs	3.94		0
Oleic acid, % of total LC	15.06	57.48	15.1
Linoleic, % of total LC	1.12	19.49	15.09
Alpha-Linolenic, % of the total LC amount	0.94	9.88	60.2
Gondoic acid, % of total LC	5.75	0	0.26
Erucic acid, % of GI amount	1.99	0.19	0
Ecosapentaenoic, % of the total LC amount	20.1	0	0.2
Nervonic, % of LC amount	0.63	0	0.06
Docosahexaenoic, % of the total GI amount	10.71	0	0.23
Acid number, mg KON/g	12.7	4.70	0.1
Peroxide number, % J/g	0.076	0.027	0.001

To assessment the physiological state of the reared fish, hematological parameters were analyzed. Blood preparations were made according to the standard technique [11], dried for 2-3 minutes and fixed in Nikiforov's mixture (1 : 1, methyl alcohol : diethyl ether). The staining was performed with azur-eosin by Romanovsky-Giemsa for 10 minutes. The following cellular elements were counted on the prepared preparations: relative number of white blood cells (leukocytes), relative number of monocytes, neutrophils, basophils and platelets.

Total serum protein (TSP) and albumin were determined by the colorimetric method [12]. Globulin was calculated mathematically. Glucose level was estimated using specialized kits Bio-Merieux (France), according to the manufacturer's instructions. Aspartaminotransferase, alanine aminotransferase, creatinine, urea, glucose, total/direct bilirubin, lactate dehydrogenase, and alkaline phosphatase in serum were determined using a CS-T240 biochemical analyzer (China) using ready reagents (kits) supplied by Spinre-

act Co (Spain), following the manufacturer's instructions. The Ritis coefficient and albumin/globulin ratio were calculated mathematically.

The results of the research were subjected to statistical analysis [13]. Statistical data were processed using Rsoftware (v3.5.2)/RStudio. The economic efficiency of cultivation was calculated according to the methodology proposed by the USSR Ministry of Agriculture and Lenin All-Union Academy of Agricultural Sciences [14].

Results and discussion

During the entire period of the experiment in the cages, the fish showed normal feeding behavior, activity, and had no pathological disorders during control rounds. On the 90th day of cultivation, the size and weight indices of rainbow trout were evaluated, and fish-biological parameters were calculated. Fish-biological parameters are given in Table 5.

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Table 5

Fish-biological parameters of growing rainbow trout on mixed feeds with replacement of fish oil and introduction of alternative protein source

Parameter	Control	Group 1	Group 2
Initial mass, g	250 ± 19.6		
Final weight, g	566 ± 34.1	582 ± 37.4	649 ± 40.8
Absolute gain, g	316	332	399
Average daily gain, g	3.51	3.68	4.43
Feeding coefficient, units	1.24	1.21	1.09
Survival rate, %	98.66	98.68	98.79

During the cultivation period, the fish reached marketable weight at which it can be sold.

In the control group, the average weight of fish reached 566 g, with a feed coefficient of 1.24. The absolute gain in the control was 316 g. Fish that received only fish oil as part of the feed, at the end of the experiment, had a mass of 582 g, with a feed coefficient of 1.21. In the second experimental group, with the addition of lupine BHTO at a concentration of 5% and the replacement of 65% of fish oil with linseed oil, the highest absolute weight gain was recorded – 399 g. The feed coefficient in this experimental group was at the level of 1.09. The survival rate in all experimental groups did not differ significantly (~ 98%).

Evaluation of clinical characteristics showed the absence of visible pathological disorders in both control and experimental groups. Pathoanatomical dissection revealed that the internal organs of the fish corresponded to species characteristics and had no disorders. A large amount of visceral fat deposits was observed in experimental individuals, covering most of the stomach and pyloric appendages. Signs of intestinal inflammation were not detected in any of the experimental groups.

Evaluation of hematological parameters of experimental rainbow trout revealed some significant differences between the control and experimental groups (Table 6).

Table 6

Hematological indices of blood of rainbow trout raised on mixed fodder with fish oil replacement and alternative protein source

Parameter	Control	Group 1	Group 2
Lymphocytes, %	83.54 ± 4.11	84.68 ± 4.22	84.21 ± 4.32
Monocyte, %	5.55 ± 2.47	4.7 ± 0.96*	4.87 ± 1.77
Neutrophil, %	5.64 ± 1.29	6.91 ± 2.18	6.71 ± 3.59
Basophil, %	5.27 ± 1.66	4.72 ± 0.93	5.81 ± 2.49
Thrombocytes, %	0.55 ± 0.21	0.44 ± 0.14	0.48 ± 0.14

In particular, a significant decrease in the relative number of monocytes was observed in the experimental groups compared to the control. Additionally, a significant decrease in the relative number of basophils was detected in experimental group 1, with a number 10.4% lower than the control. The frequency of other blood cells was similar in all experimental groups. The above data indicate that experimental feeds do not lead to the

development of a nonspecific immune response in fish. The decrease in these forms of leukocytes can be associated with the adaptation of fish to the feed formulation and, most likely, has a temporary nature.

In addition to evaluating hematological blood parameters, serum was taken from experimental rainbow trout for biochemical analysis, the results of which are shown in Table 7.

Table 7

Biochemical indices of blood serum of rainbow trout of control and experimental groups

Parameter	Control	Group 1	Group 2
Total bilirubin, kmol/L	3.11 ± 0.26	2.95 ± 0.21	3.16 ± 0.13
Bilirubin, kmol/L	0.25 ± 0.06	0.29 ± 0.05	0.31 ± 0.09
AST, units/l	531.25 ± 76.88	565.0 ± 44.62	582.6 ± 122.5
ALT, units/l	24.4 ± 4.66	34.98 ± 3.47*	26.33 ± 4.34
Urea, μmol/L	2.36 ± 0.43	2.68 ± 0.23	2.35 ± 0.28
Creatinine, μmol/L	25.60 ± 1.11	26.44 ± 5.28	28.59 ± 6.40
Total protein, g/l	34.84 ± 3.22	50.80 ± 2.1	54.72 ± 8.16
Albumin, g/l	18.16 ± 3.2	25.14 ± 1.6	32.68 ± 4.32
Alkaline phosphatase, units/L	264.4 ± 33.98	298.24 ± 73.6	265.0 ± 77.25
Glucose, μmol/L	2.52 ± 0.25	2.95 ± 0.15	2.91 ± 0.34
LDH, units/l	736.6 ± 125.6	826.6 ± 45.3	765.3 ± 124.60
Globulin, g/l	16.68 ± 1.44	24.36 ± 1.19	26.13 ± 3.44
Albumin/globulin ratio	1.08 ± 1.13	1.03 ± 1.28	1.25 ± 2.12
Ritis coefficient	21.77 ± 3.55	16.15 ± 4.64	22.10 ± 4.25

A significant increase in ALT activity in experimental group 1, up to 34.98 units/L, was found. Significant increases in protein metabolism parameters (total protein, albumin, globulin) relative to the control were observed in the fish of the experimental groups. In particular, in experimental group 2, the concentration of total protein reached 54.72 g/L, which is 57% higher than that of the control group.

A similar pattern was revealed in trout raised on mixed feed with fish oil. It is also worth noting the increase in glucose content in the blood of fish in this variant. Other blood biochemical parameters did not show significant differences and were within the normal range for this fish species under conditions of intensive fish farming [15].

To assess the economic efficiency of linseed oil use in combination with BHTO, the feed cost coefficient was applied, which has proven to be 3-5% lower than in other variants of the experiment. It is important to note that factors such as feed washing away by the flow and technical losses during hand feeding were not separately accounted for in this experiment, as these losses are already included in the indicator used, which is calculated as the ratio of the mass of used feed (kg) to fish growth (kg).

Comparison of fish growth rates showed a significant increase in the growth rate in the second experimental group compared to the control and first experimental group. As a result of control weighing at the end of the experiment, the average weight of trout in the second experimental group was 10.33% more than in the first group and 12.79% more than in the control group. Survival rates did not differ significantly between the groups. Based on the data obtained, it is

possible to conclude that positive results on the effectiveness of the use of hot-pressed linseed oil in combination with white lupine BHTO in complex formulations of mixed feed for rainbow trout have been found.

Based on the estimated cost of feed (control – 151,000 rubles per ton, experiment 1 – 188,000 rubles per ton, experiment 2 – 148,000 rubles per ton), it is possible to calculate the cost of producing one kilogram of biomass for each group. In the control group, the cost of one kilogram of biomass is 187.4 rubles/kg, in experiment 1 the cost is 227.48 rubles/kg, and in experiment 2 the cost is 161.32 rubles/kg. The cost of feed with the introduction of hot-pressed linseed oil and white lupine BHTO is 2.01% lower than the cost of feed with a complex content of rapeseed oil and fish oil and 21.28% cheaper than feed with traditional fish oil. Such an increase in economic efficiency is possible due to the high quality of linseed oil and its low cost.

Conclusions

The use of mixed feed with the introduction of white lupine (5%) and linseed oil (65%) increases the absolute weight gain of rainbow trout by 20% and reduces the feed coefficient to 1.09 units.

The replacement of 50% of fish oil with rapeseed oil led to a decrease in fish-biological parameters. The absolute growth of trout consuming rapeseed oil with feed was 30% lower than that of fish fed with barohydrothermal lupine and linseed oil.

All investigated mixed feeds allow the cultivation of rainbow trout in cage farm conditions without resulting in the development of pathological diseases and providing high marketable qualities of fish.

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