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The influence of Nu Pro® as a substitution of fish meal on the growth performance of rainbow trout (*Oncorhynchus mykiss* W.) cultivated in recirculating system

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Abstract

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The purpose of the study was to determine the effect of the addition of NuPro® as a substitution of fish meal in feeding of rainbow trout (*O. mykiss*) on the functional properties of extruded feed as well as on growth intensity, blood biochemical and meat quality parameters in experimental fish, cultivated in recirculating system. Rainbow trout received extruded feed (2.5 mm pellets), produced by extrusion process in "University of food technologies – Plovdiv". Two hundred and twenty five rainbow trout were divided into three groups: control (A), experimental B and C, each of them with three replicates, with a mean initial live weight, respectively 24.3±7.3, 24.3±7.3 and 24.4±8.5 g. The feed content for fish from experimental groups was as follows: for the control group (A) – without Nu Pro, for the group B – 33% fishmeal and 7% Nu Pro®, and that for the group C 25% fishmeal, with a content of 15% Nu Pro. The duration of the trial period was 60 days. The addition of Nu pro® on functional properties of extruded feed was retraced. At the end of the experiment, fish growth (g), their survival (%) and feed conversion ratio (K) were calculated. The blood biochemical and meat quality parameters in fish from experimental groups were also measured. The evaluation of functional properties in extruded feed showed that the use of Nu Pro® as a substitute of fishmeal has a number of advantages that include-increasing the mass flow rate during extrusion, reducing the energy consumption during extrusion, increasing shelf life due to the higher absorption index. The main disadvantage that can be indicated is the faster dissolution of the product in contact with water. At the end of the experiment, with the live weight, being highest in the rainbow trout from the experimental group (C) – 69.76±25.41 g, followed by that of fish from the experimental group (B) – 67.82±20.00 g and this one of individuals from group (A) – 64.01±17.07 g and the statistical differences haven`t been proven ($p > 0.05$). The supplementation of feed with NuPro® in the diet of rainbow trout decreased the level of alkaline phosphatase (ALP), cholesterol and triglycerides respectively with 9.29%, 16.4% and 9.47% compared with the values of these parameters in fish from control group, but the differences were not significant ($P \geq 0.05$). The partial replacement of fish meal with Nu Pro® in rainbow trout did not affect the meat quality parameters.

Keywords: rainbow trout; replacement of fishmeal; Nu Pro

Introduction

The technological parameters for the industrial production of the cultivation of fish in aquaculture are developed and improved. Therefore is need of appropriate nutrients providing rapid and environmentally friendly growth. The fish meal is the main component of extruded feed for fish (New & Wijkstrom, 2002). It gives a selected quantity of minerals, amino acids, phospholipids and fatty acids. They easily digested and lead to increase growth rates and total yields. Every year the price of fish meal increasing due to the rapid development of aquaculture and the need for its use is getting bigger (Hardy & Tacon, 2002). On the other hand agriculture in recent years increased concern animal welfare and antibiotic growth promoters are no longer accepted in some countries. For this reason, feed industry is a need for alternative products that promote growth and supply animals with protein sources without adversely affecting animal health (Mateo et al., 2004). One group of nutrients used as an alternative to animal protein sources is yeast extract, which is rich in several components, including nucleotides (Carlson et al., 2005). Nucleotides participate in cell division and consequently are involved in growth and immune response (Mateo & Stein, 2004). Therefore, nucleotides have a role in a series of vital functions of the organism (biochemical, immune, skeletal muscle protein, liver). The addition of nucleotides may help in intestinal health, bringing about a reduction in enteric diseases, especially in animals exposed to stressors such as the dietary and environmental changes (Lerner & Shamir, 2000). One such product is NuPro® which contains highly concentrated levels of essential and functional nutrients. Is rich in nucleotides, glutamic acid, inositol, amino acids and peptides. These yeast-based proteins have relatively high crude protein levels and are a source of dietary nucleotides, which can promote growth and immune function in mammals (Uauy et al., 1990) and fishes (Burrells et al., 2001). Research has shown that including NuPro® in the diets of young animals can enhance animal growth.

The purpose of the study was to determine the effect of the addition of Nu Pro® as a substitution of fish meal in feeding of rainbow trout (*O. mykiss*) on the functional properties of extruded feed as well as on growth intensity, blood biochemical and meat quality parameters in experimental fish, cultivated in recirculating system.

Material and Methods

Extrusion treatment

All experiments were carried out on a 20-mm single screw extruder – BRABENDER 20 DN- (Germany) with a screw diameter of 20 mm, equipped with a measuring device for torque Mn, N.m., die temperature, °C and die pressure MPa (Figure 1).

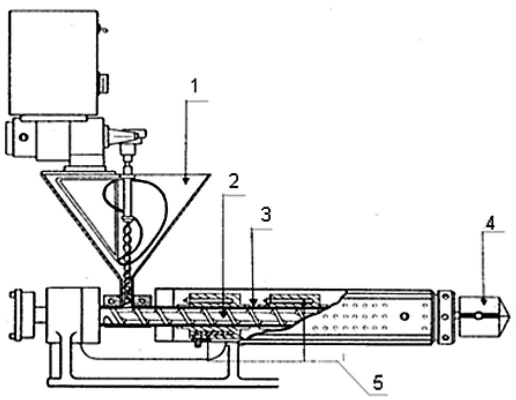

Fig. 1. Single screw laboratory extruder – BRABENDER 20DN:
***1* – Feeding device; *2* – Working screw; *3* – Cylinder;**
***4* – Die with nozzle and heater; *5* – Heating devices**

The extruder was cooled with water and air. It has a cylinder diameter of 20.05 mm and a length of 406.5 mm and is provided with a cutting mechanism. During the experiments, the following modes of operation were used:

- Temperatures in the three zones of the extruder: t_1 = 120°C; t_2 = 140°C; t_3 = 160°C;
- Working screw with a compression ratio К = 2:1;
- Nozzle with internal diameter D_o = 2 mm;
- Feeding screw speed N_f = 40 min^{-1};
- Working screw speed N_w = 140 min^{-1};

The inlet humidity of the mixtures was fixed at 28 %. It is one of the most important parameters that influence the properties the extruded feeds produced.

Functional properties of extruded feed

Mass flow rate of the extrusion process (Q, kg/h)

The mass flow was determined using the equation:

$$Q_{cp} = \frac{m}{t}, \text{ kg/h}$$

where: *m* – mass of the extruded product, kg; *t* – time, h.

Specific mechanical energy

The specific mechanical energy was determined using the equation:

$$SME = \frac{2\pi M_n n_w}{60Q} 3.6, \text{kJ/kg}$$

where: M_n – torque, N.m.; n_w – working screw speed, min^{-1}; Q – mass flow, kg/h.

Sectional expansion index (EI)

The sectional expansion index was defined as the ratio of the average diameter of the extrudate (D_e) to the die diameter (D_o):

$$E = \frac{D_e}{D_o}$$

Water solubility index, WSI, %

The extrudate was milled, then weighed 0.2 g and transferred to a centrifuge tube. The product was filled with 5 cm^3 of distilled water and then the sample was tempered in a water bath at 30°C for 30 min and stirred for some time. It was then centrifuged on a CH 90-2A centrifuge for 20 min at 3000 rpm. The supernatant liquid is separated into a glass weight and dried at a temperature of 105°C until a constant mass was reached. After tempering, the sample was weighed.

The water solubility index (WSI) is calculated using the equation:

$$WSI = \frac{m_{ds}}{m_s} 100,$$

where:

m_{ds} – mass of the dry substance after evaporation of the supernatant liquid, g;

m_s – mass of the sample, g.

Water absorption index (WAI, g/g product)

The extrudate was milled, then weighed 0.2 g and transferred to a centrifuge tube. The product was filled with 5 cm^3 of distilled water and then the sample was tempered in a water bath at 30°C for 30 min and stirred for some time. It was then centrifuged on a CH 90-2A centrifuge for 20 min at 3000 rpm. The Water Absorption Index (WAI) is calculated using the equation:

$$WAI = \frac{m_g}{m_s}, \text{g/g product} \quad (8)$$

where:

m_g – mass of the sediment, g; m_s – mass of the sample, g.

Experimental fish

The fish are cultivated in tanks with an effective volume of 0.8 m^3. Two hundred and twenty five rainboow trout were divided into three groups (control A, experimental B and experimental C) of 60, each with three replicates (A_1, A_2, A_3, B_1, B_2, B_3, C_1, C_2, C_3) and respectively with an average initial live weight 24.3±7.3, 24.3±7.3 and 24.4±8.5 g. The feed was extruded pellets with a size 2.0 mm. The Nu Pro® content as a substitution of a fish meal for rainboow trout from different groups was as follows: for the control group (A) -40% fish meal, without Nu Pro, for the experimental group B – 33% fish meal and 7% Nu Pro, and that for the experimental group C – 25% fish meal and 15% Nu Pro. Nutrients in the feed of the three groups are presented in Table. 1. The daily ration that fish received from the three replicates of the control and trial groups was up to 3% of their live weight. The duration of the trial period was 60 days.

Table 1. Nutrients in pelleted feed for rainbow trout (*Oncorhynchus mykiss* W.)

№	Indicators	Groups		
		A	B	C
1.	Fish meal, %	40	33	25
2.	Nu Pro®, %		7	15
3.	Soybean meal, %	24	26	30
4.	Wheat, %	10	15.15	12.14
5.	Dicalcium phosphate, %	2	2.25	2.25
6.	Salt, %	0.3	0.3	0.3
7.	Lysine – 98%		0.05	0.08
8.	Methionine, %		0.10	0.23
9.	Bran, %	11.20		
10.	Premix , %	0.5	0.5	0.5
11.	Sunflower meal, %	12	15	13
12.	Chalk, %		0.65	1.5
13.	Protein, %	41.46	41.53	41.41
14.	Kcal/kg	2690	2699	2630
15.	Phosphorus, %	2.04	1.92	1.79
16.	Lysine – %	2.70	2.70	2.71
17.	Methionine+ cystine, %	1.39	1.90	2.45
18.	Methionine, %	0.93	0.93	0.92
19.	Calcium, %	2.73	2.73	2.70

* 1 kg feed contains: vitamin A – 10000 IE; vitamin D_3 – 1500 IE; vitamin – E – 200 mg; vitamin K – 3 mg; thiamin – 10 mg; riboflavin– 15 mg; pyridoxin– 8 mg; vitamin B_{12} – 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

Hydrochemical Analysis

During the experimental period, the water temperature (°C) in tanks was measured daily.

The remaining hydrochemical parameters were examined in laboratory at the beginning and at the end of the experiment with the following methods:

- Nitrogen nitrite – BDS EN iso 10304-1:2009
- Nitrate nitrogen, $mg.l^{-1}$ – BDS EN ISO 10304-1:2009
- Dissolved oxygen, $mg.l^{-1}$ – BDS EN 25813:2004`
- pH – BDS EN 3424:1981
- Electrical conductivity, $\mu S.cm^{-1}$ – BDS EN 27888:2002

Intensity of fish growth

At the beginning and end of the experiment trout were individually weighed (g). To monitor the impact of the addition of Nu Pro® to extruded pellets as a substitution of a fish meal on the growth intensity of rainbow trout (*Oncorhynchus mykiss* W.), control catches were performed at a 30-day interval. The live body weight (BW, g) in the control catches was established. At the end of the trial, fish growth (g), their survival (%) and feed conversion ratio (K) were calculated according to the following equation:

Survival (%) = (final number of fish /initial number of fish) ×100

FCR = Fed feed (g) / Weight gain of fish (g)

Average individual weight gain (g) = Average final body weight in fish (g) – Average initial body weight (g)

Measurement of blood parameters

The blood from 18 individuals (6 specimens for experimental variant) was taken directly from their hearts with the aid of sterile plastic syringes with the volume of 3 ml with an appropriate for the aim needle. Heparin sodium (1%) was used as an anticoagulant. The blood was centrifuged at 3000 rpm for separating the plasma and it was used for determination of biochemical parameters. They were determined by the colorimetric method with blood analyzer (Mindray SC – 120) (Table 2).

Table 2. Biochemical parameters determined during 60 days trial

№	Biochemical parameters	Abbreviation of parameters	Units
1.	Glucose	GLU	$mmol.l^{-1}$
2.	Urea	UREA	$mmol.l^{-1}$
3.	Creatinine	CREA	$\mu mol.l^{-1}$
4.	Total Protein	TP	$g.l^{-1}$
5.	Albumin	ALB	$g.l^{-1}$
6.	Alanine Aminotransferase	ALT	$U.l^{-1}$
7.	Alkaline Phosphatase	ALP	$U.l^{-1}$
8.	Calcium	Ca	$mmol.l^{-1}$
9.	Phosphorus	P	$mmol.l^{-1}$
10.	Magnesium	Mg	$mmol.l^{-1}$
11.	Triglyceride	TG	$mmol.l^{-1}$
12.	Cholesterol	CHO	$mmol.l^{-1}$

Meat quality parameters

For the purpose of research muscle samples were taken from 18 pcs. per experimental group. The determination of the chemical composition – moisture, protein, lipids, dry matter and ash content (%) of meat of rainbow trout *(Oncorhynchus mykiss* W.), was performed in Central Scientific Laboratory at Trakia University, Stara Zagora. The meat samples for the relevant analyzes were taken from the dorsal fish muscles and prepared according to AOAC (2006, method 983.18) to determine the following parameters:

➢ Water content,% (AOAC, 1997, Method 950.46 and BDS11374-86);

➢ Protein content,% (BDS-ISO 5983, Kjeldahl method, using Kjeltec 8400 apparatus, FOSS, Sweden);

➢ Lipid content, % (BDS-ISO 6492, Soxlet method, using a Soxtec 2050 apparatus, FOSS, Sweden);

➢ Dry matter content% (BDS11374-86);

➢ Crude ash content% (BDS11374-86).

Data analysis

Statistical evaluation of the data was done by STATISTICA 6.0 software (StatSoft Inc., 2002).

Results and Discussion

Functional parameters of extruded feed

Sectional expansion index

Expansion is one of the most important properties of food products obtained at high temperatures and low humidity. This is a process that results from a variety of factors that are affected by the composition of the product to be extruded, as well as by the extrusion regimes. Higher amounts of torque, pressure and temperature inside the extruder allow the transformation of the processed products into viscous-elastic melts. The degree of transformation is strongly influenced by the moisture of the extruded product and the extrusion regimes. Expansion index is an important indicator of the crisp texture of the final product. Consequently, the expansion index provides important information on the extrudate quality (Patil et al., 2007).

Table 3 presents the mean experimental results. All experiments were performed in triplets.

In the present study, there was no significant difference in the sectional expansion index of the individual extrudates samples (Table 3). The differences range from 2 to 4%. This is due to the high humidity at which all mixtures were extruded. It leads to a decrease in the degree of gelatinization and reduction of expansion. Therefore, the final product is also of high humidity and can't increase its volume during extrusion.

Table 3. Functional parameters of extruded feed

Indicators	Mixtures		
	A $\bar{x}$ ± SD	B $\bar{x}$ ± SD	C $\bar{x}$ ± SD
EI	1.22 ± 0.03	1.19 ± 0.01	1.24 ± 0.02
Q, kg/h	5.55 ± 0.04	5.7 ± 0	6.04 ± 0.06
SME, kJ/kg	219.01 ± 6.77	179.05 ± 6.15	195.19 ± 6.79
WAI, g/g	5.63 ± 0.28	4.7 ± 0.31	3.87 ± 0.26
WSI, %	10 ± 0	16.67 ± 2.89	18.33 ± 2.89
M, N.m	23 ± 1.73	19.67 ± 0.58	22.33 ± 0.8

± Standard deviation

Mass flow rate

The mass flow rate is a major technical and economic parameter that characterizes the operation of the extruder. It often depends on the speed of the working screw, pressure and matrix design (Rosentrater et al., 2009).

The presence of Nu Pro® results in an increase in mass flow. This effect is more pronounce in mixture C, where the presence of Nu Pro® is 15%. In this case, the mass flow increases by 8%, compared to mixture A. This trend can be explained by the high levels of protein, contained in the Nu Pro® product. As a result of heat treatment, the product density increases, and consequently, increases the mass flow rate.

Specific mechanical energy

Energy consumption is an important indicator in any mechanical process in the food industry. The magnitude of the consumed energy influences the degree of macromolecular transformations and interactions occurring during extrusion, namely the degree of gelatinization of the starch and the rheological properties of the dissipated material.

The specific mechanical energy is highly influenced by the mass flow rate and the torque. As can be seen from the results, the addition of 7% Nu Pro® (mixture B) leads to lower energy consumption. In mixture C (15% Nu Pro®), the energy consumption rises by 7%, but remains much lower than mixture A, in which fish meal was used. This is due to the lower mass flow rate of mixture A and the higher amounts of torque generated during extrusion.

Water absorption index

The water absorption index of the extruded pellets reflects the ability of the starch to absorb water and is an indirect indicator of the amount of gelatinization and intact starch grains. It can be expected that when the starch structure is destroyed, more water is bound to the molecules, resulting in changes in the properties of the extruded pellets.

As can be seen from the results the presence of larger amounts of Nu Pro results in a lower water absorption index. Compared to mixture A, where fishmeal was used, absorbed water in mixture C was over 30% lower. As a result of the lower water absorption index, extruded pellets treated with Nu Pro® would stay longer on the surface of the water without sinking and would have a longer shelf life.

Water solubility index

The water solubility index is often used as an indicator for degradation of molecular structures and depends on the amount of soluble components obtained from starch after extrusion processing. It is related to the dextrinization of starch and measures the degree of its change after extrusion.

Upon addition of Nu Pro® (mixtures B and C), the WSI increased considerably. This is probably to the greater number of torn connections between the molecules during extrusion process. Therefore, the extruded pellets made from these mixtures will decompose much more quickly than those made with fish meal.

Hydrochemical parameters

During the experimental period hydrochemical parameters in the tanks were optimal for trout growing. Water temperature, dissolved oxygen, pH, nitrate, nitrite and water conductivity were regularly monitored.

The temperature values during the trout trial to determine the influence of Nu Pro® as a substitution of fish meal in the diet are presented on Table 4. In the water of tanks, where were grown fish from different experimental variants during the trial period it was from 15.5°C to 17.1°C, which is optimal for this species. The amount of dissolved oxygen in the water during the experimental period was within the range adopted for the cultivation of rainbow trout and it varies from 7.13 mg.l^{-1} to 8.42 mg.l^{-1} (Table 4). The pH values of water in the tanks range from 7.80 to 8.09, which is optimal for trout growing (Table 4). The amount of nitrate nitrogen during the experimental period ranged from 0.41 mg.l^{-1} to 0.57 mg.l^{-1}, which is below the maximum allowable value set in optimal values, (Zaykov & Staykov, 2013). The nitrite nitrogen content in the water is given in Table 4. It was less than 0.005 mg.l^{-1} to 0.009 mg.l^{-1} what is below the maximum. The water conductivity during the trout experiment is presented in Table 4 and it ranged from 546.00 µS.cm^{-1} to 685.00 µS.cm^{-1}, which is optimal for the cultivated species.

Analysis of the hydrochemical data obtained (water temperature, dissolved oxygen, pH and electrical conductivity) during the test period indicates that they are in the optimal range for growing of a rainbow trout. The same applies to the maximum permissible nitrate and nitrite concentrations in water. For trout cultivated in recirculating system, these values should be up to 2 mg/l and up to 0.01 mg/l, respec-

Table 4. Water chemical parameters in tanks during the experiment with rainbow trout

Parameter	n	Min.	Max.	Optimal values, (according Zaykov and Staykov, 2013)
Temperature, ºC	60	15.50	17.10	12.0-16.0
Dissolved oxygen, $mg.l^{-1}$	60	7.13	8.42	Not below 8
pH	60	7.80	8.09	7.0-8.0
Nitrate nitrogen, $mg.l^{-1}$	2	0.41	0.57	Up to 2.0
Nitrogen nitrite, $mg.l^{-1}$	2	<0.005	<0.009	Up to 0.01
Electrical conductivity, $\mu S.cm^{-1}$	60	546.00	685.00	–

tively, significantly higher than those maintained in water during the experimental period (Zaykov & Staykov, 2013). The maintenance of these optimal water values in all experimental variants is due to the fact that the trout from these groups is cultivated with optimized technological and technological parameters of the production technology.

Growth parameters in fish

The mean values of body live weight of trout during the beginning of the trial from the three replications of control group A and experimental groups B and C were 24.3±7.3, 24.3±7.3 and 24.4±8.5 g, respectively, with differences not statistically proven (P > 0.05) (Table 5). In the middle of the experimental period, there is a tendency for a higher live body weight in the fish of groups B and C, where extruded pellets contained 7% and 15% Nu Pro, compared to the values of this indicator of individuals in the group A, fed with granules without Nu Pro (Table 5). The same trend remained at the end of the experiment, with the live weight, being highest in the rainbow trout from the experimental group (C) – 69.76±25.41 g, followed by that of fish from the experimental group (B) – 67.82±20.00 g and this one of individuals from group (A) – 64.01±17.07 g. The differences aren't statistically proven (p > 0.05).

The survival rate of the trout from different experimental variants during the trial is presented on (Table 5). The values of this index in individuals from the repetitions of the trial groups B and C, fed with extruded pellets, containing Nu Pro® as a substitution of fish meal and fish in the control group were 98.7%, 97.3% and 98.7%, respectively.

At the end of the experiment, the average individual weight gain of fish from experimental group (C), was 45.27 ± 26.74 g and this was higher than the values of this indicator of fish from groups B and A by 4.1% and 14.4% respectively, the differences haven\`t been proven (p > 0.05) (Table 5).

During the experimental period the trout was fed four times per day. An analysis of daily fed pellets was performed on repetitions in the control and experimental groups. The feed conversion ratio in the experimental group C is 1.31 whit 15% Nu Pro®, in the experimental group with the addition of 7% Nu Pro® as is 1.37 and 1.51 – in the individuals of the control group A (Table 5). The differences are statistically proven between the values of this indicator in the fish from the control and experimental groups (P < 0.05).

The influence of Nu Pro® as a substitution of fish meal in extruded pellets on the survival rate for trout cultivated on a tank was analyzed. The data reported at the end of the trial show that this indicator in the control group was 98.7% and in experimental groups fed with 7% and 15% were respectively 97.3% and 98.7% (Table 5). This is due to optimal hydrochemical values in tanks during the trial period as a result from applied optimized technological parameters – stocking density, daily ration, and multiplicity of meals.

The analysis of the final live weight of the trout shows that it is 69.76±25.41 g in the experimental group C and this is higher with 8.98% and 2.86%, respectively than the values of this indicator of individuals from variants A and B, the differences aren't significant (P > 0.05) (Table 5).

At the end of the experimental period, the analysis of feed consumption data shows that the feed conversion ratio in trout fed with granules with a Nu Pro® content of 15% (experimental group C) is 1.31 and it is 4.7% lower than that of fish in the experimental group, fed with 7% Nu Pro® and 15.4% less in the subjects of the control group A (Table 5). Better utilization of extruded pellets, containing 15% Nu Pro® reflects positively the growth of fish from experimental group grown in tanks. At the beginning of the trial, live weight of the trout from different experimental variants was

Table 5. Growth parameters in rainbow trout during the trial

Parameter	n	A	B	C
		$\bar{x} \pm SD$	$\bar{x} \pm SD$	$\bar{x} \pm SD$
Initial body weight, g	75	24.3±7.3	24.3±7.3	24.4±8.5
Final body weight, g	75	64.01±17.07	67.82±20.00	69.76±25.41
Survival rate, %	75	98.7	97.3	98.7
Average individual weight gain, g	75	39.57±17.83	43.48±21.05	45.27±26.74
FCR		1.51	1.7	1.31

Table 6. Blood biochemical parameters in rainbow trout (*Oncorhynchus mykiss*) during the trial

	Units	n	A $\bar{x} \pm SD$	B $\bar{x} \pm SD$	C $\bar{x} \pm SD$
GLU	mmol.l^{-1}	18	6.07±1.32	6.34±1.47	6.30±2.94
UREA	mmol.l^{-1}	18	1.53±0.54	1.62±0.59	1.53±0.58
CREA	μmol.l^{-1}	18	18.79±6.80	26.87±18.87	24.47±19.05
TP	g.l^{-1}	18	40.11±7.54	40.73±7.85	36.00±8.90
ALB	g.l^{-1}	18	20.01±3.99	20.63±5.51	18.42±4.47
ALT	U.l^{-1}	18	23.50±19.70	35.13±23.56	41.67±29.36
ALP	U.l^{-1}	18	299.86±132.20	305.93±167.41	272.00±133.43
CA	mmol.l^{-1}	18	4.07±1.24	4.46±1.14	4.08±1.02
P	mmol.l^{-1}	18	6.43±1.78	7.01±1.77	5.90±1.55
Mg	mmol.l^{-1}	18	1.12±0.37	0.99±0.21	0.93±0.22
TG	mmol.l^{-1}	18	2.37±0.70	2.56±0.80	1.98±0.62
CHO	mmol.l^{-1}	18	4.01±0.7	4.19±0.81	3.63±0.83

similar (P > 0.05). At the middle of the experimental period there was a tendency for a higher live weight in fish from the experimental group C compared to this indicator in the fish from the experimental groups B and control group A.

The inclusion of 7 and 15% NuPro® in the diet of rainbow trout did not significantly affect the blood parameters. The highest average values in the quantity of glucose, urea, creatinine, total protein, albumin, alkaline phosphatase, calcium, phosphate, triglyceride and cholesterol were observed in trout from variant B and they were higher compared with the values in control variant respectively with 4.25%, 5.55%, 30.07%, 1.52%, 3.00%, 1.98%, 8.74%, 8.27%, 7.42% and 4.29%, but the differences were not proved statistically (P ≥ 0.05) (Table 6). The trout fed with feed supplemented with 14% NuPro® showed the lowest values in the following parameters: TP, ALB,ALP,P, Mg, TG and CHO and they were lower compared with the values in these parameters in fish from A respectively with 10.24%, 7.94%, 9.29%, 8.2%, 16.9%, 16.4% and 9.47% but the differences were not significant (P ≥ 0.05) (Table 6). The results received for alkaline phosphatase and cholesterol in fish from group C were in line with the study made from Kowalska et al. (2011) who found that inclusion of NuPro® in the diet for pikeperch (*Sander lucioperca*) decreased the values in these parameters. The lower level of transaminases indicated improved liver function (JarmoŁowicz et al., 2012). The received from current study results for the level for cholesterol and triglycerides level in fish fed with 14% nucleotide-rich *Saccharomyces cerevisiae* yeast protein were in confirmation made from Hassaan et al. (2018), which study found decreased quantity in these parameters when feed for Nile tilapia contained yeast extract. The quantity of albumin in our study was not significantly affected by the supplementation of feed with NuPro®. Hassaan et al. (2018) reported increased level of albumin in group fed with addition of yeast extract. This result was not confirmed in current study.

The water, protein, fat, dry matter and ash content of the trout meat from the control group (A) and experimental variants C (replacing fish meal with 7% with Nu Pro®) and C (15% replacement of fishmeal with Nu Pro®) are presented in (Table. 7). The values we received for the water content in the fish muscles of the control and trial groups were respectively 77.11 ± 0.88, 77.27 ± 0.39 and 77.35 ± 0.50. The measured result of the figure is approximately the same, they are not identified significant differences (P > 0.05). The analyzed muscles samples for raw fish protein content of the three groups are approximately the same. The value obtained for the test species in fish muscles of the control group was highest in A (20.17 ± 0.44) compared to the other two groups, but no significant differences (P > 0.05) were found. Absent and significant differences in the content of lipids in meat samples tested for all three groups. The measured values of the test indicator, were respectively – 36 ± 0.46; 1.32 ± 0.40; 1.31 ± 0.46, respectively for the A, B and C groups. Among the studied groups was no difference in the results obtained from the replacement product Nu Pro®, which shows that this substitution did not effect on the fat content in the meat of rainbow trout (P > 0.05). The results obtained are similar to the results in Danijela et al. (2011), for rainbow trout meat.

Table 7. Chemical composition of the flesh of rainbow trout

Parameters	n	A – control	B-7%	C-15%
		$\bar{x} \pm SD$	$\bar{x} \pm SD$	$\bar{x} \pm SD$
1. Water, %	18	77.11±0.88	77.27±0,39	77.35±0.50
2. Protein, %	18	20.17±0.44	20.01±0.53	19.99±0.29
3. Lipids, %	18	1.36±0.46	1.32±0.40	1.31±0.46
4. Dry matter, %	18	22.89±0.88	22.73±0.39	22.65±0.50
5. Crude ash, %	18	1.36±0.02	1.40±0.07	1.35±0.01

Rainbow trout is considered to be an average of oily fish, containing muscle lipids from 2 to 7% (Jobling, 2001). The low fat content and the relatively low cholesterol content makes the fish one of the most valuable food products for humans (Abdullahi et al., 2001; Conor, 2000; Sidhu, 2003). The detected dry matter content in the fish meat of the control group A (22.89 ± 0.88) compared to the experimental groups B (22.73 ± 0.39) and C (22.65 ± 0.50) the differences are not statistically reliable ($P > 0.05$). The crude ash content in the trout meat of the control and experimental groups is approximately the same – 1.36 ± 0.02; 1.40 ± 0.07; 1.35 ± 0.01 no reliable difference ($P \leq 0.05$) was found between the groups (Table 7).

According to the authors, in the present study, the high concentration of dry matter is at the expense of higher crude protein content in the muscles of the fish studied (Fafioye et al., 2005; Aprodu et al., 2012). Not significantly high protein content in the carp meat of the control group is probably due to the increased energy content of the fish meal ration and the appropriate fats in it, which contributes to the fullest utilization of protein from the feed (Caballero et al., 1999; Satpathy et al., 2003). The content of fat in the muscles of fish species is affected by its affiliation, live weight, season, geographical region, age, and ripeness (Piggott & Tucker, 1990).

Conclusion

The evaluation of functional properties in extruded feed showed that the use of Nu Pro® as a substitute of fishmeal has a number of advantages that include-increasing the mass flow rate during extrusion, reducing the energy consumption during extrusion, increasing shelf life due to the higher absorption index. The main disadvantage that can be indicated is the faster dissolution of the product in contact with water.

The supplementation of Nu Pro in extruded pellets for feeding of a rainbow trout, grown in recirculation system influences the investigated biological indicators as follows:

• fish fed with feed supplemented with 15% Nu Pro® showed higher final body weight and individual weight gain compared with the values in these parameters found for trout from the control group and group B (fed with feed contained 7% Nu Pro), without the differences between different groups were statistically significant ($P \geq 0.05$);

• affect feed conversion ratio, being the lowest in fish from experimental group C – 1.31 compared with average value in trout from control group – 1.51 and the difference between the values was statistically significant ($P < 0.05$);

• did not affect the survival of fish from different experimental variants;

• decreased the level of alkaline phosphatase (ALP), cholesterol and triglycerides respectively with 9.29%, 16.4% and 9.47% compared with the values of these parameters in fish from control group. Unfortunately the differences were not statistically proven ($P \geq 0.05$);

• did not affect the meat quality parameters.

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