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# Productive traits, blood biochemical parameters and meat quality of rainbow trout (*Oncorhynchus mykiss* W.) fed with supplement of nutmeg extract (*Myristica fragrans*)

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***Abstract.*** *The aim of the present study was to evaluate the effect of the dietary nutmeg extract (Myristica fragrans) supplement on the survival rate, weight gain, feed conversion ratio (FCR), economic efficiency (ECR), blood biochemical parameters, chemical composition and fatty acid profile of rainbow trout (Oncorhynchus mykiss W.), cultivated in a recirculation system. One hundred and sixty rainbow trouts were distributed in two experimental variants, each with two replications with 80 fish in one group. The average initial live weight of trouts from control (C) and experimental (E) groups was 13.43±0.33 g and 13.46±0.29 g, respectively. Fish were reared in concrete tanks with effective volume of 0.8 m$^3$, part of a recirculation system. Trouts were fed „Aqua garant UNI" extruded feed produced by Garant-Tiernahrung Gesellschaft m.b.H. - Austria, with pellet size of 2 mm. The feed of fish from the experimental group was supplemented with 1% powdered nutmeg extract (P.I.C.Co LTD), by lubricating the pellets with 5 ml sunflower oil/100 g feed. Control rainbow trouts received feed lubricated with the same amount of sunflower oil. Fish from both groups were fed 3 times daily and their ration was 3% of body weight. The experimental period lasted 60 days. The dietary supplementation of rainbow trouts with nutmeg extract had a negative impact on growth performance and economic efficiency of farming of the species. Final live weight and average individual weight gain were lower, and FCR and ECR values were inferior vs controls. The nutmeg extract had also an adverse effect on water content and dry matter of meat from supplemented fish. A positive effect was demonstrated only on meat fat content, which was substantially lower compared to that of control fish. Despite the higher SFA as well as lower UFA, MUFA, PUFA, n-6 and n-3 fatty acid content of trout meat that received nutmeg extract with feed, the PUFA/SFA and n-6/n-3 ratios remained within the favourable range for human nutrition, e.g. >0.4 and <5, respectively*.

**Keywords:** chemical composition, economic conversion ratio, fatty acid profile, feed conversion ratio, survival rate, weight gain

## Introduction

Various parts of plants and phytoextracts have been used in traditional medicine and as cooking spices for thousands of years. The ban on the use of nutritional antibiotics in aquaculture resulted in increased interest in various plant extracts for improvement of the immune status and promoting the growth of farmed fish. Numerous experiments with plant extracts - relatively inexpensive natural products that are safe for fish and for consumers were carried out (Gabor et al., 2010, 2011; Zhelyazkov et al., 2018; Georgieva et al., 2018, 2019; Sirakov et al., 2019; Velichkova et al., 2019; Koshinski, 2019, 2020). Treatment of some infectious and parasitic diseases by means of various plant extracts is described (Stratev et al., 2017). The supplementation with plant extracts, through their biologically active compounds, could have an effect on blood biochemical indices (Sirakov et al., 2019; Velichkova et al., 2019) and meat quality (Gabor et al., 2010, 2011; Georgieva et al., 2018, 2019; Koshinski, 2019, 2020), so they should also be studied.

The native land of nutmeg (*Myristica fragrans*) are the Moluccas and Spice Island of Indonesia. It is two-flowered evergreen plant the seeds of which are used as spice. It was proved to possess stimulating, anti-inflammatory, antibacterial, hepatoprotective and antioxidant properties (Charles, 2012).

The effect of *Myristica fragrans* on the growth of juvenile African catfish (*Clarias gariepinus*) (Sodamola et al., 2017) and fattened carps (*Cyprinus carpio* L.) (Zhelyazkov et al., 2018) was reported. Its anaesthetic properties in carps are also documented (Al-Niaeem et al., 2017). The presented information suggests that studies on the application of nutmeg in fish diets are relatively few. Having in mind that the metabolism of different hydrobiont species is specific, some of the biologically active compounds in nutmeg may have an adverse effect on farmed fish.

The aim of the present study was to evaluate the effect of the dietary nutmeg extract supplement on the survival rate, weight gain, feed conversion ratio (FCR), economic efficiency (ECR), blood biochemical parameters, chemical composition and fatty acid profile of rainbow trout, cultivated in a recirculation system.

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## Material and methods

*Ethical approval*

All applicable international, national, and/or institutional guidelines for the care and use of animals were followed by the authors (Directive 2010/63/EU; Regulation No 20/2012).

*Experimental design*

Rainbow trouts (n=160) were distributed in two experimental variants, each with two replications with 80 fish in one group. The average initial live weight of trouts from the control (C) and experimental (E) groups was 13.43±0.33 g and 13.46±0.29 g, respectively. Fish were reared in concrete tanks with effective volume of 0.8 $m^3$, part of a recirculation system. Trouts were fed „Aqua garant UNI" extruded feed produced by Garant-Tiernahrung Gesellschaft m.b.H. - Austria, with pellet size of 2 mm. The feed of fish from the experimental group was supplemented with 1% powdered nutmeg extract (P.I.C.Co LTD), by lubricating the pellets with 5 ml sunflower oil/100 g feed. Control rainbow trouts received feed lubricated with the same amount of sunflower oil. Fish from both groups were fed 3 times daily and their ration was 3% of body weight. The experimental period lasted 60 days. The nutritional content of fish feed is presented in Table 1.

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

| No | Item | Groups | |
|---|---|---|---|
| | | Control | Experimental |
| 1 | Crude protein, % | 45.00 | 45.00 |
| 2 | Crude lipids, % | 16.00 | 16.00 |
| 3 | Crude fiber, % | 2.40 | 2.40 |
| 4 | Crude ash, % | 8.00 | 8.00 |
| 5 | Ca, % | 1.60 | 1.60 |
| 6 | P, % | 1.20 | 1.20 |
| 7 | Nutmeg extract, % | - | 1.00 |
| 8 | ME, MJ/kg | 18.50 | 18.50 |

*1 kg feed contains: vitamin A- 10000 IE; vitamin $D_3$- 1500 IE; vitamin E- 200 mg; vitamin C- 150 mg; Fe- 62 mg; Mn- 26 mg; Cu- 5 mg; Zn- 103 mg; I- 2.6 mg; Se- 0.3 mg.

*Hydrochemical analysis*

Water chemical parameters of the recirculation system during the experiment with rainbow trout (*Oncorhynchus mykiss* W.) were determined as follows:

- Water temperature - °C; dissolved oxygen - $mg.l^{-1}$, pH and electric conductivity - $\mu S.cm^{-1}$ with MultiLineP4 equipment (Xylem Analytics Germany Sales GmbH & Co. KG, WTW);
- Ammonia, $mg.l^{-1}$ – Bulgarian State Standard (BSS) ISO 7150-1:2002;
- Nitrates, $mg.l^{-1}$ – BSS ISO 7890-3:1988;
- Nitrites, $mg.l^{-1}$ – BSS ISO 26777:1997;
- Orthophosphates, $mg.l^{-1}$ – BSS EN ISO 6878:2005.

Throughout the trial water temperature, dissolved oxygen, pH and electric conductivity were monitored on a daily basis, whereas ammonia, nitrates, nitrites and orthophosphate contents – on a weekly basis.

The role of the filtering system of the recirculation system for maintaining optimum water chemical parameters was essential. To compensate water losses, fresh water was added on a daily basis at the amount of 10% of the recirculation system volume.

*Determination of growth performance*

The effect of nutmeg extract added to extruded feed on fish weight gain and feed conversion ratio of rainbow trouts (*Oncorhynchus mykiss* W.) in the recirculation system was evaluated by weighing fish in the beginning, middle and end of the experimental period. Average body weight (g) was determined individually. By the end of the experiment, weight gain (g), survival rate (%), and feed conversion ratio (FCR) were determined.

*Economic analysis*

The analysis of the economic efficiency of dietary nutmeg extract supplementation of rainbow trouts in a recirculation system was performed with data for feed conversion ratio, survival rate and weight gain. These traits were compared between the experimental groups and extruded feed costs were calculated. The cost of 1 kg weight gain was determined. The economic conversion ratio (ECR) was calculated according to Piedecausa et al. (2007):

ECR = Cost of Diet x Feed Conversion Ratio (FCR).

*Determination of blood biochemical parameters*

Blood samples were obtained through caudal vessel puncture using EDTA containers. Glucose (GLU), urea, creatinine (CREA), total protein (TP), albumin (ALB), ASAT, ALAT, alkaline phosphatase (ALP), calcium (Ca), phosphorus (P), magnesium (Mg), triglyceride (TG), and cholesterol (CHOL) concentrations were determined by means of BS-120 Chemistry Analyzer (Mindray, China).

*Chemical composition*

Meat samples were prepared according to AOAC (2006; method 983.18) and determination of water content (%) was by air drying (AOAC, 1997; method 950.46). Crude protein content (%) was calculated by converting the nitrogen content, quantified by Kjeldahl's method, using an automatic Kjeldahl system (Kjeltec 8400, FOSS, Sweden). Lipid content (%) was determined by the method of Soxhlet, using an automatic system (Soxtec 2050, FOSS, Sweden). Ash content (%) was investigated by incineration in a muffle furnace (MLW, Germany) at 550°C for 8 h. Crucibles were brought about the room temperature and weighed.

*Fatty acid composition*

The determination of fatty acid composition (%) of the total lipids of rainbow trout is carried out by the application of a gas chromatographic method, using a gas chromatograph "Perkin Elmer" Clarus 500 with a flame ionization detector, a 60 m capillary column „Trace Gold T6-WAXMS GC Column"; column temperature – 130°C (1 min), with change 6.5°C/min to 170°C, with change 3.0°C/min to 215°C (12 min) 40.0/min to 230°C (1 min), detector temperature 280°C; injector temperature – 270°C, gas holder – hydrogen (H ), split - 1:50.

*Statistical analysis*

Statistical analysis was performed using STATISTICA 6.0 software (StatSoft Inc., 2002). Mean, standard deviation and t-test were performed. The statistical significance was determined at $p<0.05$.

## Results

*Hydrochemical analysis*

Water chemical parameters in the recirculation system throughout the experiment were within the optimum ranges for rainbow trout farming (Table 2). There were insignificant differences between groups ($p>0.05$) with respect to water parameters. The temperature of water during the 60-day period varied between 12ºC and 15ºC, or 13.50ºC on average. Dissolved oxygen was maintained above 9 $mg.l^{-1}$, and water pH in control (C) and experimental (E) groups was 7.64±0.32 and 7.58±0.27, respectively. Electrical conductivity was 486 $\mu S.cm^{-1}$. The content of ammonia, nitrates, nitrites and orthophosphates in the recirculation system was determined on a weekly basis. The measured values were significantly lower than the maximum permissible values set by Regulation No 4/2000.

**Table 2.** Water parameters in the recirculation system during the experiment with rainbow trout (*Oncorhynchus mykiss* W.)

| Parameter | n | Control group | Experimental group | Signifi-cance | Optimum values (Regulation No 4/2000) |
|---|---|---|---|---|---|
| | | $\bar{x}$ ± SD | $\bar{x}$ ± SD | | |
| Temperature, ºC | 60 | 13.50±1.25 | 13.50±1.16 | NS | 12.0-16.0 |
| Dissolved oxygen, $mg.l^{-1}$ | 60 | 9.54±0.31 | 9.42±0.30 | NS | > 9 |
| pH | 60 | 7.46±0.32 | 7.58±0.27 | NS | 6.0-9.0 |
| Electric conductivity, $\mu S.cm^{-1}$ | 60 | 486±23.51 | 486±23.83 | NS | - |
| Ammonia, $mg.l^{-1}$ | 8 | 0.61±0.28 | 0.62±0.20 | NS | < 1.0 |
| Nitrates, $mg.l^{-1}$ | 8 | 0.34±0.08 | 0.35±0.05 | NS | < 2.0 |
| Nitrites, $mg.l^{-1}$ | 8 | 0.005±0.001 | 0.006±0.001 | NS | < 0.01 |
| Orthophosphates, $mg.l^{-1}$ | 8 | 0.271±0.117 | 0.284±0.136 | NS | < 0.40 |

NS= Non–significant

*Determination of growth performance*

At the beginning of the trial, average initial body weight of rainbow trouts from all control and experimental (supplemented with 1% nutmeg extract) replications was 13.43±0.33 g and 13.46±0.29 g, respectively ($p>0.05$) (Table 3). In the middle of the trial, average individual body weight of fish from group C was 23.73±0.85 g - 1.85% higher than fish from group E ($p>0.05$). At the end of the experiment, fish from group E exhibited statistically significantly lower body weight ($p<0.05$) – 8.10% less than the control fish (43.97±1.27 g). In both groups, survival rates were equal: 97.50%. Average individual weight gain of fish from group C was 30.54±0.98 g, which was 11.76% higher than that of trouts from group E ($p<0.05$). Also, in control fish, FCR (1.27±0.04) was better compared to group E, where FCR was 14.17% poorer with statistically significantly difference vs controls ($p<0.05$).

**Table 3.** Fish production parameters

| Parameter | n | Control group | n | Experimental group | Significance |
|---|---|---|---|---|---|
| | | $\bar{x}$ ± SD | | $\bar{x}$ ± SD | |
| Initial body weight, g | 80 | 13.43±0.33 | 80 | 13.46±0.29 | NS |
| Body weight in the middle of the trial, g | 79 | 23.73±0.85 | 78 | 23.30±0.64 | NS |
| Final body weight, g | 78 | 43.97±1.27 | 78 | 40.41±1.23 | * |
| Survival rate, % | | 97.50 | | 97.50 | |
| Average individual weight gain, g | 78 | 30.54±0.98 | 78 | 26.95±0.98 | * |
| FCR | 78 | 1.27±0.04 | 78 | 1.45±0.05 | * |

Significant difference: *p<0.05, NS=Non–significant, FCR=Feed conversion ratio

*Economic analysis*

The price of extruded feed for rainbow trout was 1400.00 €/t (VAT excluded). The supplementation of this feed with 1% nutmeg extract resulted in 70 €/t, VAT excluded, more expensive feed for the experimental group by (Table 4). Better economic conversion rate of 1.78 was demonstrated in control trouts as compared to fish supplemented with 1% nutmeg extract (2.13).

**Table 4.** Economic efficiency of the nutmeg extract supplementation in the feed

| Item | Control group | Experimental group |
|---|---|---|
| Price, €/t feed (VAT excluded) | 1400.00 | 1470.00 |
| Price, €/kg feed (VAT excluded) | 1.400 | 1.470 |
| ECR | 1.78* | 2.13 |

*The lowest value shows the best Economic conversion ratio (ECR)

*Determination of blood biochemical parameters*

Blood biochemical parameters of fish are used for monitoring their health. All tested 13 parameters of control and experimental groups did not differ significantly (p>0.05) (Table 5).

**Table 5.** Blood biochemical parameters of rainbow trouts (*Oncorhynchus mykiss* W.)

| Parameters | n | Control group | Experimental group | Significance |
|---|---|---|---|---|
| | | $\bar{x}$ ± SD | $\bar{x}$ ± SD | |
| GLU, mmol/l | 6 | 4.87±0.42 | 4.94±0.26 | NS |
| UREA, mmol/l | 6 | 0.85±0.06 | 0.83±0.10 | NS |
| CREA, µmol/l | 6 | 18.17±1.58 | 20.00±2.13 | NS |
| TP, g/l | 6 | 40.43±1.31 | 38.62±2.59 | NS |
| ALB, g/l | 6 | 19.28±1.81 | 17.68±1.25 | NS |
| ASAT, U/l | 6 | 246.67±26.2 | 249.33±25.3 | NS |
| ALAT, U/l | 6 | 32.50±2.67 | 34.17±2.90 | NS |
| ALP, U/l | 6 | 590.67±90.4 | 543.17±60.6 | NS |
| Ca, mmol/l | 6 | 2.44±0.10 | 2.28±0.20 | NS |
| P, mmol/l | 6 | 5.85±0.51 | 5.60±0.42 | NS |
| Mg, mmol/l | 6 | 0.76±0.05 | 0.78±0.05 | NS |
| TG , mmol/l | 6 | 1.96±0.02 | 1.95±0.02 | NS |
| CHOL, mmol/l | 6 | 6.22±0.82 | 5.63±0.65 | NS |

NS= Non-significant; Glu- glucose; Crea- creatinine; TP- total protein; Alb- albumin;
ASAT- aspartate aminotransferase; ALAT- alanine transaminase; ALP- alkaline phosphatase;
Ca- calcium; P- phosphorus; Mg- magnesium; TG- triglycerides; CHOL- cholesterol.

*Chemical composition of meat*

The addition of 1% nutmeg extract resulted in significantly (p<0.001) increased water content of rainbow trout meat - 77.12±0.21% lower dry matter percentage (22.88±0.21%) vs control fish (75.30±0.70% and 24.70±0.70%, respectively (Table 6). Meat protein content in experimental and control fish was identical: 18.59±0.09% and 18.58±0.26% (p>0.05). Fat content of rainbow trout meat in group E was 2.59±0.21% which was statistically significantly lower (p<0.001) than that of the control fish - 4.46±0.82%. Ash content was comparable in both groups: 1.67±0.04% (control) and 1.69±0.02% (experimental) showing that the addition of 1% nutmeg extract did not have any effect on this trait (p>0.05).

**Table 6.** Chemical composition of the fillets of rainbow trout (*Oncorhynchus mykiss* W.), cultivated in a recirculation system

| Parameters | n | Control group | Experimental group | Significance |
|---|---|---|---|---|
| | | $\bar{x}$ ± SD | $\bar{x}$ ± SD | |
| Water, % | 6 | 75.30±0.70 | 77.12±0.21 | *** |
| Protein, % | 6 | 18.58±0.26 | 18.59±0.09 | NS |
| Lipids, % | 6 | 4.46±0.82 | 2.59±0.21 | *** |
| Dry matter, % | 6 | 24.70±0.70 | 22.88±0.21 | *** |
| Ash, % | 6 | 1.67±0.04 | 1.69±0.02 | NS |

Significant difference: ***p≤0.001; NS= Non–significant.

*Fatty acid composition of meat*

Meat fatty acid content of rainbow trouts reared in the recirculation system, either supplemented or not with 1% nutmeg extract, is presented in Table 7. Dietary supplementation of 1% nutmeg extract resulted in statistically significantly higher content (p<0.001) of myristic and palmitic acids. Myristic acid in control and supplemented fish was 2.49±0.17% and 4.23±0.44%, respectively, and that of palmitic acid: 17.96±0.38% and 21.15±0.04%. Stearic acid was also substantially increased (p<0.01) in fish from group E (4.58±0.42%) as compared to control fish (3.98±0.15%). SFA of trout meat supplemented with nutmeg extract was 29.95±0.05%, i.e. statistically significantly higher (p<0.001) vs controls (24.43±0.51%).

**Table 7.** Fatty acid composition of the fillets of rainbow trout (*Oncorhynchus mykiss* W.), cultivated in a recirculation system

| Parameters | n | Control group | Experimental group | Significance |
|---|---|---|---|---|
| | | $\bar{x}$ ± SD | $\bar{x}$ ± SD | |
| C14:0 Myristic | 6 | 2.49±0.17 | 4.23±0.44 | *** |
| C16:0 Palmitic | 6 | 17.96±0.38 | 21.15±0.04 | *** |
| C16:1 Palmitoleic | 6 | 2.92±0.12 | 2.97±0.11 | NS |
| C18:0 Stearic | 6 | 3.98±0.15 | 4.58±0.42 | ** |
| C18:1 Oleic | 6 | 49.68±1.03 | 47.29±1.12 | ** |
| C18:2 Linoleic | 6 | 13.83±0.48 | 12.87±0.38 | ** |
| C18:3n-3 α-linolenic | 6 | 3.21±0.26 | 2.57±0.29 | ** |
| C20:2 Eicosadienoic | 6 | 0.82±0.15 | 0.35±0.08 | *** |
| C20:3 Eicosatrienoic | 6 | 0.21±0.03 | 0.25±0.07 | NS |
| C20:5 Eicosapentaenoic | 6 | 1.71±0.30 | 2.16±0.14 | ** |
| C22:5 Docosapentaenoic | 6 | 0.64±0.12 | 0.25±0.09 | *** |
| C22:6 Docosahexaenoic | 6 | 2.57±0.28 | 1.46±0.20 | *** |
| SFA[1] | 6 | 24.43±0.51 | 29.95±0.05 | *** |
| UFA[2] | 6 | 75.58±0.51 | 70.05±0.05 | *** |
| MUFA[3] | 6 | 52.60±0.91 | 50.26±1.01 | ** |
| PUFA[4] | 6 | 22.98±0.20 | 19.89±0.96 | *** |
| n-6[5] | 6 | 14.86±0.36 | 13.46±0.53 | *** |
| n-3[6] | 6 | 8.12±0.16 | 6.43±0.44 | *** |
| PUFA/SFA | 6 | 0.94±0.01 | 0.66±0.03 | *** |
| n-6/n-3 | 6 | 1.83±0.08 | 2.10±0.06 | *** |

Significant difference: ***p≤0.001; **p≤0.01; NS= Non–significant.
[1]SFA- Saturated fatty acids; [2]UFA- Unsaturated fatty acids; [3]MUFA- Monounsaturated fatty acids;
[4]PUFA- Polyunsaturated fatty acids; [5]n-6– ΣC18:2;C20:2;C20:3;C20:4; [6]n-3–ΣC18:3n-3;C20:5;C22:6.

Palmitoleic acid content of meat of control and experimental groups was 2.92±0.12% and 2.97±0.11%, respectively, with insignificant differences between groups (p>0.05). The addition of 1% nutmeg extract to rainbow trout feed resulted in considerable reduction (p<0.01) of oleic acid content in the meat of supplemented fish from group E - 47.29±1.12% compared to controls (49.68±1.03%). Meat MUFA content in group E was 50.26±1.01%, which was statistically significantly lower (p<0.01) than the value of the non-supplemented group – 52.60±0.91%.

Nutmeg extract added to feed of rainbow trouts led to significantly lower (p<0.01) linoleic and α-linolenic acid content of their meat (12.87±0.38% and 2.57±0.29%, respectively) vs respective content in the control group (13.83±0.48% and 2.57±0.29%). Furthermore, statistically significantly lower (p<0.001) contents of eicosadienoic, docosapentaenoic and docosahexaenoic acids in fish meat was established in supplemented trouts – 0.35±0.08%, 0.25±0.09% and 1.46±0.20%, respectively. In control fish, the percentages of these fatty acids were 0.82±0.15%, 0.64±0.12% and 2.57±0.28%. Eicosapentaenoic acid in fish that received nutmeg extract was 2.16±0.14% – considerably higher (p<0.01) compared to controls: 1.71±0.30%. Eicosatrienoic acid in the experimental fish was not influenced by nutmeg supplementation (p>0.05). The tested dietary extract resulted in statistically significantly lower (p<0.01) PUFA and UFA percentages in experimental rainbow trout meat (19.89±0.96%; 70.05±0.05%) compared to the respective contents in control fish – 22.98±0.20% and 75.58±0.51%.

The percentages of n-6 and n-3 fatty acids in the meat of trouts supplemented with nutmeg extract were statistically significantly (p<0.001) decreased: 13.46±0.53% and 6.43±0.44%, vs those in control trouts – 14.86±0.36% and 8.12±0.16%.

The addition of 1% nutmeg extract to rainbow trout feed changed statistically significantly (p<0.001) PUFA/SFA and n-6/n-3 ratios. PUFA/SFA was lower in trouts supplemented with nutmeg extract (0.66±0.03) vs controls (0.94±0.01). For n-6/n-3 ratios, a higher one was found in supplemented fish (2.10±0.06) compared to non-supplemented ones: 1.83±0.08.

## Discussion

Water chemical parameters – temperature, dissolved oxygen, water pH and electrical conductivity during the trial were within respective optimum ranges for the farmed species. The same was true for maximum allowed concentrations of ammonia, nitrates, nitrites and orthophosphates in water, which, for rainbow trout farms, are set at 1 $mg.l^{-1}$, 2 mg.l-

$^{1}$, 0.01 mg.l$^{-1}$ and 0.40 mg.l$^{-1}$, respectively (Regulation No 4/2000) – throughout the experiment, the measured levels in the recirculation system water were significantly lower. There were no substantial ($p>0.05$) differences between the groups with regard to all studied water chemical parameters.

In the middle of the trial, body weight of control and experimental rainbow trouts did not differ significantly, yet at the end fish supplemented with 1% nutmeg extract demonstrated a substantially lower ($p<0.05$) live weight than controls (Table 3). This contradicted data reported by Zhelyazkov et al. (2018) in carps and Sodamola et al. (2017) in African catfish supplemented with nutmeg. Probably, our results may be due to specific features of rainbow trout metabolism.

The addition of 1% nutmeg extract to extruded feeds for rainbow trouts, reared in recirculation system, did not have any effect on survival rate, which was similar to that in controls: 97.5% (Table 3). Regarding this parameter, our data were in line with those of Zhelyazkov et al. (2018) in carps (100%) and higher that the survival rates in African catfish reported by Sodamola et al. (2017) - 63-86.3%. The differences may be attributed to the different age groups of experimental fish: fattened carps in the experiment of Zhelyazkov et al. (2018) and juvenile catfish in that of Sodamola et al. (2017); the rainbow trouts in this experiment were also in the juvenile group.

The analysis of data for average individual weight gain of rainbow trouts from group C showed that it was 11.76% higher compared to group E ($p<0.05$) (Table 3). These results are also opposite to those of Zhelyazkov et al. (2018) in fattened carps and of Sodamola et al. (2017) in juvenile catfish reporting higher weight gain in fish supplemented with nutmeg extract, as stated above, the possible cause could be the different metabolism of tested fish species.

At the end of the trial, data for consumed extruded feed showed FCR 14.17% better in control fish compared to the group that received 1% nutmeg extract ($p<0.05$) (Table 3). This disagrees with results communicated by Zhelyazkov et al. (2018) in fattened carps and Sodamola et al. (2017) in juvenile African catfish supplemented with dietary nutmeg extract.

The better economic conversion ratio in controls vs the group supplemented with 1% nutmeg extract reflects the slower growth and poorer feed conversion in the latter group and did not agree with reported better ECR in fattened carps (Zhelyazkov et al., 2018).

Blood biochemical parameters in fish are influenced by water quality, temperature, sampling technique, age, feeding, sexual maturity and photoperiod (Coşkun et al., 2016; Fazio et al., 2016). The present study confirmed that the addition of 1% nutmeg extract did not influence significantly the tested 13 blood biochemical parameters in rainbow trout (Table 5). So far, no data were reported on the effect of nutmeg extract on blood biochemistry in fish. The studies of Koshinski (2019, 2020) on addition of *Achillea millefolium* and *Taraxacum officinale* extracts to rainbow trout fish did not evidence relevant differences in blood biochemical parameters. On the contrary, Velichkova et al. (2019) reported significantly higher blood Ca concentrations in carps supplemented with *Acorus calamus*, while Sirakov et al. (2019) detected higher blood TG and CHOL in carps whose feed was supplemented with *Taraxacum officinale*.

The addition of 1% nutmeg extract resulted in statistically significantly higher water content of trout meat, while the dry matter and fat contents were lower. Similar data were documented after addition of *Achillea millefolium* and *Taraxacum officinale* extracts to the ration of rainbow trouts (Koshinski, 2019, 2020) and addition of oregano to carp feed (Georgieva et al., 2018). Yet, the supplementation of carp feed with extracts from curcumin, paprika, thyme and garlic and the same extracts to rainbow trout feed did not report any substantial influence (Georgieva et al., 2019). During the last years, consumers prefer fish with leaner meat, so the results about lower meat fat content of trouts supplemented with nutmeg extract are promising.

The supplementation of rainbow trout feed with nutmeg extract led to statistically significantly higher content of myristic, palmitic and stearic fatty acids, and to total SFA percentage in fish meat. This was not in line with the results of Georgieva et al. (2018) affirming lower levels of the same fatty acids in rainbow trouts fed feeds containing curcumin, paprika, thyme, oregano and garlic. Myristic and palmitic fatty acids are essential for humans as they lead to an increase in cholesterol, while the effect of stearic acid is neutral. Despite the higher SFA content of trout meat from group E (29.95%), it is normal for this fish species. Zhelyazkov et al. (2015) reported values from 28.28 to 29.03%

The oleic acid percentage and MUFA content in experimental group E were lower than those of the control fish, whereas according to Georgieva et al. (2018) these fatty acids in rainbow trouts supplemented with curcumin, paprika, thyme, oregano and garlic in their feed were elevated.

The addition of 1% nutmeg extract to the feed of rainbow trouts resulted in considerably lower content of linoleic, α-linolenic, eicosadienoic, docosapentaenoic and docosahexaenoic acids in meat in comparison to controls. Georgieva et al. (2018) also reported significantly lower α-linolenic acid levels in meat in trouts supplemented with curcumin and thyme, but not in fish fed paprika, oregano and garlic, yet the authors did not find significant differences with regard to the other fatty acids, unlike us. The only long chain fatty acid the percentage of which was significantly higher in the meat of experimental fish in the present study was the eicosapentaenoic acid, while the study of Georgieva et al. (2018) did not confirm any relevant effect of dietary curcumin, paprika, thyme, oregano and garlic supplementation of rainbow trouts. The addition of nutmeg extract in this experiment resulted in substantially lower PUFA, UFA, n-6 and n-3 content of trout meat contrary to the findings of Georgieva et al. (2018), observing a tendency towards higher levels of these groups of fatty acids in trouts supplemented with curcumin, paprika, thyme, oregano and garlic.

The between-group differences in values of PUFA/SFA and n-6/n-3 ratios were statistically significant. PUFA/SFA was lower in rainbow trouts supplemented with nutmeg extract compared to control fish, while n-6/n-3 was higher in fish from group E receiving nutmeg extract with feed. Regardless of the less favourable PUFA/SFA and n-6/n-3 ratios (0.66 and 2.10, respectively, in

supplemented trouts), these ratios were still within the respective desired ranges of > 0.4 and < 5 (Simopoulos, 2004).

## Conclusion

The dietary supplementation of rainbow trouts with nutmeg extract had a negative impact on growth performance and economic efficiency of farming of the species. Final live weight and average individual weight gain were lower, and feed conversion ratio (FCR) and economic conversion ratio (ECR) values were inferior vs controls. The nutmeg extract had also an adverse effect on water content and dry matter of meat from supplemented fish. A positive effect was demonstrated only on meat fat content, which was substantially lower compared to that of control fish. Despite the higher SFA as well as lower UFA, MUFA, PUFA, n-6 and n-3 fatty acid content of trout meat that received nutmeg extract with feed, the PUFA/SFA and n-6/n-3 ratios remained within the favourable range for human nutrition, e.g. >0.4 and <5, respectively.

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