Food Science and Applied Biotechnology
e-ISSN: 2603-3380

Journal home page: www.ijfsab.com
https://doi.org/10.30721/fsab2022.v5.i2

OPEN ACCESS

Research Article

Proximate and fatty acid composition of meat from rainbow trout (*Oncorhynchus mykiss* W.) after dietary supplementation with black pepper (*Piper nigrum* L.) extract

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Abstract
The study was aimed at assessing the effect of dietary supplementation with black pepper (*Piper nigrum*) on the chemical and fatty acid composition in the meat of rainbow trout (*Oncorhynchus mykiss* W.), farmed in a recirculating system.
Rainbow trout were distributed in two groups – experimental fed 1% powdered extract from black pepper (BPE) and control (C) fed without black pepper extract. After the end of the experiment, 6 fish from each group were euthanized, filleted and deboned. The 1% BPE in rainbow trout feed reduced meat water content and increased dry matter percentage and fat content. The fatty acid profile of rainbow trout meat was beneficially influenced by the dietary BPE supplement. The SFA percentage was statistically significantly decreased whereas contents of UFA, MUFA, PUFA and n-3 were considerably higher. No consistent effect was noted with respect to n-6 fatty acids. The improved PUFA/SFA and n-6/n-3 ratios in the supplemented group showed that the tested feed additive had a favourable effect on fatty acid content of rainbow trout meat. Both ratios were within the range, beneficial for human nutrition: > 0.4 and < 5, respectively.

Keywords
Oncorhynchus mykiss, *Piper nigrum*, black pepper extract, chemical composition, fatty acid profile

Abbreviations
C – control group; BPE – experimental group; SFA – saturated fatty acids; UFA – unsaturated fatty acids; MUFA – monounsaturated fatty acids; PUFA – polyunsaturated fatty acids; n-6 – omega (ω); n-3 – omega (ω)

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Article history:
Received 25 March 2022
Reviewed 9 May 2022
Accepted 31 July 2022
Available on-line 13 October 2022

https://doi.org/10.30721/fsab2022.v5.i2.183

Introduction

Fish feeds contain additives in tiny amounts along with primary components to achieve some specific goals, for instance improvement of fish meat quality, feed quality and conversion, as well as for maintenance of water quality in aquaculture farms (Bai et al. 2015). Phytoadditives are alternatives to antibiotics in aquaculture. The effects of different plants and plant extracts are known long ago as growth promoters, immunostimulants, immunomodulators, antimicrobials and antioxidants (Gabor et al. 2010). The main advantage of phytoadditives is their natural origin; they do not pose a direct threat for fish health, human consumers' health and for the environment (Gabor et al. 2011). Although the healing properties of many plants are known for centuries and they are used in folk medicine, this is yet an innovation for aquaculture, and their effects in different fish species are still not determined (Gabor et al. 2011).

Piper nigrum, black pepper is one of the most common and sold spices, used in global culinary, it is also used in medicine (Devasahayam et al. 2015). It belongs to the Piperaceae family, the fruit of the plant is used as a spice after drying (Devasahayam et al. 2015; Harrison 2016). Black pepper originates from South India; it is cultivated both there and in many other tropical regions (Sen 2004; Devasahayam et al. 2015; Hadjeski 2016). Its amazing antioxidant properties are attributed to polyphenols and tocopherols. The gastric, carminative, antioxidant, antibacterial, anti-microbial, immunomodulatory, larvicidal, anti-biotic, anti-inflammatory, antitumour, antipyretic, and antioxidant properties of black pepper are also reported (Charles 2012).

The proximate analysis of black pepper shows moisture content 2.17%, dry matter 97.83%, crude ash 12.49%, nitrogen content 1.85%, crude protein 11.56%, carbohydrates 45.16%, crude fibre 16.66% and nutritional energy 335.06 $gcal^{-1}$ (Hossain et al., 2015). Fatty acid content of black pepper comprises palmitic acid 28.57%, oleic acid 14.95%, linoleic acid 26.61% and linolenic acid 9.32% (Saini et al. 2021).

The effects of various phytoextracts were studied, mainly in carps and rainbow trout: nutmeg (Zhelyazkov et al. 2018), curcumin, paprika, thyme, oregano, *Allium sativum* (Georgieva et al. 2018, 2019), *Achillea millefolium* (Koshinski 2019), *Acorus calamus* (Velichkova et al. 2019), *Taraxacum officinale* (Sirakov et al. 2019; Koshinski 2020) etc. The effect of extract from *Piper nigrum* leaves was investigated with respect to growth performance, haematological and immune parameters in *Labeo rohita* (Ullaha et al. 2021), as well as seeds from the same plant as growth promoter in this fish species (Matiullah et al. 2016). Nevertheless, the influence of phytoextract on different fish species, their growth, health, blood biochemical analytes, and meat proximate composition is not extensively investigated. Before inclusion of a specific supplement in commercial fish feeds, its effect on respective fish species should be studied in detail (Gabor et al. 2010). The studies on chemical composition and especially on fatty acid profile of fish meat after adding plant extracts to the feeds are scarce and this has raised an increased interest to the subject.

It is considered that the chemical and fatty acid content of fish meat is appropriate for human nutrition, and the PUFA/SFA and n-6/n-3 ratios in fish meat are in the beneficial range: more than 0.4 and less than 5, respectively (Simopoulos 2004; Taşbozan and Gökçe 2017). As fish are monogastric animals, the chemical and fatty acid composition of meat depends mainly on received feed, so the former could be directed by feed composition.

The study was aimed at assessing the effect of dietary supplementation with black pepper (*Piper nigrum*) on the chemical and fatty acid composition in the meat of rainbow trout (*Oncorhynchus mykiss* W.), farmed in a recirculating system.

Materials and Methods

Ethical approval

The study has followed all listed guidelines for the care and use of animals (Directive 2010/63/EU; Regulation № 20/2012).

Experimental design

Studied 120 rainbow trouts were allotted into two groups – control (C) and experimental (BPE), each group had two replications (n=30 fish). The initial body weight of trouts was 41.55±7.76 g (group C) and 41.23±8.38 g (group BPE). The experiment was performed in a recirculation system with optimal water chemical parameters for trout farming: average water temperature 14.00°C, dissolved

oxygen >9.00 mg.l^{-1}, pH 7.62-7.64, content of ammonia, nitrates, nitrites and orthophosphates: less than 1.0 mg.l^{-1}, 2.0 mg.l^{-1}, 0.01 mg.l^{-1} and 0.40 mg.l^{-1} respectively. By lubrication of extruded feed granules („Aqua garant UNI", Garant-Tiernahrung Gesellschaft m.b.H., Austria) with sunflower oil (5 ml for 100 g feed), rainbow trout from group (BPE) received 1% powdered black pepper extract (P.I.C. Co Ltd, Sofia, Bulgaria), whereas control fish (C) received only sunflower oil-lubricated feed. The feed amount was 3% of live weight of fish, and feeding was three times per day, manually. The experiment lasted 60 days. The nutritional composition of feed of both groups was as followed: crude protein - 45%, crude lipids - 16%, crude fiber - 2.40%, crude ash - 8%, Ca - 1.60%, P - 1.20%, ME - 18.50 MJ/kg. One kg feed contained: 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.

At experiment end, the mean live weights of control and supplemented trouts were 117.25±19.15 g (C) and 124.10±19.35 g (BPE) respectively.

Sample preparation for analyses

After the experiment, 6 fish from each group were euthanized, filleted and deboned after removal of viscera and heads. The meat was minced, homogenized and submitted to chemical analyses.

Chemical and fatty acid composition was analysed in the Laboratory of Faculty of Agriculture at Trakia University, Stara Zagora.

Chemical composition

After preparation of meat samples according to AOAC (2006; method 983.18), water content (%) was determined by air drying (AOAC 1997; method 950.46). Crude protein (%) was calculated on the basis of N content, assayed using an automatic Kjeldahl system (Kjeltec 8400, FOSS, Sweden). Lipid content (%) was assayed with a Soxtec 2050 automated system (FOSS, Sweden) by the method of Soxhlet. Ash content (%) was determined after meat incineration (550°C; 8 h) in a muffle furnace (MLW, Germany). Crucibles were weighed after being brought to room temperature.

Fatty acid composition

Total lipids were extracted from muscles (Bligh and Dyer 1959) and lipid methyl esters, isolated by preparative TLC were obtained using 0.01% sulphuric acid in dry methanol for 14 h (Christie 1973). Fatty acid composition (%) of trout meat total lipids was carried out by gas chromatography using a Perkin Elmer Clarus 500 gas chromatograph with a flame ionization detector. Chromatography conditions are described in detail in a previous study of ours (Georgieva et al. 2018).

Statistical analysis

Statistical analysis was performed using STATISTICA 6.0 software (Stat Soft Inc. 2002). Data are given as mean and standard deviation. Differences between means were tested by the t-test at $p<0.05$.

Results

Chemical composition

The feed supplementation with 1% BPE resulted in lower water content of fish meat by 0.53%, respectively increased dry matter by 1.78% compared to control fish, whose water and dry matter values were 77.07±0.17% and 22.93±0.17%, respectively (P<0.01) (Table 1). The protein content of control fish meat was by 1.67% higher than that of fish from the BPE group, where it was 18.30±0.29%, yet differences were insignificant (P>0.05) (Table 1). The addition of 1% black pepper extract resulted in substantially higher (P<0.01) lipid content of supplemented fish - by 22.59% compared to that of control trout where it averaged 3.01±0.28%. Meat ash content of control fish was 1.31±0.03% - by 2.97% lower than that of experimental group BPE, with insignificant differences (P>0.05) (Table 1).

Table 1. Proximate composition of the fillets of rainbow trout farmed in a recirculation system

Parameters	n	C $\bar{x}$ ±SD	BPE $\bar{x}$ ±SD	Signific.
Water, %	6	77.07±0.17	76.66±0.13	**
Protein, %	6	18.61±0.20	18.30±0.29	NS
Lipids, %	6	3.01±0.28	3.69±0.30	**
Dry matter, %	6	22.93±0.17	23.34±0.13	**
Ash, %	6	1.31±0.03	1.35±0.03	NS

Significantly different: ** P≤0.01;
NS – Non- significant.

Fatty acid composition

The addition of 1% black pepper extract to rainbow trout feed led to statistically significantly (P<0.001) lower myristic acid content (by 26.82% vs average value of controls - 2.61±0.24% (Table 2). The palmitic acid content also changed significantly (P<0.01) being by 30.99% lower in group BPE compared to controls - 20.30±0.94% (Table 2). No statistically significant differences were found out in stearic acid contents of fish meat from both groups (Table 2). Meat SFA content of control group was 27.69±0.94% - significantly greater (P<0.001) by 23.51% than that of the supplemented group BPE (Table 2).

Palmitoleic acid content of non-supplemented trout was 3.14±0.30% which was by 39.49% lower than that of group BPE (P<0.05) (Table 2). Oleic acid content was not influenced by the addition of 1% black pepper extract to feed, so differences between groups were inconsistent (P>0.05) (Table 2). MUFA in the meat of fish from group BPE was 53.23±0.21% - by 4.38% higher than that of controls (P<0.01) (Table 2).

The addition of 1% black pepper extract to trout feed had no effect on meat linoleic acid content (P>0.05) (Table 2). A significantly higher (P<0.01) α-linolenic acid content (by 26.89%) was found out in the meat of group BPE compared to the control group (2.12±0.11% (Table 2). Eicosadienoic acid content in supplemented trout was 0.32±0.02% - by 65.63% lower vs controls (P<0.001) (Table 2). The eicosatrienoic acid of meat of treated fish was not influenced by 1% black pepper extract supplementation (P>0.05) (Table 2). The content of eicosapentaenoic acid in group BPE was statistically significantly higher (P<0.05) - by 90.10% than that of control group (0.91±0.36%; Table 2). The content of docosapentaenoic acid in the group supplemented with 1% black pepper extract was 3.2 times higher (P<0.01) than control levels (0.34±0.04%; Table 2). Docosahexaenoic acid content was also statistically significantly increased in group BPE, (P<0.001) – 2.53 times higher than in control trout whose average content was 1.26±0.71% (Table 2).

Table 2. Fatty acid composition of the fillets of rainbow trout (*Oncorhynchus mykiss* W.), farmed in a recirculation system

Fatty acids, %	n	C $\bar{x}$ ±SD	BPE $\bar{x}$ ±SD	Significance
C14:0 Myristic	6	2.61±0.24	1.91±0.15	***
C16:0 Palmitic	6	20.30±0.94	14.01±3.52	**
C16:1 Palmitoleic	6	3.14±0.30	4.38±0.99	*
C18:0 Stearic	6	4.79±0.24	5.26±0.51	NS
C18:1 Oleic	6	47.76±1.74	48.85±1.20	NS
C18:2 Linoleic	6	15.59±1.07	16.15±2.81	NS
C18:3n-3 α-linolenic	6	2.12±0.11	2.69±0.37	**
C20:2 Eicosadienoic	6	0.53±0.04	0.32±0.02	***
C20:3 Eicosatrienoic	6	0.68±0.32	0.44±0.07	NS
C20:5 Eicosapentaenoic	6	0.91±0.36	1.73±0.67	*
C22:5 Docosapentaenoic	6	0.34±0.04	1.09±0.51	**
C22:6 Docosahexaenoic	6	1.26±0.71	3.19±0.26	***
SFA[1]	6	27.69±0.94	21.18±3.16	***
UFA[2]	6	72.31±0.94	78.83±3.16	***
MUFA[3]	6	50.90±1.44	53.23±0.21	**
PUFA[4]	6	21.42±0.51	25.60±3.37	*
n-6[5]	6	16.79±1.42	16.91±2.90	NS
n-3[6]	6	4.63±0.92	8.70±0.47	***
PUFA/SFA	6	0.77±0.01	1.25±0.35	**
n-6/n-3	6	3.81±1.10	1.93±0.23	**

Significant different: *** P≤0.001; ** P≤0.01; * P≤0.05; 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

The dietary supplementation with 1% black pepper extract increased substantially UFA (P<0.001) and PUFA content (P<0.05) in experimental fish – by 9.02% and 19.51%, respectively, whereas respective average content of control fish was 72.31±0.94% and 21.42±0.51% (Table 2).

The n-3 content in the meat of fish from group BPE was significantly (P<0.001) higher (by 87.90%) than that of non-supplemented fish (4.63±0.92%) whereas n-6 meat contents of both groups did not differ considerably (P>0.05) (Table 2).

The dietary addition of 1% BPE resulted in substantial differences (P<0.01) between meat PUFA/SFA and n-6/n-3 ratios of both groups (Table 2). The PUFA/SFA ratio in supplemented fish was by 62.34% higher compared to that of controls (0.77±0.01%) along with lower n-6/n-3 ratio in group BPE (by 49.34%) as compared to the same ratio in untreated fish - 3.81±1.10% (Table 2).

Discussion

The addition of 1% black pepper extract to the feed for rainbow trout resulted in statistically significant (P<0.01) lower water meat content with respective significant elevation (P<0.01) of dry matter and fat content. Dietary supplementation of common carps (Wojno et al. 2021) and *Labeo rohita* (Matiullah et al. 2016) with black pepper increased the total fat content of their meat. Contrary to those results, the supplementation of rainbow trouts with *A. millefolium* and *T.officinale* extracts led to higher meat water content and lower fat and dry matter content (Koshinski 2019; 2020).

Comparable data were reported by Georgieva et al. (2018) following oregano supplementation of carp feed. Georgieva et al. (2018; 2019) found no consistent effect after adding curcumin, paprika, thyme and garlic extracts to the feeds for rainbow trout and carps, respectively. We have found an insignificant change (P>0.05) in meat protein and ash content after addition of 1% black pepper extract to trout feed, in support of previously reported data by Georgieva et al. (2018) with curcumin, paprika, thyme, oregano and garlic extracts added to the feed of the same fish species. Opposite data were reported by Matiullah et al. (2016) – statistically significantly higher protein and ash contents after addition of 0.5% black pepper extract to the feed of *Labeo rohita*, whereas Koshinski (2020) observed higher meat protein and lower meat ash content in rainbow trout meat after dietary supplementation with extract from *Taraxacum officinale*.

Substantial reduction of myristic (P<0.001) and palmitic (P<0.01) acids in fish meat was demonstrated after addition of 1% black pepper extract to their feed, whereas the stearic acid content did not change significantly (P>0.05). Myristic and palmitic acids increase the cholesterol, therefore they are important for human nutrition, whereas stearic acid is neutral. The total meat SFA content in rainbow trout from group BPE was significantly lower (P<0.001) than that in controls. Those data were in line with reduced content of myristic acid in the rainbow trout muscle after dietary supplemention with oregano, curcumin, garlic, paprika and thyme extracts (Georgieva et al. 2018).

The oleic acid levels in meat were not influenced by the dietary addition of 1% black pepper extract, however, the palmitoleic acid (P<0.05) and MUFA (P<0.01) contents turned out to be statistically significantly higher in the experimental group BPE vs controls. Georgieva et al. (2018) have also reported higher concentrations of these fatty acids in rainbow trout meat, fed feeds containing oregano, garlic, paprika, thyme and curcumin extracts.

The dietary supplementation of rainbow trout with 1% black pepper extract resulted in inconsistent changes (P>0.05) in linoleic and eicosatrienoic fatty acids in the meat of experimental fish in line with data of Georgieva et al. (2018) obtained in trouts fedh curcumin, oregano, thyme, paprika and garlic extracts. The statistically significantly elevated α-linolenic (P<0.01), eicosapentaenoic (P<0.05), docosapentaenoic (P<0.01) and docosahexaenoic (P<0.001) fatty acids in the meat of BPE group disagreed with data published by Georgieva et al. (2018) about considerably lower meat α-linolenic content in trout supplemented with curcumin and thyme with feed, although no differences were detected when the feed was supplemented with paprika, oregano and garlic extracts. With respect to the other four fatty acids, the research team found no significant differences after addition of those five phytoextracts, which also contradicts our findings. Eicosadienoic fatty acid was the only long-chain fatty acid, which demonstrated significantly lower concentrations (P<0.001) in the meat of supplemented fish, while Georgieva et al. (2018)

found no reliable effect from dietary addition of curcumin, paprika, thyme, oregano and garlic in rainbow trout with regard to this fatty acid. Substantially elevated UFA (P<0.001), PUFA (P<0.05) and n-3 (P<0.001) fatty acids' percentages were found out in experimental trout that received 1% black pepper extract in support to data of Georgieva et al. (2018), finding out higher levels of these fatty acid groups after trout's supplementation with extracts from curcumin, paprika, thyme, oregano and garlic. The amount of n-6 fatty acids in the meat of fish from group BPE was not altered (P>0.05) by the 1% black pepper extract added to the feed, comparable to data of Georgieva et al. (2018).

PUFA/SFA and n-6/n-3 ratios in meat exhibited statistically significant differences (P<0.01) between the two groups. PUFA/SFA was higher in trouts that received black pepper extract with the feed, whereas the n-6/n-3 ratio decreased in the supplemented groups as compared to controls. Georgieva et al. (2018) found out no significant influence in these two ratios from the addition of curcumin, paprika, thyme, oregano and garlic to the feed of rainbow trout, dissimilar to our results. The better PUFA/SFA and n-6/n-3 ratios in the group supplemented with black pepper: 1.25±0.35 and 1.93±0.23 respectively, confirmed that this feed additive had a positive effect on the fatty acid profile of rainbow trout meat. According to Simopoulos (2004), the beneficial range for these two ratios from the point of view of human nutrition was > 0.4 and < 5, respectively.

Conclusions

Adding 1% extract from black pepper (BPE) to rainbow trout feed reduced meat water content with equivalent increase in dry matter and fat contents. The rainbow trout meat fatty acids content was positively influenced by the 1% BPE supplement. The SFA percentage was significantly decreased whereas contents of UFA, MUFA, PUFA and n-3 fatty acids were significantly higher. No consistent effect was noted with respect to n-6 fatty acids. The improved PUFA/SFA and n-6/n-3 ratios in the supplemented group showed that the tested feed additive had a favourable effect on fatty acid content of rainbow trout meat. Both ratios were within the range, beneficial for human nutrition: > 0.4 and < 5, respectively.

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