



Product Quality and Safety

Effect of dietary supplementation of cinnamon (*Cinnamomum verum* Presl) extract to rainbow trout (*Oncorhynchus mykiss* W.) feed on the chemical and fatty acid profile of meat

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(Manuscript received 4 March 2024; accepted for publication 10 June 2024)

Abstract. *The aim of this study was to evaluate the effect of dietary supplementation with cinnamon (*Cinnamomum verum* Presl) on meat chemical composition and fatty acid profile in rainbow trouts (*Oncorhynchus mykiss* W.), cultivated in a recirculation system. One hundred and twenty rainbow trouts were allotted into two variants - control (C) and experimental (C.v), each in two replication with thirty fish. The average initial body weight of fish was 41.55 ± 7.76 g (control group) and 41.48 ± 7.95 g (C.v group) and final live weights on the 60th day: 117.25 ± 19.15 g (C) and 121.42 ± 19.39 g (C.v). The trial was carried out in a recirculation system that maintained water chemical parameters optimal for rainbow trout farming. The 1% powdered cinnamon extract was given to fish from the C.v. group by lubrication of extruded feed granules with 5 ml/100 g feed sunflower oil, whereas the feed of controls was lubricated only with the same amount of sunflower oil. After the end of the feeding period, six groups from each group were euthanised, heads and viscera were removed, then they were filleted and deboned. The meat was minced, homogenised and chemical and fatty acid analyses were performed. The meat water, dry matter, lipid, protein and ash contents were not changed after feed supplementation with 1% cinnamon extract. However the tested phytoextract had a beneficial effect on the meat fatty acid profile. SFA content of meat was statistically significantly reduced along with considerable increase in the content of UFA, MUFA, PUFA and n-3 fatty acid groups. The levels of n-6 fatty acids were not influenced by the dietary supplementation with cinnamon extract. The beneficial effect of this additive on fatty acid content of meat was proved by the improved PUFA/SFA and n-6/n-3 ratios in the meat of group C.v. Both ratios were within the beneficial ranges from the point of view of human nutrition: above 0.4 and less than 5, respectively.*

Keywords: *Cinnamomum verum*, cinnamon extract, *Oncorhynchus mykiss*, chemical composition, fatty acid profile

Introduction

The improvement of aquaculture efficiency depends on many factors, yet the most important

one in the used feed. The supplements to fish feeds are known to improve their quality, e.g. absorption, taste, consistency, water solubility, fish meat quality and many others (Bai et al., 2015).

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People have been familiar with the antimicrobial and antioxidant effects of various plants and plant extracts for a long time (Gabor et al., 2010), so they are believed to be suitable alternatives to antibiotics in aquaculture. Phytoadditives pose no threat either to fish and human health or to the environment, as they are natural substances (Gabor et al., 2011). Nowadays, the influence of plant extract on different fish species and on the quality of their meat is not fully investigated. A large part of available studies have investigated their effects on productive performance and blood parameters of fish, and economic results linked to feed conversion (Georgieva and Zhelyazkov, 2018; Stoyanova et al., 2018a,b; Zhelyazkov et al., 2018; Sirakov et al., 2019; Velichkova et al., 2019; Georgieva et al., 2020; Zhelyazkov and Stoev, 2020; Stoev and Zhelyazkov, 2021). Far less studies have been published on the meat chemical composition of fish supplemented with phytoadditives (Georgieva et al., 2018, 2019; Koshinski 2019, 2020; Stoev and Zhelyazkov, 2020).

Cinnamon is among the oldest spices used in global culinary (Rao and Gan, 2014). Although many species from the genus *Cinnamomum* are offered on the market as cinnamon, the inner dried bark of *Cinnamomum verum* Presl syn. *Cinnamomum zeylanicum* Blume is considered true cinnamon (Avula, 2015). *Cinnamomum verum* is a tropical shrubby evergreen tree of the Lauraceae family, native to Sri Lanka and South India, but also distributed in Southeast Asia, China, Burma, Indonesia, Madagascar, the Caribbean, Australia and Africa (Abeysekera et al., 2013; Goyal et al., 2018). Cinnamon contains a lot of vitamins, minerals, and phenolic compounds. It was reported to possess anti-inflammatory, antimicrobial, antibacterial, antitumour, cardiovascular, cholesterol lowering, immunomodulatory, larvicidal and antioxidant properties (Charles, 2012). A complete phytochemical and pharmacological overview of *Cinnamomum verum* as universal food spice was made by Singh et al. (2021). The proximate analysis of cinnamon according to this study was as follows: carbohydrates 52.0%; crude protein 3.5%, crude fat 4%, crude fibre 33.0%, moisture 5.1% and ash 2.4% (Gul and Safdar, 2009). The cinnamon fatty acid composition is characterized by 47.68%

stearic acid, 26.08% linoleic acid, 15.58% palmitic acid, 7.73% oleic acid, 2.43% undecanoic acid and 0.5% lauric acid (Jamil et al., 2016).

Before inclusion of a specific supplement in commercial fish feeds, its effect on the respective fish species should be studied in detail (Gabor et al., 2010). So far, the investigations on cinnamon as fish feed additive focused on its effects on growth performance, feed conversion, body composition, resistance to *Aeromonas hydrophila* in Nile Tilapia (Mohammad et al., 2011), growth performance and economic effect from feeding in carps (Stoyanova et al., 2018a), growth performance, blood parameters and economic efficiency in rainbow trouts (Zhelyazkov and Stoev, 2020). Data on meat proximate analysis in cinnamon-supplemented fish are few, whereas information about meat fatty acid profiles is not available. Regardless of the fact that PUFA/SFA and n-6/n-3 ratios in fish meat are within the beneficial range - >0.4 and <5 (Simopoulos, 2004; Taşbozan and Gökçe, 2017), means for their improvement in farmed fish are still sought, and this could be done through the feed.

The aim of this study was to evaluate the effect of dietary supplementation with cinnamon (*Cinnamomum verum* Presl) on meat chemical composition and fatty acid profile in rainbow trouts (*Oncorhynchus mykiss* W.), cultivated in a recirculation system.

Material and methods

Ethical approval

The study was performed in line with international, national, and/or institutional guidelines for the care and use of animals (Directive 2010/63/EU; Regulation N° 20/2012).

Experimental design

One hundred and twenty rainbow trouts were allotted into two variants - control (C) and experimental (C.v), each in two replication with thirty fish. The average initial body weight of fish was 41.55 ± 7.76 g (control group) and 41.48 ± 7.95 g (C.v group). The trial was carried out in a recirculation system that maintained water chemical parameters

optimal for rainbow trout farming: mean water temperature 14.00°C, dissolved oxygen content over 9.00 mg.l⁻¹, pH 7.64-7.72. The concentrations of ammonia, nitrates, nitrites and orthophosphates were below 1.0 mg.l⁻¹, 2.0 mg.l⁻¹, 0.01 mg.l⁻¹ and 0.40 mg.l⁻¹, respectively. The 1% powdered cinnamon extract (P.I.C.Co LTD) was given to experimental fish from the C.v. group by lubrication of extruded feed granules (Aqua garant UNI, manufactured by Garant-Tiernahrung Gesellschaft m.b.H. - Austria) with 5 ml/100 g feed sunflower oil, whereas the feed of controls was lubricated only with the same amount of sunflower oil. The daily ration was 3% of fish live weight, distributed into 3 portions, given manually. The experimental period lasted 60 days. The feed received by the 2 experimental groups contained 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 D3 - 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.

The final live weight of fish on the 60th day was 117.25±19.15 g (C) and 121.42±19.39 g (C.v), respectively.

Preparation of samples for chemical analysis

After the end of the feeding period, six subgroups from each group were euthanised, heads and viscera were removed, and then they were filleted and deboned. The meat was minced, homogenised and analysed.

Chemical composition

After preparation of fish meat samples (AOAC, 2006; method 983.18), their water content was determined by air drying (AOAC, 1997; method 950.46). Crude protein (%) was determined by Kjeldahl's method, (Kjeltec 8400, FOSS, Sweden). Meat lipid content (%) was assayed according to the method of Soxhlet (Soxtec 2050, FOSS,

Sweden). The ash content (%) was obtained after incineration at 550°C for 8 h (MLW, Germany).

Fatty acid composition

The determination of fatty acid composition (%) of the total lipids of the rainbow trout was 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 with independent-sample Student's t-test at p<0.05.

Results

Chemical composition

The chemical composition of rainbow trout meat was not influenced by the addition of 1% cinnamon extract to the feed as the between-group differences were not statistically significant (P>0.05; Table 1). Meat water content of fish from group C.v was 0.13% lower than in controls, the average value of which was 77.07±0.17%. The dry matter content of the supplemented fish meat was by 0.43% higher than that of group C (22.93±0.17%). Meat protein percentage of control rainbow trouts was 18.61±0.20%, e.g. 0.97% higher than that of fish that received 1% cinnamon extract with the feed. The fat content of the supplemented trouts was 10.30% higher compared to the control fat content of 3.01±0.28%. Meat ash of cinnamon-supplemented fish (1.28±0.06%) was reduced by 2.29% compared to the ash content of controls.

Table 1. Chemical composition of the fillets of rainbow trout (*Oncorhynchus mykiss* W.), cultivated in a recirculation system

Parameters	n	C	C.v	Significance
		$\bar{x} \pm SD$	$\bar{x} \pm SD$	
Water, %	6	77.07±0.17	76.97±0.30	NS
Protein, %	6	18.61±0.20	18.43±0.28	NS
Lipids, %	6	3.01±0.28	3.32±0.25	NS
Dry matter, %	6	22.93±0.17	23.03±0.30	NS
Ash, %	6	1.31±0.03	1.28±0.06	NS

NS – Non-significant.

Fatty acid composition

The addition of 1% cinnamon extract to the feed of rainbow trouts resulted in statistically significantly higher ($P < 0.001$) myristic acid content in fish meat – by 20.31% compared to the average control percentage of $2.61 \pm 0.24\%$ (Table 2). Palmitic acid percentage in the meat of non-supplemented controls ($20.30 \pm 0.94\%$) exceeded substantially this fatty acid in fish fed 1% cinnamon extract (C.v), the content of which was 14.93% lower ($P < 0.001$) (Table 2). There was no statistically significant difference between the groups regarding stearic acid content in meat ($P > 0.05$; Table 2). A considerably lower percentage ($P < 0.001$) – by 8.02%, was established for SFA acids in supplemented trouts in comparison to the control value of $27.69 \pm 0.94\%$ (Table 2).

The oleic and palmitoleic acids in the meat of experimental fish were not influenced by the addition of 1% cinnamon extract to the feed. The differences between both groups were inconsistent ($P > 0.05$), and the same was true for the total MUFA meat content (Table 2).

The addition of cinnamon extract to the feed did not alter the content of linoleic, α -linolenic, eicosadienoic and docosapentaenoic fatty acids ($P > 0.05$; Table 2). Considerably lower ($P < 0.05$)

eicosatrienoic acid level (by 44.12%) was found in the meat of group C.v compared to the control content of $0.68 \pm 0.32\%$ (Table 2). The eicosapentaenoic acid content in group C.v was 2.36 times higher ($P < 0.001$) vs the average control percentage of $0.91 \pm 0.36\%$ (Table 2). Also, meat docosahexaenoic acid content was substantially ($P < 0.001$) higher (3.05 times) in cinnamon-supplemented group C.v compared to controls, the average level of which was $1.26 \pm 0.71\%$ (Table 2). The addition of cinnamon extract to the feed led to statistically significantly ($P < 0.001$) higher content of UFA, PUFA and n-3 fatty acids in experimental fish – by 3.07%, 12.79% and 77.75% respectively, whereas in control trouts, the average concentrations of these fatty acid groups were $72.31 \pm 0.94\%$, $21.42 \pm 0.51\%$ and $4.63 \pm 0.92\%$. No statistically significant differences were detected with respect to n-6 fatty acids ($P > 0.05$) (Table 2).

After addition of 1% cinnamon extract to rainbow trout feed, the PUFA/SFA ratio increased by 23.38% ($P < 0.001$) in group C.v and the average level in control fish was $0.77 \pm 0.01\%$ (Table 2). The n-6/n-3 ratio also changed considerably ($P < 0.01$), but this time it was by 48.29% lower than the same ratio in the meat of non-supplemented trouts – $3.81 \pm 1.10\%$ (Table 2).

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

Parameters	n	C	C.v	Significance
		$\bar{x} \pm SD$	$\bar{x} \pm SD$	
C14:0 Myristic	6	2.61±0.24	3.14±0.04	***
C16:0 Palmitic	6	20.30±0.94	17.27±0.52	***
C16:1 Palmitoleic	6	3.14±0.30	3.63±0.95	NS
C18:0 Stearic	6	4.79±0.24	5.06±0.86	NS
C18:1 Oleic	6	47.76±1.74	46.75±1.16	NS
C18:2 Linoleic	6	15.59±1.07	14.69±1.05	NS
C18:3n-3 α -linolenic	6	2.12±0.11	2.00±1.10	NS
C20:2 Eicosadienoic	6	0.53±0.04	0.86±0.41	NS
C20:3 Eicosatrienoic	6	0.68±0.32	0.38±0.02	*
C20:5 Eicosapentaenoic	6	0.91±0.36	2.15±0.13	***
C22:5 Docosapentaenoic	6	0.34±0.04	0.26±0.13	NS
C22:6 Docosahexaenoic	6	1.26±0.71	3.84±0.43	***
SFA ¹	6	27.69±0.94	25.47±0.30	***
UFA ²	6	72.31±0.94	74.53±0.30	***
MUFA ³	6	50.90±1.44	50.38±0.21	NS
PUFA ⁴	6	21.42±0.51	24.16±0.51	***
n-6 ⁵	6	16.79±1.42	15.93±1.44	NS
n-3 ⁶	6	4.63±0.92	8.23±0.94	***
PUFA/SFA	6	0.77±0.01	0.95±0.03	***
n-6/n-3	6	3.81±1.10	1.97±0.40	**

Significant difference: *** P≤0.001; ** P≤0.01; * P≤0.05; NS – Non-significant.

¹SFA–Saturated fatty acids; ²UFA–Unsaturated fatty acids; ³MUFA–Monounsaturated fatty acids; ⁴PUFA–Polyunsaturated fatty acids; ⁵n-6–YC18:2;C20:2;C20:3;C20:4; ⁶n-3–YC18:3n-3;C20:5;C22:6.

Discussion

The meat proximate analysis results showed that the chemical composition was not changed from the addition of 1% cinnamon extract to the feed of trouts (P>0.05). This was in line with the results of Mohammad et al. (2011) who added *Cinnamomum zeylanicum* to the feed of Nile Tilapia; comparable results were obtained by Georgieva et al. (2018, 2019) with paprika, curcumin, garlic and thyme extracts added to the rainbow trout and carp feeds. On the contrary, other researchers reported increased total meat lipids in common carps (Wojno et al., 2021) and in *Labeo rohita* (Matiullah et al., 2016) supplemented with black pepper. Higher meat water content and lower fat and dry matter content were found by Koshinski (2019,

2020) with *Achillea millefolium* and *Taraxacum officinale* dietary extracts in rainbow trouts, as well as by Georgieva et al. (2018) by adding oregano to carp feed. Koshinski (2020) affirmed higher protein and lower ash content in rainbow trout meat supplemented with extract from *Taraxacum officinale*, whereas Matiullah et al. (2016) found higher meat protein and ash percentages in *Labeo rohita* that received 0.5% black pepper with the feed.

The addition of 1% cinnamon extract resulted in statistically significantly increased (P<0.001) meat myristic acid content compared to untreated fish, whereas palmitic acid in group (C.v) was substantially lower (P<0.001) than in controls. Stearic acid in the meat of rainbow trouts was not influenced by cinnamon addition. Stearic

acid is neutral from the point of view of human nutritional science, whereas both myristic and palmitic acids are important as they increase blood total cholesterol concentrations. SFA in the meat of cinnamon-fed fish was considerably ($P < 0.001$) lower than that in the control group. Unlike our results, a previous study (Georgieva et al., 2018) reported lower levels of myristic acid in the meat of trouts fed curcumin, paprika, thyme, oregano and garlic with the feed, whereas the results regarding palmitic acid and SFA were in line with our findings.

Oleic, palmitoleic and total MUFA levels in the meat of the experimental fish were not influenced by the addition of 1% cinnamon extract ($P > 0.05$) opposing to other data that evidenced higher levels of these fatty acids in trouts supplemented with curcumin, paprika, thyme, oregano and garlic (Georgieva et al., 2018).

The contents of linoleic, α -linolenic, eicosadienoic and docosapentaenoic fatty acids in the meat of rainbow trouts was not changed ($P > 0.05$) by the addition of 1% cinnamon extract to the feed. The results for linoleic, eicosadienoic and docosapentaenoic acids were comparable to those reported by Georgieva et al. (2018) for rainbow trouts fed feeds containing curcumin, paprika, thyme, oregano and garlic, yet the authors found rather lower levels of α -linolenic acid opposite to our data. A statistically significantly ($P < 0.05$) reduced amount of eicosatrienoic acid was found in the meat of fish from group C.v compared to controls, which disagreed with the results of Georgieva et al. (2018) in trouts fed curcumin, paprika, thyme, oregano and garlic with feed. The contents of eicosapentaenoic and docosahexaenoic fatty acids in the meat of trouts from group C.v was substantially higher ($P < 0.001$) than that in control fish, whereas Georgieva et al. (2018) did not report any significant effect from the addition of curcumin, paprika, thyme, oregano and garlic with regard to these fatty acids. Meat UFA, PUFA and n-3 in rainbow trout meat supplemented with cinnamon extract was considerably ($P < 0.001$) higher compared to those in controls, in line with the data of Georgieva et al. (2018), who also observed a tendency for higher concentrations of these fatty acid groups in rainbow trouts after dietary supplementation with curcumin, paprika,

thyme, oregano and garlic. The n-6 fatty acids in the meat of the experimental group C.v were not influenced by cinnamon extract supplementation of feed, similar to what was reported by Georgieva et al. (2018).

The addition of 1% cinnamon extract to rainbow trout feed led to statistically significant differences in PUFA/SFA ($P < 0.001$) and n-6/n-3 ($P < 0.01$) ratios between the groups. PUFA/SFA was higher in supplemented fish, whereas the n-6/n-3 ratio was higher in controls. Contrary to our results, dietary supplementation of rainbow trouts with curcumin, paprika, thyme, oregano and garlic showed no effect on these two ratios (Georgieva et al., 2018). The positive effect on meat fatty acid profile in rainbow trouts that received cinnamon extract was proven by the improved PUFA/SFA and n-6/n-3 ratios: 0.95 ± 0.03 and 1.97 ± 0.40 , respectively. The beneficial range for humans was reported to be > 0.4 for PUFA/SFA and < 5 for n-6/n-3 (Simopoulos, 2004).

Conclusion

The water, dry matter, lipid, protein and ash contents in the rainbow trout meat were not changed after feed supplementation with 1% cinnamon extract; however, the phytoextract had a beneficial effect on the meat fatty acid profile. SFA content of meat was statistically significantly reduced along with considerable increase in the content of UFA, PUFA and n-3 fatty acid groups. The levels of n-6 fatty acids were not influenced by the dietary supplementation with cinnamon extract. The beneficial effect of the additive on the fatty acid content of the meat was proved by the improved PUFA/SFA and n-6/n-3 ratios in the meat of group C.v as both ratios were within the beneficial ranges required by human nutritional science: above 0.4 and less than 5, respectively.

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