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Nitrogen factors for rainbow trout (*Oncorhynchus mykiss*) and brook trout (*Salvelinus fontinalis*) fillets

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Abstract: Measures for consumer protection against food adulteration and misleading labelling are integrated into EU legislation, including methods for detecting misleading practices. Verification of the meat content is available for marine products, but not for salmonid fish due to the lack of standard nitrogen factors. This study aimed to establish nitrogen factors for rainbow trout (*Oncorhynchus mykiss*) and brook trout (*Salvelinus fontinalis*). The study analysed 340 fish from Czech fisheries obtained in the summer of 2018–2020. According to the established ISO methods, fillet samples with and without skin were analysed for their nitrogen content (protein), dry matter, ash, and fat. The recommended nitrogen factor for rainbow trout fillets with and without the skin is 3.07 ± 0.12 and 3.06 ± 0.14, respectively, and the nitrogen factor for fat-free rainbow trout fillets with and without the skin is 3.33 ± 0.15 and 3.29 ± 0.15, respectively. The recommended nitrogen factor for brook trout fillets with and without the skin is 3.16 ± 0.10 and 3.12 ± 0.09, respectively, and the nitrogen factor for fat-free brook trout fillets with and without the skin is 3.42 ± 0.13 and 3.36 ± 0.12, respectively. The established nitrogen factors will enable the analysis of the meat content to ensure that consumers purchase correctly described and labelled fish products.

Keywords: adulteration; fish-food fraud; fish products; nitrogen factor; salmonid

Fish play an irreplaceable role in human nutrition, and they are a very important source of animal protein and other dietetic-valuated nutrients (FAO 2020). They provide crucial benefits for a healthy diet, such as good digestibility of fish meat, low-fat content, and its characteristic composition with

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a higher content of unsaturated fatty acids compared to farm animals and bird fat, vitamins, and minerals (Adamkova et al. 2011).

The average consumption of fish and fish products per capita in the EU was 24.4 kg in 2017 (EC 2022). The fish muscle composition is influenced by many factors, such as the fish species, breeding technology, environmental conditions, gender, age, season and others.

The base for food quality and a high level of consumer protection in relation to food information is set in the EU legislation. The general principles, requirements and responsibilities in relation to the food information disclosed to customers are mentioned in Regulation (EU) No. 1169/2011 of the European Parliament and the Council (EC 2011). The mandatory food information covers the information on the identity and composition, properties, durability, storage, safe use, and nutritional characteristics of the food according to this Regulation. For consumers, it is very important that the quantity of the main ingredients of/in the food be labelled, and the composition must be indicated on the food label (EC 2011).

The production and sale of food are inextricably linked to the attempts of their adulteration, which is primarily motivated by financial gain. It is undertaken by the substitution of some expensive ingredients with cheaper ones or the undeclared additions of water or other ingredients (Cizkova et al. 2012; Cavin et al. 2018). The adulteration mostly concerns expensive food or food that is traded in large amounts (Moore et al. 2012; Everstine et al. 2013; Cavin et al. 2018). These illegal practices can have a negative impact on the consumers' health and can endanger human lives and mental health. There are known adulteration cases, for example, the addition of melamine to milk powder used for infant formula, dyes in chilli and paprika, the addition of methanol to spirits, the horsemeat scandal, or the fipronil contamination of eggs (Cizkova et al. 2012; Cavin et al. 2018; Morin and Lee 2018). In cases where there is suspicion that animal proteins have been substituted with other proteins or other nitrogen compounds, liquid chromatography with mass detection or polymerase chain reaction (PCR) methods are used, which can detect these ingredients.

Protecting consumers against adulteration and misleading labelling is one of the main tasks of the food policy of the European Union. The main rules are contained in the base document of the food EU legislation, Regulation (EC) No. 178/2002 of the European Parliament and the Council (EC 2002a). The methods for uncovering misleading practices based on the undeclared addition of water for pork meat, chicken meat, and saltwater fish have been published in European legislation and the standards of Codex Alimentarius (EC 2002b; CA 2004; EC 2008).

Suitable methods for determining the meat content in freshwater fish products are absent. The reason for this state is the absence of species-specific nitrogen content (nitrogen factor) for freshwater fish, except for the Nile mouthbreeder (*Oreochromis niloticus*) (CA 2004), common carp (*Cyprinus carpio*) (Honzlova et al. 2021a), and European pikeperch (*Sander lucioperca*), northern pike (*Esox lucius*), and wels catfish (*Silurus glanis*) (Honzlova et al. 2021b).

This study aimed to establish nitrogen factors as determined by the Kjeldahl method (ISO 1978) within the context of the Codex standard (CA 2004) for rainbow trout (*Oncorhynchus mykiss*) and brook trout (*Salvelinus fontinalis*), the primary salmonid fish species farmed and processed in the Czech Republic for commercial markets. The established nitrogen factors can provide the basis for verifying the content of salmonid fish products and can help uncover the illegal addition of water to these products, helping to ensure that consumers buy correctly labelled or described fish products.

MATERIAL AND METHODS

Fish and experimental protocol

Two-hundred-forty market-size rainbow trout (110–900 g weight) were obtained from six Czech aquaculture facilities: The University of South Bohemia in Ceske Budejovice, Faculty of Fisheries and Protection of Waters (FFPW USB) Vodnany, and the Annin, Boskovice, Vacov, Velke Mezirici, and Reckov fisheries. One hundred market-size brook trout (170–1 085 g) were obtained from three Czech aquaculture facilities (Annin, Reckov, and Velke Mezirici fisheries). In order to assess any effect of the year and location, the fish were collected during the summer (June–August) harvesting seasons in 2018, 2019, and 2020. The fish were transported live to the Faculty

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of Fisheries and Protection of Waters laboratory, stunned by a blow to the head, and consequently killed, weighed, measured, and filleted. From the first ten fish, samples of two fillets with the skin from each fish were prepared. From the second ten fish, samples of two fillets without the skin from each fish were prepared.

The fillets from each fish were individually vacuum-packed, immediately frozen, and stored at –32 °C until the chemical analysis was performed. A total of 340 fish were chemically analysed.

Chemical analysis

One-hundred-seventy samples of fillets with skin and one-hundred-seventy samples of fillets without skin were analysed for proximate composition, protein, dry matter, fat, and ash. Before analysis, all samples were carefully homogenized by grinding on/using a knife mill Pulverisette 11 (Fritsch GmbH, Idar-Oberstein, Germany).

The protein (nitrogen) content was determined by the Kjeldahl method based on the standard method ISO 937:1978 Meat and meat products – Determination of the nitrogen content (Reference method) (ISO 1978). The homogenised samples were digested by sulfuric acid and a catalyser in a KjelROC Digestor 20 (OPSIS AB, Furulund, Sweden) digestion unit at 420 ± 10 °C. The organically bound nitrogen was determined on a KJELTEC 8400 with a KJELTEC sampler 8420 (FOSS Headquarters, Hillerød, Denmark). The protein content was calculated from the nitrogen using the conversion factor of 6.25 for the meat.

The determination of the dry matter was based on the ISO 1442:1997 Meat and meat products – Determination of the moisture content (Reference method) (ISO 1997). The homogenised samples were dried with sand to a constant weight at 103 ± 2 °C in a laboratory oven (Memmert UE 500; Memmert GmbH + Co. KG, Schwabach, Germany). The sample dried to a constant weight in approximately 3 to 4 hours.

The total fat content was determined by a method based on the ISO 1443:1973 Meat and meat products – Determination of the total fat content (ISO 1973). The homogenised samples were hydrolysed by hydrochloric acid, and then the fat was extracted by light petroleum in a SOXTEC 2050 (FOSS Headquarters, Hillerød, Denmark).

The determination of the percentage of ash was based on the standard ISO 936:1998 Meat and meat products – Determination of the total ash (ISO 1998). The homogenised samples were burned in a muffle furnace (Nabertherm A11/HR; Nabertherm GmbH, Lilienthal, Germany) at 550 ± 25 °C to a grey-white colour.

The nitrogen content (nitrogen factor) (N_f) in g/100 g was calculated from the protein content (ISO 1978):

$$N_f = \frac{protein}{6.25} \quad (1)$$

The fat-free nitrogen (N_{ff}) in g/100 g was calculated according to the formula (Colwell et al. 2011):

$$N_{ff} = \frac{100 \times N}{100 - F} \quad (2)$$

This formula was applied to the nitrogen (N) and fat (F) content for all the samples and obtained fat-free nitrogen factor (N_{ff}) for each sample.

The fish meat content based on the nitrogen factor N_f (whole fillet) in g/100 g was calculated according to the formula (CA 2004):

$$fish\ meat\ content_{N_f} = \frac{N \times 100}{N_f} \quad (3)$$

The fish meat content based on the nitrogen factor N_{ff} (fat-free basis) and defatted fish meat content (DFC) in g/100 g was calculated according to the formulas (Colwell et al. 2011):

$$fish\ meat\ content_{N_{ff}} = DFC + F \quad (4)$$

$$DFC = \frac{N \times 100}{N_{ff}} \quad (5)$$

Statistical analysis

Kolmogorov-Smirnov and Bartlett's tests were applied to assess the normal distribution data and the homoscedasticity of variance, respectively. A two-way analysis of variance (ANOVA) and Tukey's test was conducted to analyse the effects of the season, weight, fishery, and the difference between the fillets with and without skin. The significance level was set at $P < 0.05$.

Data were expressed as the mean ± SD values and range. The analysis was performed using STATISTICA v12.0 for Windows (STATSOFT, Inc.; Czech Republic).

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A redundancy analysis (RDA) with the functional traits as the response variables and sampling site and year as the categorial (explanatory) variables was applied to explain the differences among the sample distribution. The ordination plots were displayed using Canoco, Windows release, v5.10 (Biometris, the Netherlands, and Petr Šmilauer, Czech Republic).

Ethics approval

All the methods used in the present study followed the relevant guidelines and regulations. All the experimental laboratory procedures complied with the valid legislative regulations in the Czech Republic (Law No. 166/1996 and No. 246/1992); the permit was issued to No. 2293/2015-MZE-17214 and No. 55187/2016-MZE-17214.

All the samplings were carried out with the relevant permission from the Departmental Expert Committee for Authorisation of Experimental Projects of the Ministry of Education, Youth and Sports of the Czech Republic (Permit No. MSMT 5389/2018-2).

RESULTS

Rainbow trout

The proximate composition of the rainbow trout fillets is given in Table 1. The nitrogen content was significantly lower ($P < 0.01$) in the rainbow trout fillets without skin from Annin (2020), Boskovice (2018), and the fillets with skin from Boskovice (2020) compared to the fillets with and without skin from FFPW USB Vodnany (2018, 2019, 2020), Reckov (2018), Vacov (2018), Velke Mezirici (2019), the fillets with skin from Boskovice (2019) and the fillets without skin from Annin (2019). We found no significant differences ($P > 0.05$) in the nitrogen content of the fillets with and without skin at a single sampling time.

The redundancy analysis using two categorical variables produced canonical scores corresponding to the axes constrained by the explanatory variables (Figure 1). Four canonical axes explaining the variance of the response data constrained by the categorical data accounted for 43% of the total variance for the rainbow trout (Figure 1). The first canonical axis explained 25.2% of the total variance. The sampling site was found as a strong explanatory factor of the sample variability compared to the sampling year (pseudo-F 1st axis = 5.4, $P = 0.002$). All the functional traits were explained by the sample groups obtained from Vacov, Vodnany, Reckov, and Velke Mezirici separated towards the positive pole of the first axis. The sample groups obtained from Annin and Boskovice were separated near the negative pole of the first axis. They were negatively correlated with the measured functional traits of nitrogen and fat regardless of the skin presence in/on fillets. The nitrogen in fillets with skin was negatively correlated with the weight of the fish, whereas a positive relationship was observed in the same parameters in the fillets without skin. In contrast, the fat in the fillets with skin was positively correlated with the appropriate weight, but the relationship between the fat content in the fillets without skin and weight was negative.

The established nitrogen factors for the rainbow trout fillets with and without skin determined by the Kjeldahl method are calculated as 3.07 ± 0.12 and 3.06 ± 0.14, respectively, and the nitrogen factors for the fat-free rainbow trout fillets with and without the skin are 3.33 ± 0.16 and 3.29 ± 0.15, respectively. These results are presented in the summary in Table 2.

Brook trout

The proximate composition of the brook trout fillets is given in Table 3. The nitrogen content was significantly lower ($P < 0.01$) in the brook trout fillets without skin from Annin (2019) compared to the fillets with and without skin from Reckov (2018), Velke Mezirici (2019) and the fillets with skin from Annin (2020). We found no significant differences ($P > 0.05$) in the nitrogen content of the fillets with and without skin at a single sampling time. Four canonical axes explained 64.0% of the total variance, whereas the first axis explained almost 50% of the total variance (Figure 2).

The sampling sites and years were evaluated as strong explanatory variables that separated the samples along the first canonical axis (pseudo-F 1st axis = 11.2, $P = 0.002$). The redundancy analysis plot displayed no differences between the samples with and without skin, whereas the nitrogen was negatively correlated with the fat.

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Table 1. Live weight and total length of the rainbow trout (*Oncorhynchus mykiss*) and chemical composition of the fillets with and without skin

Facility	Year	Weight (g) x ± SD (min–max)	Total length (cm) x ± SD (min–max)	Fillet	Dry matter (g/100 g) x ± SD (min–max)	Ash (g/100 g) x ± SD (min–max)	Fat (g/100 g) x ± SD (min–max)	Protein (g/100 g) x ± SD (min–max)	N (g/100 g) x ± SD (min–max)
Annin	2018	143.0 ± 14.5 (110.0–160.0)	23.5 ± 1.0 (22.0–25.0)	with skin	27.1 ± 1.6$^{b(B)}$ (24.5–29.9)	1.5 ± 0.1$^{b(B)}$ (1.4–1.6)	6.3 ± 1.7$^{a(A)}$ (2.9–8.9)	18.7 ± 0.6$^{a(A)}$ (18.1–20.1)	3.0 ± 0.1$^{ab(AB)}$ (2.9–3.2)
		188.5 ± 27.4 (140.0–240.0)	25.6 ± 1.0 (23.5–27.5)	without skin	27.2 ± 1.2$^{b(B)}$ (24.6–28.7)	1.5 ± 0.1$^{b(B)}$ (1.3–1.7)	6.4 ± 1.6$^{a(A)}$ (3.4–8.7)	18.7 ± 0.4$^{a(A)}$ (18.0–19.2)	3.0 ± 0.1$^{ab(AB)}$ (2.9–3.1)
	2019	215.0 ± 69.0 (115.0–315.0)	24.9 ± 2.2 (21.5–27.5)	with skin	27.5 ± 2.0$^{b(B)}$ (24.0–30.2)	1.5 ± 0.1$^{b(B)}$ (1.3–1.6)	7.3 ± 2.1$^{ab(A)}$ 3.9–10.6	18.9 ± 0.5$^{a(A)}$ (18.2–19.8)	3.0 ± 0.1$^{ab(AB)}$ (2.9–3.2)
		245.5 ± 72.7 (155.0–405.0)	26.2 ± 1.9 (23.5–29.5)	without skin	27.0 ± 1.3$^{b(B)}$ (24.9–29.4)	1.5 ± 0.1$^{b(B)}$ (1.4–1.6)	6.4 ± 1.4$^{a(A)}$ (4.7–9.4)	19.1 ± 0.4$^{a(A)}$ (18.3–19.6)	3.1 ± 0.1$^{b(B)}$ (2.9–3.1)
	2020	257.0 ± 25.6 (225.0–295.0)	26.9 ± 0.7 (26.0–28.5)	with skin	27.6 ± 1.5$^{b(B)}$ (24.5–29.2)	1.2 ± 0.6$^{a(A)}$ (1.1–1.3)	7.9 ± 1.4$^{ab(A)}$ (5.5–10.0)	18.5 ± 0.5$^{a(A)}$ (17.6–19.3)	3.0 ± 0.1$^{ab(AB)}$ (2.8–3.1)
		243.0 ± 51.8 (180.0–345.0)	26.6 ± 2.0 (23.5–30.3)	without skin	27.4 ± 1.5$^{b(B)}$ (25.1–30.5)	1.3 ± 0.1$^{ab(AB)}$ (1.2–1.4)	7.9 ± 1.6$^{ab(A)}$ (5.1–11.1)	18.4 ± 0.5$^{a(A)}$ (17.3–19.1)	2.9 ± 0.1$^{a(A)}$ (2.8–3.1)
Boskovice	2018	354.2 ± 49.2 (276.0–418.0)	29.9 ± 2.1 (27.0–33.0)	with skin	28.8 ± 0.91$^{b(B)}$ (27.4–30.0)	1.3 ± 0.1$^{ab(A)}$ (1.2–1.5)	8.0 ± 0.8$^{ab(A)}$ (6.6–9.0)	18.8 ± 0.4$^{a(A)}$ (18.2–19.5)	3.0 ± 0.1$^{ab(AB)}$ (2.9–3.1)
		348.1 ± 21.1 (313.0–385.0)	30.3 ± 0.6 (29.0–31.0)	without skin	28.1 ± 1.8$^{b(B)}$ (24.4–30.7)	1.5 ± 0.1$^{b(A)}$ (1.4–1.6)	7.4 ± 1.6$^{ab(A)}$ (4.1–9.6)	18.3 ± 0.3$^{a(A)}$ (17.9–18.8)	2.9 ± 0.1$^{a(A)}$ (2.8–3.0)
	2019	382.3 ± 69.3 (305.0–551.0)	29.2 ± 1.5 (26.0–31.5)	with skin	27.4 ± 1.6$^{b(B)}$ (24.4–29.4)	1.5 ± 0.1$^{b(A)}$ (1.4–1.5)	6.7 ± 1.4$^{a(A)}$ (4.1–8.1)	19.1 ± 0.5$^{a(A)}$ (18.2–20.1)	3.1 ± 0.1$^{b(B)}$ (2.9–3.2)
		361.0 ± 36.1 (310.0–448.0)	30.8 ± 0.5 (30.0–32.0)	without skin	26.3 ± 08$^{b(B)}$ (24.8–27.9)	1.5 ± 0.1$^{b(A)}$ (1.3–1.8)	6.0 ± 0.9$^{a(A)}$ (4.4–7.5)	18.5 ± 0.8$^{a(A)}$ (17.3–19.7)	3.0 ± 0.1$^{ab(AB)}$ (2.8–3.2)
	2020	330.7 ± 45.7 (258.0–424.0)	30.5 ± 1.1 (29.0–32.5)	with skin	28.3 ± 0.7$^{b(B)}$ (26.9–29.6)	1.4 ± 0.1$^{ab(A)}$ (1.2–1.6)	8.3 ± 0.6$^{ab(A)}$ (7.4–9.3)	18.2 ± 0.7$^{a(A)}$ (26.9–29.6)	2.9 ± 0.1$^{a(A)}$ (2.7–3.0)
		373.1 ± 50.5 (322.0–479.0)	31.4 ± 1.2 (29.5–33.5)	without skin	27.2 ± 0.8$^{b(B)}$ (25.5–28.2)	1.5 ± 0.1$^{b(A)}$ (1.4–1.6)	7.2 ± 1.2$^{ab(A)}$ (5.5–9.5)	18.8 ± 0.6$^{a(A)}$ (17.9–19.7)	3.0 ± 0.1$^{ab(AB)}$ (2.9–3.2)
FFPW USB Vodnany	2018	198.5 ± 45.3 (130.0–265.0)	24.3 ± 1.4 (22.5–26.5)	with skin	29.1 ± 1.3$^{bc(BC)}$ (26.8–30.6)	1.2 ± 0.1$^{a(A)}$ (1.0–1.4)	7.5 ± 1.1$^{ab(AB)}$ (5.5–9.0)	20.2 ± 0.4$^{a(A)}$ (19.7–20.9)	3.2 ± 0.1$^{b(A)}$ (3.1–3.3)
		171.5 ± 34.1 (125.0–245.0)	23.6 ± 1.7 (21.5–26.0)	without skin	26.7 ± 1.6$^{b(B)}$ (24.7–29.4)	1.4 ± 0.3$^{ab(AB)}$ (1.1–2.0)	5.3 ± 1.4$^{a(A)}$ (3.5–7.8)	19.6 ± 0.4$^{a(A)}$ (19.1–20.3)	3.1 ± 0.1$^{b(A)}$ (3.0–3.3)
	2019	311.0 ± 35.6 (240.0–370.0)	28.4 ± 1.5 (25.0–30.0)	with skin	31.6 ± 1.6$^{c(C)}$ (28.9–34.3)	1.2 ± 0.1$^{a(A)}$ (1.1–1.3)	10.3 ± 1.7$^{b(B)}$ (6.8–12.4)	19.7 ± 0.3$^{a(A)}$ (19.1–20.1)	3.1 ± 0.1$^{b(A)}$ (3.1–3.2)
		425.2 ± 50.4 (355.0–505.0)	31.6 ± 1.6 (28.5–34.5)	without skin	29.5 ± 2.5$^{bc(BC)}$ (25.4–32.6)	1.5 ± 0.1$^{b(B)*}$ (1.4–1.7)	7.9 ± 2.6$^{ab(A)}$ (3.6–11.7)	19.7 ± 0.4$^{a(A)}$ (19.0–20.3)	3.2 ± 0.1$^{b(A)}$ (3.0–3.3)
	2020	317.5 ± 34.5 (265.0–395.0)	29.8 ± 1.2 (28.0–31.5)	with skin	28.1 ± 1.5$^{b(B)}$ (26.1–30.1)	1.2 ± 0.1$^{a(A)}$ (1.1–1.4)	7.1 ± 1.9$^{ab(AB)}$ (4.0–9.5)	19.6 ± 0.4$^{a(A)}$ (19.0–20.4)	3.1 ± 0.1$^{b(A)}$ (3.0–3.3)
		360.0 ± 66.3 (235.0–465.0)	29.9 ± 1.6 (27.5–32.5)	without skin	29.1 ± 1.6$^{b(B)}$ (27.0–31.8)	1.2 ± 0.2$^{a(A)}$ (1.0–1.5)	8.2 ± 1.7$^{ab(AB)}$ (6.2–11.5)	20.0 ± 0.4$^{a(A)}$ (19.3–20.6)	3.2 ± 0.1$^{b(A)}$ (3.1–3.3)
Reckov	2018	312.7 ± 26.2 (276.0–371.0)	29.7 ± 1.1 (28.5–31.0)	with skin	28.9 ± 1.3$^{b(A)}$ (26.9–31.7)	1.5 ± 0.1$^{b(A)}$ (1.3–1.6)	7.5 ± 1.9$^{ab(A)}$ (5.4–11.1)	19.9 ± 0.7$^{a(A)}$ (18.6–20.8)	3.2 ± 0.1$^{b(A)}$ (3.0–3.3)
		337.7 ± 32.3 (300.0–408.0)	30.2 ± 0.9 (28.5–32.0)	without skin	28.0 ± 1.8$^{b(A)}$ (26.0–31.0)	1.5 ± 0.1$^{b(A)}$ (1.3–1.6)	7.2 ± 2.1$^{ab(A)}$ (4.9–10.6)	19.1 ± 0.4$^{a(A)}$ (18.5–19.8)	3.1 ± 01$^{b(A)}$ (3.0–3.2)
Vacov	2018	374.5 ± 121.0 (230.0–665.0)	32.0 ± 5.2 (28.0–46.5)	with skin	29.4 ± 2.4$^{bc(A)}$ (26.2–33.3)	1.5 ± 0.2$^{b(A)}$ (1.3–1.8)	7.9 ± 2.5$^{ab(A)}$ (3.9–11.2)	19.9 ± 0.7$^{a(A)}$ (18.4–20.6)	3.2 ± 0.1$^{b(A)}$ (2.9–3.3)
		659.0 ± 147.4 (465.0–900.0)	36.9 ± 3.3 (31.0–40.5)	without skin	28.3 ± 2.2$^{b(A)}$ (25.2–32.3)$^{b(A)}$	1.4 ± 0.4$^{ab(A)}$ (1.1–1.9)	6.8 ± 2.3$^{a(A)}$ (4.3–12.2)	20.7 ± 0.8$^{a(A)}$ (19.4–21.8)	3.3 ± 0.1$^{b(A)}$ (3.1–3.5)

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Table 1 to be continued

Facility	Year	Weight (g) x ± SD (min–max)	Total length (cm) x ± SD (min–max)	Fillet	Dry matter (g/100 g) x ± SD (min–max)	Ash (g/100 g) x ± SD (min–max)	Fat (g/100 g) x ± SD (min–max)	Protein (g/100 g) x ± SD (min–max)	N (g/100 g) x ± SD (min–max)
Velke Mezirici	2019	494.5 ± 129.1 (340.0–730.0)	32.5 ± 2.3 (30.0–36.0)	with skin	29.2 ± 1.6$^{bc(A)}$ (26.7–31.3)	1.4 ± 0.1$^{ab(A)}$ (1.3–1.6)	8.3 ± 1.6$^{ab(A)}$ (5.9–10.2)	19.3 ± 0.4$^{a(A)}$ (18.5–19.9)	3.1 ± 0.1$^{b(A)}$ (3.0–3.2)
		254.5 ± 114.9 (230.0–615.0)	29.6 ± 2.2 (26.5–34.5)	without skin	26.1 ± 1.7$^{b(A)}$ (23.5–28.9)	1.2 ± 0.1$^{a(A)}$ (1.1–1.4)	5.8 ± 2.1$^{a(A)}$ (2.3–9.1)	18.9 ± 0.2$^{a(A)}$ (18.6–19.4)	3.0 ± 0.1$^{b(A)}$ (2.9–3.1)

*Significant differences between the fillets with skin and the fillets without skin at a single sampling (P < 0.01)
Data are expressed as the mean standard deviation (range), n = 10. Values with different small letters in superscripts are significantly (P < 0.01) different among the locality groups. Values with different capital letters in superscripts are significantly (P < 0.01) different among the year groups in one locality

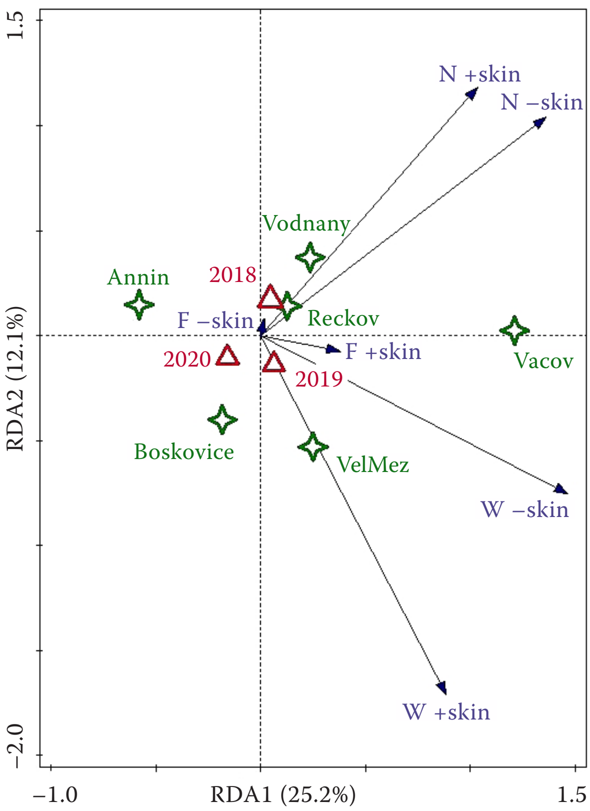

Figure 1. Ordination plots were generated by the redundancy analysis (RDA) for the rainbow trout
The functional traits, as explained data, are displayed by the arrows and sampling sites, and the years, as explanatory data, are categorised by centroids. The predicted increase in the functional trait for each sampling site and year occurs in the direction indicated by the arrow
F +skin – fat in the fillet with skin; F −skin – fat in the fillet without skin; N +skin – nitrogen in the fillet with skin; N −skin – nitrogen in the fillet without skin; VelMez – Velke Mezirici; W +skin – weight of the fish for samples with skin; W −skin – weight of the fish for samples without skin

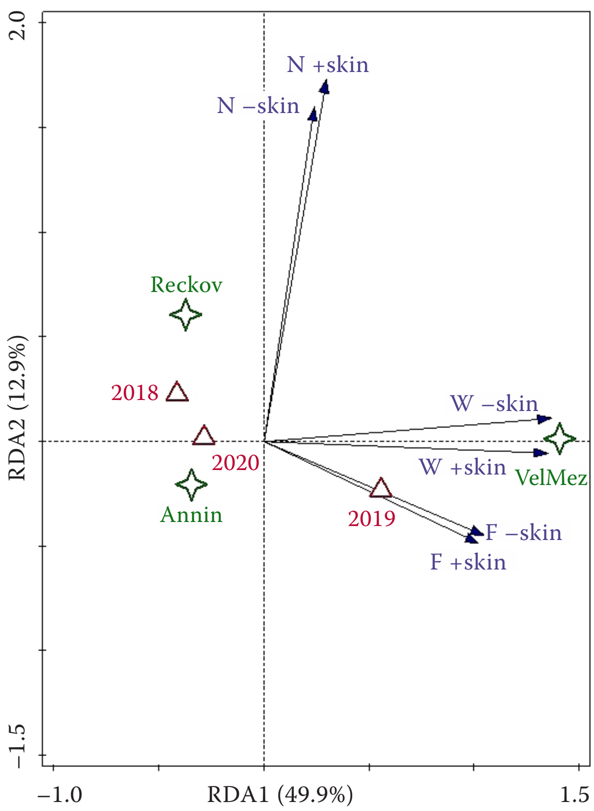

Figure 2. The ordination plots were generated by the redundancy analysis (RDA) for the brook trout
The functional traits, as explained data, are displayed by the arrows and sampling sites and the years, as explanatory data, are categorised by centroids. The predicted increase in the functional trait for each sampling site and year occurs in the direction indicated by the arrow
F +skin – fat in the fillet with skin; F −skin – fat in the fillet without skin; N +skin – nitrogen in the fillet with skin; N −skin – nitrogen in the fillet without skin; VelMez – Velke Mezirici; W +skin – weight of the fish for samples with skin; W −skin – weight of the fish for samples without skin

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Table 2. Calculated nitrogen factors for the rainbow trout (*Oncorhynchus mykiss*) and brook trout (*Salvelinus fontinalis*)

Species	*n*	Fillet	Nitrogen factor (whole fillet) N_f (Kjeldahl)	Nitrogen factor (fat-free basis) N_{ff} (Kjeldahl)
Rainbow trout	120	with skin	3.07 ± 0.13	3.33 ± 0.15
		without skin	3.06 ± 0.13	3.29 ± 0.15
Brook trout	50	with skin	3.16 ± 0.10	3.42 ± 0.13
		without skin	3.12 ± 0.09	3.36 ± 0.12

Data are expressed as the mean standard deviation (range)

Table 3. Live weight and total length of the brook trout (*Salvelinus fontinalis*) and chemical composition of the fillets with and without skin

Facility	Year	Weight (g) x ± SD (min–max)	Total length (cm) x ± SD (min–max)	Fillet	Dry matter (g/100 g) x ± SD (min–max)	Ash (g/100 g) x ± SD (min–max)	Fat (g/100 g) x ± SD (min–max)	Protein (g/100 g) x ± SD (min–max)	N (g/100 g) x ± SD (min–max)
Annin	2018	302.0 ± 24.9 (245.0–415.0)	29.1 ± 1.4 (27.5–32.5)	with skin	28.3 ± 0.7$^{b(B)}$ (26.9–29.2)	1.4 ± 0.1$^{ab(AB)}$ (1.3–1.5)	6.5 ± 1.0$^{a(A)}$ (4.5–8.2)	19.5 ± 0.4$^{a(A)}$ (18.9–20.2)	3.1 ± 0.1$^{ab(A)}$ (3.0–3.2)
		276.5 ± 42.7 (170.0–335.0)	28.8 ± 2.0 (24.0–30.5)	without skin	27.4 ± 1.0$^{b(B)}$ (25.9–29.8)	1.6 ± 0.1$^{b(B)}$ (1.5–1.8)	6.6 ± 1.0$^{a(A)}$ (5.0–8.1)	19.3 ± 0.3$^{a(A)}$ (18.8–19.6)	3.1 ± 0.1$^{ab(AB}$ (3.0–3.2)
	2019	370.0 ± 70.4 (260.0–500.0)	28.4 ± 1.3 (27.0–30.5)	with skin	28.3 ± 0.6$^{b(B)}$ (27.5–29.5)	1.2 ± 0.1$^{a(A}$) (1.1–1.3)	7.8 ± 1.2$^{a(A)}$ (6.1–9.8)	19.1 ± 0.5$^{a(A)}$ (18.5–19.9)	3.1 ± 0.1$^{ab(AB}$ (3.0–3.2)
		319.0 ± 64.5 (245.0–465.0)	26.7 ± 1.1 (25.5–28.5)	without skin	26.9 ± 0.9$^{b(B)}$ (25.3–28.3)	1.3 ± 1.0$^{ab(AB)}$ (1.2–1.5)	6.3 ± 0.5$^{a(A)}$ (5.4–7.3)	19.0 ± 0.5$^{a(A)}$ (18.2–19.8)	3.0 ± 0.1$^{a(A)}$ (2.9–3.2)
	2020	288.0 ± 54.1 (175.0–380.0)	28.3 ± 1.4 (25.5–31.0)	with skin	28.5 ± 0.9$^{b(B)}$ (27.2–30.2)	1.2 ± 0.1$^{a(A)}$ (1.0–1.4)	7.4 ± 0.7$^{a(A)}$ (6.8–8.9)	19.7 ± 0.6$^{a(A)}$ (19.0–20.5)	3.2 ± 0.1$^{b(B)}$ (3.0–3.3)
		297.5 ± 23.8 (255.0–340.0)	28.6 ± 1.1 (27.0–30.5)	without skin	28.7 ± 1.3$^{b(B)}$ (26.0–31.0)	1.1 ± 0.1$^{a(A)}$ (1.0–1.2)	7.1 ± 1.2$^{a(A)}$ (4.6–8.9)	19.6 ± 0.4$^{a(A)}$ (19.0–20.5)	3.1 ± 0.1$^{ab(A)}$ (3.0–3.3)
Reckov	2018	332.1 ± 15.4 (300.0–350.0)	30.0 ± 0.6 (28.5–31.0)	with skin	29.0 ± 0.9$^{b(A)}$ (27.4–30.2)	1.5 ± 0.1$^{b(A)}$ (1.3–1.6)	6.6 ± 0.8$^{a(A)}$ (5.4–8.0)	20.4 ± 0.6$^{a(A)}$ (19.6–21.4)	3.3 ± 0.1$^{b(A)}$ (3.1–3.4)
		324.6 ± 13.8 (304.0–345.0)	30.2 ± 0.7 (28.5–31.0)	without skin	27.4 ± 0.9$^{b(A)}$ (26.0–29.6)	1.6 ± 0.1$^{b(A)}$ (1.5–1.9)	5.4 ± 0.8$^{a(A)}$ (4.0–6.6)	20.4 ± 0.6$^{a(A)}$ (19.3–20.7)	3.2 ± 0.1$^{b(A)}$ (3.1–3.3)
Velke Mezirici	2019	924.0 ± 112.3 (715.0–1 085.0)	36.7 ± 1.8 (33.0–40.0)	with skin	31.8 ± 07$^{c(A)}$ (28.6–36.1)	1.3 ± 0.1$^{ab(A)}$ (1.1–1.5)	10.3 ± 1.9$^{b(A}$) (7.1–12.8)	20.0 ± 04$^{a(A)}$ (19.4–20.6)	3.2 ± 0.1$^{b(A)}$ (3.1–3.3)
		918.0 ± 103.1 (706.0–1 065.0)	36.1 ± 1.7 (32.2–39.8)	without skin	31.1 ± 2.1$^{c(A)}$ (26.9–34.6)	1.4 ± 0.2$^{ab(A)}$ (1.1–1.7)	10.0 ± 2.1$^{b(A)}$ (7.0–13.4)	19.7 ± 0.6$^{a(A)}$ (18.8–20.4)	3.2 ± 0.1$^{b(A)}$ (3.0–3.3)

*Significant differences between the fillets with skin and the fillets without skin at a single sampling ($P < 0.01$)
Data are expressed as the mean ± standard deviation (range), $n = 10$. Values with different small letters in superscripts are significantly ($P < 0.01$) different among the locality groups. Values with different capital letters in superscripts are significantly ($P < 0.01$) different among the year groups in one locality

The established nitrogen factors for the brook trout fillets with and without skin determined by the Kjeldahl method are calculated as 3.16 ± 0.10 and 3.12 ± 0.09, respectively, and the nitrogen factors for the fat-free brook trout fillets with and without the skin are 3.42 ± 0.13 and 3.36 0.12, respectively. These results are presented in the summary in Table 2.

DISCUSSION

The adequate number of samples, i.e., one hundred and twenty fillets with and without skin, for each in the case of the rainbow trout fillets, and fifty fillets with and without skin, for each in the case of the brook trout fillets, were analysed for

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the proximate composition, dry matter, protein, fat, and ash to determine variations between the fishery farm, years, and fillets with and without skin.

The basic nutrient values (protein, dry matter, fat, and ash) obtained in this study are similar to those reported in rainbow trout elsewhere (Ronsholdt 1995; Popelka et al. 2014; Van Doan et al. 2020; Miller et al. 2021). As the water percentage decreases, the fat and protein percentages increase, with increasing body weight and length. The ash percentage remains fairly constant for different fish sizes (Ronsholdt 1995). Our results generally agree with those reported for other fish species (Policar et al. 2016; Modzelewska-Kapitula et al. 2017; Pyz-Lukasik and Paszkiewicz 2018; Ahmed et al. 2020; Shafi et al. 2020).

On the basis of nitrogen content determined by the Kjeldahl method, the nitrogen factors for the rainbow trout fillets with and without skin and for the brook trout fillets with and without skin were established. Due to the significant variations in the fat content, in the case of the rainbow trout fillets with skin, from 2.9% to 12.4%, and from 2.3% to 12.2% without skin. As well as in the case of the brook trout fillets with skin, from 4.5% to 12.8%, and from 4.0% to 13.4% without skin. Therefore in our study, the nitrogen factors on a fat-free basis were established (N_{ff}) (Colwell et al. 2011). All the calculated nitrogen factors are given in Table 2. They are expressed as a mean with a standard deviation obtained from all the measured fillet samples. According to the Codex Alimentarius, it is recommended to use a variance value 10% (CA 2004).

The fish meat content in all the fillet samples with and without skin was calculated on the basis of the recommended nitrogen factors, N_f for the whole fillet and N_{ff} for the fat-free fillet (Table 4). In this table, the information about the numbers of samples is included, which calculated the meat content that was out of the recommended variance value of 10% (CA 2004). The number of samples that are out of the recommended variance value of 10% is low for the rainbow trout fillets for both recommended nitrogen factors, N_f and N_{ff}. For the brook trout fillets, there are no samples out of the recommended variance value of 10% for both nitrogen factors, N_f and N_{ff}. These results show that both formulas for calculating the fish meat content based on the N_f or N_{ff} lead to comparable results. Using both methods to determine the fish meat content is then possible.

The established nitrogen factors allow one to analyse samples of products from rainbow trout and brook trout for the fish meat content in accordance with the EU legislation, with the declaration of the amount of fish meat present in the product on their labels.

This information is required according to Regulation (EU) No. 1169/2011 of the European Parliament and of the Council on the provision of food information to consumers (EC 2011). It gives the possibility to check the declared information

Table 4. Meat content in the fish fillet samples taken in 2018–2020

Species	Fillet	*n*	Meat content calculated with nitrogen factor for whole fillet N_f (g/100 g) x ± SD (min–max)	Sample numbers* in pieces (%) from all the samples out of recommended variance value ± 10% (CA 2004)	Meat content calculated with nitrogen factor for fat-free fillet N_{ff} (g/100 g) x ± SD (min–max)	Sample numbers** in pieces (%) from all the samples out of recommended variance value ± 10% (CA 2004)
Rainbow trout	with skin	120	100.14 ± 4.14 (86.62–108.73)	1 (0.77)	100.07 ± 4.22 (89.17–110.27)	1 (0.77)
	without skin	120	100.16 ± 4.39 (90.51–113.95)	3 (2.31)	100.06 ± 4.34 (90.91–113.91)	2 (1.54)
Brook trout	with skin	50	99.97 ± 3.31 (93.42–108.34)	0 (0.00)	100.08 ± 3.53 (93.82–108.23)	0 (0.00)
	without skin	50	100.01 ± 2.82 (93.38–105.93)	0 (0.00)	99.94 ± 3.20 (92.68–110.55)	0 (0.00)

*For the meat content calculated with the nitrogen factor for the whole fillet, N_f; **For the meat content calculated with the nitrogen factor for the fat-free fillet, N_{ff}

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on the product labels related to the quantity of the main ingredients. It gives the opportunity to uncover any adulteration of a product with the addition of undeclared water.

Finally, it should be also noted that there are some limitations in the use of nitrogen factors for the evaluation of product adulteration. As the factors are mentioned as the average values with standard deviations, and, when deciding whether declarations of meat or fish content are fulfilled, it is important to bear in mind the possible variability of natural values (effects of the season, weight, location of the fisheries, nutritional status) and the analytical variability of their determination, and to apply the recommended variation value of 10% (EC 2011).

Conflict of interest

The authors declare no conflict of interest.

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