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INFLUENCE OF FEED COMPOSITION AND FEEDING STRATEGY ON ORGANIC BROWN TROUT (*Salmo trutta fario* L.) PRODUCTION AND FINAL PRODUCT QUALITY

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Abstract- Brown trout (*Salmo trutta fario* L.) were raised according to organic guidelines of the German association “Naturland”. Three certified organic trout feeds were tested. A control group was fed a commercial diet. Organically grown fish were larger at harvest than control fish and in better condition. The feed conversion ratio was higher for the control diet.

Specimens were analysed comparing body composition including maturation, gross energy level, carcass and fillet yield. Lipid levels of 5.5% to 6.9% were found in the fillets, associated with a sum of eicosapentaenoic acid and docosahexaenoic acid between 1.3% and 1.7%. Free amino acids varied only slightly (3.6 - 4.7 g kg^{-1} fillet). Mean values for taurine and selenium were between 0.5 mg kg^{-1} and 1.4 mg kg^{-1} and 0.10 mg kg^{-1} to 0.19 mg kg^{-1}, respectively; lowest contents were found in Brown trout raised with conventional feed. Water-binding ability and texture showed no systematic differences. Sensory evaluation rated all fillets as highly acceptable. This study demonstrates that organic feedstuffs for Brown trout production do not decrease final product quality.

Keywords- *Salmo trutta fario*, flesh quality, feed trial, organic production, body composition, quality assessment

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Introduction

The concept of organic aquaculture exists in Europe since the middle of the 1990s [1]. In 2000 the estimated global production of certified aquaculture products was about 5000 tonnes in total. In the face of increasing consumer awareness to food production and animal welfare it is believed that organic aquaculture has been and will be growing considerably in the years to come [1-3]. This interest in organic aquaculture led to the implication of various NGOs (non-governmental organisations) and official governmental organic guidelines, regulations and standards. However, universally accepted standards and accreditation have been only adopted recently [4]. Most notable in Europe, the organic implementation in aquaculture has been spearheaded by the German founded NGO “Naturland” (Association for Organic Agriculture) until finally adopted by the European Union in 2009 [5]. Highly entwined with ethical issues and consumer awareness, a clear definition of organic aquaculture guidelines is difficult. In aquaculture, the environmental and socio-economic sustainability of farming was recognised as a main aspect, powered by a growing consumer concern about product quality, sustainability and adverse environmental impacts of aquaculture and, most importantly, animal welfare [3,6-9].

All current organic guidelines encourage and require the use of organically certified feedstuffs [10], which have to be economically viable. In conventional aquaculture production, feed accounts for over 50% of the operating costs, but organic feedstuffs are up to 50% more expensive than conventional products [11]. Therefore, the price of organic aqua feeds can be expected to be the main contributor to the overall product end cost.

Due to the highly regulated nature of organic feedstuffs, relying on organic or sustainably sourced raw materials, nutritional research with organic aqua feeds has progressed slowly since the implementation of widely accepted organic standards. Modern feed formulations for carnivorous species, such as salmonids, rely on fish meal and fish oil inclusion to assure a balanced diet required for optimal health for the animals [8], and are driven by both economic and ethical concerns [12]. As agreed by most organic certification schemes and regulations for organic fish production, the use of fishmeal and fish oil from certified sustainable fishery areas, from by-products and trimmings from seafood-processing fishery, from by-catches from food fisheries or from certified organic aquaculture, but of a different species, will be required [5,10,13]. Due to the novelty of organic specifications and difficulties to source sufficient amounts of certified fish raw materials, the National Organics Standards Board (United States) suggested a 12-year phase-out schedule for the use of conventionally acquired fishmeal and fish oil in organic feed formulation [14].

Eventually, the differences in feed formulation achieved by organic guidelines compared to conventional practice can result in differences in the end product for human consumption. Currently, there is no evidence that organic feed may benefit the animals or have nutritional benefits over conventional feedstuffs [8]. No evidence exists yet that organically produced aquaculture products are superior for human health from a nutritional point of view [15,16]. However, the combination of better welfare achieved by the requested organic farming practices and "greener" raw products may reflect in a higher consumer acceptance. It has been hypothesised that organically grown animals and vegetables could be healthier due to less undesired substances or a higher nutritional benefit. Several studies have been conducted to gain information regarding the impact of the production method on the quality of organically grown fish, but significant differences between organic and conventional farming systems were not observed [17,18].

This study presents the effects of certified organic diets on grow-out performance, and final product quality as assessed in a long term commercial study on Brown trout reared in earthen ponds, testing organic guidelines recommended by the German organic certifier "Naturland" and eventually implicated by the European Union in 2009. The work conducted makes no claims regarding the nutritional equivalency or optimality of the feeds used; it aims at testing commercially available organic feed stuffs towards end product quality and overall economic feasibility of organic production in a commercial scale trout farm.

Materials and Methods

Rearing Conditions

30,000 Brown trout (*Salmo trutta fario* L.) were produced at the Institute for Fisheries of the Bavarian State Research Center for Agriculture, Starnberg, Germany, from the indigenous "Starnberg" Brown trout stock. Eggs were laid down 28th November 2007 and hatched after 52 days in water with temperature of 9ºC; the fish were grown at the breeding facilities from January 2008 (hatching) until January 2009 (start weight: 30.4 ± 0.14 g). On the 27th January 2009, the specimens were sorted with a 10 mm commercial grading sieve (AGK Kronawitter GmbH, Wallersdorf, Germany). Groups of 3,000 individuals, respectively, were transferred into the trial ponds (earthen ponds: 22 m^3 each), creating 8 equal groups of trial fish stocks. The earthen ponds were lined with gravel substrate and equipped with a water inlet with an adjustable flow rate (fixed at 2.5 l s^{-1} throughout the trial), as required by the used organic guidelines.

The fish were fed four different commercially available trout diets, of which three were organically certified (feed type "A", "B", "C") and one a conventional control diet (feed type "D"). Trial ponds were randomly allocated at trial start to create duplicates for each feed type. The fish were continuously fed during daylight by automatic surface spreaders (Pflanzer® spreading unit, 500/1000 mm x 400 mm, 80 litres; Pflanzer Fütterungssysteme, Simmozheim, Germany) (8 feed rations/day).

As required by organic guidelines (Naturland, 2012), the stocking density of the trial compartments had to be adjusted every three - four months to the required level (max 10 kg m^{-3}) over the whole production cycle. The fish were allowed to at least double in body weight, before reducing the stocking density. The required level was reached by emptying the whole compartments, using a sweep net (catch volume 30 m^3), counting out the required fish numbers and restocking of the compartments in a random order, to randomise possible environmental variations between the ponds and compartments. The water quality was monitored throughout the whole trial period and was found to be within the recommended levels for Brown trout production [19] and within the required organic thresholds.

The fish were harvested on the 16th November 2009. A complete trial department was caught using a standard pond sweep net (catch volume 30 m^3); 35 random, individual fish were subsequently sampled. The fish were killed using an electricity shock chamber followed by severing of the ventral artery, cutting along the gill bow. The weight of the fish was measured immediately, following the gill cut (final/live weight) and again after the animals were disembowelled (slaughter weight) [Table-1]. The animals were analysed for gender and maturity and subsequently filleted. A standard commercial fillet cut was carried out by hand by two certified fish processors. The weight of both fillets was measured with and without skin. Additional 30 fish per trial compartment were slaughtered, gutted, tagged, stored on ice and transported to the Max Rubner-Institut, Department of Safety and Quality of Milk and Fish Products in Hamburg, Germany. Five fish samples of each group were taken for sensory assessment (3 days *post-mortem*). The rest was stored vacuum packaged in a frozen storage chamber at -25°C and thawed, hand-filleted and skinned before analysis.

Table 1- Fish performance parameters in the trial ponds throughout the experiment

Feed type	Final weight (g)	Slaughter weight (g)	Fillet weight (g)	Fillet weight skinless (g)	Skin less fillet (%)	Condition factor	Feed conversion ratio	Specific growth rate (% / day)
A - organic	329.1 ± 7.13	290.5 ± 7.96	152.5 ± 5.38	134.8 ± 5.06	41	1.30 ± 0.020	1.25 ± 0.053	0.9
B - organic	303.9 ± 5.62	270.7 ± 6.17	140.0 ± 3.48	123.7 ± 3.21	40.7	1.32 ± 0.019	1.41 ± 0.060	0.85
C - organic	333.3 ± 5.89	280.0 ± 6.01	149.6 ± 2.90	132.6 ± 2.79	39.8	1.27 ± 0.017	1.33 ± 0.056	0.77
D - conventional (control)	302.7 ± 5.11	274.4 ± 5.53	147.1 ± 3.53	131.4 ± 3.32	43.4	1.25 ± 0.015	1.53 ± 0.065	0.76

Analytical Methods

Proximate Chemical Composition

The proximate chemical composition was determined in finely ground feed samples and homogenised fillets of 10 specimens per feed group. The water content was determined gravimetrically after drying an aliquot of the homogenate at 105°C. Fat was estimated by a modified method of Smedes [20,21]. Percentage nitrogen was measured by Dumas using a LECO TruSpecN (LECO Instruments GmbH, Mönchengladbach, Germany), and percentage protein was calculated by multiplying %N by 6.25 [22]. Mineral content was determined gravimetrically after ashing at 550°C [23]. Salt contents were obtained by titration with 0.1 n $AgNO_3$ solution [24]. The total phosphorus content was determined photometrically in a nitric acid extract of the ash, according to a modified official method of the German Food and Feed Code to measure phosphorus in meat [25].

As internal analytical quality control, the matrix meat reference material SMRD 2000 (LGC Promochem, Wesel, Germany) was used. Certified values are given for contents of ash, moisture, fat, nitrogen and phosphorus. All analysed values of the above listed components showed excellent agreement with the certified values (data not shown).

Fatty Acids and Amino Acids

Fatty acid methyl esters (FAME) were obtained from the extracted fat by trans-esterification with potassium hydroxide [26]. Fatty acid composition was determined by gas chromatography according to the DGF standard method [27]. Fatty acids in the range of C14-C22:6 were calculated as weight percentage (g/100 g total fatty acids).

HPLC determination of free amino acids (including taurine) was performed in the perchloric acid extracts of the fish muscles after precolumn derivatization with o-phthaldialdehyde. The lowest determined level of each amino acid in the fish tissue was 0.1 mg kg^{-1} wet weight. Details of both methods are described by Karl *et al.* [28].

Mineral Element Analysis

Sodium (Na), potassium (K), calcium (Ca) and magnesium (Mg) were determined in digested material by atomic absorption spectrometry with air-acetylene flame (contra AA® 700, Analytik Jena, Jena, Germany). For more details: [28].

Selenium (Se) and arsenic (As) were analysed by the continuous flow hydride system of the same analytical instrument. For total As determination, the digests were used after reduction of As (V) to As (III) with 5% KJ (w/v) and 5% ascorbic acid (w/v). Details of mineral element analysis are described in [28].

All samples were determined in duplicate. Blanks were digested during each analysis period. The commercial reference material IAEA-407 (Trace Elements and Methylmercury in Fish Tissue) of the International Atomic Energy Agency (Vienna, Austria) was used to validate the analytical methods and as quality control. The mean values obtained for analytical recovery were 101% (Na), 100% (K), 89% (Ca), 82% (Mg), 83% (Se), and 87% (As), respectively. Methods are validated in proficiency tests with certified control samples.

Physicochemical Traits

Texture measurements (15 fish/group) were carried out with a texture analyser (TA.XT2, Stable Micro Systems, Godalming, U.K.) equipped with a 25 kg load cell. 3 samples from each thawed fillet were cut out by using a cork borer (20 mm diameter) and compressed to 75% deformation with a flat-ended aluminium cylinder (50 mm diameter). Shear tests were performed at a crosshead speed of 1.7 mms^{-1} [29].

Water-binding ability (WBA) was measured under the same conditions. Samples were compressed between filter sheets (Schleicher & Schüll 2043 A, 7x7 cm) and parallel plates to 75% deformation and held at that point for 15 s. WBA was defined as the expressible moisture, calculated as % = 100 (initial weight - final weight)/ initial weight [29,30].

The pH value was measured in homogenised samples diluted to 1:10 with deionised water, using a microprocessor pH-meter (Wissenschaftlich-Technische Werkstätten, Weilheim, Germany).

Sensory Evaluation

Skinned fillets of 5 fish/group were placed in individual pouches and heated for 8 minutes in a water bath (90°C). Cooked samples were immediately served to the panel. The sessions were carried out in a sensory laboratory with separate booths. 10 trained staff members participated in the sensory profiling, using a 100 point line scale with two anchor points to rate the intensity of the sensory attributes. Assessment included descriptive terms for the appearance, odour, taste and texture, which were defined during previous sessions.

Statistical Analysis

All growth parameters were checked for normality / goodness of fit and homogeneity of variance using the Ryan-Joiner test and Levene's test, respectively. All length, weight and growth comparisons were undertaken using t-tests and ANOVA methods (Minitab, Version 16.1.0) (Minitab, Inc. State College, USA). Percentage comparisons were carried out using Fisher's exact test (Minitab, Version 16.1.0). SigmaStat version 3.5 (Systat Software Inc., San Jose, USA) was used for statistical evaluation of chemical and physical fish data. Treatments were compared using one-way ANOVA applying the Turkey´s pairwise comparison test and the Kruskal-Wallis test, respectively. The significance level in all tests was set at 0.05.

Calculations

Condition factor was calculated as: $K = W \times 100 \times L^{-3}$, where W is the individual weight of the fish and L the individual total length.

Feed conversion ratio was calculated as: $FCR = F \times G^{-1}$, where F is consumption of dry matter from feed and G is the weight gain.

Specific growth rate was calculated as: $SGR = 100 \times (\ln W_1 - \ln W_0) \times D^{-1}$, where W_0 and W_1 represent initial and final weights (pond means), respectively, and D represents the number of feeding days.

Results

Fish Performance and Carcass Yields

Fish mortalities were low (overall 1.9%) and monitored twice a day throughout the whole trial period of 11 months (mortalities/trial compartment: organic 23.5 ± 4.1 fish, control 22.5 ± 5.5 fish). No significant difference was found comparing maturity levels between organically and control fed fish with male fish being mostly mature and few females having reached maturity (male maturation: organic 99.2%, control 96.6%; female maturation: organic 3.7%, control 2.6%) (all $p > 0.50$). Fish performance throughout the trial is shown in [Table-1].

Organically grown fish were larger at trial end than fish in the control groups (weight: organic 321.5 ± 1.3 g, control 295.4 ± 1.2 g, $p < 0.001$). In line with a higher weight, organically fed fish also had a significantly better condition factor than the control animals (k-factor: organic 1.29 ± 0.02, control 1.24 ± 0.02, $p < 0.001$). Also, the feed conversion ratio was better for organically grown fish compared to the control group (FCR: organic 1.23 ± 0.05, control 1.37 ± 0.06, $p < 0.001$). The specific growth rate was significantly better in organically grown fish than in the control group (SGR: organic 0.86 ± 0.03%, control 0.79 ± 0.02%, $p < 0.001$). Corresponding to the higher live weight at trial end, the slaughter weights of the organically grown fish were significantly higher compared to the control fish (weight: organic 284.1 ± 1.4 g (88.3% of live weight), control 260.5 ± 1.2 g (88.2% of live weight), $p < 0.001$). Also, fillet yield was higher for organically grown fish (weight: organic 133.2 ± 1.7 g

(41.4% of live weight), control 123.6 ± 1.3 g (41.8% of live weight) ($p < 0.001$).

Economical Evaluation of Organic Production

The net costs for using the four feedstuffs have been calculated with only direct costs for the diets taken into account. The organic diets were more expensive than the control diet (organic diet 1.93 € kg^{-1}, control diet 1.19 € kg^{-1}). Overall the costs for the production of Brown trout fed organic diets were 43 % higher than conventional production (organic 2.30 € kg^{-1} fish growth, control 1.61 € kg^{-1} fish growth).

Proximate Composition of Feeds and Fillets

The chemical composition of the fish feeds was analysed and compared to the labelled amounts provided by the feed manufacturers [Table-2].The results for the main nutrient components were found to be in line with the labelling. A significantly reduced amount of ash was found in the conventional control diet compared to all organic diets ($p < 0.05$). Also, total phosphorus, selenium and arsenic contents were decreased (all $p < 0.05$). The other minerals were comparable.

The chemical composition of the fish muscle is given in [Table-3]. No significant differences were found in the mean water (range: 73.8% to 74.4%) and lipid contents (range: 5.5% to 6.9%). The lipid content variability within the groups was high. No statistical differences were found comparing pH values in the fillets and the raw protein contents (range: 18.4% to 19.7%). However, slightly lower protein contents were estimated in all three organic groups. The ash content in the fillets was between 0.7% and 1.1%. As result of the higher percentage of fish in the diet, the measured Se and As values in the organic diets were higher ($p < 0.05$).

The total content of free amino acids of the different feeding groups did not vary significantly (3.6 - 4.7 g kg^{-1}) with maximum values in group org C [Table-3], which had also the highest amount of indispensable amino acids (1.9 and 2.0 g kg^{-1}). In contrast, control group conv D had the lowest levels of free and indispensable amino acids. In all specimens relatively high amounts of taurine were analysed. The differences between the organic fed and the control fish were significant ($p < 0.05$).

Table 2- Chemical composition of the experimental feed (organic A, B, C and conventional control D) for the last trial phase, estimated and labelled (in brackets) amounts

	Feed type			
	A	B	C	D
	organic	organic	organic	conventional (control)
Dry matter (g/100 g)	93.7 (-)	92.9 (-)	95.4 (-)	95.5 (-)
Raw protein (g/100 g)	43.7 (40)	38.3 (40)	47.4 (48)	42.5 (42)
Raw lipid (g/100 g)	23.1 (25)	17.6 (14)	24.9 (23)	22.7 (22)
Crude fiber (g/100 g)	n.d. (1.0)	n.d. (0.9)	n.d. (4.0)	n.d. (4.0)
Ash (g/100 g)	10.1 (11.0)	12.3 (12.5)	8.5 (8.5)	5.7 (6.0)
Sodium (g/100 g)	1.13 (-)	1.26 (-)	0.64 (-)	0.21 (-)
Potassium (g/100 g)	0.79 (-)	0.72 (-)	1.10 (-)	1.04 (-)
Calcium (g/100 g)	0.81 (-)	1.06 (-)	0.65 (-)	0.53 (-)
Magnesium (g/100 g)	0.20 (-)	0.13 (-)	0.16 (-)	0.26 (-)
Phosphorus (g/100 g)	1.4 (1.6)	1.5 (1.9)	1.3 (1.2)	0.9 (1.1)
Selenium (mg/kg)	1.91 (-)	1.30 (-)	1.36 (-)	0.69 (-)
Arsenic (mg/kg)	2.88 (-)	3.12 (-)	2.92 (-)	1.88 (-)

n.d. = not determined, (-) = not specified by the manufacturer

Table 3- Chemical composition and pH-value of Brown trout fillets raised with organic feed A, B, C and conventional control feed D.

Feed type	Water (%)	Lipid (%)	Protein (%)	Free Amino acids (g kg^{-1})*	∑ indispensable Amino acids (g kg^{-1})*	Taurine (g kg^{-1})*	Ash (%)	Selenium (mg kg^{-1})	Arsenic (mg kg^{-1})	pH - value
A - organic	74.1^{a} (71.9 - 76.1)	5.9^{a} (4.2 - 9.0)	18.6^{a} (17.6 - 19.4)	4.4	1.6	1.1	0.7^{a} (0.4 - 0.9)	0.19^{a} (0.17 - 0.21)	1.20^{a} (0.82-1.50)	6.7
B - organic	74.3^{a} (72.8 - 75.1)	5.9^{a} (4.7 - 7.1)	18.6^{a} (18.2 - 19.0)	4.2	1.3	1.1	0.7^{ab} (0.6 - 0.8)	0.19^{a} (0.17 - 0.20)	1.59^{b} (1.11-1.85)	6.6
C- organic	74.6^{a} (73.3 - 76.1)	5.7^{a} (4.7 - 7.3)	18.7^{a} (17.8 - 19.5)	4.7	2	1.4	0.8^{bc} (0.7 - 1.0)	0.17^{b} (0.15 - 0.18)	0.74^{c} (0.44-0.93)	6.6
D-conventional (control)	73.9^{a} (70.8 - 75.8)	6.9^{b} (5.6 - 8.7)	19.7^{b} (18.9 - 20.3)	3.6	1	0.5	0.9^{c} (0.8 - 1.3)	0.12^{c} (0.09 - 0.16)	0.47^{d} (0.41-0.52)	6.6

Mean values and range (in brackets) of 10 fish, respectively.
*Pooled samples; Different superscript letters within rows represent significant differences ($p < 0.05$)

Fatty Acid Composition of Feed and Fillets

[Table-4] shows the fatty acid composition of the diets and the corresponding feeding groups.

Of the saturated fatty acids (SFA) palmitic acid (16:0) was highest in the feed and also in the fillets, whereby the low content in feed A is noteworthy. The presence of monounsaturated fatty acids (MUFA) varied clearly and was highest in feed B (36.3%). Except for feed B, MUFA content of all other diets was lower than the amount of polyunsaturated fatty acids (PUFA, 31.1%-38.8%). In control diet D only a very small portion of gondoic acid (20:1n-9) (1.5%) was estimated. With about 10% of analysed total fatty acids, the organic feeds contained distinctly higher amounts of this omega -9 fatty acid which is found in marine zooplankton. Oleic acid dominated in the conventional control diet. Polyunsaturated n-3 fatty acids were generally high and independent of conventional or organic raw materials. Compared to control feed D (conventional, 4.3%) and C (organic, 4.0%), the linoleic acid content of both other organic feeds was twice as high (7.2% and 8.5%). All diets contained fish oil in different percentages which is known to be dominated by the eicosapentaenoic acid (20:5n-3, EPA) and docosahexaenoic acid (22:6n-3, DHA). The sum of EPA and DHA in the diets was between 19.5% and 30.2% of the total fatty acids. This maximum amount was determined in the conventional one (control D, 30.2%), which was mainly due to the high content of EPA (22.3%). All organic diets contained more DHA than EPA. N-6 fatty acids were highest in feed A and B and lowest in feed C and control D. The n-3/n-6-ratio was between 2.5 and 6.0.

All fatty acids analysed in the diets were also found in the fillets. Kinsella *et al.* [31] reported remarkably constant saturated fatty acids in freshwater species at around 25%. Only control group D had lower values. The conventional grown specimen differed with a slightly higher content of oleic acid and linoleic acid. The higher amount of long-chain n-3 fatty acids (DHA and EPA) in the feed was not reflected in the fillet flesh. Values for EPA were lower in the muscle tissue compared to DHA. In all organic groups a better incorporation of DHA was observed ($p < 0.05$). Exclusive control D, the resulting n-3/n-6-ratio in the muscle tissue did not change remarkably.

Table 4- Fatty acids (FA) profiles of Brown trout fillets (% of total fatty acid methyl esters). Mean values of 10 fish, raised with organic feed type A, B, and C and conventional (conv) control feed D

	FA shorthand	A- organic		B- organic		C- organic		D- conventional	
		Feed	Fish	Feed	Fish	Feed	Fish	Feed	Fish
Myristic acid	14:00	7.71	5.05	6.32	4.39	8.67	5.31	7.39	3.96
Pentadecanoic acid	15:00	0.89	0.46	0.65	0.35	0.91	0.48	0.74	0.31
Palmitic acid	16:00	9.84	16.66	16.35	15.2	19.02	16.73	18.77	14.01
Heptadecanoic acid	17:00	6.48	0.41	0.36	0.29	7.75	0.59	0.54	0.34
Stearic acid	18:00	0.61	2.91	2.22	2.67	0.47	2.43	2.88	2.5
Saturated fatty acids	**ΣSFA**	**25.53**	**25.49^{a}**	**25.9**	**22.9^{bc}**	**36.82**	**25.54^{a}**	**30.32**	**21.12^{bc}**
Palmitoleic acid	16:1n-7	2.85	5.83	6.68	6.59	1.74	6.69	10.52	6.71
Oleic acid	18:1n-9c	13.24	16.8^{a}	17.73	22.38^{b}	12.24	17.27^{a}	11.73	25.29^{c}
Vaccenic acid	18-1n-7	2.48	3.08	2.4	3.29	2	2.82	2.87	3.52
Gondoic acid	20:1n-9	10.33	7.28^{a}	9.49	5.90^{ab}	10.71	7.23^{a}	1.51	2.77^{c}
Monounsaturated fatty acids	**ΣMUFA**	**28.9**	**33.00^{ac}**	**36.3**	**38.16^{b}**	**26.69**	**34.01^{ac}**	**26.63**	**38.29^{b}**
Linoleic acid	18:2n-6c	7.19	6.6	8.5	8.34	3.95	5.68	4.31	9.03
γ-Linolenic acid	18:3n-6	0.13	0.4	0.12	0.19	0.11	0.12	0.2	0.25
α-Linolenic acid	18:3n-3	2.75	1.87	3.26	2.13	2.4	1.78	1.93	2.71
Arachidonic acid	20:4n-6	0.78	0.62	0.56	0.6	0.62	0.65	0.96	0.65
Eicosapentaenoic acid (EPA)	20:5n-3	11.59	5.99^{a}	8.82	4.82^{ab}	11.46	6.32^{ac}	22.33	7.78^{c}
Docosatetraneoic acid	22:4n-6	0.18	0.13	0.1	0.06	0.1	0.12	0.13	0.08
Docosahexaenoic acid (DHA)	22:6n-3	16.15	22.33^{a}	10.68	19.06^{a}	12.47	25.07^{a}	7.88	16.77^{b}
Polyunsaturated fatty acids	**ΣPUFA**	**38.77**	**37.94^{a}**	**32.04**	**35.20^{b}**	**31.11**	**39.74^{a}**	**37.74**	**37.27^{a}**
Σn-3		30.49	30.19^{a}	22.76	26.01^{b}	26.33	30.89^{a}	32.14	27.21^{b}
Σn-6		8.28	7.51^{a}	9.18	9.00^{b}	4.78	6.25^{a}	5.4	9.76^{b}
Σn-3/Σn-6		3.6	4.0^{a}	2.5	2.9^{b}	5.5	4.9^{a}	6	2.8^{b}
ΣEPA+DHA g in 100 g lipid		27.74	28.3^{a}	19.5	23.88^{b}	23.93	31.39^{a}	30.21	24.55^{b}
ΣEPA+DHA g in 100 g fillet			1.7		1.3		1.5		1.5

Different superscript letters within rows represent significant differences (p <0.05)

Physicochemical and Sensory Fillet Quality Comparisons

Results for water-binding ability (WBA) and mean force values (N) could display possible differences in texture between the feeding groups. To our knowledge no comparable data for Brown trout are published. In assessing the statistical differences in WBA [Fig-1] and force values [Fig-2] between the feeding groups, the diversity in the composition of individual fillets must be considered. Regarding also the applied sensory texture assessment, no obvious pattern was distinguishable comparing the different feeds.

The sensory evaluation of Brown trout showed comparable results, exclusive organic group A [Fig-3], [Fig-4]. Considered as a whole, response scores demonstrated consistently high quality. Cooked Brown trout fillets had an elastic and slightly fibrous texture and a typical sweet taste. The smell was typical and reminded slightly of "cooked potatoes" (results not shown). Negative taste deviations like "musty", "metallic" or "bitter" were noticed, but only minimal. Unique trout that were fed with organic feed A were rated overall slightly less pronounced. Their smell and taste was less intense and the texture was softer.

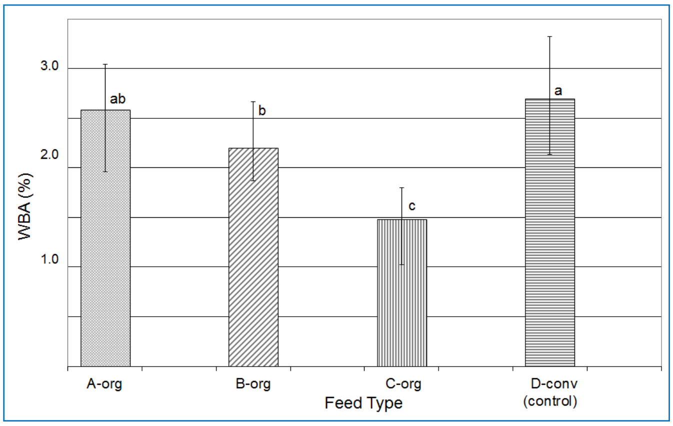

Fig. 1- Water-binding ability (WBA, %) for Brown trout fillets of the four feeding groups (organic (org) feed A, B, C and conventional (conv) control feed D). Mean values of 15 fish (± standard deviation). Different letters on the columns show significant differences between the mean values (p <0.05)

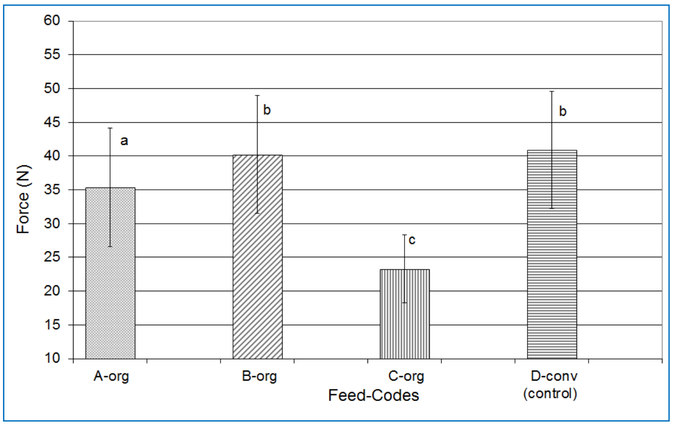

Fig. 2- Mean force values (N) for Brown trout fillets of the four feeding groups (organic (org) feed A, B, C and conventional (conv) control feed D). Mean values of 15 fish (± standard deviation). Different letters on the columns show significant differences between the mean values (p <0.05)

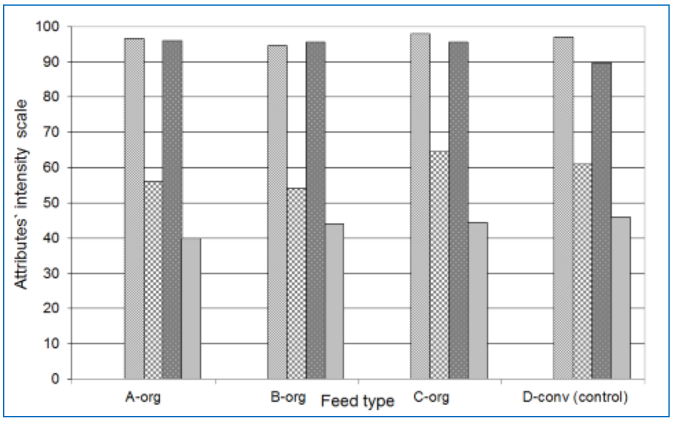

Fig. 3- Taste profiles for Brown trout fillets raised with organic (org) feed A, B, C and conventional (conv) control feed D. Mean values of 10 fillets (= 5 fish), respectively. Y-axis: 100 point line scale for intensity of the sensory attributes (0= not detected, 100= highest intensity). Column groups left to right: attributes fresh, aromatic, typical and sweet, respectively

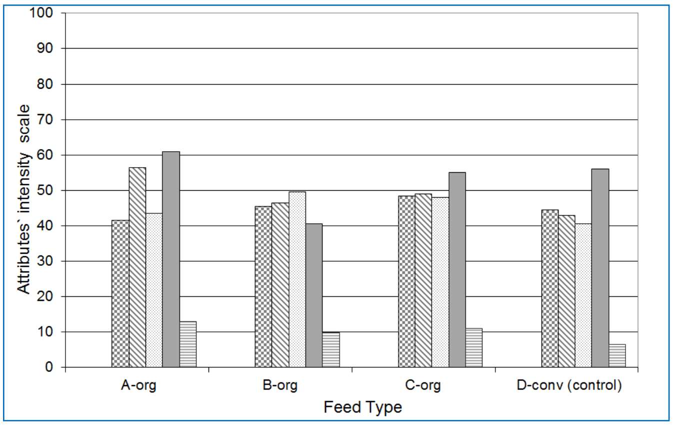

Fig. 4- Texture profiles for Brown trout fillets raised with organic (org) feed A, B, C and conventional (conv) control feed D. Mean values of 10 fillets (= 5 fish), respectively. Y-axis: 100 point line scale for intensity of the sensory attributes (0= not detected, 100= highest intensity). Column groups left to right: attributes firm, elastic, fibrous, juicy and crumbly, respectively

Discussion

Because commercially available feed was applied, the work conducted makes no claims regarding the nutritional equivalency or optimality of the formulations used. As known from previous studies, fish feed composition influences the fish muscle composition and subsequently important quality parameters [32]. The diets tested in this study differed in raw material composition and varied in protein and fat content. Raw materials used in fish nutrition have drastically changed in recent years. The compounding of the analyzed feeds followed this trend. As expected, diets with a higher raw protein and raw fat content yielded higher final fish weights (compare [Table-1] and [Table-2]). The specific growth rates (SGR) were not necessarily better in fish with a higher final harvest weight. Generally, the specific growth rates and feed conversion ratio (FCR) were better in organically fed fish compared to the control group, showing that organic diets are not lacking in performance or composition compared to established standard commercial trout diets of medium quality. Irrespective of the relatively new concept of organic aquaculture and the novelty of the developed feeds [8], it seems that organic diets are at least equal to established products with respect to performance for the production of Brown trout.

Comparing the costs for the feedstuffs, organic diets were on average 62% more expensive than the control. The higher feed price is due to the inclusion of sustainably or organically sourced raw materials in the process of producing the feed. Considering this higher feed price, the costs for feed/kg fish were 43% higher, so that an increase on final product prize needs to be included to work economically, using organic production. This is in line with previous publications [11,33]. In this study only costs of the feedstuffs are calculated. Including pre-requisites for organic production, such as the use of a natural environment (in this study earthen ponds) and a lower maximum stocking densities (in this study 10 kgm^{-3}) as well as stringent guidelines on therapeutics, the price of the organic end product can be considered to be even higher. However, it is believed that irrespective of the end product costs, consumer awareness to human nutrition and animal welfare will lead to an increase in organically grown aquaculture products [1-3].

As labelled by the producers, the organic feeds contained 53-77% fish meal and 5-14% fish oil, whereas the conventional one contained 17% fish meal and 12% fish oil, respectively.

The protein and free amino acid (FAA) content do not belong to the components which can be greatly modified by the diet [34-36]. The conditionally indispensable amino sulfonic acid taurine is regarded as important for many physiological processes in humans. Fish usually contain more FAA than terrestrial animals due to the osmoregulatory function of these compounds [37]. In all specimens relatively high levels of taurine were analysed with a clear difference between the control and the organic diets that contained more fish meal. This explains the significantly higher taurine levels in the organic Brown trout groups.

As seen in this study, the lipid content and the fatty acid profile were influenced by the diet composition which is also shown for other species [38, 39]. Although the mean lipid content of the groups was in the same range, the individual variation was large. Results for other feeding studies with Brown trout showed that this can be optimized [39]. The muscle flesh of the conventional control group D contained on average slightly more fat (p < 0.05). Nevertheless, these results are very uncertain, because of considerable variance in the lipid contents within the feeding groups. Analysed Brown trout can be classified as medium fat fish. Wild specimens contain less fat. Kaya & Erdem [40] reported a seasonal variation for wild Brown trout between 1.85% and 3.57% (mean value 2.80%).

Based on the fatty acid pattern, the nutritional quality of fish from all groups was comparable (p = 1.000). This was probably due to the fact that all finishing diets contained fish oil. Experiments with fish oil free diets have shown that positive health aspects of the flesh regarding n-3 fatty acids can drop rapidly at sub-optimal levels of fish oil inclusion [12]. Human consumption of EPA and DHA is known to have positive effects in relation to arteriosclerosis and cardiovascular diseases. The level of EPA and DHA in the fish fat depends on the amount and type of oil used. In case of EPA, the conventional control feed D had a content of 22.3%, while the fish fed with D had only 7.8% of the total fatty acid methyl esters. In comparison, feed C had only about half that amount of EPA (11.5%), while the muscle flesh had about 6.3% EPA in the fatty acid methyl esters. In this study all organic diets seem to have a higher relative retention (about half of the amount in the diet is recovered in the fillet) while the conventional diet´s retention is only about a third.

However, overall the results showed that the more expensive fish oil could be replaced partially by other oil sources without disadvantage to the quality of Brown trout fillets. The amount of EPA and DHA in the edible part was between 1.3% and 1.7%. However, lower levels of EPA and DHA can occur as consequence of marine fish oil replacement by vegetable oil. In feeding experiments with constant feed formulations Brown trout showed a high positive correlation with the PUFA and n-3/n-6 ratio of the diets [39]. When fish oil is replaced by vegetable oil, the content of n-3 PUFAs typically decreases while n-6 percentages increase. However, under commercial raising conditions these results were not equally conform. The conventional control D (12% fish oil) and the organic feed B which had the lowest declared fish oil content (5%, compared to 14% (A) and 13% (B)) led to an unfavourable relationship of the n-3 and n-6 fatty acids ($p < 0.05$).

Particularly worth mentioning is selenium as an essential micronutrient which plays a vital role in human health. Selenium is incorporated into proteins to yield selenoproteins, which are important antioxidant enzymes. Fish is an important and highly bio available source of dietary selenium. In wild freshwater fish the content can vary considerably. Between 0.05 and 2.87 mg Se kg^{-1} were reported as a result of a monitoring program [41]. Fish from the Northeast Atlantic waters contains between 0.3 and 0.6 mg kg^{-1} [42]. As shown in [Table-2], organic feeds contain between 1.3 and 1.9 mg Se kg^{-1}, while the conventional control feed does have a Se content below 1 mg kg^{-1}, which is still a distinct amount above the requirements for trout of 0.15 mg kg^{-1}, according to NRC 2011 [43]. The individual results of Brown trout fillets ranged between 0.09 and 0.21 mg kg^{-1} [Table-3]. Lowest values were estimated in the conventional control group D which contained the lowest amount of fishmeal. This explains the reduced amounts of selenium and as already explained taurine ($p < 0.05$, respectively). In comparison, Arctic charr (*Salvelinus alpinus* L.) fed with the same diets had mean Se values between 0.14 and 0.23 mg kg^{-1} (= feed D) [44].

Fish is known as the main contributor of arsenic in the diet. There is no Europe-wide regulation in food. The International Agency for Research on Cancer (IARC) listed inorganic arsenic as human carcinogen. Its organic compounds which are the most common forms of total arsenic in fish are not metabolised in humans, and are expected as not classifiable as to their carcinogenicity to humans up to now [45]. According to an EFSA study, the average total arsenic concentrations in a mix of marine and freshwater fish and other seafood ranged from 0.1 to 1.8 mg kg^{-1} [46]. The concentrations found in fish samples from Norwegian waters varied greatly between fish species, and were between 0.3 to 110 mg kg^{-1} w.w. [47]. Levels found in Brown trout were on a low level and affected by the diet.

Consumer acceptance of organically grown Brown trout was a major aspect in this trial. The investigation of the fish of market size showed that the different feeds did not influence the fillet quality in this respect remarkably. Irrespective of four different feedstuff formulations used in the trial, the sensory evaluation of Brown trout showed similar results. De Francesco *et al*. [48] described the taste of rainbow trout fed a plant protein mixture based diet as less aromatic and less sweet. Our sensory panel did not follow these findings. The partial replacement of fishmeal and fish oil with vegetable ingredients did not affect basically the taste and the odour of Brown trout. All trout flesh had an almost bright uniform colour. The muscle texture is an important attribute for the consumer acceptance, but also for the processing of the fillets. Different extraneous factor can contribute to changes in muscle structure, as shown for Brown trout subjected to exercise regimes [49]. The fish of the study presented here was grown under the same environmental conditions as required by the organic guidelines used. The firmness and the water-binding ability of the raw muscle were not systematically affected by the diet and the feeding strategy, which corresponded with the results for the sensory assessment of the cooked fillets. This indicates that the different composition of the feed was less important for the muscle characteristics.

Conclusion

The study shows that organically grown Brown trout can compete with a control group fed a standard trout diet of medium quality. Considering the higher expenses involved with growing fish organically, a higher price needs to be asked of the end consumer. This higher price is expected and in most cases appreciated by the customer, seen as a contribution to fair aquaculture production and improved animal welfare. Nor the different feed compositions neither the feeding strategies influenced the final product quality of Brown trout. No major quality differences were found between feed of organic and conventional origin. Overall the replacement of fish oil in the diet had only minor effects. The sensory quality of Brown trout was generally rated as very good. The consumer's perception of seafood as healthy because of its remarkable values of highly unsaturated omega-3 fatty acids in the fillets was also met.

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Conflict of Interest: The authors declare that there are no conflicts of interest.

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