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Effects of Dietary Mineral Supplementation on Quality of Fresh and Salt-Cured Fillets from Farmed Atlantic Cod, *Gadus morhua*

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Abstract

The aims of this study were to investigate effects of dietary mineral supplementation on chemical and sensory quality parameters of fresh farmed cod fillets and on the quality of salt-cured farmed cod. Farmed cod were fed three experimental diets with different levels of mineral supplements (no supplementation, supplementation without zinc and copper, full supplementation) for approximately 2 yr. After slaughter, one-third of the experimental fish were subjected to chemical and physical analysis, another third were used for sensory analysis and the remaining fish were salt cured. Potassium, copper, and muscle protein were higher in muscle tissue of cod fed full supplementation than cod fed without supplementation. Instrumental color analysis showed that the cut side of fresh fillets of cod fed full supplementation were slightly more green and yellow than fillets of cod fed without extra supplements. A sensory panel could, however, not detect any differences between heated fresh cod given feed with or without mineral supplements. However, the quality of salt ripened cod which had received a complete mineral supplement in the diet was reduced because of increased yellowness, probably caused by the increased level of copper in the muscle.

Wild stocks of fish in the world are generally utilized to a maximum and a further increase in the production of seafood has to come from farmed fish. Atlantic cod, *Gadus morhua* L., is a highly appreciated species caught mostly in North Atlantic waters. Some stocks of Atlantic cod, such as the Northeast Arctic cod (NEAC), have been harvested in a sustainable manner (ICES 2008), while others have been over fished, or even almost extinguished (Cook et al. 1997; Haedrich and Hamilton 2000). Supplies of wild cod vary naturally throughout the year and most NEAC is caught from December to April. Intensive farming of Atlantic cod is now being developed as a new industry in some North Atlantic countries to increase the supply of fresh cod throughout the year (Kjesbu et al. 2006; Rosenlund and Skretting 2006). Traditionally, Atlantic cod is sold fresh, as salt-cured cod or dried salt-cured cod (klipfish). The latter are traditional and still very important products made from fresh cod (Lauritzsen et al. 2004; Martínez-Alvarez and Gómez-Guillén 2006). These products are primarily consumed in Latin American and Mediterranean countries under the names bachalau or bacalao. One of the most important quality properties of salt-cured cod is the color of the flesh which should be as white as possible. A yellow color is an indication of a rancid product (Lauritszen et al. 1999).

In intensive fish farming, the feed provided is most often extruded and formulated with high-quality protein meals and oils (lipids). In addition to a binder which is often starch based, minor amounts of other ingredients such as vitamins and minerals are also included. The

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mixture of minerals used often contains magnesium, potassium, zinc, iron, manganese, and copper. The minerals are essential for growth and development (Watanabe et al. 1997), but little is known about marine fish requirements of these minerals. Minerals are, therefore, added in surplus to the formulated feed to ensure that mineral deficiency is avoided. The bioavailability of the minerals will be dependent on the feed composition. The presence of phytate in protein meals from plants can reduce bioavailability of minerals (Francis et al. 2001). Studies have shown that surplus amount of dietary minerals may affect growth and development of farmed fish (Dato-Cajegas and Yakupitiyage 1996; Berntssen et al. 1999; Kamunde et al. 2002; Nguyen et al. 2008). Effects of dietary mineral supplementation on product quality of farmed fish have been studied to a limited degree. However, Cooper and Midling (2007) have recently shown that feeding farmed cod with diets containing supplementary copper may result in reduced fresh fillet quality because of black stripes in the muscle (blood vessel melanosis). The aims of this study were to investigate effects of dietary mineral supplementation on certain chemical and sensory quality parameters of fresh farmed cod fillets and on the quality of salt-cured farmed cod.

Materials and Methods

Raw Materials

The farmed cod used in this study were kept at the Research Station in Tromsø (Norway). The eggs were provided by the Norwegian national breeding program in 2006 and the fry hatched in spring 2006. The fry received a standard formulated weaning feed without extra mineral and trace metal supplement until entering the trial-period in August 2006. This was to ensure the same baseline of mineral supplement exposure in all fish entering the experiment. The cod were kept in tanks supplied with oxygenated, filtered seawater at natural temperatures, and were fed formulated feed daily by automatic feeders during the entire experimental period.

TABLE 1. *Formulation, proximal composition, and measured mineral content of experimental feeds.*

	Diet 1	Diet 2	Diet 3
Formulation (g/kg)			
Fish meal[1]	748	748	750
Fish oil[2]	91	91	79.5
Wheat meal	136.6	133.0	10
Vitamin mix[3]	20	20	—
Mineral mix	0	3.6[4]	6.3
Inositol	0.3	0.3	U[5]
Attractant[6]	4	4	—
Proximate composition (g/kg)			
Protein	560	559	580
Lipid	154	154	150
Carbohydrate	113	111	105
Ash	107	111	99
Energy (MJ/kg)	21.4	21.4	17.2
Measured mineral content			
Cu (mg/kg)	2.8	2.9	10.1
Zn (mg/kg)	39.7	42.2	75.3
K (g/kg)	6.2	6.2	6.1

[1]Trofi, Tromsø, Norway.
[2]Nofima Ingrediens, Bergen, Norway.
[3]Vitamin premix, Norsk Mineralnæring AS, Hønefoss, Norway.
[4]Diet 2 was formulated without copper or zinc.
[5]U is a confirmed ingredient in producers' literature, and no quantitative information is available.
[6]Betafin, Danisco AS, Bryne, Norway.

In August 2006, the cod were divided into three groups and each group received one of the experimental diets. All three diets were made with fish meal and fish oil as main ingredients (Table 1). Diet 1 was formulated without extra mineral supplementation. Diet 2 contained a mineral supplement similar to Diet 3, but without copper and zinc. Diet 1 and Diet 2 were supplied by the research institute Nofima Ingrediens (Bergen, Norway). Diet 3 was a commercial cod feed (COD DAN-EX 1562, Dana Feed A/S, Horsens, Denmark).

The cod were fed the experimental diets for almost 2 yr from August 2006 until May 2008. Thirty fish were harvested in January 2008, 10 from each diet group. The fish were killed by an overdose of anaesthetic, bled and transported to the institute covered in ice. They were then gutted and washed, and biological measures were taken. The fish were stored iced in free

draining plastic boxes for 7 d prior to filleting. The right fillet was used for color (Minolta) and pH measurement as well as analysis of pH, water, protein, water-holding capacity (WHC), and metals.

In May 2008, a further 19 fish were harvested from each group. The fish were stunned by a blow to the head, followed by cutting of the isthmus and then bled in seawater for 30 min. Immediately after exsanguination, the fish were stored covered in ice and transported to the institute. The fish were gutted and washed, followed by iced storage in free draining plastic boxes. Thirty fish, 10 from each diet, were split, washed and salted 2 d post mortem. The fish were first pickle-salted by stacking layers of fish and salt (weight ratio 1:1) in a 60-L plastic tub. After 12 d the fish was removed from the brine and restacked in a free draining plastic box with surplus fresh salt (dry salting). Color was measured using a Minolta instrument after 15 wk of salting. The remaining nine fish from each group were filleted 7 d post mortem and used for sensory analysis.

Chemical and Physical Analytical Procedures

The right fillet was cut out without belly flaps. Flesh color was measured at six points on the cut side of each fillet using a Minolta Croma Metre CR 00 calibrated to a white standard. L^*a^*b measurements mode was used and whiteness was calculated as Whiteness $= (L^* - 3b^*)$ (Park 1994). The fillets were then coarsely chopped using a Stephan Blender with cooling. The minced muscle was kept on ice until further analysis. Muscle pH was measured in duplicates of 20 g of coarsely chopped muscle suspended in 0.15 M KCl (1:1; w:v) using a glass electrode. Water content was determined in duplicate samples by drying 2×10 g of coarsely chopped muscle from each fish overnight at 103 C. The water-holding capacity was determined in each fillet by measuring the liquid loss and then calculating the WHC as percentage of trapped water (muscle water - liquid loss) to muscle water content (Ofstad et al. 1993). Four replicas of 15 g minced muscle were analyzed for each sample. Centrifugation was carried out in 42-mm-diameter centrifuge tubes at 5 C for 15 min with a centrifugal force of $324g$ using a Beckman GS-6R Centrifuge. Protein was estimated based on the nitrogen content measured according to Kjeldahl using block digestion and steam distillation (AOAC, 1990).

Metals were analyzed by the method described by Cooper and Midling (2007) with some modifications. Muscle and feed samples were digested by adding 5 ml of 65% nitric acid to the samples in Teflon tubes and boiling in a microwave oven. Metal contents of the extracts were determined using a PerkinElmer AAnalyst 400 flame atomic absorption spectrometer. Extractions and measurements were performed in triplicate.

The color of the salt-cured cod was measured instrumentally at six points on the muscle surface of each split cod after 15 wk of salt curing, using a Minolta Croma Metre calibrated as described earlier.

Sensory Analysis

Sensory analysis was performed on heated samples of cod fillets fed diets 1 and 3 by an expert panel (Nofima Mat, Ås, Norway) consisting of nine trained judges. Each of the nine judges take part in the sensory analysis conducted at Nofima Mat 12 h/wk and have between 3 and 12 yr of experience using descriptive analysis on various kinds of beverages and foods, including cod. The panelists have been selected and trained according to recommendations in ISO 8586-1 (1993) and a modified quantitative descriptive method as described in ISO 6564 (1985) was used. The sensory laboratory has been designed according to guidelines in ISO 8589 (1988) with separate booths and has electronic data registration (CSA, Compusense Five, Version 3.80, Canada, 1999). Fillets of nine fish from each diet were cut into 3–4 cm pieces and packed in diffusion tight bags. The bags were heated for 7 min at 80°C prior to evaluation and served randomly to the judges. Each judge received one sample from each fish from the two diets. The samples were tested for 20 different attributes that are commonly used in sensory analysis

of fresh cod, related to odor (sourish, briny, old/stale, feed), appearance (transparency, flakiness, whiteness), taste (sweet, bitter, salt), flavor (sourish, metallic, briny, old/stale, feed) and texture (fibrousness, granularity, hardness, juiciness, chewiness).

Statistical Analysis

ANOVA and Tukeys *post hoc* test were performed using SPSS for Windows 13.0 (SPSS Inc., Chicago, IL, USA) to detect significant differences ($P < 0.05$) in chemical and physical muscle quality parameters between cod fed different diets. The same tests were also performed to detect effects of diet on the color and appearance of salted cod.

Results and Discussion

The experimental Diets (1 and 2) were formulated similarly to the commercial Diet 3 with the exception of the dietary mineral supplementation (Table 1). The diets did not appear to affect the growth rate of the fish. No differences in length or somatic (gutted) weight were detected between the dietary groups at either the sampling in January 2008 or in May 2008. The average lengths and somatic weights were 41.5 ± 3.3 cm and 738 ± 188 g in January and 45.5 ± 4.2 cm and 1000 ± 272 g in May. Furthermore, no differences were observed in the weights of the livers and gonads between dietary groups. The livers constituted as much as 9–11% of total body weight. This is in accordance with previously reported values for farmed cod and this is related to the intensive feed intake (Jobling 1988; Herland et al. 2007).

Chemical and Physical Quality Parameters

The chemical, physical, and sensory quality parameters were evaluated 7 d post mortem which is an average storage time before cod is available to consumers. For most of the chemical and physical quality parameters analyzed, there were no significant differences between the three groups. Water content and pH were the same for all three groups of fish (Table 2) and the levels of these parameters were similar to those previously reported for farmed cod (Herland et al. 2007; Solberg and Willumsen 2008). The addition of trace metals such as magnesium and manganese to cod feed did not improve the WHC as has been reported in pigs (Apple 2007). The levels of magnesium and zinc in the muscle tissue of the cod in this experiment did not appear to be directly affected by the levels in the diet. The levels of manganese in the muscle tissue were below the detection limit. Muscle potassium levels, however, were significantly higher in cod fed a fully supplemented diet (Diet 3) than in cod fed without mineral supplements (Diet 1). This may be a response to the elevated copper levels in Diet 3. Oner et al. (2008) reported increased levels of potassium in blood serum of Nile tilapia, *Oreochromis niloticus*, which had been exposed to high levels of copper in water. Copper levels were significantly higher in muscle tissue of cod fed the diet with full mineral supplementation than the two other diets without copper supplementation. Iron was not measured in this experiment as it is almost impossible to avoid blood contamination of samples.

TABLE 2. *Chemical and physical quality parameters of fresh fillets from farmed cod fed diets with different levels of mineral supplementation.*[1,2]

	Diet 1	Diet 2	Diet 3
pH	6.38 ± 0.08	6.46 ± 0.13	6.36 ± 0.13
Protein content (%)	19.7 ± 0.2[a]	19.4 ± 0.2[ab]	19.2 ± 0.7[b]
Water content (%)	79.6 ± 0.4	79.9 ± 0.3	79.8 ± 1.2
Water-holding capacity	95.7 ± 0.9	95.8 ± 1.1	96.2 ± 0.5
Minolta color			
L^*	48.4 ± 1.5	49.2 ± 2.4	50.4 ± 2.0
a^*	−1.30 ± 0.12[a]	−1.49 ± 0.21[ab]	−1.60 ± 0.20[b]
b^*	−4.68 ± 0.47[a]	−4.96 ± 0.54[a]	−3.66 ± 0.74[b]
Whiteness[3]	60.1 ± 2.9[a]	58.4 ± 3.3[ab]	56.0 ± 2.6[b]
Ca (µg/g)	64.1 ± 6.3	63.8 ± 8.9	73.0 ± 13.2
Cu (µg/g)	0.40 ± 0.13[a]	0.36 ± 0.10[a]	2.74 ± 0.54[b]
K (mg/g)	2.96 ± 0.44[a]	3.32 ± 0.29[ab]	3.77 ± 0.36[b]
Mg (µg/g)	210 ± 38	231 ± 20	241 ± 16
Mn	BD	BD	BD
Zn (µg/g)	3.43 ± 0.65	3.49 ± 0.83	3.18 ± 0.51

BD = below detection limit.

[1]Water content, WHC, pH, and Minolta color were determined after 7 d of iced storage.

[2]Different superscript letters indicate significant differences ($P > 0.05$) within the row.

[3]Whiteness = $L^* - 3b^*$.

Protein content of the muscle appeared to be influenced by diet. Cod fed the diet with full mineral supplementation (Diet 3) had significantly ($P < 0.05$) lower muscle protein content than cod fed a diet with no mineral supplementation (Diet 1). This is difficult to explain, but it has been shown that salmon fry-fed diets with elevated copper levels had reduced whole-body protein content (Berntssen et al. 1999). Hamre et al. (2004) reported reduced levels of plasma protein in salmon-fed diets containing high levels of copper. The results indicate that excess supplementation of copper could, at least in part, explain the observed reduction in muscle protein content.

The color of the fresh fillets as determined by the Minolta instrument was also influenced by diets (Table 2). The full mineral supplement group (Diet 3) had a shift in a^* value toward green and a shift in b^* value toward yellow resulting in a slightly lower Whiteness. The shift toward a more yellow color could be because of lipid oxidation that could have been evaluated by analyzing TBARS. However, lipid oxidation is not considered a problem during iced storage of lean fish muscle. Mostly because of the very low lipid content, but also because of the low proportion of dark muscle where the proxidative copper and iron content is often three times higher than in light muscle (Dulavik et al. 1998). Lauritzsen et al. (1999) have shown that the difference in yellowness of fresh cod muscle may be substantial at low levels of copper, but that the difference in TBARS was rather small. When lean fish is stored for longer periods either salt cured or frozen lipid oxidation may become more important.

Sensory Evaluation

Fresh fillets from the group fed the diet with no mineral supplementation (Diet 1) and the group fed the diet with full mineral supplementation (Diet 3) were used for sensory analysis after heating. A panel of nine trained judges evaluated 20 different quality attributes (Fig. 1). The sensory panel could not detect any differences between the fillets from the cod fed a full mineral supplement and no supplement at all. Differences in mineral and protein content, as well as color differences measured with the Minolta, had no detectable impact on the sensory quality of the cod fillet.

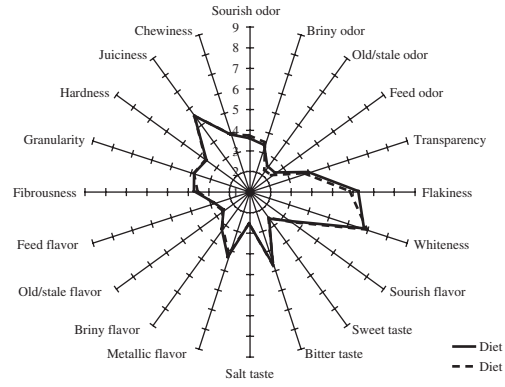

FIGURE 1. *Radar chart showing the average sensory scores given by the sensory panel. Solid line representing the farmed cod fed a diet without mineral supplement (Diet 1) and broken line representing the cod fed a commercial diet with a full mineral supplement (Diet 3). Scores are from 1 (no intensity) to 9 (high intensity).*

Salted Cod Quality

The color of the salted cod from the May sampling was analyzed by instrumental color measurements (Table 3). The results showed that the salted product from cod fed a diet with full mineral supplementation (Diet 3) was more yellow, i.e., had a higher b^* value, than the product from cod fed diets without copper and zinc supplementation (Diets 1 and 2). The product made from cod fed Diet 3 also had a lower calculated Whiteness than product made from cod fed Diet 1. This can be explained

TABLE 3. *Instrumental color measurements of split farmed cod muscle after 15 wk of salt curing.*[1,2]

	L^*	a^*	b^*	Whiteness
Diet 1	60.5	−4.33	0.14[a]	60.08[a]
Diet 2	58.4	−4.08	−0.01[a]	58.39[ab]
Diet 3	59.9	−4.24	1.32[b]	55.95[b]

[1]Minolta L^*, a^*, and b^* values and Whiteness ($L^* - 3b^*$).

[2]Different superscript letters indicate significant differences ($P < 0.05$) within the column.

by the higher levels of copper in muscle tissue from fish given Diet 3. Lauritzsen et al. (1999) have shown that copper present during salting induces lipid oxidation and subsequent yellow color in the muscle of salted cod. The quality of salt-cured cod is usually evaluated in the industry by an experienced inspector. Three such inspectors also evaluated color of the salted fish after 2 and 15 wk of salt curing. The salted cod given the diet with full mineral supplementation were evaluated as slightly more yellow than cod from on the other two diets. The fresh cod fed a fully supplemented diet was not evaluated as more yellow than the cod fed the diet without supplements. This is because of the longer storage period of the salted cod as this fish is stored for 15 wk compared to the 7-d storage of fresh cod.

The conclusions from this work are that the addition of mineral supplement to the feed of farmed cod did not affect the sensory quality of the fresh fillet. The increased level of copper in the muscle of cod receiving a diet with complete mineral supplement reduces the quality of salt-cured cod by increasing the yellowness of the muscle surface.

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