Direct measurement of nitric oxide (NO) in the gastrointestinal tract of cod (*Gadus morhua*)

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Abstract

Objective: In mammals, the biological messenger nitric oxide (NO) is generated throughout the gastrointestinal (GI) tract from the reduction of dietary nitrate and nitrite. The aim of the present study was to investigate the amount of GI NO in Atlantic cod (*Gadus morhua*) in relation to intake of food. Methods: A total of 28 cod were divided into 3 groups, fed at different times before the experiment (1 week, 1 day, and 3 h, respectively). Results: In the stomach, the measured NO concentrations were consistently higher in the group fed 3 h before the measurement, implying that the NO_3-NO_2-NO pathway is present in the stomach of cod. We also measured the NO concentration in the large intestine. Again, the values were higher in cod fed 3 h before the experiment. Conclusion: We conclude that NO is formed in the GI tract of cod, likely via the reduction of dietary nitrate and nitrite. The physiological importance of this NO production remains to be determined.

Key words: stomach, large intestine, food

Introduction

Over the years, nitrate (NO_3) and nitrite (NO_2) have been regarded as undesired residues in the food chain with potentially carcinogenic effects or as inert oxidative end products of endogenous nitric oxide (NO) metabolism, and great efforts have been made to reduce the amounts of these compounds in food and water supplies. In fish, nitrite in water is believed to be absorbed through the gills following the same pathways as sodium chloride, possibly leading to chloride depletion (1,2). Furthermore, nitrite is also believed to interact with hemoglobin (2,3). Both mechanisms might be detrimental to fish health.

However, recent comparative studies in germ-free and conventional mammals (4,5) have shown that nitrate and nitrite are both part of a physiological nitrogen recycling system in which salivary glands and the microbial flora in the digestive tract interact to reduce nitrate and nitrite to NO. Dietary nitrate is rapidly absorbed and excreted into saliva within a few minutes. Bacterial conversion of nitrate to nitrite starts in the mouth and a further, pH-dependent, conversion of nitrite to NO takes place in the stomach (5). It has also been shown that the amount of NO in the digestive tract was related to dietary intake of nitrate (5). The possible physiological impact of this microbial-derived NO production in the digestive tract has recently been reviewed (6).

To the best of our knowledge, the presence of NO in the digestive tract of fish has never been studied. The aim of the present study was to investigate whether NO is present in the stomach of Atlantic cod (*Gadus morhua*), and whether the amount is influenced by the feeding regimen. We also measured the NO concentration in the large intestine.

Material and methods

The investigation took place under field conditions at a cod farm, located north of the Polar Circle in the county of Nordland, Norway. Water temperature in the ponds was about 9°C. The conditions at the location limited the number and types of samples that could be taken for investigation.
The fish investigated were raised in captivity and fed a commercially obtained fish diet, and they were of the same size, approximate weight 3 kg. The groups studied were in three different ponds. Each cod was taken individually from the pond before investigation, thereby standardizing the time that they were out of water. The three groups were as follows. Group 1, avoided food for a week (LT = long time); group 2, avoided food for 20–24 h (MT = medium time); and group 3, avoided food for the last 2–4 h (ST = short time).

After removal from the water, the cod was immediately killed by a blow to the head, the abdominal wall was opened, and the stomach was isolated by sealing the esophagus and duodenum, using external clamps. Thereafter, 4 ml of air was inserted by means of a 5 ml syringe and a thin needle into the stomach. After about 10 s, the gas was ejected from the stomach and immediately injected into a rapid-response chemiluminescence analyzer (Aerocrines AB, Stockholm, Sweden) to determine the concentration. The instrument's detection level for NO was 1 part per billion (ppb), and the response time was < 0.5 s. Measurement of NO took place < 60 s after the cod was killed.

In some fishes, part of the large intestine was also isolated as described for the stomach, and 3 ml of air was inserted. After 10 s, gas was ejected and the content of NO was measured as described above.

Except for individual and rapid handling, the procedures for harvesting the cod and opening of abdominal wall followed the ethical rules at the farm.

**Results**

As can be seen by the data presented in Figure 1, very low levels of NO were found in the LT group (median value 1.4 ppb), whereas significantly higher values with considerable variations were found in the ST group; the values differed in the MT and ST groups (median values 8 and 65 ppb, respectively).

A few measurements of NO were also made in the large intestine (Figure 2), and here the highest values were found in the MT group.

The nitrate content measured in the food peaked at 32 μM or 2 mg/kg.

**Discussion**

As mentioned above, it is now well established in mammals that the nitrate-nitrite-nitric oxide pathway is responsible for a substantial part of NO present in the lumen of the GI tract (6). Our findings indicate that similar mechanisms are also at work in cod. The consistent differences in NO concentrations found in the three groups of cod investigated implies that also in cod, NO production in the GI tract is related to food intake. The very low values found in the LT group imply that a majority of the food had already been digested, whereas the higher values found in the MT and ST groups indicated that digestion was still going on.

The large intestine generally contained more NO in the MT than in the ST group, indicating that the food had not been properly digested yet in the ST group. This is also consistent with observations made at the site of the experiments: the variations in the amount of stomach and large intestine were

![Figure 1](image1.png)

**Figure 1.** Logarithmic values of NO concentration in stomachs of cod measured in ppb. The fish were divided into three groups: group 1 was on starvation, group 2 had been fed 24 h before the experiment, and group 3 was fed 3 h before the experiment. Mean values are indicated.

![Figure 2](image2.png)

**Figure 2.** Logarithmic values of NO concentration in the large intestine of cod measured in ppb. The fish were divided into three groups: group 1 was on starvation, group 2 had been fed 24 h before the experiment, and group 3 was fed 3 h before the experiment. Mean values are indicated.
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is likely the major substrate, and NO generation probably occurs via serial reduction to nitrite and then NO. It remains to be studied whether NO plays an equally important role in regulation of GI function in fish as it does in mammals.

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