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Short communication**Replacement of fishmeal with corn gluten meal in feeds for juvenile rainbow trout (*Oncorhynchus mykiss*) does not affect oxygen consumption during forced swimming**

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ABSTRACT

We compared oxygen consumption (MO_2 , mg/kg/h) of c. 80 g rainbow trout (*Oncorhynchus mykiss*) in an intermittent-flow swim respirometer at 15°C. Before the tests the fish were grown in flow through tanks (15°C) with either fishmeal (FM) or corn gluten meal (CGM) based diets (c. 52% protein) for a period of 3 - 4.5 months. Ten individuals from both treatment groups were fasted for 48 h before the swim test, which consisted of 18 loops of 210 s over three different periods: acclimation period (6 loops at 0.5 body lengths per s, BL/s), exercise period (8 loops at increased speed from 1 to 2.5 BL/s with recovery loops at 0.5 BL/s), and a recovery period (four loops at 0.5 BL/s). We did not observe significant differences in MO_2 between the two groups at any of the three measurement periods (repeated measures-Anova). The maximum (mean \pm SE) MO_2 values, measured during the last exercise period at 2.5 BL/s, did not differ significantly between the treatments: 404 \pm 18.7 mg/kg/h and 427 \pm 50.6 mg/kg/h in FM and CGM groups, respectively. Our result supports an earlier finding that origin of the protein does not affect MO_2 during swimming in salmonids. This is the first report of the effect of a plant protein on MO_2 of a carnivorous fish during forced swimming, and these data lend support to further development of sustainable diets to replace fishmeal with plant proteins.

Keywords: Salmonids, metabolic rate, swimming respirometer, fishmeal replacement, protein sources

Abbreviations: BL, body lengths; CF, condition factor; CGM, corn gluten meal; FM, fishmeal; MO_2 , oxygen consumption (mg/kg/h)

1. Introduction

Traditionally the feeds for carnivorous fishes have contained fishmeal (FM) as protein source. However, there is a high demand for the development of fish feeds with alternative protein sources, firstly due to dwindling marine fish stocks and a consequent rise with wide temporal variation in FM market price (Indexmundi, 2017) and secondly due to the need for more sustainable aquaculture production. The use of FM alternatives is likely to increase even more in the coming decades (Engle et al., 2017). FM in fish feeds is typically replaced with plant proteins, and the most common alternative is soybean meal but also corn gluten meal (CGM) has been used widely (Gatlin et al., 2007).

Metabolism (heat production) in fish has conventionally been estimated indirectly by measuring oxygen consumption in a respirometer. To the best of our knowledge there are no reports of experiments for swimming metabolism, *i.e.* O_2 consumption (MO_2 , mg/kg/h) during forced swimming (Cech, 1990), between the fish fed with either FM or plant protein based diets. (Wilson et al., 2007) measured MO_2 , swimming capacity and recovery from swimming of Atlantic salmon (*Salmo salar*) fed diets differing in lipid (fish oil, poultry fat and vegetable oils) and protein (FM and poultry by-product meal) source and found no significant differences between the dietary groups.

As the use of plant proteins in feeds for carnivorous fish is increasing, it would be important to know whether their use affects the swimming performance and MO_2 of fish. In this experiment, we measured swimming metabolism of juvenile rainbow trout (*Oncorhynchus mykiss*) which had been reared on two extreme experimental diets: one with FM and the other one with CGM as the only protein source. The fish fed with CGM diet did not grow to the swim test size as fast the fish fed FM diet, which may have led to changes in muscle tissues and consequently decreased the swimming capacity (Kiessling et al., 1989; Pelletier et al.,

1993; Weber et al., 2016). Our hypothesis was that the performance (lower capability to maintain high swimming speed and higher MO_2 during exercise and recovery) of the fish fed CGM diet would be inferior to that of the fish fed FM diet.

2. Materials and methods

The measurements were conducted in the wet laboratory of the University of Jyväskylä, Finland. The all-female rainbow trout originated from a commercial fish farm (Venekoski farm, Hanka-Taimen Ltd.) and they were brought to our facilities on 16 June 2016 at the size of c. 6.1 g. The fish were used in a feeding trial where they were fed the extruded FM and CGM diets (Table 1) for seven weeks. Then, fish from the replicate tanks of each dietary treatment (FM and CGM) were combined and held in two aerated 500 L flow-through tanks (temperature 14.8 - 15.2°C, oxygen 8.5 - 9.5 mg/L) where they were fed the same feed formulations but at larger pellet sizes than previously. The fish fed FM grew faster than those given CGM, and in an attempt to avoid the confounding effects of fish size on oxygen consumption the swimming tests were carried out at different times on the two groups of fish. The fish fed FM were tested on 19 - 23 September, and the fish fed CGM were tested on 31 October - 4 November 2016. There were no significant differences (*t*-test, $n=10$) between the fish in weight, total length or condition factor ($\text{CF} = \text{weight (g)} / \text{length (cm)}^3 * 100$) at the time of testing (Table 2). Before the swimming tests and measurements of oxygen consumption, the fish were held individually without food for c. 48 h in 15 L flow-through aquaria at 15°C to reduce the effect of food digestion and processing on oxygen consumption (SDA) (Jobling, 1994). Water in the laboratory was filtered water from a bore-hole, and photoperiod was 12L:12D.

The experimental diets were analysed for dry matter and moisture (ISO 6496-M), crude protein (EN ISO 5983-2), crude fat (EC Dir 98/64; EU DIR 71/393), crude fiber (ISO 5498)

and ash (ISO 5984) at Eurofins Scientific Finland Ltd. Carbohydrates were calculated by decreasing the sum of analysed components from 100 (McDonald et al., 2011); fiber was not included in this calculation because it was under the detection limit. The gross energy content of the diets was proximately calculated using standard conversion factors for carbohydrates (17 kJ/g), protein (24 kJ/g) and lipids (39 kJ/g) (Jobling, 1994) (Table 1).

The respirometer experiments were carried out using a 10 L swim tunnel respirometer, connected to an OXY-4 oxygen meter with an optical sensor (system manufacturer: Loligo Systems, Viborg, Denmark). According to the manufacturer, the 10 L swim respirometer is suitable for fishes in the range of 50 to 150 g. Before placing the fish into the swim chamber, it was netted out from the aquarium, weighed (to 0.1 g) in a bucket with c. 3 L of water, and the total length was taken (to 1 mm). Immediately after these measurements, the fish was placed into the respirometer chamber, and the computer-assisted intermittent measurement was started. Water temperature during the measurements varied between 14.6 and 15.4°C.

Each measurement in the respirometer consisted of 18 loops, 210 s each. As the measurement was intermittent, each of the loops was set to contain three stages: 1) flush period of 90 s (oxygenated water pumped into the chamber from the surrounding water bath), 2) wait period of 60 s (flush pump was stopped *i.e.* chamber was closed, and O₂ concentration was let to equilibrate) and 3) a 60 s measurement period (chamber closed). Based on the decrease of O₂ concentration during the 60 s measurement period, the AutoResp software (Loligo Systems, Viborg, Denmark) calculated MO₂ for each loop.

Water speed in the respirometer was adjusted to match the fish total length. At the start of the measurement period the fish was let to recover from handling for a period of six loops at the water speed of 0.5 body lengths/s (BL/s). After the recovery period, water speed was increased by 0.5 BL/s steps up to 2.5 BL/s, and by giving the fish a recovery loop (at 0.5

BL/s) after each exercise loop, *i.e.* 1.0, 0.5, 1.5, 0.5, 2.0, 0.5 BL/s. Finally, the speed was increased to 2.5 BL/s for two consecutive loops, after which the fish was let to recover from the exercise for a period of four loops. After the measurement was ended, the fish was netted out of the chamber and killed with a sharp blow on the head.

The rear of the chamber was equipped with an electric grid (DC 3.5 V), which would have been turned on in the case the fish started to lean with the tail against it, but this did not happen in our experiment. The experiment was done with the permission ESAVI/10412/04.10.07/2015 from the Animal Experiment Board of Finland.

The possible difference in MO_2 between the two treatments was tested by repeated measures Anova using IBM SPSS statistics 24.0 software. We tested the possible difference between the two treatments separately for the whole experimental period (18 loops), for the acclimation period (first 6 loops), for the exercise period (8 loops after acclimation) and for the final recovery period (last 4 loops). We also compared the possible difference in MO_2 at the highest test velocity (the second 2.5 BL/s loop) using Mann-Whitney U-test. Each individual was used as an observation, *i.e.* $n=10$, and $P<0.05$ was set as the level for a statistically significant difference.

3. Results and discussion

Rainbow trout of the FM and CGM groups performed equally in terms of MO_2 (Fig. 1). There were no significant differences in their MO_2 when calculated for the whole experimental period ($P=0.795$), for the acclimation period ($P=0.514$), for the exercise period ($P=0.795$) and for the final recovery period ($P=0.366$). The maximum (mean \pm SE) MO_2 during the second 2.5 BL/s velocity (loop 14) were 404 ± 18.7 mg/kg/h and 427 ± 50.6 mg/kg/h in FM and CGM groups, respectively, without being significantly different ($P=0.257$) (Fig.1).

As such, we conclude that feeding a diet based on CGM as the only protein source does not affect oxygen consumption in rainbow trout when swimming.

It must be noted that the feeds differed in terms of their composition: only the CMG diet contained added amino acids and fish protein hydrolysate. The lysine and methionine are considered as limited essential amino acids both in cereal grains and in their by-products (McDonald et al., 2011). In the present study the amino acids and fish protein hydrolysate were supplemented to avoid the typical deficiency of certain amino acids of plant proteins, especially lysine in CGM (Gatlin et al., 2007), and the decrease in feed palatability. Our unpublished experiments have shown low palatability of CGM based diets as such.

The absence of difference in oxygen consumption in rainbow trout fed FM or CGM diets concurs with the results of (Wilson et al., 2007). They grew Atlantic salmon (from 84 g to c. 0.5 kg) with feeds containing different sources for lipids and proteins and then tested the fish for oxygen consumption, swimming performance and recovery from exhaustive exercise at 9°C without finding any significant differences between the dietary treatments. The result of the current experiment and those of Wilson et al. (2007) suggest that swimming performance, and MO_2 during exercise and recovery from swimming of 24 h (Wilson et al., 2007) or 48 h (the present experiment) starved fish would not be affected by the protein source.

This is likely the first experiment looking at the potential effects of plant proteins on swimming performance and MO_2 in fish. Despite the fish fed CGM grew slower than the fish fed FM based diet, the current result regarding fish performance during forced swimming is promising for the feed industry and for further development of more sustainable fish feeds. However, it must be noted in the present experiment we tested only the effects of one plant protein (corn gluten meal) on oxygen consumption during forced swimming in juvenile rainbow trout at c. 15°C. Thus, a more comprehensive research is needed to confirm if our

finding is universal, *i.e.* whether it applies to other plant proteins and other carnivorous fish, also at sub- and supraoptimal temperatures.

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Table 1. Formulation and composition of experimental diets (CGM = corn gluten meal based diet, FM = fishmeal based diet) fed to juvenile rainbow trout before tests in the swim respirometer.

Ingredient	CGM (g/kg)	FM (g/kg)
Fish meal	0	755
Corn gluten meal	730	0
Cellulose	42	65
Corn starch	40	58
Wheat starch	0.0	20
Fish oil	82	82
Fish protein hydrolysate	10	0
Vitamin premix ^a	20	20
Methionine	5	0
Lysine	25	0
Threonine	10	0
Monocalcium phosphate	36	0
Proximate composition		
Protein (g/kg)	534±53	515±52
Fat (g/kg)	164±16	161±16
Ash (g/kg)	57±9	105±16
Fiber (g/kg)	<10	<10
Moisture (%)	5.0	6.8
Gross energy (kJ/g) ^b	22.4	21.0

^a Vitamins added to supply the following (kg⁻¹ diet): retinol acetate, 8000 IU; cholecalciferol, 3000 IU; all-race-alfa-tocopherol acetate, 300 IU; menadione sodium bisulfite, 10 mg; thiamine.HCl, 20 mg; riboflavin, 30 mg; calcium d-pantothenate, 90 mg; biotin, 0.3 mg; folic acid, 6 mg; vitamin B12, 0.04 mg; niacin, 120 mg; pyridoxine.HCl, 20 mg; ascorbic acid (Stay C), 300 mg; inositol, 200 mg. Minerals added to supply the following (mg kg⁻¹ diet): zinc, 150; manganese, 60; iodine, 4.

^b Gross energy was calculated based on energy content of protein (24 kJ/g), lipids (39 kJ/g) and carbohydrates (17 kJ/g). See text for details of calculation.

Table 2. The average \pm SE (n = 10) weight, total length, and condition factor of juvenile rainbow trout fed experimental diets (CGM = corn gluten meal based diet, FM = fishmeal based diet) and used in the swim respirometer test.

	CGM	FM
Length, cm	19.1 \pm 0.3	19.2 \pm 0.3
Weight, g	74.1 \pm 3.9	80.7 \pm 3.8
Condition factor	1.05 \pm 0.03	1.13 \pm 0.03

There were no statistically significant differences ($P > 0.05$, *t*-test) between the treatments in these variables.

Figure 1. The mean (\pm SE, $n = 10$) oxygen consumption (MO_2) of juvenile rainbow trout fed diets containing fishmeal (FM) or corn gluten meal (CGM) during the 18-loop swim respirometry test. Each loop lasted for 210 s. Swimming speed was 0.5 body lengths per s except during the loops 7, 9, 11, 13 and 14, as indicated by the values above the x-axis. There were no statistically significant differences ($P > 0.05$, RM Anova) between the treatments.

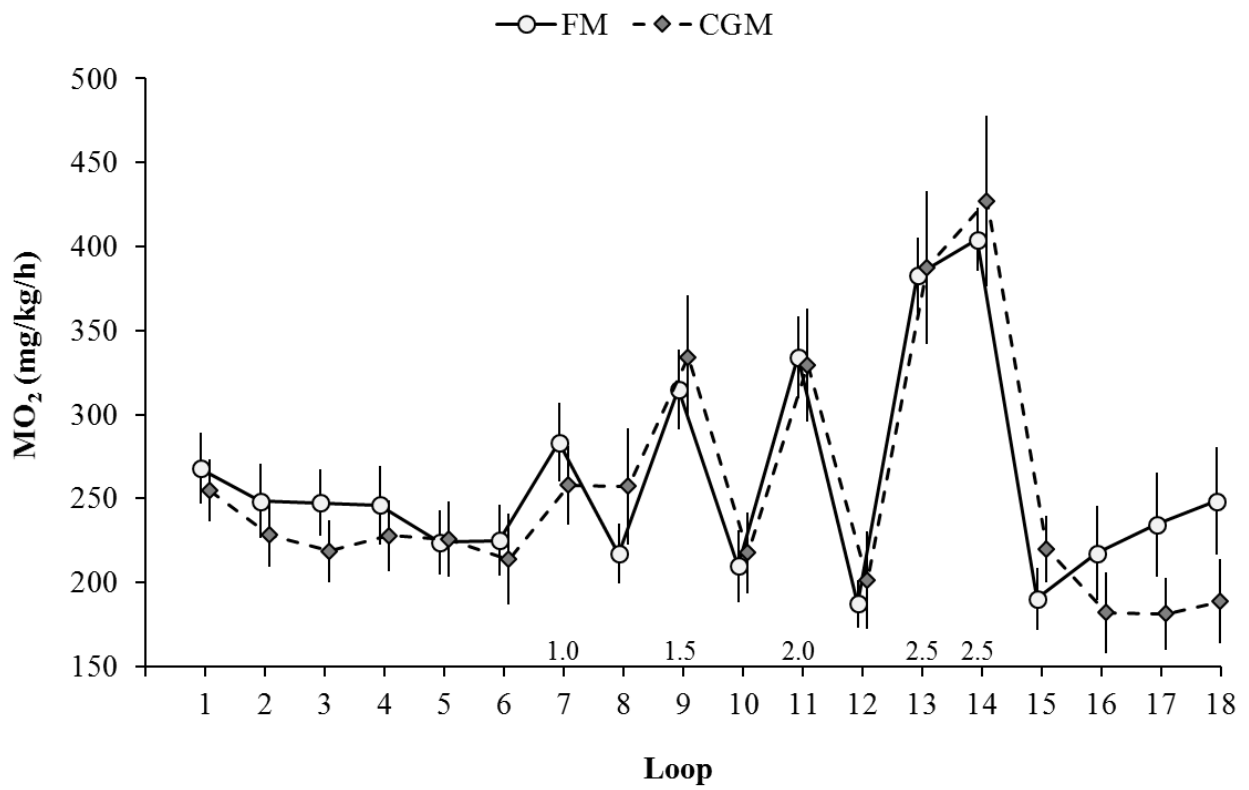


Figure 1

Highlights

- Oxygen consumption of 80 g rainbow trout in a swimming test was not affected by the type of dietary protein (fishmeal or corn gluten meal) given for the previous 3-4.5 months
- Corn gluten meal can be used in trout diets without having significant influences on the swimming performance of the fish