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DO ARCTIC CHARR, *SALVELINUS ALPINUS* (L.), HAVE SELECTIVE ABSORPTION OF DIETARY FATTY ACIDS?

By

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ABSTRACT

Arctic charr, *Salvelinus alpinus* (L.), were fed a moist pellet diet based on casein, dextrin and coconut oil enriched with 1% methyl esters of 18:2 (n-6), 18:3(n-3), 20:4(n-6), (n-3) polyunsaturated fatty acid (PUFA) mix, 20:1 (n-9) and 22:1 (n9) for 123 days. The gut contents from all rearing groups were stripped three times over a three week period. Complete absorption of 18:3, 20:4 and the dominant fatty acids in the PUFA mix (20:5 n-3 and 22:6 n-3) was observed. However, in gut contents from Arctic charr fed the 18:2 (n-6) enriched diet, small amounts (1.3%) of this fatty acid were detected. High proportions of the monene fatty acids 20:1 (n-9) (17.9%) and 22:1 (n-9) 24.3%) were detected in the gut contents from fish fed the basal diet supplemented with 20:1 and 22:1, respectively. This observation indicates low absorption of these fatty acids.

INTRODUCTION

Some information is available about the apparent digestibilities of lipid and individual dietary fatty acids in Arctic charr, (*Salvelinus alpinus* L.), and rainbow trout *(Salmo gairdneri* R) (Austreng *et al.*, 1979;1980; Ringø, 1989; Takeuchi *et al.*, 1979 a). However, no investigations have been carried out to determine the specificity of digestion and absorption of dietary fatty acids in fish by using fatty acid mehyl esters. The use of fatty acid methyl esters may circumvent the problems of lipase specificity in the hydrolysis of fatty acids in TAG described by Gjellesvik *et al.*, (1989), Lied and Lambertsen (1985) and

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Patton *et al.*, (1975). Therefore, the present investigation may give information on the selective absorption of different dietary fatty acid mehyl esters in fish. Experimental diets supplemented with marine fish oils or oils of terrestrial origin, providing a great diversity of fatty acids have been used in digestibiligy studies in fish (Austreng *et al.*, 1980). However, by using such lipid sources, the nature and the melting point of the lipid may be different and affect digestibilities of individual fatty acids. In the present study, experimental diets based on coconut oil were used. They contained only short chain saturated and monoene fatty acids, with small amounts of different polyunsaturated fatty acids (PUFA) added.

This study present some data indicating that there is selective absorption of different dietary fatty acid methyl esters in Arctic charr held in fresh water.

MATERIAL AND METHODS

Preparation of diets

The basal diet was a moist pellet diet based on casein, dextrin and coconut oil (Table 1). The addition of premixes (antioxidants and vitamins) to the basal diet are described elsewhere (Olsen *et al.*, 1990 a). The (n-3) PUFA mix was prepared by urea fractionation of a 50% concentrate of methylated PUFA from capelin *(Mallotus villosus)* oil (Martens LTD), as described by Christie (1982). The basal diet was enriched with 1 % methyl esters (95 % purity) of 18:2 (n-6), 18:3 (n-3), 20:4 (n-6), (n-3) PUFA mix, 20:1 (n-9) and 22:1 (n-9)

Table 1. Ingredients (gram per kg dry weight) of the basal diet.

Ingredients	
Caselin	706.5
Dextrin	170.0
Gelatin	17.0
Premix I	30.0
II	1.7
III	1.8
IV	20.0
Coconut oil	43.0
Lipid supplement*	10.0

* see Table 2
Composition of premixes are described by Olsen et al. (1990 a)

(Sigma), respectively. The diets were mixed thoroughly and pelleted, and stored at -80°C prior to use.

Fish and experimental condititons

Alevins of Arctic charr, (*Salvelinus alpinus* L.), were fed on a commercial feed from the initial feeding stage until a mean weight of 10 g was attained. The fish in groups of 30 were fed the diets supplemented 1% methyl esters until they had a mean weight of about 40 gram. Feed was supplied in excess (2% of body weight per day) using automatic disc feeders. Experimental design was similar to that given by Ringø and Nilsen (1987). The study was undertaken under natural photoperiod (70°N) from October to March (123 days) at an average water temperature of 6.0°C ±1.0°C.

Sampling of gut contents

Recently, Ringø found that Cr_2O_3 affected the lipid composition and bacterial flora of faeces in digestibility studies (to be published). Therefore, apparent digestibilities of lipid and individual fatty acids were not determined by using Cr_2O_3 in this study. The gut contents were stripped after the fish were anaesthetized in 0.3% benzocaine. Stripping of the fish was conducted three times over a three week period, and stripping consisted of pressing the belly of the fish in the region behind the pelvic fin to the anus as described by Ringø (1989). The gut contents from all the 30 fish in each group were pooled prior to futher treatment.

Lipid and fatty acid analyses

All analyses of diets and gut contents were carried out in triplicate. The water content of the gut contents was determined by drying samples for 48 hours at 105°C. Total lipid was determined by the method of Folch *et al.* (1957) and stored under N_2 at -80°C in hexane prior to analyses. The lipid was saponified and fatty acids esterified in 12% BCl_3 in methanol. The fatty acid composition were determined by gas chromatography (Hewlett Packard, Model 5890 A) using an SP 2330 capillary column (30 m x 0.25 mm i.d.) and helium as carrier gas. The temperature program has been described by Haug *et al.* (1988). Individual fatty acids were identified by comparison with known standards (Supelco 4-7019, 4-7042, 47033, 4-5589) using a Hewlett-Packard 3393A integrator.

Statistical signifincane

To test possible differences in the proportion of saturated fatty acids and 18:1 between the diets and the total lipid content in gut contents between the differnt rearing groups, a one way variance analysis with the program Stat View SE + Graphics ™ (1988 Abacus Concepts, INc. from Brain Power, INc. 24009 Ventura Blvd., Suite 250, Calabasas, CA 91392) was used. Significant level ($P<0.05$) was accepted.

RESULTS AND DISCUSSION

Total lipid content and fatty acid composition of the basal diet enriched with different methyl esters of fatty acids are shown in Table 2. There were no significant differences (a one way variance analysis) in the proportions of saturated fatty acids between the diets supplemented with monoenes or 18:2(n-6), 18:3(n-3) or 20:4 (n-6). Significant ($P<0.05$) higher proportion of 18:1 was found in the diet supplemented with (n-3) PUFA mix compared to the proprotion of this fatty acids in the other diets, due to the relatively high proportion of 18:1 in the (n-3) PUFA mix (results not shown). The proportions of the enriched fatty acids (18:2 (n-6), 18:3 (n-3), 20:4 (n-6), 20:1 (n-9), and 22:1 (n-9)) in the experimental diets were in the range 16-20% of total fatty acids. The proportion of 20:5 (n-3) and 22:6 (n-3) in the diet enriched with (n-3) PUFA mix accounted for about 7% each of total fatty acids. Total lipid in the gut contents from all the groups was about 2.5% of dry weight (Table 3). There were only significant differences ($P<0.05$) in the total lipid content in gut contents between group 4 and 5, and between group 4 and 6 (Table 3). No significant differences ($P>0.05$) was found between the other rearing groups.

Ringø (1989) investigated the digestibilities of individual fatty acids in Arctic charr fed commercial feed. However, no comparative absorption studies of monoene fatty acids and PUFA in fish have been carried out, where the fish are fed only one dietary fatty acid at a time. The results of the present study indicate a relative high absorption rate of 18:2 (n-6) since only a trace (1.3%) of the fatty acid was detected in the gut contents (Table 3). In gut contents from fish fed the basal diet enriched with 18:3 (n-3), 20:4 (n-6) and (n-3) PUFA mix, the enriched fatty acids were not detected. This observation indicates 100% absorption of 18:3 (n-3), 20:4 (n-6), and 20:5 (n-3) and 22:6 (n-3) in the (n-3) PUFA mix. In an earlier study, Ringø (1989) reported digestibility coffecients of 95% for (n-3) PUFA and 75 % for 20:4 (n-6).

The absorption of 20:1 (n-9) and 22:1 (n-9) was low, as high amounts of these fatty acids were detected in the gut contents (Table 3). These results are in accordance with results reported by Ringø (1989) that the digestibilities of

Table 2. Total lipid (% of dry wt) and fatty acid composition (%) of the basal diet enriched with methyl esters of; (1) 18:2 (n-6), (2) 18:3 (n-3), (3) 20:4 (n-6), (4) (n-3) PUFA mix, (5) 20:1 (n-9) and (6) 22:1 (n-9).
Values are mean of three diets samples

	Diet no.					
	(1)	(2)	(3)	(4)	(5)	(6)
Total lipid*	5.3	5.3	5.3	5.3	5.3	5.3
saturates	70.9	70.4	71.9	69.3	71.9	69.9
8:0	2.1	1.3	2.1	1.6	1.9	1.6
10:0	3.8	3.8	4.1	3.8	3.8	3.6
12:0	33.4	32.3	32.4	30.8	33.4	33.0
14:0	13.8	13.8	13.8	13.8	13.9	13.5
16:0	9.3	10.0	10.2	10.2	10.1	9.3
18:0	8.5	9.2	9.3	9.1	8.8	8.7
monoenes	6.0	5.6	5.1	9.6	24.9	24.7
16:1	0.4	0.3	0.4	0.3	0.3	0.3
18:1	5.6	5.3	4.7	8.5	5.5	5.1
20:1 (n-9)	n.d	n.d	n.d	0.8	19.1	n.d
22:1 (n-9)	n.d	n.d	n.d	n.d	n.d	19.3
(n-6) PUFA	19.7	0.7	16.8	2.2	0.9	0.6
18:2	19.7	0.7	0.9	1.3	0.9	0.6
20:4	n.d	n.d	15.9	0.9	n.d	n.d
(n-3) PUFA	n.d	20.2	n.d	15.5	n.d	n.d
18:3	n.d	20.2	n.d	0.7	n.d	n.d
20:5	n.d	n.d	n.d	7.1	n.d	n.d
22:5	n.d	n.d	n.d	1.3	n.d	n.d
22:6	n.d	n.d	n.d	6.4	n.d	n.d

n.d: not detected
* after Olsen et al. (1990 a)

the monounsaturated fatty acids 20:1 (n-9) and 22:1 (n-11) are low in Arctic charr.

The reason for the relatively high proportions of 20:1 (n-9) and 22:1 (n-9) in the gut contents from fish fed these two fatty acids compared to the complete absorption of 20:4 (n-6) and (n-3) PUFA, may be selective absorption of methyl esters of dietary fatty acids, showing preferences for 20:4 (n-6) and (n-3) PUFA.

Table 3. Total lipid (% of dry weight), statistical test and fatty acid composition (%) (± standard deviation) of gut contents stripped from Arctic charr fed basal diet enriched with methyl esters of, (1) 18:2 (n-6), (2) 18:3 (n-3), (3) 20:4 (n-6), (4) (n-3) PUFA mix, (5) 20:1 (n-9) and (6) 22:1 (n-9).
Values are mean from three gut samples taken over a three week period.

	Diet no.					
	(1)	(2)	(3)	(4)	(5)	(6)
Total lipid	2.3±0.2	2.2±0.1	2.2±0.2	2.1±0.2	2.9±0.3	3.0±0.3
Statistical test ...				*	*	*
saturates	91.3	92.1	91.4	92.0	71.4	64.2
8:0	n.d	n.d	n.d	n.d	n.d	n.d
10:0	n.d	n.d	n.d	n.d	n.d	n.d
12:0	24.4±0.2	24.2±0.8	22.9±1.0	21.5±0.7	17.5±1.1	17.8±1.3
14:0	22.2±0.5	21.8±0.7	21.2±0.8	21.7±1.2	17.3±1.1	14.3±0.7
16:0	20.2±0.5	21.1±1.0	21.4±1.2	21.6±1.2	16.6±0.5	14.7±0.8
18:0	24.5±0.5	25.0±1.1	25.9±0.7	27.2±2.3	20.0±2.0	17.4±0.6
monoenes	7.0	5.7	6.7	6.0	23.5	30.3
16:1	n.d	n.d	n.d	n.d	n.d	n.d
18:1	7.0±0.4	5.7±0.6	6.7±1.3	6.0±0.5	5.6±0.5	6.0±0.5
20:1 (n-9)	n.d	n.d	n.d	n.d	17.9±1.6	n.d
22:1 (n-9)	n.d	n.d	n.d	n.d	n.d	24.3±2.2
(n-6) PUFA	1.3	n.d	n.d	n.d	n.d	n.d
18:2	1.3±0.4	n.d	n.d	n.d	n.d	n.d
20:4	n.d	n.d	n.d	n.d	n.d	n.d
(n-3) PUFA	n.d	n.d	n.d	n.d	n.d	n.d
18:3	n.d	n.d	n.d	n.d	n.d	n.d
20:5	n.d	n.d	n.d	n.d	n.d	n.d
22:5	n.d	n.d	n.d	n.d	n.d	n.d
22:6	n.d	n.d	n.d	n.d	n.d	n.d

*; significant diferences ($P<0.05$) in total lipid content between diet groups 4/5 and 4/6.
n.d.; not detected

Severals studies have shown that the lipases preferentially hydrolyze in 1 and 3 position of the triacylglycerols (TAG) molecule, leading to the accumulation of 2-monoacylglycerols (MAG) (Brockerhoff and Hoyle, 1967; Gjellesvik *et al.*, 1989; Leger and Bauchart, 1972; Tocher and Sargent;, 1984), although a slower, but significant hydrolysis of 2-monoacylglycerols (MAG) may occur (Leger and Bauchart 1972; Patton *et al.*, 1975). According to

Brockerhoff *et al.*, (1968), most PUFA are located in the 2 position of TAG. Despite this positional disadvantage for hydrolysis, PUFA seem to preferentially hydrolyzed (Lie and Lambertsen, 1985). On the other hand, preferential hydrolysis of 18:1 from TAG has been noted irrespectively of position (Leger and Bauchart, 1972; Patton *et al.*, 1975), while shorter chain saturates seem to accumulate in diacylglycerols (DAG) and MAG (Lied and Lambertsen, 1985).

By using methyl ester derivates of PUFA to circumvent potential lipase specificity, Patton *et al.* (1975) demonstrated that 20:4 (n-6) and 20:5 (n-3) was hydrolyzed more rapidly then 18:2 (n-6) and 18:3 (n-3) in anchovy, stripped brass and pink salmon. These studies have concentrated on lipolytic activity, but, some specificity in the absorption of fatty acids may occur, since Lie *et al.*, (1987) showed that 20:1 and 22:1 accumulate in the intestinal free fatty acid (FFA) fraction when cod, (*Gadus morhua* L.), was fed a test diet with added capelin oil. This observation is in the accordance with earlier data showing that in the capelin oil treated with cod digestive juice, 20:1 (n-9) and 22:1 (n-11) was distributed in MAG, DAG and FFA, while (n-3) PUFA increaded in FFA fraction (Lied and Lambertsen, 1982).

Methyl esters are widely used in studies investigating the requirement and effects of excess amounts of essential fatty acids on growth and lipid composition of fish (Olsen *et al.*, 1990 a and b; Takeuchi and Watanabe, 1979; 1982; Takeuchi *et al.*, 1979 b). In all these studies methyl esters were regarded as TAG in terms of digestion and absorption of the component fatty acids.

The elimination of 20:4 (n-6), 20:5 (n-3) and 22:6 (n-3) from the intestinal contents in the present study, indicates the presence of specific absorption system for these fatty acids compared to the absorption of 20:1 and 22:1. Based on these results, we suggest that Arctic charr exhibits selective absorption of dietary fatty acids.

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