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The influence of temperature on the apparent lipid digestibility in Atlantic salmon (*Salmo salar*) fed *Calanus finmarchicus* oil at two dietary levels

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ABSTRACT

Oils extracted from the marine zooplankton, *Calanus finmarchicus*, have high levels of n-3 highly unsaturated fatty acids (HUFA) and are therefore of interest as an alternative lipid source in aquafeeds. Copepod lipid is composed mainly of wax esters (WE) with high levels of saturated fatty acids and monounsaturated fatty alcohols which are considered hard to digest, especially at low temperatures. This assumption has however not been verified and for this reason the present study examined the digestibility of diets containing high levels of WE and two fat levels in Atlantic salmon reared at 3 and 12 °C. The fish were acclimated for one month to 3 °C (485 g) and 12 °C (599 g) and then fed with one of four diets, high fat fish oil (33% lipid, HFFO), high fat *Calanus* oil (32% lipid, HFCO), low fat fish oil (17% lipid, LFFO) and low fat *Calanus* oil (19% lipid, LFCO). The fish meal lipid content was lowered by the use of lipid-extracted fish meal (2.3% lipid). This enabled a level of 50% WE in the LFCO and HFCO diets, compared to 0% in the LFFO and HFFO diets. The fish were then allowed to grow to around 100% of initial weight (220 days at 3 °C and 67 days at 12 °C) and then analysed for faecal lipid digestibility, bile volume, bile composition and intestinal lipolytic activity. Differences were observed in all of these parameters in relation to temperature, type of dietary oil and the lipid level in the diet. Faecal lipid content and lipid class composition were dependent on rearing temperature and the type of dietary lipid. Highest levels of undigested lipids were observed in the faeces of fish fed with CO. Wax ester-derived fatty alcohols, particularly 20:1n-9 and 22:1n-11, were less extensively digested than corresponding fatty acids from FO at both fat levels and temperatures. Fish kept at 12 °C had a significantly higher bile volume than fish at 3 °C and higher volumes were found in fish fed with CO diets compared to FO. Increased faecal holding time at lower temperature was not sufficient to ensure high digestibility since the lower bile volume and enzyme activities at 3 °C in the present trial exerted a greater effect. Although the compensatory mechanisms of increased bile volume and lipolytic activity are initiated upon feeding WE at a level of 50% of dietary lipid, these are not sufficient to compensate lipid digestibility and growth as in FO diets. Low inclusion of CO in diets during winter has to be considered as saturated fatty acids and monounsaturated fatty alcohols were poorly digested at 3 °C in fish fed with CO diets.

1. Introduction

Salmonids are commonly reared at high latitudes where large fluctuations in sea temperature occur. They have a growth optima at 12–17 °C (Brett, 1971; Koskela et al., 1997a; 1997b), but maintain feeding and growth even at temperatures approaching 0 °C (Fraser et al., 1993; Koskela et al., 1997b). However, the digestive process is influenced by temperature. In Arctic charr (*Salvelinus alpinus*) feed intake is reduced at low temperatures as are the digestive processes and the gastrointestinal holding time in an attempt to maintain optimal nutrient uptake (Olsen and Ringø, 1998). However, the results of studies with other salmonid fish are inconclusive. Although most trials have shown increased macronutrient digestibility with increasing temperature (Atherton and Aitken, 1970; Brauge et al., 1995; Olsen and Ringø, 1998; Bendiksen et al., 2003), there are also reports showing no significant effect on nutrient availability. For example, in rainbow trout (*Oncorhynchus mykiss*) reared at 3 and 11 °C (Austreng, 1978) and 7, 11 and 15 °C (Windell and Norris, 1969), temperature had no effect on lipid and fatty acid digestibility. However, rates of fatty acid digestibility are known to decrease with increasing chain length, and increase with increasing

Abbreviations: LPC, Lysophosphatidylcholine; SM, Sphingomyelin; PC, Phosphatidylcholine; PE, Phosphatidylethanolamine; UPL, Unidentified polar lipids; C, Cholesterol; FFAlc, Free fatty alcohols; FFA, Free fatty acids; UNL, Unidentified neutral lipid; TAG, Triacylglycerol; WE/SE, Wax ester/Sterol ester.

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unsaturation (Sigurgisladottir et al., 1992; Johnsen et al., 2000). This is to a large extent related to melting point (Olsen and Ringø, 1997). Thus, some studies have shown that digestibility of saturated fatty acids (SFA) is reduced at lower temperature, while the digestibility of monounsaturated fatty acids (MUFA) and polyunsaturated fatty acids (PUFA) is less affected (Olsen and Ringø, 1998; Ng et al., 2003; Ng et al., 2004). This change in lipid digestibility may be at least partly responsible for the maintenance of proper cell membrane fluidity, through homeoviscous acclimation, that occurs when ectotherms are exposed to low temperature (Wallaert and Babin, 1994; Fodor et al., 1995; Farkas et al., 2001), since low temperature acclimatized fish possess greater proportions of PUFA and reduced amounts of SFA in membranes (Hagar and Hazel, 1985; Olsen and Henderson, 1997). As such, winter performance might be affected when high levels of vegetable oils are included in feeds for salmonids that are farmed at high latitudes, due primarily to the low concentrations of n-3 highly unsaturated fatty acid (HUFA) present in these oils (Bendiksen et al., 2003).

Marine fauna from lower trophic levels could thus be a good alternative, as these sources contain naturally high levels of n-3 HUFA. The zooplankton, *Calanus finmarchicus*, is considered as an alternative lipid source in aquafeeds (Olsen et al., 2010). These animals have a high level of wax esters (WE), compared to the triacylglycerols (TAG) that are the most abundant neutral lipid in most fish species (Sargent et al., 1976). Wax esters are esters of long-chain fatty acids and fatty alcohols and are intrinsically more hydrophobic than TAG, making them harder to digest than TAG (Bauermeister and Sargent, 1979). Several trials have shown that WE levels above 40% of the dietary lipid (ca 30% lipid diets) reduce growth and lipid digestibility in Atlantic salmon, while lower levels, allow the same growth and lipid digestibility as fish fed fish oil diets (Olsen et al., 2004; Bogevik et al., 2009; Oxley et al., 2009). These previous studies have, however, been carried out at single relative high temperatures and did not make any comparisons with the situation at lower temperatures. Consequently, the intention of the current study was to study the effect of low environmental temperatures (3°) on WE utilization and digestive capability (bile volume, bile composition, midgut lipolytic activity) in Atlantic salmon.

2. Materials and methods

2.1. Fish, diets and experimental design

Three hundred and sixty Atlantic salmon (*Salmo salar* L., Mowi strain; Norwegian breeding programme, 13 month-old post-smolts) originally held at 9 °C, averaging 447 g were anaesthetized in 0.1% (w/v) MS-222 (tricaine methane sulphonate; Norwegian Medical Depot, Bergen, Norway) and measured for weight and length. The fish were then distributed equally between 24 1.5 × 1.5 × 1.0 m fibre glass tanks supplied with aerated seawater. The fish were then acclimatized to the experimental temperatures gradually over 1 month from 9 °C to either 3 or 12 °C, with twelve tanks in each temperature group. The fish grew through the acclimation period to an average of 485 g in the cold water group (3.1 ± 0.4 °C) and 599 g in the warm water group (12.3 ± 0.4 °C).

Four diets were prepared at NOFIMA (Bergen, Norway) as outlined in detail previously (Olsen et al., 2004) and contained 0.01% yttrium oxide as a marker of digestibility (Table 1). The only exception being that the fish meal was lipid-extracted fish meal obtained from TripleNine Fish protein amba (Esbjerg, Denmark) containing 2.3% lipid. The diets were designed to be low (ca 18%) and high (33%) in lipid. One low lipid diet contained oils extracted from the marine copepod *C. finmarchicus* (termed LFCO) while the other contained fish oil (LFFO). Likewise, the high lipid diets were either added *Calanus* oil (HFCO) or fish oil (HFFO). Further details on the composition are given in Table 1.

The fish were then fed with the four diets in triplicate tanks at both temperatures. In order to attain a fairly similar end weight, fish at low temperature were fed for 220 days, while those in the high temperature groups were fed for 67 days. All fish were fed to satiation twice a day using ArvoTec TD2000 feeders (Huutokoski, Finland). After the experimental period had elapsed, fish were anaesthetized in 0.1% MS-222 and measured for weight and length. Faeces were stripped from fish according to Ringø (1991), the samples from tanks were pooled, and stored at −80 °C prior to analysis. Five fish from each tank were killed by a sharp blow to the head. The luminal content of the midgut regions was then collected for analysis of lipolytic enzyme activity (Tocher and Sargent, 1984; Bogevik et al., 2008). The remaining fish were starved for 72 h. Then, five fish from each tank were anaesthetized and killed as above, and bile collected from the gall bladder with a 5 mL syringe with 0.1 mL resolution. After recording the volume, the bile was stored at −80 °C for analysis of bile salts and osmolality. Remaining fish from each triplicate group were then pooled and cross-fed with the respective opposite dietary fat source. Thus, fish previously fed with HFCO were now fed with HFFO, and those previously fed with HFFO were now fed with HFCO. The same was done for low fat fed fish, i.e. LFCO-fed fish were now given LFFO and those previously fed with LFFO were now fed with LFCO. After 1 week, the fish were anaesthetized, and the faeces were collected as described above.

Table 1
Formulation (g kg^{-1} diet) and gross composition (%) of the Atlantic salmon experimental diet.

	HFFO	HFCO	LFFO	LFCO
TripleNine fish meal[a]	417.0	417.0	575.0	575.0
Fish oil[b]	289.0	0.0	131.0	0.0
Calanus oil	0.0	289.0	0.0	131.0
Soya lecitin	5.0	5.0	5.0	5.0
Soya protein[c]	60.0	60.0	60.0	60.0
Wheat 230/05[d]	140.0	140.0	140.0	140.0
Wheat gluten 156/05[e]	80.0	80.0	80.0	80.0
Vitamin mixture[f]	10.0	10.0	10.0	10.0
Mineral mixture[g]	4.0	4.0	4.0	4.0
Charophyll Pink (10%)	0.3	0.3	0.3	0.3
Yttrium oxide (Y_2O_3)	0.1	0.1	0.1	0.1
Dry matter	93.0	93.4	91.3	90.7
Protein	43.0	44.4	54.8	55.3
Lipid	33.3	32.4	17.3	18.6
Total polar lipid	6.4	6.6	11.5	11.6
Cholesterol[h]	8.6	4.2	8.1	5.1
Free fatty acids	12.5	15.4	12.5	13.9
Triacylglycerol	62.3	21.2	55.0	19.8
Wax esters/Sterol esters	10.1	52.7	12.8	49.6

Feed codes are as follows: LF, low fat; HF, high fat; FO, fish oil; CO, *Calanus* oil.
[a] Fish meal: low fat, TripleNine, Denmark (89.1% dry matter, 76.5% crude protein, 2.3% fat (soxhlet) and 13.1% ash).
[b] Fish oil: NorSalmOil, Norsildmel, Bergen, Norway.
[c] Soya protein: soya protein concentrate (SPC 70), Sopropeche, Boulogne, France.
[d] Wheat: Norgesmøllene, Bergen, Norway.
[e] Wheat gluten: received from Ewos Innovation, Dirdal, Norway.
[f] Diets supplied with the following vitamins per kg diet: vitamin D3, 3000 I.E; vitamin E (Rovimix, 50%), 160 mg; thiamine, 20 mg; riboflavin, 30 mg; pyridoxine-HCl, 25 mg; vitamin C (Riboflavin Stay C 35%), 200 mg; calcium pantothenate, 60 mg; biotin, 1 mg; folic acid, 10 mg; niacin, 200 mg; vitamin B12, 0.05 mg; and menadione bisulphite, 20 mg.
[g] Diets supplied with the following minerals per kg diet: magnesium, 500 mg; potassium, 400 mg; zinc, 80 mg; iron, 50 mg; manganese, 10 mg; copper, 5 mg.
[h] May contain some diacylglycerol.

2.2. Analysis of diets and faeces

Diets and faeces were freeze-dried to obtain dry weight, followed by analysis of yttrium oxide according to Otterå et al. (2003). Yttrium was determined in feed and faeces by use of an ICP-MS (inductive-coupled plasma-mass spectrometry) method after wet digestion in a microwave oven (Otterå et al., 2003). Total lipid of diets and faeces