Influence of dietary conjugated linoleic acid (CLA) and tetradecylthioacetic acid (TTA) on growth, lipid composition and key enzymes of fatty acid oxidation in liver and muscle of Atlantic cod (*Gadus morhua* L.)

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Keywords: Gadus morhua; CLA; TTA; lipid; fatty acid; composition; liver; flesh; Atlantic cod

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Abstract

The aim of the present study was to determine the effects of conjugated linoleic acid (CLA) and tetradecylthioacetic acid (TTA) on growth performance, and lipid and fatty acid metabolism in Atlantic cod. The overall objective being to test the hypotheses that CLA and TTA have beneficial effects in cod culture including decreased liver size and proportion through decreased lipid content, and increased nutritional quality through effects on fatty acid compositions including accumulation of bioactive fatty acids, CLA and TTA, in flesh. Juvenile cod were fed for three months on fish meal and fish oil diets of basically commercial formulation, but containing either 0.5% or 1% CLA, or 0.5% TTA. The effects of the functional fatty acids on growth, feed efficiency, body proximate composition, liver weight and lipid composition, fatty acid compositions of flesh and liver, and key enzymes of fatty acid oxidation were determined. Dietary CLA and TTA had no effect on growth parameters in cod juveniles, but viscero- and hepato-somatic indices were increased in fish fed 0.5% CLA and TTA, respectively. Proximate composition of whole fish was not affected by CLA or TTA, and there were no major effects of either functional fatty acid on lipid contents and compositions of liver and flesh. Dietary CLA and TTA were both incorporated into tissue lipids, with CLA deposited to a greater extent in liver, whereas TTA was deposited to a greater extent in flesh. In liver, acyl CoA oxidase (ACO) activity, but not carnitine palmitoyltransferase-I (CPT-I), was increased by CLA, whereas dietary TTA increased both ACO and CPT-I activities. In contrast, ACO activity was reduced by both CLA and TTA in red and white muscle, whereas CPT-I activity was generally not affected by CLA and TTA in either muscle tissue. Therefore, the results only partially supported the hypotheses tested, as CLA and TTA had few beneficial effects in Atlantic cod and did not enhance growth parameters, or improve feed conversion or potential yield through decreased adiposity or liver lipid deposition. However, nutritional quality could be enhanced, and cod fed CLA and/or TTA could be beneficial in the human diet, through provision of bioactive fatty acids with no detrimental effects on n-3 PUFA levels.

1. Introduction

Aquaculture is the fastest growing animal-based agricultural food production sector, expanding at more than 9% per year, and currently contributes over one third of all the fish in the human food basket (Tacon, 2003). Farming of finfish in seawater is dominated by Atlantic salmon (*Salmo salar* L.), but other marine species are becoming increasingly important, including warmer water species such as gilthead sea bream (*Sparus aurata*), European sea bass (*Dicentrarchus labrax*) and turbot (*Psetta maximus*) and, in colder waters, halibut (*Hippoglossus hippoglossus*). Recently, declining catches and high market prices have seen Atlantic cod (*Gadus morhua* L.) emerge as the most promising species for culture in the northern Atlantic area (Brown and Puvanendran, 2002; Brown et al., 2003) with production expanding in Norway and Scotland. A major factor influencing the commercial success of cod culture will be the development of diets and feeding strategies to maximise growth and feed conversion efficiencies (Morais et al., 2001; Lall and Nanton 2002; Hemre et al., 2003, 2004; Rosenlund et al., 2004). One major issue pertaining to this is that cod store lipid in the liver and, in early studies, farmed fish were reported to have enlarged livers and display a hepato-somatic index (HSI) of over 12% compared to values of 2-6% in wild fish (Jobling, 1988). In early feeding trials with captive cod, the HSI increased from 9.5% to an average of 13%, and liver fat increased from around 55% to 67-70% in cod fed various dietary oils (Lie et al., 1986). Further studies have consistently shown that farmed cod have high liver lipid levels that can exceed 70% of wet weight, and have higher HSI and condition factor (K) than their wild counterparts (Dos Santos et al., 1993; Shahidi and Dunajski, 1994; Grant et al., 1998; Gildberg, 2004). Dietary formulations in aquaculture have tended to exhibit an upward trend in dietary lipid as this has successfully increased weight gains, but several studies have shown that a potential detrimental side-effect of high fat diets is the deposition of excess lipid in tissues (Sargent et al., 2002; Tocher, 2003). This may exacerbate the enlarged fatty liver

problem in farmed cod and so it is important to gain a clearer understanding of the mechanisms determining lipid and fatty acid homeostasis and deposition.

Conjugated linoleic acid (CLA) describes a group of geometric and positional conjugated isomers of linoleic acid (18:2n-6) that are found in dairy products and meat, with the two main naturally occurring isomers being cis-9,trans-11 and trans-10,cis-12 (Pariza et al., 2001). There is increasing evidence that dietary CLA decreases body fat and increases lean body mass (Thiel-Cooper et al., 2001; Tischendorf et al., 2002; Yamasaki et al., 2003), and thus attenuates obesity in several animal models (Delany and West, 2000; Wang and Jones, 2004). Proposed anti-obesity mechanisms of CLA include decreased energy/food intake and increased energy expenditure (Ohnuki et al., 2001; Terpstra et al., 2002), decreased preadipocyte differentiation and proliferation (Evans et al., 2000), decreased lipogenesis (Brown et al., 2001; Oku et al., 2003), and increased lipolysis and fatty acid oxidation (Evans et al., 2002). Thus, dietary CLA could be beneficial to cod culture if these effects on body composition and lipid metabolism could be reproduced in farmed fish.

Other bioactive fatty acids include the sulfur-substituted analogs such as the 3-thia fatty acid tetradecylthioacetic acid [CH_3-$(CH_2)_{13}$-S-CH_2-CO_2H; TTA] (Berge et al., 1989). TTA cannot be β-oxidized due to the position of the sulfur in the carbon chain and thus it is metabolized in mammals via ω-hydroxylation in the endoplasmic reticulum followed by peroxisomal oxidation from the ω-end producing short chain sulfoxy dicarboxylic acids (Skrede et al., 1997). In mammals, TTA increases both liver and muscle mitochondrial and peroxisomal fatty acid oxidation, decreases plasma lipids and adipose tissue mass, and increases free fatty acid transport from peripheral tissues to liver (Berge et al., 2002). Therefore, TTA is another fatty acid analog that could have effects on lipid and fatty acid metabolism in Atlantic cod that may be beneficial in a farming context.

The aims of the present study were to determine the effects of CLA and TTA on growth performance, lipid content, composition and metabolism in Atlantic cod. The overall objective being to

test the hypotheses that CLA and TTA have beneficial effects in cod culture including decreased liver size and proportion through decreased lipid content, and increased nutritional quality through effects on fatty acid compositions including accumulation of bioactive fatty acids, CLA and TTA, in flesh. Juvenile cod were fed for three months on fish meal and fish oil diets of basically commercial formulation, but containing either 0.5% or 1% CLA, or 0.5% TTA. The effects of the functional fatty acids on growth, feed efficiency, body proximate composition, liver weight and lipid composition, fatty acid compositions of flesh and liver, and key enzymes of fatty acid oxidation were determined.

2. Materials and methods

2.1. Diets and Animals

The dietary trial was performed at Viking Fish Farms, Ardtoe Marine Laboratory, Ardnamurchan, Scotland, between October 2005 and January 2006. Hatchery reared Atlantic cod of the 2004 year class were randomly distributed between twelve indoor, round tanks of 1.5m^3 volume (1.72m diameter). The initial stocking density was 50 fish of average fish weight 127 ± 15g per tank (5.8 kg/m^3), with 25 fish per tank individually PIT tagged (Passive Induced Transponder, Fish Eagle, Gloucestershire, England) prior to stocking. Water temperature was maintained at 12 ºC (±1 ºC) throughout the trial, with a light regime of 12L:12D. Four experimental diets were fed to triplicate tanks for three months, with feed supplied to appetite manually in one morning feed over a period of one hour. Waste feed pellets were collected and counted one hour later. The experimental diets were formulated to satisfy the nutritional requirements of marine fish (National Research Council 1993), and were formulated and manufactured by BioMar A/S, Brande, Denmark (Table 1). Isonitrogenous diets were based on fish meal and standard Northern hemisphere fish oil with CLA and TTA replacing some of the fish oil to produced diets containing 0, 0.5 and 1% CLA, and 0.5% TTA (as percentage of total

diet). Diets were identical in formulation other than fatty acid composition with CLA (LUTA-CLA^{TM} 60, containing 60% CLA methyl esters as a 50:50 mixture of c9, t11 and t10, c12 isomers; BASF AG, Ludwigshafen, Germany) and TTA (supplied by Dr Rolf Berge, Thia Medica A.S., Bergen, Norway) balanced by fish oil (capelin oil, Norsemeal Ltd., London, UK). The fatty acid compositions of the diets are presented in Table 2.

2.2. Sampling protocols

At the initiation and termination of the trial, all the fish in each tank were anaesthetized with Metomidate (50 mg/L), identified by PIT tag if present, and individually weighed and fork length recorded. At the end of the trial, 9 fish per tank (27 per dietary treatment) were killed by percussion stunning then sampled for compositional analyses, with 3 whole fish/tank frozen immediately at -20 ºC for whole body compositional (proximate) analyses. The remaining sample fish were eviscerated and used for biometric determinations (hepato-, and viscero-somatic indices) and for tissue lipid analyses. White (fast) and red (slow) muscle samples were excised from the epaxial myotomes anterior to the first dorsal fin ray (Flesh Quality Cut) and livers were taken from six fish, pooled in two samples of 3 fish each, and frozen immediately in liquid nitrogen (livers) or dry ice (flesh). All samples were subsequently stored at –80 ºC prior to analyses.

2.3. Proximate analyses

Moisture content of whole fish was determined after drying in an oven at 80 ^{0}C for a minimum of 72 h. The dried fish samples were then rigorously blended into a homogeneous crumble/meal and used for determination of whole body lipid, protein and ash contents. Lipid content in 1 g samples of dried fish

crumb was determined using the Soxhlet method with extraction in petroleum ether at 120 ºC (Avanti Soxtec 2050 Auto Extraction apparatus; Foss, Warrington, UK). Protein content (N x 6.25) was determined in the fish crumble using the automated Kjeldahl method (Tecator Kjeltec Auto 1030 Analyser; Foss, Warrington, UK). Ash contents were determined after heating portions of the fish crumble at 160 ºC for 48 h.

2.4. Lipid analyses

Liver and skinned and deboned flesh samples, each consisting of three fish, were homogenized into pooled "pates". Total lipid was extracted from diets or 1g portions of tissue pates by homogenizing in 20 volumes of chloroform/methanol (2:1, v/v) in an Ultra-Turrax tissue disrupter (Fisher Scientific, Loughborough, U.K.). Total lipid was prepared according to the method of Folch et al. (1957) and lipid content determined gravimetrically as described previously (Kennedy et al., 2005). Tissue lipid class compositions were determined by single-dimension double-development high-performance thin-layer chromatography (HPTLC) and densitometry using a Camag 3 TLC Scanner (Camag, Muttenz, Switzerland) and winCATS software as described in Kennedy et al. (2005). Fatty acid methyl esters (FAME) from diets and tissue total lipid were prepared by acid-catalyzed transesterification of total lipid similar to the method of Christie (1982) except that the reaction was performed at 80 ºC for 3 h. Extraction and purification of FAME was performed as described by Tocher and Harvie (1988). FAME were separated and quantified by gas-liquid chromatography (Carlo Erba Vega 8160, Milan, Italy) using a 30m x 0.32 mm i.d. capillary column (CP Wax 52CB, Chrompak, London, U.K.) and on-column injection. Hydrogen was used as carrier gas and temperature programming was from 50ºC to 150ºC at 40ºC min^{-1} and then to 230ºC at 2.0ºC min^{-1}. Methyl esters were identified and quantified as described previously (Kennedy et al., 2005).

2.5. Assay of carnitine palmitoyl acyltransferase I (CPT-I) and acylCoA oxidase (ACO)

Liver, red and white muscle were weighed, diced and homogenized to 20% (w/v) in 0.25 M sucrose in 10 mM N-2-hydroxyethylpiperazine-N´-2-ethanesulfonic acid (HEPES) buffer and 1 mM EDTA, pH 7.4. The homogenates were centrifuged at 1880 x g for 10 min at 4˚C, the floating fat layer aspirated and the post-nuclear fractions collected, 100 µl taken for protein determination according to Lowry et al. (1957) after incubation with 0.45 ml of 0.25% (w/v) SDS/1M NaOH for 45 min at 60^{o}C, and portions used immediately for determination of enzyme activities. CPT-I activity was estimated by determining the production of palmitoyl[^{3}H]carnitine from palmitoyl CoA and [^{3}H]carnitine essentially as described by Saggerson and Carpenter (1986). ACO activity was measured by a spectrophotometric assay based on the determination of hydrogen peroxide production coupled to the oxidation of leuco-dichlorofluorescein (DCF) in a reaction catalysed by exogenous peroxidase (Small et al., 1985).

2.6. Materials

[Methyl-^{3}H] L-carnitine hydrochloride (60-86 Ci/mmol) was obtained from GE Healthcare Bio-Sciences (Little Chalfont, Bucks, U.K.). Aminotriazole, BHT, carnitine, dichlorofluorescein diacetate, dimethylformamide, dithiothreitol, EDTA, FAF-BSA, horseradish peroxidase, leuco-DCF, N-acetylcysteine, NADP, palmitoyl-CoA and triton X-100 were obtained from Sigma Chemical Co. (Poole, U.K.). TLC (20 cm x 20 cm x 0.25 mm) and HPTLC (10 cm x 10 cm x 0.15 mm) plates, precoated with silica gel 60 (without fluorescent indicator) were obtained from Merck (Darmstadt, Germany). All solvents were HPLC grade and were obtained from Fisher Scientific UK, Loughborough, England.

2.7. Statistical analysis

All data are presented as means ± SD (n value as stated). Percentage data and data which were identified as non-homogeneous (Bartlett's test) were subjected to arcsine transformation before analysis. The effects of dietary CLA and TTA were determined by one-way analysis of variance (ANOVA) with Tukey's post-tests to determine significance of differences due to functional fatty acids. Differences were regarded as significant when $P < 0.05$ (Zar, 1984).

3. Results

3.1. Diet compositions

The control fish oil diet contained 41% total polyunsaturated fatty acids (PUFA), including 14% eicosapentaenoic acid (20:5n-3; EPA), 13% docosahexaenoic acid (22:6n-3; DHA) and almost 6% 18:2n-6, 32% total saturates, mainly 16:0, and 27% total monoenes, mainly 18:1n-9 and 16:1n-7 (Table 2). Inclusion of CLA in the diets resulted in levels of total CLA of 3.5% and 6.3% of total fatty acids at the 0.5 and 1% inclusion levels, respectively. CLA inclusion resulted in lower levels of 14:0, 16:0, EPA and DHA, but 18:1n-9 and 18:2n-6 (the other main components of the CLA mixture) increased (Table 2). Inclusion of 0.5% TTA resulted in TTA at a level of 2.8% of total fatty acids in the diet, and lower levels of 14:0 and 18:0.

3.2. Growth, biometry and whole body proximate compositions

Growth of the cod was unaffected by either CLA or TTA with neither having any significant effect on final weight, SGR or thermal growth coefficient (TGC) (Table 3). Viscero-somatic index (VSI) was lower in fish fed 0.5% CLA, and HSI was lower in fish fed TTA with condition factor slightly lower in fish fed these diets compared to fish fed fish oil. There were slight effects on feed efficiency (FCR) and gutted weight that were unlikely to be commercially important. Neither dietary CLA nor TTA had any effect on the proximate compositions of whole fish (Table 4).

3.3. Lipid contents and class compositions of liver and flesh

The lipid content of the livers, which varied between 50 and 55% of the wet weight, was not strongly affected by dietary CLA or TTA (Table 5). Neutral lipids accounted for around 97% of liver total lipid with triacylglycerol (TAG) accounting for between 91 and 93%, and these components were slightly lower in fish fed the diet containing 1% CLA. TTA had no significant effect on liver neutral lipid or TAG levels. Dietary CLA and TTA had no effect on flesh lipid contents, which were constant at 0.8% of wet weight (Table 5). Polar lipids, mainly phosphatidylcholine (PC) and phosphatidylethanolamine (PE), predominated in flesh accounting for 57 to 61% of total lipid, with neutral lipids, mainly cholesterol and free fatty acid (FFA), accounting for 39 to 43%. FFA and steryl esters were highest in fish fed 0.5% CLA, whereas flesh TAG was slightly, but significantly, higher in fish fed TTA (Table 5).

3.4. Fatty acid compositions of liver and flesh

Dietary CLA and TTA were both incorporated into tissue lipids, but it was noteworthy that CLA was deposited to a greater extent in liver lipids (Table 6), whereas TTA was deposited to a greater extent in

flesh (Table 7). Thus, CLA accounted for 1.5 and 2.9% of total fatty acids in livers of fish fed 0.5 and 1% CLA, respectively, whereas TTA accounted for 0.6% of liver fatty acids in fish fed 0.5% TTA (Table 6). In contrast, TTA accounted for 1.6% of flesh fatty acids in TTA-fed fish, compared to 0.8 and 1.9% CLA in fish fed the lower and higher CLA, respectively (Table 7). Dietary CLA had no significant effect on the proportions n-3 or n-6 PUFA in either liver or flesh, but in fish fed TTA there were decreased percentages of monoenes, n-6 PUFA and EPA, but an increased proportion of DHA in the flesh (Tables 6 and 7). Dietary CLA resulted in increased percentages of 18:0 and decreased percentages of 18:1n-9 and total monoenes in both tissues, but especially liver.

3.5. Carnitine palmitoyltransferase I (CPT-I) and acylCoA oxidase (ACO) activities in liver, red and white muscle

There was a trend for CPT-I activity in liver to be increased by dietary CLA and TTA, although it was only significant in the case of TTA (Fig.1). In red muscle, a dietary level of 1% CLA resulted in increased CPT-1 activity, but 0.5% dietary CLA or TTA had no effect. In contrast, CPT-1 activity in white muscle was not affected by any dietary treatment. ACO activity in liver was significantly increased by both dietary CLA and TTA (Fig.2). Conversely, ACO activity in both red and white muscle was decreased by CLA and TTA, with the effects being significant for CLA in red muscle and TTA in white muscle.

4. Discussion

Feeding CLA reduced liver TAG levels in rats (Rahman et al., 2002), and so the primary hypothesis we aimed to test in the present trial was that dietary CLA or TTA could have beneficial effects on lipid

metabolism in Atlantic cod, specifically that they could lower liver lipid levels, and liver size. The results show clearly that the hypothesis was not proved and that neither of these bioactive fatty acids had a major effect on liver lipid content or relative liver size as determined by HSI in cod. TTA did induce a statistically significant decrease in HSI compared to control fish fed FO alone or fish fed CLA, but the index was still over 10%, and there was no difference in liver lipid content. In contrast, TTA fed to Atlantic salmon smolts at 0.6% of the diet resulted in a slight, but significant, increase in HSI and higher liver lipid content, although the latter was not significant (Moya-Falcon et al., 2004). In the present trial, CLA at 0.5% inclusion resulted in a higher HSI in the cod, and increased HSI in response to feeding CLA had been previously reported in hybrid striped bass (*Morone saxatilis* x *M. chrysops*) (Twibell et al., 2000), yellow perch (*Perca flavescens*) (Twibell et al., 2001) and tilapia (*Oreochromis niloticus*) (Yasmin et al., 2004). Perhaps surprisingly, liver lipid content was reduced by CLA in striped bass and yellow perch despite the increased HSI (Twibell et al., 2000, 2001). In Atlantic salmon smolts there were trends of increasing HSI and liver lipid in fish fed CLA at 1 and 2% of the diet, although the data were not statistically significant (Kennedy et al., 2005). In contrast, CLA at up to 1% had no effect on HSI or liver lipid content in juvenile channel catfish (*Ictalurus punctatus*) (Twibell et al., 2003), and HSI was unaffected by dietary CLA at up to 2% in rainbow trout juveniles (Figueirdo-Silva et al., 2005).

Dietary CLA can have beneficial effects on body composition in mammals, with decreased body fat and increased lean body mass being reported in mice, rats and pigs (Wang and Jones, 2004). However, in the present trial with cod, neither CLA nor TTA had any effect on proximate composition of whole fish. Whole body proximate composition was also unaffected by dietary CLA in salmon fry and smolts (Berge et al., 2004; Kennedy et al., 2005), and rainbow trout juveniles (Figueirdo-Silva et al., 2005). Similarly, dietary CLA had no effect on carcass lipid or intraperitoneal fat in catfish (Twibell and Wilson, 2003), or on tissue lipid contents in tilapia (Yasmin et al., 2004). Furthermore,

VSI was unaffected by dietary CLA up to 2% in both Atlantic salmon smolts (Kennedy et al., 2005) and rainbow trout (Figueirdo-Silva et al., 2005). However, intraperitoneal fat was decreased by dietary CLA in hybrid striped bass (Twibell et al., 2000) and, in the present trial, visceral fat may have been reduced in cod fed 0.5% CLA as VSI was lower in this group along with higher HSI, which may suggest some redistribution of fat. However, fat redistribution has not been apparent in other trials with fish.

Studies have suggested that CLA increased fatty acid oxidation in mouse liver (Degrace et al., 2004) and a variety of tissues in rats (Rahman et al., 2001) via an increase in CPT-I activity. The present study showed that CPT-I activity in cod liver was not increased by dietary CLA, suggesting that mitochondrial β-oxidation would not be increased. Measuring this parameter would require fresh tissue but, unfortunately, assays using radioactive isotopes were not possible at the commercial farm site. In contrast, ACO in liver was significantly increased by dietary CLA in cod, suggesting that dietary CLA may have a greater effect on peroxisomal rather than mitochondrial fatty acid oxidation in cod liver. Interestingly, fatty acid β-oxidation activity was recently measured in a related gadoid, haddock *Melanogrammus aeglefinus L.*, and it was shown that peroxisomal β-oxidation predominates over mitochondrial β-oxidation in liver (Nanton et al., 2003). Similarly, fatty acid oxidation in Atlantic salmon liver is principally due to peroxisomal, rather than mitochondrial, β-oxidation (Frøyland et al., 2000). In contrast, both CPT-I and ACO were significantly increased by dietary TTA in liver, which suggests that TTA could increase fatty acid oxidation in cod liver. Previously, TTA was shown to increase β-oxidation capacity in both homogenates and the mitochondrial fraction of liver in salmon (Vegusdal et al., 2005). In contrast to liver, ACO activity was generally decreased by both CLA and TTA in the muscle tissues. Although this could indicate lower peroxisomal fatty acid oxidation, it is unlikely to have a physiologically significant effect as fatty acid oxidation activity in both red and white muscle of gadoids is predominantly mitochondrial rather than peroxisomal (Nanton et al., 2003).

However, it does not appear that dietary CLA or TTA have any significant effect on mitochondrial fatty acid oxidation in cod muscle tissues as, other than slightly increased CPT-I activity in red muscle of fish fed 1% CLA, CPT-I activity was not affected by dietary TTA or the lower level of CLA. In Atlantic salmon, fatty acid oxidation capacity was not affected by dietary CLA in either red or white muscle (Kennedy et al., 2006).

A further aim of the present trial was to determine if dietary CLA or TTA had beneficial effects on fatty acid compositions in Atlantic cod. This part of the study revealed a very interesting result with TTA accumulating in flesh to a greater extent than the equivalent dietary level of CLA and also to a greater extent than in liver. In contrast, CLA was incorporated to a greater extent in liver lipids compared to flesh. TAG predominates in liver of cod whereas polar lipids predominate in flesh, and so these data suggest that TTA may be deposited to a greater extent in polar lipids (phospholipids) and CLA to a greater extent in TAG. In Atlantic salmon fed TTA, flesh fatty acid compositions were not reported but, of the tissues investigated, the highest incorporation was found in gills with approximately equal percentages, 0.8% and 0.7% of total fatty acids in phospholipids and TAG, respectively, in fish fed TTA at 0.6% of total diet (Moya-Falcon et al., 2004). Furthermore, TTA in both heart and liver was only recovered in phospholipids and not TAG. Thus it appears that bioactive fatty acids similar to, and including, TTA could be efficiently accumulated in 'lean" fish such as cod with low flesh oil contents. It remains to be established whether fatty acid analogues such as TTA are feasible or, indeed, appropriate as supplements for the human diet (Berge et al., 2002). However, CLA may be better delivered to humans via oily fish with the level accumulating in salmon and trout fed CLA at 2% of diet reaching 7% in flesh (dietary lipid 16-17%), (Kennedy et al., 2005; Bandarra et al., 2006), or 4% in flesh of salmon smolts (dietary lipid 34%) (Kennedy et al., 2005), and 7% in whole salmon fry fed 2% CLA (24% dietary lipid) (Berge et al., 2004). Similarly, striped bass with high flesh lipid (>15%) accumulated CLA to over 7% of total fatty acids in fish fed CLA at 1% of diet, whereas the levels of CLA accumulated in yellow perch with only 3% lipid in the flesh were much lower

(Twibbell et al., 2000, 2001). Consistent with the above, the incorporation of CLA into neutral lipids was around 10-fold higher than incorporation into polar lipids in both muscle and liver in tilapia (Yasmin et al., 2004).

In mammals, CLA decreased the activity and gene expression of stearoyl coenzyme A desaturase (SCD) (Choi et al., 2001, 2002), and dietary CLA increased saturated fatty acids and decreased 18:1 and monoenes in pig muscle and fat (Ramsay et al., 2001). In the present study, increased proportions of 18:0 and decreased 18:1n-9 were observed in flesh and, especially, liver of cod fed CLA suggesting inhibition of Δ9 desaturation. Increased 18:0 and decreased 18:1n-9 was previously reported in fatty acids of Atlantic salmon fry, and liver and flesh of smolts fed CLA suggesting that SCD activity was reduced (Berge et al., 2004: Kennedy et al., 2005). Similarly, increasing levels of dietary CLA increased the proportions of 18:0 and decreased percentages of 18:1 in liver, muscle and viscera of rainbow trout juveniles (Bandarra et al., 2006), and in liver and muscle of striped bass and yellow perch (Twibell et al., 2000, 2001).

There is also evidence that CLA suppresses PUFA desaturases and elongase in cell systems (Chuang et al., 2001a,b; Eder et al., 2002), and CLA decreased C_{18} PUFA in pig muscle and fat (Ramsay et al., 2001), and DHA in chicken tissues (Yang et al., 2003). Decreased tissue PUFA levels after feeding CLA has been reported in yellow perch and tilapia (Twibell et al., 2001; Yasmin et al., 2004). In striped bass, CLA increased PUFA levels in liver but decreased PUFA in muscle (Twibell et al., 2000). Dietary CLA increased total n-3PUFA, especially DHA, in salmon fry (Berge et al., 2004), but had no effect on PUFA levels in liver, and appeared to be deposited in flesh at the expense of EPA and DHA, in salmon smolts (Kennedy et al., 2005). In the present study, CLA had no effect on PUFA levels in either liver or flesh of cod. This was perhaps not unexpected as the activity of the PUFA desaturation/elongation pathway is very low in cod (Bell et al., 2006). In contrast, however, dietary TTA significantly increased the percentage of DHA, and decreased the proportions of EPA and total n-6PUFA in flesh of cod. This could be a result of increased conversion of EPA to DHA, but there is no

evidence from previous studies to support TTA having an effect on fatty acid desaturation or elongation (Berge et al., 2002). Therefore, it may be more likely due to specificity of β-oxidation, with DHA being more resistant to oxidation than EPA (Tocher, 2003).

Although not consistently observed, some studies had suggested that CLA might enhance growth and feed efficiency in young rodents (Pariza et al., 2001). Thus, a further aim of the present trial was to determine if dietary CLA or TTA had beneficial effects on growth parameters in Atlantic cod. However, neither CLA nor TTA had any effect on growth (SGR, TGC) or feed efficiency (FCR) in the present trial. In recent studies on salmonids, no effects of dietary CLA on growth rates or FCR were observed in Atlantic salmon fry (Berge et al., 2004), or smolts (Kennedy et al., 2005), or in juvenile rainbow trout (Figuierdo-Silva et al., 2005) fed diets containing up to 2% CLA. Similarly, no effects on weight gain or feed efficiency were noted in juvenile yellow perch or catfish fed diets containing up to 1% CLA (Twibell et al., 2001; Twibell and Wilson, 2003), or in juvenile tilapia fed CLA at up to 5% of diet (Yasmin et al., 2004). However, growth of tilapia was inhibited by 10% CLA, as was growth of carp (*Cyprinus carpio*) and rockfish (*Sebastes schlegeli*) at both 5 and 10% CLA (Choi et al., 1999). Therefore, the data are consistent in suggesting that dietary CLA does not have any beneficial effects on growth performance in a variety of fish species, and can inhibit growth at high inclusion levels. In contrast, in the only other study of dietary TTA in fish, growth was inhibited in salmon smolts as evidenced by decreased final weights, SGRs and TGCs, although FCR was unaffected (Moya-Falcon et al., 2004).

5. Conclusion

The results of the present study only partially supported the hypotheses that were tested. CLA and TTA at the levels used had few beneficial effects in Atlantic cod and did not enhance growth parameters, or improve feed conversion or potential yield through decreased adiposity or liver lipid

deposition. However, nutritional quality could be enhanced, and cod fed CLA and/or TTA could be beneficial in the human diet, through provision of bioactive fatty acids with no detrimental effects on n-3 PUFA levels.

Acknowledgements

This work and SRK was supported by a Biotechnology and Biological Science Research Council (BBSRC) CASE studentship award (BioMar Ltd., Grangemouth, Scotland). We thank Dr. D. Feuerstein (BASF, Germany) for providing financial support and the CLA. We also thank Jim Treasurer (Viking Fish Farms) for performing the feeding trial and members of the Nutrition Group, Institute of Aquaculture, University of Stirling, for technical assistance.

References

Bandarra, N.M., Nunes, M.L., Andrade, A.M., Prates, J.A.M., Pereira, S., Monteiro, M., Rema, P, Valente, L.M.P. 2006. Effect of conjugated linoleic acid on muscle, liver and visceral lipid deposition in rainbow trout juveniles (*Oncorhynchus mykiss*). Aquaculture 254, 496-505.

Bell, J.G., Strachan, F., Good, J., Tocher, D.R., 2006. Effect of dietary echium oil on growth, fatty acid composition and metabolism, gill prostaglandin production and macrophage activity in Atlantic cod (*Gadus morhua* L.). Aquaculture Res. 37, 606-617.

Berge, G.M., Ruyter, B., Asgard, T., 2004. Conjugated linoleic acid in diets for juvenile Atlantic salmon (*Salmo salar*); effects on fish performance, proximate composition, fatty acid and mineral content. Aquaculture 237, 365-380.

Berge, R.K., Aarsland, A., Kryvi, H., Bremer, J., Aarsaether, N., 1989. Alkylthioacetic acids (3-thia FAs) – a new group of non-beta-oxidizable peroxisome-inducing fatty acid analogues: II. Dose-

response studies on hepatic peroxisomal- and mitochondrial changes and long-term fatty acid metabolizing enzymes in rat. Biochem. Pharmacol. 38, 3969-3979.

Berge, R.K., Skorve, J., Tronstad, K.J., Berge, K., Gudbrandsen, O.A., Grav, H., 2002. Metabolic effects of thia fatty acids. Curr. Opin. Lipidol. 13, 295-304.

Brown, J.A., Puvanendran, V., 2002. Development of Atlantic cod larviculture in Newfoundland. Bull. Aquacult. Assoc. Can. 102, 5-7.

Brown, J.A., Minkoff, G., Puvanendran, V., 2003. Larviculture of Atlantic cod (*Gadus morhua*): progress, protocols and problems. Aquaculture 227, 357-372.

Brown, J.M., Halvorsen, Y.D., Lea-Currie, Y.R., Geigerman, C., McIntosh, M., 2001. Trans-10,cis-12, but not cis-9,trans-11, conjugated linoleic acid attenuates lipogenesis in primary cultures of stromal vascular cells from human adipose tissue. J. Nutr. 131, 2316-2321.

Choi, B.-D., Kang, S.-J., Ha, Y.-L., Ackman, R.G., 1999. Accumulation of conjugated linoleic acid (CLA) in tissues of fish fed diets containing various levels of CLA. In: Xiong, Y.L., Ho, C.-T., Shahidi, F., (Eds.), Quality Attributes of Muscle Foods, Kluwer/Plenum, New York, pp 61-71.

Choi, Y., Park, Y., Pariza, M.W., Ntambi, J,M., 2001. Regulation of stearoyl-CoA desaturase activity by the trans-10,cis-12 isomer of conjugated linoleic acid in HepG2 cells. Biochem Biophy. Res. Commun. 284, 689-693.

Choi, Y., Park, Y., Storkson, J.M., Pariza, M.W., Ntambi, J,M., 2002. Inhibition of stearoyl-CoA desaturase activity by the cis-9,trans-11 isomer and the trans-10,cis-12 isomer of conjugated linoleic acid in MDA-MB-231 and MCF-7 human breast cancer cells. Biochem Biophy. Res. Commun. 294, 785-790.

Christie, W.W., 1982. Lipid Analysis, 2nd Edition, Pergamon, Oxford, pp 207.

Chuang, L.T., Leonard, A.E., Liu, J.W., Mukerji, P., Bray, T.M., Huang, Y.S., 2001. Inhibitory effect of conjugated linoleic acid on linoleic acid elongation in transformed yeast with human elongase. Lipids 36, 1099-1103.

Chuang, L.T., Thurmond, J.M., Liu, J.W., Kirchner, S.J., Mukerji, P., Bray, T.M., Huang, Y.S., 2001. Effect of conjugated linoleic acid on fungal Δ6-desaturase activity in a transformed yeast system. Lipids 36, 139-143.

Degrace, P., Demizieux, L., Gresti, J., Chardigny, J.M., Sebedio, J.L., Clouet, P., 2004. Hepatic steatosis is not due to impaired fatty acid oxidation capacities in C57BL/6J mice fed the conjugated trans-10,cis-12-isomer of linoleic acid. J. Nutr. 134, 861-867.

Delany, J.P., West, D.B., 2000. Changes in body composition with conjugated linoleic acid. J. Am. Coll. Nutr. 19, 487S-493S.

Dos Santos, J., Burkow, I.C., Jobling, M., 1993. Patterns of growth and lipid deposition in cod (*Gadus morhua* L.) fed natural prey and fish-based feeds. Aquaculture 110, 173-180.

Eder, K., Slomma, N., Becker, K., 2002. Trans-10,cis-12 conjugated linoleic acid suppresses the desaturation of linoleic and α-linolenic acids in HepG2 cells. J. Nutr. 132, 1115-1121.

Evans, M., Geigerman, C., Cook, J., Curtis, L., Kuebler, B., McIntosh, M., 2000. Conjugated linoleic acid suppresses triglyceride accumulation and induces apoptosis in 3T3-L1 preadipocytes. Lipids 35, 899-910.

Evans, M., Lin, X., Odle, J., McIntosh, M., 2000. Trans-10,cis-12 conjugated linoleic acid increases fatty acid oxidation in 3T3-L1 preadipocytes. J. Nutr. 132, 450-455.

Figueiredo-Silva, A.C., Rema, P., Bandarra, N.M, Nunes, M.L, Valente, L.M.P., 2005. Effects of dietary conjugated linoleic acid on growth, nutrient utilization, body composition, and hepatic lipogenesis in rainbow trout juveniles (*Oncorhynchus mykiss*). Aquaculture 248, 163-172.

Folch, J., Lees, M., Sloane-Stanley, G.H., 1957. A simple method for the isolation and purification of total lipids from animal tissues. J. Biol. Chem. 226, 497-509.

Frøyland, L., Lie, Ø., Berge, R.K., 2000. Mitochondrial and peroxisomal β-oxidation capacities in various tissues from Atlantic salmon *Salmo salar*. Aquaculture Nutr. 6, 85-89.

Gildberg, A., 2004. Digestive enzyme activities in starved pre-slaughter farmed and wild-captured, Atlantic cod (*Gadus morhua*). Aquaculture 238, 343-353.

Grant, S.M., Brown, J.A., Boyce, D.L., 1998. Enlarged fatty livers of small juvenile cod: a comparison of laboratory-cultured and wild juveniles. J. Fish Biol. 52, 1105-1114.

Hemre, G., Karlsen, Ø., Eckhoff, K., Tviet, K., Mangor-Jensen, A., Rosenlund, G., 2004. Effect of season, light regime and diet on muscle composition and selected parameters in farmed Atlantic cod, *Gadus morhua* L., Aquacult. Res. 35, 683-697.

Hemre, G.-I., Karlsen, Ø., Mangor-Jensen, A., Rosenlund, G., 2003. Digestibility, of dry matter, protein, starch and lipid by cod, *Gadus morhua*: comparison of sampling methods. Aquaculture 225, 25-232.

Jobling, M., 1988. A review of the physiological and nutritional energetics of cod, *Gadus morhua* L., with particular reference to growth under farmed conditions. Aquaculture 70, 1-19.

Kennedy, S.R., Campbell, P.J., Porter, A., Tocher, D.R., 2005. Influence of dietary conjugated linoleic acid (CLA) on lipid and fatty acid composition in liver and flesh of Atlantic salmon (*Salmo salar*), Comp. Biochem. Physiol. 141B, 168-178.

Kennedy, S.R., Leaver, M.J., Campbell, P.J., Zheng, X., Dick, J.R., Tocher, D.R., 2006. Influence of dietary oil content and conjugated linoleic acid (CLA) on lipid metabolism enzyme activities and gene expression in tissues of Atlantic salmon (*Salmo salar* L.). Lipids 41, 423-436.

Lall, S.P., Nanton D., 2002. Nutrition of Atlantic cod. Bull. Aquacult. Assoc. Can. 102, 23-26.

Lie, O., Lied, E., Lambertsen, G., 1986. Liver retention of fat and of fatty acids in cod (*Gadus morhua*)

fed different oils. Aquaculture 59, 187-196.

Lowry, O.H., Rosebrough, N.J., Farr, A.L., Randall, R.J., 1951. Protein measurement with the folin phenol reagent, J. Biol. Chem. 193, 265-275.

Morais, S., Bell, J.G., Robertson, D.A., Roy, W.J., Morris, P.C., 2001. Protein/lipid ratios in extruded diets for Atlantic cod (*Gadus morhua* L.): effects on growth, feed utilization, muscle composition and liver histology. Aquaculture 203, 101-119.

Moya-Falcon, C., Hvattum, E., Dyrøy, E., Skorve, J., Stefansson, S.O., Thomassen, M., Jakobsen, J.V., Berge, R.K., Ruyter, B., 2004. Effects of 3-thia fatty acids on feed intake, growth, tissue fatty acid composition, β-oxidation and Na^+,K^+-ATPase activity in Atlantic salmon. Comp. Biochem. Physiol. 139B, 657-668.

Nanton, D.A., Lall, S.P., Ross, N.W., McNiven, M.A., 2003. Effect of dietary lipid level on fatty acid β-oxidation and lipid composition in various tissues of haddock, *Melanogrammus aeglefinus* L. Comp. Biochem. Physiol. 135B, 95-108.

National Research Council, 1993. Nutrient Requirements of Fish, National Academic Press, Washington, DC.

Ohnuki, K., Haramizu, S., Ishihara, K., Fushiki, T., 2001. Increased energy metabolism and suppressed body fat accumulation in mice by a low concentration of conjugated linoleic acid. Biosci. Biotechnol. Biochem. 65, 2200-2204.

Oku, H., Wongtangtintharn, S., Iwasaki, H., Toda, T., 2003. Conjugated linoleic acid (CLA) inhibits fatty acid synthetase activity in vitro. Biosci. Biotechnol. Biochem. 67, 1584-1586.

Pariza, M.W., Park, Y., Cook, M.E., 2001. The biologically active isomers of conjugated linoleic acid. Prog. Lipid Res. 40, 283-298.

Rahman, S.M., Huda, M.N., Uddin, M.N., Akhteruzzaman, S., 2002. Short-term administration of conjugated linoleic acid reduces liver triglyceride concentration and phosphatidate phosphohydrolase activity in OLETF rats. J. Biochem. Mol. Biol. 35, 494-497.

Rahman, S.M., Wang, Y.M., Yotsumoto, H., Cha, .Y., Han, S.Y., Inoue, S., Yanagita, T., 2001. Effects of Conjugated linoleic acid on serum leptin concentration, body-fat accumulation, and β-oxidation of fatty acid in OLETF rats. Nutrition 17, 385-390.

Ramsay, T.G., Evock-Clover, C.M., Steele, N.C., Azain, M.J., 2001. Dietary conjugated linoleic acid alters fatty acid composition of pig skeletal muscle and fat. J. Anim. Sci. 79, 2152-2161.

Rosenlund, G., Karlsen, Ø., Tveit, K., Mangor-Jensen, A., Hemre, G.-I., 2004. Effect of feed composition and feeding frequency on growth, feed utilization and nutrient retention in juvenile Atlantic cod, *Gadus morhua* L. Aquacult. Nutr. 10, 371-378.

Saggerson, E.D., Carpenter, C.A., 1986. Carnitine palmitoyltransferase in liver and five extrahepatic tissues in the rat. Inhibition by DL-2-bromopalmitoyl-CoA and effect of hypothyroidism. Biochem. J. 236, 137-141.

Sargent, J.R., Tocher, D.R., Bell, J.G., 2002. The lipids. In: Halver, J.E., Hardy, R.W. (Eds.), Fish Nutrition, 3rd Edition, Academic Press, San Diego, pp 181-257.

Shahidi, F., Dunajski, E., 1994. Lipid fatty acid, growth and compositional characteristics of farmed cod (*Gadus morhua*). J. Food Lipids 1, 265-271.

Skrede, S., Sørensen, H.N., Larsen, L.N., Steineger, H.H., Høvik, K., Spydevold, Ø.S., Horn, R., Bremer, J., 1997. Thia fatty acids, metabolism and metabolic effects. Biochim. Biophys. Acta 1344, 115-131.

Small, G.M., Burdett, K., Connock, M.J., 1985. A sensitive spectrophotometric assay for peroxisomal acyl-CoA oxidase. Biochem. J. 227, 205-210.

Tacon, A.G.J., 2003. Global trends in aquaculture and compound aquafeed production. In: Tacon, A.G.J. (Ed.), Internatl. Aquafeed Directory and Buyer's Guide 2003, Turret RAI, Uxbridge, pp 8-23.

Terpstra, A.H., Beynen, A.C., Everts, H., Kocsis, S., Katan, M.B., Zock, P.L., 2002. The decrease in body fat in mice fed conjugated linoleic acid is due to increases in energy expenditure and energy loss in the excreta. J. Nutr. 132, 940-945.

Thiel-Cooper, R.L., Parrish, F.C., Sparks, J.C.,, Weigand, B.R., Ewan, R.C., 2001. Conjugated linoleic acid changes swine performance and carcass composition. J. Anim. Sci. 79, 1821-1828.

Tischendorf, F., Schone, F., Kirchheim, U., Jahreis, G., 2002. Influence of a conjugated linoleic acid mixture on growth, organ weights, carcass traits and meat quality in growing pigs. J. Anim. Physiol. Anim. Nutr. 86, 117-128.

Tocher, D.R., 2003. Metabolism and functions of lipids and fatty acids in teleost fish. Rev. Fish. Sci. 11, 107-184.

Tocher, D.R., Harvie, D.G., 1988. Fatty acid compositions of the major phosphoglycerides from fish neural tissues : (n-3) and (n-6) polyunsaturated fatty acids in rainbow trout (*Salmo gairdneri*, L.) and cod (*Gadus morhua*) brains and retinas. Fish Physiol. Biochem. 5, 229-239.

Twibell, R.G., Wilson, R.P., 2003. Effects of conjugated linoleic acids and total dietary lipid concentrations on growth responses of juvenile channel catfish, *Ictalurus punctatus*. Aquaculture 221, 621-628.

Twibell, R.G., Watkins, B.A., Brown, P.B., 2001. Dietary conjugated linoleic acids and lipid source alter fatty acid composition of juvenile yellow perch, *Perca flavescens*. J. Nutr. 131, 2322-2328.

Twibell, R.G., Watkins, B.A., Rogers, L., Brown, P.B., 2000. Effects of dietary conjugated linoleic acids on hepatic and muscle lipids in hybrid striped bass. Lipids 35, 155-161.

Vegusdal, A., Gjøen, T., Berge, R.K., Thomassen, M., Ruyter, B., 2005. Effect of 18:1n-9, 20:5n-3, and 22:6n-3 on lipid accumulation and secretion by Atlantic salmon hepatocytes. Lipids 40, 477-486.

Wang, Y.: Jones, P.J., 2004. Dietary conjugated linoleic acid and body composition. Am. J. Clin. Nutr. 79, 1153S-1158S.

Yamasaki, M., Ikeda, A., Oji, M., Tanaka, Y., Hirao, A., Kasai, M., Iwata, T., Tachibana, H., Yamada, K., 2003. Modulation of body fat, and serum leptin levels by dietary conjugated linoleic acid in Sprague-Dawley rats fed various fat-level diets. Nutrition 19, 30-35.

Yang, L., Huang, Y., Wang, H.Q., Chen, Z.Y., 2003. Isomeric distribution of conjugated linoleic acid (CLA) in the tissues of layer hens fed a CLA diet. J. Agricultural Fd. Chem. 51, 5654-5660.

Yasmin, A., Takeuchi, T., Hayashi, M., Hirota, T., Ishizuka, W., Ishida, S., 2004. Effect of conjugated linoleic and docosahexaenoic acids on growth of juvenile tilapia *Oreochromis niloticus*. Fisheries Sci. 70, 473-481.

Zar, J.H., 1984. Biostatistical Analysis 2nd Edition; Prentice-Hall: Englewood Cliffs, New Jersey.

Legends to Figures

Fig. 1. Effects of conjugated linoleic acid (CLA) and tetradecylthioacetic acid (TTA) on carnitine palmitoyltransferase-I activities in tissue homogenates of liver, red and white muscle of cod. Results are presented relative to the activity in fish fed fish oil for each tissue and are means ± SD (n = 6). Different letters denote significant differences between dietary treatments within each tissue. CLA1 and CLA2, fish fed 0.5% and 1% CLA, respectively; FO, fish fed fish oil alone; TTA, fish fed 0.5% TTA.

Fig. 2. Effects of conjugated linoleic acid (CLA) and tetradecylthioacetic acid (TTA) on acylCoA oxidase activities in tissue homogenates of liver, red and white muscle of cod. Results are presented relative to the activity in fish fed fish oil for each tissue and are means ± SD (n = 6). Different letters denote significant differences between dietary treatments within each tissue. CLA1 and CLA2, fish fed 0.5% and 1% CLA, respectively; FO, fish fed fish oil alone; TTA, fish fed 0.5% TTA.

Table 1

Formulations (percentage of dry ingredients) and proximate compositions (percentage of total diet) of the experimental diets

	FO	CLA1	CLA2	TTA
Fishmeal	53	53	53	53
Sunflower meal	17	17	17	17
Wheat gluten	5	5	5	5
Legume seeds	17	17	17	17
Micronutrients	0.4	0.4	0.4	0.4
Fish oil	8.8	8	7.1	8.3
CLA	0	0.8	1.7	0
TTA	0	0	0	0.5
Moisture	7.5 ± 0.1 [ab]	7.3 ± 0.3 [b]	8.0 ± 0.2 [a]	8.0 ± 0.2 [a]
Lipid	14.8 ± 0.3	15.4 ± 0.8	15.4 ± 0.7	15.4 ± 0.5
Protein	49.9 ± 0.4	50.2 ± 0.1	49.7 ± 0.4	49.5 ± 0.5
Ash	10.6 ± 0.0 [ab]	10.7 ± 0.1 [a]	10.5 ± 0.0 [b]	10.5 ± 0.0 [b]

Results for proximate compositions are means ± S.D. (n=3). Micronutrients, includes essential amino acids (methionine and lysine), vitamins and minerals, Biomar A/S, Brande, Denmark.

FO, control diet containing fish oil alone; CLA1 and CLA2, diets supplemented with 0.5 and 1% CLA; TTA, diet supplemented with 0.5% TTA.

Table 2

Fatty acid compositions (percentage of weight) of experimental diets containing conjugated linoleic acid (CLA) and tetradecylthioacetic acid (TTA)

	FO	CLA1	CLA2	TTA
14:0	7.2 ± 0.1 [a]	6.8 ± 0.2 [b]	6.2 ± 0.1 [c]	6.2 ± 0.0 [c]
15:0	0.6 ± 0.0	0.5 ± 0.0 [b]	0.5 ± 0.0 [b]	0.5 ± 0.0 [b]
16:0	19.7 ± 0.2 [a]	18.9 ± 0.1 [b]	18.2 ± 0.0 [c]	18.7 ± 0.1 [b]
18:0	4.0 ± 0.0	4.0 ± 0.0	4.0 ± 0.0	3.9 ± 0.0
Total saturated[1]	31.8 ± 0.3 [a]	30.5 ± 0.3 [b]	29.1 ± 0.1 [c]	29.3 ± 0.1 [c]
16:1n-7	7.8 ± 0.1 [a]	7.4 ± 0.1 [b]	7.0 ± 0.2 [c]	7.4 ± 0.0 [b]
18:1n-9	9.9 ± 0.1 [c]	10.8 ± 0.0 [b]	11.8 ± 0.2 [a]	9.8 ± 0.1 [c]
18:1n-7	3.2 ± 0.0 [a]	3.1 ± 0.1 [a]	2.8 ± 0.0 [b]	3.1 ± 0.0 [a]
20:1n-9	2.3 ± 0.0	2.2 ± 0.0	2.3 ± 0.2	2.3 ± 0.0
22:1n-11	3.2 ± 0.0 [a]	3.1 ± 0.0 [ab]	3.0 ± 0.0 [b]	3.1 ± 0.0 [a]
24:1n-9	0.6 ± 0.0	0.5 ± 0.1	0.5 ± 0.0	0.5 ± 0.0
Total monoenes[2]	27.3 ± 0.2 [a]	27.4 ± 0.2 [a]	27.6 ± 0.1 [a]	26.5 ± 0.0 [b]
CLA (9c,11t)	0.0 ± 0.0 [c]	1.8 ± 0.1 [b]	3.2 ± 0.2 [a]	0.0 ± 0.0 [c]
CLA (10t,12c)	0.0 ± 0.0 [c]	1.7 ± 0.1 [b]	3.1 ± 0.2 [a]	0.0 ± 0.0 [c]
TTA	0.0 ± 0.0 [b]	0.0 ± 0.0 [b]	0.0 ± 0.0 [b]	2.8 ± 0.2 [a]
18:2n-6	5.7 ± 0.1 [b]	5.6 ± 0.0 [b]	5.9 ± 0.0 [a]	5.6 ± 0.1 [b]
20:4n-6	1.1 ± 0.0 [a]	1.0 ± 0.0 [b]	0.9 ± 0.0 [b]	1.1 ± 0.0 [a]
Total n-6 PUFA[3]	7.8 ± 0.0 [a]	7.6 ± 0.1 [b]	7.7 ± 0.1 [ab]	7.6 ± 0.1 [b]
18:3n-3	1.0 ± 0.0 [a]	1.0 ± 0.0 [a]	0.9 ± 0.0 [b]	1.1 ± 0.0 [a]
18:4n-3	2.5 ± 0.0 [a]	2.4 ± 0.0 [b]	2.2 ± 0.0 [c]	2.5 ± 0.0 [a]
20:4n-3	0.7 ± 0.0	0.7 ± 0.0	0.6 ± 0.0	0.7 ± 0.0
20:5n-3	14.0 ± 0.0 [a]	13.2 ± 0.2 [b]	12.3 ± 0.1 [c]	14.2 ± 0.1 [a]
22:5n-3	1.9 ± 0.5	1.5 ± 0.0	1.4 ± 0.0	1.7 ± 0.0
22:6n-3	12.8 ± 0.0 [b]	12.3 ± 0.1 [c]	11.9 ± 0.1 [d]	13.4 ± 0.1 [a]
Total n-3 PUFA[4]	33.1 ± 0.5 [b]	31.1 ± 0.3 [c]	29.3 ± 0.2 [d]	33.9 ± 0.2 [a]
Total PUFA	40.9 ± 0.4 [a]	38.7 ± 0.3 [b]	37.0 ± 0.3 [c]	41.5 ± 0.3 [a]
n-3/n-6	4.2 ± 0.1 [b]	4.1 ± 0.0 [b]	3.8 ± 0.0 [c]	4.5 ± 0.0 [a]

Values are means ± SD (n = 3). Superscript letters denote significant differences between diets as determined by ANOVA as described in the Materials and Methods.

[1], includes 20:0, present in some samples at up to 0.3%;

[2], includes 120:1n-7 present in some samples at up to 0.3%;

[3], includes 18:3n-6, 20:2n-6, 20:3n-6, 22:4n-6 and 22:5n-6 present at up 0.4%;

[4], includes 20:3n-3 present at up to 0.1%; PUFA, polyunsaturated fatty acids.

Table 3

Growth and biometric parameters for Atlantic cod (*Gadus morhua*) fed diets containing conjugated linoleic acid (CLA) and tetradecylthioacetic acid (TTA) for three months

	FO	CLA1	CLA2	TTA
Initial weight (g)[1]	125 ± 14 [b]	130 ± 15 [a]	125 ± 15 [b]	127 ± 15 [ab]
Final weight (g)[1]	307 ± 50	305 ± 47	309 ± 57	303 ± 44
FCR[2]	0.80 ± 0.01 [b]	0.84 ± 0.02 [a]	0.80 ± 0.02 [b]	0.83 ± 0.00 [ab]
SGR[2]	1.03 ± 0.04	0.98 ± 0.05	1.04 ± 0.02	1.00 ± 0.02
TGC[2]	1.93 ± 0.08	1.84 ± 0.10	1.95 ± 0.04	1.87 ± 0.04
Gutted weight (%)[3]	81.5 ± 0.2 [c]	81.7 ± 0.3 [bc]	82.2 ± 0.4 [a]	81.8 ± 0.2 [b]
Condition factor (K)[3]	1.14 ± 0.04 [a]	1.12 ± 0.01 [b]	1.14 ± 0.03 [a]	1.12 ± 0.01 [b]
HSI[3]	11.0 ± 0.5 [b]	11.3 ± 0.1 [a]	10.9 ± 0.3 [b]	10.6 ± 0.1 [c]
VSI[3]	9.3 ± 1.2 [a]	8.0 ± 0.7 [b]	8.9 ± 0.2 [a]	8.8 ± 0.2 [a]
Mortality (n)	1	2	1	4

Data are presented as means ± SD, [1]n = 146-150, [2]n = 3, [3]n = 27;

Condition factor (K) = (wet weight in g) x 100)/(length in mm^3) x 1000;

FCR, feed conversion ratio = feed consumed (kg)/ weight gain (kg);

TGC, thermal growth coefficient = 100 x (final $weight^{1/3}$ - initial $weight^{1/3}$) / sum day-degrees;

HSI, Hepato-somatic index =100 x liver weight x body $weight^{-1}$;

SGR, specific growth rate (%/day) = 100 x [(Final Weight / Initial Weight)/$Days^{-1}$];

VSI, Viscero-somatic index = 100 x viscera weight x body $weight^{-1;}$

Superscript letters denote significant effects of dietary treatment as determined by ANOVA as described in the Materials and Methods.

Table 4

Proximate composition of whole Atlantic cod (*Gadus morhua*)

Diet	FO	CLA1	CLA2	TTA
Moisture	73.6 ± 0.6	73.3 ± 0.6	73.8 ± 0.6	73.9 ± 0.7
Protein	60.4 ± 1.4	59.7 ± 1.7	59.1 ± 1.7	60.6 ± 1.4
Lipid	26.2 ± 1.9	28.4 ± 2.2	27.9 ± 2.1	26.7 ± 1.8
Ash	9.6 ± 0.7	9.3 ± 0.5	9.5 ± 0.6	9.9 ± 0.4

Values are means ± SD of 9 fish. There were no significant differences between dietary treatment as determined one-way ANOVA .
CLA, conjugated linoleic acid; TTA, tetrathioacetic acid.

Table 5

Lipid content (percentage of wet weight) and class composition (percentage of total lipid) of liver and flesh of Atlantic cod (*Gadus morhua*) fed conjugated linoleic acid (CLA) and tetradecylthioacetic acid (TTA)

Lipid class	FO	CLA 1	CLA2	TTA
Liver				
Lipid content	53.8 ± 2.6 ^{ab}	50.0 ± 2.1 ^{b}	55.2 ± 2.0 ^{a}	55.2 ± 2.0 ^{a}
PC	1.5 ± 0.2 ^{b}	1.8 ± 0.1 ^{ab}	2.0 ± 0.4 ^{a}	1.5 ± 0.1 ^{b}
PE	1.3 ± 0.1 ^{b}	1.3 ± 0.2 ^{b}	1.7 ± 0.3 ^{a}	0.9 ± 0.3 ^{c}
Total polar	2.8 ± 0.3 ^{b}	3.1 ± 0.3 ^{ab}	3.7 ± 0.7 ^{a}	2.4 ± 0.3 ^{b}
Total neutral	97.2 ± 0.3 ^{a}	96.9 ± 0.3 ^{ab}	96.3 ± 0.7 ^{b}	97.6 ± 0.3 ^{a}
Cholesterol	4.4 ± 0.6 ^{ab}	4.9 ± 1.4 ^{ab}	5.4 ± 0.3 ^{a}	3.9 ± 0.6 ^{b}
Triacylglycerol	92.7 ± 0.8 ^{a}	92.0 ± 1.6 ^{ab}	90.9 ± 0.8 ^{b}	93.6 ± 0.8 ^{a}
Free fatty acid	tr	tr	tr	tr
Steryl ester	tr	tr	tr	tr
Flesh				
Lipid content	0.8 ± 0.0	0.8 ± 0.1	0.8 ± 0.1	0.8 ± 0.0
PC	30.7 ± 1.8 ^{a}	27.9 ± 1.6 ^{b}	30.8 ± 1.5 ^{a}	28.7 ± 1.2 ^{ab}
PE	17.9 ± 1.2 ^{a}	15.6 ± 0.7 ^{b}	17.1 ± 0.6 ^{a}	17.6 ± 0.5 ^{a}
PS	2.5 ± 0.6	2.8 ± 0.4	2.7 ± 0.4	3.2 ± 0.5
PI	3.8 ± 0.6	4.1 ± 0.6	3.8 ± 0.7	4.3 ± 0.5
PG/CL	2.9 ± 0.7 ^{b}	2.8 ± 0.3 ^{b}	2.8 ± 0.4 ^{b}	3.7 ± 0.2 ^{a}
Sphingomyelin	1.5 ± 0.4	1.7 ± 0.3	1.6 ± 0.2	1.6 ± 0.6
Lyso-PC	1.8 ± 0.3 ^{bc}	2.2 ± 0.2 ^{a}	2.0 ± 0.2 ^{ab}	1.5 ± 0.2 ^{c}
Total polar	61.2 ± 2.1 ^{a}	57.2 ± 1.6 ^{b}	60.8 ± 1.2 ^{a}	60.5 ± 2.1 ^{a}
Total neutral	38.8 ± 2.1 ^{b}	42.8 ± 1.6 ^{a}	39.2 ± 1.3 ^{b}	39.5 ± 2.1 ^{b}
Cholesterol	16.1 ± 0.9	15.6 ± 0.3	15.2 ± 1.8	15.7 ± 0.4
Triacylglycerol	6.9 ± 1.2 ^{b}	6.3 ± 1.6 ^{b}	5.7 ± 1.1 ^{b}	9.2 ± 0.8 ^{a}
Free fatty acid	11.9 ± 1.2 ^{b}	15.1 ± 1.6 ^{a}	13.0 ± 1.7 ^{ab}	11.5 ± 1.4 ^{b}
Steryl ester	3.9 ± 0.4 ^{b}	5.9 ± 0.9 ^{a}	5.4 ± 0.4 ^{a}	3.2 ± 0.6 ^{b}

Values are means ± SD of 6 samples each of tissue pooled from 3 fish.
Superscript letters denote significant differences between dietary treatments as determined by ANOVA as described in the Materials and Methods. CL, cardiolipin; PC, phosphatidylcholine; PE, phosphatidylethanolamine; PG, phosphatidylglycerol; PI, phosphatidylinositol; PS, phosphatidylserine; trace, < 0.5%.

Table 6

Fatty acid composition (percentage of weight) of total lipid from liver of Atlantic cod (*Gadus morhua*) fed conjugated linoleic acid (CLA) and tetradecylthioacetic acid (TTA)

	FO	CLA1	CLA2	TTA
14:0	2.9 ± 0.3 [b]	3.4 ± 0.1 [a]	3.3 ± 0.2 [a]	2.8 ± 0.1 [b]
16:0	15.7 ± 0.5 [a]	15.6 ± 0.2 [a]	15.0 ± 0.7 [ab]	14.7 ± 0.6 [b]
18:0	5.6 ± 0.2 [b]	8.3 ± 0.5 [a]	8.7 ± 0.4 [a]	4.9 ± 0.2 [c]
Total saturated[1]	24.5 ± 0.7 [b]	27.7 ± 0.6 [a]	27.3 ± 1.0	22.6 ± 0.9 [c]
16:1n-7	6.6 ± 0.2 [a]	6.2 ± 0.1 [b]	5.9 ± 0.2 [c]	6.7 ± 0.2 [a]
18:1n-9	18.8 ± 0.6 [a]	15.7 ± 0.3 [b]	15.6 ± 0.3 [b]	19.6 ± 0.7 [a]
18:1n-7	5.3 ± 0.2 [a]	4.8 ± 0.2 [b]	4.8 ± 0.1 [b]	5.7 ± 0.4 [a]
20:1n-9	5.2 ± 0.1	5.1 ± 0.2	5.1 ± 0.3	5.1 ± 0.2
22:1n-11	3.3 ± 0.2 [a]	3.2 ± 0.1 [ab]	2.9 ± 0.3 [b]	3.3 ± 0.3 [a]
24:1n-9	0.3 ± 0.1	0.3 ± 0.0	0.3 ± 0.0	0.4 ± 0.1
Total monoenes[2]	40.0 ± 1.1 [a]	35.6 ± 0.6 [b]	34.9 ± 0.5 [b]	41.0 ± 1.1 [a]
CLA (9c,11t)	0.0 ± 0.0 [c]	0.9 ± 0.2 [b]	1.6 ± 0.5 [a]	0.0 ± 0.0 [c]
CLA (10t,12c)	0.0 ± 0.0 [c]	0.6 ± 0.1 [b]	1.3 ± 0.4 [a]	0.0 ± 0.0 [c]
TTA	0.0 ± 0.0 [b]	0.0 ± 0.0 [b]	0.0 ± 0.0 [b]	0.6 ± 0.1 [a]
18:2n-6	5.4 ± 0.1	5.5 ± 0.1	5.5 ± 0.0	5.5 ± 0.2
20:4n-6	0.8 ± 0.0	0.8 ± 0.0	0.8 ± 0.0	0.8 ± 0.0
Total n-6 PUFA[3]	7.2 ± 0.2	7.2 ± 0.1	7.2 ± 0.0	7.0 ± 0.3
18:3n-3	1.0 ± 0.0	0.9 ± 0.0	0.9 ± 0.0	1.0 ± 0.0
18:4n-3	2.0 ± 0.1	2.0 ± 0.1	1.9 ± 0.0	2.0 ± 0.1
20:4n-3	0.7 ± 0.0	0.7 ± 0.0	0.6 ± 0.0	0.7 ± 0.0
20:5n-3	11.5 ± 0.6	11.2 ± 0.2	11.2 ± 0.3	11.7 ± 0.5
22:5n-3	1.5 ± 0.1	1.5 ± 0.0	1.5 ± 0.0	1.5 ± 0.1
22:6n-3	11.5 ± 0.8	11.6 ± 0.3	11.5 ± 0.4	11.8 ± 0.7
Total n-3 PUFA[4]	28.3 ± 1.6	28.0 ± 0.6	27.6 ± 0.8	28.8 ± 1.4
Total PUFA	35.5 ± 1.7	35.2 ± 0.6	34.8 ± 0.8	35.9 ± 1.6
n-3/n-6	3.9 ± 0.2	3.9 ± 0.1	3.8 ± 0.1	4.1 ± 0.1

Values are means ± SD of 6 samples each of tissue pooled from 3 fish.

Superscript letters denote significant differences between dietary treatments as determined by ANOVA as described in the Materials and Methods.

[1], includes 15:0, present in some samples at up to 0.3%;

[2], includes 20:1n-7 present in some samples at up to 0.3%;

[3], includes 18:3n-6, 20:2n-6, 20:3n-6, 22:4n-6 and 22:5n-6 present at up 0.3%;

[4], includes 20:3n-3 present at up to 0.1%; PUFA, polyunsaturated fatty acids.

Table 7

Fatty acid composition (percentage of weight) of total lipid from flesh of Atlantic cod (*Gadus morhua*) fed conjugated linoleic acid (CLA) and tetradecylthioacetic acid (TTA)

	FO	CLA1	CLA2	TTA
14:0	1.6 ± 0.2 [a]	1.3 ± 0.1 [b]	1.3 ± 0.1 [b]	1.0 ± 0.0 [c]
16:0	19.3 ± 0.8 [ab]	19.2 ± 0.8 [ab]	18.6 ± 0.5 [b]	20.1 ± 0.4 [a]
18:0	3.2 ± 0.1 [b]	4.0 ± 0.1 [a]	3.9 ± 0.2 [a]	3.4 ± 0.1 [b]
Total saturated[1]	24.4 ± 0.9	24.8 ± 0.8	24.2 ± 0.3	24.8 ± 0.5
16:1n-7	2.9 ± 0.3 [a]	2.6 ± 0.1 [ab]	2.4 ± 0.2 [b]	2.3 ± 0.2 [b]
18:1n-9	9.2 ± 0.1 [a]	8.8 ± 0.2 [ab]	8.5 ± 0.5 [b]	8.7 ± 0.4 [ab]
18:1n-7	2.9 ± 0.0 [a]	2.8 ± 0.1 [ab]	2.7 ± 0.1 [b]	2.7 ± 0.1 [b]
20:1n-9	1.0 ± 0.1	1.1 ± 0.2	1.0 ± 0.1	1.0 ± 0.2
22:1n-11	0.3 ± 0.0	0.3 ± 0.1	0.3 ± 0.0	0.3 ± 0.0
24:1n-9	0.3 ± 0.0	0.3 ± 0.0	0.3 ± 0.1	0.4 ± 0.1
Total monoenes[2]	16.7 ± 0.4 [a]	15.9 ± 0.3 [ab]	15.2 ± 0.8 [b]	15.4 ± 0.6 [b]
CLA (9c,11t)	0.0 ± 0.0 [c]	0.3 ± 0.1 [b]	0.7 ± 0.0 [a]	0.0 ± 0.0 [c]
CLA (10t,12c)	0.0 ± 0.0 [c]	0.5 ± 0.1 [b]	1.2 ± 0.1 [a]	0.0 ± 0.0 [c]
TTA	0.0 ± 0.0 [b]	0.0 ± 0.0 [b]	0.0 ± 0.0 [b]	1.6 ± 0.3 [a]
18:2n-6	4.1 ± 0.1	4.0 ± 0.4	4.1 ± 0.3	3.9 ± 0.1
20:4n-6	1.9 ± 0.0 [a]	1.9 ± 0.1 [a]	1.9 ± 0.0 [a]	1.8 ± 0.0 [b]
22:5n-6	0.6 ± 0.0	0.6 ± 0.0	0.6 ± 0.0	0.6 ± 0.0
Total n-6 PUFA[3]	7.1 ± 0.2 [a]	7.2 ± 0.3 [a]	7.0 ± 0.2 [a]	6.5 ± 0.1 [b]
18:3n-3	0.6 ± 0.0 [a]	0.6 ± 0.1 [a]	0.6 ± 0.0 [a]	0.5 ± 0.0 [b]
18:4n-3	0.9 ± 0.0 [a]	0.8 ± 0.1 [ab]	0.8 ± 0.1 [ab]	0.7 ± 0.0 [b]
20:4n-3	0.7 ± 0.0	0.7 ± 0.0	0.6 ± 0.0	0.7 ± 0.0
20:5n-3	18.6 ± 0.3 [a]	18.0 ± 0.9 [a]	18.2 ± 0.5 [a]	16.4 ± 0.2 [b]
22:5n-3	2.2 ± 0.0 [a]	2.2 ± 0.1 [a]	2.2 ± 0.0 [a]	2.1 ± 0.0 [b]
22:6n-3	28.6 ± 0.6 [b]	28.9 ± 0.8 [b]	29.4 ± 1.3 [b]	31.0 ± 0.7 [a]
Total n-3 PUFA[4]	51.7 ± 0.9	51.3 ± 0.5	51.7 ± 0.7	51.4 ± 0.8
Total PUFA	58.9 ± 0.9	58.5 ± 0.8	58.7 ± 0.5	58.3 ± 0.6
n-3/n-6	7.2 ± 0.2 [b]	7.1 ± 0.2 [b]	7.4 ± 0.3 [b]	7.8 ± 0.2 [a]

Values are means ± SD of 6 samples each of tissue pooled from 3 fish.
Superscript letters denote significant differences between dietary treatments as determined by ANOVA as described in the Materials and Methods.

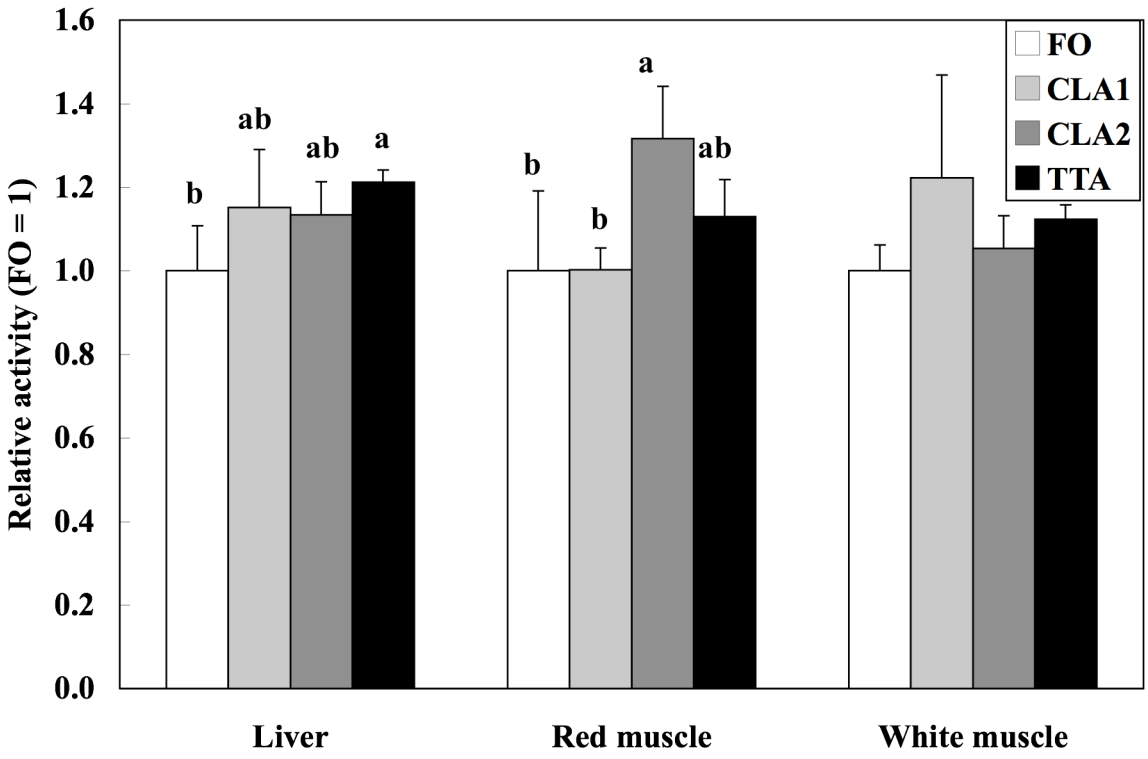
[1], includes 15:0 and 20:0, present in some samples at up to 0.3%;

[2], includes 16:1n-9 and 20:1n-11 present in some samples at up to 0.1%;

[3], includes 18:3n-6, 20:2n-6, 20:3n-6 and 22:4n-6 present in some samples at up 0.3%

[4], includes 20:3n-3 present at up to 0.1%; PUFA, polyunsaturated fatty acids.

Fig.1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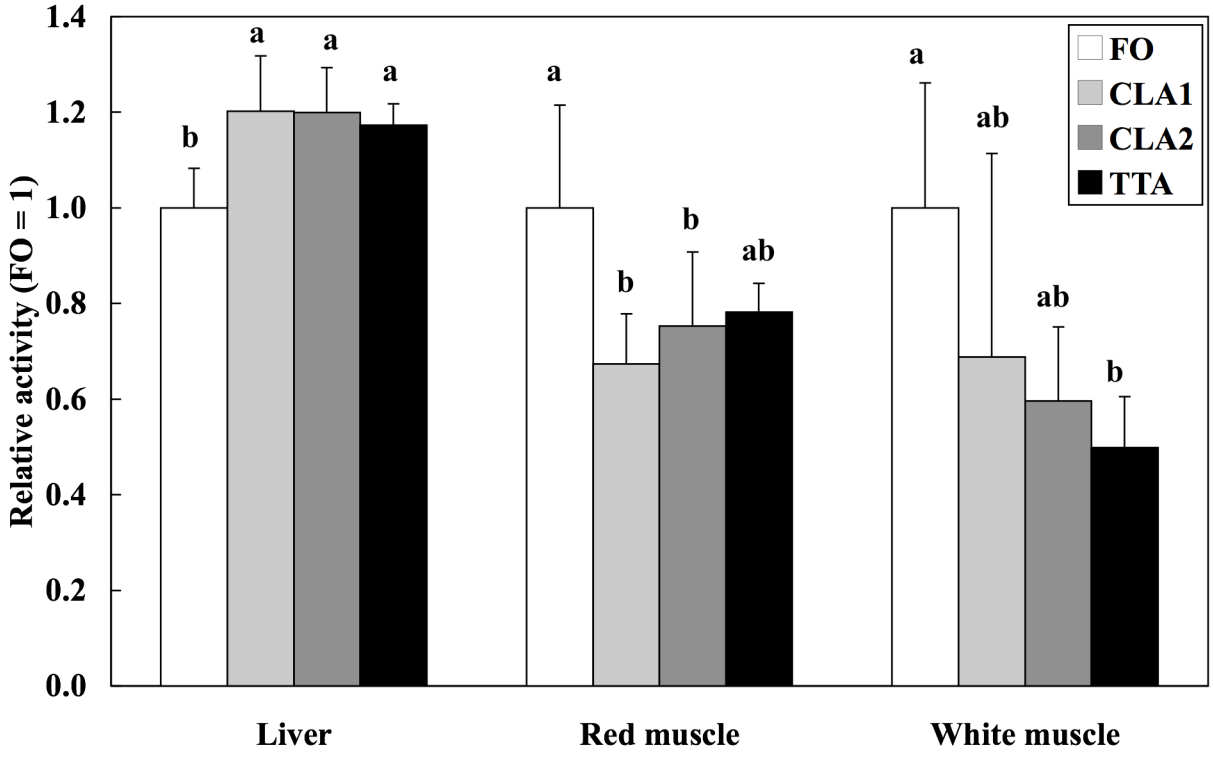
1.2
1.0
0.8
0.6
0.4
0.2
0.0
b
ab
ab
a
b
b
a
ab
FO
CLA1
CLA2
TTA
Liver
Red muscle
White muscle

Fig.2


Relative activity (FO = 1)
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0.0
FO
CLA1
CLA2
TTA
b
a
a
a
a
b
b
ab
a
ab
ab
b
Liver
Red muscle
White muscle