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Effect of lipid source and bile salts in diet of Atlantic salmon, *Salmo salar* L., on astaxanthin blood levels

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Abstract

A study was conducted to determine the effects of dietary lipid and bile acids on astaxanthin absorption in Atlantic salmon (*Salmo salar* L.). Fish with an average weight of 1500 g were fitted with a dorsal aorta cannula and fed diets containing herring oil, soybean lecithin, lard, or herring oil supplemented with taurocholic acid (2.5 g/kg diet). Each fish was fed all of the experimental diets in successive order to minimize the effect of individual variation. At a given time following the feeding of each diet, blood was collected and analyzed for astaxanthin. Soybean lecithin significantly lowered the absorption of astaxanthin compared to fish fed herring oil. A 20% ($p<0.12$) increase in blood astaxanthin was observed when the fish were fed the diet supplemented with taurocholic acid. Feeding lard significantly increased the blood astaxanthin level compared to the control group. It appears that altering the micellar structure by stimulating micellar (taurocholic acid) or mixed micellar (lecithin) systems did not increase the apparent absorption of astaxanthin. However, increasing the phospholipid level may have actually decreased the absorption possibly by lowering the astaxanthin solubility in the micelles. The increased apparent absorption of astaxanthin with lard is possibly linked to the increased content of 16:0, 18:1n−9 or 18:2n−6 fatty acids in this diet, or a reduction in very long chain monoenes (20:1n−9 and 22:1n−9). This suggests that the solubility of astaxanthin is higher in diets containing higher levels of 16:0 or 18:1n−1, or alternatively, that reductions in longer chain monoenes (20:1n−9 and 22:1n−9) increase the micellar solubility of this pigment.

Keywords: Cannulation; Atlantic salmon; Blood; Absorption; Lecithin; Lard; Astaxanthin; Taurocholic acid; Herring oil; Intestine

1. Introduction

The characteristic red colour of salmon flesh is the result of deposition of dietary carotenoids, astaxanthin and canthaxanthin since fish are unable to synthesize them *de novo*. Pigmentation of muscle is one of the

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most important quality criteria for farmed salmon, and it also determines the consumer's impression of other quality parameters such as texture and freshness (Consommateur, 2000). Pigmentation of muscle is affected by dietary carotenoid source, concentration, and duration of feeding, diet composition and genetic differences. Carotenoid pigments constitute a major cost of salmon feeds (ca. 25%), however, less than 10% of astaxanthin and/or canthaxanthin is retained in salmon flesh. A low retention may be partially caused by the low digestibility and absorption of astaxanthin in salmon diets resulting in approximately 65% to be excreted and by 50% of the absorbed astaxanthin being metabolized (Torrissen et al., 1989; Storebakken and No, 1992).

Carotenoids are hydrophobic compounds that are not easily solubilised in the aqueous environment of the gastrointestinal tract of fish. It is well established that increased levels of dietary lipids has a positive effect on astaxanthin deposition in salmonids (e.g. Einen and Roem, 1997; Nickell and Bromage, 1998; Torrissen, 2000) and that this probably relates to increased digestibility as shown for canthaxanthin (Torrissen et al., 1990; Choubert et al., 1991). The intestinal absorption of carotenoids involves several steps including disruption of the food matrix, dispersion in lipid emulsions and solubilisation into mixed bile salt micelles before being carried to the enterocyte brush border where the absorption occurs (Furr and Clark, 1997; Thyssandier et al., 2001).

Alterations of the intestinal environment may thus have a major influence on the solubility and hence absorption of dietary pigments. Dietary lipids contain a range of fatty acids that differ both in chain length and unsaturation, and accordingly in melting point and polarity. Altering the fatty acid composition will therefore significantly affect both the melting point and polarity of the lipid phase, and possibly also the solubility of astaxanthin in this phase. The observed changes in muscle pigmentation with altered dietary levels of polyunsaturated fatty acids (Bjerkeng et al., 1999; Christiansen et al., 1993) may be explained by such mechanisms.

In the digestive tract, pigments are not only mixed with partially digested lipid, but also emulsified with bile salts creating micellar-like structures that eventually pass the unstirred water layer before being absorbed through the brush border membrane. However, both the size and composition of these structures will depend on the ratios between bile salts, phospholipids and partly digested lipid. Simple micellar structures are created when the ratio of bile salts to phospholipids is high. These micelles are relatively small and sufficiently mobile to diffuse rapidly through the intestinal mucin to the epithelial surface where the absorption occurs (Wiedmann et al., 2001; Li et al., 1996). Although solutes from simple micelles are readily absorbed (Poelma et al., 1991), they are generally poor solubilisers and are therefore considered a rapid transport mechanism with low capacity. At a lower ratio of bile salts to phospholipids, mixed micelles are produced where the solubilisation power increases with the increased content of phospholipids (Cai et al., 1997). However, this also increases the size of the mixed micelle (Cabral and Small, 1989) and reduces the diffusibility of the micelle, producing a slow transport mechanism with high capacity. The extent to which astaxanthin dissolves in the different phases is unknown.

The present study was aimed at evaluating the effect of using diets that would favor the formation of different micellar phases in the fish intestine, as well as examine the effect of different polarities and saturation. Fish were fed diets supplemented with either fish oil (control), bile acids (promote simple micelles) or phospholipids (promote large mixed micelles). In addition, one diet contained lard as a lipid source, which could influence phase partition and/or the rate of triacylglycerol hydrolysis differently than that of the control group.

2. Materials and methods

Atlantic salmon (NLA strain-Norwegian breeding program, 1.5 kg) were fed a commercial diet containing no astaxanthin and canthaxanthin supplements until they reached an average weight of 1.5 kg. The carotenoid content in this diet was less than 0.5 mL kg^{-1} as analyzed by HPLC as described below. Fish were maintained in $1.5 \times 1.5 \times 1$ m standard fibreglass tanks supplied with aerated seawater (9 ± 1.5 °C) and kept under continuous simulated daylight regime at Institute of Marine Research, Matre Aquaculture Research Station, Norway. Five to ten days prior to surgery, individual fish were moved into the exper-