

# Effect of diet composition on utilisation of astaxanthin in Atlantic salmon (*Salmo salar*)

Trine Ytrestøyl<sup>1</sup>, Alexander Dikiy<sup>2</sup>, Elena Shumilina<sup>2</sup>, Alessandra Ciampa<sup>2</sup> Bente Ruyter<sup>3</sup>, Bjarne Hatlen<sup>1</sup>, Tone-Kari Østbye<sup>3</sup>, Aleksei Krasnov<sup>3</sup>

1) Nofima Sunndalsøra, 2) NTNU, Institute for biotechnology and food science, 3) Nofima Ås

Nowadays, salmon farming is experiencing problems with achieving required flesh colour and astaxantin concentrations. The gradual reduction cannot be ameliorated by adding more astaxanthin in the diets. An understanding of how dietary and environmental factors may affect astaxanthin uptake, metabolism and retention is essential, but little is known about the basal biological mechanisms that regulate the deposition of astaxanthin in the salmon muscle. Reduced inclusion of marine ingredients in the modern salmon diet has led to reduced levels of several dietary components, including phospholipids and retinol in the salmon feed. Phospholipids are important for the formation of chylomicrons in the intestine and may thus affect uptake of astaxanthin. Metabolic conversion and Salmonids metabolize astaxanthin to β-carotene and retinol. Astaxanthin is also a powerful antioxidant, and stress may thus have a negative impact on flesh pigmentation. However, the regulation of the different metabolic pathways and their importance for flesh pigmentation and health has not been much studied in Atlantic salmon.

### Experimental design

Atlantic salmon (200 g) was fed 6 diets for 12 weeks (Tab. 1). Digestibility of astaxanthin and fat were measured, and analysis of astaxanthin and metabolites were done in flesh. Liver cells were isolated from fish fed marine (1) and plant based diets (2) and incubated with <sup>13</sup>C astaxanthin and cortisol to study effects of stress on astaxanthin metabolism.

### Experimental diets

- Marine diet
- Plant diet
- Fish meal + plant oil
- Plant protein + fish oil
- Plant diet + soy lecithin
- Plant diet + marine phospholipids

Table 1: Diet composition (%)

	Diet 1 FM/FO	Diet 2 PP/PO	Diet 3 FM/PO	Diet 4 PP/FO	Diet 5 PP/PO + soy lec	Diet 6 PP/PO + MPL
Fish meal	58,7	7,5	58,7	7,5	7,5	7,5
Soy P concentrate	-	26,0	-	26,0	26,0	26,0
Wheat gluten	-	22,5	-	22,5	22,5	22,5
Wheat starch	13,5	10,0	13,5	10,0	10,0	10,0
Fish oil	22,0	6,5	5,5	25,8	6,5	6,5
Rapeseed oil	-	19,4	16,5	-	19,4	19,4
Astaxanthin (mg/kg)	42	45	42	46	48	48
Sum phosfolipid	0,5	0,3	0,6	0,3	0,8	0,6
Sum EPA/DHA	4,1	1,1	1,7	3,5	1,2	1,2

## Results

**Digestibility:** A low fish meal content in the diet lowered feed intake and growth and reduced digestibility of fat and astaxanthin. Rapeseed oil did not reduce feed intake and growth or astaxanthin digestibility (Fig. 1). Adding phospholipids to the diet improved feed intake and growth, and restored the digestibility of fat and astaxanthin. The digestibility of fat and astaxanthin was highly correlated with dietary phospholipid concentration ( $R^2 = 0.83$  and  $0.87$ ). These results suggest that the transport of fat and astaxanthin through the intestine was impaired at low dietary phospholipid concentrations. However, the solubility of astaxanthin in mixed micelles in the gut lumen could also have been affected by the dietary phospholipid content.

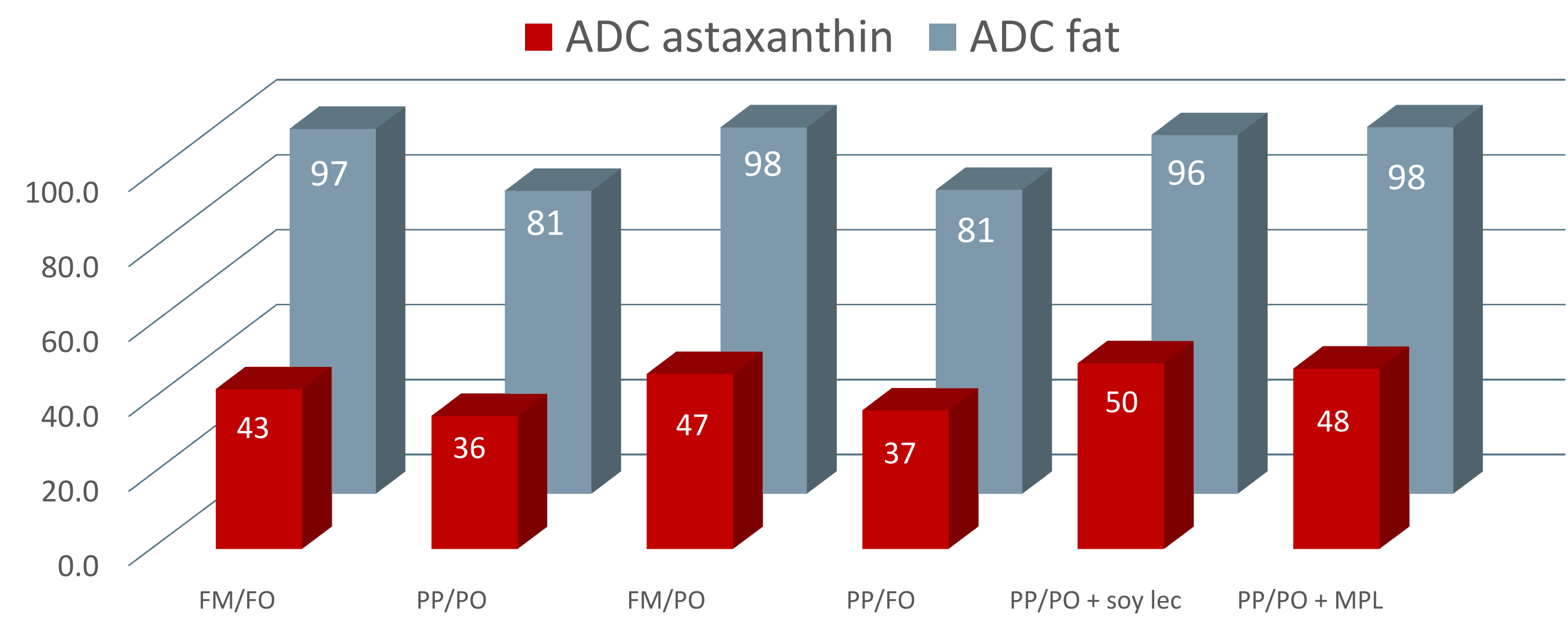


Fig 1: Digestibility (ADC, % of ingested) of fat and astaxanthin for the different diets. FM=fish meal, FO= fish oil, PP=plant protein, PO=plant oil, MPL= marine phospholipids

**Retention and metabolism:** Digestibility of astaxanthin was not correlated with flesh retention of carotenoids. Growth rate, and post-absorptive processes such as uptake and metabolism of astaxanthin in various organs are clearly important for flesh depostion of astaxanthin. A negative correlation between the flesh concentration of the metabolite idoxanthin and the retention of carotenoids was found ( $R^2 = 0.43$ ). The concentration of the astaxanthin metabolite idoxanthin was higher in flesh of salmon fed fish meal diets and the diet with marine phospholipids (Fig. 2). However, growth differences between diets make direct comparisons of astaxanthin retention challenging.

**Cell culture study:** Metabolism of astaxanthin to reductive metabolites and to retinol was higher in liver cells isolated from salmon fed the plant diet compared to the marine diet (Fig.3). Incubating cells with cortisol increased metabolism of astaxanthin to β-carotene and retinol in liver cells isolated from fish fed a plant diet, whereas cortisol did not affect astaxanthin metabolism in liver celles from fish fed a marine diet.

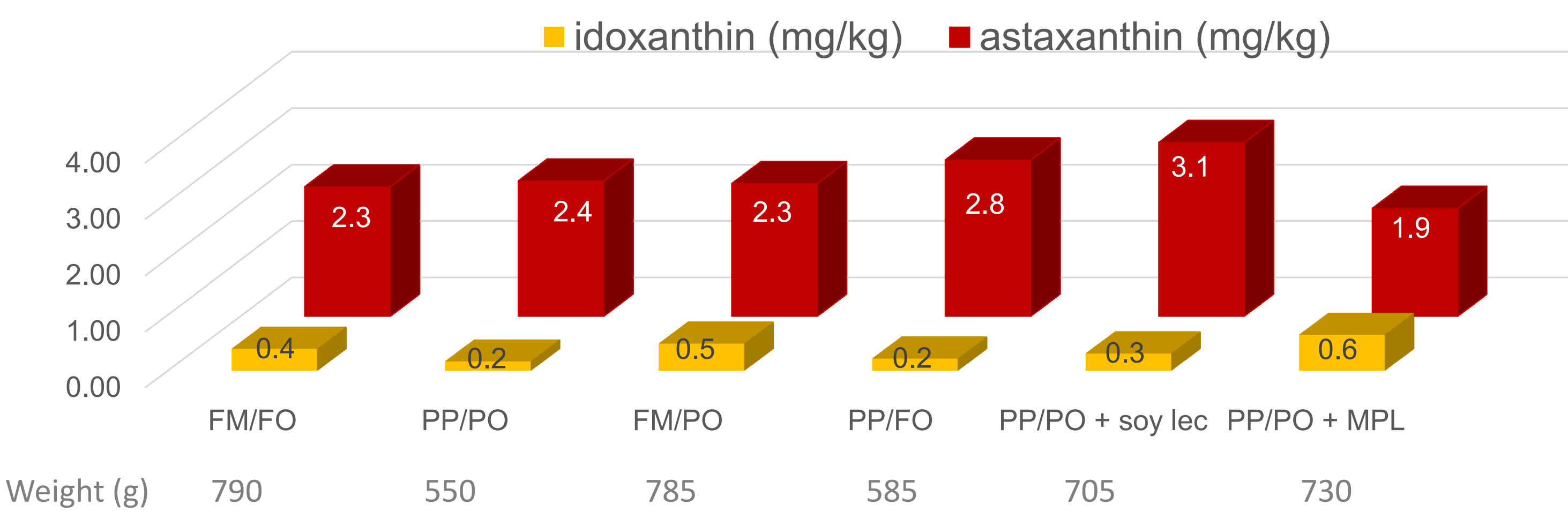


Fig 2: Flesh concentrations of astaxanthin and the metabolite idoxanthin, and bodyweight (BW) in salmon fed diets with combinations of fish meal and fish oil

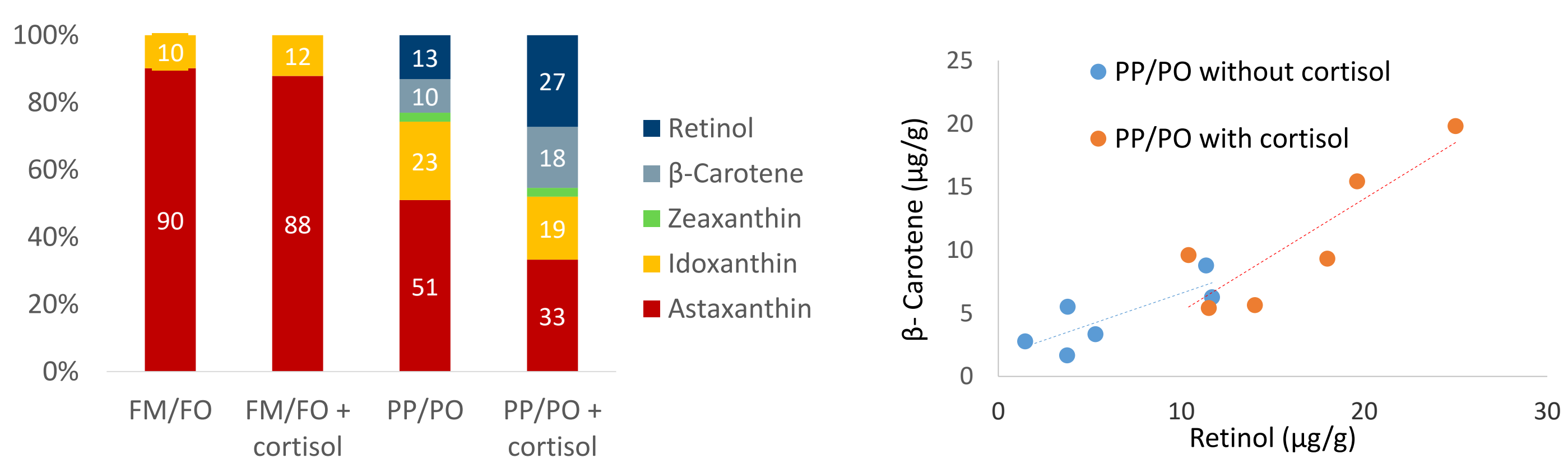


Fig 3: Astaxanthin and metabolites in liver cells isolated from salmon fed a marine diet and a plant based diet. The liver cells were incubated with <sup>13</sup>C astaxanthin for 48 h with and without cortisol.

## Summary

- Diet composition had an effect on astaxanthin uptake and metabolism
- Phospholipids in the diet were essential for growth and for digestibility of astaxanthin and fat
- Higher concentration of the metabolite idoxanthin was found in flesh of salmon fed marine protein diets
- Metabolism of astaxanthin to retinol was higher in liver cells isolated from salmon fed a plant based diet in comparison with a marine diet