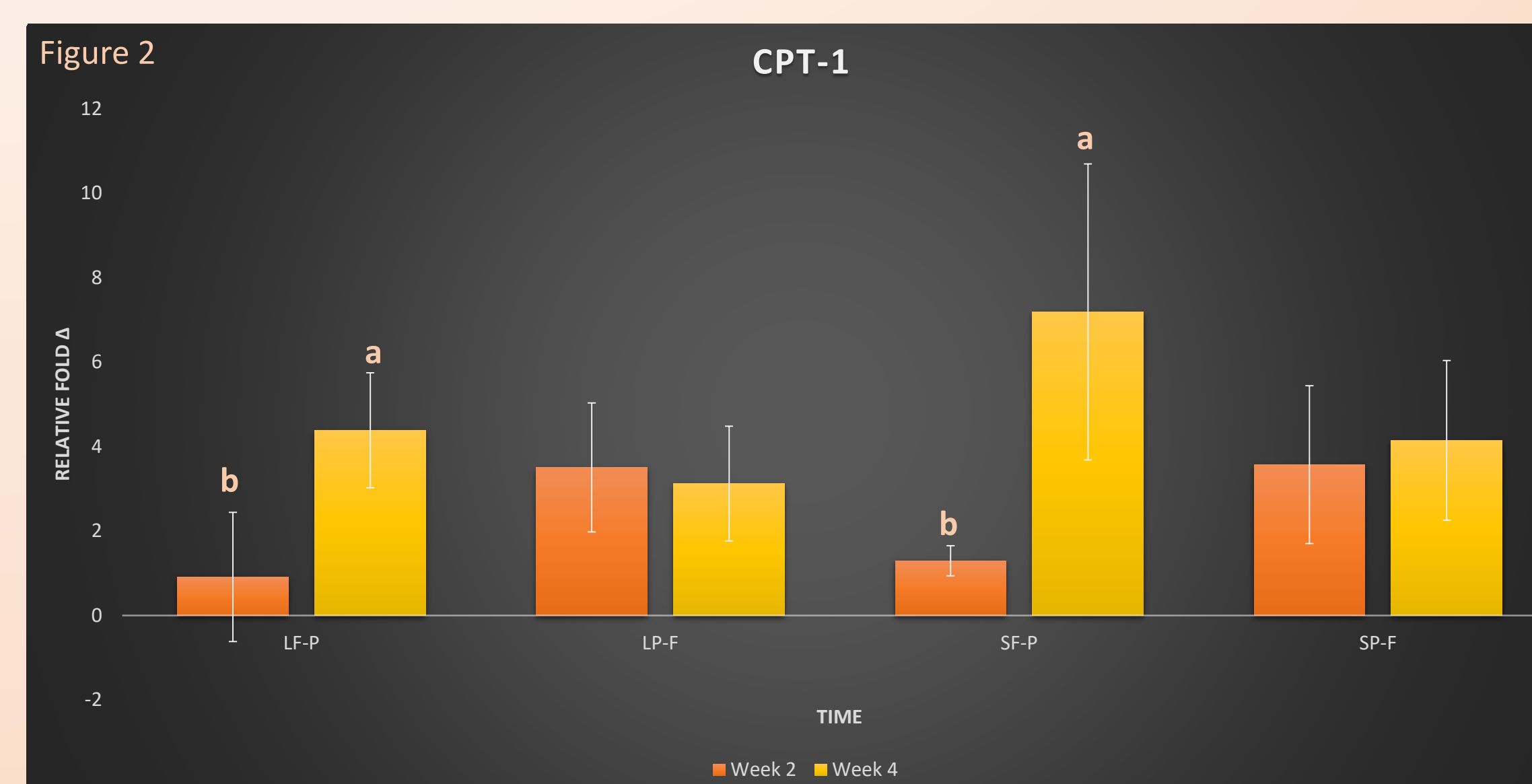
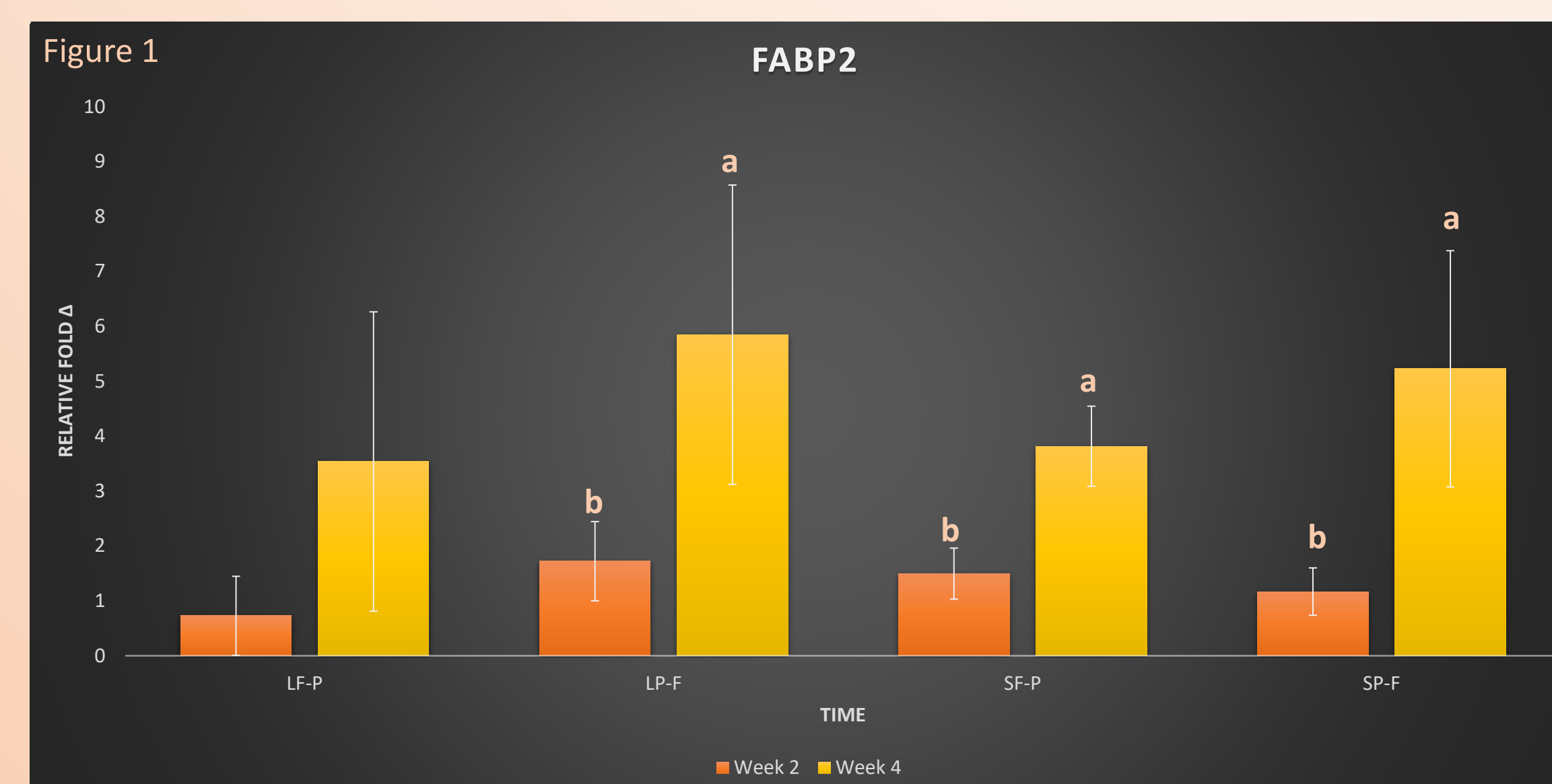


# Origin of Dietary Fat Effects Gene Expression and Fatty Acid Metabolism in Rainbow Trout

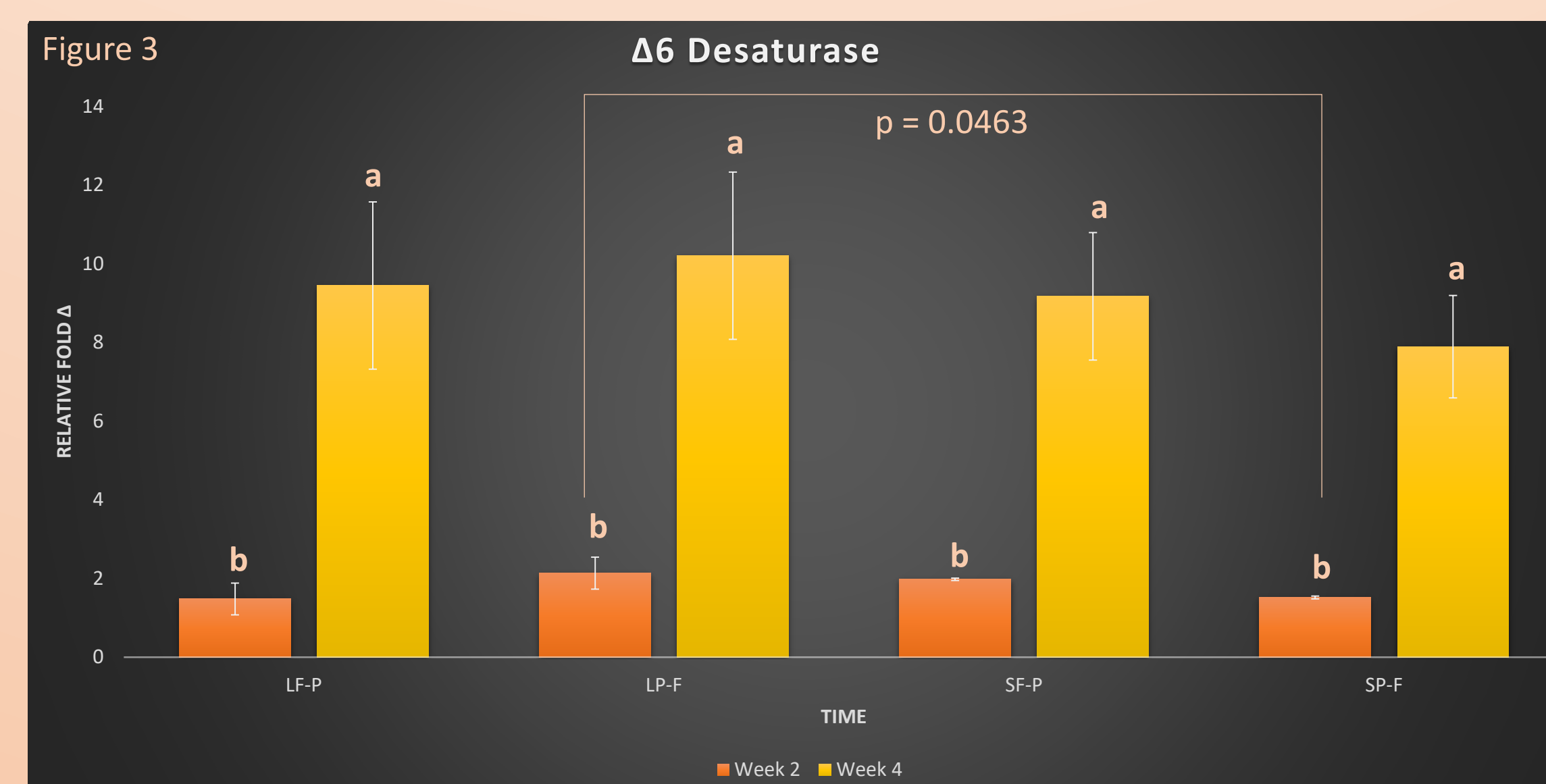
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## Abstract

We examined two lines of rainbow trout, one selected for increased growth rate, and one selected for increased lipid deposition in their fillets. Fish were fed a diet either containing fish-oil or flax-seed-oil for two weeks, then diets were switched in a cross-over design. Gene expression of fatty acid transporters, fatty acid elongases and desaturases, and  $\beta$ -oxidation were measured with quantitative PCR, while fatty acid profiles were obtained from blood plasma using LC-MS. Results show a general upregulation of fatty acid metabolism after 4 weeks regardless of diet or strain of fish. Fish selected for increased lipid deposition did show increased  $\Delta 6$  desaturase expression over fish selected for growth after 4 weeks. Levels of EPA and DHA in the blood plasma of lipid strain fish were higher than in fish selected for growth regardless of diet. These results indicate fish selected for increased lipid deposition may preferentially modify  $\alpha$ -linolenic acid in the flax seed oil diet to increase DHA and EPA content and may preferentially sequester available EPA and DHA in the fish oil diet.



LF-P: Lipid, Fish to Plant Meal Diet  
LP-F: Lipid, Plant to Fish Meal Diet  
SF-P: Select, Fish to Plant Meal Diet  
SP-F: Select, Plant to Fish Meal Diet



## Materials and Methods

Twelve tanks with two different strains of rainbow trout, select and commercial, were fed at 1% of their body mass. Utilizing a Latin square, crossover experimental design, the two strains were grown on diets of plant meal with a top coat of either fish oil or plant oil. After two weeks on this diet, samples of blood (via caudal vein puncture), liver and distal intestine were taken from three fish from each tank and diets were switched. Samples were taken again after another two weeks. During sampling, liver and distal intestine were placed immediately in .5mL of TRIzol® (Ambion-Life Technologies, Carlsbad, California) and blood samples were first spun down and the plasma removed before adding the TRIzol®. The plasma was then analyzed for EPA and DHA using LC-MS. Total RNA was isolated according to the manufacturer's instructions and quantified using a NanoDrop 2000 Spectrophotometer (Thermo Scientific, Waltham, Massachusetts). Samples were DNase treated (Dnase I, Invitrogen, Carlsbad, California) according to the manufacturer's protocol. Reverse transcription was completed using High Capacity cDNA Reverse Transcription Kits (Life Technologies). Resulting cDNAs were used in quantitative PCR reactions with SYBR Green (Life Technologies) to examine for gene expression of fatty acid transport proteins. Primer sequences for intestinal transporters (PEPT1, GLUT-4, UBQ) and liver transporters ( $\Delta 6$ DESAT,  $\Delta 5$ ELONG, FABP2, CPT-1, UBQ) were made from NCBI sequences. 50 microliters of blood plasma were run through LCMF to obtain fatty acid profiles.

## Results

- After switching diets, expression of genes related to fatty acid transport, fatty acid elongation and desaturation, and beta-oxidation were all up-regulated by the fourth week.
- Facilitated glucose transport was highly variable and inconsistent over time in this cross over trial.
- Fish selected for increased lipid deposition in their fillets did show a slight increase in  $\Delta 6$  desaturase over select line fish when switched to a fish oil diet after two weeks.
- Serum lipid analysis showed fish selected for increased lipid deposition had increased levels of EPA and DHA in their blood at 4 weeks regardless of the diet they were fed.



## Introduction

### Increasing Sustainability of Aquaculture:

The aquaculture industry currently uses the majority of fish oil (FO) and fish meal (FM) produced worldwide to manufacture fish feeds.<sup>1</sup> The reality of finite supplies of FO and FM necessitate the search for alternatives to supply increasing future demand.<sup>2</sup> Considerable work has been accomplished examining alternatives to FO and FM in aquafeeds for salmonids<sup>3</sup> and point out the need to not only explore alternative feeds but also explore ways finfish and shellfish may be genetically selected to utilize alternative feed components thereby minimizing FO and FM in aquafeeds.

Several recent studies have examined the impact of alternative feedstuffs on divergent, selected lines of rainbow trout.<sup>4</sup> These studies show rainbow trout have remarkable physiological plasticity to shift metabolic patterns in response to changes in their diet.<sup>5</sup> In turn, this variation enables alternative dietary sources such as plant oils and plant proteins to be considered in lieu of FO and FM.<sup>2</sup>

In salmonids, different physiological demands throughout their life cycle result in regulation of lipid deposition or mobilization to safeguard the availability of lipids during times of need.<sup>6</sup> Lipolysis and lipogenesis are alternatively stimulated by a number of hormones, including insulin, prolactin, growth hormone, adrenocorticotropic hormone, corticosteroids, somatostatins, and thyroid hormones.<sup>7</sup> Even so, salmonids are unable to synthesize  $\alpha$ -linolenic acid (ALA, 18:3n-3) or linoleic acid (LOA, 18:2n-6) so they must be obtained from the diet and are thus considered essential fatty acids (EFAs).<sup>8</sup> Assimilation, elongation and desaturation of fatty acids to synthesize the metabolically important lcpUFAs, eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) from ALA is a complex process involving several steps. Marine oils typically have adequate amounts of EFAs along with EPA and DHA that have been bioconcentrated at the top of the marine food chain.<sup>7</sup> Oils from terrestrialsources have different fatty acid profiles and characteristically lack EPA and DHA. However, studies have shown ALA can be converted to EPA and then to DHA in rainbow trout.<sup>9</sup>

### Human Health Impacts:

Diminishing use of FO in aquafeeds results in decreased deposition of EPA and DHA in salmonid tissues<sup>10</sup> which in turn diminishes human health-related advantages of consuming fish.<sup>11</sup> In an effort to maintain levels of DHA and EPA at levels healthy for cultured marine fish and for human consumption of fish products, FO replacement in aquafeeds has become an area of intense interest.<sup>12</sup>

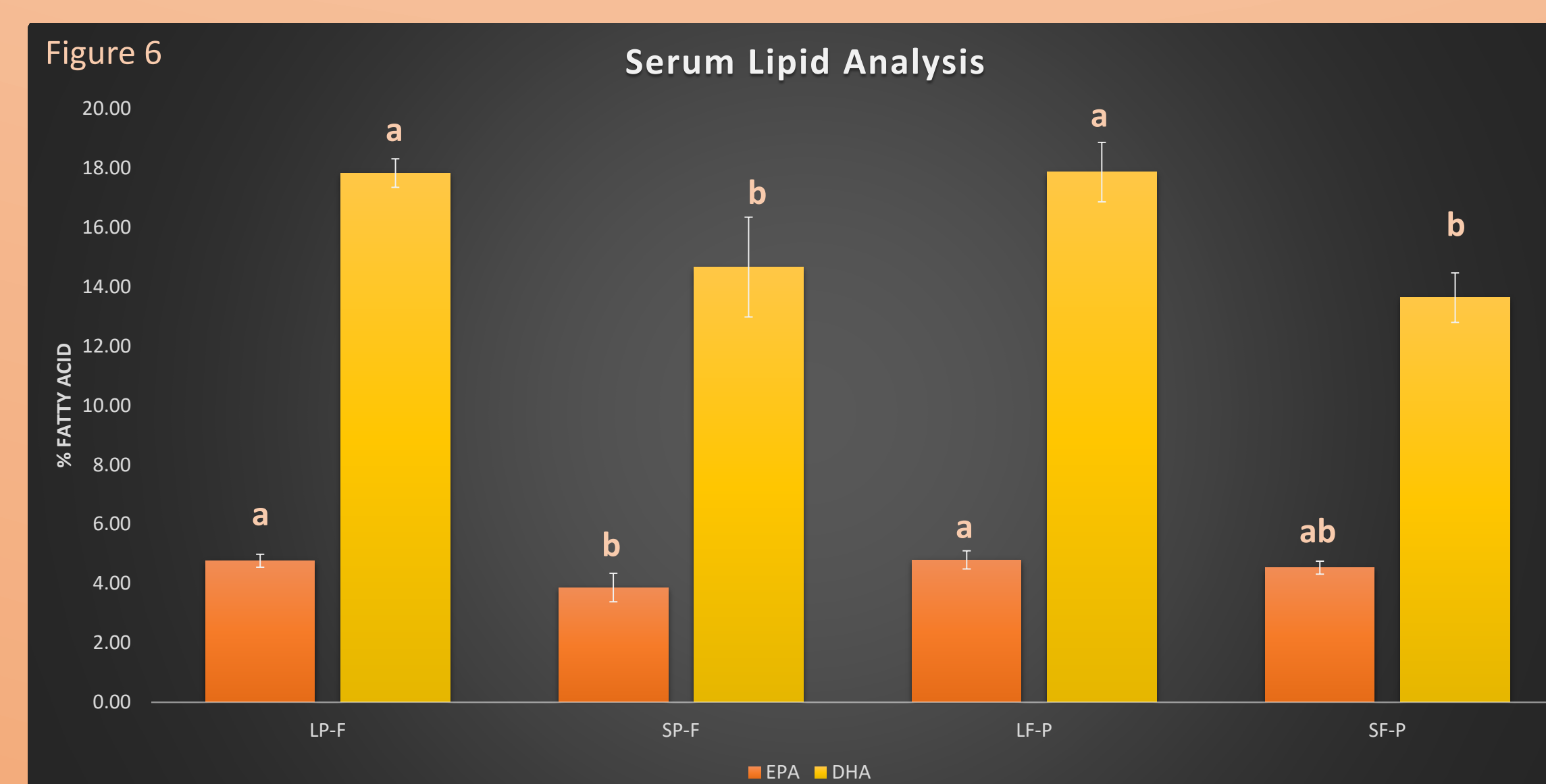
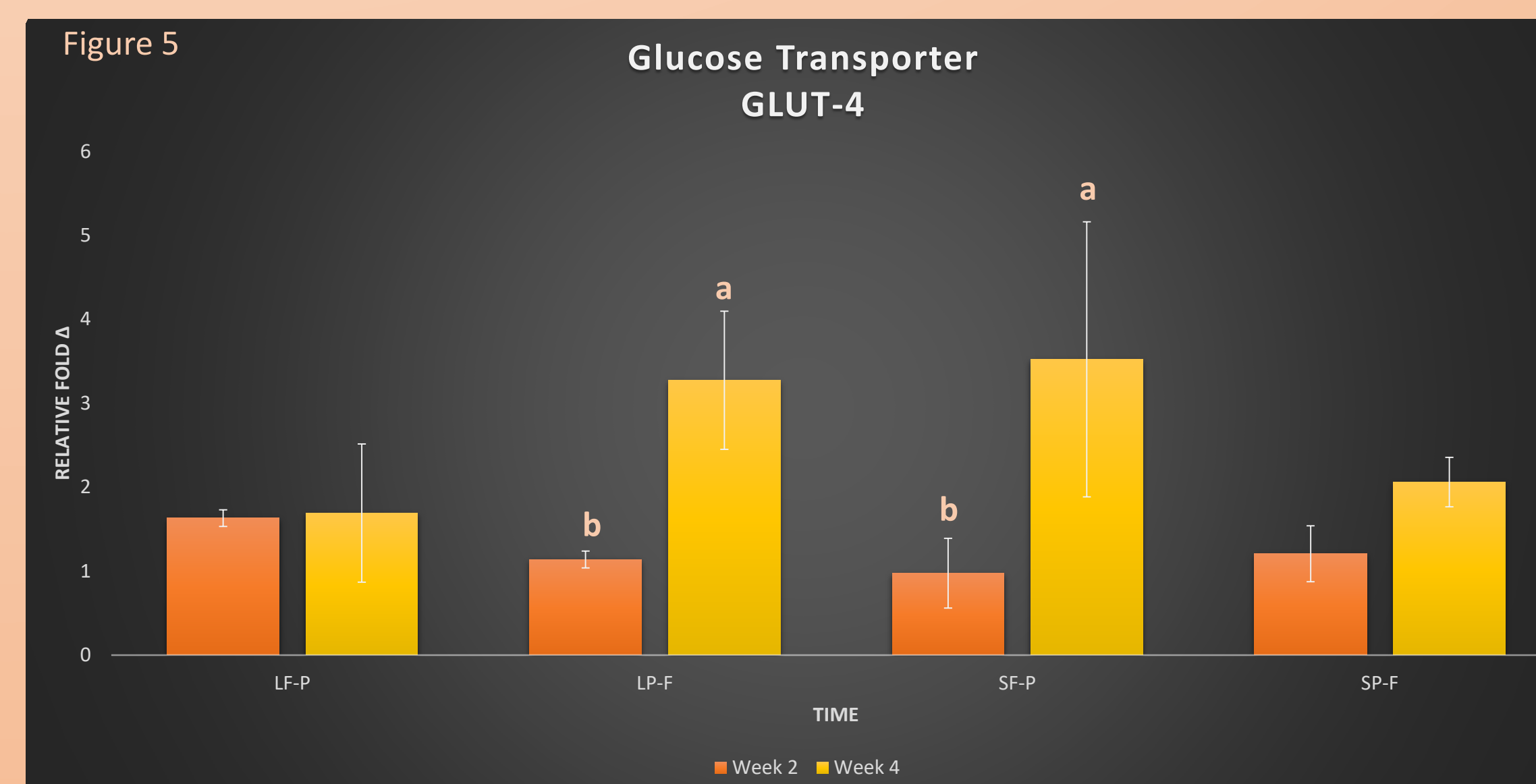
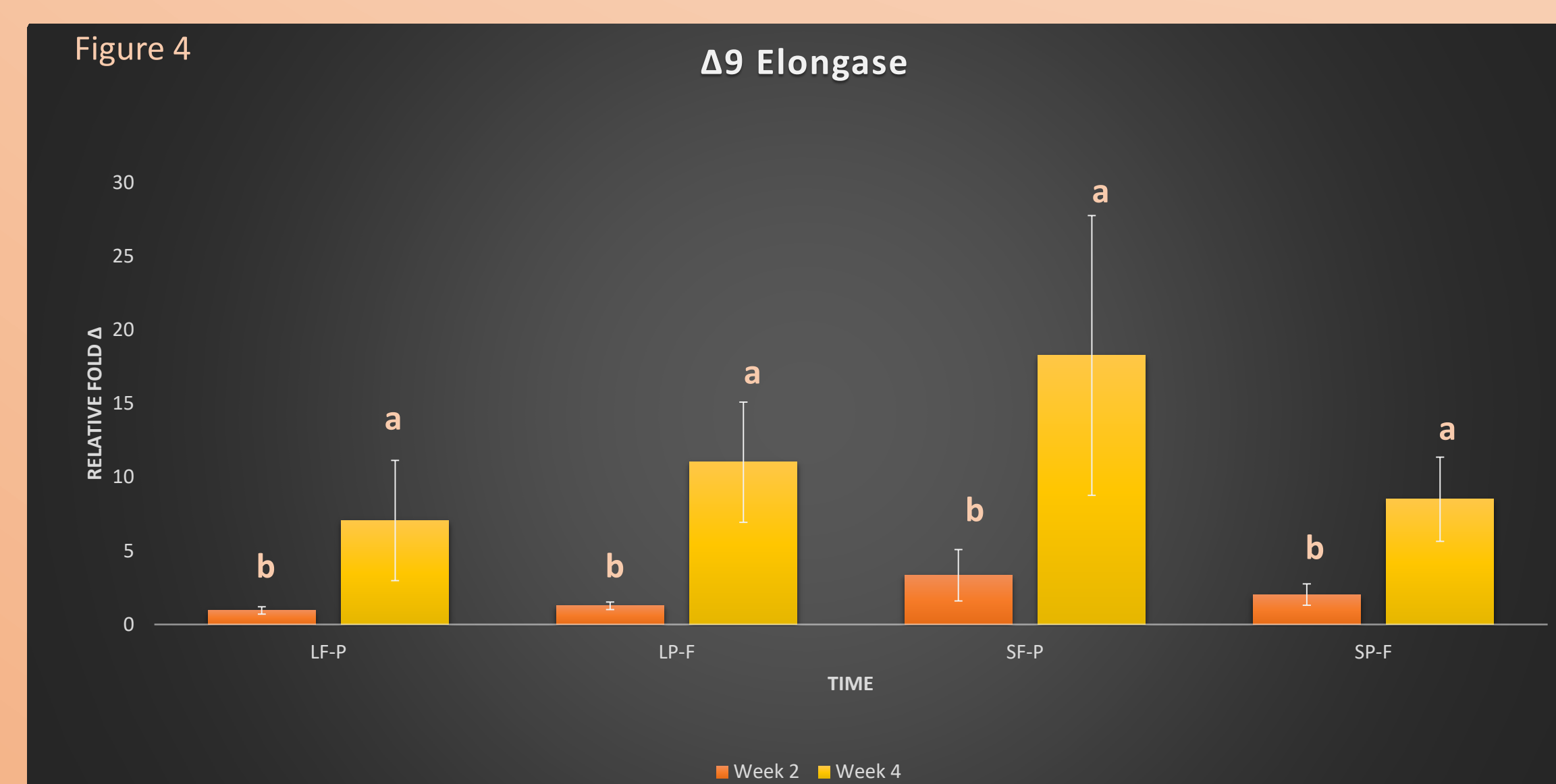
In this study we examined two lines of rainbow trout selected for fast growth (select) or for fast growth and increased lipid deposition in their fillets (lipid) to see if differences arose when a diet containing fish oil was replaced with a diet containing flaxseed oil.

## Hypotheses

- H<sub>0</sub>: Uptake of essential fatty acids will not be affected by origin of dietary lipids in selected vs. non-selected fish.  
H<sub>a</sub>: Uptake of essential fatty acids will be affected by origin of dietary lipids in selected vs. non-selected fish.

## Citations

<sup>1</sup>Food and Agriculture Organization of the United Nations (FAO) 2016. ISBN 978-92-5-109185 <sup>2</sup>Gatlin, D.M., et. al. 2007. Aquaculture Research 38:551-579 <sup>3</sup>Burr, G.S., et. al. 2011. Aquaculture 334-337:110-116. <sup>4</sup>Kalamam, B.s., et. al. 2012. Journal of Experimental Biology 215:2567-2578 <sup>5</sup>Lansard, M., et. al. 2010. Amino Acids 39(3):801-810 <sup>6</sup>Sheridan, M.A., and Kao, Y.H. 1989. American Zoologist 38(2):350-368 <sup>7</sup>Tocher, D.R. and Glencross, B.D. 2015. In: Dietary Nutrients, Additives and Fish Health. Wiley Blackwell, Hoboken, NJ. <sup>8</sup>Das, U.N. 2006. Current Pharmaceutical Biotechnology 7:467-482. <sup>9</sup>Gregory, M.K., et. al. 2016. Nutritional British Journal of Nutrition 115:1721-1729 <sup>10</sup>Bell, J.G., et. al. 2004. Lipids 39:223-232 <sup>11</sup>Harris, W.S. 2009. Current Atherosclerosis Reports 11:411-417 <sup>12</sup>leaver, M.J., et. al. 2008. Reviews in Fisheries Science 16(spl):73-94



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## Discussion

The two diets have significant differences in concentration of ALA, EPA, and DHA content. Both feed types used in this study contained 13% lipids. In the plant oil diet, ALA made up 41% of all lipids while EPA and DHA each accounted for only 0.1%. Conversely, the lipid profile of the fish oil diet contained a much lower concentration of ALA at only 4.1%, and higher concentrations of EPA, and DHA at 7.8% and 6.8% respectively. In spite of the fact that the plant oil contained much lower concentrations of EPA and DHA, the amount found within the fish remained constant in comparison to the fish on the fish oil diet. This result indicates that the fish are not only able to sequester EPA and DHA from foods, but they are also able to convert the available ALA from the plant oil into EPA and DHA.

The upregulation of  $\Delta 6$  Desaturase when the Lipid strain was on the plant oil diet gives tangential evidence of (nutritional programming) where early exposure to a diet consisting mostly of ALA lead to an increased ability to process it into usable fatty acids when given ALA later on in its life.

When comparing the two strains of rainbow trout, the Lipid strain consistently sequestered slightly higher concentrations of DHA and EPA than the Select strain as seen in Figure 6. These data suggest that the Lipid strain is more efficient at converting ALA into EPA and DHA as well as sequestering EPA and DHA directly from a food source than the Select strain regardless of the diet given to them.