Juvenile Salmon Feeding Trial Using ZipZyme™ Omega

ZipZyme™ OMEGA-3 SUPERFOOD by PHYTOSMART

DHA-enriched feed augments lipid accumulation in tissues of juvenile Atlantic salmon.

Abstract:
Docosahexaenoic acid (DHA) is an omega-3 fatty acid that plays critical roles in neurocognitive health in humans. Because salmon and other cold-water fatty fish represent a substantial source of omega-3 fatty acid intake in the human diet, supplementing the diets of salmon may increase human dietary DHA intake. We conducted a two-month feeding trial to assess DHA accumulation in the tissues of juvenile Atlantic salmon fed ZipZyme Krumble™ (ZZK) and ZipZyme™ (ZZ), two whole-cell marine microalgae products that provide high quantities of DHA and contain active DHA synthase enzymes. Salmon fed ZZK exhibited increased DHA content and percentage of total fatty acids (12.24 mg and 17.6%, respectively) versus those fed a control diet (8.94mg and 14.0%, respectively) two months after ZZK feeding ceased. Similarly, tissues of fish fed ZZ exhibited increased DHA content (9.33mg) and percentage of total fatty acids (14.4%) versus those fed a control diet. The DHA content in fish at four months of age after two months of consuming ZZK and ZZ reflected a 27% and 4% increase (respectively) over the control. Our findings suggest that DHA-enriched feed augments omega-3 fatty acid accumulation in the tissues of juvenile Atlantic salmon.

Background:
Docosahexaenoic acid (DHA) is an omega-3 fatty acid found in certain species of marine microalgae and the fish and crustaceans that ingest them. When humans consume DHA in the diet or via dietary supplements, DHA preferentially accumulates in the brain,[1] where it comprises approximately 30 percent of the brain's lipids.[2] As such, DHA plays critical roles in neurocognitive health beginning *in utero* and continuing throughout the lifespan.

For example, high-dose DHA supplementation during pregnancy has been associated with fewer maternal and neonatal serious adverse outcomes and may represent an effective strategy to decrease early preterm birth.[3]In addition, preterm infants who received supplemental DHA at birth performed better on intelligence tests later in childhood than those who did not.[4] Other evidence demonstrates that adults with higher red blood cell DHA concentrations exhibit lower levels of the proinflammatory cytokine interleukin-6 (IL-6) and less methylation of the IL-6 promoter,[5] highlighting DHA's capacity to mitigate inflammation via epigenetic mechanisms and potentially exerting profound long-term effects on human healthspan and longevity.

Finally, a growing body of evidence suggests that higher red blood cell DHA concentrations protect against Alzheimer's disease. In a study of nearly 1,500 older adults, those whose red blood cell DHA concentrations were in the top quintile were

49% less likely to develop Alzheimer's disease during follow-up than those in the lowest quintile.[6] This protective effect of DHA translated to nearly five years of life free of Alzheimer's disease. Higher DHA concentrations conferred more than seven years of protection for carriers of the *APOE4* gene, the primary genetic risk factor for Alzheimer's disease,[7] suggesting that promoting DHA intake among this susceptible group could have marked effects on their neurological health.
Cold-water fatty fish, such as salmon, represent a substantial source of omega-3 fatty acid intake in the human diet.[8] However, like other vertebrates, fish demonstrate nominal omega-3 fatty acid synthesis and must therefore obtain these essential nutrients through their diet.[9] Fish fed an omega-3 fatty acid-rich diet show marked increases in tissue omega-3 fatty acid content, potentially bolstering human dietary DHA intake.

We assessed DHA accumulation in the tissues of salmon fry fed PhytoSmart ZipZyme Krumble™ and ZipZyme™, two whole-cell marine microalgae products that provide high quantities of DHA and contain active DHA synthase enzymes.

Methods:
We conducted a small feeding study at National Cold Water Marine Aquaculture Center in Franklin, Maine, to observe DHA accumulation in juvenile salmon using PhytoSmart products, ZipZyme Krumble™ (ZZK) and ZipZyme™ (ZZ). ZZK is a flaked product containing approximately 15% moisture; ZZ is a paste product containing approximately 75% moisture, providing 20% of the effective nutrient inclusion present in ZZK.

We stocked three isolated tanks (A, B, and C) with 50 Atlantic salmon (*Salmon salar*) fry. The fish in each tank received a base feed diet of SKRETTING GEMMA Micro 150, a commercial diet commonly used in the aquaculture setting. We supplemented Tank A with ZZK at a 5% inclusion rate (on a total feed weight basis) and Tank B feed with ZZ at a 5% inclusion rate (on a total feed weight basis). Both products were tumble-coated onto the base feed for delivery. We did not supplement Tank C, which served as a control group.

The duration of the trial period was four months. Tanks A and B received their respective supplemented feeds for two months, then 100% base feed for two months. Tank C received 100% base feed for four months. We permitted all fish to eat to satiation throughout the trial duration.

At the end of the trial period, we weighed and freeze-dried the three groups of fish and prepared oil extraction samples from each tank and each feed. Using acid hydrolysis, we calculated the net fatty acid (FA) content and DHA composition in the feed mixes as well as the resulting fish grind. We analyzed the FA profile to determine DHA percentages.

Results:

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Tissues of fish fed ZZK exhibited increased DHA content and percentage of total fatty acids (12.24 mg and 17.6%, respectively) versus those fed a control diet (8.94mg and 14.0%, respectively) two months after ZZK feeding ceased. Similarly, tissues of fish fed ZZ exhibited increased DHA content (9.33mg) and percentage of total fatty acids (14.4%) versus those fed a control diet. The DHA content in fish at four months of age after two months of receiving ZZK and ZZ reflected a 27% and 4% increase (respectively) over the control.

These results are summarized in the table below.

	DHA content in diet (mg/g)	DHA content in fish (mg/g at 4 mo)	% DHA of total FA in fish (% at 4 mo)	% DHA increase (compared to control)
ZZK (5% inclusion)	12.05	12.24	17.6%	27%
ZZ (5% inclusion)	8.05	9.33	14.4%	4%
Control	9.28	8.94	14.0%	-

Abbreviations: **DHA**, docosahexaenoic acid; **mg**, milligrams; **g,** gram; **mo**, month; **FA**, fatty acid; **ZZK**, ZipZyme Krumble™; **ZZ**, ZipZyme™

Discussion:

Our findings demonstrate that DHA-enriched feed augments omega-3 fatty acid accumulation in the tissues of juvenile Atlantic salmon. Our observation of a 27% increase in tissue DHA content (versus control) following a two-month feeding trial using a 5% DHA inclusion diet raises some interesting possibilities, however.

For example, the pre-manufactured DHA in the 5% ZZK inclusion diet was likely insufficient to account for such a robust increase in end product DHA because the ZZK added to the diet did not contain that quantity of DHA. It is possible that the bioavailability of DHA in ZZK was more than 220% better than that of the DHA in the control diet. However, there are no known differences in the forms of DHA (such as triglyceride, phospholipid, or free DHA) between the control diet and the ZZK/ZZ diets that would lead to such bioavailability differences. And considering that the net DHA amount in the control diet group decreased from 9.28mg/g to 8.94mg/g, simultaneous increases in ZZK- and ZZ-fed fish would unlikely be accounted for by the DHA existing in the diet. Furthermore, the two-month period during which ZZK and ZZ were not added to the trial diets would have likely seen the same dip in DHA accumulation as the control diet without DHA synthase enzyme activity.

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We believe this study strongly demonstrates DHA synthesis in juvenile salmon due to active DHA synthase enzymes provided in ZZK and ZZ products. Future studies should involve longer trials, compare larger ZZK/ZZ inclusion rates (50% to 100%) to control diets, and assess DHA synthase enzyme validity via substrate isotope marking.

References:

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Original version (do not use):

This small-scale study was conducted at USDA ARS NCWMAC in Franklin, Maine in order to observe the benefits to DHA accumulation in Salmon using PhytoSmart products. ZipZyme Krumble™ (ZZK) and ZipZyme™ (ZZ), products were added to Salmon diets. ZZK and ZZ products contain whole cell marine microalgae, which have high quantities of DHA and contain active DHA synthase enzymes.

Three isolated tanks (A, B and C) were stocked with 50 salmon fry each. A base feed diet of SKRETTING GEMMA micro 150 was fed to all three tanks. Tank A feed was supplemented with ZZK at a 5% inclusion rate (on a total feed weight basis). Tank B feed was supplemented with ZZ at a 5% inclusion rate (on a total feed weight basis). Tank A and B supplementation was

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provided by tumble coating the ZZK or ZZ onto the base feed. Tank C feed was not supplemented, receiving 100% base feed. Tank C was used as a control group.

The total trial period was four months. Tanks A and B were fed supplemented feed for two months, then subsequently fed 100% base feed for two months. Tank C was fed 100% base feed for four months. All trial fish were fed to satiation throughout trial duration.

At the end of the trial period, the fish were weighed and freeze dried. Oil extraction samples were prepared from each tank and each feed. The net Fatty Acid (FA) content in the feed mixes as well as the resulting fish grind were calculated using acid hydrolysis. The FA profile was also analyzed to determine DHA percentages.

Results:

ZZK is a flaked product containing 15.8% moisture, while ZZ is a paste product containing ~75% moisture, therefore ZZ had an effective nutrient inclusion of ~⅛ the ZZK.

	Diet FA Content (mg)	Fish FA Content (mg at 4 mo.)	Fish DHA % in FA (4 mo.)
ZZK (5% Inclusion)	12.05	12.24	17.6%
ZZ (5% Inclusion)	8.05	9.33	14.4%
Control (100% Inclusion)	9.28	8.94	14.0%

Table 1. FA and DHA percentage analysis results on diets and resultant fish.

The data (table 1) showed a 34% increase in net DHA value in fish fed ZZK at 5% feed inclusion for 2 months followed by 100% control diet for two months.

The pre-manufactured DHA in the 5% ZZK inclusion diet is not likely large enough to account for a 34% increase in end product DHA as the ZZK added to the diet did not contain that quantity of DHA. A possibility is that the bioavailability of DHA in ZZK was more than 220% better than that of the DHA in the control diet. However, there are no known differences in the types of DHA (such as triglyceride DHA, phospholipid DHA, free DHA) between the control diet and the ZZK/ZZ diets that would lead to bioavailability differences. Furthermore, considering that the net DHA amount in the 100% control diet group decreased to 0.894% from 0.928%, simultaneous increases in ZZK, and ZZ fed fish, would unlikely be accounted for by the DHA existing in the diet. The two month period where ZZK and ZZ were not added to the trial diets would have likely seen the same dip in DHA accumulation as the control diet without DHA synthase enzyme activity. We believe this study strongly supports DHA synthesis within the fish due to active DHA synthase enzymes provided in ZZK and ZZ products.

To make the experiments conclusive, comparison in larger ZZK/ZZ inclusion rates (50%, 100%) compared to control diets, as well as the isotopic metabolomics analysis using clear spiked substrate should confirm DHA synthase enzyme validity. Also, longer trials with more replication are required for thorough statistical evaluation.

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