Microalgae-enriched feed enhances omega-3 fatty acid content of chicken eggs.

ZipZyme™
OMEGA-3 SUPERFOOD
by PHYTOSMART

Abstract:

Omega-3 fatty acids are essential long-chain polyunsaturated fatty acids that play critical roles in human health. Enrichment of commonly consumed dietary products is a viable means of increasing their intake in humans. We conducted two, small feeding trials to assess the capacity of ZipZyme™, a heterotrophic microalgae species, to increase docosahexaenoic acid (DHA) content in chicken eggs. In the first trial, we found that one month of ZipZyme™ feeding showed a decrease in DHA accumulation, but four weeks after ZipZyme™ cessation, DHA content rebounded to near baseline levels (~0.05% of total fatty acids). In the second trial, we found that 40 days after ZipZyme™ cessation, DHA levels nearly doubled, from baseline to 0.09% of total fatty acid content. These findings suggest that ZipZyme™ provides DHA-synthesizing enzymes and that these enzymes remain active after ingestion by young hens, subsequently increasing DHA content within laid eggs.

Background:

Omega-3 fatty acids are essential long-chain polyunsaturated fatty acids that play critical roles in human health. Robust evidence demonstrates that omega-3 fatty acids may prevent or ameliorate both acute and chronic health conditions, including viral infections, cardiovascular disease, neurodegenerative disorders, inflammatory conditions, and many diseases of aging.[1-4]

Omega-3 fatty acids are deemed "essential" because the human body cannot synthesize them. Alpha-linolenic acid (ALA), an omega-3 fatty acid that serves as a precursor to other essential fatty acids, can be converted in the body to the longer-chain omega-3 fatty acids eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), but the conversion rate is notably low.[4] For this reason, nutrition experts recommend eating foods high in EPA and DHA,[5] which are naturally present in certain species of marine microalgae and the cold water fatty fish that consume them.

The Food and Nutrition Board of the Institute of Medicine (now called the National Academy of Medicine) has determined that an adequate intake of ALA for adult males and females is approximately 1.6 and 1.1 grams per day, respectively, which equates to approximately 500 milligrams of EPA and DHA. However, most people living in Western countries fail to meet these intake levels. Omega-3 fatty acid enrichment of commonly consumed dietary products is a viable means of increasing their intake in humans.[6]

We sought to demonstrate that ZipZyme™, a product of concentrated marine microalgae, enhances DHA content in chicken eggs. ZipZyme™ contains *Crypthecodinium cohnii* (*C. cohnii*), heterotrophic microalgae that synthesize DHA from non-highly unsaturated fatty acid (HUFA) precursors and are therefore not reliant upon ALA conversion.[7] One 5-milliliter packet of ZipZyme™ is expected to provide at least 17 milligrams of DHA (primarily in phospholipid form) as well as active DHA synthase enzymes, which are expected to further promote DHA production from non-HUFA sources.

Methods:

We conducted two, small, consecutive feeding trials to gather preliminary data on ZipZyme™'s capacity to increase the DHA content in chicken eggs.

In the first trial (Trial 1), we isolated three healthy older hens from a large commercial flock (which served as the control group) and housed them in a separate coop. All hens received their normal feed, but the isolated hens also received one 5-milliliter packet of ZipZyme™ daily for four weeks.

We collected egg samples from the ZipZyme™-fed hens (three to six eggs per hen) during the first ("sample 1") and fourth ("sample 2") weeks of the intervention and submitted them to the New Jersey Feed Lab (NJFL) for fatty acid composition analysis. We also sent eggs from sample 2 to Creative Proteomics to confirm the NJFL findings. We collected and tested an additional sample of eggs ("sample 3") four weeks after cessation of the ZipZyme™ feeding. In addition, we collected eggs from the control group hens (which received the same feed, but without ZipZyme™) and submitted them for fatty acid composition analysis ("sample 4").

In the second trial (Trial 2), we isolated three young birds from a large flock (approximately 25 weeks old) and housed them in a separate coop. We mixed one packet of ZipZyme™ into the three birds' daily feed for 60 days. We collected sample eggs after 50 days ("sample 5") and again at approximately 60 days after cessation of the ZipZyme™ feeding ("sample 6") and submitted them for fatty acid composition analysis.

Results:

The sampling data from the first trial indicated that the DHA content in the control group eggs was consistent with USDA measurements. Less than one week of ZipZyme™ feeding was insufficient to detect changes in DHA content. One month of ZipZyme™ feeding showed a decrease in DHA accumulation. Four weeks after ZipZyme™ cessation, DHA content rebounded to near baseline levels (~0.05% of total fatty acids).

Sampling data from the second trial indicated that the DHA content after 60 days of ZipZyme™ feeding was the same as the content prior to feeding. Interestingly, at approximately 40 days after ZipZyme™ cessation, DHA levels nearly doubled, from baseline to 0.09% of total fatty acid content.

The results of the egg sample analyses are presented in the following table.

Trial 1							
Time after ZZ feeding initiation		**Baseline**		**4 weeks**		**4 weeks feeding + 4 weeks without ZZ feeding**	
		Whole egg %	**FA %**	**Whole egg %**	**FA %**	**Whole egg %**	**FA %**
	ALA	0.53	0.04	0.32	0.02	0.37	0.031
	DHA	0.71	0.05	0.45	0.02	0.55	0.047
	ALA to DHA Ratio	**0.75**	-	**0.71**	-	**0.67**	-
Trial 2							
Time after ZZ feeding initiation		**8 weeks**		**8 weeks feeding + 8 weeks without ZZ feeding**			
		Whole egg %	**FA %**	**Whole egg %**	**FA %**		
	ALA	0.5	0.045	1.18	0.1		
	DHA	0.5	0.045	**1.07**	**0.09**		
	ALA to DHA ratio	**1**	-	**1.1**	-		
Control eggs							
		Whole egg %	**FA %**				
	ALA	0.44	0.03				
	DHA	0.74	0.05				
	ALA to DHA ratio	**0.59**					

Abbreviations: ZZ, ZipZyme™; FA, fatty acid; ALA, alpha-linolenic acid; DHA, docosahexaenoic acid

Discussion:

Naturally occurring omega-3 fatty acids are typically present as either triglycerides (TG) or phospholipids (PL).[8] The reduced DHA content in eggs that we observed during our first trial may be because ZipZyme™ DHA is in PL form, and the DHA in egg yolk is typically in TG form. PL-DHA is more readily available to cells than TG-DHA, and PL-DHA in the feed may have been utilized elsewhere instead of undergoing the trans-esterification process to become TG-DHA in egg yolk.

The ZipZyme™ used in our second trial contained approximately 1020 milligrams of non-enzyme-related DHA. The three sample eggs at the end of the second trial showed a 0.045% increase in DHA compared to the control group. When we assume an average egg weight of 35 grams each, the DHA content attributed to ZipZyme™ in the final three-egg sample was 47.25 milligrams (0.045%*105 grams).

Assuming the chickens laid 60 eggs (one egg every three days) between ZipZyme™ feeding cessation and when the final egg samples were taken, we can calculate* how much DHA was produced above their baseline DHA concentrations. If we assume the DHA increase due to ZipZyme™ was linear during the 60 days of laying, we can use the average of an extra 0.0225% per egg ((0.09-0.045)/2). These assumptions indicate that a total of 472.5 milligrams of DHA accrued in the eggs (reflecting a transfer rate of approximately 46%) due to ZipZyme™ over the 60-day period compared to hens not fed ZipZyme™.

A study in which hens received an enzyme-free microalgae-supplemented feed demonstrated a DHA transfer efficiency of approximately 17% to 24% into eggs. The increased egg DHA we observed using ZipZyme™ was nearly twofold higher than this amount due to the active DHA-producing enzymes.

The proportion of ALA/DHA in fatty acid composition in the eggs from our second trial is consistent with ZipZyme™ enzyme accumulation. ZipZyme™ enzymes will not convert ALA to DHA; rather, they synthesize DHA from non-HUFA molecules. This explains the higher ALA found in sample 6 compared to the control group. This fatty acid composition suggests the presence of competition between “ALA to DHA” versus “non-HUFA to DHA” pathways and the presence of ZipZyme™'s enzyme activity.

The younger hens used in our second trial may have acquired the enzymes more readily due to their still-growing liver size, as the liver is a known enzyme storage area. A future study that entails elongating the trial period after ceasing ZipZyme™ feeding would be beneficial to determine if even higher DHA levels could be obtained, and how long the accumulated enzymatic effect remains active for its egg production.

These trials and data affirm enzymatic activity in microalgae-fed chickens to produce DHA-rich eggs. However, it raises new and further questions regarding optimization and physiology:

Optimization

- When is the best and most efficient time to achieve enzyme accumulation?
- How much ZipZyme™ is necessary to produce specific outputs?
- If the enzymatic pathway competition exists, what is the best combination of feed that optimizes DHA production with ZipZyme™?

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- ZipZyme™ is non-fattened biomass. If the birds are fed with fattened ZipZyme™ that is rich in TG-DHA in addition to PL-DHA, will the birds convert already-made TG-DHA into egg yolk? What would be the transfer efficiency of such TG-DHA?
- Economically maximizing the enzymatic production of DHA may require early accumulation of ZipZyme™ enzymes in birds before egg-laying age. In such a case, when and how much of ZipZyme™ is optimal?

Physiology

- The ZipZyme™ enzyme process uses small carbohydrates for DHA synthesis, but is there a specific intermediary or precursor molecule? What could be the direct precursor to DHA in this pathway?
- If the enzyme is functional in birds, where is the protein stored and how does it function?
- Will there be a high concentration of the protein in the liver tissue after loading?

The answers to these questions require larger scale, organized feeding, and structured data acquisitions. Future research will focus on answering these questions.

*Total DHA addition calculation:
60 Eggs at 35 grams (g) per egg = 2100g or 2,100,000 milligrams (mg)
2,100,000mg * 0.0225% = 472.5 mg

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