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SmartZYME™ DHA Synthase Enzyme Efficacy Feeding Trial on Layer Hens

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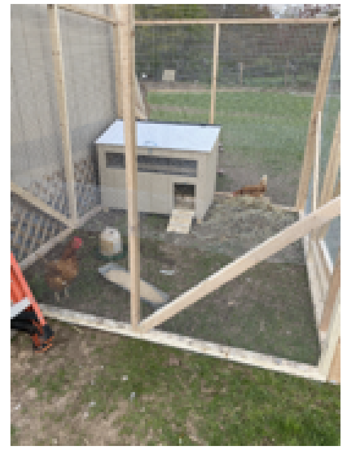

A small feeding trial to gather preliminary data on a feed supplement's ability to increase the DHA content in chicken eggs was conducted in 2020 at Fowler Farm, 208 Duncaster Rd, Bloomfield, CT 06002.

Two consecutive feeding trials were conducted commencing May 6, 2020, and ending on November 11, 2020. In each trial set, in addition to their normal feed, the birds were also fed SmartZYME™ (SZ), a product of concentrated *Crypthecodinium cohnii* that is brined with sterilized sea salt and packaged in individual 5ml quantities. Each packet of SZ is expected to contain at least 17mg of DHA, primarily in phospholipid form, and *Crypthecodinium cohnii's* active DHA synthase enzymes which were expected to further produce DHA from non HUFA (highly unsaturated fatty acids) sources.

All samples contained 3-6 eggs collected as a set. Samples were sent to New Jersey Feed Lab (NJFL) to be analyzed for Fatty Acid (FA) composition using gas chromatography (one GC data point derives from 3-6 eggs).

- Feeding Trial 1: May 6 to June 7, 2020. For the duration of this trial three chickens were isolated in a coop. One packet of SZ was mixed into the three birds' daily feed for the period of four weeks. Egg samples were taken from both Week 1(sample 1) and Week 4 (sample 2). Samples from the 4th week were also sent to Creative Proteomics for analysis to confirm NJFL findings. An additional sample (sample 3) was taken and tested four weeks after SZ feeding had stopped.
- As a control group, 6 eggs were taken from other birds at the same farm, on the same feed, but without SZ, and tested (sample 4).
- Feeding Trial 2: July 14 to Nov. 17, 2020. This trial was conducted on 3 younger birds who were beginning to lay (~25 weeks old). One packet of SZ was mixed into the three birds' daily feed for 60 days. Sample eggs were taken after 50 days (sample 5). Further egg samples were taken ~60 days after the SZ addition to feed was stopped (sample 6).

Results:

The following table shows the results of the egg sample analysis.

Trial 1: The sampling data shows that the control group is consistent with USDA measurement for DHA content in eggs. Less than one week of feeding SZ was not long enough to detect changes in DHA content. One month of SZ feeding showed a decrease in DHA accumulation. Four weeks after stopping SZ showed a rebound of the DHA content to largely base levels (~0.05% of total FA).

Trial 2: The DHA value after 60 days of feeding SZ is the same as the value prior to feeding SZ. The most interesting results come from ~40 days after stopping SZ feed, where DHA levels almost doubled from normal to 0.09% of total FA.

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	Reference USDA data	Feeding Trial 1						Control		Feeding Trial 2			
Sample #	**-**	**1**		**2**		**3**		**4**		**5**		**6**	
# days after feeding started	**-**	Older hens +4 days		Older hens 4 weeks		Olde hens 4 weeks SZ 4 weeks noSZ		No SZ		Young hen 8 weeks (50 days)		Young hen 8 weeks SZ 8 weeks noSZ	
Testing Lab	**NA**	NJFL											
NJFL Record #	**-**	0520-2453		0620-1400		0820-0373		0720-2484		0920-1200		0121-0332	
ALA	**-**	0.53	0.04	0.32	0.02	0.37	0.031	0.44	0.03	0.5	0.045	1.18	0.10
DHA	**0.058**	0.71	0.05	0.45	0.02	0.55	0.047	0.74	0.05	0.5	0.045	**1.07**	**0.09**
ALA/DHA		**0.75**		**0.71**		**0.67**		**0.59**		**1.00**		**1.10**	

Right side columns show % of whole eggs, and left side shows % against total fatty acids

Discussion:

The decreased amount of DHA in eggs during trial 1 may be because SZ DHA is primarily in Phospholipid (PL) form rather than in Triglyceride (TG) form, and the DHA in egg yolk is expected to be primarily TG form, rather than PL form. PL-DHA may not be easily converted to TG-DHA, and PL-DHA in the feed may have been metabolized to something else instead of going through the trans-esterification process to become TG-DHA in egg yolk.

The SZ used for the duration of trial 2 contained a rough total of 1020mg of non enzyme related DHA. The 3 sample eggs at the end of trial 2 showed 0.045% increase in DHA, when compared with the control group. When we assume an average egg weight of 35g each, the DHA content attributed to SZ in the final three egg sample is 47.25mg (0.045%*105g).

Assuming the chickens laid 60 eggs (one egg every three days) between when SZ feeding was stopped and the final egg samples were taken, we can calculate how much DHA was produced on top of original (not SZ related) DHA. If we assume the DHA increase due to SZ 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 calculate* a total of 472.5mg of DHA was added to the eggs due to SZ over the 60 day period as compared with hens not fed SZ.

An analysis of Alltech's TG DHA with zero enzyme feed showed a DHA transfer efficiency of ~15% into chicken eggs. The increases in egg DHA are vastly higher than 15% using SmartZYME™ feeding, suggesting endogenous activity of DHA synthase enzymes.[1]

The proportion of ALA/DHA in FA composition within trial 2 eggs is consistent with SZ enzyme build up. SZ enzymes will not convert ALA to DHA (SZ does not have any ALA in its FA composition and therefore its enzymes create DHA from non-HUFA molecules), which explains the higher ALA found in sample 6 compared to the control group. This FA composition suggests the presence of competition between "ALA to DHA", vs. "Non-HUFA to DHA" pathways and the presence of SZ's enzyme activity. The younger hens used in trial 2 may have acquired DHA synthase enzymes more readily due to their

[1] "Docosahexaenoic acid enrichment of layer hen tissues and eggs through dietary supplementation with heterotrophically grown Aurantiochytrium limacinum" C. A. Moran et.al. *Published by Elsevier Inc. on behalf of Poultry Science Association Inc.*

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still growing liver size, as the liver is a known enzyme storage area. A future study that entails elongating the trial period after ceasing SZ feed would be beneficial to determine if even higher DHA levels could be obtained, and how long the built up enzymatic effect remains active.

This experiment and set of data affirms enzymatic activity in fed chickens for the production of DHA rich eggs. However, it provides new and further questions in multiple dimensions.

1. DHA Acquisition Optimization:
    - When is the best and most efficient time to achieve enzyme build up?
    - How much SZ is necessary to produce particular outputs?
    - If the enzymatic pathway competition exists, What is the best combination of feed that optimizes DHA production with SZ?
2. Physiology:
    - The SZ 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?

The answer to these questions requires larger scale, organized feeding and structured data acquisitions.

*Total DHA addition Calculation:
60 Eggs at 35g per egg = 2100g or 2,100,000mg
2,100,000mg * 0.0225% = 472.5 mg