The effect of *Plukenetia Volubilis* residue on Omega-3 enriched eggs



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ABSTRACT

Background: Omega-3 DHA-enriched eggs provide the consumer with a value-added product that delivers a clear, functional benefit for an increasingly health conscious population. Human requires an omega-6: omega-3 ratio of 4:1. The typical Thai diet which use a lot of vegetable oil for cooking provides a dramatically high 20:1 ratio. Lowering this ratio may reduce the risk of many chronic diseases including cardiovascular disease, coronary heart disease, Alzheimer's disease and some cancers. A healthy omega-6:omega-3 ratio promotes the health benefits. The increased levels of DHA provides much needed nutrient essential to human brain functions e.g. brain activities. Aims and Objective: The purpose of this study was to study the Omega-3,6,9 concentration in raw and boiled eggs from hen feeding with Plukenetia Volubilis residue. Materials and Methods: A sample was selected from raw and boiled eggs feeding with Plukenetia Volubilis residue. Results: The result showed that total level of Omega-3 with 37869.65 mg/100mL and Omega-6 with 38490.67 mg/100mL which was not much different in Plukenetia Volubilis oil. However total Omega-9 was only 8394.74 mg/100mL compared to 37869.65 (Omega-3) and 38490.67 (Omega-6) which was almost 5 times lower than Omega-3,6.In addition, the comparison of Omega-3,6,9 concentration between raw and boiled eggs from hen feeding by Plukenetia Volubilis residue were also analyzed. The total level of Omega-3,6,9 in boiled eggs was higher than in raw eggs (Omega-3: 122.85 mg/100g (raw egg), 158.12 mg/100g (boiled eggs); Omega-6: 1479.18 mg/100g (raw egg), 1766.53 mg/100g (boiled eggs); Omega-9: 3009.16 mg/100g (raw egg), 3375.23 mg/100g (boiled eggs)), respectively. Conclusion: There is constantly growing demand from consumers for food products of superior health quality. We can increase the omega-3 content of eggs through the enrichment of the layers' diet with Plukenetia Volubilis. However, the relationship of Plukenetia Volubilis residue in chicken egg is still needed for further study.

Key words: Egg; Sacha Inchi; Plukenetia Volubilis residue

INTRODUCTION

Functional foods have become a popular topic in the food industry due to the push for healthier food products on supermarket shelves. There are many different types of fats in the foods we eat. Most Western diets contain too much saturated fat or saturated fatty acids (SFA). Many of us eat 20% more than the recommended maximum amount of SFA. The changing of omega-6/omega-3 polyunsaturated fatty acids (PUFA) in the food supply of Western societies occurred over the last 150 years is thought to promote the pathogenesis of many inflammatory-related diseases.1-4

DHA (Docosahexaenoic acid) is an essential omega-3 fatty acid that the body cannot efficiently produce itself - it must be taken in through diet. DHA is delivered to infants via their mother during pregnancy, and after birth through breast milk or enriched formula. When infants grow into toddlers and transition to eating and drinking at the table, a nutritional gap is created. Dietary sources of DHA such as salmon and mackerel are not commonly found on the

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plates of children, which leads to less than optimal levels of DHA intake.⁵⁻⁹ Children are regularly consuming only 25% of the daily amount of DHA recommended by experts. The opportunity exists to fill this nutritional gap with naturally DHA-enriched foods that are without all of the additives and no"fishy" taste found in DHA-added products currently on the market. 6-7,9-11 DHA is a long chain fatty acid which is found in the tissues of salmon, mackerel and other fish. It is the only kind of omega-3 that is efficiently absorbed and taken directly into the cell membranes, and is an essential nutrient that helps support brain, eye and heart health.^{5,11} The human population can no longer rely on fish consumption as an adequate supply of DHA Omega-3 given that DHA and EPA have decreased in farmed fish due to the shift in raw materials used in aquaculture diets. 5-6,10-11

The process to producing DHA-enriched foods is simple. When chickens are fed a DHA-enriched diet, the DHA is passed along to consumers via the eggs, providing additional nutrition to the consumer in each bite. 9-11 DHAenriched functional eggs can be consumed in a tasty and convenient delivery system - the foods we already more enjoy eating compare with fish oil supplementation. Food products are now being fortified or enriched with DHA omega-3 in order to meet consumers' nutritional requirements. 5,9-11 Omega-3 DHA-enriched eggs provide the consumer with a value-added product that delivers a clear, functional benefit for an increasingly health conscious population, while offering the retailer differentiation from competitors. The importance of DHA does not become any less significant with age. Thai foods use too much vegetable oil or omega-6 for stir fried and deep-fried cooking. Levels of omega-3 fatty acids, such as DHA and eicosapentaenoic acid (EPA) have decreased in fish due to the shift in raw materials used in aquaculture diets.^{5,9-11} Adult consumers are not receiving an adequate amount of dietary DHA to balance the high ratio of omega-6 to omega-3 fatty acids found in today's Thai diet. This disproportionate ratio may contribute to excess inflammation in the body, increasing the incidence of chronic disease. Plentiful levels of dietary DHA are shown to reduce the risk of coronary heart disease, depression, cancer, age-related mental decline and improve brain function.1-9,12-14

Human requires an omega-6:omega-3 ratio of 4:1. The typical Thai diet which use a lot of vegetable oil for cooking provides a dramatically high 20:1 ratio. Lowering this ratio may reduce the risk of many chronic diseases including cardiovascular disease, coronary heart disease, Alzheimer's disease and some cancers. In addition, a healthy omega-6:omega-3 ratio promotes the health benefits. The increased levels of DHA provide much needed nutrient essential to human brain functions e.g. brain activities.^{5,7-13} The daily

intake of omega-3 fatty acid should be in the range of 140 mg/day to 667 mg/day. However, a few countries reach the intake of only 250 mg per day; therefore, the trend of enriched food products with omega-3 fatty acids have emerged. Eggs are the potential source of n-3 fatty acids because they can be easily enriched with omega-3 PUFA through dietary modifications of the laying hens. Thus, the aim of this study was to study the Omega-3, 6, 9 concentration in raw and boiled eggs feeding with *Plukenetia Volubilis* residue.

MATERIALS AND METHODS

Tools and equipments

- Sample: Plukenetia Volubilis oil was extracted by Sakda Sacha Inchi Inca (Thailand) Co., Ltd., Khongchai Sub-district, Khongchai District, Kalasin Province, Thailand.
- 2. Sample examination: The nutrient specification and fatty acid profile in *Plukenetia Volubilis* oil to prepare for developing DHA enriched eggs; raw and boiled eggs.
- 3. Research Protocol: This study was designed to compare the nutrient specification and fatty acid profile in *Plukenetia Volubilis* oil. Eggs were collected to test for nutrient specification and fatty acid profile at Central Laboratory (Thailand) Co., Ltd., KhonKhaen, Thailand.

Materials

Sample Type: Sacha Inchi Oil; Raw and Boiled Eggs Packaging: Glass Bottle Plastic Lid Quantity: 1 bottle, Weight/Volume: 3000mL Temperature: Room temperature, in good condition when received

Reference methods

- Energy in-house method TE-CH-169 based on Compendium of Methods for Food Analysis Thailand, 1st edition, 2003
- 2. Protein in-house method TE-CH-042 based on AOAC (2012), 981.10
- Carbohydrate in-house method TE-CH-169 based on Compendium of Methods for Food Analysis Thailand, 1st edition, 2003
- 4. Of which sugars in-house method TE-CH-169 based on Compendium of Methods for Food Analysis Thailand, 1st edition, 2003
- 5. Fat IAOAC (2012), 922.06
- 6. Of which saturates in-house method TE-CH-208 based on AOAC (2012) 996.06
- Fibre in-house method TE-CH-076 based on AOAC (2012) 985.29

- 8. Sodium in-house method TE-CH-134 based on AOAC (2012) 984.27 by ICP-OES
- 9. Omega-3 in-house method TE-CH-208 based on AOAC (2012) 996.06
- 10. Omega-6 in-house method TE-CH-208 based on AOAC (2012) 996.06
- 11. Omega-9 in-house method TE-CH-208 based on AOAC (2012) 996.06.

RESULTS

In the present study, the *Plukenetia Volubilis* oil and eggs sample were sent to test for nutrient specification and fatty acid profile. A comparison nutrient profile for *Plukenetia Volubilis* Oil is presented in Tables 1-3.

Table 1: Nutrient specification of Plukenetia Volubilis oil					
Typical value	Per serving (15 mL)	Per 100mL			
Energy (kJ)	519	3460			
Energy (kCal)	126	842			
Protein (g) (%N x 6.25)	0.0	0.0			
Carbohydrate (g) of	0.0	0.0			
which sugars (g)	0.0	0.0			
Fat (g) of which	14.0	93.5			
saturates (g)	1.1	7.4			
Fibre (g)	0.0	0.0			
Sodium (a)	0.00	0.0			

Table 1 shows total level of energy as 3460 kJ/100 mL and 842 kCal/100mL. However total fat is only 93.5/100mL while of which saturates as 7.4/100mL, respectively.

Table 2: Omega-3,6,9 concentration in Plukenetia Volubilis oil				
Typical value	Test Results (mg/100mL)	LOD		
Omega-3	37869.65	-		
Omega-6	38490.67	-		
Omega-9	8394.74	-		

Table 2 shows total level of Omega-3 with 37869.65 mg/100mL and Omega-6 with 38490.67 mg/100mL which is not much different. However total Omega-9 is only 8394.74 mg/100mL compared to 37869.65 (Omega-3) and 38490.67 (Omega-6) which is almost 5 times lower than Omega-3,6.

Table 4 and Figure 1 show the comparison of Omega-3,6,9 concentrationbetween raw and boiled eggs from hen feeding by *Plukenetia Volubilis* residue. The total level of Omega-3,6,9 in boiled eggs is higher than in raw eggs (Omega-3: 122.85 mg/100g (raw egg), 158.12 mg/100g (boiled eggs); Omega-6: 1479.18 mg/100g (raw egg), 1766.53 mg/100g (boiled eggs); Omega-9: 3009.16 mg/100g (raw egg), 3375.23 mg/100g (boiled eggs)), respectively.

DISCUSSION

DHA is an essential omega-3 fatty acid that human or animal cannot efficiently produce themselves; it must be taken through diet. Dietary sources of DHA such as salmon and mackerel are not commonly found on the plates of children and teenagers, which leads to less than optimal levels of DHA intake.¹⁷⁻¹⁸ Daily intake of omega-3 fatty acid should be in the range of 140 mg/day to 667 mg/day. However, a few countries reach the optimal intake of 250 mg per day; therefore, the trend of enriched food products with omega-3 fatty acids have emerged. 6-8,19-22 The omega 6:3 ratio is considered to be the factor that determines whether a diet is healthy or not. The ratio of n-6/n-3 is critical in achieving an appropriate balance of highly unsaturated fatty acid derived function in the human body. This study produced the result showing a balance of the omega 6:3 ratio in Plukenetia Volubilis oil. 5-6,11,23-26

The omega 6:3 ratio is considered to be the factor that determines whether a diet is healthy or not. The ratio of n-6/n-3 is critical in achieving an appropriate balance of highly unsaturated fatty acid derived function in the human body. Pipidemiology and dietary intervention studies have concluded that while an exceptionally high omega 6:3 ratio promotes the development of many chronic diseases, a reduced omega-6:omega-3 ratio can prevent or reverse these diseases. The present study produced the result that adding *Plukenetia Volubilis* residue shows an increase in the omega 6:3 ratio in eggs (Table 4 and Figure 1).

Modification of yolk fatty acid through feeding of layers with different fat supplements has been reported previously.9 In the present study, supplementation of Plukenetia Volubilis residue resulted in increased DHA level in the egg yolk, and the concentration was found to have increased in boiled eggs compared to raw eggs (Figure 1). However, the overall performance of the layers, in terms of feed intake, egg production, egg weight, egg mass, albumin height, and haugh unit of the eggs, was not observed to have been greatly affected by feeding Plukenetia Volubilis residue. However, some previous studied revealed that feeding with fish oil could provide an enrichment of EPA and, especially, DHA in the egg yolk. Additionally, 3% of dietary menhaden oil could increase yolk DHA to 252 mg/egg.^{9,28-29} Adding 4% fish oil to the hen diets for 8 weeks resulted in the increasing of the yolk DHA from 10.61 ± 1.30 mg, of the control group, to 32.51 ± 4.72 mg per egg.9 In addition, in Kaewsutas, et al.'s study, It showed that eggs from the hens fed with 1% or 2% of microalgae were observed to have a higher DHA level per egg (1% $algae = 75.49 \pm 18.75 \text{ mg}, 2\% \text{ algae} = 114.35 \pm 16.66 \text{ mg},$ which was significantly different (p<0.05) from both the control group and the fish oil group. Eggs from hens

Fatty acid composition	Raw e	Raw eggs		Boiled eggs	
	g/100g	LOD	g/100g	LOD	
Saturated Fatty Acid	2.64	-	3.17	-	
Butyric acid (C4:0)	-	0.01	-	0.01	
Caproic acid (C6:0)	-	0.01	-	0.01	
Caprylic acid (C8:0)	-	0.01	-	0.01	
Capric acid (C10:0)	-	0.01	-	0.01	
Undecanoic acid (C11:0)	-	0.01	-	0.01	
_auric acid (C12:0)	-	0.01	-	0.01	
Triecanoic acid (13:0)	-	0.01	-	0.01	
Myristic acid (C14:0)	0.03	-	0.03	-	
Pentadecanoic acid (C15:0)	-	0.01	-	0.01	
Palmitic acid (C16:0)	1.92	-	2.26	-	
Heptadecanoic acid (17:0)	0.02	-	0.02	-	
Stearic acid (C18:0)	0.67	-	0.86	-	
Arachidic acid (C20:0)	-	0.01	-	0.01	
Heneicosanoic acid (C21:0)	-	0.01	-	0.01	
Behenic acid (C22:0)	-	0.01	-	0.01	
Tricosanoic acid (C23:0)	-	0.01	-	0.01	
Lignoceric acid (C24:0)	-	0.01	-	0.01	

4.82

0.21

2.98

0.02

1.24

1.24

0.04

0.02

0.02

0.20

0.08

0.01

3.22

1.60

0.01

0.01

0.01

0.01

0.01

0.01

0.01

0.01

0.01

0.01

0.01

Table 4: Omega-3,6,9 concentration compared between raw and boiled eggs from hen feeding by *Plukenetia Volubilis* residue

Unsaturated Fat

Myristoleic acid (C14:1)

Palmitoleic acid (C16:1)

Pentadecenoic acid (C15:1)

Heptadecenoic acid (C17:1)

Oleic acid (C18:1, Omega-9)

Eicosenoic acid (C20:1, Omega-9)

Linoleic acid (C18:2, Omega-6)

Nervonic acid (C24:1, Omega-9)

Linoleic acid (C18:2, Omega-6)

g-Linolenic acid (C18:3, Omega-6)

Linoleic acid (C18:3, ALA, Omega-3)

Eicosadienoic acid (C20:2, Omega-6)

Eicosatrienoic acid (C20:3, Omega-3)

Docosadienoic acid (C22:2, Omega-6)

Elaidic acid (C18:1 trans)

Monounsaturated fatty acid Polyunsaturated fatty acid

Trans Fat

Linolelaidic acid (C18:2, trans)

Arachidonic acid (C20:4, ARA, Omega-6)

Eicosapentaenoic acid (C20:5, Omega-3)

cis-8,11,14-Eicosatrienoic acid (C20:3, Omega-6)

Docosahexaenoic acid (C22:6, DHA, Omega-3)

Erucic acid (C22:1, Omega-9)

Typical value	Raw eggs	Boiled eggs	
	Test results (mg/100g)	Test results (mg/100g)	
Omega-3	122.85	158.12	
Omega-6	1479.18	1766.53	
Omega-9	3009.16	3375.23	

fed with 4% fish oil in the diet were found to have a DHA level of 232.50±31.19 mg/100 gram of egg yolk or 57.45±8.24 mg/100 gram of whole egg, which gives the result as 32.51±4.72 mg/egg.9 The incorporation of

yolk n-3 fatty acid by up to 200 mg/yolk is significant as this amount of n-3 FA is comparable with that found in a 100 g serving of lean cold-water fish.²⁹ In general, the fatty acid profile of the egg is dependent on the type of diet the hens are fed. The level of essential fatty acids in the egg yolk achieved by the inclusion of different sources of fatty acids in the diet varies considerably.²⁹

5.55

0.23

3.35

0.03

1.44

1.44

0.01

0.05

0.02

0.02

0.28

0.11

0.01

3.62

1.93

0.01

0.01

0.01

0.01

0.01

0.01

0.01

0.01

0.01

0.01

There is constantly growing demand from consumers for food products of superior health quality. As the approved ratio is actually between 4:1 and 10:1,³⁰ supplementation of omega-3 fatty acid in the diet promotes a qualitative change in the fatty acid profile in the egg yolk and reduces the n-6/n-3 ratio to a more beneficial level with regard to

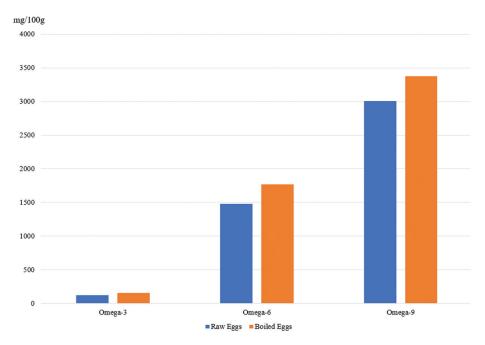


Figure 1: Omega-3,6,9 concentration compared between raw and boiled eggs from hen feeding by Plukenetia Volubilis residue

human nutritional needs. 9,31 The present study can increase the omega-3 content of eggs through the enrichment of the layers' diet with *Plukenetia Volubilis* residue. This supplement can equal the omega 6:3 ratio in the egg yolk to the optimal ratio. The fatty acid composition of an ingredient has a direct effect on fat utilization or deposition in poultry. 32 Longer chain fatty acids including omega-3 fatty acids EPA and DHA are almost exclusively deposited for storage in the form of phospholipids, particularly phosphatidylethanolamine in egg yolk. 33 Taking advantage of dietary omega-3 fatty acid deposition into yolk, producers are able to create value-added ALA, EPA, and DHA enriched eggs. 47,11-14,17,33-34

According to the European Union Guidelines (2010), the developing DHA-enriched egg has to be at least 180 mg/100-gram whole egg. The panel of the European Union Food Safety Authority (2010) considers that the intake of EPA and DHA of about 250 mg/day is required to obtain the claimed effect as regards eye, brain, and heart health. Considering that most egg consumers eat two eggs as a serving, the equivalent of >228 mg DHA could be obtained from a typical serving of these eggs. Therefore, the consumption of microalgae DHA-enriched egg could be beneficial to the eye, brain, and heart of the consumers.9 Feeding Plukenetia Volubilis residue might promote DHA deposition in the egg. The panel of European Union Food Safety Authority (2010) considers that the intake of EPA and DHA of about 250 mg/day is required to obtain the claimed effect as regards eyes, brain, and heart health. Supplementation with Plukenetia Volubilis residue might equal the DHA concentration to egg. Therefore, the

consumption of *Plukenetia Volubilis* residue from DHA-enriched egg could be beneficial to eyes, brain, and heart of the consumers. 1,5-8,11,32,35-36

CONCLUSION

Modification of the fatty acid profile in *Plukenetia Volubilis* residue to equal concentration of DHA omega-3 fatty acids of the omega 6:3 ratio has the potential for improving brain health of consumers. The results of this study revealed that manipulation of Omega-3 fatty acids to optimal level could be possible by supplementing specific methods.

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PS- Concept and design of the study, statistically analyzed and interpreted, manuscript preparation, critical revision of the manuscript.

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