

## Topographical distribution of the human brain to *Plukenetia volubilis*-based omega-3,6,9 enriched egg consumption

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### ABSTRACT

Docosahexaenoic acid (DHA) is related to cognitive development. Consumers' brain health could benefit from changes in the omega 6:3 ratio. This study aimed to examine how the *Plukenetia volubilis*-based omega-3,6,9 enriched egg supplementation affects electrical brain activity during the attention/inhibition (Go/NoGo) test. Healthy subjects (n = 20) were chosen at random to eat 2 boiled *Plukenetia volubilis*-based omega-3,6,9 enriched eggs for 12 weeks. The ePrime v.3.0 application recorded behavioral performance during the Go/NoGo test during the electroencephalographic recording. The finding of this study was that twelve weeks of boiled *Plukenetia volubilis*-based omega-3,6,9 enriched egg consumption significantly decreased the reaction time responses compared to the baseline. The topographical distribution revealed that the mean amplitude of N1 produced a slightly larger amplitude in the 12<sup>th</sup> week compared to the baseline. The P3 component, following N1, was also larger in the 12<sup>th</sup> week compared to the baseline. After 12 weeks of consuming the *Plukenetia volubilis*-based omega-3,6,9 enriched eggs, the central nervous system activities during a Go/NoGo test were believed to be enhanced.

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## 1. INTRODUCTION

As dietary consumption is an important factor, the brain's function and development require adequate amounts of eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) [1]. The HS-Omega-3 Index® can be used to determine their levels in erythrocytes. Individual Omega-3 indices vary between 2 and 20% in individuals. An insufficient amount of omega-3 fatty acids has been related to decreased brain volume, memory loss, dementia progression, and other cognitive problems [1]. In several observational studies, fish consumption has been linked to several biomarkers, including omega-3 polyunsaturated fatty acids (PUFAs), urinary iodine concentration, 1-methylhistidine, and trimethylamine N-oxide. However, sufficient information derived from the randomized controlled trials study on children is still lacking. One prior study evaluated the impact of fatty fish consumption vs. meat consumption on several biomarkers in preschool children. It was found that people who eat a lot of fatty fish have lower levels of several biomarkers, including n-3 PUFAs, urinary iodine concentration (UIC), hair mercury, and plasma 1-MH levels [2].

The main omega-3 unsaturated lipid in mammalian grey matter is DHA, a dietary supply of essential unsaturated omega-3 fatty acids [3]. In the frontal cortex, saturated fat accounts for about 15% of total fat [4], [5]. We know that DHA is given to babies by their mothers during pregnancy via the placenta and then by breast milk or supplemented formula. When infants become toddlers, they are allowed to eat and drink at the table [4]. DHA-rich foods like salmon and mackerel are not very common in kids' diets, so they do not get enough DHA [4]–[6]. DHA may play a role in autonomic, attentiveness, and inhibitory functions, all of which are influenced by frontal brain development [4]. Previously, DHA has been linked to cognitive development, particularly on cognitive function tests [4]–[7]. It is thought that the docosahexaenoic acid-rich frontal cortex is in charge of things like executive function, higher-order psychological tasks, and attention [8]. In 2003, Haag discovered a relationship between omega-3 fatty acids and central nervous system functions [9]. Because supplementing with omega-3 fatty acids in healthy people has not been thoroughly investigated, there are some uncertainties over whether DHA can help improve cognitive function in young people. Generally, the DHA-rich frontal cortex is responsible for planning, critical thinking, problem-solving, and focused attention [8]. Executive function, which encompasses working memory, planning, and mental flexibility, is based on the development of the frontal lobe and includes autonomic function, attention, and inhibition [4]. DHA could be involved in these processes. Dietary consumption determines the status of docosahexaenoic acid, which is essential for brain function. A diet lacking in omega-3 polyunsaturated fatty acids has been shown to benefit school-aged children in rural China. However, there was no effect of 300 mg DHA per day for 6 months on executive functioning such as working memory and cognitive flexibility [10], [11].

Dietary supplements are widely accessible and offer a variety of benefits. In contrast, the usefulness and safety of dietary supplements in children are largely uncertain. Knowing how dietary supplements work is essential for developing public health policies. In an online cross-sectional questionnaire survey conducted by Ishitsuka *et al.* [13], they looked into the types of dietary supplements that Japanese primary school students had. A mother-reported questionnaire was used to assess nutritional supplement use, sociodemographics, and wellbeing behaviors. Around 333 (6.8%) of the 4933 children were confirmed to be supplement users. Among n-3 fatty acids or fish oil, probiotics (1.0%), vitamin supplements (0.9%), multivitamin-minerals (0.8%), and herbs (0.8%) were the most popular supplements. Non-vitamin and non-mineral supplements have been found to be the most popular among Japanese primary school students [12]. Although sacha inchi seed contains 85% polyunsaturated fatty acids (x-3 and x-6) [13], little research has been done on the use of seed residues. Chemists have identified key phenolic families in Sacha inchi shells and assessed their antioxidant activities. The varieties of Sacha inchi tested had different amounts of x3, x6, and other phytochemicals ( $p < 0.05$ ). The potential of Sacha Inchi shell as a new and alternative resource for phenolic component antioxidant properties in the nutritional and/or functional food sectors was also proposed in previous studies [14], [15].

An electroencephalogram (EEG) is utilized to determine a patient's brain health. Manual EEG detection is time-consuming and problematic. The device's strong signaling makes detection difficult. As a result, a new method of detection is required. Multiple techniques were used to identify EEG signals, speeding up medical diagnosis. An early detection using the entire frequency band or spectrum Sameer *et al.* employed high-frequency EEG bands to detect seizures. The gamma band (30–60 Hz) detected seizures using Haralick's features and method. The computational load was reduced when a specific band was used. It also emphasizes the use of the gamma band in seizure detection. The measurable EEG component aids in seizure detection [14]. Munirathinam and his team came up with a new way to look for normal and epileptic signals by using autoassociative neural network model (AANN). The study indicated that an oppositional crow search algorithm (OCSA) detected seizures better than other methods [13]. One such method was developed by Gupta and colleagues [13]. P300 was detectable in neonates and appeared to represent the same cognitive processes as in adults, but its peak latency was much slower. Teenagers' and adults' electrical activities to novel stimuli differed from those of infants, and there was no P300 component. At present, P300 is being studied in babies; how it changes in people who aren't developing properly; how it's linked to behavior; and how it's unique to each person.

*Plukenetia volubilis* (Sacha inchi) seed oil is found to be rich in polyunsaturated fatty acids x-3 and x-6 (85%) [16]. Chirinos *et al.* [14] optimized sacha inchi shell phenolic compound extraction and identified key phenolic components, including antioxidant capacity. The phenolic compounds and antioxidants found in sacha inchi shell are regarded as health-promoting phytochemicals [16], [17]. To our knowledge, no scientific reports on the impact of omega-3-rich *Plukenetia volubilis* residue on cognitive functions exist. Thus, the goal of this study was to see if having *Plukenetia volubilis*-based omega-3,6,9 enriched eggs could have an effect on the cognitive abilities of healthy school children by looking at their behavior, electroencephalogram, and topographical distribution.

## 2. RESEARCH METHOD

### 2.1. Study design

Prior to beginning, the protocol was approved by the Institute's Human Ethical Committee under the number 201/2021, dated April 9, 2021, at Maha Chulalongkorn Rajavidyalaya University, Thailand. This study was carried out in accordance with the Helsinki Declaration. Informed consent from all subjects was provided before being enrolled in the investigation. Upon enrollment, subjects were assigned identification numbers (I.D.) in ascending order and randomly assigned in equal proportions to two groups using a blocked randomized list: the normal egg group (NG) and the *Plukenetia volubilis*-based omega-3,6,9 enriched egg group (OG). Prior to the start of the study, research assistants affixed subject identifiers to all investigational products, and the study site staff received a list of subject identifiers that had been concealed. The assignment was blind to both the subjects and the investigators. Throughout the 12-week trial period, subjects were told to take one boiled investigational product once a day at the same time in the morning. One boiled egg was randomly distributed to each subject as either OG or NG for 12 weeks. As a result, the OG and NG seem identical. All subjects were instructed to control their normal food and exercise habits. A computerized psychological battery was used to evaluate each subject's cognitive abilities at baseline, prior to the use of investigational products, and at 12 weeks.

### 2.2. Subjects

Subjects were chosen at random. Subjects with chronic, malignant, mental, or neurological problems were excluded from the study. Subjects were seen at screening, 0 week (baseline), and 12 weeks. All individuals' demographic data was obtained, including education, gender, and age. This study included 40 children ages 6 to 12 years old who had not consumed omega-3 enriched eggs in the previous three months. All subjects spoke Thai as their primary language, and were examined by the project's physician. There was also no brain injury or omega-3 enriched egg allergy. All subjects had normal hearing and eyesight. In terms of demographical data, no significant variations between the two groups were found. During the 12-week study, all subjects ate the same basic meal plus investigational products. Six subjects withdrew due to inconvenient transportation; five from OG and two from NG groups, respectively. The flowchart for study enrollment and completion as well as the timeline of the study are shown in Figures 1, 2, and Table 1.

### 2.3. Investigational products

The purpose of this study was to explore how the fatty acid specifications and nutritional requirements of omega-enriched eggs affect cognitive performance. The Sakda Sacha Inchi Inca (Thailand) company extracted the *Plukenetia volubilis*-based omega-3,6,9 enriched eggs. All boiled omega-3,6,9 enriched eggs were prepared and evaluated for fatty acid and other nutrient specifications at the Central Laboratory (Thailand) company using the in-house method TE-CH-208 based on Association of Official Agricultural Chemists (AOAC 2012) 996.06. The nutritional specification of a boiled *Plukenetia volubilis*-based omega-3,6,9 enriched egg was determined. The DHA (C22:6, omega-3) was 0.11 g/100 g of egg. The linolenic acids (C18:2 and C18:3) account for 1.44 and 0.05 g/100 g of egg. The gamma-linolenic acid content was 0.01 g/100 g of egg. The saturated and unsaturated fats were 3.17 and 5.55 g/100 g of egg, respectively. The total omega-3,6,9 concentrations from the investigational egg were 158.12, 1766.53, and 3375.23 g/100g of egg, respectively. The boiled *Plukenetia volubilis*-based omega-3,6,9 enriched egg contained no eicosapentaenoic acid or trans fat as shown in Table 1.

Table 1. The investigational products nutritional content\*

Typical value	Test Results (g/100g)	LOD
Docosahexaenoic acid (C22:6, DHA, Omega-3)	0.11	-
Eicosapentanoic acid (C20:5, EPA, Omega-3)	Not Detected	0.01
Linolenic acid (C18:3, ALA, Omega-3)	0.05	-
Gamma-linolenic acid (C18:3, Omega-6)	0.01	-
Linoleic acid (C18:2, Omega-6)	1.44	-
Arachidonic acid 20:4 (n-6)	0	-
Omega 6:3	0	-
Saturated Fat	3.17	-
Unsaturated Fat	5.55	-
Trans Fat	Not Detected	0.01
Omega-3	158.12	-
Omega-6	1766.53	-
Omega-9	3375.23	-

\*In-house method TE-CH-208 based on AOAC (2016) 996.06

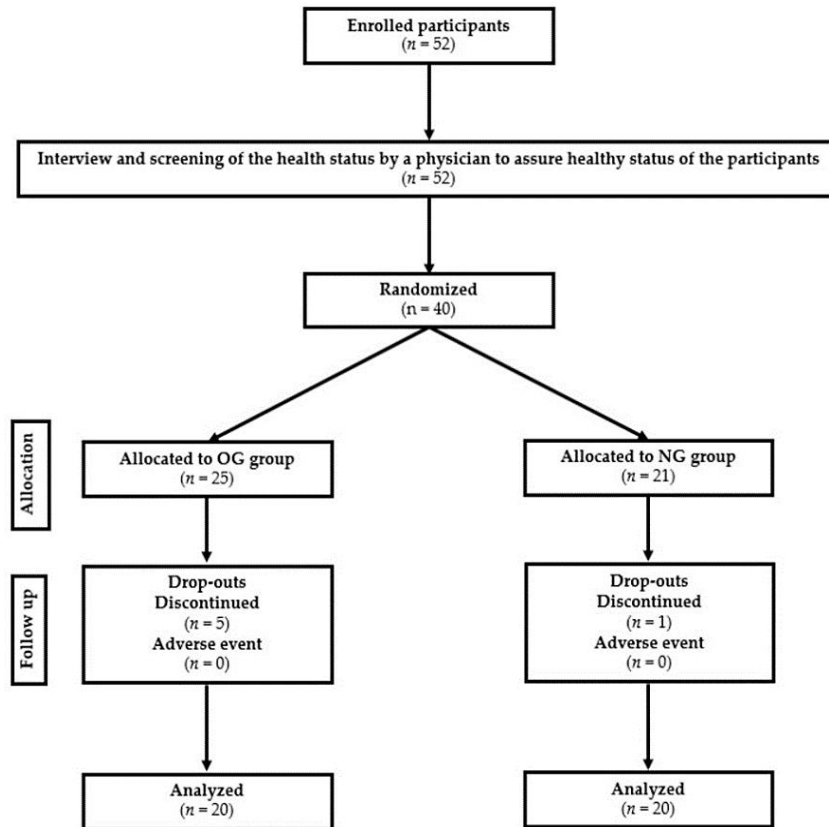


Figure 1. The study's intervention and subjects are depicted in this diagram. NG: Normal egg group ( $n = 20$ ); OG: *Plukenetia volubilis*-based omega enriched-3,6,9 enriched egg group ( $n = 20$ )

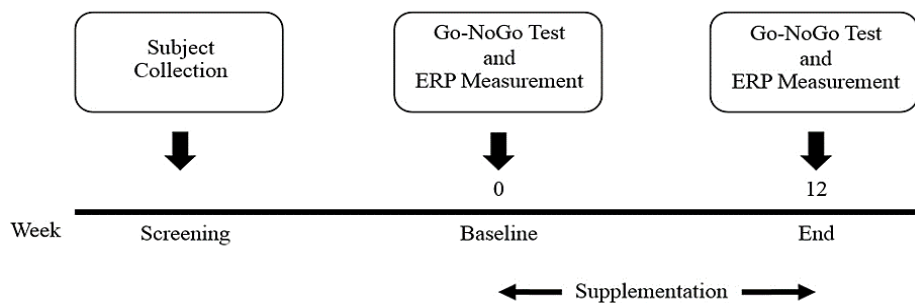


Figure 2. The timeline of both Go/NoGo test and event-related potential (ERP) measurement in the study

Table 1 provides a nutritional specification for the boiled *Plukenetia volubilis*-based omega-3,6,9 enriched egg used in this study. The level of docosahexaenoic acid (C22:6, DHA, omega-3) was 0.11g/100g, linolenic acid (C18:3, ALA, omega-3) was 0.05g/100g, gamma-linolenic acid (C18:3, omega-6) was 0.01g/100g, and linoleic acid (C18:2, omega-6) was 1.44g/100g, respectively. However, total unsaturated fat is only 5.55g/100g, while saturated fat was 3.1 g/100g. On the other hand, eicosapentanoic acid (C20:5, EPA, omega-3) was not detected in boiled omega-3,6,9 enriched eggs. Furthermore, total omega-3 concentrations of 158.12g/100g and omega-6 concentrations of 1766.53g/100g were significant. However, total omega-9 was 3375.23g/100g, compared to 158.12 g/100g for omega-3 and 1766.53g/100g for omega-6, almost three times higher than omega-3,6.

**2.4. Cognitive ability evaluation**

**2.4.1. Cognitive function batettery**

A Go/NoGo task was employed in this study. This study used Go/NoGo task to track working memory updating, shifting, and inhibition. The subjects were assessed by qualified research assistants who

were blinded to the treatment assignment and were graduate students in educational psychology. The first author, a cognitive neuroscientist, and the second author, a psychologist, co-investigated in the study. The accuracy, response, and feedback of the Go/NoGo task were monitored and shown in percentage and milliseconds.

#### 2.4.2. Task and experimental procedure

The visual oddball paradigm and the cognitive psychological battery, both given on a computer, were used to test the cognitive abilities. A modified version of the Go/No-Go test was performed on the subjects. Alphabets serving as Go and No-Go signals were displayed for 300 milliseconds throughout this test. On a monitor around 150 cm away from the subjects' eyes, the inter-stimulus interval was 1,500 milliseconds. The deviant was the 'Go' condition with an 80 percent probability, and the standard was 'NoGo' with a 20 percent probability. The experiment was divided into two blocks, each with 100 trials. The task was composed of 200 stimuli (2 blocks; 20% NoGo signals). In the task, the alphabet X was used as Go and other alphabets as NoGo signals. Subjects were required to hold their reaction to a single NoGo letter and a series of Go stimuli. The reaction buttons were placed under the subjects' palms in a soundproofed and electrically protected chamber. When the frequent (Go) stimuli (alphabet X) were shown on the computer screen, subjects had to press a response keyboard as rapidly as they could (with their dominant hand) and withhold their replies to the infrequent (NoGo) stimuli (other alphabets). Subjects were given the conditions in reverse order. Prior to the experimental session, all subjects were given a practice block to check that they understood the Go/NoGo test. At the end of each block, subjects were given feedback. For stimulus presentation and behavioral response recording, a stimulus system (ePrime, Psychological Software Tools, Inc., Pittsburgh, PA, USA) was employed as shown in Figure 3.

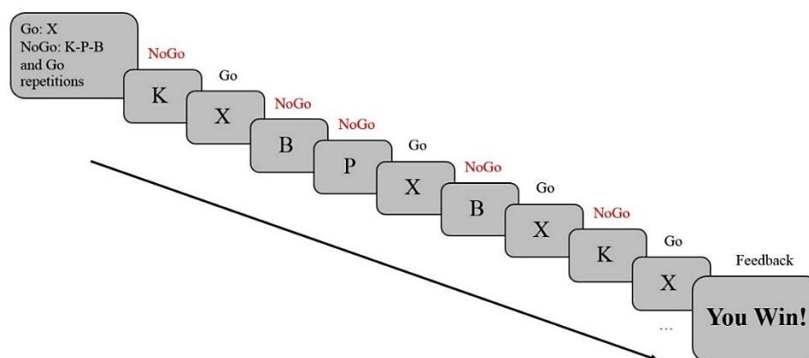


Figure 3. Parametric Go/NoGo task in the study

#### 2.4.3. Behavioral recording and analyses

The cognitive function test assessed the subjects' accuracy and reaction times by hitting buttons. Because of the test's two-force choice structure (no button press), button presses were classified as accurate (button code matched stimulus type) or erroneous (button code did not match stimulus type). The reaction times were calculated using the time difference between the button codes and the commencement of the stimuli. We only included trials with reaction times of 100–1500 msec. This removed inadvertent and severely delayed button presses caused by distraction or cognitive exhaustion. Each subject's reaction times were averaged. These tests used one-way analysis of variance (ANOVA) to compare accuracy and reaction times. On significant ANOVA results, we applied Tukey's post hoc analysis. The cognitive function exam used student t-tests for accuracy and reaction speeds. The *p*-value of 0.05 was chosen as the statistical significance level [18].

#### 2.5. Electroencephalographic recordings and signal processing

For electroencephalographic (EEG) recording, a 32-channel active set of electrodes was pre-mounted in an elastic Electro-Cap (Waveguard™ original Electro-cap, ANT Neuro, Hengelo, Netherlands) based on the international 10-20 electrode positioning system. Due to the use of very thin electrode wires, the waveguard original EEG cap was incredibly light, and the flexible, breathable cap fabric allowed for comfortable recordings even over longer periods of time. A ground electrode was inserted between the



electrodes Fz and Cz. For ocular artifact identification, manual reference electrodes were inserted on ipsilateral mastoids (M1 and M2), with Fp1 and Fp2 electrodes. All electrodes had an impedance of less than 10 k $\Omega$ . With a 0.05 to 100 Hz band pass, the EEG was amplified, captured at 500 Hz, and, during the experiment, the data was saved on a hard disk for off-line processing. A 0.1–30 Hz band pass was used to digitally filter event-related potentials (ERPs) waveforms. The epoch on which the average was calculated was 500 milliseconds, and the baseline was 100 milliseconds before the commencement of the presenting stimuli. Before extracting ERPs waveforms, all artifacts, including ocular artifacts, were eliminated from the continuous recording EEG. Each epoch was also subjected to a baseline correction. Any voltage variations of less than 0.1  $\mu$ V or greater than 70  $\mu$ V were rejected from further investigation. After registration, the data was corrected, and the artifacts were rejected. EEG epochs with absolute amplitudes of over 100  $\mu$ V were automatically detected and excluded. All active channels were artifact rejected with a threshold of 100  $\mu$ V before averaging. The target stimuli triggered reactions of frequent stimuli with an 80% frequency. Non-target stimuli were provided randomly with a 20% chance (oddball paradigm). Measurements were made of the amplitude ( $\mu$ V) and latency (msec) of the ERP signals with a 1000 ms interstimulus interval. Each cognitive test took 5 minutes to record using the ASA<sup>TM</sup> 4.0 analytical software (ANT Neuro, PE Hengelo, Netherlands). Source reconstruction, signal analysis, and magnetic resonance imaging (MRI) processing techniques were all included in the ASA<sup>TM</sup> software package. Both brainwave latencies and amplitudes were measured where the P300 was the positive peak between 250 and 400 msec as shown in Figure 4 [9], [16].

Global field power (GFP) peak measure was used to determine the brain electric field map's electric strength (hilliness) with its spatial layout. On one spontaneous EEG map, all voltage estimations' spatial standard deviation was measured [19], [20]. The spatial standard deviation of global field power is a technique of determining how much activity is present in the field at any one time. This allows us to characterize the potential field in a form that is independent of the object under consideration. The latencies of evoked potential components are calculated using the occurrence times of global field power maxima [21]. Visual inputs triggered the computation of global field power, component latency, global dissimilarity of potential field distributions, and a topographical temporal segmentation technique provided with multichannel data. Averaging the ERPs over all scalp channels, excluding electrooculographic channels, gave us GFP for each subject. The mean GFP peak amplitudes were obtained for each subject. The subjects' grand mean GFP peak amplitudes were then computed [18]–[20]. We analyzed the GFP waveforms based on the cognitive function tests as shown in Figure 4.

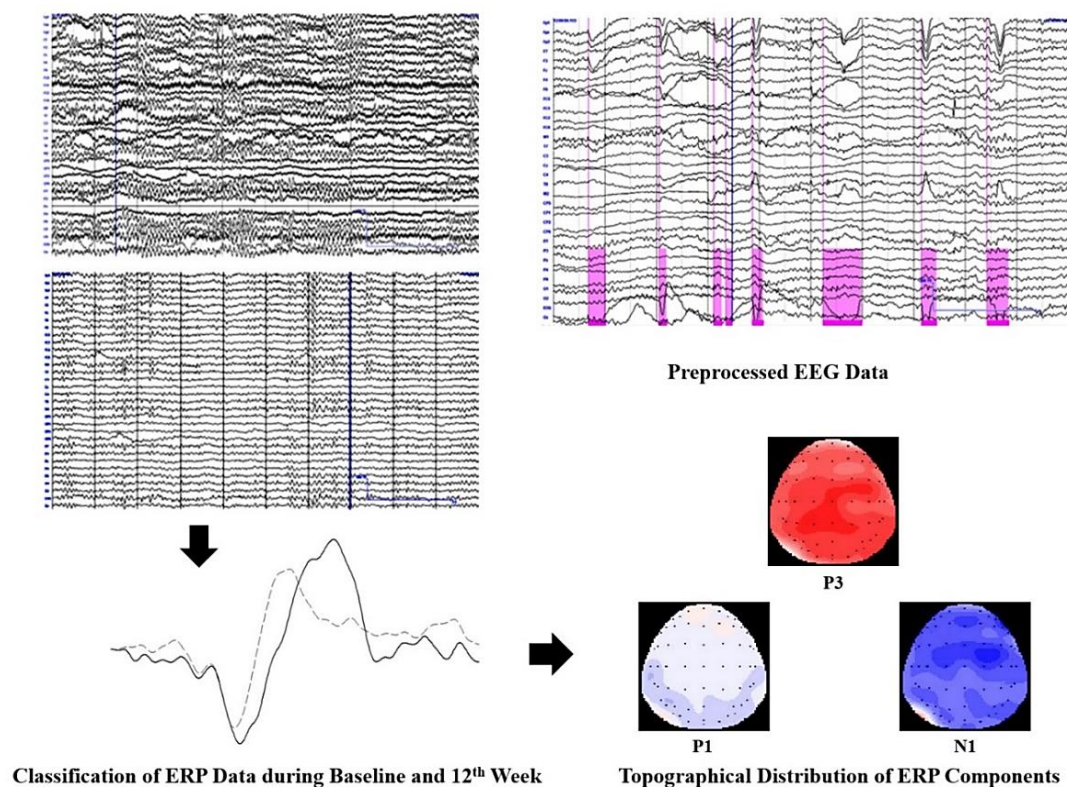


Figure 4. Electroencephalographic processing and topographical distribution analysis

## 2.6. Statistical analysis

The means and standard deviations for quantitative data were supplied. The data was analyzed with the SPSS program (Mae Fah Luang University) version 21.0, Renewal Quote Number: 26500879, and Passport Advantage Site Number: 3547818. To see how boiled omega-3,6,9 enhanced egg supplementation affects cognitive function over the time periods (baseline and 12<sup>th</sup> week), mean response times (RTs) and correct responses were analyzed by means of a compared *t*-test. RTs to cognitive function tests were measured with Go signals, and only correct replies were measured. The percentage of correct reactions was used to assess response accuracy in Go trials. Furthermore, repeated measures ANOVA was used to explore the effects of different time periods on the amplitudes of each peak for the Go-P1, Go-N2, and Go-P3 ERP components. A *p*-value of less than 0.05 was used to determine significant differences.

## 3. RESULTS AND DISCUSSION

### 3.1. Demographics of subjects

Subjects were recruited between June and September 2021, until the desired sample size was reached. A total of 20 subjects were allocated to the 3<sup>rd</sup> grade ( $n = 4$ ), 4<sup>th</sup> grade ( $n = 13$ ), 5<sup>th</sup> grade ( $n = 6$ ), and 6<sup>th</sup> grade ( $n = 2$ ) student groups, respectively. The study dropout rate was low ( $n = 5$ , 20.0%), with two, two, and one dropout from the 3<sup>rd</sup> grade, 4<sup>th</sup> grade, and 5<sup>th</sup> grade student groups, respectively as shown in Figure 1. No subject was non-compliant with the treatment schedule, which was defined in the protocol as missing three or more consecutive doses of the investigational medication. Table 2 shows the subjects' characteristics in each group ( $n = 2$ , 3<sup>rd</sup> grade,  $n = 11$ , 4<sup>th</sup> grade,  $n = 5$ , 5<sup>th</sup> grade, and  $n = 2$ , 6<sup>th</sup> grade). Subjects in the OG group were 8.25 ( $\pm 1.36$ ) years old, whereas those in the NG group were 8.73 ( $\pm 1.22$ ) years old. Demographic factors did not differ statistically significantly across the groups. Subjects were male/female (OG: 13/7; NG: 11/9), and the education of subjects was primary school. No past serious medical conditions were reported by the subjects' parents.

Table 2. The demographic data of subjects in both OG and NG groups

Demographic and Baseline Characteristics	OG ( $n = 20$ )	NG ( $n = 20$ )
Age (years), mean (SD)	8.25 ( $\pm 1.36$ )	8.73 ( $\pm 1.22$ )
Male	13	11
Female	7	9
Weight	22.25 ( $\pm 1.06$ )	23.41 ( $\pm 1.77$ )
Height	117.67 ( $\pm 0.47$ )	118.33 ( $\pm 1.92$ )
BMI	21.11 ( $\pm 1.52$ )	20.15 ( $\pm 1.31$ )
Educationnal background		
1 <sup>st</sup> level	-	-
2 <sup>nd</sup> level	-	-
3 <sup>rd</sup> level	3	5
4 <sup>th</sup> level	11	9
5 <sup>th</sup> level	4	4
6 <sup>th</sup> level	2	2

### 3.2. Cognitive engancing effect of the *Plukenetia volubilis*-based omega-3,6,9 eggs

The effect of the *Plukenetia volubilis*-based omega-3,6,9 enriched egg on the cognitive function battery test was conducted on 20 subjects who had the *Plukenetia volubilis*-based omega-3,6,9 enriched eggs and 20 subjects who had normal eggs. Table 3 shows the baseline and change scores from the baseline and 12<sup>th</sup> weeks of cognitive function battery assessments. RTs and correct responses (mean standard deviation: SD) are shown in Table 2. There was a main effect in behavioral performance scores ( $F_{3,79} = 4.821$ ;  $p = 0.0046$ ). The boiled *Plukenetia volubilis*-based omega-3,6,9 enriched egg supplementation for twelve weeks reduced response time considerably ( $p < 0.05$ ). Moreover, the speed of response time was much faster in the 12<sup>th</sup> week than in the baseline (baseline:  $570.70 \pm 67.83$  msec; 12<sup>th</sup> week:  $511.48 \pm 76.57$  msec;  $t(19) = 3.191$ ;  $r = 0.9739$ ;  $p = 0.0048$ ). On the other hand, normal boiled egg supplementation for twelve weeks reduced response time, but not statistically significantly (baseline:  $580.32 \pm 64.18$  msec; 12<sup>th</sup> week:  $576.87 \pm 67.62$  msec;  $t(19) = 1.00$ ;  $r = 0.3441$ ;  $p = 0.3299$ ). However, the correct responses of both OG and NG groups showed no statistical significance between baseline and 12<sup>th</sup> week (OG group:  $98.16 \pm 0.37$  (baseline);  $99.14 \pm 0.15$  (12<sup>th</sup> week); n.s.; NG group:  $97.33 \pm 0.97$  (baseline);  $98.73 \pm 1.09$  (12<sup>th</sup> week); n.s.) as shown in Table 3.

Table 3. Comparing the effect of the *Plukenetia volubilis*-based omega-3,6,9 enriched eggs on a cognitive function battery test at baseline and after 12 weeks

Cognitive Function Test <sup>a</sup>	Mean (SD) Scores at Baseline	Mean (SD) Scores at 12 <sup>th</sup> Week	p-value
OG			
(%) Accuracy (Go)	98.16	99.14	NS
(%) Error (Go)	1.84	0.86	NS
Response time (ms) (Go)	570.70±67.83	511.48±76.57	0.0048
NG			
(%) Accuracy (Go)	97.33	98.73	NS
(%) Error (Go)	2.67	1.27	NS
Response time (ms) (Go)	580.32±64.18	576.87±67.62	0.3299

<sup>a</sup>Test parameters: Go/NoGo Accuracy: Go/NoGo response task accuracy scores; Go/NoGo Error: Go/NoGo response task incorrect and omission scores; Go/NoGo Response Time: Go/NoGo response tasks mean reaction time in milliseconds (ms); NG: Normal egg group ( $n = 20$ ); OG: *Plukenetia volubilis*-based omega-3,6,9 enriched egg group ( $n = 20$ )

### 3.3. Electroencephalographic and topographical distribution of the *Plukenetia volubilis*-based omega-3,6,9 enriched eggs

Table 4 illustrates the effect of the *Plukenetia volubilis*-based omega-3,6,9 enriched egg on cognitive function as measured by electroencephalography at the beginning and end of the study. The GFP was shown as a function of time, and the occurrence timings of maximum global field power were utilized to calculate the latencies of ERPs components. According to the cognitive function tests, the grand mean GFP peak amplitude and latency of ERPs were shown for all subjects. The P3 ERP component was measured at around 280-375 msec [10]. The P3 amplitude of the OG group [ $F(1,39) = 7.54, p < 0.0001$ ] was statistically significant while compared between baseline and 12<sup>th</sup> week values (baseline:  $2.933 \pm 1.54 \mu\text{V}$ ; 12<sup>th</sup> week:  $6.563 \pm 1.41 \mu\text{V}$ ;  $t(39) = 8.114, p < 0.0001$ ). The P3 amplitude of the NG group [ $F(1,39) = 6.14, p < 0.05$ ] was statistically significant while compared between baseline and 12<sup>th</sup> week values (baseline:  $3.68 \pm 2.37 \mu\text{V}$ ; 12<sup>th</sup> week:  $4.26 \pm 2.38 \mu\text{V}$ ;  $t(39) = 7.31, p < 0.0001$ ). The OG group's mean P3 latency was slightly faster at the 12<sup>th</sup> week compared to baseline (478.78 ( $\pm 44.80$ ) msec; 12<sup>th</sup> week: 437.31 ( $\pm 78.61$ ) msec;  $t(39) = 2.73, p = 0.0105$ ). For the NG group, the latency of P3 was also faster at the 12<sup>th</sup> week compared to baseline (baseline: 493.56 ( $\pm 39.64$ ) msec; 12<sup>th</sup> week: 481.07 ( $\pm 42.09$ ) msec;  $t(39) = 3.74, p = 0.0177$ ) as shown in Table 4 and Figure 5.

Table 4. Comparing the effect of the *Plukenetia volubilis*-based omega-3,6,9 enriched eggs on a cognitive function battery test assessed by the electroencephalography at baseline and after 12 weeks

Cognitive Function Test <sup>a</sup>	Mean (SD) Scores at Baseline	Mean (SD) Scores at 12 <sup>th</sup> Week	p-value
OG			
Latency (ms)	478.78±44.80	437.31±78.61	0.0105
Amplitude ( $\mu\text{V}$ )	2.93±1.54	6.56±1.41	<0.0001
NG			
Latency (ms)	493.56±39.64	481.07±42.09	0.0177
Amplitude ( $\mu\text{V}$ )	3.68±2.37	4.26±2.38	<0.0001

<sup>a</sup>Test parameters: Latency: Go response task mean reaction time in milliseconds (ms); Amplitude: Go response task mean amplitude in microvolt ( $\mu\text{V}$ ); NG: Normal egg group ( $n = 20$ ); OG: *Plukenetia volubilis*-based omega-3,6,9 enriched egg group ( $n = 20$ ); P3 ERP component around 280-375 msec

Several dietary supplements are available. Adults can take supplements, but children cannot. Dietary supplement use must be considered in public health policies. Supplements that were not vitamins or minerals were more popular among elementary school students [22]. *Plukenetia volubilis* Linneo, or Sacha inchi, is an oleaginous plant. It has been grown in the Peruvian Amazon lowlands for millennia [23], [24]. Sacha inchi seeds are abundant in x-3 and x-6 polyunsaturated fatty acids (85% of total fatty acids). The nutraceutical and/or functional food industries will benefit from an alternative supply of phenolic compounds and antioxidants. Phytochemicals are abundant in Sacha inchi seeds [14], [15].

The essential fatty acids EPA and DHA are essential for brain growth and function. The levels of EPA and DHA in erythrocytes should be determined using a reliable method (HS-Omega-3 Index®). It varies between 2 and 20%, with an optimal range of 8–11%. An unhealthy omega-3 index has been linked to an increased risk of total mortality, ischemic stroke, shrinkage of brain capacity, and other brain issues [1]. During the last trimester of pregnancy, when the fetal brain is rapidly growing, it develops in neural tissue. DHA, which is required for brain development, is absent in embryos born at 29 weeks of gestation. DHA deficiency after birth might cause cognitive impairment. Dietary sources of DHA and EPA are essential for school-age children's cognitive and behavioral development. Previous research found a link between dietary



DHA consumption and plasma DHA levels in school-aged children. The fact that school-age kids don't eat enough omega-3 PUFAs shows how important it is to get more through food and supplements [25].

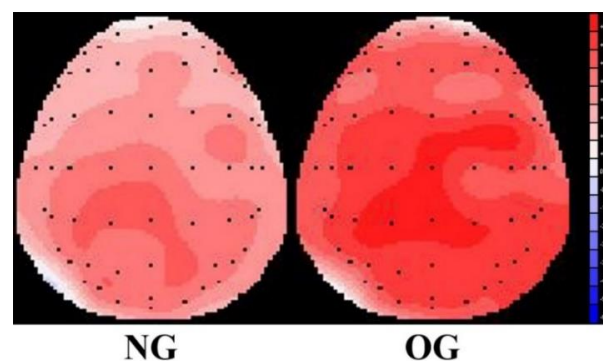


Figure 5. Topographical distribution of P300 ERP component after 12 weeks of investigational products supplementation. P300 between 280-375 msec; The red color represents positivity power ( $\mu\text{V}$ ), while the blue color represents negativity power ( $\mu\text{V}$ )

The European Food Safety Authority [16], [17] believes that a daily intake of roughly 250 mg of EPA and DHA will have a positive influence on eye, brain, and heart health. Dietary consumption determines DHA levels because it is a critical component of the brain. DHA supplementation may benefit school-aged children whose diets are deficient in omega-3. Healthy rural school-aged children were given either 300 mg of DHA per day or a placebo for six months after receiving DHA supplementation. The Digit Span Backwards and Wisconsin Card Sorting Tests were used to test working memory and cognitive flexibility of executive skills at three separate points in time: baseline, three months, and six months. The Wisconsin card sorting and Digit Span Backwards tests both demonstrated a considerable improvement in performance. However, there was no significant intervention effect on executive function scores. Furthermore, there was no connection between erythrocyte DHA levels and executive function in the linear regression analysis. Giving 300 mg of DHA daily to healthy school-aged children for 6 months had no effect on executive functions such as working memory and cognitive flexibility [10]. DHA is a dietary source of DHA, which is essential for brain function. On the other hand, 6 months of 300 mg/d DHA had no effect on executive abilities such as working memory and cognitive flexibility [11]. DHA is required for autonomic, inhibitory, and attentional functions of executive function. The development of the frontal lobe is essential for the development of working memory, mental flexibility, and planning. The most abundant fatty acid in mammalian grey matter is DHA. Unsaturated fat makes up about 15-20% of the total unsaturated fat concentration in the mature human frontal cortex [4], [5], [8], [26]. DHA-rich frontal lobes are known to facilitate planned behavior and cognitive skills such as problem solving and creative thinking. The prefrontal cortex was discovered to have a significant role in the initiation of Go and NoGo circumstances [26]. According to earlier studies, P3 has been detected in infants and appears to represent the same cognitive processes as adults. However, P3's peak latency is substantially longer.

The body cannot produce DHA, an omega-3 unsaturated lipid, on its own. It must be consumed regularly. DHA-rich foods like salmon and mackerel are lacking in children's diets, resulting in a DHA deficiency [6]. Microalgae can produce DHA-enhanced eggs that are a safe and long-term replacement for fish oil [6]. Given that most people eat two eggs per serving, a typical egg meal contains over 228 mg of DHA. Consumption of microalgae DHA-enriched eggs may therefore be beneficial to brain health [10]. Furthermore, previous blood studies showed that 35 days of omega-3 treatment significantly reduced the arachidonic acid/eicosapentanoic acid ratio (AA/EPA) [11]. By eating n-3 fatty acid enhanced eggs (6 eggs per week) for 8 weeks, lacto-ovo vegetarians can boost DHA content in erythrocyte membranes [22]. To show that eating eggs with DHA improves brain function, there must be adequate DHA in the erythrocyte membrane [4], [6], [11], [27].

Several studies have related omega-3 PUFAs, urinary iodine, 1-methylhistidine, and trimethylamine N-oxide to fish consumption. When fatty fish intake was compared to meat intake in preschool children, it had the greatest impact on omega-3 (n-3) PUFAs, urinary iodine content, hair mercury, and plasma 1-methylhistidine. However, biomarkers related to micronutrient status, inflammation, essential amino acids, choline oxidation, and tryptophan pathways were not found in this earlier study [2]. However, a recent

study [11] found that omega-3 has an effect on central nervous system activity. This study found that taking omega-3 supplements (EPA and DHA) for 35 days made people more alert and capable, especially in complex cortical processing [4]–[11]. However, our current research demonstrated that supplementing with cooked omega-3,6,9 enriched eggs from *Plukenetia volubilis*-residue for twelve weeks reduced the response time reactions significantly. When compared to the baseline, the reaction time in the 12th week was considerably faster. Topographical distribution also showed that the N1 component produced a slightly larger amplitude in the 12th week after consumption compared to the baseline. Moreover, the P3 component, following N1, was larger in the 12th week after consumption compared to the baseline. Therefore, the result of our recent study is associated with a lower reaction time measured by the Go/NoGo test and has an influence on reactivity, which has been previously reported in several previous studies [4]–[11].

After 6 months of treatment with omega-3 supplements, antisocial behavior was dramatically reduced. Children with a strong psychopathic-like personality in the omega-3 group showed significant antisocial behavior modifications after therapy. Omega-3 supplementation in children may prevent antisocial and violent conduct in females [28]. The role of cognition in everyday and social conduct is now well acknowledged. Following the success of mulberry fruit in animal research, mulberry milk has been shown to provide cognitive benefits. Anthocyanin-rich mulberry milk increased P300 amplitude at 1.5 and 3 hours after administration in clinical trials. Digit updating had a faster response time at 1.5 and 3 hours after the treatment, but picture updating did not. Mulberry milk improves attention, cognition, and working memory in school-aged children [29]. Krill oil, which is abundant in omega-3 polyunsaturated fatty acids and phosphatidylcholine, has many advantages. The reason for this is that oxyhemoglobin concentrations change more than medium-chain triglyceride concentrations when working memory is used at week 12. At week 12, krill oil had a lower P300 latency difference than medium-chain triglycerides. After 12 weeks, oxyhemoglobin went up a lot more in the krill oil group than in the medium-chain triglyceride group [30].

Executive functions are required to manage and regulate behavior. Executive dysfunction can occur in a variety of illnesses, and executive functions can assist us in planning, solving issues, and achieving our objectives [31]–[33]. These characteristics are required for academic success and later job success [34], as well as planning, organization, problem-solving, and performance monitoring [35]. An early executive function called "inhibition of improper motor responses" is important because it sets the stage for later executive functions like planning and problem-solving, which are also important. Executive functioning subcomponents may be separated in infancy or come from a system that isn't very well-defined at first. Despite the widespread belief that the fundamental structure of executive functions remains relatively stable from approximately the age of 7 [36], [37], executive functions are affected by illness, unfavorable circumstances, and developmental defects [32], [33]. While executive functions are vulnerable as they mature, their malleability may allow for treatment [31], [36].

Electroencephalography (EEG) assesses cognitive neuronal activation. EEG and ERPs are commonly employed in investigations of child and adolescent psychopathology and neurological substrates. ERPs are non-invasive ways to research brain synaptic processes. As a biomarker for human cognitive and behavioral functioning, ERP may be used in pediatric psychiatry. The P300, error-related negativity, and reward positivity are three parts of the ERP that have been used to test for psychiatric illnesses in children and teenagers. When comparing attention-deficit/hyperactivity disorder (ADHD) patients to healthy people, P300 latency and ERN were considerably different [38]. Cognitive processes can be monitored in real time using EEG [39]. The better time resolution of EEG allows tracking of rapid neural events and source analysis of linked brain regions [39]. It is capable of directly detecting brain activity dynamics associated with cognitive activities [40]. This is because the EEG was the first tool used to measure neuronal excitability in kids, and ERPs have been used in a lot of studies to look at child psychopathology and the neurological foundations of psychiatric conditions in kids [39], [41]. ERPs are time-locked voltage changes measured on the scalp [42]. In other words, ERPs allow us to quantify coordinated neuronal activity by averaging time-locked parts of electrical recordings [43]. As a result, ERPs were used to evaluate several executive functions, including perceptual processes, sensory-motor integration, and cognitive functioning [44]. ERPs are a great tool for studying brain function in children [38].

In children with epilepsy, cognitive impairment is common. When compared to non-epileptic children, children with idiopathic epilepsy had lower levels of omega-3 fatty acids, higher levels of omega-6 fatty acids, and also an abnormal omega-6/omega-3 ratio. In children with epilepsy, these data demonstrated a clear positive relationship between blood omega-3 levels, cognitive function scores, and P300 latency. Thus, serum omega-6 to omega-3 fatty acid ratios is abnormally high in children with epilepsy, which is linked to poor cognitive performance [22]. The P300 component was utilized to investigate dyslexic children's post-attentive processing and topographic voltage distribution. For both right parietal and left occipital areas, the dyslexia group evoked higher P300 amplitudes and intensities than the control group. Post-attentional integration is higher in dyslexic children, involving the parietal and occipital regions. P300 latency showed no significant variations [45]. A previous study investigated the impact of the fatty acid ratio

of linoleic (omega-6) to alpha-linolenic (omega-3) on cognitive performance in 2019. The P300 components and the Arabic-language Stanford-Binet exam were used to look at omega-3 and omega-6 fatty acid levels and how they were linked to cognitive performance [22]. It was more difficult for children with epilepsy to get enough omega-3 fatty acids and less difficult for them to get enough omega-6 fatty acids than it was for children without epilepsy. There is also a considerable positive connection between serum omega-6 levels and P3 latency in children with epilepsy [22]. According to Ali *et al.*, the dyslexic group's P3 amplitudes at the T4 electrode were greater than the control group's in the right parietal and left occipital areas. In dyslexic children, both parietal and occipital areas were functional for the post-attentive process [45]. P300 is detectable in infants and appears to represent identical cognitive processes as in adults, although its peak latency is greatly delayed [12]. Latency of the auditory nogo-N200 was found to be affected by age-related variables [46]. Developing cognitive therapies for ADHD generally necessitates numerous cognitive assessments. In both memory tasks and response control, the combination of n-back and nogo paradigm showed the same kind of electrical activity in the brain. According to this study, ADHD patients reported lower n-back and nogo P3 amplitudes, increased omission mistakes, and reaction times. Memory tasks and response control impairments in ADHD patients can be assessed using the n-back/nogo task [47].

#### 4. CONCLUSION

The cognitive function test and electroencephalographic measurements demonstrated that after 12 weeks of supplementation, the effect of the *Plukenetia volubilis*-based omega-3,6,9 enriched eggs on central nervous system activity could be seen. N1 and P3 ERP components that were related to *Plukenetia volubilis*-based omega-3,6,9 enriched egg supplementation had different topographical distribution. This suggests that attention-related rechecking analyses are taking place in the central nervous system. We hypothesized that the changes in time and physical parameters that docosahexaenoic acid from *Plukenetia volubilis*-based omega-3,6,9 enriched egg supplementation might demonstrate the central nervous system functions.

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