THE EFFECT OF DIETARY OIL ON THE GROWTH AND INTELLECTUAL CAPACITY OF MICE

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ABSTRACT

The nutritional values of three vegetable oils were evaluated on the basis of the growth response, the food efficiency ratio and the effect on mental activity. Avocado, sesame and coconut oil were the only sources of fat in the experimental diets fed to three groups of albino mice. Mice fed with avocado and sesame yielded higher body weight gains and food efficiency ratios than mice fed with coconut oil. This agrees with the reports made on the study of these oils by the biological assay. The values for the mental activity of mice fed with avocado and sesame diets were also higher. However, mice fed with avocado diet showed relatively higher values than those fed with sesame. It is possible that the content of essential fatty acids among others could be directly related to intellectual performance.

I. INTRODUCTION

Fats should be regarded as essential dietary requirement of man. The human diet is often low in fats of any kind and that when fats are added they usually contain little of the acids more unsaturated than oleic acid. Butter and coconut oil are the chief table fats and animal fat is probably equally poor as source of unsaturated fatty acids.

It is possible that our higher carbohydrate and protein diets, carrying little of the unsaturated fatty acid are contributing factors to poor health. The addition of egg yolk and cod liver oil to diets may improve the patient because of the fatty acid rather than the vitamin content. The prevalence of dry skins, abnormal kidneys and hearts may be attributed to improper fat intake. The nerve tissue, kidneys and other organs contain several unsaturated acids. If the liver and nerve tissue are limited in their ability to produce these acids, they should be sufficiently supplied through the diet.

Majority of the people in the Philippines as elsewhere depend on domestic animals for their fat requirements. The Philippines, being an agricultural country, abounds in agricultural crops from which quality fats can be derived. Progress is being made in laboratories towards the production of oils and the determination of essential fatty acids.

The main aspect of this study is the evaluation of avocado, sesame and coconut oil on their fat quality. Biological assays have proven that a number of essential fatty acids are present in these oils (See Table I). Several studies have shown that the use of vegetable sources of fats in the diet leads to better growth response which can be an overall effect of a number of metabolic process. It has also been shown that metabolism of essential fatty acids affect the development of brain in animals.

The purpose of this work is to determine the nutritional value of the three vegetable oils and its effect on the mental activity of mice.

II. REVIEW OF LITERATURE

The term "essential fatty acids" was introduced in 1930 (4) and has been used since that time to designate a group of unsaturated acids which will bring about a renewal of growth of rats. This property is characteristic of the acids in the "linoleic acid series" (linoleic, linolenic and arachidonic). Their detailed structures are shown below:

$$\underset{}{CH_3}\text{-}(CH_2)_4\text{-}\underset{15}{CH}{=}\underset{12}{CH}\text{-}CH_2\text{-}\underset{10}{CH}{=}\underset{9}{CH}\text{-}(CH_2)\text{-}COOH$$

LINOLEIC ACID

CH_3-$(CH_2)_4$-CH=CH-CH_2-CH=CH-CH_2-CH=CH-$(CH_2)_7$-COOH

16 15 13 12 10 9

LINOLENIC ACID

CH_3-$(CH_2)_4$-CH=CH-CH_2-CH=CH-CH_2-CH=CH-CH_2-CH=

15 14 12 11 9 8 6

CH-$(CH_2)_3$-COOH

ARACHIDONIC ACID

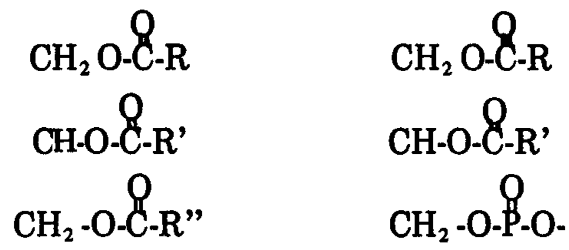
The importance of an adequate supply of essential fatty acids in the diet gained impetus towards the determination of the nutritive values of fats and oils. A number of different criteria have been employed for making this evaluation but growth-promoting effect has been the principal method for attacking this problem. This is supplemented by other experiments among which are pregnancy and lactation method (5), the effect of multi-generation experiments(6) and the "survival factor" method(7).

It would be expected that if the importance of fat in these methods were to be ascribed to the presence of essential fatty acids, vegetable oils would function equally well as a nutritional requirement.

Lipids, a general category which include fats and other related compounds, are of particular interest not only in physiology but also in neurochemistry since they constitute about 10% of the brain tissue (8). The chemical composition of whole brain tissue is indicated in Table II. The values presented do not take into account variations that occur for different animals and different regions of the brain.

The fatty acids are the building blocks of several classes of lipids including phosphatides. The phosphatides form a large part of the structural elements of the brain as shown in Table III. They are said to contain essential fatty acids.

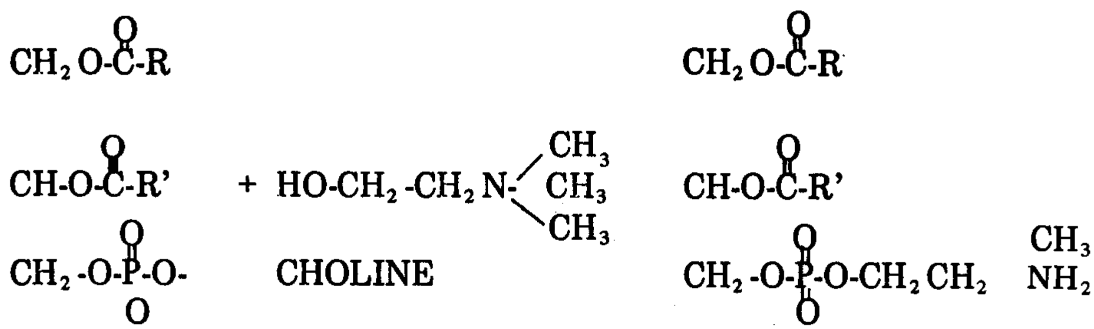
The phosphatides are also known as phosphoglycerides in that one of the primary hydroxyl groups of glycerol is esterified to phospheric acid instead of fatty acid (10).



TRIGLYCERIDE   PHOSPHATIDIC ACID

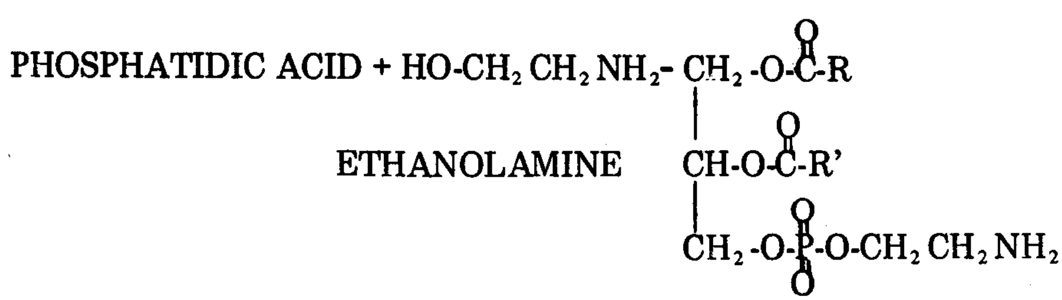
where R, R', R" = fatty acids

When the phosphate group of phosphatidic acid is bound to a nitrogeneous compound such as choline, ethanolamine and serine phosphatidic derivatives are formed:



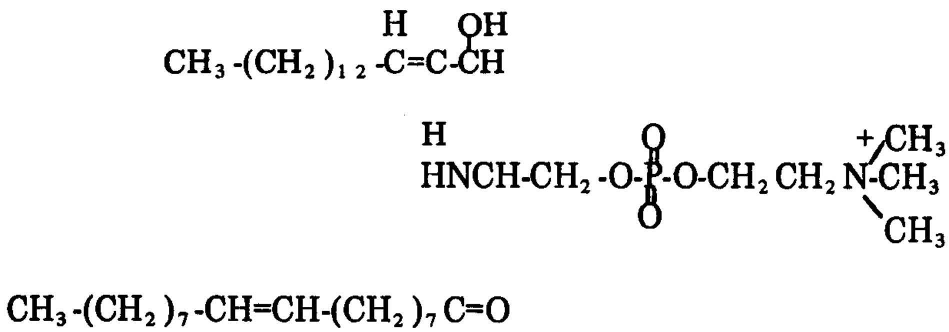
PHOSPHATIDIC ACID

LECITHIN OR
PHOSPHATIDYL CHOLINE



CEPHALIN OR
PHOSPHATIDYL ETHANOLAMINE

Sphingomyelin does not contain glycerol. Its alcohol portion is either an aminoalcohol or sphingosine. It contains choline at the phosphate end.



SPHINGOMYELIN

In contrast with the wealth of information regarding the physiological effects of essential fatty acids, not much information can be obtained regarding the metabolism of essential fatty acids in the central nervous system. Their importance lies in their participation as components of myelin sheaths, cell membranes and enzyme complexes (9a).

The turnover of fatty acids in brain has been studied by the use of isotapes. For example, in newborn animals maintained on deuterium oxide, the percentage in cholesterol and fatty acid fractions of brain are higher in the first weeks of life(9a). The conclusion is that lipids are synthesized in the central nervous system and not delivered from other sources. This conclusion is supported by the ability of brain tissue to synthesize phosphatides in vitro, using P^{32} studies.

The biochemical importance of the fatty acid composition of the phospolipids has drawn attention in recent years. Phosphate exchange in phosphatidic acids has been studied in relation to cholinergic agents. They found that the fatty acid composition of the phosphatidic acid may be a determinant in the rate of active transport across a membrane (11).

Whiting (11) also made a study on the essential fatty acid content of erythrocyte and mitochondria of the brain of rats and found that it contained approximately 25% to 35% essential fatty acids. The approximate composition of brain mitochondrial lipids has been found to be 22% cholesterol, 7% cholesterol ester and 71% phospholipid. The lipids of brain mitochondria from weanling rats contained 1.7% linoleic acid, 11% arachidonic acid and 9% docosahexaenoic acid. By varying the fatty acid composition of the dietary lipid, brain mitochondrial lipids were more resistant to change than were the lipids of either of the tissues studied.

The question of direct incorporation of linoleic acid into the brain of fully grown adult animals has been studied by Dhopeshwarkar(12). In his conclusion, he said that arachidonic acid is the direct metabolic product of linoleic acid. He concluded further that the animal does not have to depend on the supply of essential fatty acid it received during the early period after birth before myelination. However, it is still not known whether this normal uptake of essential long chain fatty acid is affected in degenerative nervous conditions.

Rats participate in a variety of metabolic pathways to provide energy to the brain. Complex natural fats are broken down into their component fatty acids to form acetyl coenzyme A and enter into the Kreb cycle.

Moreover, fats serve as transport form of neurotransmitters and receptors of nerve ending membranes(10a). One of the neurotransmitters synthesized at the expense of energy-rich phosphate bonds is the neurohumor acetylcholine. The formation of acetylcholine requires the aid of both the enzyme choline acetylase and coenzyme A. The enzymes that catalyze the breakdown of fatty acids to acetylcoenzyme A are located in the mitochondria while choline is released along with an extracellular fluid by an active membrane mechanism. The reactions are summarized below (10b, 10c):

$$CH_3(CH_2)_nCH_2\overset{O}{\overset{\|}{C}}\text{-}OH \xrightarrow[\text{thickinase}]{\text{ATP, CoASH}} CH_3(CH_2)_nCH_2\text{-}\overset{O}{\overset{\|}{C}}\text{-}SCoA$$

fatty acid

fatty acyl
dehydrogenase
FAD
FADH$_2$

$$CH_3(CH_2)_{n\text{-}1}CH{=}CH\text{-}\overset{O}{\overset{\|}{C}}\text{-}SCoA$$

HOH
enoylhydratase

$$CH_3(CH_2)_{n\text{-}1}\overset{OH}{\overset{|}{C}}H\text{-}CH_2\overset{O}{\overset{\|}{C}}\text{-}SCoA$$

hydroxyl fatty
acyl CoA dehy-
drogenase
NAD NADH

$$CH_3(CH_2)_{n\text{-}1}\overset{O}{\overset{\|}{C}}\text{-}CH_2\overset{O}{\overset{\|}{C}}\text{-}SCoA$$

thiolase
CoASH

$$CH_3(CH_2)_{n1}\text{-}\overset{O}{\overset{\|}{C}}\text{-}SCoA + CH_3\text{-}\overset{O}{\overset{\|}{C}}\text{-}SCoA$$

ACETYL COENZYME A

$$\underset{\text{ACETYL COENZYME A}}{CH_3\text{-}C\text{-}SCoA} + \underset{\text{CHOLINE}}{HOCH_2CH_2\text{-}\overset{+\ CH_3}{N}\text{-}CH_3} \; CH_3 \xrightarrow[\text{ACETYLASE}]{\text{CHOLINE}} \underset{\text{ACETYLCHOLINE}}{CH_3\text{-}C\text{-}O\text{-}CH_2CH_2\text{-}\overset{+\ CH_3}{N}\text{-}CH_3}\; CH_3$$

"BOUND" ACETYLCHOLINE

ACETYLCHOLINE
(FREE, ACTIVE)

CHOLINESTERASE

CHOLINE + ACETIC ACID

ACETYLCHOLINE
(RECEPTOR COMPLEX)

It is firmly established that acetylcholine is released at the neuromuscular junction during activity(13). However, accumulation of acetylcholine beyond a certain concentration paralyzes transmission and alters the behavior. An enzyme, acetylcholine esterase or cholinesterase was discovered to inactivate acetylcholine rapidly after its release by hydrolyzing it instantaneously(10d):

$$CH_3\text{-}\overset{\overset{O}{\|}}{C}\text{-}O\text{-}CH_2CH_2\text{-}\overset{+}{N}\begin{matrix} \diagup CN_3 \\ \text{-}CH_3 \\ \diagdown CH_3 \end{matrix} \xrightarrow[HOH]{CHOLINESTERASE} CH_3COOH + HOCH_2CH_2\overset{+}{N}\begin{matrix} \diagup CH_3 \\ \text{-}CH_3 \\ \diagdown CH_3 \end{matrix}$$

ACETYLCHOLINE ACETIC ACID CHOLINE

Synapses in which one neuron stimulate another by the release of acetylcholine have been studied. Repeated synaptic "use" during learning could increase the efficiency of synapses by leading to the release of more acetylcholine. It is believe that the difference between fast and slow learners could be caused by a biochemical difference in the acetylcholine-cholinesterase ratio (Ach/AChE) rather than by anatomical difference in the brain(14).

Learning is generally considered to involve chemical changes in the nervous system. It also involves a change in response to stimuli, hence, behavioral techniques are necessary to relate the biochemical and behavioral sequences that occur in the brain. Studies involving laboratory animals trained in a maze are often employed. Mice trained in a Y and T maze, pigeons trained to press a bar in a skinner box and rats trained on a discrimination task have been utilized in experiments involving behavioral performance.

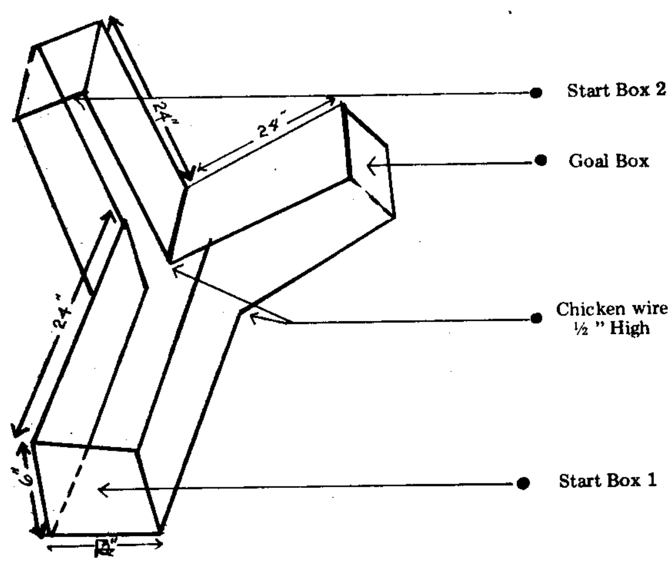
III. MATERIALS AND METHODS

The avocado and sesame oils were obtained from Prof. Aurea Aparato at National Science Research Center, University of the Philippines, Diliman, Quezon City. The coconut oil used was Baguio Edible Oil. The Y maze was constructed out of plywood and was painted brown although gray is often used in most experiments.

A preliminary study was undertaken to determine the best fat level of the experimental diet for the albino mice (15). The albino mice used in this study were male and female weanlings and adults taken from the animal colony in the College of Veterinary Medicine, University of the Philippines, Diliman, Quezon City and from Dr. Gloria L. Enriquez of the Department of Zoology, U.P. They were housed in individual cages and given their respective diets and water *ad libitum.* Three diets were prepared. The composition of these diets are given in Table IV. Every 100 grams of diet was fortified with 0.14 gram of vitamin supplement, the composition of which is shown in Table V (16). Each diet was fed to a group of 5 mice for 4 weeks. Their weights and food intakes were recorded every 4 days.

At the end of 4 weeks, the mice were starved for a variable number of days (about 1-2 days). They were run in a Y maze and fed *ad libitum* after the test. This experiment lasted for two weeks.

The figure below illustrates the floor plan of the Y maze used for the behavioral test (17):



At the goal box, a watch glass filled with the experimental diet and a drinking bottle filled with tap water were placed as stimuli. The two alleys were left empty and served as start boxes. The reason for the presence of two start boxes is to eliminate the "right and left hypotheses"(18). That is, an animal may systematically choose the alley on the right or the alley on the left. What was done during the test was to put the animal at start box 1 for the first run and at start box 2 for the second run. For 12 trials at most, the two start boxes were alternately used.

The hypotheses that were followed in this experiment are the "Perseverative Spatial" and the "Alternating Spatial" hypotheses (18). An animal is credited with displaying a "Perseverative Spatial" hypothesis when it chooses the goal box because the previous alley it went through had proved to be correct. Conversely, the animal may show "Alternating Spatial" hypothesis when it chooses the goal box after going through the wrong alley. The author assumed the "Perseverative Spatial" hypothesis to be correct because it shows the ability of the animal to discriminate the correct alley from the wrong.

Before the mice were tested, they were pretrained for one whole day in the Y maze. Each mouse learned to leave the start box and run through the alley leading to the goal box, as well as to eat and drink in the goal box. The mouse was tested after going through a 48-hour starvation period or more with 12 trials at most a day. A trial was eliminated or the test was stopped when the animal did not respond to the stimuli. Responses like getting out of the box, displaying a feeling of exhaustion after several trials were not counted.

During the testing period, the only food the mouse obtained was in the goal box of the apparatus. It ate there for 15 seconds (a stopwatch was used) after each trial and was later taken to the other start box. A piece of wood in the start box was used to block the animal while resting. After 15 seconds, the block is released so that the animal could run through the alley. This process was repeated until the maximum of 12 trials. The mouse was put back to its cage and fed *ad libitum* with the experimental diet. After two days the mice were ready to be tested in the maze again. The test was done for 5 "running" days when they were able to "solve" the maze almost perfectly.

An example of the score sheet taken per mouse in one "running" day is shown below:

TRIALS

Diet	1	2	3	4	5	6	7	8	9	10	11	12
Coconut	A	P	P	A	P	P	P	P	A	A	A	P
Avocado	P	P	P	P	P	P	P	P	A	A	P	P
Sesame	A	P	P	P	P	P	A	P	A	P	P	P

P—Perseverative Spatial Hypothesis
A—Alternating Spatial Hypothesis

IV. RESULTS AND DISCUSSION

Mice fed with avocado and sesame diets yielded higher body weight gains and food efficiency ratios than those fed with coconut diet as can be seen in Table VI and Figure 1. This upholds the reports of Jamieson and Deuel (19,20) who assayed the fatty acid content of these oils by analytical and microbiological method, that avocado and sesame oils are higher in essential fatty acid content than coconut oil.

Figure 1 shows the growth curves of the mice fed with different diets. It should be noted that the mice used were of different ages and weights, hence, their growth rates varied. Note that mice fed with coconut and avocado diet started almost at the same weight while mice fed with sesame diet had a lower weight than the two. When gains in body weight were calculated for, mice fed with sesame diet gave the highest growth response followed by mice fed with avocado diet and lastly the coconut group.

The results obtained in food efficiency ratios and body weight gains of the mice fed with different oils are parallel such that the greater the food consumption, the greater is the growth response. This exploration is supported by Deuel (21).

The overall results are best explained on the basis that essential fatty acids provide a necessary factor to permit growth by providing a required component for certain enzymes. The functional mechanism of these acids are not yet known. The mechanism of action appears to resemble that of the vitamins and they have often been referred to as Vitamin F(22).

From the data in Table VII, it is apparent that the values for the mental activity or "intellectual performance" of mice fed with avocado and sesame diets are higher than the result obtained from the coconut fed group. This is in agreement with the findings of Paoletti(23) that essential fatty acid deficient rats show impairment of performance when subjected to a specific behavioral test. The mice employed in this experiment however were not deficient in essential fatty acids. The different oils fed them had varying amounts of essential fatty acids particularly in linoleic acid (see Table I). It is possible that acetylcholine which is a by-product of fatty acid metabolism could be a factor for the difference in the "maze-bright and maze-dull" mice.

The working hypothesis of the author is that when less than the optimum requirements of essential fatty acids is ingested, the concentration of acetylcholine produced is low, nerve transmission is rendered inefficient and therefore "intellectual performance" would be lower than when adequate amount of essential fatty acids is taken in. Since the nutritional requirements of the brain remain constant (8), an excess of essential fatty acids may cause damage to the brain.

The results obtained are open to question. What the author is leading into is that essential fatty acids play an important role in the development of intellectual ability. The use of a behavioral technique in this thesis is justified and equally necessary since one of the variables involved is "learning."

It should be noted that the experiment started with 5 mice per group which were gradually reduced to 3. The reason is that the condition imposed during the starvation period was too abrupt. That is, a 24-hour starvation period was not enough to effect hunger on the mice so it was prolonged to 48 hours or more. Some mice died while others were accidentally injured in the cage. The data obtained in Table VII was based on the number of mice that survived and participated in the test.

The experiment may be improved by using animals that are of the same age or weaned at the age of one week or less after birth. Modified feeding instruments are necessary if baby animals are to be used. The duration of the feeding experiment must be longer to get maximum results. Moreover, the use of complicated maze is also advisable.

V. CONCLUSION

Body weight gains and food efficiency ratios of mice fed with avocado and sesame diet are higher than those fed with coconut diet. The mental activities of mice fed with avocado and sesame diets are also higher with the avocado group showing relatively higher values than the sesame group. These indicate the direct relation of these oils to the essential fatty acid content. Avocado and sesame oils have higher essential fatty acid content than coconut oil and, hence, have higher nutritive values.

Table I
Essential Fatty Acids in avocado oil

Avocado Oil (1)	Sesame Oil (2)	Coconut Oil (3)
Moisture content of the fruit – 71%		Average nut yields 0.5 lbs. of dried copra
Oil content of the fruit 29%		

		Dried copra yields 60-65% oil
Specific gravity of of the oil at 25°C-0.9132		Lauric acid- 48.0%
Unsaponifiable matter-1.6%		Capric acid-7.2%
Unsaturated acids-84-3%		Caprylic acid-7.9%
Oleic acid-74.0%		Oleic acid-5.7%
Linoleic acid-10.3%		Linoleic acid-2.6%
Myristic acid-0.05%	Linoleic acid-40.4%	Myristic acid-17.5%
Palmitic acid-6.62%	Linolenic acid-0%	Palmitic acid-.9.0%
Stearic acid-0.55%	Total essential fatty acids by biological assay-28.2%	Stearic acid-2.1%
Arachidic acid-trace		Caproic acid-trace

TABLE II
Approximate Composition of Brain(8)

Component	Whole Brain (%)
Water	78
Lipids	10
Protein	8
Carbohydrate	1
Inorganic Salts	1
Others	2

TABLE III
Concentration of Lipids in Human Brain(% dry weight) (9)

	Adult		Infant	
	Gray	White Matter	Gray	White Matter
Cholesterol (free)	6.3	14.3	5.1	7.0
Phosphatides	21.0	23.8	19.6	22.1
lecithin	6.3	4.6	7.8	9.1
cephalin	11.7	12.4	10.5	11.6
sphingomyelin	3.0	6.8	1.3	1.4
Cerebrosides	5.5	16.3	5.6	6.2

TABLE IV
Percentage Composition of the Basal Diets

Diet Number	I	II	III
"Vitamin-free" casein	18.0	18.0	18.0
Starch	45.7	45.7	45.7
Sucrose	10.0	10.	10.0
Salt Mixture (24)	4.0	4.0	4.0
Liver Extract	1.0	1.0	1.0
Methionine	0.2	0.2	0.2
Choline Chloride	0.2	0.2	0.2
Vitamin Mixture*	0.14	0.14	0.14
Coconut Oil	20.8	—	—
Avocado Oil		20.8	—
Sesame Oil	—	—	20.8

*See Table V for composition

TABLE V
Composition of Vitamin Supplement per 100 grams of Diet(16)

Vitamins	Gram
Inositol	0.1000
Thiamine hydrochloride	0.0005
Pyridoxine hydrochloride	0.0005
Nicotinic acid	0.0005
Calcium pantothenate	0.0025
p-Aminobenzoic acid	0.0300
2-Tocopherol acetate	0.0025
2-methyl-1, 4 napthoquinone	0.0002
Biotin	0.00001
Folic acid	0.00001
Vitamin B_{12} (in mannitol)	0.0015
Riboflavin	0.0010

TABLE VI
The Effect of Dietary Oil on Body Weight Gains and Foof Efficiency Ratios

Diet	Food Intake Gram	Body Weight Grain Gram	Food Efficiency Ratio* Gram Gain/Gram Food
Coconut	70.5	6.2 ± 0.6**	0.09 ± 0.01**
Avo cado	71.0	7.3 ± 1.5	0.10 ± 0.02
Sesame	70.9	8.7 ± 1.6	0.12 ± 0.02

*Average of 5 mice per group
** Mean ± average deviation

TABLE VII
The Effect of Dietary Oil on the Mental
Activity of Mice

Diet	% Correct Response*
Coconut	55.3 ± 8.0**
Avocado	75.2 ± 9.9
Sesame	63.6 ± 9.3

* $\frac{\text{TOTAL No. of Perseverative Spatial Hypothesis}}{\text{Total No. of Trials}} \times 100$

**Mean ± average deviation
Note: Number of Mice per group at the Start-5
Number of Mice per group in the End-3

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