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ANALYSIS OF SIALIC ACID AND SPHINGOMYELIN OF BRAIN OF MICE BY USING SPECTROPHOTOMETER AND HPLC

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ABSTRACT

Sialic acid is an important component of brain which is needed to form ganglioside molecule of brain's membrane, while sphingomyelin has a role in myelination of central nervous system during the growth development of babies. The aims of this research were to analyze the sialic acid and sphingomyelin contents of the brain of mice and compared with their contents in the ration of mice. The study was carried out in three phases as follows: i.e., ration formulation, analysis of sialic acid and sphingomyelin contents in the ration, and analysis of sialic acid and sphingomyelin contents of the brain of mice. The mice used were Swiss Webster strain, male, weaned, aged 28 days, with a total number of 32. Analysis of sialic acid content was conducted using UV spectrophotometer, while sphingomyelin analysis was done by using HPLC. The data obtained were tested statistically using SPSS 17.0 software. The results showed that sialic acid and sphingomyelin contents of brain of mice fed F1, F2 and F3 rations (supplemented with egg yolk) were greater than that of mice fed F0 (control) ration.

Key Words: Sialic acid, Sphingomyelin, Brain, Mice

INTRODUCTION

Chemically and structurally sphingomyelin can be grouped as phospholipid, however, because its backbone is a sphingosine, it is also called sphingolipid (Gurr et al, 2002); while sialic acid is a derivative of N- or O-acyl of neuraminic acid (Schauer et al, 1995).

According to Oshida et al (2003), Sherwood (1996) and Ganong (1983), the main components of brain are fatty acids such as DHA and ARA, and sialic acid and sphingomyelin. ARA and DHA

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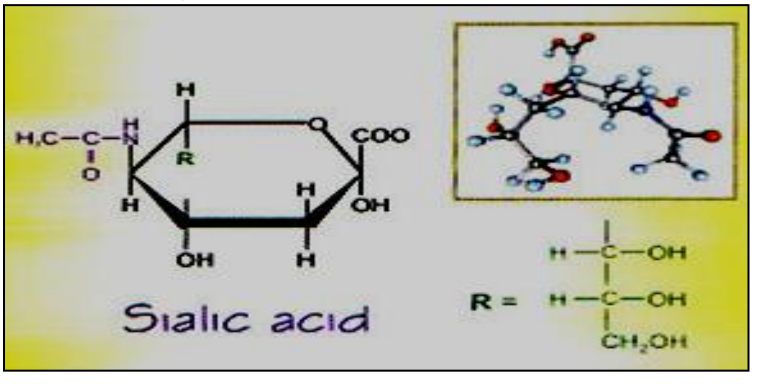
function to help communication among nerve cells by releasing neurotransmitters, elongation of nerve cell branches and completing the relation among nerve cells.

SIALIC ACID

Sialic acid is an important component of brain which is needed to form ganglioside molecules of brain cell membrane. It's present is very important in brain development, to increase learning ability and memory formation (Wainscot, 2004; Tram et al., 1997).

Figure-1. Sialic Acid (Wainscot, 2004)



Early human milk is a rich source of sialic acid, N-Acetylneuraminic acid (NANA) (Carlson,1985) but the metabolic fate and nutritional significance of sialic acid are currently unknown. There is evidence from animal models that exogenous sialic acid infulences brain growth and learning ability (Morgan BLG and Winick M, 1980), Neural cell membranes contain 20 times more sialic acid than do other types of membranes (Schauer, 1982) and brain gangliosides are an especially rich source. The adult human brain contains concentrations of sialic acid that are 2-to 4 fold higher than those of other mammals, including chimpanzess (Wang et al., 1998).

Sialic acid is thought to play a role in the structural and functional establishment of synaptic pathways : > 40% of sialic acid in the brain is found in the synaptosomal faction and contributes to the negative charge of the membrane (Schauer, 1982). Becauce most neurotransmitters are positively charged, sialic acid may assist neurotransmission by facilitating the binding of transmitter molecules to the synaptic membrane (Morgan dan Winick, 1980).

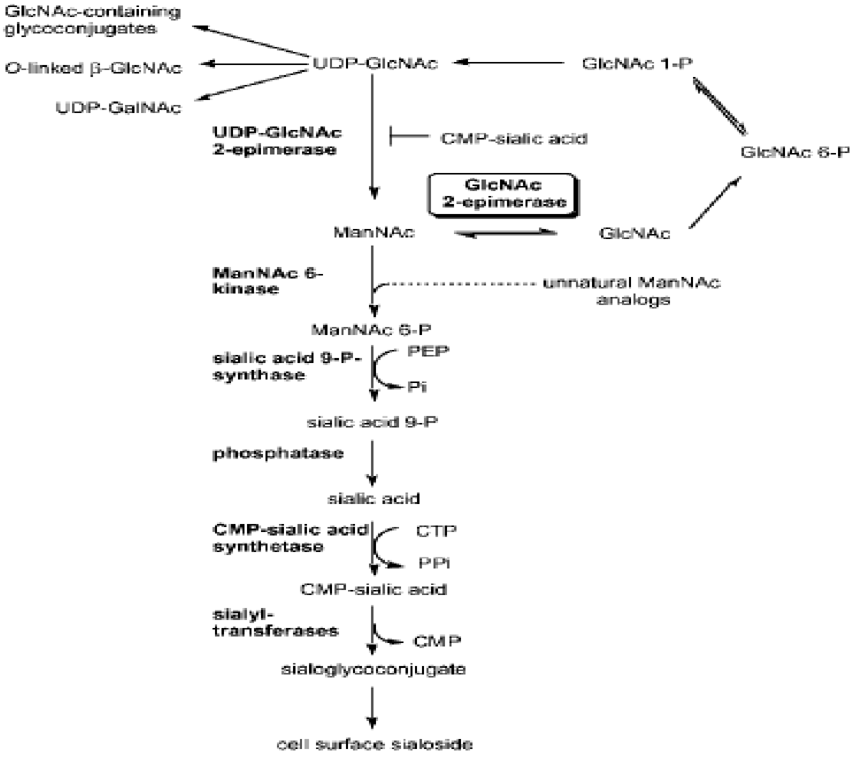
Morgan BLG and Winick M, (1980) showed that exogenous sialic acid administered by intraperitoneal injection increased the production of ganglioside sialic acid in the brain and improved learning ability in well nourished and malnourished rat pups. They also found that these changes persisted into maturity.

Carlson SE and House SG, (1986) showed that both intraperitoneally and orally administered sialic acid increased brain ganglioside sialic acid in young rats. Timing appears to be critical, however, because studies in older animals do not show a significant incorporation of labeled sialic acid after acute dosing (Nohle U and Schauer R, 1981).

The liver of all mammals, including those of humans, have the capacity to synthesize sialic acid from simple sugar precursors. However, the liver of new born infants is relatively immature and the rapid growth and development of the brain, especially inpreterm infants, may be compromised by a limited rate of de novo synthesis (Carlson, 1985). Thus, dietary sources of sialic acid may pay a role in determining the final concentration of sialic acid in the brain and may possibly affect the learning ability of human infants.

Sialic acid is found in especially high concentrations in brain gangliosides, and supplementary sialic acid is associated with increased learning behaviour in animals .

Figure-2. Sialic Acid Biosynthesis. (Sarah J. Luchansky, et al. 2003)

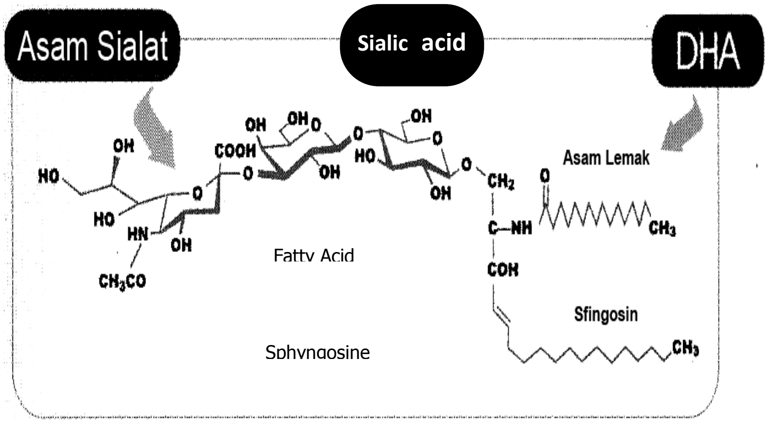


Sialic acid biosynthesis : ManNAc is synthesized from UDP-GlcNAc by UDP-GlcNAc 2-epimerase, but the role of GlcNAc 2-epimerase is unclear. ManNAc or a ManNAc analog is phosphorylated by ManNAc 6-kinase to yield ManNAc 6-phosphate (ManNAc 6-P). ManNAc 6-phosphate is subsequently condensed with phosphoenolpyruvate (PEP) to yield sialic acid 9-phosphate (sialic acid 9-P) in a reaction catalyzed by sialic acid-9-phosphate synthase. Dephosphorylation of sialic acid 9-phosphate by an unknown phosphatase and transport to the nucleus enables CMP-sialic acid synthetase to produce CMP-sialic acid. Following transport into the Golgi compartment, CMP sialic acid is utilized by the sialyltransferases that append the sialic acid to glycoconjugates ultimately destined for the cell surface or secretion. (Sarah J. Luchansky, Kevin J. Yarema, Saori Takahashi, and Carolyn R. Bertozzi. 2003)

Experimental studies in animals have shown that brain structure and function are permanently affected by early nutrition. In the rat brain, malnutrition during the first few weeks of life produces a reduction in the extent of dendritic arborization, a decreased content of ganglioside and glycoprotein sialic acid,and corresponding deficits in learning behavior (Morgan BL, Winick M. 1981). Gangliosides are neuronal membrane glycosphingolipids that are concentrated in the synaptosomes and are important for differentiation (Rosner H. 1998), synaptogenesis (Ledeen RW, et al. 1998), and neurotransmission (Rahmann H, Kortje KH, Seybold V, Rosner H**,** 1990). Gangliosides interact with membrane-bound functional glycoproteins, which modulates their activity and influences the membrane-mediated transfer of information (Fishman PH, Brady RO. 1976.). Brain growth and maturation are associated with an increase in gangliosides and sialoglycoproteins (Kracun I, Rosner H, Drnovsek V, et al. 1992).

In the early process of brain development, bonding sialat acid on gangliosida gangliosida strongly associated with DHA and total omega-3 fatty acids, both structurally and functionally (Wang and Miller, 2003).

Figure-3. Bound Gangliosides GM3 Sialic Acid (Wainscot,2004)



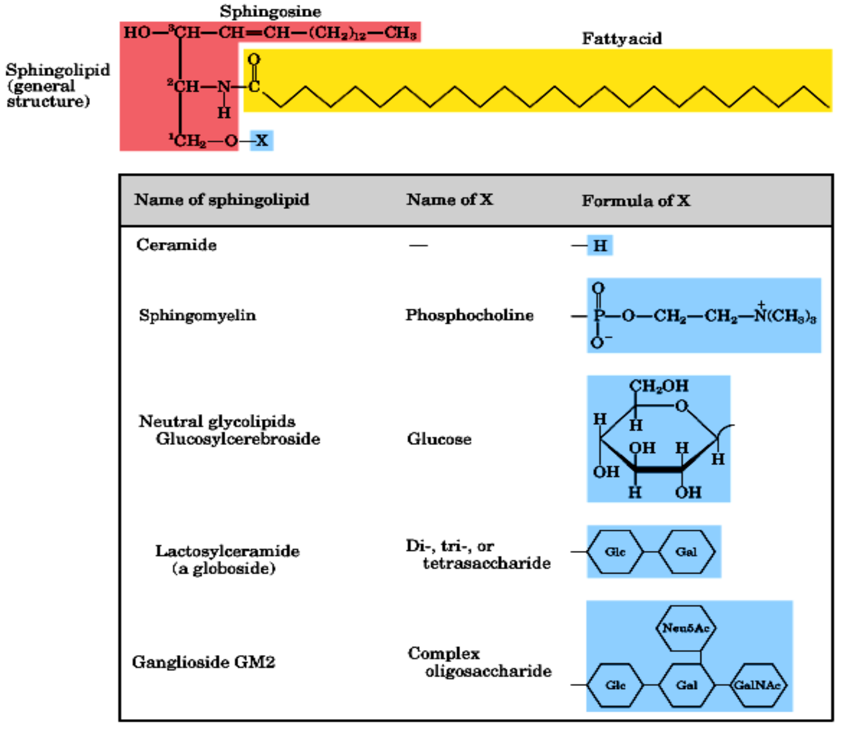
Carbohydrate compounds that play a role in the construction of complex oligosaccharides gangliosida or free (HMOS), are: Glucose (GLC), galactose (Gal), N-acetylglucosamine (GlcNAc), N-acetylgalactosamine (GalNAc), fucosa (FUC), sialat acid (NeuAc or NeuGc) (Christie, 2007 dan Fishman, 1976).

All of the human brain gangliosida sialat acid in the form of Neu5Ac. Gangliosides contained in the cell membrane at the end of the neuron, on the formation of synapses, and amino sugar components sialic is located at the reactive end of the carbohydrates in gangliosida (Wainscot, 2004).

SPHINGOMYELIN

Sphingomyelin has an important role in myelination of the central nervous system during the growth of infants. The addition of one molecule of fatty acid on the amine group of sfingosin will produce adhesion molecules and subsequent seramida fosfokolin the hydroxyl group at the end will produce sphingomyelin, as well as one type of phospholipid fosfatidilkolin, a molecule containing one molecule of choline sphingomyelin. Sphingomyelin in addition to having a function as protective membrane as well as choline carrier (Oshida et al., 2003).

Figure-4. Structure Sfingolipid and Sphingomyelin (Gurr, et.al.,2002)



Sphingomyelin did not contain glycerol, but ceramid, because all of ceramid sphingolipid made, it can be classified as well as sphingomyelin sfingolipid (Jumpsen & Clandinin, 1995). ceramid cerebrosida which subsequently form a universal marker of myelination (myelin-forming) in the brain, catalyzed UDP-galaktosiltransferase. Myelin of the central nervous system (CNS) contains cerebrosida the highest compared with other tissues. cerebrosida resulting from the enzyme catalyzed UDP-ceramida galaktosil-transferase:

a. Synthesis de novo from serine and palmitoyl CoA catalyzed enzyme SPT (serine palmitoyl transferase).

b. Sphingomyelin in the food enzyme catalyzed sfingomielinase. Sphingomyelin can be synthesized by the body from the amino acid serine with the help of co-factors such as palmitic acid, vitamin B6 (pyridoxine), vitamin B3 (niacin) and vitamin B2 (riboflavin) so we get sphingosin (Vesper et al., 1999).

Process of myelination of nerve cells can be inhibited when the activity of enzymes that act to synthesize sfingomielinnya inhibited. At SPT inhibited the enzyme activity, inhibited myelination process (Oshida et al., 2003),

Low nutrient conditions is one factor that can lead to inhibition of the sphingomyelin-forming enzyme activity, but by consuming foods containing sphingomyelin inhibited myelination process will re-activated (Oshida et al., 2003).

Sphingomyelin is an important structural and bioactive lipid (1–3). It is found in eggs, milk, meat, fish and soybeans (Vesper et al. 1999).

Watson, (2002), Egg yolks contain triglycerides (65.5%), phospholipids (28.3%), and cholesterol (5.2%). Composition of phospholipids in egg yolk are phosphatidylcholine (73%), fosfatidil-etanolamin (15%), lisofosfatidilkolin (5.8%), sphingomyelin (2.5%), lisofosfatidiletanolamin (2.1%), plasma-logen (0.9%), and fosfolidil inositol (0.6%). Egg yolks also contain 2.5 mg of GM4, GM3 8.5 mg and 1.5 mg GD3 (Li, et al., 1978).

MICE

Mice is one of the basic model (prototype) to study human behavior. Mice are commonly used to study human behavior of adult mice or rats are weaning off (Zutphen *et al*, 1993).

Mice were able to adapt in various environmental conditions, are omnivorous and includes an easy to breed animals. Mice have evening activities. Daily activity of mice consisted of a period of rest and active periods alternating with 14 hours each day and 10 hours of the night (Zutphen *et al*, 1993).

Table-1. Biological Data Mice

	size
Adult weight (grams) :	
male	20 - 40
female	25 - 40
Weight at birth (g)	0.5 - 1.5
Time of weaning weight (g)	10
Time of weaning age (days)	21 - 28
Breeding age (weeks)	8 - 10
Lifetime (years)	1 - 2

(Zutphen *et al*, 1993)

Vesper et al (1999) stated that sialic acid and sphingomyelin from food consumed has an important role in myelination of central nervous system during development process. Noh and Koo (2003) found that giving exogenous sialic acid and sphingomyelin intraperitoneally or orally could increase the learning ability of rats.

The aims of this research were to analyze the sialic acid and sphingomyelin contents of the brain of mice and compared with their contents in the ration of mice.

MATERIALS AND METHODS

The mice used were Swiss Webster strain, male, weaned, aged 28 days, with a total number of 32. The ration of mice was composed of corn starch, corn oil, casein (plus egg yolk), vitamin and mineral mixes, and fiber (CMC). The egg yolk was obtained from the egg of local hens. In this case, egg yolk was the sole source of sialic acid and sphingomyelin in the ration of mice.

Chemicals used for sialic acid and sphingomyelin analysis were acetonitril, methanol, sulfuric acid, DMSO, TCA, sphingomyelin standard (EC No. 2860972) and sialic acid standard (EC No. 205-023-1).
Instruments used were mini UV spectrophotometer (Shimadzu) and HPLC (Hitachi).
Formulation of rations was conducted by making a variation of the quantity of casein and egg yolk as shown on Table 1. The formulas had similar protein and calorie contents.

Mice were raised during 4 weeks, and at the last day of experiment, the brains of mice were taken to be analyzed for their sialic acid and sphingomyelin contents.

Table-2. Composition of mice rations (per 100 grams)

Component	Materials	F0 (g)	F1 (g)	F2 (g)	F3 (g)
Protein	Egg yolk	0	5	10	15
	Casein	22,1	21,2	20,3	19,4
Lemak	Corn oil	6,5	6,5	6,5	6,5
Vitamin	Vit mix	1	1	1	1
Mineral	Min mix	5	5	5	5
Fiber	CMC	5	5	5	5
Water	Water	13	13	13	13
Carbo-hydrate	Corn starch	47,4	43,3	39,2	35,1
Total		100	100	100	100

Analysis of sialic acid content was conducted using UV spectrophotometer (Modified from Wang et al, 2001a), while sphingomyelin analysis was done by using HPLC (Noh and Koo, 2003) :

Preparation of standard sphingomyelin.
Preparation of Egg Yolk
HPLC conditions :
Column Brand: Hitachi
Column: C18
Column size: 12.5 cm x 4 mm.
Eluent : mixture of MeOH : ACN

= 5: 95 (v/v)

Flow rate through the column :

= 0.7 mL / min.

Pressure: 32 bars

Temperature: 40°C

Detector: ultraviolet light (λ = 202 nm)

Sphingomyelin standard retention time: 1.7 minutes.

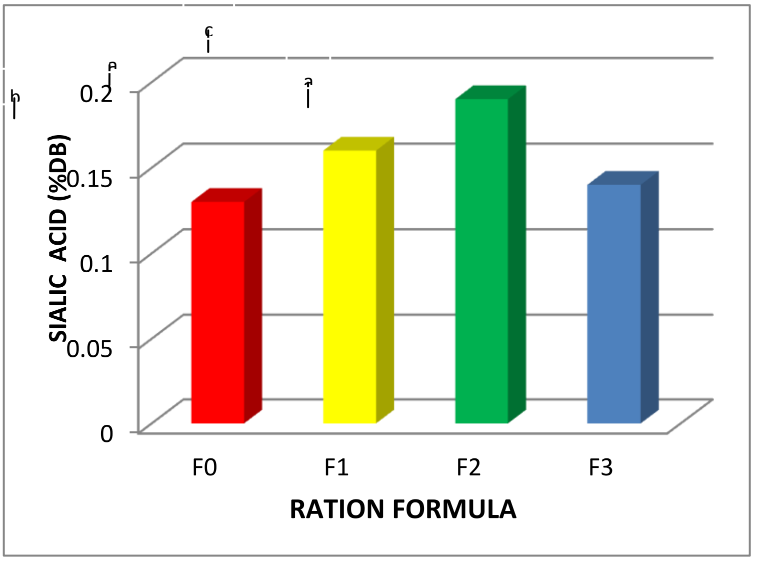
Observations made of the parameters relative retention times compared to the sample chromatogram with a retention time relative standard chromatogram sphingomyelin.

The data obtained were tested statistically using SPSS 17.0 software (Walpole dan Myers, 1995).

RESULTS AND DISCUSSION

Sialic acid contents of brain of mice are depicted on Figure 5. It is shown that the contents of sialic acid of brain of mice given ration of F0, F1, F2 and F3 were 0.13 %, 0.16 %, 0.19 %, and 0.14 % dry basis, respectively.

Figure-5. Sialic Acid Contents of Brain of Mice

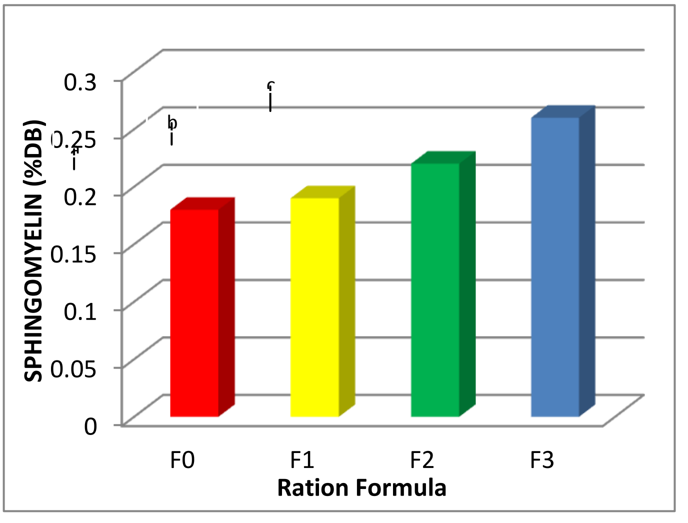


The highest sialic acid content of brain of mice is found on the brain of mice fed ration of F2 (i.e., 0.19 % db), and it is significantly different from others. Wainscot (2004) found that giving exogenous sialic acid intraperitoneally or orally could increase the sialic acid content permanently in the ganglioside of the brain of rats, and these rats have greater learning ability.

Sialat acid is an important content in the brain needed to form a molecule gangliosida of brain cell membranes. Its existence is a very important role in brain development, learning, and memory formation (Wainscot 2004 and Tram et al., 1997).

Sphingomyelin contents of brain of mice is depicted on Figure 6. It is shown that the contents of sphingomyelin of brain of mice given ration of F0, F1, F2 and F3 were 0.18 %, 0.19 %, 0.22 %, and 0.26 % dry basis, respectively.

Figure-6. Sphingomyelin Contents of Brain of Mice



The highest sphingomyelin content of brain of mice is found on the brain of mice fed ration of F4 (i.e., 0.26 % db), and it is significantly different from others. Oshida et al (2003) stated that sphingomyelin contains phosphate and choline. Choline is part of lecithine which is a phospholipid found abundant in brain as a component of nerve cell membrane. Choline is also needed in the formation of acetylcholine which is an important neurotransmitter.

Eggs are one of Neurotransmitter-forming foods, ie molecules that served as a messenger in the brain. The stimulation of the nerve will be forwarded to other nerve cells through neurotransmitters, and neurotransmitter is derived primarily from the protein we eat. The smooth delivery of stimuli from one nerve cell to other nerve cells, among others, determined by the availability of a chemical compound that served as conductor of stimuli. Nerve cells can only produce the neurotransmitter as needed if there is enough food consumed by children (Winarno and Ong, 2007).

Neurotransmitters have a very important role in the mechanism of learning, memory. One factor that plays an important shift from short term memory phases of memory into long term memory, namely N-methyl-D-aspartate (NMDA). NMDA plays a role in controlling the influx of Ca^{2+} ions (Carlson, 2004). Ca^{2+} ions play an important role in the process of changing impulses of electrical signals into chemical signals in the form of neurotransmitters produced by synaptic vesicles (Campbell dan Reece, 2002).

At the time of Ca^{2+} ions into the presynaptic membrane, Neurotransmitters released through the membrane vesicles postsinaptik then received through the NMDA receptor membrane postsinaptik (Carlson, 2004).

Comparison of sialic acid contents of the ration and of the brain of mice is presented on Table 3, while comparison of sphingomyelin contents is shown on Table 4.

Table-3. Comparison of sialic acid contentsof the ration and of the brain of mice

Ration	Silaic acid (% dry basis)	
	Egg Yolk	Brain
F0	0.00	0.13
F1	0.55	0.16
F2	0.54	0.19
F3	0.54	0.14

Table-4. Comparison of sphingomyelin contents of the ration and of the brain of mice

Ration	Sphingomyelin (% dry basis)	
	Egg Yolk	Brain
F0	0.00	0.18
F1	0.27	0.19
F2	0.26	0.22
F3	0.27	0.26

It is shown on Table 3 and Table 4 that the brains of mice fed ration of F1, F2 and F3 have greater sialic acid and sphingomyelin contents than the brain of mice fed ration of F0 (not supplemented with egg yolk). These data indicate that egg yolk could be used as a source of sialic acid and sphingomyelin.

Eggs are animal food lipidnya component is dominated by sphingomyelin, serebrosida, globosida, gangliosida, and sulfatida. Sphingomyelin-containing phosphate and choline: Choline is part of lecithin is a phospholipid found in the brain much as forming the membrane (wall) of nerve cells. Choline is required for the formation of acetylcholine, a major neurotransmitter (Oshida et al., 2003). Sialiloligosakarida / sialat acid from egg yolk may improve learning ability and inhibits the growth of infants rotravirus (Sasaki, et al., 1998).

Even though sialic acid and sphingomyelin contents of brain of mice fed F1, F2 and F3 rations were greater than that of mice fed F0 (control) ration. However, there is not a good relation between the concentrations of sialic acid and sphingomyelin of the ration and that of the brain of mice.

CONCLUSION

Egg yolk could be used as a source of sialic acid and sphingomyelin. Sialic acid and sphingomyelin contents of brain of mice fed F1, F2 and F3 rations (supplemented with egg yolk) were greater than that of mice fed F0 (control) ration. However, there is not a good relation between the concentrations of sialic acid and sphingomyelin of the ration and that of the brain of mice.

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