Malaysian Journal of Medicine and Health Sciences (eISSN 2636-9346)

ORIGINAL ARTICLE

# Effect of Functional Feed Catfish (*Clarias gariepinus*) Oil and Probiotic *Enterococcus faecium* IS-27526 on Lipid Profile of Aged Atherogenic Female Cynomolgus Monkey (*Macaca fascicularis*)

Mahmud Aditya Rifqi[1], Clara M. Koesharto[2], Ingrid S Surono[3], Sri Anna Marliyati[2]

[1] Department of Nutrition and Health, Faculty of Public Health, Universitas Airlangga, Surabaya 60115, Indonesia
[2] Department of Community Nutrition, Faculty of Human Ecology, IPB University, Bogor 16680, Indonesia
[3] Department of Food Technology, Faculty of Engineering, Bina Nusantara University, Jakarta 11480, Indonesia

## ABSTRACT

**Introduction:** Catfish oil contains saturated and unsaturated fatty acids. Polyunsaturated fatty acid contributes to maintain the lipid profile. *Enterococcus faecium* IS-27526 is a novel indigenous probiotic of dadih origin. The aim of this study was to find out the effect of probiotic and catfish (*Clarias gariepinus*) based functional feed on lipid profile of aged female Cynomolgus monkey/CM (*Macaca fascicularis*) fed with atherogenic diet. **Methods:** Nine CMs were randomly devided into 3 groups, in single blind intervention study for 90 days , namely: A1= atherogenic diet as control positive, A2= probiotic, A3= probiotic + catfish oil after 45 days adaptation period. The average of CM's bodyweight was in a range of 3 to 4 kg. The main ingredients of atherogenic diet were catfish flour, egg, sugar, egg yolk flour, and condiments. Serum lipid profile was assessed monthly by *Cholesterol Oxidase-Peroxidase Aminoantipyrine* (CHOP-POD) methods for cholesterol, LDL and HDL, while *Glycerol-3-Phosphate Oxidase Peroxidase-Antiperoxidase* (GPO-PAP) method for Triglyceride. **Results:** Atherogenic diet did not significantly affect appetite and bodyweight of the animals (p=0,12). The atherogenic diet significantly increased cholesterol and LDL level of animals (p=0.03 and p = 0.04) after 90 days administration . The CMs fed with probiotic *E. faecium* IS-27526 and catfish oil showed significant higher serum cholesterol (p=0,03) and LDL levels (p=0,04) compared to atherogenic diet alone and atherogic diet with probiotic *E. faecium* IS-27526. Probiotic *E. faecium* IS-27526 at $10^8$ cfu/day stabilized the cholesterol and LDL level after 90 days administration compared with other groups of intervention. The TG and HDL of each group showed within normal range during the whole intervention study. **Conclusion:** Atherogenic feed significantly increased cholesterol and LDL level of CMs. Probiotic *E. faecium* IS-27526 showed a potential beneficial effect in maintaining lipid profile and could prevent dyslipidemia.

**Keywords:** *Clarias gariepinus*, Catfish oil, *Enterococcus faecium* IS-27526, Cholesterol, LDL

**Corresponding Author:**
Mahmud Aditya Rifqi, MSc
Email: mahmudraditya@fkm.unair.ac.id
Tel: +62 852 4404 6825

## INTRODUCTION

Rapid demographic change has occurred since people are now living longer and healthier lives than ever before, as shown by the fastest-growing segment of the world population being those aged 60 and over. In 2010, the number of people aged 60 years and above represents 7.6 percent of the total population, and in 2015, there was 8.2 % elderly population, or 21 million elderly, so that Indonesia becomes the largest elderly population in Southeast Asia, and represent the World's 8th largest conglomeration of elder persons. The proportion of the elederly population in Indonesia is projected to reach 11.8 percent, or reaching 33.7 million in 2025 and it will continue to grow to nearly 74 million in 2050. Hence, during the next few decades, Indonesia will be in population ageing stage (1).

The elderly belongs to vulnerable groups of population due to the decrease in physical fitness and the body's physiological function. Changes in BMI (Body Mass Index) is one of the consequences of being older. Aging is related to increase in visceral fat and decreased of peripheral fat free mass due to lost of muscle and bone mass. The enhancement of visceral fat, such as the intramuscular and intrahepatic fat is related to insulin resistance (2). Insulin resistance is also responsible for dyslipidemia. While in Indonesia, cardiovascular disease (CVD) is responsible for 37% of deaths where hyperlipidemia is one of the dominant contributing risk factors (Dalal and Robbins, 2002; Hussain et al., 2016)

(3, 4).

Fried catfish is widely consume in Indonesia as one of the affordable and popular protein source foods. However, larger fish is often rejected because the fried fish product is often less crispy. Thus these larger fish is potential for the production of catfish flour with a by-product of catfish oil. Catfish powder as functional food ingredient significantly improved nutritional status of undernourished children younger than 5 (5,6). Meanwhile, catfish oil contains essential fatty acids, namely linoleic acid (omega 6) (C18:2) and alpha-linolenic acid (omega 3) (C18:3), at the amount of 17.79% and 1.21%, respectively (7). Several studies revealed that essential fatty acids have anti-inflammatory and anti-atherosclerosis effects (8).

*Enterococcus faecium* IS-27526 is a novel indigenous probiotic of dadih origin, a traditional fermented milk of West Sumatera (9,10). Surono (2003) and Dharmawan et al. (2006) reported that several lactic acid bacteria isolates had probiotic properties as shown by acid and bile tolerance and ability to adhere to both mucus layer and Caco-2 cells (11). Moreover, Collado et al. (2007) found that *E. faecium* IS-27526 reported to be able to adhere to the intestinal mucosa (9). *E. faecium* IS-27526 has also shown an immunomodulatory property (12). Further, administration of microencapsulated *E. faecium* IS-27526 in pasta cream form has been proven to increase body weight and fecal lactic acid bacteria in mice (13). Based on this evidences, this study aimed to evaluate the effect of catfish oil and probiotic *E. faecium* IS-27526 on lipid profile in aged female Cynomolgus monkey (*Macaca fascicularis*) fed with atherogenic feed.

## MATERIALS AND METHODS

### Animal model

The ethical clearance has already approved by Animal Ethics Commission of Faculty of Veterinary Medicine IPB University with number P.01-13-IR IPB. Three years old Cynolmolgus monkey (CM), were used as animal model, and were quarantined in the Center of the Primate study of the IPB University for 135 days. Adaptation period was 45 days (30 days cage adaptation, fed with commercial monkey chow, followed by providing commercial monkey chow in the morning and atherogenic diet in the afternoon for 15 days during feed adaptation). The average of CM's bodyweight were in a range of 3-4 kg. Female monkeys were chosen as simulation subjects since elderly women will be the human subject in further clinical study.

### Experimental Design and diets

A 90 days single blind intervention study on female Cynolmolgus monkey (CM) were conducted. Nine CMs were randomly assigned into three groups (n = 3 in each group), namely: A1= atherogenic diet, A2= atherogenic diet+ probiotic, A3= atherogenic diet + probiotics + catfish oil. Diets were given based on 120 calories/kg of body weight per day, and supplemented twice a day, morning and afternoon, while the drink was supplied ad libitum.

All animals were fed with atherogenic feed, with the main ingredients were catfish flour, egg, sugar, 4% egg yolk flour, and condiments. The total energy intake from the atherogenic diet was 410 Cal/day including 18.76% fat intake for A1 and 18.84% for A2. The catfish oil contributed an additional 25 Cal/day and 3.10 % fat to the feed, replacing butter in other groups, thus the A3 diet contained energy 435 Cal/day and fat 21, 86%. Microencapsulated probiotic *E. faecium* IS-27526 (GenBank accession no. EFO68251) was administrated in powder form at $10^8$ cfu/day in A2 and A3 groups. A veterinarian was responsible for monitoring the intake of daily feed.

### Data Collection and analyses

Animals were sedated at the baseline and every month to measure the body weight and lipid profile with Ketamine HCl (10 mg/kg bodyweight) intramuscularly. Venous blood samples were obtained at baseline, month one, month two and at the end of the 90 days intervention from the femoral vein (Table 2). Three ml of blood was collected with minimal stasis by using a 0.9-mm needle (PrecisionGlide; Becton-Dickinson Vacutainer systems, Plymouth, United Kingdom). For cholesterol and lipoprotein analyses, 10 mL blood was collected into a serum tube (Corvac; Becton Dickinson Vacutainer Systems). At least 1 h after venipuncture, serum was obtained by centrifugation at 3500 x g for 30 min at 4 °C and stored at - 80 °C. Serum total cholesterol, LDL and HDL cholesterol were assessed by enzymatic colorimetric test with CHOP-POD method, and triglyceride with GPO-PAP method. The reagent and test kit were produced by PT. Rajawali Nusindo, Indonesia.

### Statistics

Analysis of Variance (ANOVA) was utilized to test the comparison of lipid profile within and between-groups. Duncan test was performed as the advanced test.

## RESULTS

The feed consumption of nine female monkeys (*Macaca fascicularis*) and their mean bodyweight are shown in Table I. All groups consumed more than 85% of feed, and the difference in feed consumption between groups were not significant ($p < 0.05$), likewise with the bodyweight of the animals in each group of treatment.
The level of plasma lipids during the experiment are shown in Table II. The atherogenic diet significantly increased cholesterol and LDL of animals in two-three month intervention. At baseline and one month intervention there were no significant increase in cholesterol level. The atherogenic and probiotic diet

**Table I: Average body weight and feed consumption of Cynomolgus Monkeys**

| Groups | Average of CM's Bodyweight (kg) | Feed Consumption (gram) | P-value |
|---|---|---|---|
| A1 | 3.41±0.29 | 90.91 ± 0.31 | <0.05 |
| A2 | 3.28±1.22 | 85.75 ± 0.36 | |
| A3 | 3.30±0.58 | 89.18 ± 0.44 | |

Note: (A1) Positive Control (Atherogenic diet), (A2) Probiotics (Atherogenic diet + probiotics), (A3) Probiotics + catfish oil (Atherogenic diet + probiotics + catfish oil).

**Table II: Average of the CM's lipid profile (mg/dl) during intervention**

| Variable | Mo | A1 | A2 | A3 |
|---|---|---|---|---|
| Cholesterol | 0 | 124.00±19.3$^{a}$ | 103.00±4.04$^{a}$ | 138.00±20.5$^{a}$ |
| | 1 | 215.00±51.8$^{ab}$ | 189.00±37.9$^{ab}$ | 411.00±126$^{cd}$ |
| | 2 | 452.00±136$^{d}$ | 378.00±114$^{bcd}$ | 846.00±200$^{e}$ |
| | 3 | 344.00±138$^{bcd}$ | 224.00±91.5$^{abc}$ | 697.00±91.4$^{e}$ |
| LDL | 0 | 68.100±20.0$^{ab}$ | 49.800±18.1$^{a}$ | 78.000±28.1$^{ab}$ |
| | 1 | 126.00±32.9$^{abc}$ | 117.00±58.4$^{abc}$ | 330.00±167$^{cd}$ |
| | 2 | 383.00±135$^{d}$ | 170.00±99.4$^{cd}$ | 785.00±231$^{e}$ |
| | 3 | 280.00±153$^{bcd}$ | 170.00±99.4$^{abcd}$ | 659.00±111$^{e}$ |
| Triglyceride | 0 | 36.60±6.65$^{a}$ | 30.60±15.9$^{a}$ | 50.00±11.2$^{ab}$ |
| | 1 | 86.00±38.7$^{c}$ | 50.00±8.88$^{ab}$ | 73.00±32.9$^{bc}$ |
| | 2 | 59.00±7.54$^{abc}$ | 42.30±6.80$^{ab}$ | 39.60±6.50$^{a}$ |
| | 3 | 64.00±11.2$^{abc}$ | 52.30±11.0$^{ab}$ | 46.60±4.04$^{ab}$ |
| HDL | 0 | 48.60±22.9$^{a}$ | 47.60±11.2$^{a}$ | 49.60±7.23$^{a}$ |
| | 1 | 71.30±44.1$^{a}$ | 62.00±21.3$^{a}$ | 66.30±35.1$^{a}$ |
| | 2 | 56.60±23.1$^{a}$ | 48.60±18.3$^{a}$ | 53.30±81.8$^{a}$ |
| | 3 | 50.60±30.2$^{a}$ | 44.00±6.08$^{a}$ | 28.00±21.1$^{a}$ |

Difference subset means statistically significant (p<0.05) among group
(A1) Positive Control (Atherogenic diet), (A2) Probiotics (Atherogenic diet + probiotics), (A3) Probiotics + catfish oil (Atherogenic diet + probiotics + catfish oil).

did not significantly increase lipid cholesterol during 3 months intervention. While, catfish diet significantly increase cholesterol level since one month until the end of intervention. All of intervention did not significantly increase triglyceride and LDL level during intervention. Atherogenic feed significantly increased cholesterol and LDL level after 90 days administration (p =0,04 and p=0,04, respectively). The presence of catfish oil in the diet significantly inreased lipid profile, as shown by significant higher cholesterol (p=0,03) and LDL level (p=0,04) as compared to atherogenic diet as well as atherogenic diet wih probiotic *E. faecium* IS-27526. The normal range of cholesterol level and normal LDL level of monkey is in a range of 106-148 mg/dl, and 48-89 mg/dl, respectively (14). Our study found that the average plasma cholesterol level after 90 days of intervention was 344.00±138.00 mg/dl, 224.00±9.15 mg/dl, and 697.00±91.40 mg/dl, and LDL level was 280.00±153 mg/dl, 170.00±99.4 mg/dl, and 659.00±111 mg/dl in monkey fed with atherogenic diet, atherogenic and probiotic diet, and atherogenic, probiotic and catfish oil diet, respectively. Probiotic tended to normalize the high cholesterol due to atherogenic feed, while atherogenic feed as well as its combination with catfish oil resulted in hyperlipidemia, as shown by significant increased of serum cholesterol and LDL level. (p<0.05).

Thus, the probiotic *Enterococcus faecium* IS-27526 tended to diminish the increment of the cholesterol and LDL level in animal subjects fed with atherogenic diet. On the contrary, significant increased of cholesterol and LDL level (p<0.05) were observed while the animal fed with catfish oil combined with probiotic in addition to atherogenic diet.

The normal triglyceride level for the animal subjects is in a range of 44 to 75 mg/dl. Triglyceride level of animals at the end of this study were 64.0±11.2 mg/dl, 52.3±11.0 mg/dl, and 46.6±4.04 mg/dl in monkey fed with atherogenic diet, atherogenic and probiotic diet, and atherogenic together with probiotic and catfish oil diet, respectively. Triglyceride level of animals in all groups were within the normal range and no significant different after 90 days of intervention (p>0.05).

The normal HDL level for the animal subjects is in a range of 19 to 103 mg/dl (15). The HDL level of animals at the end of this study were 50.60±30.2 mg/dl, 44.0±6.08 mg/dl, and 28.0±21.1 mg/dl in monkey fed with atherogenic diet, atherogenic and probiotic diet and atherogenic together with probiotic and catfish oil diet respectively. The HDL level of animals in all groups were within the normal range, and showed no significant different. There was no significant effect of each type of animal feed on HDL level between time point (p>0.05).

Figure 1 shows changes of lipid profile after 90 days of intervention (delta values from baseline to end-line). Probiotic *E. faecium* IS-27526 tended to normalize and stabilize the cholesterol and LDL level of animals.

## DISCUSSION

Consumption level in animal subjects is affected by palatability, form, and type of diet (16). During the first seven days of adaptation period, the animal subjects consumed Monkey chow, which is a commercial feed for monkeys, and no significant effect on body weight and lipid profile of Cynomolgus monkeys (CM's). Addition of egg yolk flour in atherogenic diet significantly increased

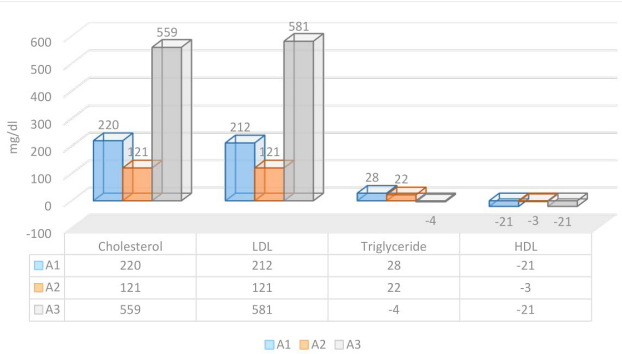


**Figure 1: Lipid profile changes during the intervention (delta values before and after 90 days administration)**

Note: A1 = atherogenic diet, A2 = probiotic, A3 = probiotic and catfish oil blood cholesterol and LDL level of the CMs compared to monkey chow (16). Another study also reported increase in lipid profile due to intake of egg yolk flour in rabbit (17). Excessive intake of egg yolk strongly correlated with hyperlipidemia (18). Hypercholesterolemia is strongly associated with coronary heart disease and arteriosclerosis (19,20,21,22) and decreasing serum cholesterol is an important strategy for its prevention. Lipid profile represents lipid disorder in a human's body, thus elevated lipid profile is an indicator of metabolic syndrome (23).

Our study observed that the probiotic *E. faecium* IS 25576 tended to diminish the increase of serum cholesterol and LDL level due to atherogenic diet (Table II). Cholesterol and LDL level in CMs fed with probiotics *E.Faecium* IS 25576 was significantly lower compared to other groups after 90 days administration. Previous studies revealed that indigenous probiotics of dadih origin were proven to decrease cholesterol levels (24, 25).

The increased of cholesterol and LDL level by atherogenic diet was in accordance with study of Garg (26). Addition of catfish oil significantly increased the cholesterol and LDL of animals to higher level. Our result is in line with a research conducted with the same female aged CM, and reported that catfish oil significantly increased levels of total cholesterol and LDL cholesterol in blood serum, but not in the plasma HDL (29). Catfish oil has a high content of saturated fatty acid (SFA), monounsaturated fatty acid (MUFA) and polyunsaturated fatty acid (PUFA) (30). The different effect of fish oil from catfish (freshwater fish) and deep sea water fish might be due to high 20% of saturated fatty acids, dominated by lauric acid and palmitic acid (28.8%). Lauric and palmitic acids are hypercholesterolemic compared with oleic acid. Lauric acid raises total cholesterol concentrations more than palmitic acid. The proportion of n-3 PUFA of seawater fish is in a range of 23-43,7% and higher than those of n-3 PUFA found in catfish (17.79 %) (31).

Another possibile factor to explain the correlation between catfish oil and hypercholesterolemic effect is oxidized fatty acid that occurs during catfish oil preparation. Fat that contains oxidized fatty acids in the diet might contribute to the presence of oxidized components in circulating lipoproteins (30). Both *in vitro* and epidemiology studies indicate that oxidized lipids may be involved in atherosclerosis. Dietary oxidized fatty acids will be absorbed by the intestine and incorporated into lipoproteins and could potentially impose an oxidative stress and exacerbate atherogenesis. Small quantities of oxidized linoleic acid promotes an atherogenic lipoprotein profile and atherosclerosis in the presence of a cholesterol-rich diet. This combination of dietary cholesterol and oxidized fatty acids may lead atherosclerosis (32,33,34).

This study has highlighted the potential role of probiotic *E. faecium* IS-27526 administration at a dose of $10^8$ cfu/day for 90 days diminishes the increment, hence, stabilizing cholesterol and LDL in atherogenic animal model.

## CONCLUSION

The present study found that female aged Cynomolgous Monkeys given atherogenic diet and catfish oil for 90 days showed a significant increase in total cholesterol and LDL cholesterol level ($p < 0.05$). The administration of probiotic *E. faecium* IS-27526 to female aged Cynomolgus Monkeys fed with atherogenic diet showed a potential beneficial effect in maintaining lipid profile and prevention of dyslipidemia. Further clinical trial is needed to warrant the efficacy of probiotic *E. faecium* IS-27526 in suppressing the cholesterol and LDL atherogenic human subjects, and also to find out the effect of catfish oil alone in profil lipid.

## ACKNOWLEDGEMENT

The authors thank to Directorate General of Higher Education, Indonesian Ministry of National Education for a grant in aid of Competitive Research Grant (HIKOM DIKTI 2008-2010). The authors also would like to thank PT Carmelitha Lestari, Primate Research Center, Microbiology Laboratory of Atmajaya University Jakarta, and Microbiology Laboratory SEAFAST Centre, Faculty of Agricultural Engineering and Technology, IPB University, and PT Ultra Jaya Milk Co for facilitating the research.

## REFERENCES

1. United Nations. World population aging 2019 Highlight. New York: Department of Economic and Social Affairs UN; 2019.
2. Villareal DT, Apovian CM, Kushner RF, Klein S. Obesity in older adults: technical review and position statement of the American Society for nutrition and NAASO, The Obesity Society. American Journal of Clinical Nutrition. 2005; 82 (5): 923-34
3. Flood M, Newman A (2007). Obesity in older adults: Synthesis of findings and recommendations for clinical practice. Journal of Gerontological Nursing. 33:19-35.
4. Mukhopadhyay SK. Study of lipid profile in obese individuals and the effect of cholesterol-lowering agents on them. Al Ameen J Med Sci. 2007; 5(2):147-151.
5. Kusharto C, Marliyati S, Surono, Dainy N. Functional clarias biscuit riched protein, mineral, and fiber, added fish oil as nutritious and emergency food for elderly. Bogor (ID): Institut Pertanian Bogor; 2012
6. Adi AC. Efficacy of Biscuit Enriched with the Catfish

Protein Flour (*Clarias gariepinus*), Soy Protein Isolate, and *Enterococcus faecium* IS-27526 Microencapsulated Probiotic as a Supplementary Food on Underweight Under-five Children (2-5 years old) [Thesis]. Bogor: Bogor Agricultural University; 2010.
7. Srimiati M, Kusharto CM, Tanziha I, Suseno SH. Effect of different bleaching temperatures on the quality of refined catfish (*Clarias gariepinus*) oil. Procedia Food Science. 2015; 3, 223-230.
8. Demonty I, Chan Y, Pelled D, Jones P. Fish-oil esters of plant sterol improve the lipid profile of dyslipidemic subjects more than do fish-oil or sunflower oil esters of plant sterols. Am J Clin Nutr. 2006; 84:1534-42.
9. Collado MC, Surono IS, Meriluoto J, Salminen S. Potential probiotic characteristics of Lactobacillus and Enterococcus strains isolated from traditional dadih fermented milk against pathogen intestinal colonization. Journal of Food Protection. 2007; 70(3):700-705.
10. Surono, IS. 2015. Traditional Indonesian dairy foods. Asia Pacific Journal of Clinical Nutrition 24, pp. S26-S30
11. Dharmawan, J., Surono, I.S., Kun, L.Y. Adhesion properties of indigenous dadih lactic acid bacteria on human intestinal mucosal surface. 2006 Asian-Australasian Journal of Animal Sciences 19(5), 2006, pp. 751-755
12. Surono IS, Koestomo PF, Novitasari N, Zakaria FR, Yulianasari, Koesnandar. Novel probiotic *Enterococcus faecium* IS-27536 supplementation increased total salivary sIgA level and bodyweight of pre-school children: A pilot study. Elsevier Ltd. 2011; 17: 496-500.
13. Harianti R. The effect of high protein biscuits added with probiotic cream administration on the profile of fecal microbiota and body weight of rats [thesis]. Bogor (ID): Bogor Agricultural University; 2009.
14. Fortman JD, Hewett TA, Bennett. The Laboratory Non-human Primate. Florida (US): CRC Pr; 2002.
15. Bennet BT, Abee CR, Henrickson R. Non-Human Primates in Biomedical Research: Biology and Management. Academic Press: New York (US); 1996.
16. Astuti D, Mansjoer, Sajuthi. Blood lipid profile of Cynomolgus monkey (*Macaca fascicularis*) induced by a high-fat diet. Jurnal Primatologi Indonesia. 7(1): 16-20; 2010.
17. Srilatha B, Adaikan PG, Ng SC, Aulkumaran S. Effect of feeding egg yolk on the serum lipid levels in rabbits. Methods Find Exp Clin Pharmacol. 1997;19(7):489-92
18. Djouss'e L, Gaziano JM, Buring JE, Lee I. Egg consumption and risk of type 2 diabetes in men and women. Diab. Care. 2009, 32:295-300
19. Lee YW, Roh WS, Kim JG. Benefits of fermented milk in rats fed by hypercholesterolemic diet (II) Kor J Food Hyg. 1992;7:123–135.
20. Anderson JW, Gilliland SE. Effect of fermented milk (yogurt) containing Lactobacillus acidophilus L1 on serum cholesterol in hypercholesterolemic humans. J Am Coll Nutr. 1999;18:43–50.
21. Law MR, Wald NJ, Wu T, Hackshaw A, Bailey A. Systematic underestimation of association between serum cholesterol concentration and ischaemic heart disease in observational studies: data from the BUPA study. BMJ . 1994;308:363–366.
22. Ross R. The pathogenesis of atherosclerosis: a perspective for 1990s. Nature. 1993;362:801–809. doi: 10.1038/362801a0.
23. McGuire M, Beerman KA. Nutritional sciences from fundamentals to food. Belmont (US): Thomson Wadsworth; 2009.
24. Pato, U., Surono, I.S., Koesnandar, Hosono, A. Hypocholesterolemic effect of indigenous dadih lactic acid bacteria by deconjugation of bile salts. Asian-Australasian Journal of Animal Sciences. 2004 17(12), pp. 1741-1745.
25. Surono, I. S. 2003. Probiotic properties of indigenous dadih lactic acid bacteria. Asian-Aust. J. Anim. Sci. 16:726-731
26. Garg A, Simha. Update on dyslipidemia. J.Clin Endocrinology Metabolism. 2007; 92: 1581-1589.
27. Ngadiarti I, Kusharto CM, Briawan D, Marliyati SA, Sajuthi D. Effect of catfish oil intervention on lipid profile in female aged Cynomolgus Monkey (Macaca fascicularis). Jurnal Sains Kasihatan Malaysia. 2014; 12 (02): 17-26.
28. E H Temme , R P Mensink, G Hornstra. 1996. Comparison of the Effects of Diets Enriched in Lauric, Palmitic, or Oleic Acids on Serum Lipids and Lipoproteins in Healthy Women and Men. Am J Clin Nutr . 1996 Jun;63(6):897-903
29. Srimiati M, Kusharto CM, Tanziha I, Suseno SH. 2015. Effect of different bleaching temperatures on the quality of refined catfish (Clarias gariepinus) oil. Procedia Food Science. 2015; 2 : 223-230.
30. Jessup W, Kritharides L, Stocker R. Lipid oxidation in atherogenic: an overview. Biochemical Society Transactions. 2004 Vol 32 (1)
31. Merchant NK, Penumetcha M, Meilhac O. Oxidized fatty acids promote atheroslerosis only in the presence of dietary cholesterol in low-density lipoprotein receptor knockout mice. Biochemical and molecular actions of nutrients. 2002.
32. Penumetcha M, Khan N, Parthasaraty S. Dietary oxidixed fatty acids: an atherogenic risk?. Journal of Lipid Research. 2000, Vol 41.