

Effect of Catfish' (*Clarias gariepinus*) Flour and Oil with Probiotic *Enterococcus faecium* IS-27526 Based Functional Feed Provision on Body Weight and C-reactive Protein (CRP) of Aged Atherogenic Female Cynomolgus Monkey

MAHMUD ADITYA RIFQI¹, CLARA M KUSHARTO^{2*},
INGRID S SURONO³, AND SRI ANNA MARLIYATI²

¹Department of Nutrition and Health, Universitas Airlangga, Jalan Mulyosari, Surabaya 60286, Indonesia;

²Department of Community Nutrition, Institut Pertanian Bogor, Kampus IPB Dramaga, Bogor 16680, Indonesia;

³Department of Food Technology, Universitas Bina Nusantara, Jalan Jalur Sutera Barat Kav. 21, Alam Sutera, Tangerang 15143, Indonesia

The aim of the research was to study the effect of provision of catfish' flour, oil and probiotic *Enterococcus faecium* IS-27526 on body weight and CRP (C-reactive protein) of aged female Cynomolgus monkey (*Macaca fascicularis*). Nine aged female Cynomolgus monkeys were randomly divided into three groups. The age is determined by dentition, with body weight in a range of 2 - 4 kg. Animals were placed in individual cages in the position where they can interact audiovisually. Feed composition consists of sugar, egg, soy flour, wheat flour, sweet potatoes flour, butter, egg yolk flour, catfish' flour and oil, and microencapsulated probiotic *E. faecium* IS-27526 and administered for 90 days effective. Evaluation of body weight and CRP were conducted. The study showed that there is no significant effect of experimental diets on body weight in each group. probiotic tends to suppress the body weight gain where the body weight of cynomolgus fed with probiotic diet has more stable than others. The body weight of cynomolgus in probiotic diet is shown more stable than others. There is no effect of experimental diets on CRP which is marked by negative result of CRP test. Probiotic *E. faecium* IS-27526 is potential for body weight homeostasis regulation to reduce the risk of overweight and obesity.

Key words : body weight, *Clarias gariepinus*, CRP, *Enterococcus faecium* IS-27526, *Macaca fascicularis*

Tujuan dari penelitian adalah untuk mempelajari pengaruh pakan fungsional berbasis tepung, minyak ikan lele dan probiotik *Enterococcus faecium* IS-27526 terhadap berat badan, dan CRP (C-reactive protein) pada monyet ekor panjang (*Macaca fascicularis*) betina usia tua. Sembilan ekor monyet ekor panjang dibagi dalam tiga kelompok secara acak. Usia ditentukan melalui pergigian (dentisi), dengan berat badan antara 2-4 kg. Hewan coba ditempatkan pada kandang individu dengan posisi masih dapat berinteraksi secara audiovisual satu dengan yang lain. Pakan yang diberikan terdiri atas gula, telur, tepung kedelai, tepung terigu, tepung ubi jalar, butter, tepung kuning telur, tepung ikan lele, minyak ikan lele, dan probiotik *E. faecium* IS-27526. Pengukuran berat badan (BB) dan CRP dilakukan setiap bulan. Tidak ada pengaruh signifikan pemberian pakan terhadap berat badan hewan coba pada setiap kelompok. Namun, probiotik memiliki kecenderungan menekan peningkatan berat badan. Tidak ada pengaruh pakan terhadap CRP ditandai dengan hasil negatif pada uji CRP. Probiotik *E. faecium* IS-27526 berpotensi dalam regulasi homeostasis berat badan untuk menurunkan risiko kelebihan berat badan dan obesitas.

Kata kunci: berat badan, *Clarias gariepinus*, CRP, *Macaca fascicularis*, probiotik *Enterococcus faecium* IS-27526

Since the industrial revolution in the middle of the 19th century, average female life expectancy has increased in Western societies from 45 years to 80 years, which corresponds to an increase of 2.3 years per decade (Oppen *et al.* 2002). The increase in average life span observed in all developed countries is accompanied by an incremental burden of age associated diseases.

Elderly are among vulnerable groups due to weakening immune system. The aging process is related to changes of BMI (Body Mass Index). Aging is also associated with chronic, low-grade inflammatory

activity. Systemic chronic inflammation has been found to be related to mortality risk from all causes. Age-related diseases such as Alzheimer, atherosclerosis, diabetes mellitus, sarcopenia, and osteoporosis are initiated or worsened by systemic inflammation, (Westendorp 2012).

Catfish' flour has been proven to be able to improve the nutritional status of undernourished five years old children (Kusharto *et al.* 2008; Adi 2010). Catfish flour is produced, while catfish oil as by product. Catfish' oil contains essential fatty acids, i.e., 22.65%, 17.79%, 1.21% of oleic acid (C18:1), linoleic acid (C18:2) (omega 6), and linolenic (C18:3) (omega 3) respectively (Srimati 2011). Various studies revealed that the essential fatty acid has anti-inflammatory and anti-

*Corresponding author; Phone: +62-11116178/+62-251-8624907, Email: kcl_51@yahoo.co.id

atherosclerosis effects (Demonty *et al.* 2006).

Enterococcus faecium IS-27526 was isolated from dadih fermented buffalo milk in West Sumatera. Based on *in vitro* and *in vivo* studies, *E. faecium* IS-27526 has been proven to have the ability of adherence to the intestinal mucosa (Collado *et al.* 2007). Novel probiotic *E. faecium* IS-27526 has significant positive effects on humoral immune response, salivary sIgA, in underweight preschool children, and on body weight gain of pre-school children (Suroño *et al.* 2011). Administration of microencapsulated *E. faecium* IS-27526 in the pasta cream has been proven to increase the body weight and fecal lactic acid bacteria of mice (Harianti 2009).

This study was conducted to the aged female atherogenic Cynomolgus monkey (*Macaca fascicularis*) to evaluate the effect of functional feed of catfish' flour, oil and probiotic *E. faecium* IS-27526 based on body weight and CRP (C-reactive protein)

MATERIALS AND METHODS

Strain and Probiotic Preparation. *E. faecium* IS-27526 was isolated from dadih fermented milk and was identified by 16S rRNA gene sequencing as *E. faecium* (GenBank accession no. EFO68251) (Suroño *et al.* 2011). The probiotic culture was cultivated in MRS Broth in a 10 L fermentor for 22 h at 37 °C under aerobic condition, harvested by centrifugation (3200 × g, 4 °C, 20 min), washed twice with phosphate-buffered saline (PBS, pH 7.0) and frozen. Purity and viability of probiotic was tested on MRS Agar plates before and during the study period.

Experimental Animals. Nine aged female Cynomolgus monkeys (*M. fascicularis*) or CM of 2-4 kg quarantined in the Center of Primate Study of the Institut Pertanian Bogor. Animals were adapted in the individual cages in the position where they can interact audiovisually each other. Diets were given for 120 kalori kg⁻¹ of body weight per day, while the drink was

supplied ad libitum. The ethical clearance had been obtained from Animal Care and Use Committee (ACUC) of PT Bimana Indomedical number P.01-13-IR.

Experimental Design. This preliminary study was conducted in Randomized Factorial (RAF) design. The trial required oral consumption of intervention diet over a total period of 90 d. The diet formula is based on previous research of Kusharto *et al.* (2012). The diets provision to animals were divided into three groups (n=3). The groups namely A1 standard diet (410 Cal 100 g⁻¹, fat 18.76%), A2 standard diet + probiotic *E. faecium* IS-27526 (10⁸ cfu g⁻¹), (425 Cal 100 g⁻¹ and fat of 19.84%), A3 standard of diet + probiotic *E. faecium* IS-27526 (10⁸ cfu g⁻¹) + fish oil (7 mL 100 g⁻¹) (energy 435 Cal 100 g⁻¹, fat 21.86%). Egg yolk flour was added as atherogenic diet (4% of diet).

The subjects were conditioned to abstain from other probiotic and prebiotic during intervention. Compliance of intake were monitored and assured by veterinarian. Body weight and CRP were collected at baseline and monthly during 3 months intervention.

Analysis of Samples. Body weight weighing-scale was conducted. The CRP test was agglutination test. The reagent/test kit was produced by PT. Rajawali Nusindo, Indonesia.

Analysis of Data. Comparison of body weight and lipid profile within and between group were tested by Analysis of Variance (ANOVA). Further test the difference between the group was analyzed using Duncan Multi Range Range test (DMRT) with 5% significance level.

RESULTS

Subject. Nine apparently healthy monkeys (*M. fascicularis*) were included in the research and no adverse effect was found. Percentage of feed consumption is shown in Table 1.

The percentage of animals diet consumptions more

Table 1 Percentage of feed consumption

Groups	Body weight average (kg)	Consumption average (gram)	Consumption perentation (%)
A1	3.41 ± 0.29	90.91 ± 0.31	90.91
A2	3.28 ± 1.22	85.75 ± 0.36	85.75
A3	3.30 ± 0.58	89.18 ± 0.44	89.18

Note: (A1) Standard diet, (A2) Standard diet+probiotics, (A3) Standard diet+probiotics + catfish oil

than 85%, not significant difference exist within the groups.

Body Weight. The variety of diets affect of animal body weight. Measurement of body weight was performed during the intervention. The percentage of body weight is shown in Fig 1. Based on ANOVA, the difference of body weight not significant ($p>0.05$) between groups. All diets tend to increase the animal body weight during intervention. Body weight in probiotic diet more stable than others.

CRP (C-Reactive Protein). CRP is an important component of the immune system, collection of protein that is made by body when a major infection or trauma. CRP can be an indicator of inflammation in the body. Many studies have found that CRP as marker of inflammatory process are elevated among individuals at high risk for future heart disease. Inflammation is important in all phases of heart disease, including the early initiation of atherosclerotic plaques within the arteries, as well as the acute rupturing of these plaques that results in heart attack and, all too often, sudden death (Ridker 2003).

Table 2 shows the negative results of CRP during intervention. This result indicates no inflammation as an indicator of atherosclerosis in all interventions

which are characterized with negative results.

DISCUSSION

Factors that influencing the animals' consumption are palatability, form and type of diet (Bennet *et al.* 1996). In adaptation period the animal consumes the Monkey chow which is common feed of monkeys. Monkey chow diet didn't significantly affect CM body weight and lipid profile. While, high fat diet with the addition of egg yolk flour is able to raise the palatability of feed and blood cholesterol of CM (Astuti *et al.* 2010; Pelletier 1996).

Measurement of body weight during intervention is required as the indicator of health of animals (Fortman *et al.* 2002). The growth of animal depends on the nutrition content of diet. Egg yolk flour is added to improve the palatability affecting the body weight (Astuti *et al.* 2010). Oktarina (2009) revealed that yolk flour (fat content $\pm 19.62\%$) tended to cause more obesity compared with monkey chow (fat 5.55%). It is due to higher fat compared with protein and carbohydrate.

Probiotic tends to suppress the body weight gain. Nugraha (2013) revealed that administration of probiotic *E. faecium* IS-27526 increased fecal lactic acid

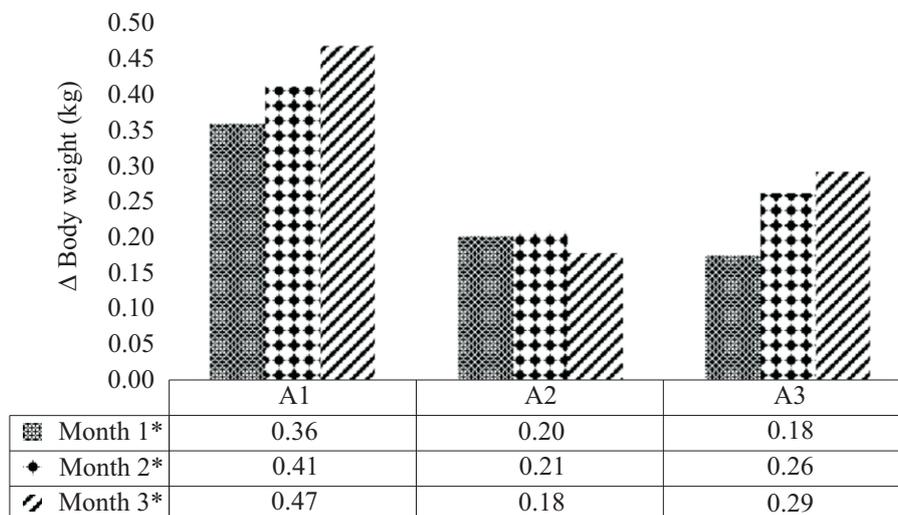


Fig 1 Alteration of *Macaca fascicularis* body weight

Table 2 Evaluation of CRP of *Macaca fascicularis* during intervention

Treatment	Month*				
	N	1	2	3	4
Standard (A1)	3	(-)	(-)	(-)	(-)
Standard + Probiotics (A2)	3	(-)	(-)	(-)	(-)
Standard + Probiotics + Fish oil(A3)	3	(-)	(-)	(-)	(-)

*Note: (1) Month 0 (baseline data), (2) Month 1, (3) Month 2, (4) Month 4

bacteria and decreased fecal coliform bacteria of macaca significantly ($p < 0,05$). Probiotic causes the balance of microflora in gastrointestinal tract, that suppressing the pathogenic bacterial growth. *Lactobacillus* and *Enterococcus* strain significantly reduced pathogen adhesion to mucus (Collado *et al.* 2007) The healthy intestinal tract optimizes the absorption and causes the beneficial to health (Harianti 2009).

Probiotic *E. faecium* IS-27526 is potential for body weight homeostasis regulation to reduce the risk of over weight and obesity supported by significantly lowering LDL and total cholesterol (Rifqi 2014).

Inflammation is measured by CRP in this research. The result shows the negative result (-) in all treatment groups. CRP is a powerful predictor of risk, particularly when combined with cholesterol evaluation. A persistently elevated CRP level is indicative of the risk of heart disease and of the accelerated atherosclerosis that affects individuals with diabetes and others metabolic syndrome,

The older adult who are over weight/obese are more likely than those who are not obese to the symptoms of metabolic syndrome. Overweight as a major underlying factor contributing to atherosclerotic cardiovascular disease, including abnormal lipid profile. Probiotic *E. faecium* IS-27526 is potential of body weight homeostasis regulation to reduce the risk of over weight and obesity.

ACKNOWLEDGMENT

This research was supported by The grant from Competitive Research Grant of Directorate General of Higher Education. Social Special thanks delivered to Director of PT. Carmelitha Lestari, Primate Research Centre, Department of Microbiology, Atmajaya University and Microbiology of SEAFast Centre, LPPM-IPB, PT Ultra Jaya Milk Co. for their support and advices.

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