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LIST OF CONTENT

Volume 14, Number 2, May 2012

Utilization of Cow Milk Enriched with Conjugated Linoleic Acid to Decrease Body Weight, Cholesterol, Low Density Lipoprotein and to Increase Blood High Density Lipoprotein. <i>FM Suhartati, W Suryapratama and S Rahayu</i>	70-76
Intake, Rumen Degradation and Utilisation of Urea-Ammoniated Grass Hay by Kacang Goats as Affected by Supplementation of Sun-dried Fish or Fishmeal <i>IGN Jelantik, MR Weibsjerg and J Madsen</i>	77-86
The Growth, Yield and Quality of Elephant Grass (<i>Pennisetum purpureum</i>) Spesific Toleran of Acid Soils by Mutagenesis with Ethylmethane Sulfonate <i>Munasik, CI Sutrisno, S. Anwar and CH Prayitno</i>	87-91
Morphometric Characterization and Genetic Distance among Four Breeds of Rabbit (<i>Oryctolagus cuniculus</i>) <i>A Setiaji, Sutopo and E Kurnianto</i>	92-98
Assessment of Endometritis in Arabian Mare <i>MA Hamouda, FA Al-Hizab, IM Ghoneim, AM Al-Dughaym and HJ Al-Hashim</i> ...	99-103
In Vitro Motility, Velocity and Capacitation Status of Merino Ram Spermatozoa <i>Ismaya, L Fitzpatrick and P Summers</i>	104-110
Different Haematological Condition, Immune System and Comfort of Muscovy Duck and Local Duck Reared in Dry and Wet Seasons <i>Ismoyowati, M Samsi and M Mufti</i>	111-117
Identification of Local Chicken Farmers' Behavior in Adopting Innovation of Technology Intensification <i>L Setiana, Isbandi, Atmomarsono and U Waridin</i>	118-122
Simulation Analysis Impact of Transaction Cost on Economic Behavior of Cattle-Coconut Farmers' Household in Bolaang Mongondow <i>FH Elly, BM Sinaga, SU Kuntjoro and N Kusradi</i>	123-130
Rabbit Production in Selected Urban Areas of Southern Ghana: Status and Implications for Policy and Research <i>DY Osei, SO Apori and ELK Osafo</i>	131-140

Utilization of Cow Milk Enriched with Conjugated Linoleic Acid to Decrease Body Weight, Cholesterol, Low Density Lipoprotein and to Increase Blood High Density Lipoprotein

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Abstract. An experiment to investigate the ability of cow milk enriched with conjugated linoleic acid to decrease body weight, total cholesterol, blood Low Density Lipoprotein (LDL), and to increase blood High Density Lipoprotein (HDL) has been conducted using in vivo experimental method. Research material consisted of 40 8-week-old white female rats (*Rattus norvegicus*) of Wistar strain (as an animal model). The method used was an experimental method with a Completely Randomized Design. The treatments tested were P1 = high-fat ration containing 27.66% fat (HF), P2 = HF + 5 ml of milk/head/day, P3 = HF + 10 ml of milk/head/day, P4 = low-fat ration containing 5% fat (LF). Each treatment was repeated five times to make 20 experiment units, each consisted of two rats. Body weight gain, total cholesterol, LDL-cholesterol and HDL-cholesterol were observed. The data obtained were then analyzed using analysis of variance followed by orthogonal contrast test. Orthogonal polynomials tests was applied to evaluate the response variables. The results showed that 10 ml/head/day of cow milk was needed to decrease body weight of hypercholesterolemic rats and 5 ml/head/day of cow milk was needed to decrease total cholesterol, LDL-cholesterol and to increase blood HDL-cholesterol of hypercholesterolemic rats.

Keywords: cow milk, conjugated linoleic acid, body weight gain, cholesterol.

Abstrak. Eksperimen dilakukan untuk mengetahui kemampuan susu sapi yang diperkaya dengan asam linoleat konjugasi untuk menurunkan bobot tubuh, kolesterol total LDL darah, dan untuk meningkatkan HDL darah dilakukan menggunakan metode eksperimental in vivo. Materi penelitian terdiri dari 40 tikus betina berumur 8 minggu (*Rattus norvegicus*) dari keturunan Wistar (sebagai hewan model). Metode yang digunakan adalah eksperimen dengan Rancangan Acak Lengkap. Perlakuan yang diuji adalah P1 = pakan tinggi lemak mengandung 27,66% lemak (HF), P2 = HF + 5 ml susu/ekor/hari, P3 = HF + 10 ml susu/ekor/hari, P4 = pakan rendah lemak mengandung 5% lemak (LF). Setiap perlakuan diulang lima kali sehingga ada 20 eksperimen unit yang masing-masing terdiri dari dua tikus. Peningkatan bobot tubuh, kolesterol total, kolesterol LDL dan kolesterol DHL dicermati. Data yang diperoleh dianalisa menggunakan analisis variansi dilanjutkan dengan uji kontras orthogonal. Uji orthogonal polinomial dilakukan untuk menilai respon peubah. Hasil penelitian menunjukkan bahwa 10 ml susu sapi/ekor/hari dibutuhkan untuk mengurangi bobot tubuh tikus hypercholesterolemic dan 5 ml susu sapi/ekor/hari dibutuhkan untuk menurunkan kolesterol total, kolesterol LDL dan untuk meningkatkan kolesterol HDL darah pada tikus hypercholesterolemic

Kata kunci: susu sapi, asam linoleat konjugasi, kenaikan bobot tubuh, kolesterol

Introduction

Fresh milk is one of the best functional foods because it has naturally full of nutrients. Milk is said to be a functional food because in addition to containing protein, fat, lactose, nonfat dry matter and water also contains conjugated

linoleic fatty acids crucial to human health (Suhartati and Subagyo, 2010), such as depressing cholesterol and increasing High Density Lipoprotein (HDL) blood plasma (Choi et al., 2006), lowering adipose fat (Gillis et al., 2004), working as anti-obesity with hypolipidemic activity (Yeung et al., 2000).

Obesity is one of the major risk factors for cardio-vascular diseases which is one of several degenerative diseases that now occupies the number one cause of death in Indonesia. Various studies have shown association between obesity and coronary heart disease (Anwar, 2004). Incidence of obesity, high total cholesterol and LDL and low HDL can be overcome by taking conjugated linoleic fatty acids. The main source of conjugated linoleic fatty acids are from ruminants products (Desroches et al., 2005), among others, milk (Meng et al., 2008).

Suhartati and Subagyo (2010) conducted an engineering-dairy cows feed experiment and were able to increase the conjugated linoleic fatty acid content as much as 130%. To prove the ability of these fatty acids in losing body weight, total cholesterol, LDL-cholesterol and increasing HDL-cholesterol, study on white mice as the experimental animals is necessary

Materials and Methods

Four female dairy cows were used to produce milk. Cow's ration consisted of grass and concentrate with a ratio of 60:40. The concentrate consisted of 30% rice bran and 20% tapioca residue fermented using *Saccharomyces cerevisiae*, 20% palm kernel cake, 20% ground corn, 9% pollard and 1% minerals, added with 3% soybean oil (based on concentrate dry matter). The milk produced was tested on 40 female white rats (*Rattus norvegicus*) of Wistar strain aged 8 weeks.

In vivo experimental method with Completely Randomized Design were applied. The treatments were P1 = high-fat ration containing 27.66% fat (HF), P2 = HF + 5 ml of milk/head/day, P3 = HF + 10 ml of milk/head/day, P4 = feed containing 5% fat (low fat; LF). Each treatment was repeated 5 times to make 20 experimental units, each consisted of two white rats, involving 40 rats as a whole.

Nutrient content of high fat and low fat feed are presented in Table 1.

The rats were put into individual wire cages; 10 rats were used as negative control (non-hypercholesterolemia), 30 rats were made hypercholesterolemia by giving high fat feed (the feed consists of BR 2 feed, egg yolks, lard and bovine brain (Table 2). Egg yolk and lard were given through the syringe and then let the rats lick it off. The blood was sampled every two weeks to measure the cholesterol. Hypercholesterolemia has been achieved after a month of experiment. The rats were fed on appropriate ration every morning for two months. The milk was given through nipple and waited until all suckled. To assess the body weight gain, the rats were weighed every week and repeated until the end of the study. At the end of the study, the blood was collected via orbital sinus using the method proposed by Gurr (2006), centrifuged to get the serum and then analyzed for the levels of total cholesterol, LDL-cholesterol and HDL-cholesterol, using CHOD-PAP method, reagent Bavaria/DIASYS, Photometer HD 5010. Body weight gain was obtained by subtracting the initial body weight from the final body weight.

The experiment was conducted at three locations: (1) cow milk was produced at Margomulyo Dairy Farmers group of Kemutug Lor Village, Baturaden District, Purwokerto; (2) In vivo experiments using white rats were conducted at Merdeka Street 38 Purwokerto; (3) total cholesterol, LDL and HDL were analyzed at the Faculty of Medicine and Health Sciences, University of Jenderal Soedirman, Purwokerto.

The data were analyzed using analysis of variance followed by orthogonal contrast test. To evaluate the response variable, orthogonal polynomials tests were applied (Steel and Torrie, 1993).

Table 1. Nutrient content of food used in the study

Nutrient Content	High fat feed ^{a)}	Low fat feed ^{b)}
Dry Matter (%)	86.77	87.00
Protein (% BK ^c)	20.11	20.00
Fat (% BK)	27.66	5.00
Fibre (% BK)	8.59	5.00
Ash (% BK)	4.19	4.00

^{a)} Analyzed at the Food Science Laboratory of Animal Husbandry Faculty (2011)

^{b)} According to the label provided of BR2 chicken feed (a product of Charoen Pokphand^R)

^{c)} DM = Dry Matter

Results and Discussion

The hypercholesterolemic rats, both of which were given or not given additional milk, resulted in non-significant body weight gain ($P>0.05$) compared to the negative control rats (non-hypercholesterolemic). It was proven that the cow milk was able to maintain the body weight of hypercholesterolemic rats steady. The provision of cow milk was able to reduce 31.44% body weight gain of the hypercholesterolemic rats and the addition of 10 ml of milk/head/day was able to reduce body weight gain of 62.31% compared to the addition of 5 ml of milk/head/day ($P<0.01$). These results indicated that the addition of cow milk was able to inhibit the rate of body weight gain, resulting in decreased body weight gain. The addition of 10 ml of milk/head/day on the rats not only inhibited body weight gain, but also decreased 40% of the rats in their body weight. The addition of cow milk resulted in a negative linear response ($P<0.01$) on body weight gain of rats following the equation $Y = 12.23 - 0.77X$, with the coefficient of determination (R^2) of 0.24 (Figure 1). Although only 24% of body weight gain was due to the addition of milk, this effect was highly significant ($P<0.01$).

Bauman et al. (1999) suggested that CLA body fat content could be increased by CLA supplementation. Feeding of CLA in non-ruminants resulted in an increase of CLA levels in muscle and fat tissues (Boles et al., 2005).

The results of this study using white rats had shown that CLA intake lowered body fat and increased lean body mass. Studies using various animal models have shown that administration of CLA isomers cis 9 - trans 11 and trans 10 - cis-12, resulted in a very dramatic condition for which rats are sensitive animals to CLA in the fat loss (Whale et al., 2004; Bhattacharya et al., 2006; Wang and Jones., 2004 and Reiner et al., 2004).

According to Bauman et al. (1999), isomers cis-9, trans-11 is the major isomer, comprising 80-90% of total milk fat CLA (Gurr, 2009). Thus, providing cow milk in rats ration increases the supply of isomer cis-9, trans-11CLA which plays role in reducing body fat. Because body fat has decreased, then the body weight gain decreased.

The highest decrease in blood cholesterol (88.4 ± 48.75 mg/dl) was achieved by rats fed on hypercholesterol ration added with cow milk of 5 ml/head/day. Blood cholesterol rats fed on hypercholesterol ration without cow milk decreased by 1.8 ± 18.54 mg/dl and hypercholesterol ration added with 10 ml milk/head/day cow milk decreased by 17.6 ± 14.83 mg/dl, while rats fed on low-fat ration increased blood cholesterol by 4.6 ± 20.85 mg/dl.

Adding cow milk had significant effect on the blood cholesterol reduction ($P<0.01$). The reduction in blood cholesterol of the hypercholesterol rats, with or without cow milk addition, was higher than those fed on negative

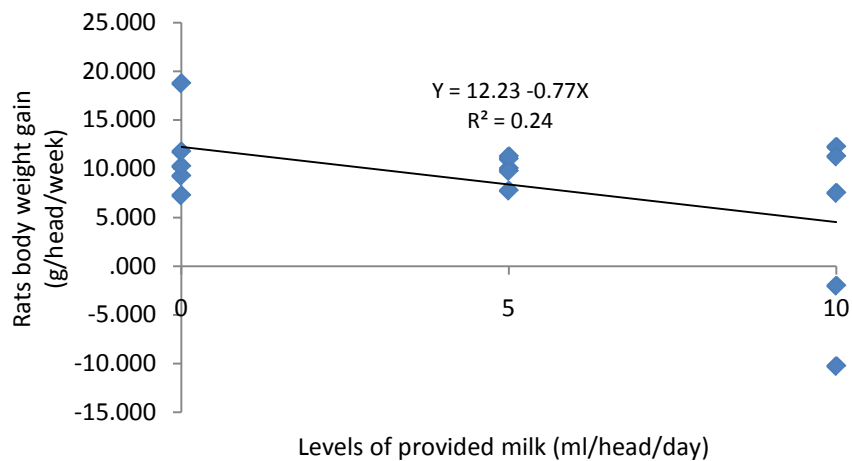


Figure 1. Effect of cow milk on the reduction of rats body weight

control (low fat) ($P < 0.01$); the blood cholesterol of rats fed on negative control diet even increased. These results are consistent with the results of research done in East Africa by Gurr (2009), that the consumption of milk in significant amounts is able to maintain plasma cholesterol in a very low level. In addition, Gurr (2009) also suggested that cholesterol synthesis in liver was under feedback control of intake cholesterol in the diet. Too much cholesterol in the diet caused cholesterol biosynthesis in the liver to stop. Conversely, low-cholesterol in the diet caused the activity of enzymes involved in cholesterol biosynthesis increased to maintain the supply of cholesterol in the membrane structure, synthesis of bile acids and steroid hormones. At a high cholesterol intake, the process of its absorption will decline to be in critical limit. Cholesterol is relatively less absorbed; only about half amount in the diet is absorbed into the blood. However, there is a significant difference in capacity of individuals to absorb and metabolize cholesterol. Capacity of some individuals to the regulation of cholesterol metabolism is not perfect, which leads to over production.

The addition of cow milk of 5 ml/head/day was able to lower cholesterol five times

compared to that given 10 ml/head/day of cow milk. Cow milk addition gave quadratic response to the decline of blood cholesterol, following the equation $Y = -18 - 33.06X + 3.148X^2$ with coefficient of determination (R^2) = 0.64 and point P (5.25; -8.5) (Figure 2), showing that the highest cholesterol reduction was 8.5 mg/dl achieved in the provision of 5.25 ml of ml/head/day.

The reduction of blood LDL-cholesterol was only observed on rats that received 5 ml/head/day of cow milk, reduced by 44.4 ± 27.17 mg/dl, whereas hypercholesterolemic rats that were not given milk showed LDL increase by 26.2 ± 38.83 mg/dl and those given 10 ml of milk/head/day showed LDL increase by $30.6 \pm 13.24\%$. The LDL-cholesterol increased 33.8 ± 15.77 mg/dl on rats consuming low-fat diet (negative control).

There was effect of giving cow milk to the content of LDL-cholesterol ($P < 0.01$). The increase in LDL-cholesterol in the blood of rats that received low-fat feed (negative control) was markedly higher than the increase in LDL-cholesterol of hypercholesterolemic rats ($P < 0.01$). In addition, the provision of cow milk is able to lower blood LDL-cholesterol of rats and the addition of 10 ml/head/day of cow milk

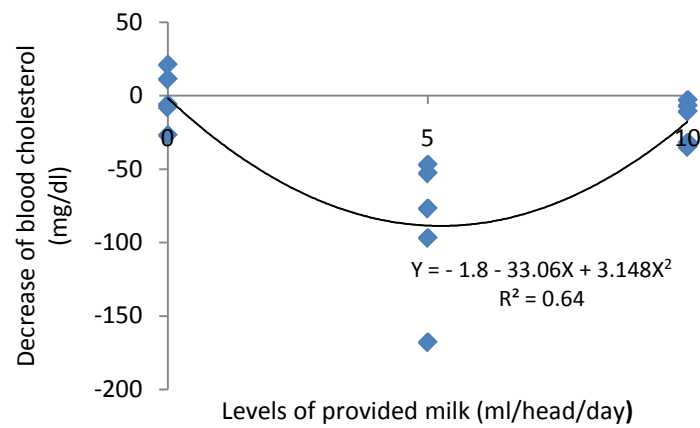


Figure 2. Effect of cow milk addition on the decrease of blood cholesterol in rats

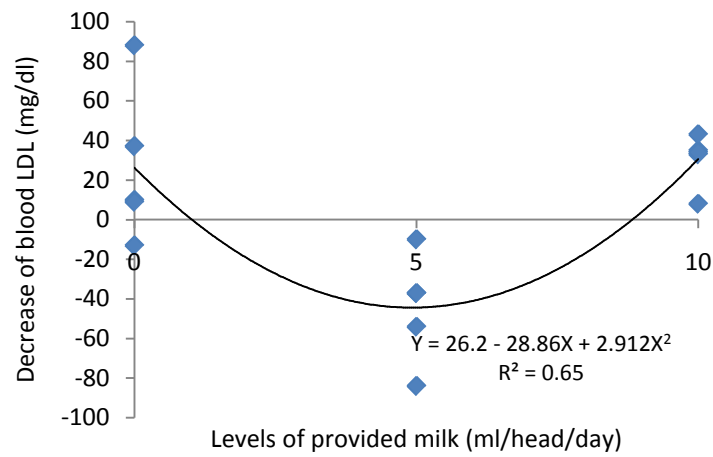


Figure 3. Effect of cow milk on the decreasing of low density lipoprotein in blood rats

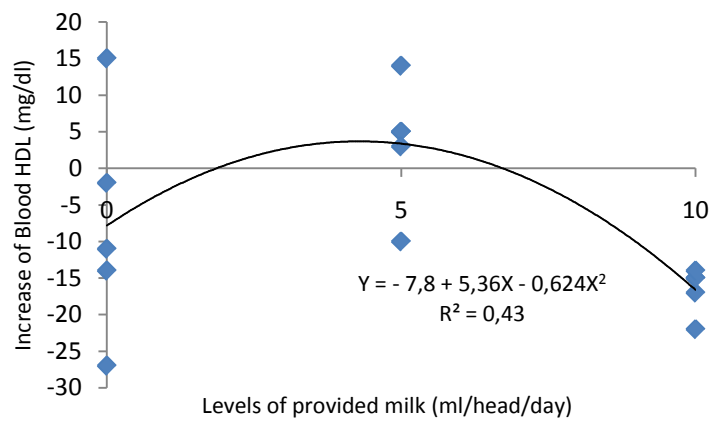


Figure 4. Effect of cow milk on increase of blood high density lipoprotein in rats

conversely improve blood LDL-cholesterol ($P < 0.01$).

The provision of cow milk has quadratic effect ($P < 0.01$) on LDL-cholesterol reduction following the equation $Y = 26.2 - 28.86X + 2.91X^2$, coefficient of determination (R^2) = 0.65, with the point P (5; -45.35) (Figure 3). This point illustrates that the highest decrease in LDL, which declined 45.35 mg/dl occurred in the provision of 5 ml/head/day of cow milk. In both equations, cholesterol and LDL-cholesterol described that a decrease in LDL-cholesterol was in line with cholesterol reduction. Gurr (2006) stated that total cholesterol could be legitimately used as a substitute for LDL cholesterol. Based on this statement, it is expected that both curves are alike and the highest decrease in both total cholesterol and LDL-cholesterol were observed in the provision of cow milk of about 5 mg/head/day.

High Density Lipoprotein (HDL-cholesterol) of hypercholesterolemic rats fed on high-fat without milk decreased by 7.8 ± 15.58 mg/dl; those added with 5 ml/head/day of cow milk increased 3.4 ± 8.62 mg/dl; those added with 10 ml milk/head/day of cow milk decreased 16.6 ± 3.21 mg/dl, whereas HDL-cholesterol of rats fed on low fat (negative control) decreased by 15.4 ± 2.30 mg/dl.

Provision of cow milk had highly significant effect ($P < 0.01$) on levels of HDL-cholesterol. Blood HDL-cholesterol of rats fed on low fat (declining 15.4 ± 2.30 mg/dl) revealed the ability to reduce twice of the blood HDL-cholesterol of hyperlipidemic rats fed on high fat ration (declining 7 ± 12.86 mg/dl). HDL-cholesterol of hyperlipidemic rats fed on high-fat milk plus 5 ml of milk (increasing 3.4 ± 8.62) was significantly higher ($P < 0.01$) than rats fed on milk 10 ml/head/day (declining 16.6 ± 3.21 mg/dl). Cow milk provided quadratic response to the decreased levels of HDL-cholesterol by the equation $Y = -7.8 + 5.36 X - 0.624 X^2$,

coefficient of determination (R^2) = 0.43, the highest point of P (4.3; 3.71) (Figure 4).

As described earlier that whenever cholesterol in diet was low, the activity of enzymes involved in cholesterol biosynthesis increased to maintain the supply of cholesterol in the membrane structure, synthesis of bile acids and steroid hormones. At a high cholesterol intake, the absorption would decline, which tended to be on limit absorption, thus the cholesterol content on hyperlipidation slightly decreased. Mathematically, because HDL-cholesterol was the difference between total cholesterol and LDL-cholesterol, the response given was opposite or inversely proportional to the LDL-cholesterol response.

Conclusions

To reduce body weight of rats that have undergone hypercholesterolemia, 10 ml of milk/head/day was required and 5 ml of cow milk/head/day was required to reduce total cholesterol, LDL-cholesterol and to increase HDL-cholesterol in the blood of rats that have undergone hypercholesterolemia.

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