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Milk Chemical Composition of Dairy Cows Fed Rations Containing Protected Omega-3 Fatty Acids and Fermented Rice Bran

Sudibya* & S. H. Purnomo
Department of Animal Science, Faculty of Agriculture, Sebelas Maret University
Jln. Ir. Sutami 36 A, Surakarta 57126, Indonesia
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ABSTRACT

The research was conducted to investigate the effect of ration containing protected omega-3 and fermented rice bran on chemical composition of dairy milk. The research employed 10 female PFH dairy cows of 2-4 years old with body weight 300-375 kg. The research was assigned in randomized complete block design. The treatment consisted of P0= control ration, P1= P0 + 20% fermented rice bran, P2= P1 + 4% soya bean oil, P3= P1 + 4% protected tuna fish oil and P4= P1 + 4% protected lemuru fish oil. The results showed that the effects of fish oil supplementation in the rations significantly (P<0.01) decreased feed consumption, cholesterol, low density lipoprotein, lipids, and saturated fatty acids. Meanwhile, it increased milk production, content of high density lipoprotein, omega-3, omega-6 and unsaturated fatty acids in the dairy cows milk. It is concluded that the inclusion of 4% protected fish oil in the rations can produce healthy milk by decreasing milk cholesterol and increasing omega-3 fatty acids content.

Key words: dairy milk, fermented rice bran, fish oil protected, omega-3 fatty acid

ABSTRAK

Penelitian ini bertujuan untuk menguji pengaruh ransum yang mengandung omega-3 terproteksi dan dedak padi fermentasi terhadap komposisi kimia susu sapi. Penelitian menggunakan 10 ekor sapi perah betina berumur 2-4 tahun dengan bobot badan 300-375 kg. Penelitian menggunakan rancangan acak kelompok. Perlakuan terdiri atas P0= ransum kontrol, P1= P0 + 20% dedak padi fermentasi, P2= P1 + 4% minyak kacang kedelai, P3= P1 + 4% minyak ikan tuna terproteksi, dan P4= P1 + 4% minyak ikan lemuru terproteksi. Hasil penelitian menunjukkan bahwa efek suplementasi minyak ikan dalam ransum secara nyata (P<0,01) menurunkan konsumsi pakan, kolesterol, LDL (low density lipoprotein), lipid, dan asam lemak jenuh. Namun demikian, meningkatkan produksi susu, kandungan HDL (high density lipoprotein), omega-3, omega-6, dan asam lemak tidak jenuh dalam susu sapi. Dapat disimpulkan bahwa penambahan minyak ikan terproteksi sebanyak 4% dalam ransum mampu menghasilkan susu yang sehat dengan menurunkan kandungan kolesterol susu dan meningkatkan kandungan asam lemak omega-3.

Kata kunci: susu sapi, dedak padi fermentasi, minyak ikan terproteksi, asam lemak omega-3

INTRODUCTION

Dairy milk is a source of animal protein which is needed by the community at all age levels. Therefore, we need a dairy product that can meet the nutritional value of all age levels. Dairy milk product which is rich in omega-3 fatty acids and low in cholesterol is a breakthrough to produce healthy animal product. The content of omega-3 is very beneficial for the children especially in infancy, while for adults are very useful for those who have heart problem high blood pressure and cholesterol. Recent research from O'Rourke (2013) found that supplementation of omega-3 was able to active the process of autophagy, which is a kind of recycling mechanism that is able to increase the body's cell. Furthermore, it can increase cell resistance in order to slow cell aging. Therefore, we need to study dairy cows which produce milk containing high omega-3 and low cholesterol. These products can be made by manipulating the soap supplementation protected with extracts containing omega-3 fatty acids which mixed with fermented rice bran in rations. Research on milk products with high omega-3 fatty acids has not been much reported. Sudibya *et al*. (2013) reported that supplementation of tuna and lemuru fish oil in the rations can increased omega-3 fatty acids and decreased cholesterol of goats'

*Corresponding author:
E-mail: sudibya_uns@yahoo.com

milk. Therefore, the researchers assume that supplementation of fish oil on the ration of dairy cow will get the same impact.

Omega-3 fatty acids often found in marine fish mainly lemuru, tuna and shark. Lemuru fish when it is extracted will generate a lot of fish oil containing omega-3 fatty acids especially EPA (eicosapentaenoic acid) 34.17% and DHA (docosahexaenoic acid) around 17.40% and fat content 6% and TDN 182 kcal/kg fish oil, while tuna fish will generate fish oil containing omega-3 fatty acids especially EPA (eicosapentaenoic acid) 33.6% to 44.85% and DHA (docosahexaenoic acid) around 14.64% and 5.8% fat and 178 kcal TDN/kg (Sudibya *et al*., 2008, 2011). Because of content differences between two fish, therefore it needs to be examined.

A manipulation of fat metabolism in the rumen is intended to control antimicrobial effects of fatty acids to minimize disturbance of rumen fermentation. Therefore the highest fat level can be included in the diet. Secondly, it's to control biohidrogenation to increase the absorption of desired fatty acids to improve the nutritional quality of livestock products (Chillard, 1993). Fish oil supplementation in the diet should be in a particular done so as not to disrupt the activity of rumen microorganisms. Jenkins (1993) stated that the addition of fish oils in ruminant feed should not exceed 6%-7% of ration dry matter as it will affect rumen fermentation. Sudibya *et al*. (2010, 2011) stated that functions of omega-3 fatty acids in decreasing cholesterol levels in two ways namely 1) stimulates the excretion of cholesterol through the bile from the liver into the intestine and 2) stimulate the catabolism of HDL cholesterol by the liver back into bile acids and not regenerated again. It's excreted through feces. Dairy cows milk usually consumed by humans in a state of cooked, so, we need organoleptic (taste, odor and color). Furthermore, we need to examine the content of omega-3 fatty acids as well as fat oxidation products with peroxide levels and the levels of malonaldehyde.

In light of such issues, the study aims to produce feed formula of dairy cows which containing protected fish oil and fermented rice bran. The second purpose was to examine the milk chemical composition particularly omega-3 fatty acids and cholesterol.

MATERIALS AND METHODS

This research was experimental design conducted in Glagah village, Jatinom District, Klaten Regency. The laboratory analysis conducted in Veterinary Medicine Laboratory of Bogor Agricultural University, Chemistry Laboratory of Gadjah Mada University and Laboratory of Agriculture Technology and the Laboratory of Feed Nutrition, Department of Animal Husbandry, Sebelas Maret University, Surakarta.

Rice Bran Fermentation

Fermentation of rice bran was done by yeast with duration of 0, 1.5, 3, and 4.5 d. Furthermore, the fermented rice bran was analyzed proximate in order to reveal the content of crude protein and crude fiber.

Formation of Fatty Acid Soap

Protected fat products were made through a combination process of saponification and encapsulation of lemuru fish oil and tuna oil using 10% NaOH, 10% starch, and saturated CaCl2 solution. Saponification and encapsulation were done by heating lemuru and tuna fish oil at a temperature of 60-80 oC for 10 min and then mixed with 10% NaOH solution with stirring and 10% starch solution was added to form a paste of clay. Clumps were settled for one night in order to make it hard. Clumps of crystallized soap obtained by soaking with saturated CaCl2 solution for 2 h. Fatty acid soap crystals formed was filtered and pressed then dried in the oven or in the sun. Fatty acid soap crystals that have been dried were used in the digestibility trials and the resistance from biohydrogenation process by rumen microbes.

Experimental Design and Data Analysis

The study was conducted as a randomized completely block design with 5 dietary treatments namely P0= Control ration, P1= P0 + 20% fermented rice bran replacing rice bran in the ration, P2= P1 + 4% soya bean oil, P3= P1 + 4% protected tuna fish oil, P4= P1 + 4% protected lemuru fish oil and using 2 blocks of body weight as replications. The data were analyzed with analyses of variance (ANOVA) and were continued with orthogonal contrast test (Steel & Torrie, 1980).

Farming Management of Dairy Cows

Farming of dairy cows carried out for 90 d. Feeding was given twice a day in the morning and evening. Milking was done also twice a day in the morning and afternoon. The amount of feed given was 20 kg of fresh forage and 9 kg concentrate per day per cow. Milk samples were first taken on day 21th after feeding treatment, cooked at temperature of 60 ^{o}C and were analyzed in the laboratory. Analysis in the laboratory was taken three times on day 45th, 60th, and 90th. The composition of the ration can be seen in Table 1, 2, and 3.

Parameter Analyses

Variables measured were: cholesterol content of cooked dairy milk with the method of Kleiner & Dotti (1962), lipid content of cooked dairy milk with the method of AOAC (2001), LDL (Low Density Lipoprotein) and HDL (High Density Lipoprotein) of cooked dairy milk with the method of Assman (1982), contents of linolenic fatty acid (omega-3) and linoleic fatty acid (omega-6) of dairy milk with the method of AOAC (1990).

RESULTS AND DISCUSION

Consumption of Feed

The lowest consumption of feed was in P4 9.37 kg (DM/head/day), whereas the highest one was in P0 11.1 kg/head/day. The complete data can be seen in Table 4.

Table 1. Ingredient of feed formula (%)

Name	Water	Ash	Crude protein	Crude lipid	Crude fiber	TDN	Ca	P
King grass	84.10	8.80	8.72	3.68	33.60	52.10	0.16	0.19
Rice bran	12.72	7.70	11.99	10.70	11.61	67.90	0.04	0.34
Fermented rice bran	12.97	2.59	14.20	5.58	8.10	69.40	0.24	0.32
Cassava dregs	12.97	2.59	2.20	5.58	24.10	54.40	0.24	0.32
Coconut meal	11.97	7.94	23.03	15.30	13.50	78.70	0.02	0.36
Soya bean meal	12.20	6.84	44.60	6.70	9.80	56.40	0.03	0.24
Mineral premix	-	-	-	-	-	-	50.00	25.00
Tuna fish oil*	-	-	-	5.80	-	178.00	-	-
Lemuru fish oil*	-	-	-	6.00	-	182.00	-	-

Source: *) Laboratory analysis on LAKFIP UGM (2010).

Table 2. Nutrient content of feed formula

Nutrient content	P0	P1	P2	P3	P4
Crude protein (%)	13.80	14.00	14.20	14.26	14.35
TDN (kcal/kg)	64.00	64.00	65.00	67.00	67.00
Crude lipids (%)	4.82	5.02	5.02	5.02	5.02
Crude fiber (%)	18.90	16.90	16.90	16.90	16.9
Calcium (%)	2.58	2.58	2.59	2.58	2.58
Phospor (%)	0.78	0.78	0.78	0.78	0.78
Omega-3 (%)	0.00	0.00	0.12	3.13	3.15

Note: P0= Control ration, P1= P0 + 20% fermented rice bran replacing rice bran in the ration, P2= P1 + 4% soya bean oil, P3= P1 + 4% protected tuna fish oil, P4= P1 + 4% protected lemuru fish oil

Table 3. Composition of ration

	P0	P1	P2	P3	P4
King grass	50	50	50	50	50
Soya bean meal	4	4	4	4	4
Rice bran	30	10	10	10	10
Fermented rice bran	0	20	20	20	20
Yellow corn	12	12	12	12	12
Coconut meal	4	4	4	4	4
Soya bean oil	0	0	4	0	0
Tuna fish oil	0	0	0	4	0
Lemuru fish oil	0	0	0	0	4
Total	100	100	104	104	104

Note: P0= Control ration, P1= P0 + 20% fermented rice bran replacing rice bran in the ration, P2= P1 + 4% soya bean oil, P3= P1 + 4% protected tuna fish oil, P4= P1 + 4% protected lemuru fish oil

Table 4. Average feed consumption and production of dairy milk

Treatment	Parameter	
	Production of milk dairy cow (L/head/day)	Feed cunsumption (kg DM/head/day)
P0	8.147±0.106[a]	11.190±0.121[a]
P1	8.520±0.330[a]	11.090±0.163[a]
P2	8.800±0.199[a]	10.800±0.199[a]
P3	9.360±0.178[b]	9.500±0.100[b]
P4	9.500±0.100[b]	9.370±0.178[b]

Note: P0= Control ration, P1= P0 + 20% fermented rice bran replacing rice bran in the ration, P2= P1 + 4% soya bean oil, P3= P1 + 4% protected tuna fish oil, P4= P1 + 4% protected lemuru fish oil

The treatments had significant (P<0.01) difference on feed consumption. P0 (control), P1, and P2 treatments had lower feed consumption than those of P3 and P4 treatments, but among P0, P1, and P2 were not significantly different. This is due to the equal nutrition contained in the ration.

The addition of tuna fish oil (P3) and lemuru fish oil (P4) decreased feed consumption since fish oil serves as the source of energy in addition to the source of unsaturated fatty acids leads to the raise of TDN content in the feed. The increase of TDN would consequently improve rumen microbial growth and activity producing higher VFA as energy source for the host. High VFA production caused less feed was required to fulfill the animal requirement.

The effect of unsaturated fatty acid addition in the feed was decreasing production of methane (CH4) and in the ratio of acetate to propionate formed in the rumen (Rebollar & Blas, 2005). Oil or fat in the feed will immediately undergo lipolysis and biohydrogenation. The hydrogen used in the biohydreganation process of unsaturated fatty acids was the side product of synthesis of acetic and butyric acids (Tiven *et al*., 2011). The formation of methane gas from hydrogen and CO2 can be minimized as a result of hydrogen shifting to saturate the fat. Organic matter is the major component of dry matter. Ash contains mineral which does not have energy. So, the digestibility of organic matter is closely related to the energy content available in the feed (Orskov, 1987).

Production of Milk

The milk production given different treatments is presented in Table 4. Fish oil supplementation had a significant (P<0.01) effect on the milk production. The productions of milk in P1 and P2 did not increase in comparison to the production in P0 due to the insignificant different in feed consumption. The addition of tuna fish oil (P3) and lemuru fish oil (P2) could increase the production of milk since fish oil serves not only as the source of unsaturated fatty acid but also the source of energy. Therefore, fish oil addition could increase feed consumption which eventually increased production of milk. This result was inline with the study of Fatahnia *et al*. (2008) that supplementation of fish oil in the diet could produce more milk than soybean oil.

Lipid Content of Milk

The lipid content of P0 was significantly (P<0.01) different with P3 and P4, but was not significantly different with P1 and P2 (Table 5). Treatment of P1 and P2 was significantly (P<0.01) different with P3 and P4. Abughazaleh *et al*. (2002) and Fatahnia *et al*. (2008) reported that addition of fish oil and soybean oil or its combination on the diets of dairy cows could increase milk fat composition. That was inline with this research that the addition of protected lemuru and tuna fish oil (P3 and P4) could increase lipids content of dairy cow milk. That was because fish oil is energy source and source of unsaturated fatty acids. It can increase lipids content of dairy cow milk. Suarez *et al*. (1996) reported that supplementation of polyunsaturated fatty acids in the ration can increase lipids content in the body tissue. Furthermore, Vahmani *et al*. (2013) found that supplementation of dairy cows with marine oils stimulates increasing fatty acids content of milk.

Low Density Lipoprotein Content of Milk

Low density lipoprotein content of P0, P1, and P2 was significantly (P<0.01) different with P3 and P4. However, it was not significantly different among P0, P1, and P2, also between P3 and P4 (Table 5). Sudibya *et al*. (2010) reported that supplementation of soya bean oil could not decrease LDL content of dairy milk. Meanwhile, in this research the addition of protected lemuru and tuna fish oil (P3 and P4) could decrease LDL content of dairy milk. That was because fish oil stimulates the excretion of cholesterol through the bile from the liver into the intestine. It was inline with Komari (1994) and Sinclair (1996) that supplementation of polyunsaturated fatty acids in the ration could decrease LDL content in the body tissue. In addition, Gebauer *et al*. (2007) and Shingfield *et al*. (2008) reported that feeding fish oil and marine oil decreased low density lipoprotein.

High Density Lipoprotein Content of Milk

High density lipoprotein content of P0 was significantly (P<0.01) different with P3 and P4, but was not significantly different with P1 and P2 (Table 5). Gebauer *et al*. (2007) and Shingfield *et al*. (2008) reported that feeding fish oil and marine oil increased high density lipoprotein. That finding was inline with this research that the addition of protected lemuru and tuna fish oil (P3 and P4) could increase HDL content of dairy milk. That was because fish oil stimulates the catabolism of HDL cholesterol by the liver back into bile acids and not regenerated again. It is excreted through feces. It can increase HDL content of dairy milk. Komari (1994) and Sinclair *et al*. (1996) reported that supplementation of polyunsaturated fatty acids in the ration could increase HDL content in the body tissue.

Cholesterol Content of Milk

Cholesterol content of P0 was significantly (P<0.01) different with P3 and P4, but was not significantly different with P1 and P2 (Table 5). Supplementation of soya bean oil (P2) did not decrease the content of cholesterol in dairy cow milk. That is in line with the result of research from Sudibya *et al*. (2010) which states that soya bean oil supplementation can not be used to decrease cholesterol levels. The addition of tuna and lemuru fish oil can decrease cholesterol content of dairy cow milk in P3 and P4. It was concurred with Sudibya *et al*. (2010, 2013) that cholesterol levels in dairy cows milk decrease due to the transfer of omega-3 fatty acids. This can be explained that the omega-3 fatty acids work by stimulating the catabolism of HDL cholesterol to the liver back into bile acids and not regenerated again. It was excreted through feces. So that the cholesterol content in excreta

Table 5. Average lipids, low density lipoprotein (LDL), high density lipoprotein (HDL), and cholesterol content of dairy milk

Treatment	Parameter			
	Lipids (%)	LDL (mg/dl)	HDL (mg/dl)	Cholesterol (%)
P0	3.340±0.140[a]	37.380±0.967[a]	62.620±0.967[a]	0.186±0.019[a]
P1	3.366±0.169[a]	36.280±1.010[a]	63.720±1.010[a]	0.170±0.020[a]
P2	3.823±0.263[a]	36.270±0.950[a]	63.730±0.951[a]	0.163±0.021[a]
P3	4.016±0.142[b]	25.190±0.932[b]	74.810±0.932[b]	0.132±0.009[b]
P4	4.023±0.264[b]	25.150±0.853[b]	74.850±0.853[b]	0.124±0.007[b]

Note: Means in the same column with different superscript differ significantly (P<0.01). P0= Control ration, P1= P0 + 20% fermented rice bran replacing rice bran in the ration, P2= P1 + 4% soya bean oil, P3= P1 + 4% protected tuna fish oil, P4= P1 + 4% protected lemuru fish oil.

increased due to decreasing cholesterol levels in cow milk. Furthermore, insignificantly different between P3 and P4 was probably caused by the content of unsaturated fatty acids of protected lemuru and tuna fish oil is relatively the same so that the effects is similar.

Omega-3 Fatty Acids (Linolenic) Content of Milk

The omega-3 content of the milk was presented in Table 6. Omega-3 content of P0 significantly (P<0.01) different with P3 and P4, but was not significantly different with P1 and P2. However, it was not significantly different between P3 and P4. Baumgard *et al*. (2000) reported that concentration of total omega-3 fatty acids increased in the milk of animals fed diet containing fish oil compared with the other oil supplementation. It means that protected unsaturated fatty acids did not undergo biohydrogenation process by rumen microbes. It was digested and absorbed in the intestine so that omega-3 fatty acids were deposited in milk. Goodridge *et al*. (2001) reported that supplementing dairy cow diets with a formaldehyde-treated flax product could increase a milk fat high in omega-3-linolenic acid. In addition, Fatahnia *et al*. (2008) found that the concentration of omega-3 fatty acids increased in milk of animals given diet containing fish oil compared with soybean oil or combination of fish oil and soybean oil.

Omega-6 Fatty Acids (Linoleic) Content of Dairy Milk

Omega-6 content of P0 was significantly (P<0.01) different with P3 and P4, but omega-3 content of P1 and P2 was not significantly different (Table 6). Sudibya *et al*. (2010) reported that supplementation of soya bean oil could not increase omega-6 content of dairy milk. Meanwhile, in this research the addition of protected lemuru and tuna fish oil could increase omega-6 content of dairy milk. It means that protected unsaturated fatty acids did not undergo biohydrogenation process by rumen microbes. It was digested and absorbed in the intestine so that the omega-6 fatty acids were deposited in milk. Komari (1994), Sinclair (1996) and Suarez *et al.* (1996) reported that supplementation of unsaturated fatty acids in the ration could decrease saturated fatty acids content in the animal tissue. Furthermore, Fatahnia *et al*. (2008) reported that feeding fish oil and soybean oil and or its combination decreased the proportion of saturated fatty acids in milk fat.

Unsaturated Fatty Acids Content of Dairy Milk

Unsaturated fatty acids content of P0 was significantly (P<0.01) different with P3 and P4, but it was not significantly different unsaturated fatty acids content of P1 and P2 (Table 6). This research revealed that supplementation of fish oil in the diet could increase unsaturated fatty acids. That was inline with Estiasih (2009) who found that combination of fish oil and peanut oil may increase the levels of long chain unsaturated fatty acid. It can increase unsaturated fatty acids content of dairy milk. Goodridge *et al*. (2001) reported that milk linoleic acid was 10.3% significantly (P<0.05) higher in the milk of cows fed protected linola vs the control. Furthermore, Fatahnia *et al*. (2008) strengthened that feeding fish oil and soybean oil or its combination could increase unsaturated fatty acid concentrations. The incorporation of dietary unsaturated fats in milk would improve the nutritional value and possible human health benefits.

Saturated Fatty Acids Content of Dairy Milk

Saturated fatty acids content of P0 was significantly (P<0.01) different with P3 and P4, but was not significantly different with P1 and P2. Treatment of P1 and P2 were significanty (P<0.01) different with P3 and P4 (Table 6).

Fahtania *et al*. (2008) reported that feeding fish oil and soybean oil or its combination decreased the proportion of saturated fatty acids in milk fat. Furthermore, Estiasih (2009) reported that combination of fish oil and peanut oil may decrease the levels of long chain unsaturated fatty acid from 5.9% to 3.4%. That was because fish oil is energy source and source of unsaturated fatty acids. It can decrease saturated fatty acids content of dairy milk. That was inline with the study of Komari (1994), Sinclair *et al*. (1996) and Suarez *et al*. (1996) who reported that supplementation of unsaturated fatty acids in the ration could decrease saturated fatty acids content in the body tissue.

Table 6. Average of omega-3, omega-6, unsaturated fatty acid, and saturated fatty acid content of dairy milk (%)

Treatment	Parameter			
	Omega-3	Omega-6	Unsaturated fatty acid	Saturated fatty acid
P0	0.903±0.100[a]	0.550±0.105[a]	44.890±2.219[a]	55.110±4.891[a]
P1	1.017±0.081[a]	0.673±0.095[a]	46.540±2.567[a]	53.460±4.161[a]
P2	1.003±0.084[a]	0.677±0.093[a]	46.550±2.159[a]	53.450±2.159[a]
P3	3.597±0.203[b]	20.630±2.765[b]	71.530±2.825[b]	27.470±1.784[b]
P4	3.873±0.371[b]	19.760±0.923[b]	72.290±1.408[b]	27.710±1.408[b]

Note: Means in the same column with different superscript differ significantly (P<0.01). P0= Control ration, P1= P0 + 20% fermented rice bran replacing rice bran in the ration, P2= P1 + 4% soya bean oil, P3= P1 + 4% protected tuna fish oil, P4= P1 + 4% protected lemuru fish oil.

CONCLUSION

Supplementation of protected PUFA and fermented rice bran decreases feed consumption and increases production of cow milk. The supplementation of protected tuna and lemuru fish oil up to 4% in the fermented rice bran feed of dairy cows increases the content of omega-3, omega-6 and unsaturated fatty acids but it decreases the content of saturated fatty acids of cow milk.

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