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Composition and Isolation of Lactoferrin from Colostrum and Milk of Various Goat Breeds

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Abstract

Dairy goats colostrum is one of lactoferrin source which has various benefits such as antimicrobial activity. The samples of colostrum and milk are samples of three breeds of goats. The highest concentration of lactoferrin in colostrum and milk is 88.70±12.11 mg/l for saanen goats crosses with PE goats (SAPE), with approximately molecular mass of lactoferrin is 83,513.3 Dalton and the last is 72.57±23.61 mg/l for peranakan etawah (PE), with approximately molecular mass of lactoferrin is 86,075.4 Dalton. These results have important information as a basic knowledge of the value of the concentration of lactoferrin in goat breeds.

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Keywords: Isolation, Lactoferrin, Colostrum, Milk, Goat, Breeds

Introduction

Goats are one of the types of animals that potentially developed into a producer of meat and milk in Indonesia. Several types of dairy goats breeds that can be developed in Indonesia, among others Peranakan Etawah goats (PE), Jawarandu goat and Saanen goats crosses with PE goats

* Corresponding author. Email : <u>agusrachman@ung.ac.id</u> (SAPE). Maintenance of dairy goats could provide valuable animal protein of high biological and essential minerals and vitamins from cattle. According to comprehensive studies it was established [12] that goat's milk has a nutritional value similar to cow's milk and can be used as an alternative to cow's milk for the rehabilitation of children, who suffer from poor nutrition. Goat colostrum lactoferrin concentration is higher than cow's milk, which ranged from 455.8 to 2058.3 mg / dl on goat colostrum and 575.0 mg / dl in bovine colostrum [7]. Goat colostrum lactoferrin concentration is high due to the quantity of goat colostrum produced. The higher the quantity of colostrum, then the quality will be reduced, in terms of dry matter high goat colostrum, which ranged between 25.43 to 38.96%, while the dry matter 18.30% bovine colostrum[11].

Goat's milk has a milk components such as lactoferrin, immunoglobulins and lactoperoxidase as an anti-microbial. Lactoferrin in milk are able to bind metal ions on the microbes that inhibit microbial growth. Lactoferrin can be used for enrichment or fortification of milk. High levels of lactoferrin in milk will improve the quality of milk, especially microbiological quality and value to milk as a functional food. Based on the function of proteins chemically, then there are two possible physiological functions of lactoferrin, namely: 1) as a source of iron for infants / children and 2) a potential antimicrobial factors in the milk ducts in the digestive tract and baby / children [2,5,6]

Colostrum also called "mother's milk" is a light yellow solution produced by glands udder during the first hour after birth, usually began to be produced before birth and collected over the last few weeks of pregnancy [3]. Colostrum is stored by the udder glands around 2-3 last day of gestation and secreted approximately 2-3 first days after birth. Colostrum is no longer produced at 4-5 days after birth, then there will be changes in the colostrum into milk completely [3] Colostrum has a serum protein content is very high and often still contained blood [16]. The things that affect levels of lactoferrin in colostrum generally include 1) day milking, colostrum has a higher lactoferrin levels (575.0 mg / dl in colostrum of cows and 459.4 mg / dl in cow's milk) [7], 2) presence or absence of bacterial infection [15], and 3) Genetic animal itself [14].

Preliminary studies that identify the presence of lactoferrin in milk goat "kacang", and goat colostrum and milk have been conducted [10]. The results of these studies indicate that lactoferrin band has gained a molecular weight of 73.441 kDa for goat milk and "kacang' goat milk whereas for colostrum peranakan etawah (PE) goats molecular weight of 74.991 kDa were obtained. However, information on the isolation of lactoferrin contained in colostrum and milk from various dairy goats breeds is still very limited. This study aims to (1) Studying the influence of the chemical composition of colostrum and milk of goats breeds Peranakan Etawah (PE), Jawarandu and Saanen goats crosses with PE goats (SAPE), including dry matter content, non fat dry matter, protein, specific gravity, and pH on lactoferrin to be identified, (2) Perform insulation lactoferrin from colostrum and goat milk to obtain isolates lactoferrin, and (3) Identify the presence of lactoferrin to determine the concentration and lactoferrin content in colostrum and milk goats were studied.

Materials & Methods

This research used colostrum and milk from the Peranakan Etawah goats (PE), Jawarandu, and Saanen goats crosses with PE goats (SAPE) that were obtained from local farm. The method of research was Completely Randomize Design (RAL) factorial pattern (3×8) with three repetition treatments to the various species of goats and different days of milking. Colostrum

samples used 150 ml with the result of milking days 1 (H1), day 2 (H2) and day 3 (H3), whereas for the samples of milk used 250 ml with the results of milking day 4 (H4) up to day 8 (H8), stored at 5° C for 6 months were studied. The methods of research such as milk and colostrum quality test (test of specific gravity, test of milk fat content (methods gerber), test of levels of protein milk, and test of pH value); separation fat and casein; isolate of whey protein, lactoferrin concentration calculation, determination of content lactoferrin by sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE)

Results and Discussions

Effect of Chemical Composition of Colostrum and Milk from Peranakan Etawah (PE) Goats, Jawarandu Goats and Saanen Goats Crosses with PE Goats (SAPE) against Lactoferrin

The chemical composition of colostrum and milk include levels of dry matter (DM), non fat dry matter (NFDM), fat, protein, specific gravity (BJ) and pH.

Day	Day Milking		Goat Breeds (n)		
Milking		PE (3)	Jawarandu (3)	SAPE (3)	
1 st day	1 st day	38.96±3.62 ^a	37.49±10.48 ^a	47.09±9.05 ^a	
2 nd day	2 nd day	$25.52{\pm}0.98^{ab}$	30.62±5.26 ^{ab}	28.63±1.74 ^{ab}	
3 rd day	3 rd day	26.34±2.09 °	28.61±0.91 °	23.86±5.16 ^c	
4 th day	4 th day	23.51±1.95 ^{ab}	25.48±2.14 ^{ab}	19.28±3.61 ^{ab}	
5 th day	5 th day	17.76±1.84 ^{ab}	27.49±2.27 ^{ab}	17.16±0.24 ^{ab}	
6 th day	6 th day	16.79±4.02 ^{ab}	17.14±0.85 ^{ab}	16.57±0.06 ^{ab}	
7 th day	7 th day	15.56±2.81 ^{ab}	16.86±1.36 ^{ab}	16.11±0.39 ^{ab}	
8 th day	8 th day	13.58±0.31 ^{ab}	16.21±0.95 ab	15.63±0.49 ^{ab}	

Table 1. Mean and Standard Deviation Dry Matter (DM) (%) Colostrum and Goat Milk

Description: Superscript with different letters in the same row or column shows significant differences (P < 0.05); PE: Peranakan Etawah goats; SAPE: Saanen goats crosses with PE goats

Day	Day Milking		Goat Breeds (n)		
Milking		PE (3)	Jawarandu (3)	SAPE (3)	
1 st day	1 st day	17.63±1.10 ^a	15,32±1,93 ^a	18,59±3,25 ^a	
2 nd day	2 nd day	12.36±1.13 ab	13,87±0,88 ^{ab}	14,12±1,32 ^{ab}	
3 rd day	3 rd day	13.00±2.43 °	12.69±0.61 °	12.59±1.64 °	
4 th day	4 th day	$11.51{\pm}0.49^{ab}$	11.65±1.16 ^{ab}	10.94±0.98 ^{ab}	
5 th day	5 th day	$10.01 {\pm} 0.73^{ab}$	12.59±0.26 ^{ab}	11.09±0.07 ^{ab}	
6 th day	6 th day	9.62±1.14 ^{ab}	10.37±0.69 ^{ab}	10.81±0.23 ^{ab}	
7 th day	7 th day	9.60±1.12 ^{ab}	10.26±0.45 ^{ab}	10.66±0.17 ^{ab}	
8 th day	8 th day	9.26±0.66 ^{ab}	9.86±0.15 ^{ab}	10.58±0.21 ^{ab}	

Table 2. Mean and Standard Deviation Non Fat Dry Matter (NFDM) (%) Colostrum and Goat Milk

Description: Superscript with different letters in the same row or column shows significant differences (P <0.05); PE: Peranakan Etawah goats; SAPE: Saanen goats crosses with PE goats

Table 1 shows, the content of dry matter or DM (%) colostrum and goat milk produced in the milking day 1 to day 8 consecutive decline. Based on the analysis of variance, breeds and the goat milking effected on dry matter (DM) (P <0.05). The highest content of colostrum dry matter (DM) found in saanen goats crosses with PE goats (SAPE) (milking day 1 i.e. 38.96%) and the highest content of milk dry matter (DM) found in Jawarandu goats (milking day 5 is 27.49%). It is established [10], that the content of colostrum dry matter is higher than milk, associated with higher total solids in colostrum.

The data of Table 2 shows, non fat dry matter or NFDM (%) colostrum and goat milk produced in the milking day 1 to day 8 consecutive decline. Non fat dry matter or NFDM levels are influenced by the fat content of colostrum and milk goats. Based on the analysis of variance showed that differences in the breeds and the goat milking effect on non fat dry matter or NFDM (P < 0.05) with non fat dry matter shown by the number of different and looks of each day decreased milking. The data in the table shows that the highest content of colostrum non fat dry matter or NFDM saanen goats crosses with PE goats (SAPE) found in goats (milking day 1 ie 18.59%) while the highest levels found in milk non fat dry matter or NFDM Jawarandu goats (milking day 5 is 12.59%). Marked difference in the content of this non fat dry matter or NFDM caused by genetic differences, feed management (feed intake, feed quality and type of feed given). It is established [13] that the content non fat dry matter or NFDM determined by the components of protein, lactose, minerals, vitamins and enzymes.

Milking Day		Goat Breeds (n)		
Wilking Day	PE (3)	Jawarandu (3)	SAPE (3)	
1 st day	21.33±2.57 ^a	22.17±8.55 ^a	28.5±5.82 ^a	
2 nd day	13.17±1.44 ^{ab}	16.75±4.38 ^{ab}	14.51±0.88 ab	
3 rd day	13.33±0.58 ^c	15.92±0.58 °	11.26±3.67 °	
4 th day	12±2.16 ^{ab}	13.83±1.01 ^{ab}	8.33±3.22 ^{ab}	
5 th day	7.17±2.92 ^{ab}	14.83±2.56 ^{ab}	6.066±0.30 ^{ab}	
6 th day	16.79±4.02 ^{ab}	6.67±0.96 ^{ab}	5.76±0.20 ^{ab}	
7 th day	5.96±1.76 ^{ab}	6.6±1.08 ^{ab}	5.45±0.39 ^{ab}	
8 th day	4.31±0.64 ^{ab}	6.35±1.01 ^{ab}	5.05±0.43 ^{ab}	

Table 3. Mean and Standard Deviation Fat (%) Colostrum and Goat Milk

Description: Superscript with different letters in the same row or column shows significant differences (P < 0.05); PE: Peranakan Etawah goats; SAPE : Saanen goats crosses with PE goats

Milking Day		Goat Breeds (n)	
Miking Day	PE (3)	Jawarandu (3)	SAPE (3)
1 st day	5.96±2.30 ^a	9.00±0.32 ^a	8.90±0.56 ^a
2 nd day	4.51±1.48 ^{ab}	7.38±0.78 ^{ab}	8.28±0.94 ^{ab}
3 rd day	4.68±1.09 °	6.27±0.83 °	6.86±0.31 °
4 th day	3.92±1.06 ^{ab}	5.91±1.51 ^{ab}	6.20±0.24 ^{ab}
5 th day	4.29±0.33 ^{ab}	5.00±0.83 ^{ab}	5.88±0.31 ^{ab}
6 th day	4.15±0.59 ^{ab}	4.75±0.63 ^{ab}	5.71±0.28 ^{ab}
7 th day	4.32±0.49 ^{ab}	4.68±0.85 ^{ab}	5.49±0.14 ^{ab}
8 th day	3.47±1.28 ^{ab}	4.71±0.90 ^{ab}	4.77±0.19 ^{ab}

Table 4. Mean and Standard Deviation Protein (%) Colostrum and Goat Milk

Description: Superscript with different letters in the same row or column shows significant differences (P < 0.05); PE: Peranakan Etawah goats; SAPE : Saanen goats crosses with PE goats

As seen on Table 3, the content of fat (%) colostrum and goat milk produced in the milking day 1 to day 8 consecutive decline. Colostrum and milk fat content is a critical component that determines the quality of the colostrum and milk goats. Based on the analysis of variance showed that differences in the breeds and the goat milking effect on fat content (P < 0.05) with the

amount indicated by the different fat content of each breeds and seen goats decreased on each day milking. The data in the table show that the fat content of colostrum is highest in saanen goat crosses with PE goats (SAPE) (milking day 1 ie 28.50%) while the highest content of milk fat contained in Jawarandu goats (milking day 5 is 14.83%). It can be caused by a change in the normal colostrum into milk so the longer the time milking the fat content decreased. It is estbalished [3] that colostrum is not produced again 4-5 days after birth, then there will be changes in the colostrum into milk completely. It is established [9] that a milk fat content and dry matter less than colostrum.

The data in Table 4 shows, the content of protein (%) colostrum and goat milk produced in the milking day 1 to day 8 consecutive decline. The protein content is an important component that determines the concentration of lactoferrin in colostrum and milk goats. Based on the analysis of variance showed that differences in the breeds and the milking goats influenced the protein content (P < 0.05) indicated by the number of different protein content of each breeds and seen goats decreased on each day milking. The data in the table show that colostrum contains the highest protein levels found in Jawarandu goats (milking day 1 which is 9%) while the highest content of milk protein content found in saanen goats crosses PE goats (SAPE) (milking day 5 is 14.83%). It is established [9] that the levels of protein in the initial milking higher than normal milk completely. This is due to the protein content of forages is quite low.

		Goat Breeds (n)	
Milking Day	PE (3)	Jawarandu (3)	SAPE (3)
1 st day	1.0493±0.00 ^a	1.0392±0.00 °	1.0465±0.0079 ^a
2 nd day	1.0421±0.00 ^{ab}	1.0384±0.00 ^{ab}	1.0415±0.0052 ^{ab}
3 rd day	1.0317±0.00 ^c	1.0345±0.00 ^c	1.0376±0.0048 ^c
4 th day	1.0334±0.003 ab	1.0323±0.003 ab	1.0362±0.0037 ^{ab}
5 th day	1.031±0.004 ^{ab}	1.035±0.003 ^{ab}	1.0368±0.0057 ^{ab}
6 th day	1.030±0.002 ^{ab}	1.034±0.003 ^{ab}	1.0360±0.0010 ^{ab}
7 th day	1.031±0.003 ^{ab}	1.033±0.001 ^{ab}	1.0360±0.00080 ^{ab}
8 th day	1.0315±0.003 ab	1.0320±0.001 ^{ab}	1.0360±0.00089 ^{ab}

Table 5. Mean and Standard Deviation Specific Gravity (BJ) (g/ml) Colostrum and Goat Milk

Description: Superscript with different letters in the same row or column shows significant differences (P < 0.05); PE: Peranakan Etawah goats; SAPE: Saanen goats crosses with PE goats

	PE (3)	Jawarandu (3)	SAPE (3)
1 st day	6.69±0.14 ^ª	6.75±0.10 ^ª	6.47±0.023 [°]
2 nd day	6.72±0.16 ^{ab}	6.71±0.05 ^{ab}	6.48±0.111 ^{ab}
3 rd day	6.74±0.15 ^c	6.58±0.12 ^c	6.37±0.06 ^c
4 th day	6.71±0.14 ^{ab}	6.64±0.06 ^{ab}	6.39±0.07 ^{ab}
5 th day	6.67±0.12 ^{ab}	6.65±0.07 ^{ab}	6.37±0.06 ^{ab}
6 th day	6.67±0.15 ^{ab}	6.64±0.07 ^{ab}	6.36±0.05 ^{ab}
7 th day	6.69±0.13 ^{ab}	6.65±0.13 ^{ab}	6.33±0.03 ^{ab}
8 th day	6.70±0.15 ^{ab}	6.63±0.05 ^{ab}	6.40±0.03 ^{ab}

Table 6. Mean and Standard Deviation pH Colostrum and Goat Milk

Description: Superscript with different letters in the same row or column shows significant differences (P < 0.05); PE: Peranakan Etawah goats; SAPE: Saanen goats crosses with PE goats

In Table 5 looks, content specific gravity or BJ density (g / ml) colostrum and goat milk produced in the milking day 1 to day 8 consecutive decline. Specific gravity of colostrum and milk depend on the total solids in colostrum and milk goats. Higher total solids causing the higher the specific gravity of colostrum and milk, while the total solids of lower and higher water content causes the specific gravity of colostrum and milk be low. Based on the analysis of variance showed that differences in the breeds and the goat milking affect the specific gravity (P <0.05) with the weight indicated by the number of different types of each breeds and seen goats decreased on each day milking. The data in the table show that colostrum contains the highest density found in goat (milking day 1 is 1.0493 g / ml) while the content of the highest density found in saanen goats crosses with PE goats (SAPE) (milking day 5 is 1.0368 g / ml). Increased density is due terbebaskannya gases such as CO and N₂ are contained in the newly acquired milk from milking [4]. It is established [1] that ranges from 1,030 to 1,056.

The data in Table 6 shows, the value of the pH effect on protein levels in colostrum and milk goats. Protein is denatured at low pH plays an important role so as to maintain the quality of protein that can affect the concentration of lactoferrin in colostrum and milk goats. Based on the analysis of variance showed that differences in the breeds and the goat milking effect on pH (P <0.05) indicated by different pH values of each breeds goat. The results were obtained pH of goat milk, and peranakan etawah goats (PE) 6.67 ± 0.12 to 6.74 ± 0.15 ; saanen goats crosses with PE goats (SAPE) 6.58 ± 0.12 to 6.75 ± 0.10 and jawarandu goats 6.33 ± 0.03 to 6.48 ± 0.11 ; this case shows that the pH of normal milk.

Identification of Lactoferrin from Colostrum and Milk from Peranakan Etawah (PE) Goats, Jawarandu Goats and Saanen Goats Crosses with PE Goats (SAPE) with Spectrophotometric Method

The results of the mean and standard deviation of colostrum and milk lactoferrin are presented in Table 7.

In Table 7 shows, lactoferrin concentration (mg / l) colostrum and goat milk produced in the milking day 1 to day 8 consecutive decline. Based on the analysis of variance showed that differences in the breeds and the goat milking affect the concentration of lactoferrin (P <0.05) with the concentration of lactoferrin was shown by the number of different breeds each goat and visible decrease in the daily milking. The data in the table show that the higher the concentration of lactoferrin in goat colostrums saanen goats crosses with PE goats (SAPE) (milking day 1 is 207 mg / l), whereas higher concentrations of lactoferrin in goat milk Jawarandu (6th day milking is 63.16 mg / l). It is established [17] that the content of lactoferrin in colostrum and milk cows differed among individuals and also during the period of lactation. Goat colostrum lactoferrin concentration is higher than cow's milk, which ranged from 455.8 to 2058.3 mg / dl on goat colostrum and 575.0 mg / dl in bovine colostrums [7].

Lactoferin Concentration (mg/l) in	Goat Breeds (n)			
	PE (3)	Jawarandu (3)	SAPE (3)	
1 st day	156,36±107,66ª	197,39±58,94 °	207,39±58,94°	
2 nd day	154,82±53,92 ^{ab}	205,83±32,30 ^{ab}	204,83±32,30 ^{ab}	
3 rd day	48,58±3,46 ^c	67,23±33,54 [°]	66,23±33,54 [°]	
4 th day	47,58±3,46 ^{ab}	58,58±3,46 ^{ab}	58,58±3,46 ^{ab}	
5 th day	43,24±10,06 ^{ab}	48,45±12,60 ^{ab}	47,45±12,60 ^{ab}	
6 th day	46,10±2,86 ^{ab}	63,16±43,30 ^{ab}	42,16±43,30 ^{ab}	
7 th day	42,66±5,48 ^{ab}	30,51±5,86 ^{ab}	36,51±5,86 ^{ab}	
8 th day	41,24±1,22 ^{ab}	25,22±2,21 ^{ab}	32,25±2,20 ^{ab}	

Table 7. Mean and Standard Deviation Lactoferin Concentration (mg/ l) Colostrum and Goat Milk PE,
Jawarandu and SAPE in Difference Milking Day

Description: Superscript with different letters in the same row or column shows significant differences (P < 0.05); PE : Peranakan Etawah goats; SAPE : Saanen goats crosses with PE goats

Identification of lactoferrin Content of Colostrum and Milk from Peranakan Etawah (PE) Goats, Jawarandu Goats and Saanen goats crosses with PE Goats (SAPE)

The test results by electrophoresis shown in Figure 1, 2 and 3, shows a tape that allegedly lactoferrin in colostrum and goat milk.



Fig 1. The test results by electrophoresis shown Colostrum and Milk from saanen goats crosses with PE goats



Fig 2. The test results by electrophoresis shown Colostrum and Milk from Peranakan Etawah (PE) Goat

Protein bands were seen in colostrum and goat milk SAPE, PE and Jawarandu located between β Galactosidase protein bands (116,000 Daltons) and Bovine Serum Albumin (66,200 Dalton). The results of the study, the estimated molecular weight of colostrum and goat milk lactoferrin SAPE is 83513.8 Dalton; molecular weight of colostrum and goat milk lactoferrin PE is 86075.4 Dalton; and molecular weight of colostrum and goat milk lactoferrin Jawarandu is 80292.16 Dalton. It is established [8] that the approximate molecular weight of lactoferrin from

cow udder secretion glands approaching 83 and 87 kDa. It is established [17] that lactoferrin a predicted molecular weight of 84,000 Da to 80,000 Da for lactoferrin.



Fig 3. The test results by electrophoresis shown Colostrum and Milk from Jawarandu Goat

Conclusion

Differences in the breeds and the goat milking affected the chemical composition of colostrum and milk (dry matter content, non fat dry matter, fat, protein, specific gravity and pH). Composition of colostrum (milking the 1st, 2nd and 3rd) has a value higher than the levels of milk (milking the 4th to the 8th day milking) the goat. Differences in the breeds and the goat milking affected the concentration of lactoferrin in colostrum and milk.

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