



# Isolation and Identification of Lactoferrin and Lactoperoxidase from the Colostrum of Indonesian Ettawa Crossbred Goat

Dian Wahyu Harjanti<sup>1,\*</sup>, Rizke Ciptaningtyas<sup>2</sup>, Ahmad Ni'matullah Al-Baarri<sup>3</sup>, and Endang Kusumanti<sup>1</sup>

<sup>1</sup>Faculty of Animal and Agricultural Sciences, Diponegoro University, Indonesia

<sup>2</sup>Faculty of Medicine, Diponegoro University, Indonesia

*Background:* Lactoferrin is a glycoprotein of the transferrin family which has multifunctional properties and useful for clinical and commercial application. It has some advantages as an immune system modulation, antibacterial activity and antioxidant in infant and adult as well as in animal. Lactoperoxidase is one of the most prominent enzymes in milk and catalyzes the inactivation of a wide range of microorganisms. Both lactoferrin and lactoperoxidase are expressed in most biological fluids, including colostrum. Chemical components of colostrum may vary along the days after parturition. The study of lactoferrin and lactoperoxidase from dairy goat is very limited. This study aimed to isolate and identify characteristics (the molecular weight and concentrations) of lactoferrin and lactoperoxidase from Indonesian native Ettawa crossbred goat colostrum. *Method:* The isolation and purification of lactoferrin and lactoperoxidase was conducted by an ion-exchange chromatography (SP-sepharose). Other compositions of colostrum, protein, fat and lactose concentration, were also determined. *Results:* The result showed that lactoferrin and lactoperoxidase concentrations in goat colostrum on the first day (317.3 mg/L and 204 mg/L) were higher than colostrum on the second day (190.5 mg/L and 61.9 mg/L) postpartum. The molecular weight of lactoferrin and lactoperoxidase were 75 and 72 kDa. Protein, fat and lactose concentrations of colostrum on the first day postpartum (6.5%, 5.7% and 4.9%) were higher than those on the second day (4.9%, 4.4% and 4.1%). *Conclusion:* A high amount of lactoferrin and lactoperoxidase can be isolated from the goat colostrum on its first day of postpartum.

**Keywords:** Ettawa Goat, Lactoferrin, Lactoperoxidase, Colostrum.

## 1. INTRODUCTION

Milk is the most nutritious food that has long been and will always be consumed as part of a healthy balance diet. Milk consumption plays an important role in assisting individuals to meet their nutrient requirements. Nowadays, nutrition science moves beyond the study of essential nutrients in milk. Many research over the last twenty years focused only on bovine milk, meanwhile the exploration of dairy goat is still limited. Colostrum has become popular as a product for human consumption, because of its excellence of bioactive proteins source. Colostrum means a fluid secreted by the mammary glands of milk-producing animals up to three or five days post parturition that is rich in essential nutrients.<sup>1</sup> Recently, many studies conduct to explore an active biological substances in colostrum that is useful for human and animal to against the pathogenic agents. Lactoferrin and lactoperoxidase are substances found mainly in milk and believed to have a health benefit to human and animal.

Lactoperoxidase is a peroxidase enzyme secreted from mammary gland, salivary and other mucosal glands. Many research on the clinical application in human reported that lactoperoxidase was useful for the treatments of breast cancer,<sup>2</sup> and oral disease.<sup>3,4</sup>

Lactoferrin is an iron-binding glycoprotein of transferrin family that possess antimicrobial activity. Lactoferrin is mainly present in colostrum and other biological fluids such as blood and mucous secretions of mammals. In human and cow, the antibacterial power of lactoferrin against bacteria, some yeast, fungi, viruses and parasites has been investigated.<sup>5,6</sup> In addition, the modulatory effect of bovine and human lactoferrin on inflammatory response and activation of the immune system have been reported previously.<sup>7</sup> Lactoferrin works as an antimicrobial compound through chelating the iron ion and making this essential ion unavailable to invading the pathogens.

Lactoferrin concentration in milk varies depending on the species and lactation stages.<sup>8,9</sup> The lactoferrin concentration in cow's milk is lower than in human milk.<sup>10</sup> To our knowledge, there is no study about the changes of lactoperoxidase

\* Author to whom correspondence should be addressed.

concentrations throughout lactation period in dairy animals. Moreover, a report about both lactoferrin and lactoperoxidase produced from native dairy goat in the tropic is also limited. Hence, this study aimed to isolate and identify the lactoferrin and lactoperoxidase produced from Indonesian native Ettawa crossbred goats.

## 2. MATERIAL AND METHOD

### 2.1. Goat Colostrum

Colostrum samples were collected from 6 healthy individual Ettawa crossbred goats at the beginning of lactation. The samples were immediately frozen at  $-18^{\circ}\text{C}$  before analysis.

### 2.2. Lactoperoxidase and Lactoferrin Production from Whey

Two liters of colostrum samples were placed into a waterbath at  $35^{\circ}\text{C}$ . The colostrum was defatted by adding 50 ml 0.02% rennet (w/v), followed by incubation for 30 min. Colostrum was then filtered using sterile cloth and centrifuged at 60 rpm at  $10^{\circ}\text{C}$  for 15 min to obtain its whey.<sup>11</sup> The whey was loaded into SP Sepharose Fast Flow Column (GE Healthcare Bio-Science AB, Sweden). A total of 300 ml of 0.4 M NaCl was diluted in 300 mL of 0.1 M Phosphate buffer (pH 7.0). The solution was flowed into SP Sepharose Fast Flow to generate lactoperoxidase solution as described previously.<sup>12</sup> A total of 300 ml of 1 M NaCl in 0.1 phosphate buffer (pH 7.0) was then poured to produce lactoferrin solution.

The eluates of both lactoferrin and lactoperoxidase were analyzed separately to measure its concentration using spectrophotometer at 280 nm. The purity of lactoferrin and lactoperoxidase in the eluate were tested using dodecyl sulfate-polyacrilamide gel electrophoresis (SDS-PAGE). The sample (lactoferrin or lactoperoxidase eluate) was diluted in 1:4 (v/v) ratio with buffer (0.0248 M Tris, 0.19 M Glycine, 0.1% SDS 10%, pH 6.8). Samples were heated at  $100^{\circ}\text{C}$  for 5 min and then loaded into the gel electrophoresis wells. ExactPro Broad Range (10–245 kDa) 4–20% tris-glycine (1st BASE, Singapore) was used as protein ladder for lactoferrin, whereas Thermo Scientific PageRuler Prestained Protein Ladder (10–180 kDa) 4–20% tris-glycine (Thermo, Germany) was used as protein ladder for lactoperoxidase. The remaining of each lactoferrin and lactoperoxidase solution was filtrated through a  $0.22\text{-}\mu\text{m}$ -pore-size filter unit and stored at  $-30^{\circ}\text{C}$ .

### 2.3. Nutrient Composition of Colostrum

A total of 5 ml colostrum was poured into the crucible and heated in the air oven at  $100^{\circ}\text{C}$  for 4 h. Its first and final weights were then calculated to measure the total solid content. The protein

concentration of colostrum was determined following the method of Bradford.<sup>13</sup> Lactose content was analyzed using the method of AOAC 930.28–1930. Fat content was measured by mixing 5 ml of colostrum sample with 3 ml of *n*-hexane. The sample was shaken for 2 min until 2 layers formed. The first layer (the bottom portion) was taken and dried by heating and calculated for the fat content. Specific gravity of the sample was measured using lactodensimeter (Funke Gerber, Germany).

## 3. RESULTS

Colostrum on the first day postpartum contained a high content of total solids (19.5%) and it was composed of 6.5% of protein, 4.9% lactose, 5.6% of fat, and 13.9% of solid non fat. The nutrient compositions decreased as later postpartum days. On the second day of lactation, the content of total solid was 12.8%, containing 4.9% of protein, 4.1% of lactose, 4.0% of fat and 8.8% of solid non fat.

The concentration of lactoferrin and lactoperoxidase of goat colostrum on the first day postpartum were 317.3 and 204 mg/L. The concentrations were decreased on the second day postpartum (Fig. 1). The concentrations were 190.5 and 61.9 mg/L for

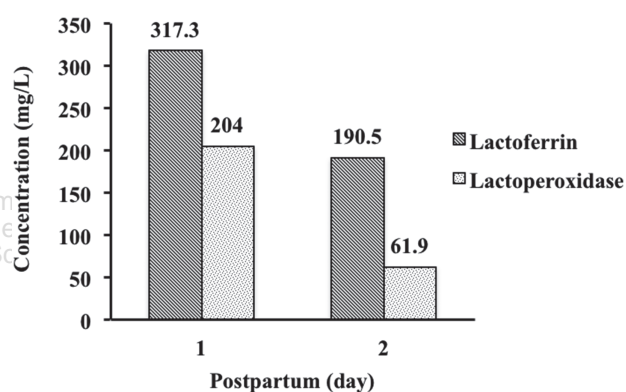


Fig. 1. Lactoferrin and lactoperoxidase concentrations in goat colostrum postpartum.

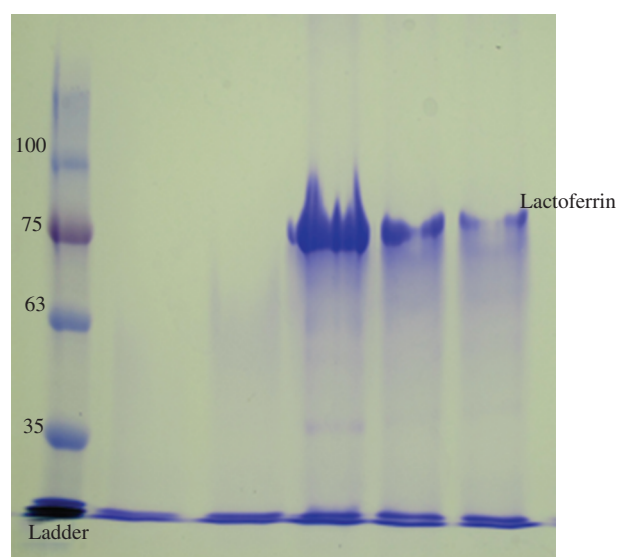


Fig. 2. Electrophoresis result of lactoferrin from goat colostrum.

Table I. Nutrient composition of Ettawa crossbred goat colostrum.

Nutrient composition	Days postpartum	
	1	2
Total solid (%)	29.5	24.8
Specific gravity (g/ml)	1.041	1.034
Protein (%)	6.5	4.9
Lactose (%)	4.9	4.1
Fat (%)	9.6	7.0
Solid non fat (%)	13.9	8.8

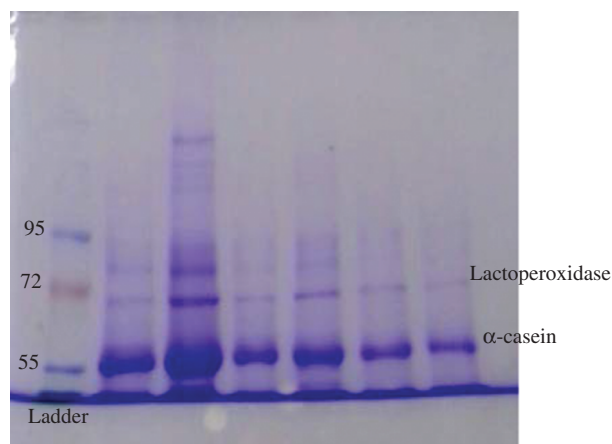


Fig. 3. Electrophoresis result of lactoperoxidase.

lactoferrin and lactoperoxidase. The molecular weight of goat lactoferrin is 75 kDa (Fig. 2) and lactoperoxidase is 72 kDa (Fig. 3).

#### 4. DISCUSSION

Colostrum formation in the pregnant animal is initiated about 3–4 weeks before parturition when a limited amount of fluid, growth factors and other substances are released into the developing mammary tissue.<sup>14</sup> When the placenta is eliminated at birth, mother's progesterone levels fall dramatically and its inhibitory control of the fluid secretions into mammary gland is removed. The cells inside the mammary gland are filled by a protein-based substances that essentially blocks any further transfer of substances from the mother's blood into the gland.<sup>15</sup> Therefore, the colostrum reflects the functional change that have occurred in the gland up to the time, such as having a high content of protein (most of which is IgG), fat and low in the lactose content. After birth, there is a physical removal of fluid from the mammary gland by milking. Since the transfer of substances from mother's blood is blocked, replacement fluid will contain primarily substances synthesized by the secretory cells in the mammary gland. Hence, the fluid composition after birth (normal milk) is different with the fluid originally contained in the mammary gland at birth (colostrum).

Similar with our findings, previous report in cow also demonstrated that the nutrient composition of colostrum (total solid, solid not fat extract, lactose, milk fat and protein) changed rapidly in time and it was suggested that, on the third day postpartum, the nutrient contents was already similar to that of normal milk.<sup>16</sup> Meanwhile, other report suggested that the changes to normal milk occurred on the fifth days postpartum.<sup>9</sup> Dry matter, protein and fat content change and the value were similar to those provided by a cow milk.<sup>17</sup> Other author found a higher percentage of protein<sup>18,19</sup> on the first day of lactation, as the breed of goat were different with those in this study. In Saanen goat, the chemical composition of colostrum at 3 h postpartum were 21.23%, 7.73%, 1.57%, 1.93% and 10.24% for total solid, fat, ash, lactose and protein, respectively.<sup>20</sup> In human studies, colostrum ingestion contributes to the improvement of health status and it assists the healing of immune deficiency status.<sup>21</sup>

Goat colostrum contains also with lower concentration of various trace components, including lactoferrin and lactoperoxidase.

The concentrations of lactoferrin and lactoperoxidase on the second day were lower than those on the first day postpartum. This is mostly due to the sharp decrease of protein fractions, that the concentrations was highest on the first day colostrum portion. Despite they had low concentrations, lactoferrin and lactoperoxidase are physiologically important for protection of mammary gland and the growth of neonates.<sup>5,22</sup> Lactoferrin molecular weight for Indonesian dairy Ettawa crossbred goat is 75 kDa. It seems that different animal and breed had different characteristic of lactoferrin. Indonesian meat-producing goat namely Kacang goat has 73.14 kDa of lactoferrin molecular weight.<sup>23</sup> In the other countries, the molecular weight of lactoferrin from Pakistani native goat colostrum was approximately 80 kDa,<sup>24</sup> Meanwhile in Korean native goat, the lactoferrin molecular weight from colostrum was 82 kDa.<sup>25</sup> Furthermore, molecular weight of lactoferrin was 80 kDa in dairy cow,<sup>26</sup> 79.5 kDa in camel<sup>27</sup> and 77 kDa in human.<sup>10</sup> Lactoferrin is an important host defense molecule.<sup>5,22,26</sup> It has become an evident that oral administration of lactoferrin exerts several beneficial effects in both infant and adult human and animal health as anticancer, antiviral, antibacterial and anti-inflammation. The inhibitory activity of lactoferrin to bacteria is believed to be the result of the powerful iron chelating ability of lactoferrin, making iron unavailable to bacteria.<sup>5</sup> The specific receptors of lactoferrin on the cell surface of microorganisms also affect the antibacterial ability of lactoferrin. Lactoferrin is binding to lipopolysaccharide of bacterial walls and oxidizing the iron part of bacteria to form peroxides. It causes the impairment of bacteria membrane permeability and cell breakdown (lysis).<sup>28</sup> Lactoferrin has potential as a therapeutic agent against methicillin-resistant *Staphylococcus aureus* (MRSA) either alone or combination with other antimicrobial drug.<sup>29</sup>

Similar with lactoferrin, the molecular weight of lactoperoxidase is also varies according to species. Previous study reported the molecular weights of lactoperoxidase in camel and bovine milk are 78 kDa and 72.5 kDa, respectively.<sup>27</sup> In the human mother's milk, purification and quantification of lactoperoxidase, using polyclonal antibodies technique against recombinant human lactoperoxidase, found two bands of lactoperoxidase with molecular weight 80 and 100 kDa.<sup>32</sup> Lactoperoxidase is the most abundant enzymes in milk. This enzyme, combination with H<sub>2</sub>O<sub>2</sub> and SCN<sup>-</sup> known as the Lactoperoxidase system (LPOS), exerts antibacterial activity through hypothiocyanite (OSCN<sup>-</sup>), a product of an enzymatic reaction.<sup>11</sup> This substrate may damage the sulfhydryl groups of protein in the cytoplasmic membrane of microbes through protein oxidation, thus causing the death of microbes.<sup>30</sup> In food industry, LPOS has been used as a preservative for dairy products and non-dairy products. LPOS also known to be efficacious against the microorganisms found in the vegetable<sup>31</sup> and fruits.<sup>11</sup>

In the future, lactoferrin may be useful as an alternative to antibiotic to prevent the antibiotic resistance in human and animal. Lactoperoxidase is very potential for industry as bio-preservative in food and other products. It needs a further research to study a potency of goat colostrum lactoferrin as natural drug, antimicrobial and immunomodulatory agent.

#### 5. CONCLUSION

The highest concentration of lactoferrin and lactoperoxidase, of Indonesian Ettawa crossbred goat colostrum, can be isolated from

the colostrum on the first day postpartum. The molecular weight of Indonesian Ettawa crossbred goat lactoferrin and lactoperoxidase are 75 and 72 kDa.

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