Antimicrobial activity of whey mare’s milk against *Salmonella enteritidis*

A Detha¹, A Saputra², A Ola³

¹Department of Animal Diseases and Veterinary Public Health, Faculty of Veterinary Medicine, Nusa Cendana University, Kupang, Indonesia
²Division of pharmacology and biochemistry, Faculty of Veterinary Medicine, Nusa Cendana University
³Faculty of Engineering and Science, Nusa Cendana University, Kupang, Indonesia.

*Corresponding author: detha.air@staf.undana.ac.id

Abstract. Mare's milk has good nutritional value with a balanced protein, fat and lactose content and therapeutic food sources because of high lactoferrin content in whey protein. Lactoferrin, lysozyme, immunoglobulins are important compounds contained in milk whey protein. As previous, *Salmonella* Enteritidis infection as a major cause of salmonellosis, globally. The test was conducted at the Veterinary Public Health Laboratory of the Faculty of Veterinary Medicine, Nusa Cendana University. Pathogenic bacteria was used *Salmonella* Enteritidis ATCC® 13076 ™, obtained from the Veterinary Public Health Section, Faculty of Veterinary Medicine, Bogor Agricultural University. The results show that Lactoferrin has antimicrobial activity against pathogenic bacteria of *Salmonella* Enteritidis. Results of this research may be basic research to the development of lactoferrin from Sumba mare’s milk as an antimicrobial agent potential in treating Salmonellosis.

1. Introduction

Protein in milk is an organic component composed of amino acids. There are two main types of milk protein, casein and whey which form a colloidal substance in milk. Compounds in whey protein in the form of α lactoalbumin, β lactoglobulin, lactoferrin, serum albumin, and lactoperoxidase, have an important role in the prevention of diseases caused by antimicrobial, anti-cancer and immunostimulatory [1,2]. Whey protein in mare’s milk, more than whey in cow's milk. The balance between casein and whey is thought to be an important factor in determining the level of cow's milk protein allergy in humans and this balance modification can reduce allergenicity to cow's milk [3]. Mare’s milk has a high biological value due to antimicrobial factors such as lysozyme, lactoferrin (Lf), lysozyme and immunoglobulins in milk whey. Major bioactive components (α-lactalbumin, β-lactoglobulin, serum bovine albumin, immunoglobulin, glycomacropeptides) and minor components of milk (lysozyme, lactoperoxidase and lactoferrin) [4]. Antimicrobial perspective of milk as a reference nutraceutical for health promotion and disease barriers [5]. Protein in horse milk whey can inhibit the growth of bacteria that cause subclinical
mastitis between *Streptococcus agalactiae* and *Streptococcus pyogenes* [6]. Some important diseases that are currently of concern are Salmonellosis caused by *Salmonella enterica* serovar Enteritidis (*Salmonella Enteritidis*). In developing countries, these pathogens have become a major cause of morbidity and mortality in children [7,8]. *Salmonella Enteritidis* infection has a major impact on health with high mortality as a major cause of salmonellosis globally [9]. *Salmonella Enteritidis* has also been a major cause of outbreaks of human salmonellosis in Indonesia, the United States and Europe which are related to the consumption of poultry products contaminated with this pathogen [10,11]. The purpose of this study was to find the antimicrobial activity of Whey mare’s milk against pathogenic bacteria *Salmonella Enteritidis*.

2. **Material and Methods**

The Antimicrobial Activity of Whey Mare’s Milk was conducted at the Veterinary Public Health Laboratory of the Faculty of Veterinary Medicine, Nusa Cendana University. Pathogenic bacteria were used *Salmonella Enteritidis* ATCC® 13076™, obtained from the Veterinary Public Health Section, Faculty of Veterinary Medicine, Bogor Agricultural University. Mare colostrum was obtained from East Sumba, East Indonesia.

2.1 Preparation of Whey Proteins

The milk was centrifuged at 3,000 × g for 1 h at 4°C, to produce skim milk (defat). Skim milk that has been obtained is then added HCL 2 N to reach pH 4.6 to separate casein through a centrifugation process at 20,000 × g, 4°C for 30 min. After the casein is deposited on the base of the tube, an acid whey is obtained which is then neutralized to reach pH 6.8 by adding 2N NaOH. Whey neutral was centrifuged in 10000 × g, 30 min at 4°C [12,13].

2.2 Antibacterial Activity of Whey Against *S. Enteritidis*

Pathogenic bacteria was used *Salmonella Enteritidis* ATCC® 13076™. Antimicrobial activity of whey was conducted by disc diffusion method. In this process, pure fresh cultures from the *Salmonella Enteritidis* ATCC® 13076™ pathogen were planted on Muller-Hinton agar media on a plate. Furthermore, sterile round discs (6 mm) were soaked in 100μl of each whey extracted and placed on a plate with repetition replicated 4 times. Antibiotics were used as positive controls and sterile deionized water as a negative control. The cup was incubated for 24-48 hours at 37 °C [4,14]. The diameter of the zone of inhibition in the cup was measured and antimicrobial activity was expressed in mm and the results were expressed as mean [15].

3. **Result and Discussion**

The test was conducted at the Veterinary Public Health Laboratory of the Faculty of Veterinary Medicine, Nusa Cendana University. Pathogenic bacteria used are *Salmonella Enteritidis* ATCC® 13076™, obtained from the Veterinary Public Health Section, Faculty of Veterinary Medicine, Bogor Agricultural University. The results show that diameter of the zone of inhibition of penicillin is 19,7 mm and Whey protein is 19,2 mm (Table 1). This result indicate that whey has antimicrobial activity against pathogenic bacteria *Salmonella Enteritidis*. Whey has an inhibitory effect on S. Enteritidis up to 19 mm.
Table 1. Whey inhibitory activity against S. Enteritidis bacteria

| Repeat | Penicillin | Whey Protein | Sterile deionized water |
|--------|------------|--------------|-------------------------|
| 1      | 22         | 19           | 6                       |
| 2      | 21         | 20           | 6                       |
| 3      | 19         | 19           | 6                       |
| 4      | 1,7        | 19           | 6                       |
| Mean   | 19,7       | 19,2         | 6                       |

These results are in line with various studies that show the presence of bioactive proteins in whey which are antimicrobial. Whey protein as a source of antibacterial peptides. The composition of whey protein in horse milk is β lactoglobulin, α lactalbumin, immunoglobulin, blood serum albumin, lactoferrin and lysozyme which are similar to cow’s milk. Peptides in α-la have immunomodulatory effects, including stimulating phagocytosis from macrophages so that α-la is considered a compound that has important antimicrobial power. Based on the action target, bacterial growth is inhibited by the ability of lactoferrin to absorb iron and also for the permeability of bacterial cell walls by binding to lipopolysaccharide through its N terminal [16].

Lactoferrin and lysozyme work synergistically to remove Gram negative bacteria effectively. The synergistic process causes inactivation of Gram negative, including S. Enteritidis [17]. Horse milk, the main antimicrobial is lysozyme, lactoferrin is higher than cow’s milk, but lactoferrin is more dominant in human milk [18]. Antimicrobials in milk whey namely IgA, IgG, IgM, lactoferrin and lysozyme provide immunity to the neonatal body and protection against infection [16]. Results of this research may be basic research for the development of whey from Sumba mare’s milk as an antimicrobial agent potential in treating Salmonellosis.

4. Conclusion
The results show that Lactoferrin has antimicrobial activity against pathogenic bacteria Salmonella Enteritidis. Results of this research may be basic research to the development of whey from Sumba mare’s milk as a potential candidate natural antimicrobial agent potential in treating Salmonellosis.

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References
[1] Ebringer L, Ferencik M and Krajcovic J 2008 Review: Beneficial health effects of milk and fermented dairy products. *Folia Microbiol.* **53**(5):378-394.
[2] El-Fakharany, Tabil E M, Abd El-Wahab, Haroun A, and Redwan E M 2008 Potential activity of camel milk-amylase and lactoferrin against hepatitis C virus infectivity in HepG2 and lymphocytes. *Hepatitis Monthly* **8**: 101-109.
[3] El-Agamy E I 2000 Effect of heat treatment on camel milk proteins with respect to antimicrobial factors: A comparison with cows’ and buffalo milk proteins. *Fd. Chem* **2**: 227-232.
[4] Niaz B, Zahoor T, Randhawa M and Jamil A. 2017. Isolation of Lactoferrin from Camel Milk through Fast Protein Liquid Chromatography and its Antagonistic Activity against Escherichia coli and Staphylococcus aureus. *Pakistan J. Zool.* **49**(4): 1307-1313.
[5] El-Hafez, S.M.A., Ismael, A.B., Mahmoud, M.B and Elaraby, A.K.A., 2013. Development of new strategy for non-antibiotic therapy: bovine lactoferrin has a potent antimicrobial and immunomodulator effects. *Adv. Infect. Dis* 3: 185-192. [12] Braden CR. 2006. Salmonella enterica serotype Enteritidis and eggs: *A national epidemic in the United States. Clin Infect Dis* 43: 512-517.

[6] Detha A, Sudarwanto M, Latif H, Datta FUD and Puji L. 2013a. Fractionation and Identification Antimicrobial Activity of Sumba Mare’s Milk Protein Against Subclinical Mastitis Bacteria in Dairy Cattle. *Global Veterinaria* 11(5):674-680.

[7] Tarabees R, Elsayed M, Shawish R, Basioni S and Shehata A. 2017. Isolation and characterization of Salmonella Enteritidis and Salmonella Typhimurium from chicken meat in Egypt. *J Infect Dev Ctries* 11(4):314-319.

[8] Graham SM. Salmonellosis in children in developing and developed countries and populations 2002 *Curr Opin Infect Dis.* 15(5):507-512.

[9] Majowicz S E, Musto J, Scallan E, Angulo F J, Kirk M, O’Brien S J, et al. 2010 The global burden of nontyphoidal Salmonella gastroenteritis *Clin Infect Dis* 50(6) 882-889.

[10] Braden CR 2006 Salmonella enterica serotype Enteritidis and eggs: A national epidemic in the United States. *Clin Infect Dis* 43: 512-517.

[11] Much P, Pichler J, Kasper S, Lassnig H, Kornschob er C, Buchner A 2008. A foodborne outbreak of Salmonella Enteritidis phage type 6 in Austria*Wien Klin Wochenschr* 121: 132-136.

[12] Yoshida S Z, Wei Y, Shinmura and Fukunaga N Separation of lactoferrin-a and –b from Bovine colostrums *J. Dairy Science* 83 2211-2215

[13] Kim KS, Kim JS, Shin AS, Noh HW, Lim SD, Suv D and Alimaa J. 2009. Purification and Characterization of Mongolian Mare Lactoferrin. *Korean J. Food Sci. Ani. Resour.* 19(2): 164-167.

[14] Moradian, F., Sharbafi, R. and Rafiei, A., 2014. Lactoferrin, isolation, purification and antimicrobial effects. *J. med. Bioeng.*, 3: 203-206.

[15] Nazzaro F, Orlando P, Fratanni F, Coppola R 2010 Isolation of Components with Antimicrobial Property from the Donkey Milk: A Preliminary Study. *The Open Food Science Journal* 4 43-47

[16] Baldi A, Politis I, Pecorini C, Fusi E, Roubini C and Dell’Orto V 2005 Biological effects of milk proteins and their peptides with emphasis on those related to the gastrointestinal ecosystem. *J Dairy Res.* 72:66-72.

[17] Uniacke-Lowe T, Huppertz T and Fox PF 2010 Equine milkproteins: Chemistry, structure and nutritional significance. *Int Dairy J.* 20:609-629.

[18] Malacarne M, Martuzzi F, Summer A and Mariani P 2002 Review: Protein and fat composition of mare’s milk: some nutritional remarks with reference to human and cow’s milk. *Int Dairy J.* 12:869-877.

[19] Baldi A, Politis I, Pecorini C, Fusi E, Roubini C, Dell’Orto V 2005 Biological effects of milk proteins and their peptides with emphasis on those related to the gastrointestinal ecosystem. *J Dairy Res.* 72:66-72.