Localization and Molecular Size of Mucin2 Glycoproteins Forming the Gut Mucosal Barrier in the Indonesian Indigenous Naked Neck and Normal Feathered Chickens

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ABSTRACT: The cecum of chicken gut may be more susceptible to pathogens because of colonization of microbes. Lower segment of gut is also the primary tissue where microorganisms may invade from external environment colonizing in the cloaca. Mucin composed of glycoproteins play significant roles in the barrier against infection on the mucosal surface. The aim of this study was to determine the differences in the mucosal barrier formation in the lower segment of gut between Indonesian naked neck and normal feathered chickens. The lower segments of gut (rectum, colon, and cecal tonsil) of Indonesian indigenous naked neck and normal chickens were collected. The expression of mucin2 gene in the gut mucosa was analyzed by reverse-transcription-polymerase chain reaction (RT-PCR). Localization and molecular size of the mucosal glycoproteins were analyzed by western blot methods. WGA and Jacalin lectins were used for western blot analysis. Mucin-2 gene was expressed in mucosal gut of rectum, colon, and cecal tonsil in both naked neck and normal chickens. Western blot analysis showed single band in both WGA and Jacalin in mucosal gut of rectum, colon, and cecal tonsil in both naked neck and normal chickens. These results suggest that mucin2 gene as well as glycoprotein containing WGA and Jacalin positive sugars covers the surface of mucosal gut in both naked neck and normal chickens, probably to form mucosal barrier.

Keywords: Indonesian naked neck chickens, Mucosal gut, Mucin-2, Glycoprotein.

INTRODUCTION

In general, mucosal barrier systems formed by mucus gel, epithelial cell junctional structures, and leukocyte activity, play important role to prevent infection by pathogenic agents in mucosal tissues. Mucins have the ability to form a physical barrier and act as adhesion decoys to invading agents (Linden *et al.*, 2008a), and they may prevent pathogen penetrance by inhibiting bacterial adhesion to the mucosal epithelium surface (Berry *et al.*, 2002). Mucins either have direct antimicrobial activity or carry other antimicrobial molecules (Linden *et al.*, 2008b). Cell surface mucins may also initiate intracellular signaling in response to bacteria, and thus they have both a barrier and reporting function on the apical surface of mucosal epithelial cells (Linden *et al.,* 2008a). If microorganisms cross an epithelial barrier and begin to replicate in the mucosal tissues, phagocytic cells including the monocytes or macrophages, or polymorphonuclear leukocytes (PMNs) recognize, ingest, and destroy them (Murphy *et al.,* 2007; Macia *et al.*, 2012). Thus, it is of great importance to identify the mechanism by which mucin is synthesized and epithelial tight junctions are formed in the oviduct of hens to prevent infection of this organ and contamination of eggs by pathogenic agents.

Glycoprotein sugar-residues could be identified and characterized by lectins. Lectins bind to a specific sugar residue of glycoprotein with high affinity. WGA, a lectin from wheat germ agglutinin (*Triticum vulgaris*), binds specifically to N-acetylglucosamine (GlcNAc) and N-acetylneuraminic

acid (sialic acid). Jacalin lectin, the major protein from jackfruit (A*rtocarpus heterophylus*) seeds, shows highly specific binding to galactose (Gal) and N-acetylgalactosamine (GalNAc) (Kabir, 1998; Tatsuzuki *et al.,* 2009; Fallis *et al.*, 2010).

Reports of mucin glycoprotein expression in the Indonesian naked neck and normal feathered chickens were very limited. Therefore, the aim of this study was to determine the differences in the mucosal barrier formation in the lower segment of gut between Indonesian naked neck and normal feathered chickens.

MATERIALS AND METHODS

Experimental birds

Indonesian native naked neck and normal fethered chicken with the age and weight of the relatively uniform were used in this study. All chickens were identified according to non feather distribution, namely naked neck and normal feathered chickens. The lower segments of gut (rectum, colon, and cecal tonsil) of Indonesian indigenous naked neck and normal chickens were collected. The expression of mucin2 gene in the gut mucosa was analyzed by reverse-transcription polymerase chain reaction (PCR). Localization and molecular size of the mucosal glycoproteins were analyzed by SDS-PAGE and western blot methods. WGA and Jacalin lectins were used for western blot analysis.

PCR analysis for expression of mucin2

Total RNA was extracted from the mucosal tissues of rectum, colon, and cecal tonsil using Sepasol RNA I Super. They were treated with DNase to remove genomic DNA and were reverse-transcribed using ReverTra Ace according to the manufacturer's instructions. PCR was performed using Takara Ex Taq. Primers used for mucin2 analysis were as follows (forward: 5'-GTC GAT TGT CAC TCA CGC CTT-3'; reverse: 5'-ACT TGC CTG AAT CAC AGG TGC-3'). PCR products of mucin2 were separated by electrophoresis. PCR was performed as described by Ariyadi *et al.* (2012).

SDS-PAGE and Western blot

SDS-PAGE and Western blot was performed as described by Abdelsalam *et al*., 2010. Rectum, colon, and cecal tonsil tissue was homogenized separately in a 5 times volume of homogenization buffer. The samples were centrifuged at 12,000 X g for 20 min at 4 °C. The supernatant was collected and the protein concentration was measured using a protein assay reagent (Bio-Rad Lab, Hercules, CA, USA) using bovine serum albumen as the standard protein. The samples were separated by Tricine-sodium dodecyl sulphate-polyacrylamide gel electrophoresis (Tricine SDS-PAGE; 16% separating gel and 4% stacking gel) as described by Abdelsalam *et al.* (2011). Samples were mixed with sample buffer. Each 10 µl sample mixture was run on gels. After SDS-PAGE, the proteins in the gel were electrophoretically transferred onto a PVDF membrane (Bio-Rad Lab.) at 270 mA for 1 h. The membrane was soaked in methanol for 10 min and then washed briefly with Tris-buffered saline containing 0.1 % Tween20 (TBS-T) (20 mM Tris HCl, pH 7.6, 0.8 % (w/v) sodium chloride and 0.1 % (v/v) Tween 20). It was incubated with 5% (w/v) casein milk solution in TBS-T for 60 min and then incubated with biotinylated-WGA or Jacalin lectins diluted at a concentration of 10 µg/ml in TBS-T overnight at 4 °C. The membrane was then washed in TBS-T

for 30 min (10 min X 3) before incubation with avidin-peroxidase complex diluted at 1:5,000 in TBS-T for 1 h at room temperature. The membrane was washed with TBS-T for 30 min (10 min X 3 times) and the lectin-precipitates on the membrane were visualized by DAB solutions for 1 min.

RESULTS AND DISCUSSION

Figure 1 shows the expression of mucin2 gene in the rectum, colon, and cecal tonsil of both Indonesian indigenous naked neck and normal feathered chickens. Mucin2 gene was expressed in the rectum, colon, and cecal tonsil of both Indonesian indigenous naked neck and normal feathered chickens. Electrophoresis of PCR product showed that mucin2 gene was expressed at 441 bp, where the band of mucin2 was denser in the naked neck than of the normal feathered chickens.

It was supported by Smirnov *et al.* (2005) that the mucin glycoprotein was expressed in the chicken jejenum and ileum. Rajkumar *et al.* (2010) that the immune competence was higher in the naked neck chickens than of the normal feathered chickens.

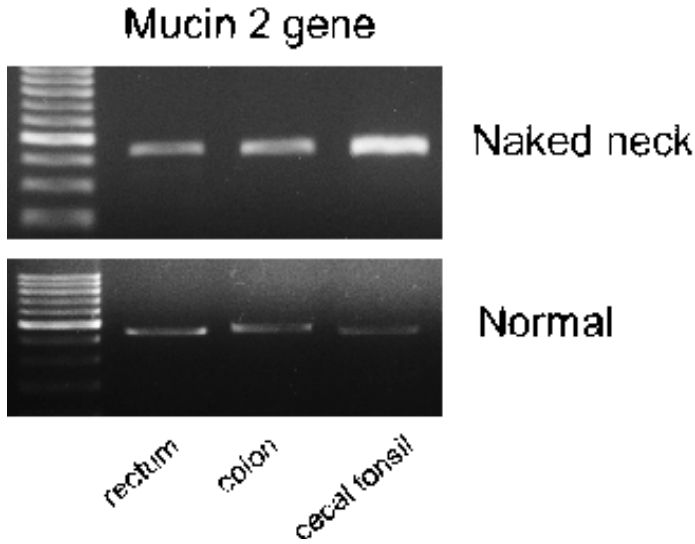

Figure 1. Expression of mucin2 gene in the rectum, colon, and cecal tonsil of both Indonesian indigenous naked neck and normal feathered chickens. Electrophoresis of PCR product showed that mucin2 gene was expressed.

Western blot analysis by WGA lectin showed the single band in the rectum, colon, and cecal tonsil of both Indonesian indigenous naked neck and normal feathered chickens. The molecular size of glycoprotein containing sugar residue was approximately 66.2 kDa. Western blot analysis by Jacalin lectin showed the single band in the rectum, colon, and cecal tonsil of both Indonesian indigenous naked neck and normal feathered chickens. The molecular size of glycoprotein containing sugar residue was approximately 66.2 kDa.

CONCLUSIONS

These results suggest that mucin2 gene as well as glycoprotein containing WGA and Jacalin positive sugars covers the surface of mucosal gut in both naked neck and normal feathered chickens, probably to form mucosal barrier.

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