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Research Article
Expression of Mucin 2 Glycoproteins Forming the Mucosal Barrier of the Gut in Indonesian Indigenous Naked Neck Chickens and Normal Feathered Chickens

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Abstract

Background: The cecum of the chicken gut may be susceptible to pathogens because it is readily colonized by microbes. The lower segment of the gut is also the primary tissue that permits the invasion of microorganisms from the external environment and the cloaca. Mucins, which are composed of glycoproteins, play significant roles in forming the barrier against infection on the mucosal surface. **Objective:** The aim of this study was to determine the differences in the mucosal barrier of the lower segment of the gut between Indonesian naked neck chickens and normal feathered chickens. **Methodology:** The lower segments of the gut (rectum, colon and cecal tonsil) of Indonesian indigenous naked neck chickens and normal chickens were collected. The expression of the mucin 2 gene in the gut mucosa was analyzed by reverse-transcription-polymerase chain reaction (RT-PCR). The localization and molecular sizes of the mucosal glycoproteins were analyzed by Western blot. Wheat Germ Agglutinin (WGA) and jacalin lectins were used for Western blot analysis. **Results:** The mucin 2 gene was expressed in the mucosal gut of the rectum, colon and cecal tonsil in both naked neck chickens and normal chickens. Western blot analysis showed a single band for both WGA and jacalin from the mucosal gut of the rectum, colon and cecal tonsil in both naked neck chickens and normal chickens. **Conclusion:** These results suggest that the mucin 2 gene and glycoproteins containing WGA and jacalin positive sugars cover the surface of mucosal gut in both naked neck chickens and normal chickens, most likely to form a mucosa barrier.

Key words: Indonesian naked neck chickens, mucosal gut, mucin 2, glycoprotein, WGA

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

In general, mucosal barrier systems are composed of mucus, epithelial cell junctions and leukocyte activity. These systems play an important role in preventing infection of mucosal tissues. Mucins form a physical barrier and act as adhesion decoys to invading agents[1] and they may prevent pathogen penetrance by inhibiting bacterial adhesion to the mucosal epithelium[2]. Mucins either have direct antimicrobial activity or carry other antimicrobial molecules[3]. Cell-surface mucins may also initiate intracellular signaling in response to bacteria and they thus have both a barrier and reporting function on the apical surface of mucosal epithelial cells[1]. If microorganisms cross an epithelial barrier and begin to replicate in the mucosal tissues, phagocytic cells, such as monocytes or macrophages or polymorphonuclear leukocytes (PMNs) recognize, ingest and destroy them[4,5]. Thus, it is important to identify the mechanisms by which mucin is synthesized and epithelial tight junctions are formed in the oviduct to prevent infection of this organ and contamination of eggs by pathogens.

Glycoprotein sugar residues can be identified and characterized using specific lectins. Lectins bind to a specific glycoprotein sugar residue with high affinity. The 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 (*Artocarpus heterophyllus*) seeds, shows highly specific binding to galactose (Gal) and N-acetylgalactosamine (GalNAc)[6-8].

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

MATERIALS AND METHODS

Experimental birds: Indonesian native naked neck chickens and normal feathered chickens of relatively uniform age and weight were used in this study. Each chicken was assigned to a breed according to feather distribution. The lower segments of the gut (rectum, colon and cecal tonsil) were collected from each bird. The expression of the mucin 2 gene in the gut mucosa was analyzed by reverse-transcription Polymerase Chain Reaction (PCR). The localization and molecular size of the mucosal glycoproteins were analyzed by SDS-PAGE and Western blot. The WGA and jacalin lectins were used for Western blot analysis.

PCR analysis for expression of mucins: Quantitative reverse-transcription PCR analysis was performed as described previously[9]. Briefly, total RNA was extracted from the mucosal tissues of the ileum using Sepasol RNA I super (Nacalai Tesque Inc., Kyoto, Japan). The extracted total RNA samples were dissolved in TE buffer (10 mM tris, pH 8.0 with 1 mM EDTA). They were treated with 1 U of RQ1 RNase-free DNase (Promega Co., Madison, WI) on a PTC-100 programmable thermal controller (MJ Research Inc., Waltham, MA) at 37°C for 45 min and 65°C for 10 min. The concentration of RNA in each sample was measured using Gene Quant Pro (Amersham Pharmacia Biotech, Cambridge, UK).

The RNA samples were reverse-transcribed using ReverTra Ace (Toyobo Co., Ltd., Osaka, Japan) according to the manufacturer's instructions. The reaction mixture (10 μL) consisted of 1 μg of the total RNA, 1x RT buffer, 1 mM dNTP mixture, 20 U of RNase inhibitor, 0.5 μg oligo (dT) 20 primer and 50 U ReverTra Ace. Reverse transcription was performed at 42°C for 30 min, followed by heat inactivation for 5 min at 99°C using the PTC-100 programmable thermal controller (MJ Research Inc.).

The PCR was performed using Takara Taq (Takara Bio Inc., Shiga, Japan) according to the manufacturer's protocol. The primers were as follows (forward: 5'-GTC GAT TGT CAC TCA CGC CTT-3', reverse: 5'-ACT TGC CTG AAT CAC AGG TGC-3'). The PCR mixture (25 μL) contained 0.5 μL cDNA, 1x PCR buffer, 1.5 mM $MgCl_2$, 0.2 mM of each dNTP, 1.25 U Takara Taq and 0.5 μM of each primer. The mucin gene was amplified in a PTC-100 programmable thermal controller (MJ Research Inc.) under the following conditions: 94°C for 30 sec, then denaturation at 34 cycles at 95°C for 30 sec, annealing at 58°C for 60 sec and extension at 72°C for 60 sec. The PCR products were separated by electrophoresis on a 2% (w/v) agarose gel containing 0.4% (w/v) ethidium bromide.

SDS-PAGE and Western blot analysis: The SDS-PAGE and a Western blot were performed as described by Abdelsalam *et al.*[10]. Rectum, colon and cecal tonsil tissues were homogenized separately in a 5-fold volume of homogenization buffer consisting of 20 mM tris-HCl (pH 7.4), 150 mM NaCl, 5 mM EDTA, 1% (v/v) triton X-100, 10% (v/v) glycerol, 0.1% (w/v) SDS and 1 mM phenylmethylsulfonyl fluoride using a polytron homogenizer (Polytron PT1200c, Kinematica AG, Switzerland). The samples were centrifuged at 12,000×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) with bovine serum albumin as the standard protein.

The samples were separated by tricine-sodium dodecyl sulfate-polyacrylamide gel electrophoresis (Tricine

SDS-PAGE, 16% separating gel and 4% stacking gel) as described by Abdelsalam *et al.*[10]. Samples were mixed with sample buffer composed of 30% (v/v) glycerol, 5% (v/v) mercaptoethanol, 4% (w/v) SDS, 0.06% (w/v) bromophenol blue and 150 mM tris-HCl, pH 7.0 at a sample protein concentration of 1 μg μL^{-1} and boiled for 5 min. Each 10 μL sample mixture was run on gels. After SDS-PAGE was complete, 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% tween 20 (TBS-T) (20 mM tris HCl, pH 7.6, 0.8% (w/v) sodium chloride and 0.1% (v/v) tween 20). The membrane was incubated with a 5% (w/v) casein milk (Roche, Mannheim, Germany) solution in TBS-T for 60 min and then incubated with rabbit anti-chicken AvBD12 polyclonal antibody Abdel Mageed *et al.*[11] diluted to a concentration of 10 μg mL^{-1} in TBS-T or mouse anti-chicken β-actin monoclonal antibody (Santa Cruz Biotechnology, Santa Cruz, CA) diluted 1:5,000 in TBS-T overnight at 4°C. The membrane was then washed in TBS-T for 30 min (10 min X3) before incubation with peroxidase-labeled anti-rabbit IgG for AvBD12 or anti-mouse IgG for β-actin (GE Healthcare, Buckinghamshire, UK) 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 X3 times) and the lectin precipitates on the membrane were visualized using DAB solutions for 1 min.

Statistical analysis: Fold changes in mucin expression were expressed as the Mean±SEM. The data were analyzed by Completely Randomized Designs (CRD) one-way ANOVA followed by Duncan's new multiple range test (DMRT). Differences were considered significant at $p<0.05$.

RESULTS

Figure 1 shows the expression of the mucin 2 gene in the rectum, colon and cecal tonsil of Indonesian indigenous naked neck chickens and normal feathered chickens. The mucin 2 gene was expressed in the rectum, colon and cecal tonsil of Indonesian indigenous naked neck chickens and normal feathered chickens. Electrophoresis of the PCR product showed that the mucin 2 gene was expressed at 441 bp, with a denser band for the naked neck chickens than for the normal feathered chickens.

Figure 2 shows a Western blot analysis of WGA lectin in the rectum, colon and cecal tonsil of Indonesian indigenous

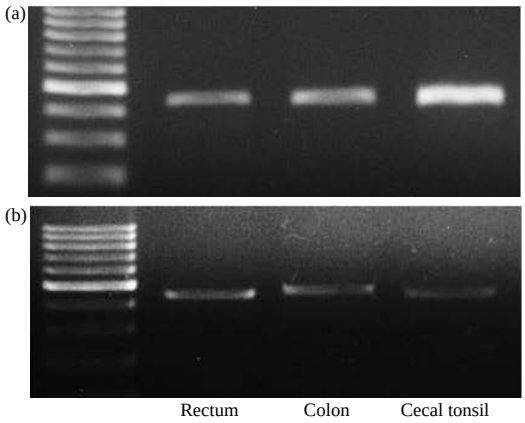

Fig. 1(a-b): Expression of the mucin 2 gene in the rectum, colon and cecal tonsil of Indonesian indigenous (a) Naked neck chickens and (b) Normal feathered chickens. Electrophoresis of the PCR product showed that the mucin 2 gene was expressed

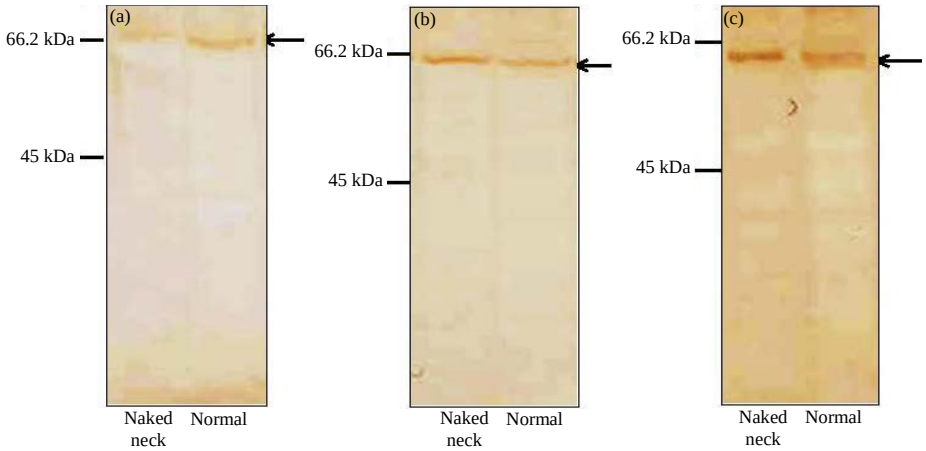

Fig. 2(a-c): Western blot analysis using WGA lectin in the (a) Rectum, (b) Colon and (c) Cecal tonsil of Indonesian indigenous naked neck chickens and normal feathered chickens. The molecular size of the glycoprotein containing the sugar residue in the rectum, colon and cecal tonsil was approximately 66.2 kDa (arrows)

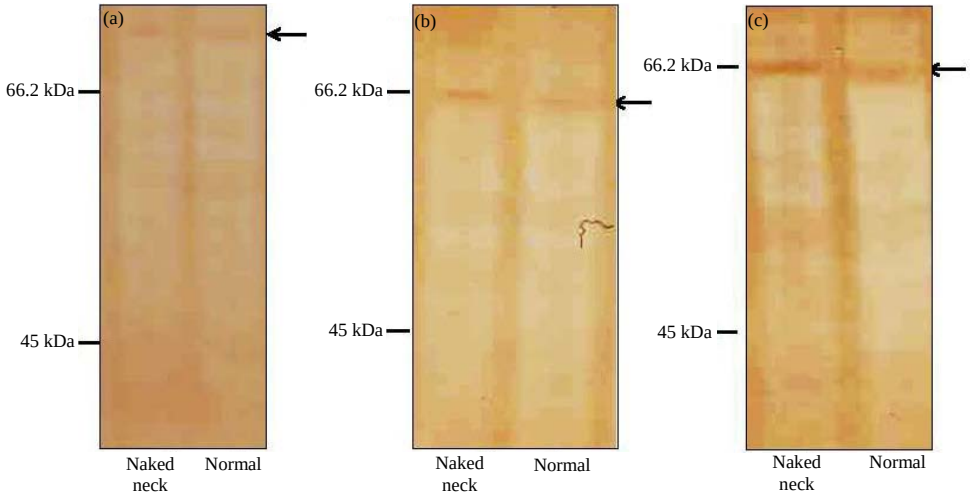

Fig. 3(a-c): Western blot analysis using jacalin lectin in the (a) Rectum, (b) Colon and (c) Cecal tonsil of Indonesian indigenous naked neck chickens and normal feathered chickens. The molecular size of glycoprotein containing sugar residue in the colon and cecal tonsil was approximately 66.2 kDa (arrows)

naked neck chickens and normal feathered chickens. The molecular size of the glycoprotein containing the sugar residue was approximately 66.2 kDa.

Figure 3 shows a Western blot conducted with jacalin lectin in the rectum, colon and cecal tonsil of Indonesian indigenous naked neck chickens and normal feathered chickens. The molecular size of glycoprotein containing the sugar residue was approximately 66.2 kDa.

DISCUSSION

We here identify the differences in the mucosal barrier formation in the lower segment of the gut between Indonesian naked neck chickens and normal feathered chickens. Significant findings included the discoveries of the mucin 2 gene and the glycoproteins containing WGA and Jacalin positive sugars that cover the surface of mucosal gut in both naked neck chickens and normal chickens.

The mucin 2 gene is expressed in the rectum, colon and cecal tonsil of Indonesian indigenous naked neck chickens and normal feathered chickens. Electrophoresis of the PCR product showed that the mucin 2 gene was expressed at 441 bp with a denser band for the naked neck than for the normal feathered chickens. Smirnov *et al.*[12] previously found that the mucin glycoprotein was expressed in the chicken jejunum and ileum. Rajkumar *et al.*[13] revealed that the immune competence is higher in naked neck chickens than in normal feathered chickens. Ariyadi *et al.*[9] showed that expression of mucin mRNA was higher and immunoreactive mucin 5 AC on the surface of the mucosal epithelium was denser in laying hens than in molting hens.

Western blot analysis using WGA lectin revealed a single band in the rectum, colon and cecal tonsil of Indonesian indigenous naked neck chickens and normal feathered chickens. The molecular size of the glycoprotein containing the sugar residue was approximately 66.2 kDa. Western blot analysis using jacalin lectin revealed a single band in the rectum, colon and cecal tonsil of Indonesian indigenous naked neck chickens and normal feathered chickens. The molecular size of the glycoprotein containing the sugar residue was approximately 66.2 kDa. Ariyadi *et al.*[14] showed that estrogen upregulates mucin expression and increases the number of mucosal glycoproteins in the oviduct of laying hens.

CONCLUSION

These results suggest that mucin 2 and glycoproteins containing WGA- and jacalin-positive sugars cover the surface of mucosal gut in both naked neck chickens and normal feathered chickens, most likely forming a mucosal barrier.

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