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E-mail: editorijps@gmail.com

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Research Article
Effects of *Ascaridia galli* Infection on Mucin-Producing Goblet Cells in the Mucosal Duodenum of Indonesian Local Chickens (*Gallus domesticus*)

[1]J. Prastowo and [2]B. Ariyadi

[1]Faculty of Veterinary Medicine, Universitas Gadjah Mada, Jalan Fauna 3, Bulaksumur, Yogyakarta 55281, Indonesia
[2]Faculty of Animal Science, Universitas Gadjah Mada, Jalan Fauna 3, Bulaksumur, Yogyakarta 55281, Indonesia

Abstract

Background and Objective: Chickens infected with *Ascaridia galli* worms (*A. galli*) suffer from slow growth and decreased weight gain. Infection with A. galli usually causes severe damage to the intestines due to migration of the worms in the tissue phase of the intestinal mucosa layer, which affects the proliferation of goblet cells in the duodenums of affected chickens. It is necessary to conduct a study that evaluates the effects of *A. galli* infestation on the number of goblet cells in the duodenal epithelium of infected chickens. **Methodology:** In this study, we used a total of 20day-old chicks (DOC). The A. galli worms used in this study were obtained from local chicken slaughterhouses. The worm eggs were embryonized to the L2 stage and inoculated with as many as 5000 eggs/chicken. Samples of the duodenum were taken after the chickens were 6 weeks old, at which time Periodic Acid Schiff (PAS) staining was performed. The elimination rate of the number of worm eggs after the infection was observed every once a week up to week 6 of the research period. Results were analyzed using Student's t-test and descriptive analysis. **Results:** There tended to be a higher number of mucin-producing goblet cells in the treatment group than in the control group. The number of worm eggs found increased until the 21st day after infection. The peak of worm egg elimination occurred on the 21st day after infection, with a mean of 2000±250 eggs per gram (EPG); however, the number decreased again on the 28th day after infection. **Conclusion:** Based on the results of this study, it can be concluded that A. galli infection has a significant effect ($p<0.05$) on the increased number of goblet cells in the duodenal epithelium of broiler chickens. This worm infection also affects the length of the duodenum and the increase in chicken body weight on a weekly basis.

Key words: *Ascaridia galli*, goblet cells, Indonesian local chickens, mucus, PAS staining

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Corresponding Author: Bambang Ariyadi, Faculty of Animal Science, Universitas Gadjah Mada, Jalan Fauna 3, Bulaksumur, Yogyakarta 55281, Indonesia
Tel: +62 274 513363

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

INTRODUCTION

The mucus produced by goblet cells provides intestinal surface protection from the threat of invading *A. galli*. The mucus released by goblet cells confines the movement of the worms by covering their cuticles, so they are unable to attach to the intestinal mucosa; with the help of peristalsis, the worm is then removed from the body[1]. Goblet cells synthesize and secrete glycoprotein-shaped mucus to protect intestinal epithelial cells from different kinds of invasion, including worm invasion by *A. galli*. Cytokine activation stimulates goblet cell proliferation[2].

Ascaridia galli worm infection in birds is widespread throughout the world and can be seen in both domestic and wild poultry birds[3]. Infection with *A. galli* often leads to decreased growth rates and weight loss[4]. This is likely associated with intestinal mucosal damage, which causes blood loss and secondary infections[5]. The severity of intestinal mucosal damage depends on the number of worms present in the intestines[6]. Worm infection causes chronic hemorrhage because migratory larvae cause gastrointestinal damage, including gastritis, enteritis and ulceration of the tractus digestivus,which eventually leads to a condition called chronic blood loss[7]. Anwar andZia-ur-Rahman[8] stated that there was a decrease in serum electrolytes, sodium, potassium and calcium levels on days 21 and 40 after infection with 350 embryonic *A. galli* eggs. However, the serum electrolytes, magnesium and phosphorus levels did not change significantly.

Worm infection also causes the occurrence of food fluid and intestinal blockage by roundworms and tapeworms and the inner lining damage ofthe intestine. *Ascaridia galli* worm infection usually causes severe damage to the intestinum at the tissue phase during worm migration. This migration occurs in the intestinal mucosal lining and causes hemorrhagic enteritis, along with impaired digestion and nutrient absorption, which therefore affect mucin production by the epithelium[9]. It is necessary to investigate how to evaluate the effects of *A. galli* worm infestation on the goblet cells that produce mucin in the mucosal duodenum of Indonesian local chickens (*Gallus domesticus*).

MATERIALS AND METHODS

Research design: In this study, we used a total of 20 Indonesian local day-old chicks (DOC). The *A. galli* worms were obtained from local chicken slaughterhouses. Chickens were adapted to the experimental cages for 2 weeks; prior to infection with *A. galli*, all of the chickens were treated with commercial worm medicines. The chickens were grouped in to a control and an infected group, each consisting of 10 birds.

Preparation of infective *A. galli* eggs: The *A. galli* worm was put into a beaker containing 0.85% physiological NaCl. All the worms were washed with solution several times until they were clean of dirt. The selected female worms were marked by a large body size and a straight tail end. The female worms were then placed into a beaker containing physiological NaCl. The collected female worm was cut in the posterior genitals, which is the boundary between dark and light and then the eggs and uterus were obtained by massaging the worm's body. The eggs were inserted into a glass containing 50 mL 0.5 N NaOH and were then stirred using a magnetic stirrer for 30 min. Then, eggs were allowed to stand for 10 min to settle and the supernatant was discarded. This process was repeated up to 3 times. The solution laden eggs were centrifuged 3 times prior to the incubation (embryonization). The centrifuged eggs were inserted into an Erlenmeyer tube containing 150 mL of aquadest. An oxygenator pipe was inserted into the tube, then the mouth of the tube was covered with cotton and the oxygenator was turned on. The eggs were muddy at room temperature for 2 weeks. After 2 weeks, the eggs contained stage II larvae and were ready to infect the chickens[10].

***Ascaridia galli* infection:** The chicken treatment group was infected with as many as 5000 eggs/chicken orally, while the control group was given peroral aquades.

Duodenal sampling: Duodenal samples were taken at the end of the 6th week. Ten chickens from each treatment group were euthanized using a sharp knife that cut the carotid artery, jugular vein, trachea and esophagus. Duodenums and pancreases were obtained and the length of the duodenum was measured using a ruler[11].

Periodic acid schiff (PAS) staining: Duodenal samples were fixed with formalin solution, dehydrated and immersed in paraffin. A sample of tissue was minced at a thickness of 4 μm, gently pressed against the tissue surface and allowed to dry on to a slide/object glass. The tissue on the object glass was then immersed into the Periodic Acid Schiff (PAS) solution (Merck, Darmstadt, Germany). Data were determined by summing all of the active goblet cells found in the duodenal villi, which was done by performing calculations per 1000 absorptive cells in the villi[12].

Data analysis: The data for the weight of the chickens during maintenance, as well as the number of *A. galli* worms found in the lumen and duodenal mucosa, the number of *A. galli* eggs per gram of feces, duodenal length and number of goblet cells with staining of Periodic Acid Schiff (PAS) were analyzed using Student's t-test and descriptive analysis.

RESULTS AND DISCUSSION

We identified *Ascaridia galli* infection in goblet cells of the mucosal duodenum of Indonesian local chickens. Our significant findings were: (1) The number of mucin producing cells (goblet cells) of chickens in the treatment group were significantly higher than those in the control group, (2) The number of worm eggs found increased until the 21st day after infection and (3) The peak of worm egg elimination occurred on the 21st day after infection and then decreased again on the 28th day after infection.

The goblet cell numbers, number of worms and length of the duodenum in the control group and treatment groups are presented in Table 1. The average number of goblet cells in the control group was 235±31.98 and the average number of goblet cells in the treatment group was 399±53.31. The result of the t-test shows that there is a significant difference ($p<0.05$) between the groups, indicating that treatment of the worm infection had a positive effect on the number of goblet cells present in the treatment group. There tended to be a higher number of goblet cells in the treatment group than in the control group.

Ascaridia galli worm infection causes an increase in the number of goblet cells that produce mucin. This is also influenced by the life cycle of the worm, which undergoes molting in the duodenum[13]. The above conditions indicate that infection with *A. galli* stimulates the defense response of the mucous membranes in the intestines of infected chickens. The basis of the immune response comes from mucus derived from goblet cell secretions located in the surface of the small intestine. This mucus responds to the presence of parasites in the intestine by catching worm larvae in the intestinal lumen. Increased mucus can be caused by increased numbers of goblet cells. After four moltings, the young *A. galli*, or L5, have grown and reached adulthood in the duodenal lumen, leading to an increase in the number of goblet cells that can be seen in the duodenum.

Athaillah[13] stated that the prepatent period of worming occurs in the duodenum within 11-15 weeks; however, Urquhart *et al.*[14] stated that the prepatent period for *A. galli* worms is 5-6 weeks. This distinction is one manifestation of the competition among individual worms in the fight for habitat and nutrition. Additionally, the defensive response of each individual chicken is different, which potentially caused variation in the number of goblet cells and made inaccuracy calculation.

Localization of Periodic Acid Schiff (PAS) positive substances in the duodenum with or without *A. galli* infection is presented in Fig. 1. Observations of intestinal preparations not infected with *A. galli* show that the divisions between the tunica mucosa, submucosal tunica and tunica

Table 1: Influence of *Ascaridia galli* worm infection against local chickens

	Control group	Treatment group
No. of goblet cells	235±31.98	399±53.31*
No. or worm	0±0	0.5±0.92*
Length of the duodenum (cm)	27.75±0.76	19.5±2.31*

*$p<0.05$

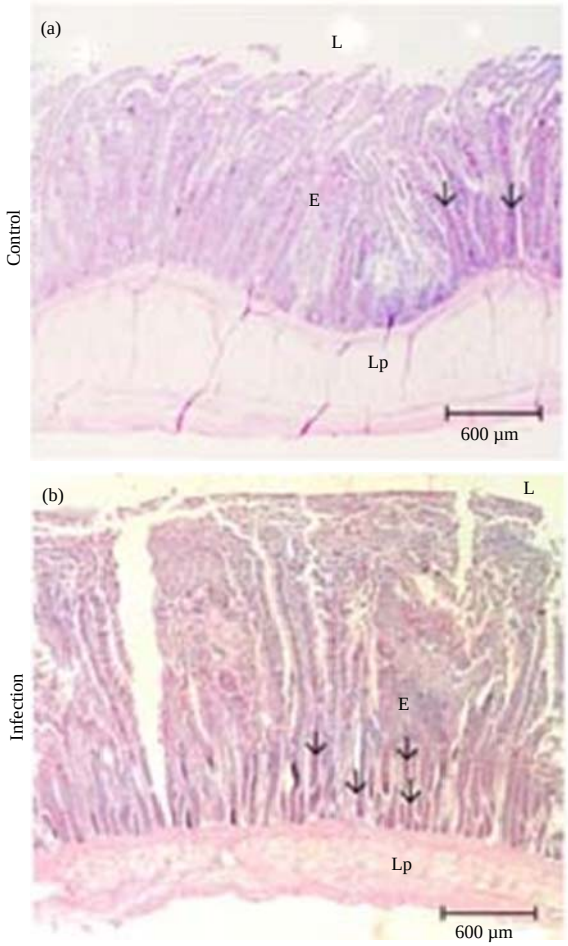

Fig. 1(a-b): Localization of Periodic Acid Schiff (PAS) positive substances in the duodenum with or without *Ascaridia galli* infection

Note that PAS- positive substances are localized in surface epithelium (arrow). E: Mucosal epithelium, L: Lumen of intestine, Lp: Lamina propria

muscularis is still clear. The villi are regular, blunt, wide and distinguishable. A simple columnar villous cell with a 1/3 basalt core is still visible and there are not many Lieberkuhn crypts .Observations of intestines that have been infected with *A. galli* showed villi degeneration. In the duodenal histopaths of the treatment group, the intestinal villi tended to appear longer than the villi of the control group. The duodenal villi in chickens from the treatment group also tended to be irregular with necrosis in the villous epithelium, which made it hard to distinguish from the surrounding tissue. Within the intestine, the worm infection obviously damaged a number of Lieberkuhn crypts that stayed in the initial phase of regeneration.

The presence of worm infection often causes goblet cells to increase and the submucosal tunica layer of the bowel to be thinner. Zalizar *et al*.[15] stated that infection with *A. galli* usually stayed in mild level and will lead to degeneration and mild necrosis of villous epithelial cells, as well as the small intestinal crypts. Epithelial cells in the digestive tract play a role in the digestion of food by producing various enzymes that digest different types of nutrients and altered them in a form that can be easily absorbed by the chicken intestine.

Large numbers of goblet cells can be found in intestines infected with *A. galli* (Fig. 1b). This infection causes the goblet cells to experience a proliferation, which aims to allow the animal to survive the infection. Darmawi *et al*.[16] revealed that goblet cell proliferation plays a role in the mechanism by which *A. galli* larvae are excreted, which occurs through the secretion and release of mucin into the intestinal lumen to increase the mucus capacity so that the larvae can quickly be removed from the host's body. This research agrees with Morrow[17], who stated that *A. galli* infection may cause a loss in production of disaccharidase enzymes (enzymes that digest disaccharide carbohydrates) in the apical part of the villi due to changes in the villi that cause a decrease in digestion of metabolic energy. Extensive damage to epithelial cells in the gastrointestinal tract of infected chickens may result in the replacement of functional cells with immature and non-functional cells, forming a friable intracellular complex.

Balqis *et al*.[18] stated the higher the number of *A. galli* infections in the duodenum, the higher the proliferation of goblet cells. Goblet cell proliferation provides intestinal surface protection from the threat of invading *A. galli* and the mucus released by goblet cells confines a worm's movement by covering the cuticle making it unable to attach to the intestinal mucosa and, with the help of intestinal peristalsis, to be excreted with the feces.

Based on the results in Table 1, it can be seen that at 4 weeks after infection the mean number of worms living in the duodenum was 0.5±0.92 tail (0.08%). *Ascaridia galli* worms that were alive on the 28th day post-infection were only found in the duodenums of two chickens: B7 (2 worms) and B8 (2 worms), which amounted to a total of 4 worms. The presence of worms in the chickens' duodenal lumen indicates that the dose of egg infection performed is in the value of the *A. galli* antigen, which allowed the worms to survive. According to Athaillah[13] a significant dose of infection is seen with a dose of 4000 infective eggs. This dose appears to be just below the antigen's threshold value, which can evoke a protective immune response in chickens.

In this study, the average length of the duodenum 4 weeks after infection was 27.75±0.76 cm for the control group and 19.5±2.31 cm for the treatment group. *Ascaridia galli* worm infection tends to affect the length of the duodenum, with lengths from chickens in the treatment group being shorter. The results of the t-test shows that there is a significant difference ($p<0.05$) between the lengths of the duodenum, indicating that worm infection had an effect on the length of the duodenum. The length of the chicken duodenums in the treatment group tended to be shorter than in the control group. In adult chickens, the length of the small intestine is approximately 62 inches or 1.5 m. The anatomical small intestine is divided into three parts, namely, the duodenum, jejunum and ileum. The duodenum is located at the very top of the small intestine and reaches a length of 24 cm[19]. According to Michel[20], the tissue phase that occurs from day 1-26 of infection causes the network to experience developmental resistance (retained) due to larvae entering the intestinal lining membrane. *Ascaridia galli* worms live on the duodenal lender membrane from day 8-17 after infection. Larvae 5 (L5) (young worms) return to the duodenal lumen on the 17th-18th day after infection.

Infected chickens further were monitored every seven days during the research period to investigate the elimination of worm eggs (Table 2). It has been accepted that the number

Table 2: Mean of elimination of worm eggs of treatment group (egg per g EPG^{-1})

Days after infection	No. of worm eggs (egg per g EPG^{-1})
7	950±118.75
14	1800±225
21	2000±250
28	700±87.5

Table 3: Weight gain of control group and treatment group of day 28 after infection

Week after infection	control group (g)	treatment group (g)
Week 1	101.25±14.5	101.0±15.5
Week 2	102.4±15.4	57.5±14.5*
Week 3	102.5±15.7	55.0±8.8*
Week 4	112.5±9.5	25.0±4.5*

*($p<0.05$)

of worm eggs found increases until the 21st day after infection. The peak of worm egg elimination occurred on the 21st day after infection with a mean of 2000±250 eggs per gram (EPG), with the number decreasing again on the 28th day of infection.

Table 3 demonstrates that the mean amounts of weight gained in control-group chickens and treatment-groups chicken were almost the same in the first week. In week 2 there was an average difference in chicken weight gain, with the control group gaining an average of 102.4±15.4 g week^{-1} and the infected group gaining 57.5±14.5 g week^{-1}. In week 3 there was an average difference of chicken weight gain with the control group gaining 102.5±15.7 g week^{-1} and the infected group gaining 55.0±8.8 g week^{-1}. The differences of chickens' weight of both groups measured in week 4 were significant. The average of weight in the control group was higher than those in the treatment group with value 112.5±9.5 and 25.0±4.5 g week^{-1}, respectively.

The results of the t-test show a significant difference ($p<0.05$), meaning that treatment of the worm infection had an effect on the increase in chicken weight seen in the treatment group. The increase in chicken weight in the treatment group tended to be lower than the increase seen in the control group. This is similar to what was observed by Athaillah[13], where there was an increase in body weight in the treatment group but this remained below the body weight of the controls. The body weight of the treatment group doesn't match the body weight of the control group due to the worm infection. *A. galli* causes slowing of growth and intestinum mucosal damage that disrupts the absorption of nutrients for infected chickens[9].

CONCLUSION

Based on the results of this study, it can be concluded that infection with the worm *A. galli* significantly influences the increase of goblet cell numbers in the duodenal epithelium of chickens. This worm infection also affects the length of the duodenum and weight gain of the infected chickens.

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