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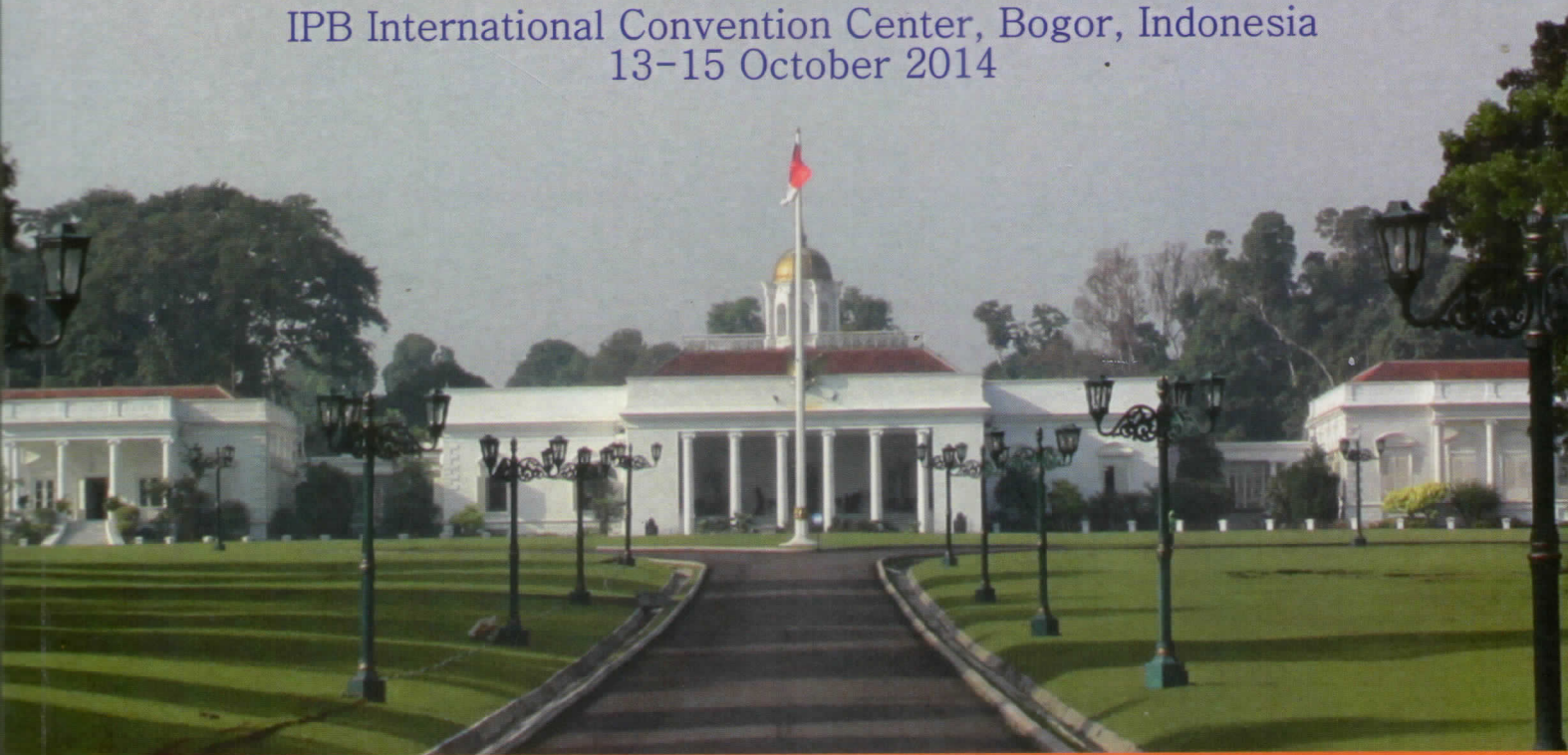
3 JOINT INTERNATIONAL MEETINGS 2014

THE 14TH ANNUAL WORKSHOP OF THE REGIONAL NETWORK ON ASIAN SCHISTOSOMIASIS AND OTHER HELMINTH ZOOZOSIS

THE 5TH ANNUAL MEETING OF SOUTH EAST ASIA VETERINARY SCHOOL ASSOCIATION

THE 3RD SCIENTIFIC MEETING OF INDONESIAN VETERINARY SCHOOL ASSOCIATION

IPB International Convention Center, Bogor, Indonesia
13-15 October 2014



Faculty of Veterinary Medicine
Bogor Agricultural University



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The Effect of Pepsin and Trypsin Enzym on Anti H5N1 IgG Titer of Colostrum from Bovine Vaccinated with H5N1 Vaccine

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Key words: IgG, pepsin, trypsin, colostrums

INTRODUCTION

Passive immunization is one of choice to cure of H5N1 virus infection due to lack of H5N1 vaccine production for human. The ability of H5N1 virus mutation proved to be much faster than a human's ability to produce vaccines on time [1]. Research of Itoh *et al.* [2] showed that passive immunization with MAb ch61 one day before or after challenge with a lethal dose of the virus completely protected mice, and partial protection was achieved when mice were treated 3 days after the challenge. Passive immunization using monoclonal antibody has to be done through injection and not applicable per-oral. Therefore, passive immunization approach through the use of colostrum hiperimun can be applied in efforts to control bird flu (Avian Influenza / AI).

However, per-oral administration of colostrum will expose the IgG of colostrum to h enzymes in the digestive tract such as pepsin and trypsin. This research was conducted to study the effects of exposure to anti-H5N1 IgG in the colostrum of the enzyme pepsin and trypsin.

MATERIALS AND METHODS

Colostrums were derived from pregnant Holstein Friesian cows vaccinated with subtype H5N1 vaccines 3 times at the final trimester. The vaccine were administrated subcutaneous with 2 weeks interval. Colostrum samples were collected immediately after birth of the cow. Colostrums were prepared using methods Esfandiari *et al.* [3]. Analysis for the presence of IgG anti-AI H5N1 in colostrum performed using indirect Enzyme Linked Immunosorbent Assay (ELISA) method. IgG anti-AI H5N1 exposure to pepsin and trypsin was performed using the method of Soejoedono *et al.* [4]. IgG subsequently titrated with haemagglutination inhibition test (HI test).

RESULT AND DISCUSSION

The result of ELISA of colostrum samples were showed that the colostrums contain IgG anti H5N1 AIV as much as 1.05 ELISA Unit. The HI test showed that the titer of IgG anti H5N1 in colostrums were very high i.e 2⁸. According to Angi [5], antibody anti H5N1 AIV with Titer 2⁵ until 2⁸ were able to neutralize H5N1 AIV in the field. In chicken, the minimum standart antibody that able to prevent AIV H5N1 infection is 4 (log₂) or 2⁴ by HI test [6].

The exposure of trypsin to IgG of colostrum for 30 and 60 minutes were decreased the antibody titer (Table 1). It's showed that trypsin caused antibody damaged, indicated by decreasing antibody titers. Trypsin is one of the three proteinase digestive tract enzym, other enzym are pepsin and chymotrypsin. Along with other proteinase enzym in the digestive tract trypsin broke down protein molecules into the component peptides and amino acids, so the glycoprotein IgG molecules also broken down by the enzyme trypsin. IgG hydrolysis by trypsin lead IgG structure split into three fragments. Fragmentation is located near the disulfide bonds in the flexible region of IgG. The fragmentation of trypsin was similar to that formed by the papain exposure. This indicates that the structure of the IgG fraction due to trypsin occurs in the same area as a result of the influence of papain [7].

Exposure of pepsin also caused the decrease of in antibody titer. Titers of antibodies in colostrum only 2³ after for 30 minutes of pepsin incubation and lower i.e only 2² after 60 minutes of

incubation (Table 1). As well as trypsin, pepsin also show the damaged of antibody molecules. Several studies have showed that antibodies will remain after digested by pepsin and trypsin. Pepsin and trypsin caused the structural damage and separate IgG immunoglobulin fragments into Fab, Fc, and Fab'2. Fab'2 and Fab fragments still have the ability to bind antigens and demonstrated the ability of neutralization [8]. Pepsin exposure caused the isolation of Fab' but IgG molecule still has the ability to precipitate antigen. Fc portion of immunoglobulin structure will be further degraded. Degradation resulted in the loss of the Fc portion of the immunoglobulin binding site. The loss of the "arms" of the Y structure affects the ability of IgG in the IgG antigen binding, which means the ability to bind IgG in the antigen may be reduced or even disappear [9]. Immunoglobulins, both IgG and IgY are sensitive to high temperature, pH and enzymes. IgG will experience a decrease in activity in the digestive tract, one of which is caused by the influence of the enzyme pepsin and trypsin, in addition to the influence of the pH and temperature. Treatment of IgY at pH 2 and pepsin exposure shows the degradation of proteins, resistant to pH 4 but not resistant to trypsin activity [4]. Pepsin and trypsin is a proteolytic enzyme that plays a role in the digestive tract. Pepsin is found specifically in the stomach, it has activity to break down the protein molecules into smaller molecules which peptone and proteosa. Pepsin is known as milk coagulated. The presence of this enzyme that affects immunoglobulin in the digestive tract can be damaged of Immunoglobulin. Immunoglobulin can be applied orally with liposomes packaged in capsules, coated with polymer or with the addition of sugar so that the antibody is able to withstand the proteases when administered orally [8].

Table 1 Antibody titers of colostrum samples which treated with enzymes.

Colostrum antibody titers before treatment (2 ⁿ)	Colostrum antibody titers after treatment (2 ⁿ)			
	Trypsin		Pepsin	
	30'	60'	30'	60'
8	0	0	3	2
8	0	0	3	2
8	0	0	3	2

CONCLUSION

IgG anti H5N1 from colostrum were decreased of titre after typsin and pepsin exposure, so for application per oral in passive immunization, IgG need to be coated by substance that able to protected IgG from enzym.

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