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ENTOMOLOGY

Entomology contribution in animal immunity: Determination of the crude thoraxial glandular protein extract of *Stomoxys calcitrans* as an antibody production enhancer in young horses

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Abstract

This experiment was conducted to evaluate the level of antigens protein contained in the crude thoraxial glandular protein (TGP) extract of *Stomoxys calcitrans* which function as immunity enhancer in young horses. The detection of protein content of the thoraxial glandular samples was performed by using a spectrophotometer Nano Drop-1000. This result showed that the lowest level of antigen protein was 0.54 mg/mL, the highest was 72 mg/mL, and the average was 0.675 mg/mL. Six foals were used and divided into two groups. The first group was treated with a solution of 100 µg of TGP by subcutaneous injection, the other group acted as control. The TGP extract was injected on the first day of the experiment. Three ml of blood were sampled from the jugular vein on the 14th day after TGP injection. The blood sampled was centrifuged and its serum placed in micro-tubes to observe the IgG level. The injection of TGP had a significant effect on the IgG level of the experiment animals ($P<0.05$). This experiment emphasized an important relation between entomology and animal husbandry; health improvement in the young animals was observed after the injection of the insect antigen, so it can be concluded that crude thoraxial glandular proteins of *S. calcitrans* can be used to improve the immunoglobulin-G circulation in foals.

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Introduction

The flies, especially the ones belonging to the family *Muscidae,* are often considered as bio-transmitters of pathogen microbes both in animals (Brits *et al.*, 2016; Iqbal *et al.*, 2014; Rumokoy *et al.*, 2017) and experiment plants (Kalaikesar *et al.*, 2016; Wasala *et al.*, 2013; Talley *et al.,* 2009). The importance of research work in entomology in relation with mammalian immunity was already described (Obame-Nkoghe *et al.*, 2017; Toar *et al.,* 2017); the stable fly *Stomoxys calcitrans* (*Diptera, Muscidae*) plays an important role as disease vector in humans and animals. *S. calcitrans* is a blood feeding insect, injecting its saliva produced in the salivary glandular located in the thoraxial segment (Baldachino *et al.,* 2013).

The organic components of saliva include digestive enzymes, helping the insect to acquire a meal from the blood. Anticlotting proteins with multiple isoforms were found in the saliva of horn flies (Cupp *et al.,* 2009; Untalan *et al.,* 2006). The saliva of *S. calcitrans* has been used for farm animal defense against the insect itself as pest. The injection of crude saliva-gland extract of *S. calcitrans* in cows showed an increase of IgG antibodies (Ameri *et al.*, 2008) minimizing the damage caused by *S. calcitrans* to experiment animals.

These scientific results in entomology gave a solution against the hypo-globulinemia in young horses. Young animals affected by hypo-globulinemia were easily infected by the pathogenic microbes and parasites from the *ex-utero* environment (Rumokoy & Toar, 2014). According to Grongnet (1996) this condition in neonates often followed a failure of passive transfer that caused a high mortality rate.

Based on this background we carried out this study to evaluate the antigen proteins in the crude thoraxial glandular protein

extract and the consequences of the TGP treatment on the serum IgG level of foals.

Materials and methods

The sampling of *C. calcitrans* was carried out at the farm of *Sentrum Agraris Lotta* (SAL) in Manado, North Sulawesi, Indonesia. Six experiment foals were reared in traditional farms in the Minahasa region, North Sulawesi, Indonesia. The identification of the antigen protein was conducted at the Laboratory of Immunology and Parasitology at the University of Salamanca, Spain.

Preparation of the thoraxial glandular protein extract

The extraction of the crude TGP of *S. calcitrans* was adapted from procedure of Swist *et al*. (2002). The dissection of *Stomoxys calcitrans* was run in a petri. This dissection was realized under a photonic microscope, model Meiji EMZ-TR. After obtaining TGP, we proceeded to its identification with a ND-1000 spectrophotometer and the separation of protein molecules by a SDS-PAGE.

Animals and analysis of the immunoglobulin-G serum

Six foals of local breed, under traditional rearing were used in this experiment. The blood serum IgG level of the animals was analyzed by using the *Single Radial Immuno-Diffusion* method, starting with the following procedures: filling four wells with 15 µL of standard solution, and then filling the trench (well) gel with a 15 µL sample of blood plasma. Then the plate was moved into the incubator box at a temperature of 30-40°C, left there for about 16 hours so that the antibodies could diffuse in the gel containing the anti-IgG antigen. After that, the plate was filled with a solution of 2% acetic acid and incubated for one minute. The following stage involved rinsing the drain plate and the gel twice using deionized water. Then, for the last time, the plate was filled with deionized water or distilled water and incubated for approximately ten to fifteen minutes. The last step was measuring the IgG content based on the radius precipitation.

Research procedures

The foals were divided into two groups: one group was treated with a subcutaneous injection of 100 µg of crude TGP, the other group acted as control. Fourteen days after the injection, approximately 3 ml blood samples were collected through the jugular vein. The blood was centrifuged immediately and then serum was collected in an Eppendorf tube to be prepared for the IgG analysis.

Statistical analysis

To evaluate the effect of TGP on the IgG level of the foal serum, the means of sampling used with the control group were compared to those of the treated group by a t-test (Zar, 1996).

Results

The TGP substances indicated on the SDS-PAGE a content of protein antigen with a molecular weight of 27 kDal (Figure 1). The fore-mentioned identified weight of the protein substance belonging to the TGP of the stable fly (*S. calcitrans*) appeared similar to the one reported by Wang *et al.* (2009) and Ueti *et al*. (2007)

The analysis of protein content of the thoraxial glandular extract carried out by using a spectrophotometer ND-1000 showed that the lowest level of the antigen (Figure 2) protein was 0.54 mg/mL, the highest was 72 mg/mL, and the average was 0.675 mg/mL with SEM 0.118.

The effect of the treatment with the crude TGP of *S. calcitrans* on the IgG levels in the serum of foals is presented in Figure 3. The data in Figure 3 above shows that the serum IgG level in experiment animals treated with crude TGP of *S. calcitrans* was significantly higher ($P<0.05$) than the serum IgG level of the control animals.

Discussion

Crude TGP of *S. calcitrans* collected from populations in the area of *Sentrum Agraris Lotta*, Minahasa, Indonesia, contained an average of 0.675 mg/mL of antigen-5 protein. It was used as an

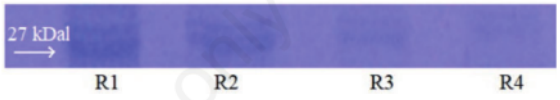

Figure 1. Identification of the antigen-5 of crude thoraxial gland protein (TGP) extract of *Stomoxys calcitrans* by using electrophoresis with four R (replications).

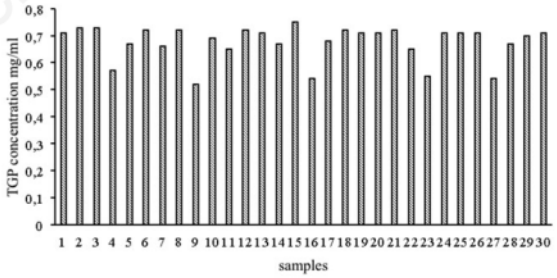

Figure 2. Levels of antigen protein in the crude thoraxial glandular protein (TGP) extract of *Stomoxys calcitrans*.

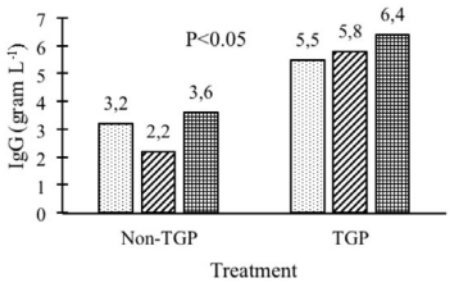

Figure 3. Levels of IgG in the serum of foals observed in the group treated with thoraxial glandular protein (TGP) compared those who did not receive the TGP treatment (non-TGP).

immunogen for the experiment animals. This performance emphasized that substances from the saliva gland of flies are dominated by proteins of 27 kD (Swist *et al.*, 2002), and play an important role in the immune reaction in cattle (Torr & Mangwiro, 2000).

The mean value of the serum IgG was higher in animals treated with a TGP injection than in control animals, showing that the effect of the TGP substance is to enhance the production and circulation of IgG antibodies, specifically illustrating the immune response to the antigen-5 protein as described by Ueti *et al.* (2007). When an animal is treated with an antigen, this antigen can trigger the primary immune response causing the serum IgG to reach a peak 10 to 14 days after antigen exposure (Kresno, 1996), whereas if the antigen is absent, the primary immune response cannot occur.

The influence of TGP immunization by injection on IgG levels in the serum of foals in this research can provide a new hope to overcome the infection potency of pathogenic bacteria coming from the environment of the foals. This immune response is a consequence of the neonatal foals that are always born in hypo-globulinemia conditions, so that the effect of a TGP application as an immunogen has a positive role in increasing the neonatal serum antibody level. The result of this study on neonatal foals is in line with the work described by Ameri *et al.* (2008), which was done on calves. The efforts to increase such neonatal serum IgG antibody level are urgently needed in small-scale farming with traditionally-bred animals as we can find it in many areas today (Joern & Laws, 2013). The use of crude thoraxial glandular protein extract from these flies can be promoted as a means to overcome neonatal foals' high mortality rates, resulting from the failure in passive transfer (Rumokoy & Toar, 2014). The effect of the antigen-5 of *S. calcitrans* in enhancing the production of antibodies which circulate in the blood allows to minimize the diseases caused by pathogen bacteria distributed by flies or other insects to other animals as reported by Pava-Ripoll *et al.* (2015) and Barro *et al.* (2006).

Conclusions

The crude thoraxial glandular protein extract obtained from *S. calcitrans* can be used to improve the IgG antibody circulation in foals. However it will be important to continue the research by evaluating the role of the thoraxial glandular protein on the specific IgG antibody production in foals.

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