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Application of Thoracic Immunogen of *Musca domestica* on Immunoglobulin-G Level of Goats Detected Through a Single Radial Immuno-Diffusion Test

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Abstract. The purpose of this study was to detect the IgG level of goats treated with immunogen thoracic of *Musca domestica* (ITMd). We reared twelve young goats aged two months old under extensive treatments, divided into two equal groups for a comparison experimental design. The animals in the first group (R0) served as the control without immunogen injection, and the second group (R1) was the treatment group receiving a subcutaneous injection of 10 mL of thoracic antigens per head. The parameter observed was IgG (immunoglobulin-G) antibody serum level. The quantification of goat immunoglobulins was carried out using a single radial immuno-diffusion (SRID) technique. The data obtained from the two groups were analyzed with a t-student test. The results showed that the total IgG antibody serum of goats in the treatment group was significantly higher than that of the control group ($P< 0.05$). This difference may be due to the thoracic *M. domestica* immunogen response which increased antibody synthesis of experiment goats. We concluded that the treatment of thoracic immunogen of *M. domestica* at a level of 10 µL injected subcutaneously could significantly increase IgG antibodies in goat blood which were detected using a single radial immuno-diffusion method.

Keywords: Musca domestica, immunogen, goats

Abstrak. Tujuan penelitian ini adalah untuk mendeteksi pengaruh ekstrak kasar *thoracic immunogen M. domestica* (ITMd) terhadap kadar antibodi IgG dalam darah kambing. Penelitian dilakukan dengan menggunakan dua belas ekor kambing muda berumur dua bulan yang dipelihara secara ekstensif, dibagi menjadi dua kelompok dengan jumlah hewan yang sama. Penelitian ini direalisasikan dengan rancangan eksperimen perbandingan rata-rata dua kelompok. Hewan pada kelompok pertama (R0) digunakan sebagai kontrol tanpa menerima injeksi imunogen sedangkan kelompok kedua (R1) bertindak sebagai kelompok perlakuan yang disuntik secara subkutan dengan larutan 10 µL antigen thorax per kepala. Parameter yang diamati adalah kadar antibodi IgG serum (imunoglobulin-G). Kuantifikasi imunoglobulin kambing dilakukan dengan menggunakan teknik *single radial immuno-diffusion* (SRID). Data yang diperoleh dari kedua kelompok dianalisis dengan uji t-student. Hasil penelitian menunjukkan bahwa total serum antibodi IgG kambing pada kelompok perlakuan lebih tinggi secara signifikan dibandingkan dengan kelompok kontrol ($P<0,05$). Perbedaan ini diduga disebabkan respon imunogen toraksial *M. domestica* yang meningkatkan sintesis antibodi kambing percobaan. Disimpulkan bahwa aplikasi imunogen toraksial M. domestica pada level 10 µL yang disuntikkan secara subkutan dapat meningkatkan secara signifikan antibodi IgG dalam darah kambing yang dideteksi menggunakan metode *single radial immuno-diffusion*.

Kata kunci: *Musca domestica*, imunogen, kambing

Introduction

This research was conducted to complete our previous preliminary-trial work on adult *M. domestica* insect immunogens on the proportion of immunoglobulin serum protein level tested to two-month-old goats through an observation using a Portable Refractometer Brix. The goats, especially the young ones, often experienced health problems that led to stunted growth and even death. When the serum immunoglobulin level of animals is low, they are prone to health issues (Kamada et al., 2013). Detection of the level of serum immunoglobulin in goats is important because it could indicate the body's ability to fight infections of pathogenic microbes as related to Hurley et al. (2011).

The application of immunogen extracted from insects to enhance animals' immunoglobulin levels has been reported by some previous authors. The use of crude salivary gland extract of a stable fly as a member of the Muscidae family was related to the works of Toar et al. (2017) who reported the role of insect immunogens on goat immunity enhancement and then Rumokoy et al. (2020) conducted a preliminary trial of subcutaneous injection of larvae antigen crude extract on the development of serum protein quantity in goats. Breijo et al., (2018) used bioactive salivary protein of *Haematobia irritans*. Furthermore, Chernysh and Kozuharova (2013) indicated that the immunogen of alloferon isolated from insects can play a role in stimulating the immune system of mice and humans against cancer problems.

The potency to increase goat production today in the covid-19 pandemic period is currently needed to support food security to ensure the fulfillment of food demand in the community. One of the efforts related to this need is to maintain the health of goats in a small-scale business. In Indonesia, this type of livestock is very important, because it is spread in almost all regions, besides being a source of food, it is also an object of livelihood to support breeders' household economy.

This situation could slow down goat production, consequently is not easy to develop the breeding goats. On the other hand, accumulatively in various countries such as Indonesia, a lot of people are interested in raising goats could be an income source for the families. Health problems in goat kids are still an important limiting factor in the development of goat farms which is indicated by a mortality rate level as a serious threat in this animal husbandry business. The utilization of insect immunogen substances could be an alternative solution to overcome the above problems. This natural ingredient supports the development of organic food production because its application is not based on industrial synthesis materials. Several reports of previous research indicated a positive role of antigens derived from insect immunogens for mammals.

Materials and Methods

Flies Rearing

Musca domestica flies were bred from the larval stage (maggot) in a mixture of rice bran, coconut dregs being fermented process, and fish meal with a ratio of 4:1:1, mixed evenly. Pupa formation was observed from day four, then the pupae that had formed were transferred to a transparent tube placed in a rearing box equipped with a cover of porous paper and moistened to keep the box moist until the pupa developed and turned into adults.

Immunogen Extract

Procedure of immunogen Thoracic of *M. domestica* (ITMd) crude extract preparation was performed after a collection of *M. domestica* from a rearing box and then 15 flies were placed in a net bag after the insects were killed in a beaker glass with a volume capacity of 1 liter and placed in a refrigerator at -4 °C for 10 minutes. Dissection of thoracic cavity used a spatula and tweezers on a petri dish by separating the exoskeleton, realized under a photonic microscope. The thoracic substance obtained was added with a 10% phosphate-buffered saline (PBS) solution of 0.2 mL with a pH of 7.4 continued with a refinement, filtering, centrifugation, and elimination of floating substances. Then, the solution was centrifuged at 5000 rpm for 3 minutes, followed by sediment accumulation, dilution, and filtration using a 0.22 µm filter to sterilize the ITMd and to avoid contamination of microbial-pathogenic and other micro substances.

Animal Experimental, Vaccination, Blood Collection, and Serum Creation

A total of 12 young goats after weaning, aging of 2 months, without sex distinguishing, bodyweight between 3-5 kg, were used in this

experiment. The animals were divided into two groups: the first six as control and others treated with ITMd and immunized subcutaneously with a dose of 10 µL of ITMd immunogen per head. After the 14th day of immunization, a blood sampling was collected by using venipuncture through the jugular vein. The blood flowed into a vacuum container with a volume of 4 mL. The blood (2 mL per animal) was drawn and soon centrifuged in a Vitesse of 7500 rpm for eight munites to get the serum substance, placed in a micro-tube of 0.5 mL, then moved in a refrigerator to be frozen until further analysis.

IgG Quantification

The quantification of goats' IgG antibody serum was performed by using a single radial immuno-diffusion (SRID) kit produced by Kent Laboratories 777 Jorgensen Place Bellingham, WA 98226 USA.

The procedure of quantification was based on a plate containing specific antiserum gel, 0.1M phosphate buffer pH 7.0 with 0.1 sodium azide as a bacteriostatic agent, 1µg/mL amphotericin B as an antifungal agent. Specimen preparation and handling: Collect blood sample without anticoagulant and allow to clot at room temperature and then make a separation by centrifugation as mentioned above.

Fill the well with a 5 µL sample. A circle on precipitated antigen and antibody will be formed and continues to grow until equilibrium is reached. The incubation was left overnight then the diameter of a ring formed corresponding to the level of IgG was measured. The zone diameters of reference sera were plotted against the logarithm (base 10) of the antigen concentration.

Qualification of Immunogobuline Levels

The results of goats' IgG levels obtained were divided into four qualifications of immunoglobulin level indication (A, B, C, and D) based on the diameter ring (ø) formed in the agar plate observed in Single Radial Immuno-diffusion test. Qualifications 'A' as a relatively low level of IgG in goat serum if appeared a ø less than 5.4 mm; qualification 'B' as a baseline IgG level in goat serum if $ø \geq 5.4 \leq 6.6$ mm; qualification 'C' as an average IgG serum level of goats if the ø was $\geq 6.7 \leq 7.9$ mm and qualification D as high IgG serum level in goats if $ø \geq 8$ mm. The ring formed according to the reaction of IgG antibody incorporated in the agar plate containing antigens.

Statistical Analysis

The variable measured was the serum immunoglobulin-G level of the animals experimental. The data obtained were analyzed with a t-test according to the procedure of Zar (2010) to compare the IgG value level between two groups of R0 and R1.

Results and Discussion

The immunity performance of experimental goats was as follows: The treatment animal group that received ITMd showed a significant immune response ($P<0.05$) higher than the control group as shown in Figure 1. The IgG concentration found in the control group (R0) varied from 1.208 to 1.398 mg IgG/dL serum which was lower than in the treatment group (R1) varied from 1.334 to 1.949 mg IgG/dL serum. This performance could be due to the immune sentinel cells in an individual that initiate the immune response by the ITMd exposed to enhance antibody production om 1.208 to 1.398 mg IgG/dL serum which was lower than in the treatment group (R1) varied from 1.334 to 1.949 mg IgG/dL serum. This performance could be due to the immune sentinel cells in an individual that initiate the immune response by the ITMd exposed to enhance antibody production as linked to the report of Luecke et al. (2021) and Chen et al. (2015). Another scientific piece of information by Adamski et al. (2019) explained that insects (beetles) could be used as an organism model in

biomedical research. Various sources indicate that peptides derived from insects have the potential for diseases treatment (Chowanski et al., 2017; Rumokoy et al., 2017; Ariantini et al., 2019; Cherniack, 2010; Chernysh and Kozuharova, 2013).

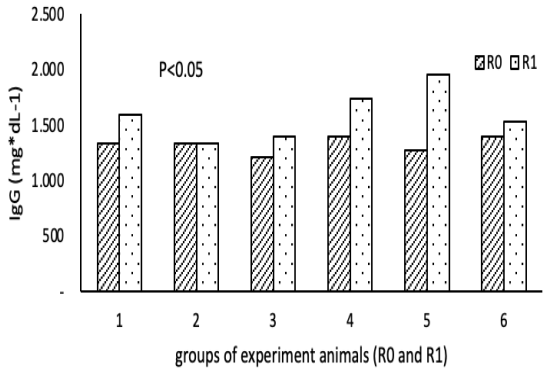

Figure 1. IgG Level of Experiment Animals

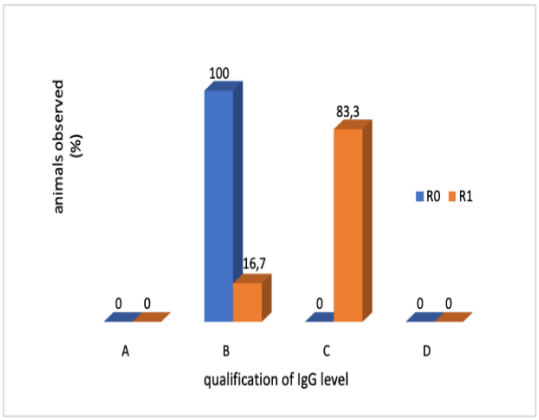

Figure 2. Immunity Performance of Experiment Goats

The immunity performance of experimental animals (Figure 2) was achieved through the qualification of IgG level serum detection. All animals (100%) in the control group (R0) received 'B' qualification as the baseline IgG level in goats, and their ø was > 5.4 < 6.6 mm. Meanwhile, the treatment group (R1) had two qualifications of IgG levels detected in the treatment goats: 16.7% of animals had a B qualification (ø > 5.4 < 6.6 mm) while the majority had a 'C' qualification which 83,3% of the animals in this group having a ø > 6.7 < 7.9 mm reached a level C.

The level of IgG in serum was determined by various factors, including the success of the passive transfer of immunoglobulin, health, and environmental condition (Rumokoy and Toar, 2014). The immunoglobulins in the body of goats were relatively stable (Duysburgh et al., 2021) and could still be detected after several months as linked to the study of Czopowicz et al. (2018). Although there is no detailed explanation regarding the mechanism of the role of insect antigens in stimulating immunoglobulin production, there are various indications that this insect immunogen can improve the immune system in human and mouse cells as reported by Chernysh and Kozuharova (2013), and those tested on goat livestock (Toar et al., 2019). Ai et al. (2013) reported a protein-enriched fraction from larvae of the *M. Domestica* acted as antiviral and immunomodulatory.

Conclusions

We concluded that the application of thoracic immunogen of *M. domestica* in this experiment at a level of 10 µL per head which was injected subcutaneously significantly increased IgG serum in goat blood treatment which was detected using a single radial immuno-diffusion method.

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