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Expressions of Rat Antibodies in the Serum of Laying Hens Repeatedly Immunized with *Rattus rattus* Meat Extracts: Potential Antibodies for Development of Kits in the Prevention of Meat Adulteration

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***Abstract*: In the development of immunoassay-based diagnostics such as enzyme-linked immunosorbent assays (ELISA) and immunochromatography (IC), antibodies play a crucial role. In this study, we succeeded in producing antibodies against house rat (*Rattus rattus*) meat extract (RME) by immunizing adult laying hens using (RME) repeatedly. Adult laying hens were immunized four times, with a dose of 200 µg/head in the first injection, 100 µg/head in subsequent immunizations with a two-week interval between vaccinations. The results of ELISA analysis showed that the antibodies produced could be used to distinguish between rat meat and beef, goat, chicken, as well as pork ($p < 0.0002$). Therefore, it can be concluded that antibodies against rat meat extract produced in laying hens in this study have high potential as raw material for the development of kits for meat authentication between animal species. Further research is needed for application in the prevention of meat adulteration.**

***Keywords*: antibody, chicken serum, ELISA, meat adulteration, rat meat extract.**

1. Introduction

Since the COVID-19 pandemic, people have become increasingly concerned about the safety and quality of food, i.e. food is non-contaminated with harmful biological and non-biological materials, as well as containing healthy nutrition [1]. For certain consumers, the food should be processed according to the provisions of halal i.e. free from any component that Muslims are prohibited from eating according to Islamic law. Consumer demand for halal products has become a strategic issue not only in the country such as Indonesia, but also at the regional of Asia and even international levels [2].

There are three types of animals commonly used to counterfeit halal processed food products, namely pigs, rats, and dogs. This is carried out by "sneaky" traders to make a profit. It is acknowledged that for some consumers, these three species are not a problem from socio-cultural or religious aspects. However, if the processing is carried out imperfectly, these animals that are classified as wild, such as the house rats, are known to be vectors of several zoonotic pathogens such as *Leptospira interrogans*, *Streptobacillus moniliformis* [3], [4], and hepatitis E virus [5] can pose a threat to public health and can have fatal consequences [6], [7].

For this reason, in the last decade or so, research on the development of tools to authenticate halal food products or food free of harmful biological contaminants has grown rapidly, including Indonesia. Among these detection kits are rapid tests based on lateral flow assay (LFA) and enzyme linkage immunosorbent assay (ELISA) [8]. Both diagnostic methods are based on immunoassays in which success is mostly determined by the characteristics of antibodies to the analyte (antigens) to be tested. In the development of the kits, the antibody as the raw materials can be bought or be produced as an in-house antibody. Efforts to procure them have been made through several studies, including antibodies against pork components [9], [10] but still need to be optimized.

In this study, the successful production of antibodies against rat meat extract using laying hens is reported. Antibodies obtained in this study have the potential to be used in the development of immunodiagnostics.

2. Materials and Methods

A. Biological Materials and their Preparations

Biological materials including beef, mutton, pork, and chicken were purchased from wet markets in Mataram city. Meanwhile, rat meat came from house mice that were caught and processed according to applicable regulations. The meat extraction and immunization process were based on Nurhaerani et al. [9] with some modifications. The laying hens used were two hens of the Hy-Line Brown breed. This study was conducted in accordance with animal ethics No: 361/UN18.F7/ETIK/2023.

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B. Immunizations and Serum Collections

Immunization of laying hens with RME was carried out four times. The first immunization was at a dose of 200 µg/head in an emulsified mixture of RME and complete Freund's adjuvants 1:1 (v/v), followed by three consecutive injections at two-week intervals, at a dose of 100 µg/head in an emulsified mixture of incomplete Freund's adjuvants 1:1 (v/v). All injections were made by the sub-cutaneous route, in the neck area of the chickens.

Ten to 14 days after the last immunization, chicken serum was collected from the wing veins (vena pectorales). The serum was then pooled, and antibodies were isolated and purified using 50% (v/v) ammonium sulphate and analyzed using ELISA technique.

C. ELISA

ELISA was performed as reported by [11], in general, each well of ELISA plates was coated with rat, pig, cow, goat, and chicken meat extracts, each well of 5 ng, i.e. 10 µg/ml, 100 µl per well in phosphate buffered saline (PBS), pH 7.4 then incubated overnight at 4°C. After incubation, blocking and washing, 50 µl of chicken antibody against RME was dripped into each well with a final antibody concentration of 1 ng per well. Following incubation for 60 minutes at 37°C and washing, 50 µl of secondary antibody (HRP conjugated Goat Anti-Chicken IgY) was added per well at a final dilution of 20,000 times according to the manufacturer (Invitrogen, USA). After incubation for 60 minutes at 37°C and washing, a further reaction was performed by adding 50 µl of TMB substrate per well, incubated at 37°C for 10-15 minutes in a dark room. The reaction was stopped with sulfuric acid, then read at optical density (OD) 450 nm.

3. Results and Discussion

This study aims to produce antibodies against rat meat extract to be used for the development of diagnostic tools to authenticate meat that is free from rat meat mixture. Diagnostics for authentication is needed especially for consumers who for certain considerations do not consume food contaminated with components of animal species, such as rats.

In this study, laying hens were used as antibody producers because when the target antibodies have been obtained, the antibodies can be harvested from the eggs every day. This is a non-invasive animal utilization model and has been of particular interest in recent years [12], [13]. At the time of this report, post-immunization eggs are being processed to isolate yolk immunoglobulin (IgY). In parallel, the expression of antibodies against RME was analyzed by ELISA method and the results are presented in Fig. 1.

The results of statistical analysis (n=3) showed that the antibodies produced in this study had high specificity which was significantly different ($p < 0.0002$) when compared to the reaction to meat from other species such as those from pigs, cows, goats, and chickens. These results suggest that this antibody has a strong potential to be used as a raw material for diagnostic development to distinguish between animal species.

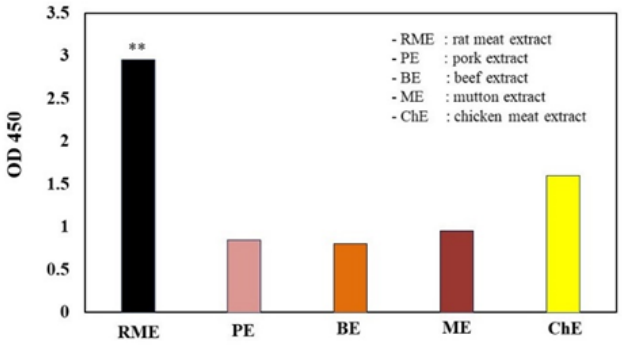

Fig. 1. Results of antibody (chicken anti-rat antibody) specification analysis against rat meat extract (RME) that differed significantly (** $p < 0.0002$) compared to pork (PE), beef (BE), goat (ME), and chicken (ChE) meat extracts

Although statistically significant differences were observed, Figure 1 still shows the presence of non-specific reactions, especially against chicken species. This is understandable because the secondary antibody used in this study was goat-anti chicken IgY whereas the primary antibody produced in this study was derived from laying hens. Non-specific binding (NSB) and cross-reaction in ELISA are common, however an accurate and reproducible ELISA without cross-reaction is an obligation. Hence false immunodetection results must be prevented [14]. In the future, these non-specific reactions should be suppressed or eliminated either by improving the inhibitor or blocking system, or by developing secondary antibodies specific to rat meat, including the use of monoclonal antibodies.

4. Conclusion

In this study we successfully produced antibodies against REM expressed in laying hen serum. These antibodies have potential for the development of ELISA to control meat adulteration between animal species. However, efforts to eliminate cross-reactions between species still need to be carried out.

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