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PREFACE

In the name of Allaah the Most Gracious and Merciful. We would like to express our deep gratitude to our God, we could finish Proceedings of International Seminar. This proceedings was set of articles or papers that has been presented at International Seminar on *From Ocean for Food Security, Energy, and Sustainable Resources and Environment*. This seminar is organized by cooperation between Fisheries and Marine Faculty, Airlangga University, Surabaya and Research Center for Marine and Fisheries Socio Economics, Agency for Marine and Fisheries Research, Ministry of Marine Affairs and Fisheries, Jakarta, and Agrotechnology and Food Science Faculty, Universiti Malaysia Terengganu, Malaysia. The seminar is attended by researchers, lecturers, students of undergraduate, master and doctoral degrees, and also by government official. The papers cover broad topics about food production technology, product value improvement, resources and environment biophysics, alternative energy and environment biophysics, and socio-economic.

We would like to express our sincere thanks to Rector and Vice Rector of Airlangga University, Vice Counsellor and Dean of Agrotechnology and Food Science Faculty of Universiti Malaysia Terengganu, Head of Research Center for Marine and Fisheries Socio Economics, Dean of Fisheries and Marine Faculty, Airlangga University, keynote speakers: Prof. Dr. Gunawan Sumodiningrat from Gadjah Mada University, Prof. Dr. Sakri Ibrahim from Universiti Malaysia Terengganu, and Prof. Hassan Hj. Mohd Daud, DVM., Ph.D. from Universiti Putra Malaysia, moderators, presenters, participants, and colleague for supporting and kind help in the seminar. We also wish to thank to all sponsorships: Vice President of PT. CP Prima, Director of PT. Sufie Bahari Lines, Head of Fisheries and Marine Office, Regency of Tuban, Head of Fisheries and Marine Office, Regency of Pasuruan, General Manager of PT. Sanbe Farma, Director of PT. Petrokimia Gresik, Director of CV. Antika, Coordinator of Education Fish Pond, Fisheries and Marine Faculty, Airlangga University, and Director of PT. SIER for good contributions and partnership in the seminar. Finally, we would like to express our sincere thanks to the Steering Committee and Organizing Committee either staff and students from Faculty of Fisheries and Marine, University of Airlangga or staff from Research Center for Marine and Fisheries Socio Economics, Agency for Marine and Fisheries Research, Ministry of Marine Affairs and Fisheries, Reviewer: Prof. Ir. Sukoso, M.Sc., Ph.D from Fisheries and Marine Science Faculty, Brawijaya University, Ir. Murwantoko, M.Sc., Ph.D. and Ir. Triyanto, M.Si., Ph.D. from Department of Fisheries, Agriculture Faculty, Gadjag Mada University, Ir. Agung Sudaryanto, M.Sc., Ph.D. from Fisheries and Marine Science Faculty, Diponegoro University, Mohammad Yunus, DVM., M.Kes., Ph.D. from Department of Parasithology, Veterinary Medicine Faculty, Airlangga University, Ir. H. M. Pujoyuwono, M.Sc. from Research Center for Marine and Fisheries Socio Economics, Agency for Marine and Fisheries Research, Ministry of Marine Affairs and Fisheries, and Prof. Sayed Mohd Zain Hasan, Ph.D. from Agrotechnology and Food Science Faculty, Universiti Malaysia Terengganu.

Surabaya, 1 December 2009

Editors

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OPTIMIZATION ENZYME LINKED IMMUNOSORBENT ASSAY (ELISA) USED FOR TESTING COW'S PROTEIN LEVELS IN VACCINE MEDIUM

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somespondence.

ABSTRACT

This study generally aim was to create a system-specific testing to determine the rest of the protein derived from cows on the medium used in producing the vaccine. While specific objective was to optimize Enzyme Linked immunosorbent assay (ELISA) that was optimized levels of antigen, antibody, conjugate and blocking materials used in the ELISA tests used to test the cow's protein content in the vaccine medium. Examination of the rest of the cow proteins are necessary because if the vaccine manufacturing using beef bullion good medium still have the rest of the protein can cause allergy if injected into the body. These tests should be sensitive and specific so that the chosen method of ELISA sandwich technique. ELISA with a sandwich technique is one of the ELISA technique in which antibodies attached on the specific surface hole tied with antigen to form Ab-Ag complex. Complex is then reacted with specific antibodies labeled with enzymes (conjugates) to form Ab-Ag complex. If the added substrate, this complex hydrolyze substrate is an indication of antigen. Substrate hydrolysis is usually held within a certain time and the reaction was stopped by adding a strong acid. The results showed that the use of blocking Bovine Serum Albumin (BSA) for 3% levels, antibody levels of 40 mcg/mL, the concentrations of antigen from 10, 5, 2.5, and 1.25 mcg/mL and IgG biotin conjugate with 20 times dilution gave optimal results and can be used as a standard to measure the cow's protein content in the vaccine medium.

Keywords: medium, vaccine, cow, ELISA, BSA

INTRODUCTION

Proteins are organic compounds that is a polypeptide with more than 50 units of amino acids by forming a complex structure. Molecular mass could even reach 10,000 and most of naturally occurring and found in all living things (Fessenden, 1997). Most proteins are immunogenic and are generally multi-determinant and univalent. Making vaccines using medium with beef broth base materials that have been added papain which is an enzyme to be become protein amino acids. Sometimes the protein in the medium is not completely broken down into amino acids, so the rest of the protein cow testing is very important because if there are still cows in the rest of the media proteins can cause allergies if the vaccine is injected in the body (Baratawijaya, 2000).

Many methods can be used to test the protein in sample. In immunology, among others with immunobloting, radioimunoassay, elektroforesa, Enzyme Linked Immunoassay (ELISA) and many others. The use of ELISA methods because this method is easy to do, do not contain radioactive materials, more specific and can measure the quantity of protein (Charles et al., 2001). ELISA is a method to determine the amount of antigen or antibody levels are low (Graham, 1998). Standard procedures are usually investigation by ELISA using Bovine Serum Albumin (BSA) as blocking materials. Because the BSA expensive alternatives sought as a blocking material that is used gelatine, with the assumption that the same gelatin protein derived from cow's gelatine prices far cheaper, easer available and can be stored at room temperature, so the gelatine can be expected to give results as good with the BSA in the ELISA tests are used to test the protein content of cows in the vaccine medium.

Gelatine in the nature insoluble in cold water but is soluble water temperature above 40°C. The solubility of gelatine is influenced a variety of factors such as temperature, concentration and particle size. The pH of gelatine ranges 4.5-6.5. Gelatine is widely used as a food preservative (jelly, jam, candy), the pharmaceutical industry (ointments, capsules), wrapping paper money, making it lighter and cosmetics (Watkiss, 2000). The purpose of this study is the optimization ELISA by comparing the use of gelatine and BSA as a blocking material in the ELISA tests used to test the cow's protein content in the vaccine medium. The results of this study are expected to be useful to create a specific test system to determine the rest of the protein derived from cows on the medium used in producing the vaccine.

MATERIALS AND METHODS

Determination of protein standard curve created using BSA is the concentration of initially 10 mg/mL, then serially diluted so that the obtained concentration 78, 39, 19.5, 9.8, and 4.9 mcg/mL. After serial dilutions and solution of BSA with various concentrations was measured using a spectrophotometer U-2001 at wavelength λ =200 nm to measure absorbance, then the resulting data entered into the computer to know the linear regression equation.

Preparation Materials Used in Testing

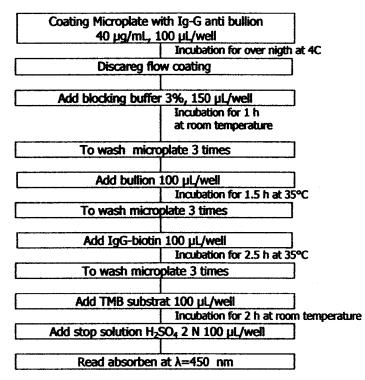
The manufacture of blocking with 3% concentration blocking material is made by dissolving 1.5 g of BSA or gelatine to 50 mL solution in PBS pH 7.2, stirring with a magnetic stirer. Blocking the manufacture of gelatine, stirring frequently, this solution should be heated too quickly to gelatine late.

PBS Diluen

On the use of BSA as a blocking material, PBS solution made by dissolving 3 g BSA in 100 mL of PBS pH 7.2 stirred using magnetic stirrer and then added 100 μ L. Tween 80 as much as 80 it serves to avoid

non-specific absorption on the tube wall or particles. Similarly, in making gelatine diluen using as material bloking, who replaced his BSA only be gelatine.

Dilution concentration bullion and IgG-biotin conjugate in series beef bullion as serially diluted antigen using diluen PBS. Initial concentration of protein bullion 60 mg/mL serially diluted. At the plate using BSA blocking materials created by the concentration of starting 10, 5, and 2.5 μ g/mL, was on the plate 1.25 μ g/mL using gelatine as a blocking material, made with a concentration dilution start 5, 2.5, 1.25, and 0.63 μ g/mL. The dilution of IgG-biotin conjugate serially was 10, 20, 30, and 40 times, respectively. Determination of protein content of beef bullion using ELISA method.



RESULTS AND DISCUSSION

Before the first experiment conducted among IgG-conjugates with biotin antibullion to be used as conjugates to be attached to the antigen, the bullion. These conjugates are useful as a labeled antibody enzime. Determination of protein concentration standard curve using BSA. Protein concentration measurements performed using spectrophotometry. Absorbance protein in beef bullion measured at wavelength λ =190-320 nm. The results showed that the wavelength λ =200 nm, absorbance well enough that measurements performed on protein concentration in these wavelengths.

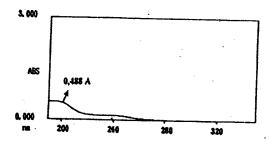


Figure 1. Beef bullion protein absorption at 500 times dilution

This optimization was performed to determine the amount of required antigen. Calculation of protein concentration was performed Then the protein content was measured using beef bullion curve made from

BSA with linear regression equation obtained: Y = 0062 + 0.00035x.

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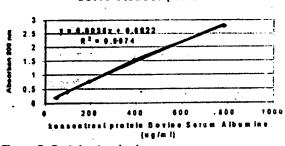


Figure 2. Protein standard curve

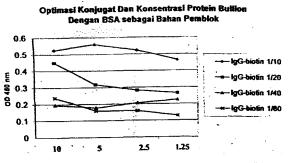
Determination of Protein Content in Cow Bullion

ELISA reaction order can be used to measure the quantity of antigen. It would require an optimal amount of antigen. After the curve is made and known to the linear regression equation can be calculated on the protein content of the bullion absorban measurements obtained at wavelength λ =200 nm the concentration can be calculated using a linear regression equation above is 120 mcg/mL in dilution 500 times or 60 mg/mL without dilution. Usability is the determination of protein concentration to determine the existing concentration on orotein beef bullion as an antigen. Protein content optimization beef bullion. In this study, the proteins specified in bullion as an antigen that is done by way of dilution story. Once known concentration of bullion is carried bullion protein content optimization using BSA and gelatin as blocking the function to avoid non-specific reactions with the same concentration of 3%, which were both carried out in order to obtain optimal results and fewer errors in final results.

In this study, the concentration of antibodies attached to each surface of the same hole, which is 40 mcg/mL, and then attached to bullion as an antigen with a concentration different. After the antigen is attached to the IgG antibody conjugated with biotin as a conjugate with a concentration different. It's shown bullion as antigens will provide optimal results at a certain concentration.

Table 1. Optimal density (OD) of protein concentration beef bullion at various conjugates with BSA as a blocking

No	conjugate	The concentration of protein conjugates beef bullion (µg/mL)				
		10	5	2.5	1.25	
1	IgG-biotin 1/10	0.525	0.559	0.544	0.523	
2	IgG-biotin 1/20	0.450	0.369	0.314	0.298	
3	IgG-biotin 1/40	0.195	0.162	0.205	0.212	
4	IgG-biotin 1/80	0.238	0.218	0.158	0.114	

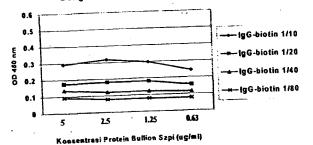


Konsentrasi Protein Bullion (ug/ml)

Table 2. Optimal density (OD) of protein concentration beef bullion at various conjugates with gelatine as a blocking

No	conjugate	The concentration of protein conjugates beef bullion (µg/mL)				
		5	2.5	1.25	0.63	
1	IgG-biotin 1/10	0.292	0.317	0.297	0.303	
2	IgG-biotin 1/20	0.175	0.193	0.181	0.166	
3	IgG-biotin 1/40	0.136	0.139	0.121	0.121	
4	IgG-biotin 1/80	0.093	0.086	0.078	0.093	

Optimasi Konjugat Dan Konsentrasi Protein Bullion Dengan Gelatin Sebagai Bahan Pemblok



Based on the results were show in Table 1 and graphs can be seen that the conjugate with 20 times dilution gave optimal results of the smaller concentration of the smaller OD values so that the resulting linear lines can be used as a standard to measure protein samples containing beef bullion. In Table 2 and graphs of the use of gelatine as a blocking material, ODnya value lower than the use of BSA as a blocking material, although decrease trends but does not provide optimal results. So to measure samples containing beef protein bullion still using BSA as a blocking material with a concentration of 3%. 40% levels of antibodies, conjugates at 20 times concentrations range dilution and antigen 10-1.25 mcg/mL is used as a standard curve. If the concentration is too high, the sample must be diluted and vice versa if the sample is too low concentration should be concentrated to be measured on the standard curve was created.

CONCLUSIONS

The results showed that the use of blocking BSA for 3% levels, antibody levels of 40 mcg/mL, the concentration of antigen from 10, 5, 2.5, and 1.25 mcg/mL and IgG biotin conjugate for 20 times dilution gave optimal results and can be used as a standard to measure the protein content of cows in the media vaccine.

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