

Appendix 16

THE EFFECTS OF SHEARING AND ASCORBYL PALMITATE SUPPLEMENTATION ON PHYSIOLOGICAL, HEMATOLOGICAL, BLOOD BIOCHEMISTRY, OXIDATIVE STRESS AND HSP70 LEVEL OF PADJADJARAN SHEEP

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ABSTRACT

Indonesia has very hot ambient temperatures and high humidity, it is not favourable for organism whose bodies are covered long fleece. Shearing increase heat dissipation from the sheep's body. Antioxidants administration reduce the negative effects of excessive free radical production. Ascorbic acid is popular as an antioxidant but is easily degrading in rumen. Ascorbyl palmitate is ascorbic acid in the form of an ester which is insoluble in water. The objective of research are to analyzed of shearing and supplementation of *ascorbyl palmitate* (AP) as antioxidant to physiological profile, blood metabolite, oxidative stress and level of HSP 70 of Padjadjaran sheep exposed to heat shock. The shearing treatments are un-shear (c_0) and shearing (c_1), while supplementation of AP were un-supplemented (a_0) and supplemented (a_1). The combination of treatments are un-shearing and un-supplemented (c_0a_0), only shearing (c_1a_0), only supplemented AP (c_0a_1) and shearing with supplemented AP (c_1a_1). The heat shock was expose the sheep under sunlight. The measure of physiological profile are respiration rate (RR), pulse rate (PR) and rectal temperature (RT); hematological profile measure as red blood cells (RBC), hemoglobin (Hb), hematocrit (Hct), white blood cells (WBC), mean corpuscular value (MCV), mean corpuscular hemoglobin (MCH) and mean corpuscular hemoglobin concentration (MCHC); The blood metabolite measure of glucose, cholesterol and total protein level; the oxidative stress measure by concentration of superoxide dismutase (SOD), Catalase (CAT), glutathione peroxidase (GPx) and Malone dialdehyde (MDA); and the level of HSP70. The results showed that shearing had a significant effect ($p < 0.05$) on RR and RT; the administration of ascorbyl palmitate had a significant effect ($p < 0.05$) on the PR of Padjadjaran sheep heated stress. The combination of shearing and administration of ascorbyl palmitate treatment significantly ($p < 0.05$) on WBC; shearing treatment had a significant effect ($p < 0.05$) on Hct and MCHC; while the administration of ascorbyl palmitate had a significant effect ($p < 0.05$) on the RBC of Padjadjaran sheep heated stress. The combination of shearing and the administration of ascorbyl palmytate treatment had a significant effect ($p < 0.05$) on the total protein level of Padjadjaran sheep heated stress. The combination of shearing and the administration of ascorbyl palmytate treatment had a significant effect ($p < 0.05$) on the levels of SOD, CAT and GPx of Padjadjaran sheep heated stress. No significant effect ($p > 0,05$) of treatment of shearing and administration of ascorbyl palmytate on HSP70 levels in Padjadjaran sheep heated stress. The conclusion, shearing and *Ascorbyl Palmitate* supplementation have benefit effects to Padjadjaran sheep.

Keyword: Padjadjaran sheep, heat stress, antioxidant

DEVELOPMENT OF BONE SUBSTITUTE MATERIALS BASED ON CARBONATE APATITE USING SIMPLE SOLUTION AND FINE BUBBLE CARBONATION METHOD

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ABSTRACT

The development of bone replacement biomaterials aims to overcome the shortcomings and drawbacks of conventional bone grafts such as complicated surgical procedures, chronic inflammation, the body's immune rejection, bone donors limitation and others. Currently, the most widely developed biomaterial as a bone replacement material is calcium carbonate (CaCO_3), due to its biocompatibility and biodegradability that meet the requirements of biomaterial applications, for instance as a scaffold for maxillofacial bone regeneration, deficiency in the maxillary and mandibular ridges. The pure and porous CaCO_3 vaterite polymorph is used as a precursor in the manufacture of carbonate apatite (CO3Ap)-based scaffolds. This study produced a CO3Ap-based bone replacement scaffold that has bioactivity as a bone tissue. This study consists of three stages, namely: the first stage is the synthesis of CaCO_3 with a simple solution and CO_2 bubbling method using a fine bubble generator and diffuser, the second stage is the manufacture of CO3Ap scaffold using CaCO_3 precursor obtained from the synthesis of CO_2 bubbling with a diffuser, and the third stage is a Ca^{2+} ions release test from the selected scaffold by immersion in a simulated body fluids (SBF) solution. The first stage of the study was the synthesis of CaCO_3 using a simple solution method at three variations of the synthesis, namely temperature (200 C and 270 C), stirring speed (800 and 1000 rpm), and variations in reaction time (5, 10, 15, 30, and 60 minutes). In the optimal synthesis variation, CaCO_3 was obtained in the vaterite phase with an average percentage above 90% and has a spherical and porous morphology. However, the secondary CaCO_3 particle size is still quite large, ranging from 2-5 μm . Therefore, the next step of synthesis was carried out to obtain nano-sized particles using the CO_2 bubbling method. Synthesis using the bubbling method begins by using a fine bubble generator (FBG) with two variations of precursors, namely pro-analytical materials and natural materials. Synthesis using pro-analytical materials was carried out by varying the molarity of the pro-analytical $\text{Ca}(\text{OH})_2$ precursor (0,25; 0,5; 0,75; 1; and 1,25 M) and the initial pH of the reaction (8, 9, and 10), while the synthesis using natural calcium oxide precursors used smaller variations in molarity (0,01; 0,03; and 0,05 M). Synthesis using pro-analytical precursors produced CaCO_3 in the calcite phase with a particle size of 1-4 μm , while natural materials produced CaCO_3 in the calcite phase with a smaller particle size (650,7; 659,4; and 769,1 nm). Presumably, the CaCO_3 is obtained in a calcite phase due to a lack of CO_2 concentration and an increase in temperature during the carbonation process. Therefore, in order to obtain a vaterite phase, the synthesis was continued by using a diffuser type of CO_2 bubble generator. In the synthesis optimization of CaCO_3 vaterite, a fine bubble diffuser was used with variations in the molarity of the pro-analytical $\text{Ca}(\text{OH})_2$ precursor (0,1; 0,5; and 1,25 M), solvent mixture of methanol and water, reaction temperature ± 200 C, rate of CO_2 gas flow is 1 L/min,

and the carbonation time is 120 minutes. In this synthesis, CaCO₃ was obtained in the vaterite phase based on the identification of X-ray Diffraction that showed 2 theta at 24,90°; 27,04°; 32,78°; 43,85°; 50,07°; and 55,80° and is also supported by the results of the Fourier Transform Infra Red (FTIR) test at wave numbers 744 and 745 cm⁻¹. The results of SEM observations, the morphology of the CaCO₃ is round and is 605 nm in size. In the second stage, the best CaCO₃ powder from the synthesis using a fine bubble diffuser was used as a precursor in the manufacture of the CO₃Ap scaffold using a polyurethane foam method. CaCO₃ vaterite precursors and dicalcium phosphate anhydrous (DCPA) with a ratio of 30:70 wt% were mixed with 0,2 M Na₂HPO₄ solvent with a solvent:powder ratio = 0,6 to obtain a paste consistency. Then the paste was molded with polyurethane foam and continued by burning in the furnace at temperatures of 500, 600, 700, 900, and 1100 °C for 2 hours. The process produced a scaffold with open pores that is interconnected according to the polyurethane foam mold. The CO₃Ap scaffold was obtained at a combustion temperature of 500 °C which was shown by the Ca /P molar ratio of 1,51 from the EDS observations. In addition, the FTIR test on the scaffold strengthened the mentioned statement by showing a finding of; phosphate groups at 1029, 936, 605, and 560 cm⁻¹, and carbonate groups at 1435, 876, and 715 cm⁻¹. The apatite carbonate formed in the polyurethane foam mold is able to produce a porous scaffold according to its mold with an average pore size of 254.5µm. The third stage is the bioactivity test by measuring the release of Ca²⁺ ions from the selected CO₃Ap scaffold by immersion in SBF solution. The produced CO₃Ap scaffold showed Ca²⁺ ions release into the SBF solution, and there was an increase in Ca²⁺ ions released from the CO₃Ap scaffold for 28 days of immersion. Based on the results of all the carried out stages of this study, it can be concluded that the majority of CaCO₃ powder in the vaterite phase can be obtained by synthesis using a fine bubble diffuser and that it meets the criteria as a precursor for the manufacture of CO₃Ap-based scaffolds using the polyurethane foam method, and the scaffold obtained has bioactivity based on the Ca²⁺ ion release test using immersion test in SBF solution.

Keywords : calcium carbonate, vaterite, pore, scaffold, carbonate apatite, bioactivity, ion Ca²⁺ release

MODEL DEVELOPMENT OF SUPER INTENSIVE FRY STRIPED CATFISH CULTURE WITH THE APPLICATION OF ULTRAFINE BUBBLES IN RESIRCULATING AQUACULTURE SYSTEM

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ABSTRACT

Striped catfish (*Pangasianodon hypophthalmus*) is one of the leading commodities in Indonesia and world trading with an increasing production of 15-20% of the total global catfish production of 2.8 million tons. One of the important factors for achieving catfish production targets is the availability of seeds of good quality and quantity. Improvement efforts made in this research are controlling the environment of catfish larvae rearing media through the application of Ultrafine Bubbles (UFBs) technology. It can already be produced in the Laboratory of the Center for Institutional Excellence Functional Nano Powder (FiNder U-CoE) Universitas Padjadjaran. The result of UFBs test on the bubble size with different oxygen gas sources showed that the bubble size ranged from 607.6 to 518.5 nm. It was not affected by the oxygen source, while the zeta potential was different between air (-11.5 mv to -16,7 mv) and pure oxygen (- 21.4 mv to -25.2 mv). Using pure oxygen is very significant in increasing dissolved oxygen to super saturation (345.33% or 25.39 mg/L at the air saturation 7.5 mg/L). Furthermore, the gas oxygen in ultrafine bubbles in the form of reserved oxygen potential (ROP) of 39% (2.95 mg/L) within 45 minutes of bubbling. This oxygen removed ammonia from synthetic sources by 83.33%, while ammonia from effluent (fish waste) is 39.93%. The results of the second stage of the study showed that the use of UFBs-RAS using pure oxygen in the rearing of striped catfish larvae in the endogenous feeding phase resulted in the absorption rate of egg yolk, larval growth rate and the efficiency of egg yolk utilization which were not significantly different. The value of growth performances were 3.743 ± 0.385 - 4.82 ± 0.359 %/hour, 1.77 ± 0.281 - 2.029 ± 0.153 %/hour and 50.634 ± 2.827 - $47,245 \pm 4.34$ % respectively. Where as in the eksogenous feeding phase, UFB-RAS was able to enhance growth performance at a stocking density of 100 fish/L with a survival value of 42.11 ± 1.87 %, biomass weight rate of 14.69 ± 2.24 %/day and feeding efficiency of 43.36 ± 4.40 %. In addition, based on the calculation of the water balance analysis, it showed that the condition of the larval rearing media both in the endogenous and exogenous feeding phases were in optimal conditions. The dissolved oxygen and ammonia were 128.97 - 128.73% (hyperoxia) and 0.006 - 0.007 mg/L respectively. The results of further research on the role of UFBs-RAS on the production performance of catfish reared for 5 days with tubifex feeding showed that UFBs-RAS was able to increase productivity. The values of growth performances were stocking density of 800 fish/15L, survival rate of 100%, biomass growth rate of 56.13 ± 2 , 82%/day, biomass weight of 9.674g/L, feeding efficiency of 70.41 ± 11.92 % and size uniformity of 95.35%. Furthermore, based on hematological parameters, the harvested striped catfish fry showed no stress conditions with a glucose content of 49.75 ± 8.84 mg/dl (glucose level > 60 mg/dl) and a red blood cell count of 1.51 ± 0.07 million cells (normal).

Keywords : UFBs-RAS, striped catfish, larva phase, growth, hematological

ANTIOXIDANT ACTIVITY OF THE RED GINGER-BREAD AND ITS EFFECT ON THE LIPID PROFIL AND BLOOD PRESSURE OF HEALTHY VOLUNTEERS

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ABSTRACT

The aim of the study was to obtain the antioxidant activity of red ginger extract (*Zingiber officinale* var. Rubrum) and red ginger bread extract and its effect on the lipid profile and blood pressure adjustment on healthy volunteers. Proximate characteristics on red ginger were analysed by applying the AOAC method of analysis resulted in 3.37% protein content, 1.72% fat, 11.90% carbohydrates, while red ginger bread gave 8.18% protein content, 8.86% fat and 67.05% carbohydrates. The chemical composition was analysed using a Spectrophotometer for the ethanol extract of red ginger, giving an antioxidant activity value of IC₅₀ as much as 125.94 ppm with moderate indication; red ginger flour 55.66 ppm with strong indication; and red ginger bread 6% as much as 142.93 ppm that considered to be medium strength. High Performance Liquid Chromatography (HPLC) method was used to determine 10-gingerol content, giving 19.52 ppm for red ginger powder ethanolic extract and 6.35 ppm for the 6% red ginger bread extract. Organoleptic assessment of with hedonic test for 6% red ginger bread was considered to be quite like the aroma of bread by panellist with the value of 3.63 and like for the taste of ginger on bread with the value of 4.3. The addition of red ginger flour 6% extends the shelf life of bread to an average of 8.83 days, compared to bread that does not use red ginger flour, which is an average 4.25 days. Anthropometric test showed that red ginger bread 6% that was given to healthy volunteers (n=20) for as long as 14 days can reduce belly circumference 4.25% (p = 0.004), but it showed no significant effect on the bodyweight reduction as 0.33% (p=0.387). The result also showed that consumption of red ginger bread could lowering systolic blood pressure as 9.42% (p = 0.000) and lowering diastolic blood pressure as much as 9.09% (p = 0.000). In terms to the lipid profile, red ginger bread showed significant increasing of HDL as much as 24.48% (p = 0.000), lowering total cholesterol to 8.20 (p = 0.032); but it showed no significant different in lowering LDL as 1.88% (p = 0.765) and changes in triglyceride as much as 7.87% (p = 0.346). Therefore, it is concluded that 6% red ginger bread has antioxidant activity that gives beneficial to health. The addition of red ginger to bread dough can synergize with yeast to become a good bread and it could extend the shelf life and has an effect on lipid profile and blood pressure.

Keyword: antioxidant, *Z. officinale* var. Rubrum, cholesterol, HDL, systolis, diastolic.

MODIFICATION OF POROUS CARBON MATERIAL FROM WATER HYACINTH FOR APPLICATIONS OF HIGH CAPACITY LITHIUM SULFUR BATTERY COMPOSITES

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ABSTRACT

Porous carbon has been successfully made from water hyacinth plants with the stages of carbonization, activation and calcination processes. There are two treatments in the process of making porous carbon, namely without doping and with N doping. The purpose of N doping is to increase the electrical conductivity and polarity of the porous carbon. Non-doped and N-doped porous carbon composites have also been successfully prepared by mixing sulfur-porous carbon in two different ratios. From the test results with N₂ adsorption-desorption, the surface area of porous carbon is 642.39 m²/g and the total pore volume is 0.713 cm³/g. The results of the composite test showed that the sulfur content in the composite for a ratio of 1:2.5 and 1:3.5, respectively, was 60.6 wt% and 78.9 wt%. For N-doped porous carbon, the sulfur content in the composite was 66.95 wt% and 68.98 wt%. The results of the test using the four line probe (FLP) method obtained the electrical conductivity of porous carbon and N-doped porous carbon of 3.93 x 10⁻² S/cm and 17.16 x 10⁻² S/cm, composites and N-doped composites of 5.4 x 10⁻⁴ S/cm and 14.61 x 10⁻⁴ S/cm for the ratio 1:2.5, 2.24 x 10⁻⁴ S/cm and 5.0 x 10⁻⁵ S/cm for 1:3.5 ratio. Lithium sulfur battery cathodes have been successfully made from composites with various thicknesses of 100 μm and 200 μm. The sulfur content at the cathode was 2.80 mg/cm² and 3.57 mg/cm² for non-doped porous carbon and 4.93 mg/cm² for N-doped porous carbon. -The sulfur content values are 264 mAh/g and 312 mAh/g respectively. While the N-doped porous carbon battery has an initial discharge capacity of 584 mAh/g. Doping N has been successful in increasing the initial discharge capacity of the battery.

Keywords: lithium-sulfur battery, water hyacinth, porous carbon, capacity, composite

DEVELOPMENT OF SARS-CoV-2 ANTIGEN DETECTION BASED ON IMMUNOGLOBULIN Y (IgY) USING SURFACE PLASMON RESONANCE (SPR)

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ABSTRACT

SARS disease reappeared at the end of 2019 with a new name as Coronavirus Disease 2019 (COVID-19) which was caused by a new virus called SARS-CoV-2. This virus has spread throughout the world until recently and caused massive deaths and losses. The nucleic acid test in the form of real-time reverse transcriptase polymerase chain reaction (RT-PCR) is very important to diagnose COVID-19 in patients but this method has several drawbacks such as operators who have to be trained, the diagnosis results appear in a relatively long time, and the examination price relatively expensive. This research was conducted to produce immunoglobulin Y (IgY) extracted from chicken egg yolk targeting the S-protein receptor-binding domain (RBD) on SARS-CoV-2 as a component of the surface plasmon resonance (SPR) SARS-CoV-2 antigen detection kit. This research was started by extracting IgY from hyperimmune chicken egg yolks with the polyethylen glycol (PEG) precipitation method and continued with dialysis. The extracted IgY was further purified using thiophilic adsorption chromatography and concentrated by using Amicon® Ultra-15 ultrafiltration. The IgY activity against SARS-CoV-2 RBD was tested qualitatively using the agar gel precipitation test (AGPT) technique and the total protein content was determined using the Lowry method. IgY was tested for its affinity against SARS-CoV-2 RBD using SPR. The IgY concentration obtained was 11 mg/mL. The AGPT result showed the presence of IgY activity against SARS-CoV-2 RBD isolated from egg yolk and chicken serum after 8 weeks after the first vaccination of chickens. The SDS-PAGE results showed a very clear band of IgY characters. The obtained IgY showed adequate interaction with commercial SARS-CoV-2 RBD on a SPR device. The purified IgY was able to bind with protein-S RBD and showed a fairly good affinity for the SARS-CoV-2 antigen sample. The results of these observations indicate that IgY anti-S-protein SARS-CoV-2 can be produced and purified from chicken egg yolk and can be used as a diagnostic component to detect SARS-CoV-2 antigen, especially on SPR.

Keywords: COVID-19, SARS-CoV-2, immunoglobulin Y (IgY), surface plasmon resonance (SPR).