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Preface

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Preface

The 2nd International Conference on Agriculture and Rural Development (The 2nd ICARD)

The 2nd International Conference on Agriculture and Rural Development (The 2nd ICARD) had a theme “The Challenges of Sustainable Agriculture in the New Normal Era”. The conference was still held during the pandemic of covid-19 due to the great enthusiasm to have an event discussing the covid-19 impacts on agriculture, food, and rural livelihood. The travel restriction was also the reason to carry out the conference via online platform. Thus, it proceeded as the original agenda.

The 2nd ICARD was held on 16 November 2020 and organized by the Faculty of Agriculture, University of Sultan Ageng Tirtayasa (Untirta), Indonesia. It also became the framework of collaboration among the Faculty of Agriculture Untirta, Kazan National Research Technological University-Rusia, Gorgan University of Agricultural Sciences and Natural Resources-Iran and Universiti Teknologi MARA-Malaysia. The main sponsors of this conference were the Ministry of Education and Culture, and Indonesia agronomist association (PERAGI).

The 2nd ICARD was managed properly with virtual meeting and arranged into two sessions; they were oral presentations from keynote speakers as a plenary session and scholar presenters as a parallel session. In the first session, the respective keynote speaker was each allocated 40 minutes to hold the speech consist of 30 minutes for presentation and 10 minutes for discussion (Q and A). Four keynote speakers contributed to the plenary session. Assist. Prof. Dr. Yudi L.A. Salampessy from Untirta was talking about *Mining and Biodiversity Comics Publishing to the Elementary School Students around Mine Areas*. Also, our beloved keynote speaker Assoc. Prof. Dr. Saed Gorgin, from Gorgan University of Agricultural Sciences and Natural Resources, Iran, was presenting his study on *the Strategy of Shelf-Life Extension to Keep the Quality of Conical Lift net Catch for Sustainability in New Normal Era*. Furthermore, the third keynote speaker from Malaysia, Assoc. Prof. Dr. Asmah Awal (Universiti Teknologi MARA) was giving a great valuable presentation about *Sugar Palm (Arenga pinnata Wurmb Merr.): A Review on Plant Tissue Culture Techniques for Effective Breeding*. Moreover, our last keynote speaker Assoc. Prof. Elena Nikitina, Ph.D from Kazan National Research Technological University, Rusia, shared her study about *Potato Starch as a Component Increasing the Antioxidant Potential of Yogurt*. In the second session, the scholar presenters whose had submitted the selected paper were conferred 15 minutes including Q and A to speech the presentation one by one. They had divided into different rooms based on their scope of study.

The 2nd ICARD was attended by presenters from five countries, Indonesia, Germany, Malaysia, Russia, and Iran. Four keynote speakers and 80 scholar presenters attended to this conference. Research topics were divided into five main scopes focusing on Agricultural and



Environmental Sciences, Agribusiness and Agricultural Resource Economics, Fishery and Marine Sciences, Forestry/Silviculture, and Food Science and Agricultural Product Technology.

The 2nd ICARD was successfully run via a “Zoom” application meeting. All participants joined in a plenary room when the keynote speakers presented their research. As soon as the plenary session was over, the participants were assigned into a “breakout” room based on their topic of interests. Finally, they joined in the plenary room for the closing ceremony. We would like to mention that all of the participants were satisfied with both the conference performance on the D-day and the organizer responses during the paper submission process.

Lastly, we would like to thank to the chairman, scientific committees, keynote speakers, and all participants. We greatly thanks to the publisher for publishing the 2nd ICARD selected papers. We are looking forward to more scholars to join in this conference next year.

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AGRICULTURE AND RURAL DEVELOPMENT (THE 2nd
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16 November 2020, Serang City, Indonesia

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Table of contents

Volume 715

2021

◀ Previous issue Next issue ▶

**The 2nd International Conference on Agriculture and Rural Development 16 November 2020,
Serang City, Banten, Indonesia**

Accepted papers received: 10 March 2021

Published online: 07 April 2021

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Preface

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Visitors' Perceptions of Cultivation Technology Innovation in Agro Innovation Park, BPTP
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S Edi and Rustam

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Farm Business Analysis of Crops in Tidal Land (Case Study in Rantau Makmur Village, Rantau Rasau District) 012002

Adri and Yardha

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Communication Networks of On-Farm Rubber in Riau Province, Indonesia

R Yulida, R Rosnita, Y Andriani and M Ikhwan

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Quantifying the Reliable Discharges as an Incipient Analysis of Agricultural Planning and Developing in Ciujung Watershed

N P Purnaditya and S Asyiah

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Revenue and Feasibility Analysis of Celery (*Apium Graveolens* L.) in Lingkar Selatan Village Paal Merah District Jambi City

Suharyon and S Edi

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Distribution Mapping of Rice, Corn, and Soybean Production Based on Geographic Information Systems in Pandeglang Regency

N Hermita, A T Sumantri, R A Riyanto and A Mulyaningsih

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Environmental Carrying Capacity for Food Availability in Pandeglang Regency, Banten Province

Mirajiani

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Possible Use of Food Security and Vulnerability Atlas (Fsva) to Detect Problem on Poverty and Stunting, The Case of Banten Province

Y Budiawati, R Z Islami, T Perdana and R S Natawidjaja

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Analysis on Factors Affecting The Risk of Rice Farming Production in West Tanjung Jabung Regency

Yardha, E Wahyudi and A Wafi

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012010

Risk Analysis of Rice Farming in Alue Merbau Village, East Langsa Sub-District, Langsa City

F H Saragih, Hanisah and F Alham

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The Role of the Sawangan Organic Rice Farmers Association in Increasing the Economic Value of Organic Rice: Case Study in Sawangan, Magelang

A Setiawati, M Wijaya and R Setyowati

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Demand for Agricultural Product and Sustainable Development Issues: Empirical Studies from Household Survey

M Rasyid

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Analysis of the Role of Livestock Sub-Sector in Economic Growth in West Sumatra

A Suresti, F Tan, Adrimas and U Dinata

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012014

The Effect of Personal and Situational Factors on The Performance of Agriculture Extention Worker on the Behavior of Seeking Information as an Intervening Variabel (Study Case in Lebak Regency Banten Province)

L Nurhayati, Nurmayulis and Y L A Salampessy

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Tuba root (*Derris elliptica* Benth) flour extract concentration assay to control the corn cob borer (*Helicoverpa armigera* Hubn.)

R Rustam, D Salbiah, R Rismawanto and R Saputra

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Improvement of shallots (*Allium ascalonicum*) cultivation on paddy fields to increase shallots yields and farmers' income during the Covid-19 pandemic

D Haryani, M C Hadiatry, S Yuniarti and R Purba

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012019

The study of composting system and its use in supporting vegetable cultivation in Kepulauan Seribu - Jakarta

A F C Irawati, Y Sastro, Ikrarwati and S Sutardi

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012020

The success factor in developing an energy independent village in Klaten Central Java

D W Ningtyas, Suwanto and E Lestari

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Correlation study of soil test on phosphorus in ultisol soil for shallots (*Allium ascalonicum* l.)

I P Lestari, A D Susila, A Sutandi and D Nursyamsi

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Morphological characterization and development potential of beneng variety (*Xanthosoma undipes* K. Koch) Pandeglang - Banten

Z Yursak, I Hidayah, A Saryoko, S Kurniawati, O Ripasonah and P N Susilawati

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Heavy metal uptake and transition across the links of soil-plant-animal-food chain

R Volkov and D Samigullin

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012024

Potassium nutrition differentially affect early growth and leaf greenness of shallot under micro sprinkle fertigation in dry land

A Laila, M Sari, N I Muztahidin, A A Fatmawaty and N Hermita

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012025

Increasing shelf life of rhizobacteria formula with alginate in encapsulation during storage

J E R Rumbiak and S Hilal

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Mapping Distribution Approach in Various Types of Use Sub-Optimal Dry Land in Aceh Besar District

H A Umar, S Sufardi, S Syafruddin and T Arabia

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Efficacy of Red Betel Leaf's (*Piper crocatum*) Chloroform Extract as Repellent Against Rice Bugs *Leptocorisa acuta* Thunberg, 1783 (Hemiptera: Alydidae)

F Saraswati, A S Herdiannanta and R C H Soesilohadi

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012028

A Model for Accelerating Rice Planting in Paddy Fields to Provide Food in Banten Province during the Covid-19 Pandemic

Kardiyono, T Mulyaqin, P N Susilawati, D Haryani, I Setyowati and H. Ismatul

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012029

Yield Performance of Shade Tolerant Soybean Cultivars under Shaded Environment at Various Planting Densities

A Saryoko, Z Yursak, Y Astuti, S Kurniawati, S Kusumawati and T Mulyaqin

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Yield ability and grain quality of upland rice in Sukabumi and Lampung

R Hermanasari, A P Lestari, Yullianida, A Hairmansis, Santoso and A Nasution

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Improving Rice Productivity Through the Implementation of Jajar Legowo Super (Jarwo Super) Technology in Jambi Province

Endrizal, Jumakir and J Bobihoe

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012032

Screening of Rizoplan Rhizobacteria for Suppression of Bacterial Wilt (*Ralstonia solanacearum*) and Promoting the Growth on Chili (*Capsicum annum*)

J E R Rumbiak and S Hilal

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012033

Characterization of Performance 12 Superior Lines of Upland Rice Planted in Two Environmental Condition

E S Mulyaningsih, Y G D Anggraheni, E B M Adi, N Burhana and L Santun

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012034

Hybridization and In Vitro Seed Germination of a Commercial Hybrid *Oncidium* Orchid in Indonesia

S Rianawati, Suryanah, Syafni, Musalamah, K Dwiatmini and B Marwoto

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The performance of the brown planthopper (*Nilaparvata lugens*) population and predators on endemic lowland rice areas of Banten Province

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Yield of Soybean and Corn Intercropping Farming in Rainfed Lowland in Central Lampung, Lampung Province
Slameto, Meidaliantisyah, Agung Lasmono and Wahyu Wibawa
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-
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Increasing Planting Density of Maize Trough Double Row Cropping System to Improves Seed Yield
A Saryoko, Y Giamerti, Z Yursak, P N Susilawati, S Kusumawati, Kardiyono and N Winanti
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The Effect of Basic Fertilizer Doses On Soybean Growth And Production
T Liana, D. Irwandy and A Bhermana
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-
- OPEN ACCESS** 012039
Quality of Growth Media and Yields of *Allium ascolanicum* L. on Ultisol Soil Combined with Rabbit Manure
Ikrarwati, N A Syamsi, Y Sastro, T B Rusbana, N R Sudolar and Y Romadhonah
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- OPEN ACCESS** 012040
Optimization of Annealing Cycle and Temperature SNAP T12 Primer Distinguishing Markers for Male, Female and Hermaphrodite Plants in Papaya (*Carica papaya* L)
Noflindawati, A Anwar, A Sutanto and Yusniwati
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Implementation of Eco-friendly Technologies to Control Bacterial Leaf Blight of Rice Disease (*Xanthomonas oryzae* pv. *oryzae*)
S Kurniawati, Y Astuti, Y S Hidayat and E Y Susanti
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Application of New Superior Varieties of Irrigated Land Rice within Integrated Crop Management
R Yusuf, F Fuadi, A S Alim, P N Istina and N Sutrisna
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Interaction of Genetic and Zn Fertilizer Application on Rice Yield and Grain Zinc Content

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Studies on Cultivation of Several Varieties of Onion (*Allium ascalonicum* L.) under Plastic Shade during Rainy Season in Jakarta

E Sugiartini, F R Eris, E Pancaningsih, O Nurviani and N Herawati

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012045

Diversity Assessment of Mango (*Mangifera* spp) Plant Collection of Cibinong Germplasm Garden Based on Leaves Morphology and RAPD Markers

Y G D Anggraheni and E S Mulyaningsih

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012046

Genetic diversity of Some Indonesian Local Rice Varieties based on Simple Sequence Repeat (SSR) marker related to Aromatic Genes

Susiyanti, Nopiasari, I Rohmawati, P Nursusilawati, Sjaifuddin and S Abdullah

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Characterization of Sugar Plant Seeds (*Arenga pinnata* Merr.) from Several Locations in Banten Province and Their Growth Response to the Provision of Liquid Organic Fertilizers

AM Kartina, Susiyanti and W A Rusadi

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012048

The Symptomp Rate of Cocoa Pod Borer (*Conopomorpha cramerella*) due to the Combination of Several Concentrations of Neem (*Azadiracta indica*) Solution Given the Biosurfactant of Diethanolamide Olein Palm and Different Fruit Sizes

Nurmayulis, D Hastuti, F R Eris and Mujahidah

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012049

Combination of fish oil with rubber seed oil to the growth performance of catfish (*Clarias* sp.)

M B Syamsunarno, I D G Tambunan, A Munandar, Mustahal, A N Putra and M Herjayanto

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012050

Characteristics of Oil in Water (o/w) Type Lotions Incorporated with Kappa/Iota Carrageenan

D Fransiska, M Darmawan, E Sinurat, B B Sedayu, Y W Wardhana, Y Herdiana and G P Setiana

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012051

Study of The Effect of Light on The Length-Frequency and Catch Rate of Caspian Sea Sprat Conical Lift Net in Amirabad Fishing Ports

S Nodehshrifi, S Gorgin, S Y Paighambari, HA Khoshbavar-Rostami, M Babanezhad and H Fazli

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Aquaculture in The World and Russia: State and Prospects

T E Marinchenko

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012053

Combination Effect of Solid Waste of *Gracilaria* Extraction and *Sargassum* Powder on Nitrogen Content of Bio Fertilizer

J Basmal, R Kusumawati, N Rahman and Z Hanafia

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012054

The Effect of Addition Glycerol Against Nori Characterization from *Gracilaria* sp and *Ulva* sp Seaweeds

E Sinurat, D Fransiska and Livia

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012055

Comparison of Gel Preparation Methods on Gel Strength Measurement of Carrageenan

Fateha, N Ulya, Asmanah and Agusman

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Measurement of Fish Gelatin using Rotational Viscometer: An Alternative to Conventional Pipette Method

Agusman, Suryanti, Nurhayati, Murdinah and T Wahyuni

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012057

Effects of Water on Hydrophobization and Mechanical Properties of Thermoplastic Agar

Agusman, D Fransiska, Nurhayati, H E Irianto, P Priambudi, A H D Abdullah, R C Nissa, P P P Asri, N Masruchin, B B Sedayu *et al*[+ Open abstract](#) [View article](#) [PDF](#)

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012058

The Effect of Adding *Bacillus* NP5 to Feed on Growth, Survival Rate, and Protection Against *Aeromonas hydrophila* of Catfish (*Clarias* sp.)

Mustahal, Sevia, M Herjayanto, M B Syamsunarno and A N Putra

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012059

Effect of *Trichoderma* Addition on *Sargassum* Organic Fertilizer

R Kusumawati, Nurhayati, H E Pangestu and J Basmal

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012060

Development of seed mass production of snakehead (*Channa striata*) in Indonesia

A Saputra, M B Syamsunarno and M T D Sunarno

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012061

Penetration of Microplastics (Polyethylene) to Several Organs of Nile Tilapia (*Oreochromis niloticus*)

D Aryani, M A Khalifa, M Herjayanto, E A Solahudin, E M Rizki, W Halwadiyah, H Istiqomah, S H Maharani, H Wahyudin and G Pratama

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012062

Economic Resilience of Fishermen Community During Covid-19 Pandemic

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012063

The Development Design of Venturi Type Protein Skimmer for Mariculture Land Base System

A Susanto, M Herjayanto, Alimudin, W Budiaji, E Priyantono and N A Guna

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012064

Business Analysis of Fish Sauce from Goldspotted Anchovy (*Coilia dussumieri*) in Bagansiapiapi, Riau Province

K S Harahap, R S Mardiah and S A Ikhsan

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012065

Hazard analysis and critical control point of milkfish floss production as indigenous food from Banten province

W Nurtiana, Z Najah, D Anggraeni and N A Putri

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012066

The use of plant proteins in the technology of fermented dairy-free products

D Khrundin, V Ponomarev, E Yunusov and G Ezhkova

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012067

Development of functional beverages from herbs: aspect of nutrition, processing and safety

V Y Pamela, S Kusumasari and B Meindrawan

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012068

Quality of red tuna (*Yellowfin tuna*) fishball, white oyster mushroom (*Pleurotus ostreatus*) on different types of packaging and storage time

I K Budaraga, V Saibuma and L Hermalena

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Analysis of glutamine synthetase activity from *Lactobacillus hilgardii* LMG 7934

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Design formula and product prototype of beverage made from tamarillo (*Solanum betaceum*) fruit and sappan wood (*Caesalpinia sappan*) using Kano method

C Angelica, O O Pangestu, J Kurniawan, B Meindrawan and D J C Hutabarat

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012071

Whole genome sequence data of *Lactobacillus fermentum* AG8, the producer of antibacterial peptides

A Pavlova, G Ozhegov, M N Yahia, N Gogoleva, E Shagimardanova, E Nikitina and A Kayumov

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012072

Potato starch as a component increasing the antioxidant potential of yogurt

E Nikitina

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012073

Effect of the formulation of fermented white corn flour and glutinous rice flour on the quality of instant cream soup powder

N A Pratiwi, R Rahmawati, R R Maulani, D Hunaefi, D Saputra and Tj Muhandri

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012074

Performance test and economic analyses of Semi Mechanic Corn Sheller

T Hidayat, Yahumri, Miswarti, H Iswadi, T Rahman, S Yuliasari and W Mikasari

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Shrimp Paste Crackers as Potential Product Development for Small and Medium Enterprise (SMEs)

C Retnaningsih, B Bektir, O Ruenda and Meiliana

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012076

Mitigation of Human-Orangutan Conflict in Orangutan Reintroduction Area at Suo-suo Village, Buffer Zone of Bukit Tigapuluh National Park

Maskulino, A H Harianja and W Kuswanda

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012077

Ethnopharmacological Properties of Essential Oils from Natural Forests in Northern Sumatra

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012078

The Consumer Preferences for New Sumatran Camphor Essential Oil-based Products using a Conjoint Analysis Approach

C R Kholibrina and A Aswandi

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012079

Gender Adaptation Strategy to The Impact of Forest and Land Fire in Kelampangan Village Peatland, Sebangau, Central Kalimantan

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Combination of fish oil with rubber seed oil to the growth performance of catfish (*Clarias* sp.)

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Abstract. The rubber seed oil contains essential fatty acids, which may be required by cultured freshwater fish. Therefore, the research aimed to determine a combination of fish oil and rubber seed oil for catfish's best growth performance (*Clarias* sp.). Isonitrogenous ($33.23 \pm 0.19\%$) and isoenergy (266.50 ± 0.09 DE kcal/100g) tested diets were used in the experiment. The fish oil was replaced by rubber seed oil at 0%, 15%, 25%, 35%, and 50%, respectively. Cyanide acid of rubber seed oil was reduced its content by stirring at 110 °C for 90 minutes to have as high as 0.47 ppm. Catfish fingerlings of 6.91 ± 0.04 g in individual weight were randomly distributed into 15 aquariums (60x40x40cm) each at a rate of 20 fingerlings and fed on the tested diet ad satiation for 40 days during the experimental period. The results showed that the increase in rubber seed oil to replace fish oil in the feed was not significantly different from the survival rate and nutrient retention in body catfish ($P > 0.05$). Furthermore, catfish were not poisoned by cyanide acid, indicated by catfish blood profile in the normal range at the end of rearing ($P > 0.05$). The combination of fish oil and rubber seed oil by 50% gives optimal growth in catfish.

1. Introduction

Catfish require a nutritionally complete and balanced diet for growth. The quality of fish feed is determined by the nutrients available to support basic metabolic, health status, and optimal growth [1]. Lipid is one of the macronutrients that must be available in the feed. It serves as a source of essential fatty acids, and if insufficient amounts in the feed, it can reduce the use of protein as an energy source [2]. Fish oil is a major component of fat in food because of its complete fatty acid profile and can be a source of fish's metabolic energy [3]. However, its use is currently limited to meet the growing demand for the aquafeed industry [4]. That leads to increased feed prices affecting profit freshwater fish farmers, especially in catfish [5].

Vegetable oil is a potential source of lipid to replace fish oil in the diet because of its abundant availability, acceptable price, relatively low organic contamination, and high fatty acid [6]. Furthermore, types of agro-industrial by-products have the potential as alternative feed ingredients [7]. Rubber seed is a by-product in rubber cultivation that has not been utilized, with an oil content of around 40 - 50%



[8]. Rubber seed oil is a by-product of processing rubber seed meal, and it is estimating that rubber seed oil production in Indonesia is in the range of 192.2 - 199.08 million tons per year [9].

Rubber seed oil is a semi-drying oil containing essential fatty acids, namely, oleic acid (22.95%), linoleic acid (37.28%), and linolenic acid (19.22%) [10]. Similarly, the result was found by [11], who reports that oleic acid levels, linoleic acid, and linolenic acid were 25.33%, 37.50%, and 14.21%, respectively. The rubber seed oil has a higher fatty acid than soybean oil [12] and almost close to corn oil's fatty acid composition [13]. The rubber seed oil's essential fatty acid composition is 52% of the fatty acid composition [14].

Unprocessed rubber seed oil in the diet will limit feed intake, lower growth and survival rates, affecting blood profiles of fish [9] [15]—that is thought to be due to an anti-nutritional substance in cyanide acid-containing rubber seed oil. The most prominent anti-nutritional content in rubber seeds is cyanide acid [16]. There are also phytic, oxalic, tannin, saponins, and trypsin inhibitors [17] [18]. Several studies have reported that cyanide acid content can reduce through physical treatment, including soaking, heating, boiling, and roasting [19] [20]. Furthermore, [21] reported that stirring at 100°C could reduce cyanide acid in rubber seed oil. Therefore, the research aimed to determine a combination of fish oil and rubber seed oil for catfish's best growth performance (*Clarias* sp.).

2. Material and Methods

2.1. Rubber seed oil preparation

The number of 18 kg of rubber fruit was taken from smallholder plantations in Semerangkai Village, Sanggau Regency, West Kalimantan, and broken down to get a seed. The rubber seed was then boiled for 20 minutes, dried in an oven at 60°C for 60 minutes, and ground into meal form. The meal was oiled out using a pressing machine at 80°C for 30 minutes [22]. Cyanide acid of rubber seed oil was reduced its content by stirring at 110°C for 90 minutes to have as high as 0.47 ppm. [23] recommends that cyanide acid content in food products is 1 ppm. The process of removal of cyanide acid refers to [21].

2.2. Preparation experimental diet

Isonitrogenous ($33.23 \pm 0.19\%$) and isoenergy (266.50 ± 0.09 DE kcal/100g) tested diets were used in the experiment. The primary sources of protein in the experimental diet were fish meal and soybean meal. The total lipid in the test diet was 5% [24], from a combination of fish oil and rubber seed oil, namely 0%, 15%, 25%, 35%, and 50%. Raw materials are weighed according to the formula and mixed evenly and molded-in pellets with a 3 mm diameter, then oven at 60°C [9]. After drying, the experimental diet was put into a plastic bag labeled, and the samples were taken as the subject of proximate and fatty acid analysis. The formulation and analysis results of the experimental diets are present in Table 1 [25].

2.3. Experimental design

Catfish of 6.91 ± 0.04 g/fish in initial weight were reared at 20 individuals/aquarium. The fishes were raised in 15 aquariums of 60 x 40 x 40 cm in dimension size, each for 40 days. After acclimatized for seven days, the fish was fasted for 24 hours to eliminate the body's remaining feed [26]. Feeding was carried out three times a day at satiation. Water changes were carried out as much as 50% of the aquarium's total volume, aiming to maintain water quality during the study. At harvest, several fish samples were taken as subjects for proximate analysis and blood profiles.

2.4. Growth parameters

At the end of the study, the growth parameters of catfish were analyzed. The specific growth rate (SGR), feed efficiency (FE), survival rate (SR), and protein/lipid retention (PR/LR) were calculated using the formula according to [25].

$$\begin{aligned} \text{SGR (\%)} &= 100 \times (\ln W_t - W_o) / \text{time (days)} & (1) \\ \text{FE (\%)} &= 100 \times [\text{wet weight gain (g)} / \text{dry feed intake (g)}] & (2) \\ \text{SR (\%)} &= \text{NT}/\text{N0} \times 100 & (3) \\ \text{PR/LR (\%)} &= ((F - I) / P) \times 100 & (4) \end{aligned}$$

Where W_t and W_o indicate the final and initial weight (g), respectively, NT is the number of fish at the end (individuals), N0 is the number at the beginning (individuals). F is total protein/lipid in the final body (g), I is the total protein/lipid in the initial body (g), and P is total protein/lipid consumption (g).

Table 1. Composition of tested diets, and their proximate analysis and fatty acids

Composition	Experimental diet (%)				
	0	15	25	35	50
Fish meal	30.00	30.00	30.00	30.00	30.00
Soybean meal	27.50	27.50	27.50	27.50	27.50
Tapioca	13.00	13.00	13.00	13.00	13.00
Pollard	9.45	9.45	9.45	9.45	9.45
Wheat flour	7.50	7.50	7.50	7.50	7.50
Fish oil	5.00	4.25	3.75	3.25	2.50
Rubber seed oil	0.00	0.75	1.25	1.75	2.50
Vitamin and mineral premix	5.00	5.00	5.00	5.00	5.00
Casein	2.00	2.00	2.00	2.00	2.00
Vitamin C	0.05	0.05	0.05	0.05	0.05
PMC	0.10	0.10	0.10	0.10	0.10
Total	100.00	100.00	100.00	100.00	100.00
Proximate compositions (dry base)					
Crude protein (%)	33.07	33.16	33.48	33.39	33.06
Crude lipid (%)	6.43	6.56	6.46	6.44	6.45
Ash (%)	13.62	13.92	13.80	13.74	13.63
Crude fiber (%)	7.44	7.45	7.41	7.42	7.48
Free extract nitrogen (%)	39.44	38.91	38.85	39.01	39.38
DE (kcal/100g)*	266.43	266.47	266.63	266.55	266.41
Fatty acids (%)					
Σ SFA**	18.72	18.59	18.51	18.42	18.29
Σ MUFA***	19.84	19.57	19.38	19.20	18.92
Σ n-6	20.46	20.71	20.88	21.05	21.31
Σ n-3	10.83	10.65	10.53	10.40	10.22
Σ n-3/ Σ n-6	0.53	0.51	0.50	0.49	0.48

Noted: * DE= Digestible energy = 1 g protein: 3.0 kkal DE; 1 g lipid: 8.1 g kkal DE; 1 g carbohydrate: 2.5 kkal DE [27]

** SFA= Saturated fatty acids

*** MUFA = Monounsaturated fatty acids

2.5. Blood profiles

At harvest, three individuals from each treatment were taken out for collecting blood samples of fish. The blood samples were taken out using a syringe already containing anti-coagulant 0.1 mL of 3.8% Na citrate [28]. Furthermore, the blood profile consisting of total red blood cells (RBC), white blood cells (WBC), haemoglobin, and haematocrit were calculated with a microscope's aid. Determination of total

RBC and WBC according to the method [29], measurement of haemoglobin (Hb) levels by the Sahli method according to [30], and measure of haematocrit (Ht) levels according to the procedure [31].

2.6. Chemical analysis

The proximate composition of raw materials, experimental diet, and fish body samples were analyzed according to the standard method [32]. The proximate composition is crude protein, crude fat, ash content, crude fiber, and nitrogen-free extract.

2.7. Statistical analysis

Statistical analysis of growth parameters and blood profiles perform using the Statistical Package for the Social Sciences (SPSS) program for Windows (v.16.0). If a significant difference, then proceeds with the Tukey test at a level of 95% [33].

3. Results and Discussion

Based on the results of the research in Table 2, it was known that the increase in rubber seed oil in feed to replace fish oil could increase feed intake in catfish ($P < 0.05$). This increase is thought to be due to rubber seed oil, which can increase feed palatability. Similar results are reported in juvenile Malaysian mahseer (*Tor tambroides*) fed a vegetable oil-based feed to replace fish oil [34]. Unsaturated fatty acids influence feed palatability. According to [15], the decrease of unsaturated fatty acids results in poor taste and the feed aroma, limiting feed intake by fish. In this study, the unsaturated fatty acid content in the test feed (Table 1) is thought to have increased feed consumption.

Table 2. The growth performance of catfish fed the experimental diet during the rearing period

Parameters**	Experimental diet (%)*				
	0	15	25	35	50
FI (g)	165.00±3.61 ^a	167.33±4.04 ^a	176.67±5.51 ^{ab}	181.67±5.86 ^b	183.67±2.08 ^b
FE (%)	40.31±1.78 ^a	41.35±2.87 ^{ab}	44.56±5.86 ^{ab}	49.08±2.80 ^{ab}	49.08±1.58 ^b
SGR (%)	1.00±0.06 ^a	1.03±0.06 ^a	1.14±0.12 ^{ab}	1.24±0.03 ^b	1.30±0.05 ^b
Protein retention (%)	67.09±2.44	67.61±2.72	71.59±2.84	72.40±3.14	73.95±3.41
Lipid retention (%)	64.03±5.34	62.42±3.21	63.12±5.62	64.05±5.33	65.61±5.02
SR (%)	96.67±2.89	96.67±2.89	98.33±2.89	100.00±0.00	96.67±2.89

Noted: *The values in the same row followed by similar superscript are not significantly different ($p > 0.05$).

** feed intake (FI), feed efficiency (FE), specific growth rate (SGR), survival rate (SR)

The increased composition of rubber seed oil in the feed affects feed efficiency and can further increase growth. The results of statistical tests showed that the feed without rubber seed oil (0%) was significantly different on feed efficiency and specific growth rate at 50% treatment ($P < 0.05$). The combination of fish oil with rubber seed oil as much as 50% produces an adequate supply of essential fatty acids to increase catfish growth. The use of vegetable oil to replace fish oil in aquafeed can be done more than 50% if the fatty acids found in the feed are sufficient to meet the needs of essential fatty acids in fish [4]. Based on Table 1, the n-6 fatty acids increased with the additional rubber seed oil in the diet. In general, freshwater fish need linoleic acid (18: 2n-6), or linolenic acid (18: 3n-3), or both to support optimum growth [1]. According to [35], catfish need more n-6 than n-3 fatty acids to accelerate their growth. The increasing growth of catfish is thought to be related to the decreasing ratio of n-3/n-6 in the diet. According to [36], the n-3 and n-6 fatty acid requirements in the lower n-3/n-6 rate promote better growth at the striped catfish (*Pangasius hypophthalmus*).

Protein and lipid retention at the end of the study had no significant effect with different ratios of rubber seed oil and fish oil ($P > 0.05$). The presence of saturated fatty acids in the appropriate test diet

required by catfish causes the energy derived from these fatty acids to be used as an energy source to maintain the fish's body. According to [29], higher saturated fatty acids can be easily oxidized. The energy derived from these fatty acids can be used to maintain body maintenance and protein for growth. This study's results are following the results of a research according to [37] [36] [38].

Table 3. The value of blood profiles in catfish fed the experimental diet at the end of the study

Blood profile**	Experimental diet (%)*				
	0	15	25	35	50
RBC ($\times 10^6$ cell/mm ³)	1.49±0.14	1.56±0.12	1.56±0.11	1.55±0.13	1.57±0.13
RWC ($\times 10^4$ cell/mm ³)	3.20±0.06	3.21±0.08	3.28±0.15	3.26±0.03	3.28±0.07
Hb (g%)	6.47±0.15	6.50±0.20	6.43±0.15	6.50±0.30	6.43±0.21
Ht (%)	25.60±0.44	25.40±0.17	25.83±0.31	25.73±0.65	25.47±0.31

Noted: *The values in the same row followed by similar superscript are not significantly different ($p > 0.05$).

** red blood cell (RBC), white blood cell (WBC), Haemoglobin (Hb), Ht (Haematocrit)

Table 3 shows that the blood profiles did not significantly differ between treatments ($P > 0.05$). The level of cyanide acid will affect fish health and fish blood profile. According to [15], fish exposed to cyanide acid in their body causes the fish to be under metabolic stress, making it susceptible to disease. The process of cyanide acid poisoning in fish involves cellular cytochrome enzymes so that haemoglobin in the blood cannot bind to oxygen and causes low haemoglobin [39]. High cyanide acid will affect blood chemistry and blood profile, such as low haematocrit, low total red blood cells and white blood cells [40] [15]. The study results [9] reported a low fish survival rate due to high cyanide acid, but in this study, the survival rate was not different, namely between 96.67-100% ($P > 0.05$). The content of cyanide acid of rubber seed oil used in this study can make tolerant of catfish. Cyanide acid below 5.05 ppm in the feed does not affect fish survival rate [41]. Reducing cyanide acid to fish tolerance levels can improve rubber seed oil quality combined with fish oil up to 50% (1:1). These results suggest that rubber seed oil can be used as an alternative source of vegetable oil mixed with fish oil in feed, such as corn oil and palm oil. The combination of fish oil and corn oil is the best as a lipid source in the diet [27] [1]. According to [4], the vegetable oils most commonly used for fish feed production include soybeans, corn, rapeseed, sunflower, palm oil and olive oil.

4. Conclusion

The best combination of fish oil and rubber seed oil at 1:1 (50%) in the diet composition resulted in catfish's optimal growth performance.

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