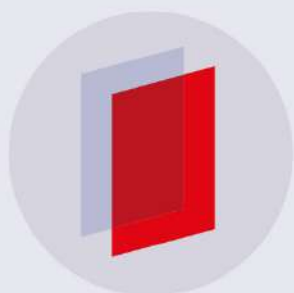


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Nowadays, the population all over the world is about 7.4 billion. Huge quantities of high quality food and agricultural products are needed every day. Therefore, forceful agricultural knowledge and innovation systems assure that the global food system contribute to decent supplies of high quality food and agricultural products, in a sustainable way. The International Conference on Food and Agriculture (ICoFA) will be a paramount and valuable event for scientists, researchers, students and practitioners to meet and discuss recent issues in food and agriculture sectors. The conference will cover a series of presentations and discussions in plenary, concurrent and poster sessions. ICoFA is dedicated to present high quality researches delivered by reputable Keynote Speakers and invited speakers from Asia region.

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Table of contents

Volume 207

2018

◀ Previous issue Next issue ▶

**1st International Conference on Food and Agriculture 2018
20-21 October 2018, Bali, Indonesia**

[View all abstracts](#)

Accepted papers received: 12 November 2018

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Preface

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011001

1st International Conference on Food and Agriculture 2018

[+ View abstract](#) [View article](#) [PDF](#)

OPEN ACCESS

011002

Peer review statement

[+ View abstract](#) [View article](#) [PDF](#)

Papers

Agriculture Engineering and Biotechnology

OPEN ACCESS

012001

Potential of *Beauveria bassiana* Lowland Isolates against *Spodoptera litura* in Tobacco Plant

D N Erawati, I Wardati and S Humaida

[+ View abstract](#) [View article](#) [PDF](#)

-
- OPEN ACCESS** 012002
- The effects of different concentration of sucrose and various auxin on in vitro shoot and microtuber formation of red potato (*Solanum tuberosum*, L. var Desiree)
- S L Asmono, V K Sari and Djenal
- [+](#) View abstract [View article](#) [PDF](#)
-
- OPEN ACCESS** 012003
- Design and performance test of a batch system rotary vacuum dryer with a 50-liter capacity to dry basidiomycota class mushrooms
- S Djamilia, Iswahyono and A Bahariawan
- [+](#) View abstract [View article](#) [PDF](#)
-
- OPEN ACCESS** 012004
- Optimization of Design Heat Exchanger to Reduce Fouling Resistance in Milk Pasteurization
- T Budiati and T R Biyanto
- [+](#) View abstract [View article](#) [PDF](#)
-
- OPEN ACCESS** 012005
- The impact of land use changes on carrying capacity of sampean watershed in Bondowoso Regency
- S Sugiyarto, B Hariono, R Wijaya, P Destarianto and A Novawan
- [+](#) View abstract [View article](#) [PDF](#)
-
- OPEN ACCESS** 012006
- Characterization of Morphology from Orchid Vanda sp. as a Genetic Information Source for Preservation and Agribusiness of Orchids in Indonesia
- K Kasutjaningati and R Firgiyanto
- [+](#) View abstract [View article](#) [PDF](#)
-
- OPEN ACCESS** 012007
- Mathematical Model of the Water Quality in Kalibaru Watershed
- B Hariono, R Wijaya, M F Kurnianto, Sugiyarto, M J Wibowo and S Anwar
- [+](#) View abstract [View article](#) [PDF](#)
-
- OPEN ACCESS** 012008
- Effect of ultraviolet and ultrasonic on potential antidiabetic activity of in vitro shoot cultures of *Orthosiphon aristatus*
- D. Hunaefi, N.D. Yuliana, I. Smetanska and N. Gruda

[+ View abstract](#)

[View article](#)

[PDF](#)

Agroindustry and Agribusiness

OPEN ACCESS

012009

Determination of Water Quality Status of Stored Method in Tlogo Ampel Watershed

Saiful Anwar, M Joko Wibowo, Sugiyarto, Budi Hariono and Rizza Wijaya

[+ View abstract](#)

[View article](#)

[PDF](#)

OPEN ACCESS

012010

Determination of Agro-Industry Area Based on Cassava Commodity in Bondowoso Regency

D. Hermanuadi, A. Brilliantina and E.K. Novitasari

[+ View abstract](#)

[View article](#)

[PDF](#)

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012011

Quality Improvement of Vannamei Shrimp Production Process Using ISO 9001:2015

R Iskandar, W Dhamayanthi and I A A Pongoh

[+ View abstract](#)

[View article](#)

[PDF](#)

OPEN ACCESS

012012

The main problems of na-oogst tobacco agribusiness in Jember

Muksin, R Hari, K Tanti and I Titik

[+ View abstract](#)

[View article](#)

[PDF](#)

OPEN ACCESS

012013

Modeling of rainfall and fertilization factor of sugarcane productivity in Asembagus sugar factory Situbondo

I Harlianingtyas, D Hartatie and A Salim

[+ View abstract](#)

[View article](#)

[PDF](#)

OPEN ACCESS

012014

Deficiency factors in rehabilitation and capacity building of local farmers national parks through interpretative structural modeling approaches

H Rujito, M M D Utami, Rizal and H Y Riskiawan

[+ View abstract](#)

[View article](#)

[PDF](#)

OPEN ACCESS

012015

Development of Agroindustry Based on Region Superiority in The Efforts to Accelerate Economic Growth in Arjasa District

D E Putra and A M Ismail

[+](#) [View abstract](#) [View article](#) [PDF](#)

OPEN ACCESS

012016

Design of Agroindustry Development Strategy Based on Fisheries Cacthe of Merauke Regency

R A Djamali and P Betaubun

[+](#) [View abstract](#) [View article](#) [PDF](#)

Animal Nutrition, Animal Production, and Veterinary Science

OPEN ACCESS

012017

Effects of zeolite in aflatoxin B1 contaminated diet on aflatoxin residues and liver histopathology of laying duck

I Sumantri, H Herliani, M Yuliani and N Nuryono

[+](#) [View abstract](#) [View article](#) [PDF](#)

OPEN ACCESS

012018

In Vitro Studies: Potential Of Natural Source Herbal As Anti Cholesterol Agent

R Widyani, MH Hermawan and K Tirtodihardjo

[+](#) [View abstract](#) [View article](#) [PDF](#)

OPEN ACCESS

012019

Probiotics based on Local Microorganism as a substitute of Antibiotic Growth Promotor (AGP) on Broiler productivity

U Suryadi and A F Prasetyo

[+](#) [View abstract](#) [View article](#) [PDF](#)

OPEN ACCESS

012020

Personality assessment of different horse breeds trained for military purposes

T S M Widi, G A Rakasiwi, T Nugroho and N Widias

[+](#) [View abstract](#) [View article](#) [PDF](#)

OPEN ACCESS

012021

Effect of supplement Maggot Black Soldier Fly live on the percentage of carcass and weight of carcass of male Alabio ducks

A. Gunawan, S. Erlina, R. Samudera, M. Syarif D, M.Y. Noor and A.X. Lantu

[+](#) [View abstract](#) [View article](#) [PDF](#)

OPEN ACCESS

012022

Precaution in Introducing Double-Muscled Exotic Breeds into Indonesian Cattle Population

N Widyas, S Prastowo, T S M Widi and I G S Budisatria

[+](#) View abstract [View article](#) [PDF](#)

OPEN ACCESS

012023

The effects of rice hull supplementation or inclusion on performance and gastrointestinal weight of broiler chickens

S Hartini, DD Rahardjo and H Sasongko

[+](#) View abstract [View article](#) [PDF](#)

OPEN ACCESS

012024

Supplementation of probiotic and prebiotic on the performance of broilers

M M D Utami and N D Wahyono

[+](#) View abstract [View article](#) [PDF](#)

OPEN ACCESS

012025

Sustainability status of integrated rice-corn and beef cattle farming agriculture business in Jember regency

N B E Sulistyono, Z Fanani and M M D Utami

[+](#) View abstract [View article](#) [PDF](#)

OPEN ACCESS

012026

An analysis of marketing channels on broiler carcass in Jember Regency

N D Wahyono and M M D Utami

[+](#) View abstract [View article](#) [PDF](#)

OPEN ACCESS

012027

Current and future goat production in Jembrana Regency, Bali Province

L Doloksaribu, B P McLachlan, R S Copland and P J Murray

[+](#) View abstract [View article](#) [PDF](#)

OPEN ACCESS

012028

Body dimensions of primiparous Bali cattle (*Bibos sondaicus*) supplemented with concentrate at their first three month of pregnancy

A A Oka, N N Suryani, M Dewantari, N P Sarini and N P Mariani

[+](#) View abstract [View article](#) [PDF](#)

OPEN ACCESS

012029

Increasing antioxidant activity of quail (*cortunix-cortunix japonica*) eggs with the addition of sweet flag (*acorus calamus*) powder as a feed additive

Y F Nuningtyas and E Widodo

[+](#) View abstract [View article](#) [PDF](#)

OPEN ACCESS

012030

Effects of Mount Agung eruption on botanical composition and nutritive value of ration fed and rumen performance of Bali cattle in evacuation zones

N N Suryani, I W Suarna and I G Mahardika

[+](#) View abstract [View article](#) [PDF](#)

OPEN ACCESS

012031

Allele frequency estimation of BLAD (Bovine Leukocyte Adhesion Deficiency) in dairy cattle in Enrekang regency South Sulawesi Indonesia

M I A Dagong, L Rahim, R R S R Aprilita Bugiwati and Nurmulyaningsih

[+](#) View abstract [View article](#) [PDF](#)

OPEN ACCESS

012032

Identify the diversity of helminth parasites in cattle in Jember district (East Java - Indonesia)

A Awaludin, Nurkholis and S Nusantoro

[+](#) View abstract [View article](#) [PDF](#)

OPEN ACCESS

012033

The blood haematological profile on laying hens that treated by different levels of yeast supplementation

D Pantaya and M M D Utami

[+](#) View abstract [View article](#) [PDF](#)

OPEN ACCESS

012034

The effect of adding probiotic *Saccharomyces cerevisiae* on dietary antibiotic-free on production performance and intestinal lactic acid bacteria growth of broiler chicken

S Wulandari and T M Syahniar

[+](#) View abstract [View article](#) [PDF](#)

OPEN ACCESS

012035

Addition garlic extract in ration on fat deposition of broiler

M M D Utami, D Pantaya, N Nofida, N H D Larasati and A Agus

[+](#) View abstract [View article](#) [PDF](#)

Food Safety

OPEN ACCESS

012036

Antimicrobial activity of essential oil from Indonesian medicinal plants against food-borne pathogens

T Budiati, W Suryaningsih, S. Umaroh, B. Poerwanto, A Bakri and E Kurniawati

[+ View abstract](#)

[View article](#)

[PDF](#)

OPEN ACCESS

012037

Increasing of fish quality using ice-sterof foam container for paseban fisherman at Jember Regency East Java, Indonesia

D E Rahmanto and N Nurhayati

[+ View abstract](#)

[View article](#)

[PDF](#)

Food Science and Technology

OPEN ACCESS

012038

The Potency of betacyanins extract from a peel of dragon fruits as a source of colourimetric indicator to develop intelligent packaging for fish freshness monitoring

Ardiyansyah, Mulia W Apriliyanti, A Wahyono, M Fatoni, B Poerwanto and W Suryaningsih

[+ View abstract](#)

[View article](#)

[PDF](#)

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012039

Utilization of edamame soybean (*Glycine max (L) Merril*) as modified of enteral formula high calories

A. Widiyawati and Y. Susindra

[+ View abstract](#)

[View article](#)

[PDF](#)

OPEN ACCESS

012040

Full Trap Method in Handling Warehouse Pests in Ledokombo, Jember

I Erdiansyah, F Mayasari, S U Putri, V Kartikasari and Eliyatningsih

[+ View abstract](#)

[View article](#)

[PDF](#)

OPEN ACCESS

012041

Performance of Resistance of Rice Varieties Recommendation of Jember Regency to Brown Planthopper Pest (*Nilaparvata lugens Stal.*)

I Erdiansyah and Damanhuri

[+ View abstract](#)

[View article](#)

[PDF](#)

OPEN ACCESS

012042

Exploring the potency of gathotan and gathot as diabetes functional food: resistant starch analysis

D E Puspaningtyas, P M Sari and R J Kusuma

[+](#) View abstract [View article](#) [PDF](#)

OPEN ACCESS

012043

Purple Yam Flour (*Dioscorea alata* L.) Processing Effect on Anthocyanin and Antioxidant Capacity in Traditional Cake "Bolu Cukke" Making

R. Larief, A. Dirpan and Theresia

[+](#) View abstract [View article](#) [PDF](#)

OPEN ACCESS

012044

Antioxidant Activity, Total Phenol, and Sensory Properties of Melinjo Peel Tea with Pre-Treatment

M W Apriliyanti, M Ardiyansyah and A M Handayani

[+](#) View abstract [View article](#) [PDF](#)

OPEN ACCESS

012045

The effect of blanching and drying temperature upon proximate composition of okra flour

R Rindiani, H Warsito and N Maria Rosiana

[+](#) View abstract [View article](#) [PDF](#)

OPEN ACCESS

012046

Surimi from Freshwater Fish with Cryoprotectant Sucrose, Sorbitol, and Sodium Tripolyphosphate

Fahrizal, N Arpi, S Rohaya and R Febriani

[+](#) View abstract [View article](#) [PDF](#)

OPEN ACCESS

012047

Determination of Water Content Of Intact Sapodilla Using Near Infrared Spectroscopy

Kusumiyati, Yuda Hadiwijaya and Ine Elisa Putri

[+](#) View abstract [View article](#) [PDF](#)

OPEN ACCESS

012048

Improvement of Sensory and Chemistry Quality of Fried Edamame by Freezing

B Hariono, MF Kurnianto, A Bakri, M Ardiansyah and R Wijaya

[+](#) View abstract [View article](#) [PDF](#)

OPEN ACCESS

012049

Utilization of different nitrogen sources for the growth of microalgae isolated from mangrove leaves in Banda Aceh - Indonesia

S H Anwar, S Harzaki, M I Sulaiman and T Rinanda

[+](#) View abstract [View article](#) [PDF](#)

OPEN ACCESS

012050

Preparation and characterization of biodegradable film based on skin and bone fish gelatin

N Arpi, Fahrizal, Martunis and E Hardianti

[+](#) View abstract [View article](#) [PDF](#)

OPEN ACCESS

012051

The characteristics of Aloe vera gel as an edible coating

L Suriati, I G P Mangku and I N Rudianta

[+](#) View abstract [View article](#) [PDF](#)

OPEN ACCESS

012052

Thermal penetration study for the purpose of formulating sterilization procedures of yellowfin tuna canning

H Hasan, S H Anwar, S Rohaya and Martunis

[+](#) View abstract [View article](#) [PDF](#)

OPEN ACCESS

012053

The effect of native chicken legskin gelatin concentration on physical characteristics and molecular weight of edible film

M Sompie, S E Surtijono and Ch Junus

[+](#) View abstract [View article](#) [PDF](#)

OPEN ACCESS

012054

Physical properties and cellular structure of bread enriched with pumpkin flour

Agung Wahyono, A Z Tifania, E Kurniawati, Kasutjianingati, W W Kang and S K Chung

[+](#) View abstract [View article](#) [PDF](#)

OPEN ACCESS

012055

Formulation of Artificial Rice Cereal by using Fermentation of *L Plantarum* POLIJE 15420 for Diabetes Mellitus Patients

H Warsito, A Santoso and Y Wibisono

[+](#) View abstract [View article](#) [PDF](#)

Organic Agriculture

OPEN ACCESS

012056

Improving Soil Fertilizer Through Application of Organic Fertilizer Humid Acid and Mikoriza in Supporting Growth and Production of Chilli Plants In Sand Land

M Z Sukri, V K Sari and R Firgiyanto

[+ View abstract](#) [View article](#) [PDF](#)

IT for Agriculture

OPEN ACCESS

012057

Going Digital: A New Concept of Indonesian Food Composition Databases

M Iqbal and M R Permadi

[+ View abstract](#) [View article](#) [PDF](#)

OPEN ACCESS

012058

Development of Low Cost Toxic Gas Explosive Modeling System using Wireless Array Sensor Network

D P S Setyohadi, H Y Riskiawan, S Kautsar and P Destarianto

[+ View abstract](#) [View article](#) [PDF](#)

OPEN ACCESS

012059

The Precision Agriculture Based on Wireless Sensor Network with MQTT Protocol

Y Syafarinda, F Akhadin, Z E Fitri, Yogiswara, B Widiawan¹ and E Rosdiana

[+ View abstract](#) [View article](#) [PDF](#)

Renewable and Novel Energy Sources

OPEN ACCESS

012060

An investigation of dummy load energy in gunung sawur 1 microhydro power plant - Lumajang East Java

DE Rahmanto and V Femintasari

[+ View abstract](#) [View article](#) [PDF](#)

OPEN ACCESS

012061

Bioethanol Production from Sugarcane Molasses with Simultaneous Saccharification and Fermentation (SSF) Method using *Saccaromyces cerevisiae*-*Pichia stipitis* Consortium

Audiananti Meganandi Kartini and Yeny Dhokhikah

[+ View abstract](#) [View article](#) [PDF](#)

A novelty design of GHG Emission Reduction Cost for the Province of Nusa Tenggara Timur, Indonesia: A quantitativebased scientific view

A Amheka and Y Higano

[+ View abstract](#) [View article](#) [PDF](#)

Implementation microcontroller-based bioethanol levels measurement tool on bioethanol purification equipment

E Antika and Y Susmiati

[+ View abstract](#) [View article](#) [PDF](#)

The Other

Studies on inhibition of *Morinda citrifolia* leaf extract (*Morinda citrifolia* L) against the growth of *Aeromonas hydrophilla* in vitro

I A A Pongoh and A A Gemaputri

[+ View abstract](#) [View article](#) [PDF](#)

Ethyl Acetate Fraction Of Secang As Anti Cervical Cancer By Inducing p53 and Caspase 9

N P L Laksmiani, N M W Astuti, C I S Arisanti and N L P V Paramita

[+ View abstract](#) [View article](#) [PDF](#)

The Influence of Sediment Loads on the Irrigation Discharge in The Upstream and Downstream of the Major River in Banyuwangi Regency

Z Erwanto, D Dwi Pranowo, D S W P J Widakdo and N S R Wilujeng

[+ View abstract](#) [View article](#) [PDF](#)

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Addition of Garlic Extract in Ration to Reduce Cholesterol Level of Broiler

M M D Utami¹, D Pantaya¹, A Agus³

¹Department of Animal Husbandry, Politeknik Negeri Jember

²Faculty of Animal Husbandry, Universitas Gadjah Mada

merry.mdu@polije.ac.id

Abstract. The purpose of this research is to know the effect of garlic extract (GE) in reducing cholesterol level of broiler chicken by analyzing cholesterol level of broiler chicken blood. Two hundred one day broiler age were used in this study for 35 days. The chickens were randomly divided into four treatments, each treatment consist of five replications and each repetition consist of ten chickens. This research is used completely randomized design, such as: T0: 0% EBP, T1: 2%, T2: 4% and T3: 6%. Furthermore, at age 35 days each chicken was taken blood to be analyzed cholesterol levels, low density lipoprotein (LDL), high density lipoprotein (HDL) and calculated the ratio of LDL and HDL levels. The data obtained were analyzed using software from Statistical Product and Service Solution (SPSS 16.0). The results of significant analysis continued by Duncan's New Multiple Range Test. Addition of GE from the 2% level decreases ($P < 0.05$) of LDL and total cholesterol, and increases HDL and HDL-LDL ratio. The conclusions is obtained garlic extract plays an important role in lowering cholesterol levels of broiler meat.

Key words: garlic extract, cholesterol, broiler

1. Introduction

Cholesterol is a component of fat that plays a role in the formation of hormones. Normally, the human body produces cholesterol synthesized in the liver in the right amount, but the number can increase due to the addition of food derived from animal fat [1].

In recent years, the education and research on food safety, increasing public awareness of health. Food of animal origin, such as egg yolk, meat, liver, and brain of farm animal contains high cholesterol. Several studies have already reported the increasing of metabolic disease because the consumption of high cholesterol, such as fatty liver, coronary heart disease, pancreatitis, atherosclerosis, hypertension and stroke [2].

The potential of Indonesian medicinal plants is very high, about 1260 species of plants that live in tropical rainforests as medicinal plants. Depend on these number, about 180 species are used for the medicine industry and herbal medicine industries, and only a few species have been intensively cultivated [3].

Garlic contains of high sulfur levels, including allicin, diallyl disulfide (DDS), and diallyl trisulfide (DTS), which is a volatile oil and S-allyl cysteine (SAC), a water soluble amino acid. Sulfur-containing compounds are responsible for the taste, aroma, and pharmacological properties of garlic [4]. The majority of the sulfur-containing compounds in garlic are γ - glutamyl-S-allyl-L-cysteines and S-allyl-L-cysteine sulfoxides (aliciin) which are the major compound of amino acids. All sulfoxides, exception for cycloalliin, are converted to thiosulfonates so that no thiosulfonates are found in the intact garlic. Continued γ -glutamyl-S-allyl-L-cysteines be subsequently converted to S-allyl-L-cysteines (SAC) through enzymatic transformation with γ -Glutamyltranspeptidase when garlic extracted with a liquid solvent. The SAC, which is the main product of γ -Glutamyl-S-allyl-L-cysteines, is a detectable sulfur of amino acids in the blood, proven to be biologically active and bioavailable [5].



The compound of γ -glutamate-S-alk (en) il-L-cysteine compound is an intermediate of biosynthesis compound of other organosulfurs, including alliin. This compound is formed from amino acids biosynthesis pathways. γ -glutamyl-Salk (en) il-L-cysteine enzymatic reaction will produce many derivatives, by two branches of the reaction, those are the formation pathways of thiosulfinat and S-allyl cysteine (SAC).

The process of slicing, changed γ -glutamyl-S-alk (en) il- L-cysteine took place that be held by the enzyme γ - glutamyl - transpeptidase and γ -glutamyl-peptidase oxidase, and also produce alliin. At the time of garlic bulbs sliced and mashed in the process of making the extracts or seasoning, allinase enzyme becomes active and hydrolyze alliin produces allyl sulfenic. Condensation of the acid produces alliin, pyruvic acid, and NH_4^+ ions. One milligram of alliin is equivalent to 0.45 mg alliin [6]. Heating can inhibit the activity of allinase enzyme. At temperatures above 60°C , this enzyme will convert alliin into alliin [7]. Therefore, it easy to to lead into further reactions, depending on processing conditions or other external factors such as storage, temperature, and others.

In fresh form, the biological activity of garlic is very low, as it is known to several kinds of garlic preparations, such as garlic powder, garlic oil, and garlic extract (GE) [7]. Furthermore, based on several studies that have been done, there were no side effects and toxicity of garlic, as a result it is safe to use without causing undesirable effects [8]. The safety of using GE has been recommended based on several studies that have been done. Extraction of garlic bulbs with ethanol at temperatures below 0°C , produce alliin. Extraction with ethanol and water at 25°C produced alliin and does not produce alliin, while extraction by steam distillation method (100°C) causes all ingredients of alliin turned into allyl sulfide compounds [6]. Futhermore, the extraction process need to be done at room temperature. Garlic extract in ethanol with concentration of 15-20% can be stored up to 20 months [9]. In the form of GE, all alisin is converted to alylsulfide, the sequence based on the percentage is DTS of 73%, 8% DDS, and dialylsulfide (DS) 8% [10].

Some reports from clinical studies of using garlic found a cholesterol-lowering effect [7]. Several studies also reported that garlic inhibits the oxidation of cholesterol, thereby reducing the atherosclerotic, inhibiting the calcium deposits that harden the arteries, lower blood pressure, inhibit platelet aggregation to form blood clots, and reduce homocysteine (an amino acid that increases the risk of cardiovascular disease and stroke).

The research of [11] showed that giving of garlic with dose of 2 - 3mg /broiler/day was able to decrease cholesterol level contained in the meat and increase the carcass percentage of broiler better than broiler without garlic. Decreasing cholesterol levels in broiler meat is caused by the hypocholesterolemic effect of the active compound of garlic is alliin.

Based on explanation above, it is necessary to produce nutritious broiler meat but low cholesterol, with the addition of garlic extract on ration of broiler. The purpose of this study is to comprehensively examine the extent to know the effectiveness of the addition of garlic extract on ration to reduce cholesterol levels of broiler meat. The specific benefit of this research is giving recommendation the utilization of garlic extraction, especially applied to broiler.

2. Method

The research was divided into two steps: the first step was extraction of garlic, and the second step was in vivo test with the addition of garlic extract in ration and tested the cholesterol level of broiler. Equipment that used in this research include: shaker, autoclave, blender, vacuum rotary evaporator, Erlenmeyer, and beaker glass. Extraction steps of garlic were as follows: 250 grams of garlic mixed with 96% ethanol as much as 500 mL. Garlic solvent obtained was filtered with 2 layers of gauze, then filtered again with whittman paper number 2 obtained filtrate (crude extract). The filtrate is accommodated in an erlenmeyer tube. Subsequently entered it into a vacuum rotary evaporator for to evaporate ethanol and obtain garlic extract. Garlic extract was obtained and taken it as much as 100 mL. The concentration of garlic extract obtained through the above process is 280 mg / 1 mL.

Garlic extract obtained by the process with immune response of broiler. A total of 200 chickens were used in this study. Chicken grouped according to treatment as much as four treatment groups. Each treatment consisted of five replicates and each replication consisted of 10 chickens. The treatments were T1 = control, T1 = 2% garlic extract, T2 = 4% garlic extract and T3 = garlic extract 6%.

Blood sampling test was done on day 35, each replication was taken 3 blood samples, then each chicken was taken as much as 10 ml from axillary vein in the central nerve by using disposable syringe with volume of 10 mL. Blood sample inserted into vacum tubes containing anticoagulant ethylene diamine tetra acetic acid (EDTA). Procedure of determining total cholesterol level of chicken blood using photometer with method of cholesterol oxidase-peroxidase amino antipyrine phenol (CHOD-PAP) with wavelength 546 and temperature 37°C, while the procedure of calculating white blood cell (leucocytes) by hemocytometer method using Turk solution. The nutrient feed composition is shown in Table 1.

Table 1. Nutrient composition of feed treatment

Ingredients	Experimental Diets			
	1	2	3	4
Energy Metabolism (Kcal.Kg)	3200	3200	3200	3200
Protein (%)	23	23	23	23
Lipid (%)	4	4	4	4
Crude Fiber (%)	4.6	4.6	4.6	4.6
Calcium (%)	0.99	0.99	0.99	0.99
Phosphor (%)	0.35	0.35	0.35	0.35
Garlic Extract (%)	0%	2%	4%	6%

The parameters observed in this research were high density lipoprotein (HDL), low density lipoprotein (LDL), HDL and LDL ratio, and total cholesterol.

3. Results and Discussion

The results of the measurement of total cholesterol, HDL, LDL are presented in Table 1. The results of statistical analysis showed that GE addition in ration had significant effect ($P < 0,05$).

Table 2. The level of LDL, HDL, and cholesterol

Parameters	Level of Garlic Extract			
	1	2	3	4
LDL (mg/dl)	101,44 ^c	86,21 ^b	78,24 ^a	72,13 ^a
HDL (mg/dl)	38,21 ^a	55,84 ^b	60,43 ^c	63,91 ^c
Kolesterol total (mg/dl)	186,66 ^c	173,31 ^b	151,11 ^a	146,22 ^a

Liver produces bile salts that play an important role in the process of digestion of fat. Bile salts are stored in the gallbladder. During digestion, the gallbladder contracts and rapidly transfers bile salts into the duodenum through the ductus choledocus. Bile salts are synthesized in the liver from cholesterol, secreted into bile and usually back to the liver through reabsorption in the small intestine (enterohepatic cycle). The use of GE in the diet significantly decreased ($P < 0.05$) total cholesterol levels compared to controls.

Decreasing cholesterol levels due to using of GE on the diet can be explained as follows: in addition to improving the performance of digestive enzymes, GF also decreases cholesterol formation. The synthesis of cholesterol occurs in the liver, which is divided into four stages, the first stage of acetic acid is converted into mevalonate derivatives with enzyme 3-hydroxy-3 methyl glutaryl coA reductase (HMGCoA-reductase), the second stage is the change of mevalonate derivatives into squalene derivatives, the third stage is conversion of the squalene compound into demosterol by the steps of

lanosterol; zimosterol, and kholestadienol, and the fourth stage, the change of demosterol into cholesterol [12].

The effectiveness of GE in lowering total cholesterol levels follows as a mechanism as follows: the primary target of GE is the HMGCoA-reductase enzyme which is the initial enzyme for cholesterol synthesis. Alisin is able to bind to the sulfhydryl group which is the functional part of co-enzyme A in the process of formation the cholesterol [13]. Furthermore alisin, diallyl disulfide, and alilmercaptan inhibit the enzyme HMGCoA-reductase and accumulation of lanosterol [14]. Constraints on the HMGCoA-reductase enzyme are an indication of the absence of cholesterol synthesis and simultaneously inhibit the mevalonate. The last products of fat digestion in the intestine are monoglycerides, fatty acids, cholesterol, phospholipids and triglycerides.

Fat is insoluble in water, so it requires a special carrier to be able to flow with blood throughout the body, in order to soluble the fat bound by proteins, this bond is called lipoprotein. According [12] LDL contains 43% of cholesterol to be sent throughout the body. The main protein that forms LDL is apo-b (apoprotein b) that is easily attached to the blood vessels. Apo-b binds to lipoprotein b that has a high cholesterol content.

The treatments of garlic extract on ration lowers LDL levels (T1, T2 and T3). To determine the percentage decrease in LDL with using GE on ration, calculations were performed by comparing LDL levels of treatments to control. The calculation result of LDL more lower than control, decreasing LDL level 14,58% (GE 2%), 25,82% (GE 4%), and 28,89% (GE 6%). The LDL data of 4% and 6% were close to [15]. Garlic extract supplementation on ration effectively reduced LDL levels by 30% compared to controls.

From the research results can be observed a decrease in LDL levels which with the increasing use of GE on ration. This phenomenon raises the assumption that GE accelerated LDL catabolism followed by cholesterol translocation into cells. Cholesterol in-cell inhibition inhibits cholesterol synthesis [5]. Studies in rats showed that GE of 200 mg/Kg body weight decreased LDL levels significantly [16].

High Density Lipoprotein (HDL) is a high protein content lipoprotein [17] the major proteins that make up HDL are apo-a (apolipoprotein a), low fat content and high density. Statistical analysis showed a significant increase in HDL ($P < 0.005$) in EBP treatment versus control. Decreased HMG-CoA activity by GE affects LDL receptor activity that is central to cholesterol metabolism and increases the apo-a activity binding to HDL. Increased apo-a activity binding to HDL will lower LDL concentrations in the blood.

The HDL level of control was 38.21 mg/dL, while GE treatment resulted in HDL levels between 55.84-63.91 mg/dL, the results of which are close to [18] HDL chickens range from 40.5 to 50.4 mg / dl. Increased HDL levels indicate a response to the treatment. High levels of HDL prevent the risk of atherosclerosis by transporting cholesterol from peripheral tissue to the liver and reducing excessive cholesterol. According to [1], HDL is a lipoprotein that transports lipids from the periphery to the liver.

The HDL molecule is relatively small compared to other lipoproteins, so it can pass through the vascular endothelial cells and enter the intima to transport back the cholesterol collected in the macrophages. In addition, HDL also has anti-oxidant properties that can prevent the occurrence of LDL oxidation. The use of EBP affects HDL levels. In the T1 treatment (GE 2%) HDL levels increased 11.21%, followed by an increase of 20.35% (T2) and 27.28% (T3) compared to controls. According to [19], EBP can raise HDL levels up to 15%.

Based on the results of the study the higher the use of GE in the feed will increase the ratio of HDL and LDL. Reduced LDL in the blood will raise HDL, whereas HDL serves to transport cholesterol from the tissues and blood vessel walls to the liver to be metabolized. The higher level of HDL in the blood will be more cholesterol that can be taken, so that cholesterol levels in the blood will decrease. It is further explained that not only total cholesterol is taken into account but also the ratio of LDL and HDL, ideally LDL as low as possible and HDL as high as possible.

4. Conclusions

From the results of the study concluded that the application of garlic extract in the diet to reduce levels of LDL and cholesterol, raising levels of HDL in broiler chickens.

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