



# Proceeding

## The 2<sup>nd</sup> International Seminar Feed Safety for Healthy Food

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The 2<sup>nd</sup> International Seminar  
“Feed Safety for Healty Food”

Technical Editors :  
Secretariat of The International Seminar  
“Feed Safety for Healty Food”

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The 2<sup>nd</sup> International Seminar  
“Feed Safety for Healty Food”

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The 2<sup>nd</sup> International Seminar  
“Feed Safety for Healty Food”

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## **FOREWORD**

We thank the Almighty Allah, the Most Gracious and the Most Merciful that the proceedings of the 2<sup>nd</sup> International Seminar, the 8<sup>th</sup> Biannual Meeting and 3<sup>rd</sup> Congress and Workshop of AINI with the theme “Feed Safety for Healthy Food” organized by Indonesian Association of Nutrition and Feed Science, Faculty of Animal Husbandry, Universitas Padjadjaran on 6 - 7 July 2011 have been completed.

These activities were to collect variety of scientific information with the purpose to collect scientific information about feed for a healthy food, to produce a draft policy on a national feed system and to make a scientific forum for Academics, Researchers, Practitioners of animal husbandry, Health and Policy makers. Scientific papers that were presented either in oral or poster stated in the proceedings.

Thanks go to all those who have provided both moral support or material so that this seminar can be carried out and the proceeding can be issued.

Jatinangor, 6 Mei 2012

**Committee**

**CONTENTS**

TECHNICAL EDITORS .....	i
KEYNOTE AND MAIN SPEAKERS .....	ii
SUBJECT EDITORS .....	iii
FOREWORD .....	iv
CONTENTS .....	v
DIETARY STRATEGIES OF AMMONIA MITIGATION AT POULTRY FARMS IN INDONESIA	
Adrizal, P. Patterson, and Nelson .....	1
EFFECTS OF FEEDING FORAGES LEAF MEAL ON THE PERFORMANCES OF LAYING HENS	
Ahmad Windu Bahari and Osfar Sjoftjan .....	18
THE PROTEOGLICAN QUALITY FROM PRODUCT NATURATED OF CHITOSAN EXTRACT WHICH DIGESTIBILLITY AND HEMATOLOGIC MEASURED	
A b u n, Denny Rusmana, and Kiki Haetami .....	29
RUMINANTS FEED CHAIN DEVELOPMENT IN INDONESIA: REVIEWING AND A VALUE ANALYSIS	
Achmad Firman, Andre R Daud, Hasni Arief, dan Anita Fitriani .....	38
BEEF CATTLE DEVELOPMENT: LIVESTOCK PRODUCTION AND FEEDING SYSTEM AND ANIMAL PERFORMANCE UNDER FARMER GROUP OF BEEF CATTLE DEVELOPMENT PROGRAM	
Akhmad Sodiq .....	44
BLOOD ALBUMIN AND YOLK CHOLESTEROL OF DUCK ( <i>Anas sp.</i> ) POLLUTED BY LEAD (Pb) TEXTILE INDUSTRY WASTE	
Andi Mushawwir and Diding Latipudin .....	54
FEED SAFETY: ISSUES AND CHALLENGES FOR RUMINANT INDUSTRY IN INDONESIA	
Andre R. Daud, A. Firman .....	59
EFFECT OF UREA ADDITION AND INCUBATION TIME IN PALM FIBER FERMENTATION ON CHEMICAL COMPOSITION AND GAS PRODUCTION IN-VITRO	
Asih Kurniawati, Chusnul Hanim, and Syaiful Anwar Malik .....	66

EFFECT OF COMMERCIAL TANNIN AND <i>Leucaena Leucocephala</i> ON THE RUMEN METHANOGENIC BACTERIA OF CATTLE AND CARABAO Bambang Suwignyo, Medino G. N. Yebron, Jr and Cesar C. Sevilla .....	74
<i>Saccharomyces cerevisiae</i> IN GOAT FEEDS AFFECTED RUMEN FERMENTATION PATTERN BUT DID'NOT AFFECTED METHANE CONCENTRATION Caribu Hadi Prayitno, Tri Rahardjo Sutadi dan Suwarno .....	84
EFFECT OF FLUSHING ON SPERM QUALITY IN NATIVE ROOSTERS ( <i>Gallus Gallus Domesticus L</i> ) Dadang Mulyadi Saleh .....	90
THE EFFECT OF PRE-CONDITION AND WATER SOLUBLE CARBOHYDRATE SOURCES ADDITION ON NAPIER GRASS SILAGE QUALITY Despal and Permana, I.G. ....	94
ISOLATION AND SCREENING OF FUNGI PRODUCING CELLOBIOSE DEHYDROGENASE: "ENZYMES FOR ANIMAL FEED PREPARATIONS BASED ON ENZYMATIC PROCESS" Desriani, Bambang Prasetya, Puspita Lisdiyanti, Wiwit Amrinola, Neneng Hasanah, Rivai .....	101
TOXIC DOSE METHANOL EXTRACT AND RESIDUE OF <i>Jatropha curcas L.</i> MEAL ON MICE ( <i>Mus musculus</i> ) Dewi Apri Astuti, Sumiati and P. C. Nanlohy .....	106
EFFECT OF INCREASING ENERGY CONTENT IN DIET ON THE PRODUCTIVITY OF SUMATERA COMPOSITE BREED EWES DURING LACTATION Dwi Yulistiani .....	115
VARIOUS METHOD OF PROCESSING TO INCREASE THE UTILIZATION OF CASSAVA PEEL AS RUMINANTS FEED Dwi Yulistiani, I.W. Mathius and Santi Ananda.A.A. ....	121
THE EFFECT OF TEMULAWAK ( <i>Curcuma xanthorrhiza Roxb</i> ) AND COMBINATION OF VITAMIN C AND VITAMIN E SUPPLEMENTATIONS ON PERFORMANCE OF HEAT- STRESSED BROILERS E.Kusnadi, A.Rahmat, A.Djulardi .....	128
EFFECT OF USING BY-PRODUCT OF VIRGIN COCONUT OIL PROCESSING (BLONDO) IN RATION ON DUCK PERFORMANCE E. Martinelly, Husmaini, A. Salim and R. Lubis .....	135



DETECTION OF ANTIBIOTICS RESIDUAL IN PIG AND CHICKEN PREMIX THROUGH TEST MICROBIOLOGICAL Ellin Harlia .....	140
DETERMINATION OF UTILIZATION LEVEL OF <i>Curcuma zedoaria</i> <i>Rosc.</i> TO IMPROVE RUMEN ECOLOGY OF MASTITIS DAIRY COWS ( <i>in-vitro</i> ) Ellyza Nurdin and Hilda Susanti .....	143
AVAILABILITY OF RICE STRAWS AS FEED RESOURCE IN SUPPORTING CROP LIVESTOCK SYSTEM (Beef Cattle-Paddy) BASED ON ECO-FARMING IN JAMBI PROVINCE Evi Frimawaty, Adi Basukriadi, Jasmal A.Syamsu, T.E.Budhi Soesilo .....	150
EFFECTS OF SUPPLEMENTAL ORGANIC CHROMIUM AND FUNGI <i>Ganoderma lucidum</i> ON MILK PRODUCTION AND IMMUNE RESPONSE IN LACTATING COWS F.Agustin, T.Toharmat, D.Evvyernie, D.Taniwiryo, S.Tarigan .....	156
THE EFFECTS OF RUMINAL INFUSION OF UREA TO DRY MATTER AND CRUDE PROTEIN INTAKES WITH UTILIZATION OF LEUCAENA ( <i>Leucaena leucocephala</i> ) IN BUFFALO ( <i>Bubalus bubalis</i> Linn.) F.F. Munier and C.C. Sevilla .....	164
THE REQUIREMENT OF ENERGY AS WELL AS DIGESTIBLE PROTEIN OF MILKING BEEF COW F. Rahim .....	172
EFFECTS OF VITAMIN E SUPPLEMENTATION ON PRODUCTION AND REPRODUCTION PERFORMANCE OF MUSCOPY DUCK ( <i>Cairina moschata</i> ) Hafsah, Rosmiaty Arief, and Mulyati .....	179
THE EFFECT OF <i>HIBISCUS ROSA-SINENSIS L</i> LEAVES AS SAPONIN SOURCES ON PROTOZOA POPULATION, GAS PRODUCTION AND RUMEN FLUID FERMENTATION CHARACTERIZATION IN VITRO Hendra Herdian, Lusty Istiqomah, Andi Febrisiantosa, Sigit Wahyu Hartanto .....	186
BLOOD MEAL USAGE IN DIET OF AMMONIATED RICE STRAW BASIS FOR SIMMENTAL CATTLE Hermon .....	194
RESPONSE OF NATIVE CHICKENS ON FEED FORMULATIONS USING LOCAL UNCONVENTIONAL FEEDSTUFFS Heti Resnawati .....	200



EARTHWORMS AS SOURCE OF PROTEIN ALTERNATIVE FOR POULTRY FEED Heti Resnawati .....	206
EFFECT OF SHEEP URINE ON DRY MATTER YIELD AND FORAGE QUALITY AND CORN YIELD Iin Susilawati, Nyimas Popi Indriani, Lizah Khairani, Mansyur, Romi Zamhir Islami .....	211
EFFECT OF FEED RESTRICTION ON FEED EFFICIENCY, CARCASS QUALITY AND DIGESTIVE ORGANS CHARACTERISTICS OF BROILER J.J.M.R. Londok, B. Tulung, Y.H.S. Kowel, and John E.G.Rompis .....	216
STRATEGIC UTILIZATION OF RICE STRAW AS FEED FOR RUMINANTS IN THE BANTAENG DISTRICT : SWOT ANALYSIS APPROACH Jasmal A. Syamsu and Hasmida Karim .....	227
THE EFFECT OF PHYTATE IN DIET AND LEAD (Pb) IN DRINKING WATER ON LEAD OF BLOOD, MEAT, BONE AND EXCRETA OF STARTING DUCK Kamil K.A., R. Kartasudjana, S. Iskandar .....	236
THE EFFECT OF PHYTATE IN DIET AND LEAD (Pb) IN DRINKING WATER ON HEMATOLOGICAL INDICATORS OF STARTING DUCK Kamil, K.A. ....	244
PEMANFAATAN BIO-MOS ( <i>Mannan oligosakarida</i> ) HASIL BIOPROSES LIMBAH INTI SAWIT DALAM PAKAN IKAN NILA Kiki Haetami, Junianto, dan Abun .....	250
THE ADDITION OF COCOA ( <i>Theobroma cacao</i> ) POWDER IN MILK FERMENTED TO REDUCE THE URIC ACID LEVEL ON HYPERLIPIDEMI RATS Lovita Adriani .....	260
THE EFFECT OF SUPPLEMENTATION FERMENTED KOMBUCHA TEA ON URIC ACID LEVELS IN THE DUCK BLOODS Lovita Adriani .....	266
IMPROVING THE NUTRIENT QUALITY OF JUICE WASTE MIXTURE BY STEAM PRESSURE FOR POULTRY DIET Maria Endo Mahata, Yose Rizal and Guoyao Wu .....	270
PERFORMANCES AND HAEMATOLOGY CHARACTERISTICS OF BROILER CHICKS FED VARYING MODIFIED PALM KERNEL CAKE M Tafsin, ND Hanafi, Z Siregar .....	277

EFFICACY OF GARLIC EXTRACT ON PERFORMANCE AND FAT DEPOSIT OF BROILER Merry Muspita Dyah Utami .....	284
IMPROVING THE QUALITY OF PALM KERNEL CAKE CONTENT AS POULTRY FEED THROUGH FERMENTATION BY COMBINATION WITH VARIOUS MICROBE, AND HUMIC ACID DOSAGE Mirnawati, Yose Rizal, Yetti Marlida and I. Putu Kompiang .....	290
EFFECTS OF PLANT PROPORTIONS OF <i>Panicum maximum</i> AND <i>Centrosema pubescens</i> APPLIED WITH PHOSPHATE FERTILIZERS AND DEFOLIATED AT DIFFERENT INTERVALS ON DRY MATTER YIELD, YIELD ADVANTAGE AND NUTRITIONAL QUALITY Muhammad Rusdy .....	301
THE FORAGE COMPOSITION OF SHEEP AND CUT AND CARRY SYSTEM CAPACITY IN THE PALM GARDENS SUB CIBADAK, DISTRICT SUKABUMI Muhammad Setiana .....	308
DETERMINATION OF UTILIZATION OF LEVEL SHRIMP BY PRODUCT ON BROILER PERFORMANCE Muhtarudin, Tintin Kurtini, Dian Septinova .....	311
ENZYME SUPPLEMENTATION ON LOCAL FEEDS (PELLETED OR MASH) FOR BROILER CHICKENS GROWTH: TECHNOLOGY INNOVATION TO SUPPORT FOOD SUSTAINABILITY N.G.A. Mulyantini .....	315
PENGUNAAN LUMPUR SAWIT FERMENTASI DALAM PAKAN TERHADAP PROFIL DARAH DAN LEMAK AYAM BROILER Ning Iriyanti dan Bambang Hartoyo .....	319
THE EFFECT OF FEEDING PRODUCT FERMENTED WITH <i>Monascus purpureus</i> ON PERFORMANCES AND QUAIL EGG QUALITY Nuraini, Sabrina dan Suslina A Latif .....	327
THE EFFECT OF PHYTOGENIC FEED ADDITIVES FOR BROILER CHICKEN Nurita Thiasari and Osfar Sjöfjan .....	334
EFFECT OF CORN MEAL SUBSTITUTION WITH NOODLE WASTE AND FORTIFIED NOODLE WASTE IN DIET ON BROILER PERFORMANCE Osfar Sjöfjan <sup>1</sup> and Ahmad Windu Bahari <sup>2</sup> .....	342



SUPPLEMENTATION <i>Curcuma longa</i> OR <i>Curcuma xanthorrhiza</i> ON CARCASS TRAIT AND CHOLESTEROL CONTENT OF BROILER R. Mutia and Sumiati .....	349
SUPPLEMENTATION <i>Curcuma longa</i> OR <i>Curcuma xanthorrhiza</i> ON BROILER PERFORMANCE R. Mutia and Sumiati .....	355
INFLUENCE of PARE FRUIT EXTRACT ( <i>Momordica charantia</i> L.) TO VISCERAL FAT WEIGHT, FEMUR MUSCLE AND LIVER MIDDLE-AGED FEMALE MICE SWISS WEBSTER Rita Shintawati, Hernawati .....	361
EFFECTIVITY OF SILAGE AND PROBIOTIC ON THE RUMEN METABOLISM OF ONGOLE CATTLE IN VIVO EXPERIMENT Ridwan, R, Y. Widyastuti, S. Budiarti, A. Dinoto .....	368
EFFECT OF EDAMAME SOYBEAN ISOFLAVONE CONCENTRATE ON BROILERS GROWTH PERFORMANCE Rosa Tri Hertamawati, Ujang Suryadi dan Dadik Pantaya .....	378
THE EFFECT OF ADDING “TAPE SINGKONG” (FERMENTED CASSAVA) JUICE ON THE CHARACTERISTICS OF FERMENTED MILK Salam N.Aritonang, Elly Roza, Sri Novalina .....	383
PROTEIN MOLECULAR STRUCTURE OF CANOLA SEED AFFECTED BY HEAT PROCESSING METHOD IN RELATION TO PROTEIN AVAILABILITY: AUTOCLAVED HEATING VS. DRY HEATING: A NOVEL APPROACH Samadi .....	389
THE EFFECT OF CONDENSED TANNIN OF MIMOSA BARK ADDED TO SOYBEAN MEAL ON <i>IN VITRO</i> GAS PRODUCTION Siti Chuzaemi, Mashudi .....	402
<i>In vitro</i> RUMEN ENZYME ACTIVITIES ON DIFFERENT RATIO OF FORAGE AND CONCENTRATE SUPPLEMENTED BY LERAK ( <i>Sapindus rarak</i> ) EXTRACT Sri Suharti, Dewi Apri Astuti, Elizabeth Wina, K.G. Wiryawan and Toto Toharmat .....	408
THE USE OF <i>Squilla empusa</i> FERMENTATION IN THE DIET LAYERS THE EFFECTS YOLK EGGS Sri Suhermiyati, Roesdiyanto, Winarto Hadi .....	415



TRANSFER OF OMEGA-3 PROTECTED AND L-CARNITINE IN THE DIETS OF FERMENTED RUBBISH MARKET ITS EFFECT ON FATTY ACID COMPOSITION OF CHEMIST SIMENTAL MEAT CATLLE Sudibya .....	420
THE EFFECT OF CHEMICAL AND BIOLOGICAL TREATMENTS ON WEIGHT LOSS, NUTRIENTS CONTENT, TRYPSIN INHIBITOR AND LECTIN ACTIVITIES OF <i>Jatropha curcas</i> L. MEAL Sumiati , D. A. Astuti , and R. Rahmasari .....	430
FORAGES FOR GOAT PRODUCTION UNDER CITRUS ECOSYSTEM IN NORTH SUMATRA Tatang M. Ibrahim .....	438
ENVIRONMENTAL MANIPULATION MICROINTESTINAL USING LECTIN JATROPHA SEED MEAL AS MEDIA ATTACHMENT LACTIC ACID BACTERIA AND ITS INFLUENCE ON THE HAEMATOLOGICAL PROFILE OF POULTRY Titin Widiyastuti and Caribu Hadi Prayitno .....	447
THE EFFECT OF MIXED COMMERCIAL YEAST CULTURE FERMENTATION FOR CASSAVA WASTE ON ITS PROXIMATE COMPONENTS Tri Agus Sartono, Nurwantoro, and Joelal Achmadi .....	451
CORRELATION BETWEEN THE PUBLIC UNDERSTANDINGS OF AVIAN INFLUENZA WITH LEVEL OF WILLINGNESS TO CONSUME POULTRY PRODUCT Unang Yunasaf dan Adjat Sudradjat M. ....	456
UTILIZATION OF UREA AND FISH MEAL IN COCOA POD SILAGE BASED RATIONS TO INCREASE THE GROWTH OF ETAWAH CROSSBRED GOATS Wisri Puastuti and Dwi Yulistiani .....	463
ACTIVITY OF CELLULASE FROM SELECTED ACTINOMYCETES <i>Streptomyces rimosus</i> sp. ID05-A0911 Wulansih Dwi Astuti, Roni Ridwan, Yantyati Widyastuti .....	470
IMPROVING THE NUTRIENT QUALITY OF JUICE WASTES MIXTURE THROUGH FERMENTATION BY USING <i>Trichoderma viride</i> FOR POULTRY DIET Yose Rizal, Maria Endo Mahata and Indra Joli .....	482

THE EVALUATION OF FERMENTATIVE CAPABILITY OF CELLULOTIC FUNGI FROM COW RUMEN FLUID AGAINST DECREASE IN CRUDE FIBER AND READY AVAILABLE CARBOHYDRATE IN CASSAVE PEEL WASTE Yuli Andriani *, Ratu Safitri **,Abun*** .....	492
THE EFFECT OF WASHING AND FERMENTATION OF CASSAVA PEEL ON HCN CONCENTRATION AND RUMEN VFA PRODUCTION Yuni Suranindyah, Andriyani Astuti .....	502
PARITY RELATIONS WITH THE MINERAL CONTENT OF BLOOD ON THE PARENT CATTLE ARTIFICIAL INSEMINATION (AI) IN WEST SUMATRA Zaituni Udin and Zesfin BP .....	508
EFFECT OF FEEDING A TRADITIONAL TOWARDS THE DEVELOPMENT OF LIVESTOCK REPRODUCTION BUFFALO THE DISTRICTS OF KAMPAR PROVINCE RIAU Zespin BP, Ferry Lismanto Syaiful and Yendraliza .....	516
EFFECT OF SAPONIN ( <i>Sapindus rarak</i> fruit) ON MEAT CHOLESTEROL FROM BROILER CHICKENS Chusnul Hanim, Lies Mira Yusiati, and Rahma Fitriastuti .....	520
BODY WEIGHT GAIN OF ETAWWA CROSSBREED GOATS MALE FED LOCAL FEED IN WEST JAVA Denie Heriyadi .....	526
TESTING FEED OF SUGAR CANE PULP AMMONIATION WITH UREA AND AMMONIUM SULFATE ADMINISTRATION BY MEASURING TOTAL VFA CONCENTRATION AND BACTERIA AND PROTOZOA POPULATION OF SHEEP RUMEN FLUID Diding Latipudin, An-An Yulianti, Ronnie Permana .....	532
UTILIZED BIO-MOS (Mannan Oligosaccharide) FROM BIOPROCESSED OF PALM KERNEL CAKE ON FEED OF NILE TILAPIA Kiki Haetami, Junianto, and Abun .....	542
UTILIZATION OF ENCAPSULATED EARTHWORM EXTRACT ( <i>Lumbricus rubellus</i> ) AS FEED ADDITIVE ON BROILER PERFORMANCE AND MEAT QUALITY Lusty Istiqomah, Hardi Julendra, Ema Damayanti, Septi Nur Hayati and Hendra Herdian .....	550

PERFORMANCES AND HAEMATOLOGY CHARACTERISTICS OF BROILER CHICKS FED VARYING MODIFIED PALM KERNEL CAKE M Tafsir, ND Hanafi, Z Siregar .....	559
EFFECT OF KOMBUCHA FERMENTATION ON HEMATOLOGY STATUS AND CARCASS WEIGHT IN DUCK Novi Mayasari, Lovita Adriani and Angga Kurniawan.....	566
UTILIZATION OF VEGETABLE CROPS RESIDUES AS ELEPHANT GRASS SUBSTITUTE IN COMPLETE FEED ON BODY COMPOSITION OF SHEEP Umi Muyasaroh, Limbang K Nuswantara dan Eko Pangestu .....	572
THE EFFECT OF WASHING AND FERMENTATION OF CASSAVA PEEL ON THE CONCENTRATION OF HCN AND RUMEN VFA PRODUCTION Yuni Suranindyah, Andriyani Astuti .....	577
AUTHOR INDEKS .....	583



**THE ADDITION OF COCOA (*Theobroma cacao*) POWDER IN MILK  
FERMENTED TO REDUCE THE URIC ACID LEVEL ON HYPERLIPIDEMI  
RATS****Lovita Adriani***Animal Husbandry Faculty, Universitas Padjadjaran, Bandung-Indonesia*  
Corresponden Email : lovita\_yoghurt@yahoo.co.id**ABSTRACT**

Chocolate can undergo fortification process to be added into other food such as probiotic yoghurt. Fortification is usually regarded as the deliberate addition of one or more micronutrients to particular foods, so to increase the intake of these micronutrients in order to correct or prevent a demonstrated deficiency and provide a health benefit.

The study object was to investigate the effect of addition cocoa powder in milk fermented milk mixture to reduce the uric acid level on hyperlipidemia rats.

In this research was used a Completely Randomized Design experimental method with five treatments, i.e. R0 = control, high fat diet, R1 = high fat diet + 4 ml yoghurt, R2 = high fat diet + 0,35 g cocoa, R3 = high fat diet + 4 ml yoghurt + cocoa 0,23 g, R4 = high fat diet + 4 ml yoghurt + cocoa 0,35g, R5 = high fat diet + 4 ml yoghurt + cocoa 47g; each treatment was repeated five times. From the statistical analysis it was indicated that the effect from the addition of cocoa and probiotic in ration, showed a significant effect ( $P < 0.05$ ) on decreased the uric acid level on hyperlipidemi rats

The results showed that addition cocoa into probiotic in all treatment has reduced the uric acid level, i.e. R1= 34,03%, R3= 29,9 %, R4= 29,9 %. The highest for reducing uric acid is R5= 37,11%, and the lowest is R2= 14,03%, compare to control

Keywords : *hyperlipidemi rat, probiotic, cocoa, uric acid*

**INTRODUCTION**

Cocoa was named *Theobroma* by Linnaeus, which means 'food of the gods'. It was so called from the goodness of its seeds (Grieve M. A., 2010). Cocoa beans are used in chocolate production. Chocolate/Cocoa contains flavonoids, a type of polyphenol antioxidant. Antioxidant can reduce cancer by attacking actively oxygen compounds which are carcinogen to our body. Some researchers found that chocolate may lower blood pressure of people with hypertension. Beside that, it also can reduce LDL cholesterol level (Gloria Tsang R, 2006). Flavonoids in cocoa are flavanols (epicatechins and catechins), anthocyanins and proanthocyanidins. The cocoa epicatechins and procyanidins make stabilize the overall blood sugars. Consume smaller amounts of very dark chocolate (providing 30 mg of polyphenols per day) for a much

longer period of time, there is an improvement in endothelial cell relaxation, but without a reduction of blood pressure

Probiotic yoghurt is basically yoghurt with live and active cultures. It can promote and maximize digestion of certain nutrients in the human body. Yoghurt contains such as: energy, protein, fat, carbohydrates, minerals and has a fairly complete content of vitamins are: vitamin A, B complex, B1 (thiamin ), B2 (riboflavin), B6 (pyridoxine), B12 (cyanocobalamin), vitamin C, vitamin D, E, folic acid, nicotinic acid, pantothenic acid, biotin and choline (Lovita A, 2005). Normally, the body eliminates enough uric acid in the urine or through the intestines to keep its concentration at a healthy level.. Only 5% of those with hyperuricaemia develop clinical symptoms of gout. Uric acid is an end-product compound from the breakdown of purines. About two thirds of purine is generated from within the body, while one third comes from the diet (Fam 2005)

Yoghurt can help to reduce uric acid levels. Also help to reduce the risk of gout. Uric acid is a divalent acid, but the second dissociation constant is so small that at around pH 7 only the monobasic salts are formed. Uric acid is a purine synthesized by a series of reactions that also are used for synthesis for other purine such as adenine and guanine , which are component of DNA. The final step the uric acid synthesis is controlled by the enzyme xanthine oxidase, a molybdenum containing enzyme. Xanthine oxidase is an enzyme that responsible for converting purine become uric acid. The most important structure in purine biochemistry is the nucleotide consisting of a purine base, ribose or deoxyribose, and phosphoric acid, but free hypox.

Theobromine, serotonin, in cocoa give the additional energy. Cocoa also stabilizes blood sugar and makes people feel stronger more quickly.

Raw cocoa contains calcium, phosphorus, iron, thiamine (vit B1), riboflavin (vit B2), niacin, nicotinamide (vit B3), pantothenic acid (vit B5), pyridoxine (vit B6), ascorbic acid (vit C), magnesium, copper, zinc, manganese, and vitamin E.

Food fortification is usually regarded as the deliberate addition of one or more micronutrients into particular foods to increase the intake of these micronutrients in order to correct or prevent a demonstrated deficiency and provide a health benefit (Frederic W. et al, 2006). Uric acid is the final product or waste products resulting from metabolism / breakdown of purines. Uric acid is antioxidants in animals, but when amounts in blood increase or reach the saturation level will experience a crystallization The content of uric acid on rat uric acid in, whereas in female mice at  $2.92 \pm 0.241$  mg / dl (Taconic Technical Laboratory, 1998 in Kusmiyati, 2008).

Uric acid is a derivate of purine alkaloid compounds (xanthine). Uric acid are semisolid organic compounds consisting of carbon, oxygen, nitrogen, and hydrogen with the formula  $C_5H_4N_4O_3$ , which is the end of protein and purines metabolism

Xanthine oxidase catalytic is an enzyme that catalyzes hipoxanthin and xanthin to uric acid, which is a purine degradation pathway.in normal tissue. xanthin oxidase is dehydrogenase In normal tissue, xanthin oxidase is a dehydrogenase that uses NAD as an electron acceptor in the purine degradation pathway.



## MATERIALS AND METHODS

In this experiment was used animal sample, which consisted of 36 adult male Wistar rats. Those rats are about 2-3 months old and weigh about 200-250 gram. They get treatment for 37 days, which consisted of 2 days adaptation, 7 days of pre-condition with hypercholesterol feed, and 28 days of treatment. In this study, was used Completely Randomized Design . The 36 rats are randomly divided into 6 groups which consists of 3 rats in each group, and repeated twice for each treatment.

At the end of the treatment, the sufficient amount of rat blood was taken by cutting the edge of its tail to analyze the uric acid level using the microhematocrite pipet.

### Material Used In Experiment

This study used the fortified cocoa (*Theobroma cacao*) powder in probiotic yoghurt as a preventive medicine to reduce the uric acid level in rats. The cocoa powder was obtained from pure cocoa from Ceres, a commercial food factory in Bandung. Rat's standard food is pellet and aquadest , also probiotic yoghurt is taken from product of Lovita Yoghurt Unpad.

The cocoa concentration of 12%, 15% and 18% were used, along with a constant amount of probiotic yoghurt. According to Lovita, Unpad, a person should consume about 250 ml per day. Those values are converted to be given to the rats which weigh 200 gram. Based on the comparison of body weight of human towards the rats, the dosage for the rats is 4 ml per day. The concentration of probiotic yoghurt that will be given to the rats will be calculated following the research of Lovita (2005)

Normal consumption of cocoa powder for human according to the research of *Ochanomizu University, Japan, is from 13g – 36g per day. Based on the research, was decided to use 3 different concentrations, each value 13 g, 19.5 g, and 26 g.* Those values are converted to be given to the rats based on the comparison of body surface areas of human towards the rats:

$$\begin{aligned} \text{Rat dosage (200 gram)} &= \text{Human dosage (70 kg)} \times \text{body surface area} \\ &\quad \text{comparison of rat to human (conversion factor)} \\ &= 13\text{g/day} \times 0.018 = 0.23 \text{ g/day} \end{aligned}$$

$$\text{Empirical concentration} = 0.23 \text{ g} \rightarrow 0.23 \text{ mL}$$

$$= \frac{0.23}{4 \text{ mL}} \times 100 = 5,8 \%$$

With the same formula, the empirical concentration for dosage of 19.5 g is 8.8%, and for dosage of 26 g is 11.8%. This concentration was given to the rat in the study. Feed composition followed the theory of Reeves, et al. (1993), where a value of 1% cholesterol was added for hypercholesterolemic condition.

The formula treatments are:

Group 1: hypercholesterol feed

Group 2: hypercholesterol feed + probiotic yoghurt 4 ml

Group 3: hypercholesterol feed + cocoa 0,23 gr

Group 4: hypercholesterol feed + probiotic yoghurt 4 ml + cocoa 0.23 gr

Group 5: hypercholesterol feed + probiotic yoghurt 4 ml + cocoa 0.35 gr

Group 6: hypercholesterol feed + probiotic yoghurt 4 ml + cocoa 0.46 gr



## RESULTS AND DISCUSSIONS

### Effect Treatments on Uric Acid Level

Table 1. Effect of treatment on uric acid level (mg/dl)

R0	R1	R2	R3	R4	R5
a	ab	a	ab	ab	b
4,85	3,2	4.17	3,4	3,4	3,05

R0 = high fat diet

R1= high fat diet + 4 ml yoghurt ,

R2= high fat diet + 0,35 g cocoa,,

R3= high fat diet + 4 ml yoghurt + cocoa 0,23 g

R4 = high fat diet + 4 ml yoghurt + cocoa 0,35g

R5 = high fat diet + 4 ml yoghurt + cocoa 47g

In Table 1 and Figure 1. showed that the level of uric acid in tested animal blood was reduced after consuming mixed or fortified cocoa in probiotic yoghurt. The effect almost the same for consuming only cocoa (R2) and R0 (control). R1 (consuming only probiotic), R3(probiotic with little dose cocoa) and R4 (probiotic with medium dose cocoa) seemed better than R0 and R2, for decreasing uric acid level. Consume only yoghurt probiotic is better than consume only cocoa for reducing uric acid level. R5 showed significantly different compared to all treatments, which means that the use of cocoa mixed with yoghurt can reduce the uric acid levels than using cocoa or yoghurt separately The main contributors for reducing uric acid is antioksidan The cocoa's epicatechins and procyanidins make compounds inhibit the activity of the reaction of xanthine oxidase and superoxide so that the levels of uric acid reduce (*Pittman JR. and Bross MH., 1999*) Polyphenol compounds is also a diuretic, so it will dissolve and uric acid in urine wasted, like work allopurinol in lowering uric acid level using an inhibitory pathway enzyme xanthin oxidase.

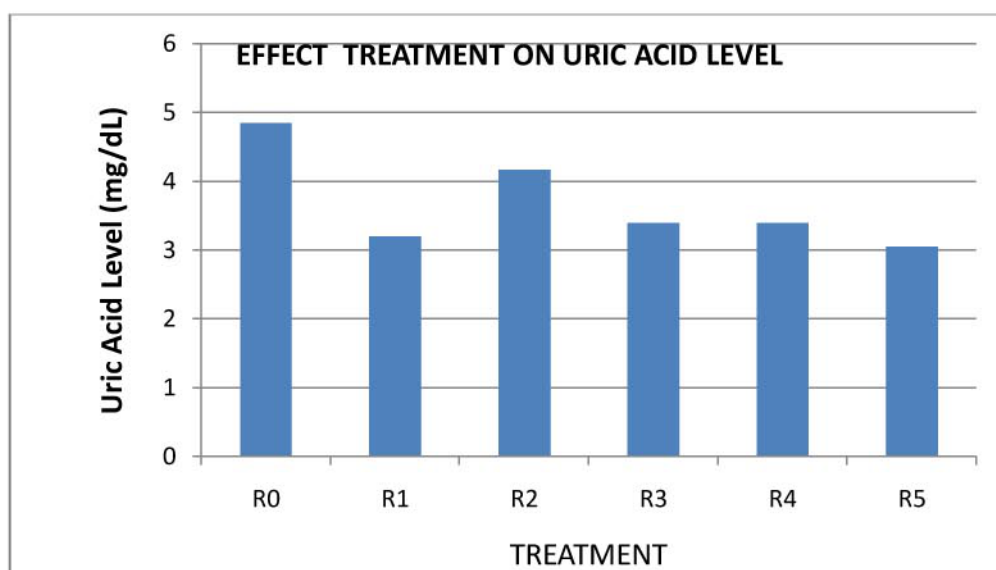


Figure 1. Effect of Treatment on Uric Acid Level

### CONCLUSION

1. Consuming mixed cocoa in probiotic yoghurt proved to be better than only the probiotic yoghurt or cocoa itself in reducing the level of uric acid
2. Consuming only cocoa is almost the same with control, only reduce 14,03% uric acid level
3. R1, R3, R4 mixed cocoa in yoghurt can reduce uric acid until 34.03%, 29.9%, 29.9% respectively
4. R5 is the highest for reducing uric acid level until 37.11 % compare to control

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