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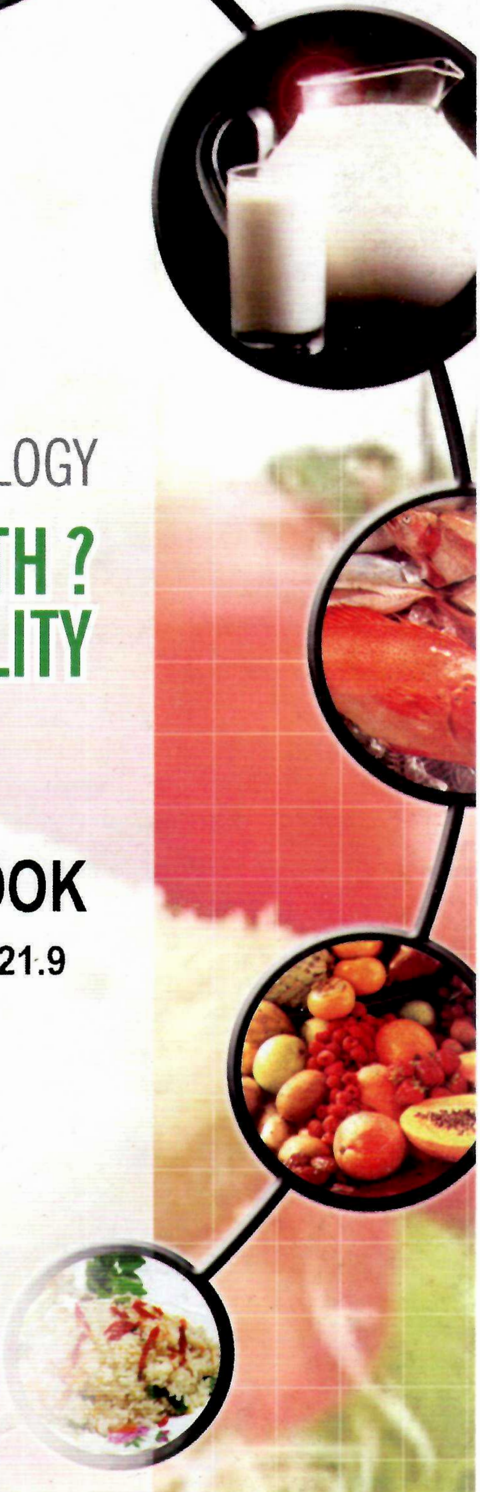
THOMAS AQUINAS BUILDING UNIKA SOEGIJAPRANATA

the 7th national student conference



ON FOOD SCIENCE AND TECHNOLOGY

HEALTHY FOOD OR FOOD FOR HEALTH ? FROM NUTRITION TO FUNCTIONALITY

PROCEEDING BOOK

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PREFACE

This is the 7th National Student Conference on Food Science and Technology organized by Department of Food Technology, Faculty of Agricultural Technology, Soegijapranata Catholic University, Semarang. The main theme of this conference, **"Healthy Food or Food for Health? From Nutrition to Functionality"**, implies the new efforts for documenting as well as improving the students research culture on food science and technology in Indonesia.

This conference was designed especially for students to share their research findings and vision on food science and technology. Furthermore they could improve their presentation capability in English. Presentation covered following topics :

Food Safety and Quality
Food Processing and Engineering
Nutrition and Functional Food
Food Microbiology and Biotechnology

In order to response the newest trend on food issues and encourage the students with an international academic atmosphere, there are plenary presentation about **"Developing Functional Food in Indonesia"**, **"Recent Trend in Health and Functional Food"**, and **"Flavor Trend for Health Food : Citrus"** that delivered by the expert and competence academicians.

The Organizing Committee is gratefull to all the honorable speakers, participants and sponsor companies for joining this gathering and for their valuable contribiton to the Conference

Semarang, June 2007

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THE PROSPECT OF HERBAL-ORIGIN ANTIVIRAL IN PROVIDING SAFE CHICKEN MEAT

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ABSTRACT

The needs of free antibiotics and free vaccine foods are increasing today, but under our geographical situation, it is getting more and more difficult to meet, because it is a populous country, have stable temperature and humidity all year around which favoring to the growth of bacteria and viruses. In order to combat with these two microbias, both our chicken breeders and growers tend to use antibiotics and vaccines respectively. Consequently, our chicken cannot be accepted by other countries, because antibiotics and vaccines contents in the chicken meat are beyond the international standard. However, some of our herbal contains various antibacterial and antiviral agents, which if they are treated in the right manner can be more effective than antibiotics and vaccines. This paper states the effectiveness of some herbal extracts to inhibit bacteria and some viruses which up to these days has resulting serious damages in our chicken farm. Three compounds namely 6-metil-4-kromanon, limonene, marmelin extracted from *Aegle marmelos*, L. Corr. effectively inhibit the growth of *E. coli*. The compounds also increased antibody titter of deadly virus Infectious Bursal Disease (IBD) and Newcastle Disease (ND), up to 11.3 and 4.95 respectively. These levels of titter allow chicken safe from these viruses diseases in order to provide safe food.

Keywords: *herbal antiviral, herbal antibacterial, chicken broiler, safe food*

INTRODUCTION

Indonesia is an agricultural country. Besides other agricultural products, livestock and livestock products play important role in national income and become more significant year by year. The government promotes livestock production with the aim to increase national stock to meet national demand. Within the livestock sector, poultry farming seems more attractive than others and the progress has brought about a meat surplus, while other meat products far below national needs. Attempts to export poultry meat, however, is still facing with poultry diseases which reducing productivities. Some diseases significantly taking major economic depletion are colibacillosis, Newcastle Disease (ND), and Infectious Bursal Disease (IBD) or Gumboro.

Colibacillosis is a common bacterial diarrhea, which causes economic losses in chicken production. It can spread to human and also causes diarrhea (Nataro and Kaper, 1998). Harmless strains of *E. coli* normally can be found widespread in nature, including in the intestinal tracts of humans and other vertebrates (Poonsuk et al., 1986). Disease causing types are a frequent cause of both intestinal and urinary-genital infections. Some strains of *E. coli* produce toxins that are structurally similar and have effects like those produced by Shigella dysenteriae (NIAID, 2003).

Under our farm business circumstances, neither growers nor breeders did not normally conduct preventive treatments against bacteria or virus before their chickens are infected. Once the infection occurs, "heavy treatments" of high dosage vaccines and/or antibiotics is employed to combat the diseases. Consequently, the concentration of both medicines in the meat are always higher than the international standards. FAO and WHO established Maximum Residue Limits (MRLs) for chlortetracycline and tetracycline of 600 microgram/kg in kidney; 300 in liver; 100 in muscle; 100 in milk; 200 in egg and 100 in fat for all poultry (JECFA, 1995; FAO-WHO, 2000). Indonesian poultry products normally contain 20 – 120% more than these levels (Collignon, 2002) and the broiler also contain tylosin, penicillin, oxytetracycline, and canamycin (Kompas, 2004)

On the other hands, Indonesia is rich in natural herbs and the utilization of medicinal herbs has a long history of successful use in the treatment of numerous conditions. The progress ini science and technology over the last two decades, have proven these traditional beliefs scientifically. Herbs contain many active components that have a wide range of pharmacological properties. Based on an extensive exploration, at least 550 species have been identified to have antibacterial as well as antifungal agents (Goun et al., 2003). Xanthorrhizol of *Curcuma xanthorrhiza* has been established to have strong potential as antibacterial agents (Hwang, and Shim, 2000a;b).

Other agent, chromanone (Cottiglia, 2002; Ibrahim and Abul-Hajj, 1990) in *C. xanthorrhiza* Roxb. and in bael fruit (*Aegle marmelos* Corr.) (Subrata and Playford, 2003) have also been documented as antibacterial agent. Zerumbon in *Z. aromaticum* Val. has also been reported having strong antibacterial activity (Novianti, 1990; Partini, 1993) and been believed by Javanese communities for generations to have antiviral activities against some viral diseases like skin problem, nasal ilness, influenza, and the likes. Recent studies reported that zerumbon has wide antibacterial spectrum to both gram positive and gram negative bacterial (Ficker et al., 2003; Goun et al, 2003). *A. marmelos* Corr for centuries has been accepted as fruit of god Shiva, due to its affectivity in controlling such diseases, includes viral diseases.

The progress in phytopharmaceutical as well as in biochemical sciences over the last three decades, documented that bael paper contains three antiviral compounds, namely marmeline, 6-methyl-4-chromanon and limonene (Sabu et al., 2004; Ahmed et al., 1998). The fruit hawever also contain some toxic compounds, namely cuminaldehyde, butyl hydroxyianisol, alloimperratorin methyl ester, butil-p-tolil sulphide, marmelosine, and marmeline itself. At low dosage, marmeline as well as marmelosine, is an antiviral agent but at high dosage is toxic (Badam et al., 2002). Marmeline, is an alcaloid and firstly identified and isolated in 1981 as N-2-hidroksy-2-[4-(3′,3′-dimethylalyloxy) fenyl] ethyl cinamide (Sharma et al, 1981). Others compounds, i.e. luvangetine and aegeline also have been reported having antiviral activities (Kamalakkannan and Prince, 2004; Arul et al., 2004).

The active compound of 6-methyl-4-chromanon, has been identified having intra-molecule (Doreswamy et al., 2004), and the position of the carbon rings are binding the functional chains and can be subtituted by other compound which has stronger ellectronegativity (Fillion, 2006). Referring to these chemical charracteristics, some compounds have been identified to be chromanone derivates, and have wide pharmacocinetics spectrums such as anthraquinon-chromanon that has strong antimicrobacterial effecta (Kanokmedhakul et al, 2002; Cottiglia, 2002), 1,4 chromanon that used as bioinscticides (Yang et al, 2002), 2,4 chromanon-deamine that has been widely used as bioherbicides, having strong antiproliferative effect against tumor cells (Lampronti et al, 2003) and cancer cells (Lambertini et al, 2004). The synthesis had been succesfully be made biologically, using a beta-alkyl-stirene to both its allyphatic and aromatic chains (Jang et al., 2004),

The third compound is limonen, which has been identified to be an active antiviral agent due to the easiness conversion to be limonen ephoxide when it is reacting with unstructured nitrogen, such as unstructuctured nitrogen-chains (Davis and Boyer, 2001) and when it is hydrolized to become limonen stereoisomer (Chen et al, 1993). Due to the easiness in hydrolysis and the changing in mthyl chain (– CH_3), limonen is given various names, for instance 1-methyl-4-isopenthyl-1-cyclo heksane, 1,8(9)-menthadien (Moonen et al., 1999). Under biochemical nomenclature, limonen is identified as a derivate of α-terphenyl cation (van der Werf, 2000), which until this day, it has been identified 16 homologs hydrocarbon isophentyl (De Vincenzi, 2000). Limonen is a group of lignan and the carbon structure is monocyclic monotherpene (da Cruz and Sivik, 2002).

Based on the reviews and previous researches of these three active compounds, it was suggested that the compounds will potentially effective to strengthen chicken farm against viruses and bacterial effects. This study explores both antiviral and antibacterial effects of the thee compounds aplied in chicken broiler farm, managed in commercial manner.

MATERIALS AND METHODS

The research was run since July 2006 until February 2007, comprised of two studies; laboratory and field studies. The first one included preparation of active compounds and microbial evaluation. The active compound was made by extraction technique, conducted at Department of Production, PT Indoherb Sains Medika, Semarang, Indonesia. The microbial evaluation included antibacterial testing which was organized at the Veterinary Research Center in Wates, Yogyakarta, whereas the antiviral testing both for ND and IB were run at Laboratory of Pathology and Pharmacology, PT. Agrinusa Unggul Jaya, Jakarta. Field studies were managed in twice chicken growing; the first one was in August – September 2006 and the second one was in November – December 2006. All field studies were run at Farm Station of PT. Agrinusa Unggul Jaya, Gunung Pati, Semarang.

Laboratorial Studies

Active compounds preparation. Antibacterial active compounds were extracted from "temu ireng" (*Curcuma aeruginosa* Roxb.) rhyzomes, "temu Lawak" (*Curcuma xanthorriza* Roxb.) rhyzomes, "cabe jawa" (*Piper retrofractum* Vahl.) seeds, and "lempuyang wangi" (*Zingiber aromaticum*, Vahl.) rhyzomes, using procedures as described in Indonesia Patent No. P00200500691, and was formulated following the procedures of Indonesia Patent No. P 00200500690. Whereas antiviral compounds were extracted from "lempuyang wangi" (*Zingiber aromaticum*, Vahl.) rhyzomes and "Mojo" or bael fruit (*Aegle marmelos* L. Corr.), as decribed in Indonesia Patent No. P 00200500692 and the extracts were then formulated using procedures of Indonesia Patent No. P 00200500689. The formula was then commercially branded as Vet-i.

Microbial Studies. *E. coli* bacteria were grown in Petri dishes with Mueller-Hinton Agar (MHA). The inoculums suspensions were compared with McFarland standard No. 0.5. The bacteria were spread over the surface of the MHA disks using sterilized cotton buds, the treated dishes were provided with a drop of Vet-i, whereas the control dishes were left untreated. The dishes were then cultured for 72 hours. The diameter of Vet-i's inhibition zone was measures and was made in ten replicates. Blood collection and serum separation for ND and IB viruses was conducted following this procedure. Blood was collected from each chicken via brachial vein at day 30th of age. Sera were separated, labeled and stored at -20oC until further analysis. Batches of sera were subjected to serological test. Antibody titers against ND virus were measured using ELISA technique described by Snyder et al. (1984) and ND virus antibody test kit (Synbiotics Corporation, San Diego, USA). Hindrance for IBD virus isolation is that most of very virulent field isolates do not replicate in common tissue culture (van den Berg et al., 1991), where as for virus neutralization test, the field strains was adapted in order to evaluate its titer both for IB and ND (Mitta et al., 2005).

Field Studies

Some 8.000 chickens were grown in two separate farm houses, for two levels of trials, i.e. Vet-i treatment and control; 4000 chickens each. The control was treated with antibiotic normally local farmers apply to control *E. coli*, i.e. oxytetracycline (OTC). Control hens were also given with ND and IB vaccines.-The chickens were managed under the same conditions with the same bedding technique, to give an equal coccidiosis pressure as on other farms. To assure that the chicken were heavily infected; colibacilosis were collected from a flock of domestic chicken where E. coli was diagnosed by a veterinarian at the Veterinary Research Center in Wates, Yogyakarta. The bedding of these chickens with faeces from 7 days was collected as the bacterial inoculums.

When the chickens reached three weeks of age, *E. coli* was collected for laboratory analysis. At the age of 30th days old, 20 chickens were chosen randomly from each flock, and the blood was collected for its antiviral analysis. The number of chicken depletion was recorded and the chicken weight was recorded using 20 chickens randomly chosen from each treatment group.

RESULTS AND DISCUSSIONS

Antibacterial active compounds significantly inhibit the growth of *E. coli* (Figure 1.). The diameter of inhibition zone was 23,01 ± 0.37 mm, or at 95% degree of confident the zone was ranging from 22,64 mm to 23,38 mm. These levels of inhibition zones indicate that the treated active compound has a strong bacteriostatic. The zone was higher than those documented by previous studies using some rhyzomes the member of Zingiberaceae family, extracted using alcohol, 16,66 mm (Naufalin et al, 2006), and was stronger than hydrobenzene groups isolated from pangola (*Digitaria decumbens*) which documented the diameter at 14 – 18 mm (Rahayu et al., 2006). These level of inhibition diameter were also stronger than those resulted by *Andrographis paniculata* (Burm. F.) aqueous extract, either extracted with 70% or 85% alcoholic extract, which documented only at 12 mm and 10 mm diameter respectively (Tipakorn, 2002).

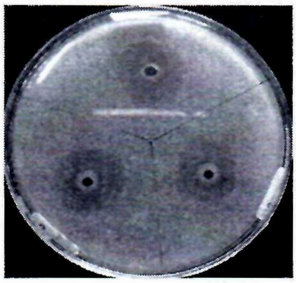
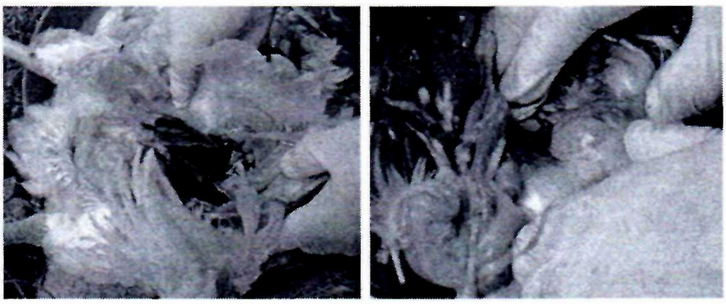


Figure 1. The inhibition zones of herbal-origin bacteriostatic to *E coli.*

The results were in line with field findings which showed less damage to chickens body (Figure 2.). The figure showed that the treated chicken contained less fester than that of control one. The higher fester will lead to the lower prices of the chicken because of lower in net chicken body weight as well as due to ethical aspect. The fester itself is destroyed erythrocytes, which mean higher fester content indicated the higher erythrocyte destroyed. Since erythrocyte naturally plays role in controlling chicken natural immunity, then the higher erythrocyte destroyed means the lower chicken immunity. Broilers selected for erythrocyte deficiency have been found to have increased susceptibility to various non-infectious diseases (Boersma, 2001).



A). *E. coli* in treated B). *E. coli* in control

Figure 2. The comparison between treated and control treatment infected by *E coli.*

The active compounds also performed significant antiviral activities against IB and ND viruses, shown in high antibody titer of both viruses. The antiviral activities against the two viruses were shown by high antibody titer of the two viruses. The IB antibody titer recorded in treated chickens was 11.3 and was higher than antibody titer produced by vaccine, i.e. 8.5 (Table 1.). Although there is no established figure on the level of protectiveness of IB antibody titer, this highly antibody titer indicates that the treated active compound significantly effective to increasing chicken immunity against IB virus. Since IB virus is an immunodepletor, the highly antibody titer will help chicken's immunity against other disease (Huff et al, 2005), which in term will reduce chicken mortality rate and increase body weight due to the less stress caused by the diseases.

Antibody IB titer in treated chicken

Titer group	Titer Range	No. of Sample	
		Vaccine	Vet-i
Negative	0 - 288	14	4
Suspect	288 - 355	0	1
1	356 - 1000	2	3
2	1000 - 2000	0	1
3	2000 - 3000	2	2
4	3000 - 4000	2	2
5	4000 - 5000	0	3
6	5000 - 6000	0	4
7	6000 - 8000	0	0
8	8000 - 10000	0	0
9	>10000	0	0
Total Sample		20	20
Log2 titer		8.5	11.3

Table 2. shows the higher total of antibody ND titer in treated chicken sera, i.e. 4.95, compared to chicken control, 3.60. As what found in IB titer, there is not available the level of protectiveness of ND antibody titer. However, this level of antibody titer indicates the treated active compound significantly effective increasing chicken immunity against ND virus.

Antibody ND titer in treated chicken

Titer HI by Log 2	Number of treated chicken	
	Control	Treated
2	2	
3	7	
4	8	4
5	3	13
6		3
Titer average	**3,60**	**4,95**

Since the treatment was employing active compounds, without providing neither IB nor NDV into the chicken body, the observed antibody titer of both viruses then indicated that the treated chicken producing its own vaccine, using the viruses infecting them. More detailed investigation is needed to explore the mechanism of the formation of these antibody titer, particularly in finding the critical stage (s) of virus inactivation and is an open opportunity to further research.

These laboratorial findings lead to the increase final field performance of chicken-farm compared to control as shown on Table 3. While there was no serious differences between the two farms in term of chicken health, day-to-day chicken activities, and daily performance, the increase in field performance might be due to the less stress of the treated chicken compared to control. The stress might be due to the stronger effects of antibiotic and vaccines applied to the control chickens farm (Huff et al, 2005). This can be seen in the lower feed intake during few days after the medicines were applied, which might resulting in the lower body weight and higher chicken depletion.

Comparison of field performance of chicken-farm between treated and the control chickens

Parameter	Control	Treatment
DOC Weight (g)	42.07	40.46
Day to harvest	35.20	32.65
Feed intake (g/hen)	2,122.51	2,071.11
Body wt (g/hen)	1,779.18	1,843.75
Depletion (%)	4.65	3.53
FCR	1.68	1.52
IP	323.08	348.71

The other possible stress factors were weather and physical handling during the cultivation period. The main effects of antibiotic and vaccines were to combating bacteria and viruses respectively, whereas the active compounds treated have been documented to reducing chicken stress against both physical handling and weather, due to lower concentration of sugar and fat (ISM, 2007).

The antibiotics applied were tetracycline (TCs), derived from *Streptomyces* spp. The antibiotics have broad spectrum activity against many gram-positive and -negative bacteria (Vakharia et al, 1992). TCs are actively transported into the cells of susceptible bacteria where they exert a bacteriostatic effect by inhibiting protein biosynthesis after binding to the 30 S ribosomal subparticle (Tipakorn, 2002). Other TCs, Oxytetracycline (OTC), tetracycline (TC), CTC and doxycycline (DC) are commonly applied to food-producing animals as drugs and feed additives because of their broad spectrum activity and cost effectiveness (Hisiau et al., 1995). The residues of antibiotics in animal meat can enter human food and increase the risk of ill health in persons who consume products from treated animals (Niu et al, 2006; Liu et al, 2005; JECFA, 1995).

The findings of tetracycline residues in animal products have been widely published. Al-Ghamdi et al. (2000) documented residues of tetracycline compounds in poultry products produced in the eastern province of Saudi Arabia. Studies on Indonesian poultry products also reported that excessive use of antibiotics including virginiacin, spiramycin, bacitracin and avopacin leads to residue contamination of the meat products (Bunyapraphatsara, 2000).

Infectious bursal disease (IBD) is an acute, highly contagious viral disease in chickens. The virus belongs to genus Avibirnavirus in the family of Birnaviridae (Dobos et al., 1979), is a nonenveloped icosahedral, bisegmented, double stranded RNA virus with a diameter of about 55-60 nm (Ismail and Saif, 1990). This virus can be differentiated into two serotypes; serotype 1 contains pathogenic strains, whereas serotype 2 strains are not pathogenic one (Ismail and Saif, 1990). Pathogenic serotype 1 IBD virus field strains can be grouped into classical, antigenic variant and very virulent strains (Brown et al., 1994). IBD is becoming increasingly important in the broiler industry, because of its immuno-depletion effect, which suppresses the immune reaction of poultry against other diseases such as ND, infectious bronchitis, necrotic enteritis and coccidiosis.

Similarly to this, some strains of vaccine virus (IB or ND) can produce tissue reactions of the respiratory organs and mucous membranes, which may develop into a chronic respiratory disease (Alexander, 1991) Vaccines, especially live vaccines, may also have more generalized detrimental effects (King and Cavanagh, 1991). Vaccination of broiler flocks in the face of an outbreak has mixed results; in some situations, increased rather than decreased the mortality. USA produces about 18 – 20 billion doses of ND vaccine per year, and Indonesia with about 190 million chicken is a good vaccine buyer. However, the US government until this day "has to reject" any chicken product to from Indonesia, because almost all poultry products produced by Indonesian poultry contain "too much vaccine".

CONCLUSSIONS

- Antimicrobial active compounds extracted from *C. aeruginosa* Roxb., *C. xanthorriza* Roxb. and *Z. aromaticum*, Vahl. rhyzomes, and *A. marmelos* L. Corr. provided 23,01± 0.37 mm diameter of inhibition zone to *E coli*.
- Antiviral effects of the active compounds were providing antibody titer of ND and IB virus 4.95 and 11.3 respectively which were higher than those produced by the vaccines, i.e. 3.60 and 8.5.
- The evaluation of these antibacterial and antiviral laboratorial resulted in significant field performance of the chicken particularly in improving feed intake, i.e. from 2,122.51 to 2,071.11 g/hen, increasing chicken body weight from 1,779.18 to 1,843.75 g/hen, reducing depletion rate from 4.65% to 3.53% and FCR from 1.68 to 1.52, and increasing IP from 323.08 to 348.71
- The herbal-origin antibacterial and antiviral is therefore potential to produce healthier chicken meat compared to antibiotics and vaccines applications, which have been widely documented resulting residues in the chicken meat.

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