

The Lempuyang Gajah [*Zingiber Zerumbet* (L.) Smith] Extract Supplementation in Broilers Feed to Suppress Foodborne Disease “Salmonellosis” for Consumers’ Health Safety Effort

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Abstract. The study aims to control Salmonellosis through the addition of *Z. zerumbet* extract in broilers feed. The experiment consisted of 5 treatments namely, T0 (standard diet), T1 (standard diet + infection of *S. enteritidis*), T2 (standard diet + infection of *S. enteritidis* + 0.33% *Z. zerumbet* extract), T3 (standard diet + infection of *S. enteritidis* + 0.67% *Z. zerumbet* extract), and T4 (standard diet + infection of *S. enteritidis* + 1% *Z. zerumbet* extract). Each treatment was repeated five times. The parameters measured were the number of *S. enteritidis* colonies, small intestinal morphology, and digestive enzymes activity. The results showed that no *S. enteritidis* colonies were found in T3 and T4 treatments, whereas T2, T3 and T4 had a high ratio of villi / Liberkuhn crypt depth and higher enzyme activity as compared to that of T1. It was concluded that the addition of *Z. zerumbet* extract at a concentration of 0.67% to 1% has the potential as a feed additive for controlling Salmonellosis in broiler chickens. Thus, the extract can replace AGP as a feed additive that is safe for the health of broilers as well as humans as the consumers.

Keywords: *Z. zerumbet*, Salmonellosis, foodborne disease, feed additive, Antibiotic Growth Promoters (AGP)

INTRODUCTION

Salmonella enteritidis (*S. enteritidis*) is currently the only Salmonella serotype that causes diseases in humans. This serovar infection has increased worldwide from the early 1970s. Moreover, in 1990, it replaced *Salmonella typhimurium* (*S. Typhimurium*) as the main cause of Salmonellosis in the world [1].

Salmonella enteritidis is commonly found in chicken farms. It causes the contamination of egg and chicken consumption. As a result, cases of foodborne disease in various parts of the world, including Indonesia, emerge. Poultry products, especially from chickens, are required to be *Salmonella*-free [2]. Concerning this matter, the government has established regulations stating that meat, eggs, and processed products for consumption must be free of Salmonella. In other words, the amount of Salmonella sp must be negative in every 25 grams of egg, meat, and its processed products [3].

There are 150 serotypes of Salmonella causing gastroenteritis in humans, and *S. enteritidis* is the predominant causative agent. Clinical symptoms occur between 18-48 hours after bacterial infection, with a duration of about 2-5 days. These symptoms include watery stools, sometimes containing blood, accompanied by fever and abdominal pain [4].

Intestine mucosa is the primary defense against *Salmonella sp*. The colonization of *Salmonella sp* occurs in the secum branching, before entering the colon. Furthermore, through the lymph system, *Salmonella sp* may infect the spleen and liver organs [5].

The Antibiotic Growth Promoters (AGP) usage in broilers feeds often causes the inhibition of colonization of beneficial intestinal bacteria, such as *Lactobacillus* and *Bifidobacteria*, as well as the increased resistance of *Salmonella sp* [6]. There has been resistance to the antibiotics vancomycin, erythromycin, and penicillin in *Salmonella sp* isolated from broiler meat, with resistance approaching 100 percent [7].

The phytobiotic substitute for AGP in broiler chicken feed serves as an alternative because it contains antibacterials that can be extracted and used as feed additives [8]. One of such phytobiotics is

Lempuyang Gajah (*Zingiber zerumbet* L. Smith). The extract contains various antibacterials, including alkaloids, saponins, flavonoids, lipids, polyphenols, and terpenoids, which can inhibit cell wall synthesis, membrane function cell, and protein synthesis [9]-[11].

The current study examines the ethanol extract of powder from *Z. zerumbet* L. Smith as a feed additive substituting AGP in controlling Salmonellosis in broilers to prevent the *Salmonella sp* contamination in poultry products, with the hope of minimizing the number of cases of foodborne disease and the content of antibiotic residues in poultry products, which will further manifest the health of the user community.

METHODS

Plant Materials and Preparation

The rhizomes are cleaned of dirt, washed and cut into thin strips, then dried in an oven at 45°C to reduce the moisture content, then milled until smooth. The powder is immersed in a jar containing 95% ethanol while stirring at a speed of 50 rpm for 24 hours. The extract was filtered, then evaporated for 1 hour using a rotary evaporator and for 12 hours with a water bath. The thick extract (100%) was diluted to 10% by adding a sterile aqua dest. This extract was dried in an oven at 40°C for two days, and then a 5% starch was added to obtain a powder form [12].

Care System

A total of 125 One-Day-Old-Chick (DOC) broilers, with an initial weight range of 34 grams to 47 grams, were maintained for 35 days. At the age of 4, 17 and 24 chickens were vaccinated with the ND vaccine, while artificial infection with *S. enteritidis* ATCC 31194 was carried out at the age of 10 days orally at a dose of 10^{10} CFU. *Zingiber zerumbet* extract supplementation was carried out at the age of 7 days until the end of maintenance [13].

Research Design

In vivo experiments on broilers were carried out with 5 treatments, among others T0 (standard diet), T1 (standard diet + infection of *S. enteritidis*), T2 (standard diet + infection of *S. enteritidis* + 0.33% *Z. zerumbet* extract), T3 (standard diet + infection of *S. enteritidis* + 0.67% *Z. zerumbet* extract), and T4 (standard diet + infection of *S. enteritidis* + 1% *Z. zerumbet* extract) [13].

Measured Variables

The variables in this study were the number of *S. enteritidis* colonies, small intestine morphology, and enzyme activity, including lipase, amylase, and protease. Feces samples for measuring the number of

bacterial colonies were taken from 28 days old chickens, while intestinal samples for measuring intestinal morphology and enzyme activity were taken at the end of maintenance.

Measurement

Numbers of Salmonella sp Colony

The number of *Salmonella sp* colonies in the broilers' feces was sampled on the 10th day, shortly before being infected, and on the 28th day. The measuring procedure employed the petri dish count method. The total number of colonies was obtained by multiplying the number of colonies from the calculation with the dilution factor.

Intestinal Morphology

Intestinal samples for histological observation were taken by cutting one centimeter from the distal duodenum (before the descending loop), jejunum (before Meckel's diverticulum), and ileum (before the ileocecal junction). Fixation was carried out using saline buffered formalin (10%), formed paraffin inclusions, and cut using a microtome with a thickness of 5 micrometers. The staining of the preparations was carried out by the Hematoxylin-Eosin method. The villi height and depth of Lieberkuhn's crypt were observed using a Carl Zeiss microscope (Jena model 742797 RDA) and measured using a multilevel optical lens (Olympus 10x / 18 [14].

The activity of the Digestive Enzymes

The method of measuring the protease activity employed a modification of the Walter method [15]. Meanwhile, lipase testing was carried out according to the method initiated by Lindfield et al. [16]. And, the amylase activity was measured according to the Bernfeld method [17].

Data Analysis

The data applied a descriptive quantitative analysis.

RESULTS & DISCUSSION

Numbers of Colony of S. enteritidis in Feces

The colony numbers of *S. enteritidis* in 28-day-old chickens in T0, T1, T2, T3, and T4 treatments were 179×10^2 CFU, 271×10^2 CFU, 242×10^2 CFU, 0 CFU, and 0 CFU, respectively. No bacterial colony was found in T3 and T4 treatments. It indicates that the addition of *Z. zerumbet* extract by 0.67% to 1% significantly reduces the number of *S. enteritidis* in feces to zero colonies. This fact proves the high efficacy features of the antimicrobial contained in the extract of *Z. zerumbet* against *S. enteritidis* as it can

maintain its activity up to the back of the digestive tract, namely the cecum, the locus where the colonization of *S. enteritidis* is formed. Thus, *Z. zerumbet* extract has a vital role in inhibiting the horizontal transmission of bacteria to the environment as well as preventing the contamination of eggshells by feces[18]. Consequently, it can reduce the case of the foodborne disease and guarantee the health of the poultry product consumers.

It is apparent that *zerumbon*, as the dominant essential oil in *Z. zerumbet*, is capable of damaging the cell walls and structural integrity of *S. enteritidis* membranes as well as penetrating the complex gram-negative bacterial cell walls that consist of the outer layer (lipoprotein), middle layer (lipopolysaccharide), which acts to block the entry of antibacterials, and inner layers in the form of peptidoglycan [19]. The membrane damage causes the leakage of ions, reduction of membrane potentials, and collapse of the proton pump. It inhibits the enzyme activities so that it may interfere with the cell metabolism and deplete the energy acquisition for the cells; this eventually leads to the death of the cells [20], [21].

The Small Intestinal Morphology

The effects of the treatment on small intestinal morphology, including the height of the villi (VH), the depth of the Lieberkhu crypt (CD), and the comparison of both, can be seen in Table 1.

Table 1. Morphology of the small intestine

Small Intestine	Villi Height (VH) (µm)	Crypt of Lieberkhu Debt (CD) (µm)	Villi Height/Crypt Debt Ratio (VH/CD ratio)
Duodenum			
T0	87.47 ± 2.42	10.15 ± 0.90	8.68 ± 0.87
T1	75.58 ± 13.94	15.86 ± 2.14	4.76 ± 0.62
T2	85.37 ± 1.40	10.73 ± 0.40	7.96 ± 0.32
T3	74.64 ± 10.12	10.96 ± 1.07	6.90 ± 1.51
T4	74.88 ± 5.28	10.26 ± 0.40	7.32 ± 0.80
Jejunum			
T0	97.27 ± 1.81	9.45 ± 2.10	10.70 ± 2.44
T1	101.94 ± 14.95	17.49 ± 2.10	5.87 ± 0.95
T2	105.90 ± 7.18	12.60 ± 1.40	8.50 ± 1.35
T3	83.74 ± 0.40	13.06 ± 0.81	6.43 ± 0.37
T4	96.34 ± 12.97	14.00 ± 1.40	6.93 ± 0.67
Ileum			
T0	41.11 ± 3.85	7.70 ± 1.81	5.56 ± 1.27
T1	33.82 ± 7.28	10.50 ± 0.70	3.26 ± 0.92
T2	44.32 ± 3.98	10.26 ± 1.07	4.35 ± 0.61
T3	52.02 ± 4.22	11.43 ± 1.07	4.58 ± 0.60
T4	47.35 ± 1.76	11.66 ± 1.07	4.09 ± 0.50

Note: T0 (standard diet), T1 (standard diet + infection of *S. enteritidis*), T2 (standard diet + infection of *S. enteritidis* + 0.33% *Z. zerumbet* extract), T3 (standard diet + infection of *S. enteritidis* + 0.67% *Z. zerumbet* extract), and T4 (standard diet + infection of *S. enteritidis* + 1% *Z. zerumbet* extract).

A high VH / CD ratio indicates a healthy intestine, the highest value in all intestine parts is achieved in the negative control group (T0), and meanwhile, the lowest value is obtained in the positive control group (T1). It indicates that *S. enteritidis* infection has caused changes in the intestinal mucosal structures, which interferes with the process of digestion and

absorption of nutrients. Intestinal mucosa plays a role in the intestinal fluid secretion and absorption of food substances. The fluid is produced by glands along the mucosa, namely the crypt of Lieberkhu and Bruner glands. The addition of *Z. zerumbet* extract 0.33% - 1% to the broilers infected with *S. enteritidis* can increase the VH / CD ratio in the duodenum, jejunum, and ileum, although this value is still below T0. This fact shows that there is a phytobiotic role in the *Z. zerumbet* extract as anti-inflammatory antibacterial and stimulating digestive enzyme activity[22]-[24]. *Zingiber zerumbet* extract supplementation in broiler feed can be used as an alternative to AGP to treat foodborne diseases caused by *Salmonella sp.*, especially *S. enteritidis*, to ensure the health of the public who use poultry products.

Digestive Enzyme Activity in the Small Intestine

In general, the T2, T3, and T4 treatments show higher lipase, protease, and amylase enzyme activity in the small intestine than T0 and T1. Data on digestive enzyme activity is shown in Table 2.

Table 2. Digestive Enzyme Activity in Broilers' Small Intestine

Treatment	Enzyme Activity		
	Lipase (µ mol/minute)	Protease (IU/mL)	Amylase (IU/mL)
T0	1.057	0.132	0.161
T1	1.056	0.227	0.122
T2	1.042	0.274	0.144
T3	1.346	0.375	0.227
T4	1.207	0.295	0.321

zerumbet extract), and T4 (standard diet + infection of *S. enteritidis* + 1% *Z. zerumbet* extract).

The increasing activity of enzymes is attributed to the essential oils that contain the antibacterial agents against *S. enteritidis* in the infected broilers (T1). These essential oils stimulate the activity of the enzymes (lipase, protease, and amylase) in the normal broilers (T0). *Zerumbon* is the most common compound found in *Z. zerumbet* crude ethanol extract, it has an antibacterial power against *Salmonella sp* [25], by inhibiting the cell wall synthesis, membrane function, and protein synthesis [9],[11]. The results of this study indicate the role of *Z. zerumbet* extracts in controlling the Salmonellosis disease. It is also revealed that the extract can simultaneously stimulate the activity of digestive enzymes; therefore, it can replace the role of AGP as a feed additive in the broilers' feed.

CONCLUSION

It was concluded that the *Z. zerumbet* extract as supplementation in broiler feed at a concentration of

0.67% to 1% has the potential as a feed additive to control Salmonellosis in broiler chickens. Phytobiotic extracts have high efficacy as an antibacterial agent against *S. enteritidis*, which causes foodborne diseases. It can repair intestinal damage and digestive enzyme activity due to Salmonellosis so that *Z. zerumbet* extract can replace AGP, which is safe for the health of broiler chickens and consumers.

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