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Phytobiotic Properties of Garlic, Red Ginger, Turmeric and Kencur in Growing Ducks

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Abstract. Phytobiotic properties of garlic (*Allium sativum*), turmeric (*Curcuma domestica*), red ginger (*Zingiber officinale*) and kencur (*Kaempferia galangal*) were studied using standard *in vitro* antibacterial test and *in vivo* feeding trial with ducklings. In the in vitro experiment, potency of aqueous extract of these phytobiotic agents were tested against *Salmonella pullorum* and *Escherichia coli*. Feeding trial was carried out for 6 week starting at day 28 using ducklings fed diets supplemented with 1% of each of four phytobiotic agents. The highest antibacterial activity against *S. pullorum* and *E. coli* was observed with garlic and no additive effect when mixture of phytobiotics was used. Weight gain, fed intake and feed conversion ratio of ducklings were not affected by inclusion of garlic, red ginger and kencur. However, 1% turmeric supplementation significantly reduced growth performance to ducklings.

Key words: phytobiotic, antibiotic, duck, medicinal plants

Abstrak. Penelitian karakteristik fitobiotik dari bawang putih (*Allium sativum*), kunyit (*Curcuma domestica*), jahe merah (*Zingiber officinale*) dan kencur (*Kaempferia galangal*) telah dilakukan secara in vitro melalui uji aktivitas antibakteri dan secara in vivo dengan perlakuan suplemetasi fitobiotik didalam pakan anak itik. Pada percobaan in vitro, potensi aktivitas antibakteri dari ektrak fitobiotik diuji menggunakan *Salmonella pullorum* and *Escherichia coli*. Percobaan suplementasi fitobiotik diberikan masing-masing sebesar 1% didalam pakan anak itik. Pemberian pakan perlakuan dilakukan selama 6 minggu, dimulai pada saat anak itik berumur 28 hari. Hasil penelitian menunjukkan aktivitas antibakteri terhadap *S. pullorum dan E. coli* paling tinggi adalah ektrak bawang putih dan tidak ada pengaruh yang lebih baik apabila dicampur dengan ektrak fitobiotik lainnya. Pertambahan bobot badan, konsumsi pakan dan konversi pakan anak itik tidak dipengaruhi oleh penambahan bawang putih, kunyit, jahe merah dan kencur. Akan tetapi, suplementasi kunyit nyata menurunkan performan pertumbuhan anak itik.

Kata kunci: fitobiotik, antibiotik, itik, tanaman obat-obatan.

Introduction

Duck producers are still largely using traditional approach of growing and feeding with minimum profit margin and low production efficiency in Indonesia. Many breeders use antibiotics for prevention and treatment of disease as well as growth promoting purposes. Antibiotics has long been under the spotlight as food and feed safety issue in scientific community because of antibiotic residues and resistance development. Therefore, duck producers are increasingly enforced to adopt application of other antibiotics replacement strategies (Conway and Wang, 2000). Utilization of phytobiotics as locally available natural growth promotors (NGPs) considered as a practical and achievable way (Vidanarachchi et al., 2010; Grashorn, 2010; Yang et al., 2015). Among many medicinal plant available as phytobiotics, garlic, turmeric, red ginger and kaempferia are widely available and used as food item in South and South-East Asia.

Garlic (*Allium sativum*) contains alliin that convert to allicin upon crushing the bulbs. Allicin is reported to have strong antibacterial effect against *Campylobacter jejuni* and improves performance in broiler chickens (Tollba and Hassan, 2003; Royban et al., 2013). Turmeric (*Curcuma domestica*) contains active substances including terpenoids, alkaloids, flavonoids, essential oils, phenols and curcuminoid which often serve as antimicrobial agents against *Escherichia coli and Staphylococcus aureus* (Niamsa and Sittiwet, 2009). Kencur (*Kaempferia galangal*) is reported to have antimicrobial effect on gram-positive and negative bacteria (Kochuthressia, et al., 2012; Tewtrakul et al., 2005), but is not used in poultry feeding studies. Red ginger (*Zingiberofficinale*) contain phytochemicals such as zingiberol, zingiberine, bisabolene and gingerol that are potent antimicrobial agents against *Salmonella enteritidis and* of *E. coli*. (Indu et al., 2006; Akintobi et al., 2013).

To date, no study has been reported on the use of garlic, red ginger, turmeric and kencur as phytobiotic agent in duck production. Accordingly, this study was aimed to examine the antibacterial potency of garlic, red ginger, turmeric and kencur aqueous extract against *Salmonella pullorum* and *E. coli* and their effectiveness to improve growth performance in growing duck.

Materials and Methods

Fresh bulbs of *Allium sativum* and rhizomes of *Curcuma domestica*, *Zingiberofficinale*and *Kaempferia galangal* are originated from Indonesia and available in traditional market. The plants were cleaned, sliced and washed with sterile distilled water and then oven-dried and milled.

In vitro experiment

Appropriate amount of each plant powder (0.5, 1, 1.5 or 2 g) was placed in a flask, and added with 100 ml distilled water, the flasks were stirred and the extracts were obtained after filtration using filter paper. Filter paper discs of 6 mm diameter were prepared, sterilized and soaked in each of the prepared filtrates for 10 min. The saturated discs were aseptically placed over Salmonella Shigella Agar (SS) plates seeded with *S. pullorum* (Gram-positive) and Eosin Methylene Blue Agar(EMB) plates seeded with *E. coli* (Gram-negative). All steps were conducted under strict laboratory procedures for microbiology. The plates were incubated at 37˚C for 24 h. The diameter of inhibition zones was measured in mm using caliper. The tested microorganisms were also assayed for their sensitivity against the antibiotics ampicillin (0.5g/500µl/disk) and chloramphenicol (0.5g/500µl/disk) using standard Kirby Baeur disc diffusion method. The 8 treatment groups that tested were garlic 0.5-2% (GA); red ginger 0.5-2% (RG); turmeric 0.5-2% (TU); kencur 0.5-2% (KA); garlic + red ginger 0.5-2% (GR); garlic + turmeric 0.5-2% (GT); garlic + kencur 0.5-2% (GK).

In vivo experiment

This experiment was conducted to determine the effect of phytobiotic supplementation on duck growth performance. A total of 128 day-old male duck (Anas platyrhynchos) with 37.5g average weight were obtained from a local hatchery and distributed randomly in $1m^2$-sized slat floor pens (4 birds/pen) in a conventional open-sided house with 27-29^{0}C housing temperature and 60-80% relative humidity. The birds were fed standard diet with broiler feed (BR1 code Ex), product of PT Cargill Indonesia from DOD to 3 weeks old. The feed contained metabolizable energy of 3000 kcal/ kg, 20.5% crude protein, 5% crude fat, 5.75% crude fiber, 0.95% Ca and 0.8% P. Preliminary basal feed was given for 1 week then fed treatments from 4-10 weeks later. The basal diet composition and nutrient content are presented on Table 1. Each experiment units

Table 1. Composition and nutrient content of basal diet (%)

Ingredient	The percentage of feed material
Corn	40
Rice bran	39
Soybean meal	12
Fish meal	8
Vitamin-mineral premix	1
Total	100
Calculated analysis	
Crude protein (%)	16.10
ME (kcal/kg)	2905
Crude fiber (%)	3.91
Crude fat (%)	4.36
Calcium(%)	1.82
Total Phospor (%)	1.33

consisted of 4 ducklings each assigned to one of eight dietary treatments with 4 replicates, or 32 pens in total. The dietary treatments were BD: basal feed (control); GA: basal feed + garlic 1%; RG: basal feed + red ginger 1%; TU: basal feed + turmeric 1%; KA: basal feed + kencur 1%; GARG: basal feed + garlic 0.5% + red ginger 0.5%; GATU: basal feed + garlic 0.5% + turmeric 0.5%; GAKA: basal feed + garlic 0.5% + kencur 0.5%. No antimicrobial, anticoccidial drugs or feed additives were included in the basal diets. Ducklings were weighed individually on 4th week until 10th week. Feed intake was recorded and feed conversion ratios (FCR) were calculated. Mortality was recorded in each subgroup. Data were subject to completely randomized design (CRD) using ANOVA general linear models procedure (GLM) of SAS software. Significant effects were separated by Honest Significant Difference (HSD). Mortality data were subject to chi-square analysis and statistical significance was considered at $P \leq 0.05$ test.

Results and Discussion

The results of in vitro experiment show that among the four herbal plants studied the aqueous extract of garlic showed considerable growth-inhibiting activity against *E. coli* and *S. pullorum* with all concentration (0.5-2%) (Figure 1). Increase in the inhibitory zone was observed by increasing the concentration of the extract. Kencur and particularly turmeric and red ginger were appeared to have almost no to negligible antibacterial effect on *S. pullorum*. However, they were observed to inhibit E. coli at least with concentration higher than 0.5%. Combination of garlic extract with three other extracts demonstrated no superior beneficial effect compared to the sole garlic extract.

In vivo experiment result showed the effects of feeding diets supplemented with dried powder form of the four phytobiotics and the combinations on feed intake, body weight (BW), weight gain (WG), feed conversion ratio (FCR) and mortality in Table 2. Following 6 weeks of feeding trial, no significant positive or negative effects were observed by supplementation of garlic, red ginger, kaempferia, garlic+red ginger, garlic+turmeric and garlic + kencur on all performance parameters measured. Supplementation of diet with 1% turmeric had detrimental effects on WG, feed intake and FCR compare to control birds. Mortality rate was not affected by any of the dietary treatments.

The results of different studies provide evidence that some medicinal plants might indeed be potential sources of new antibacterial agents even against some antibiotic-resistant strains (Indu et al., 2006). The findings of the current study are consistent with those of Safithria et al. (2011) who found garlic extract inhibited the growth of *E. coli.* Other studies also showed that phytochemicals in garlic have strong antibacterial properties to combat *E. coli*, *Salmonella*, (Johnson and Vaughn, 1969), *C. botulinum* (DeWit et al., 1979), and other pathogenic species. Garlic (*A. sativum*) has a phytochemical content of alkaloids, tannins, phylobatanin, anthraquinone and saponin (Akintobi et al., 2013). Allicin is

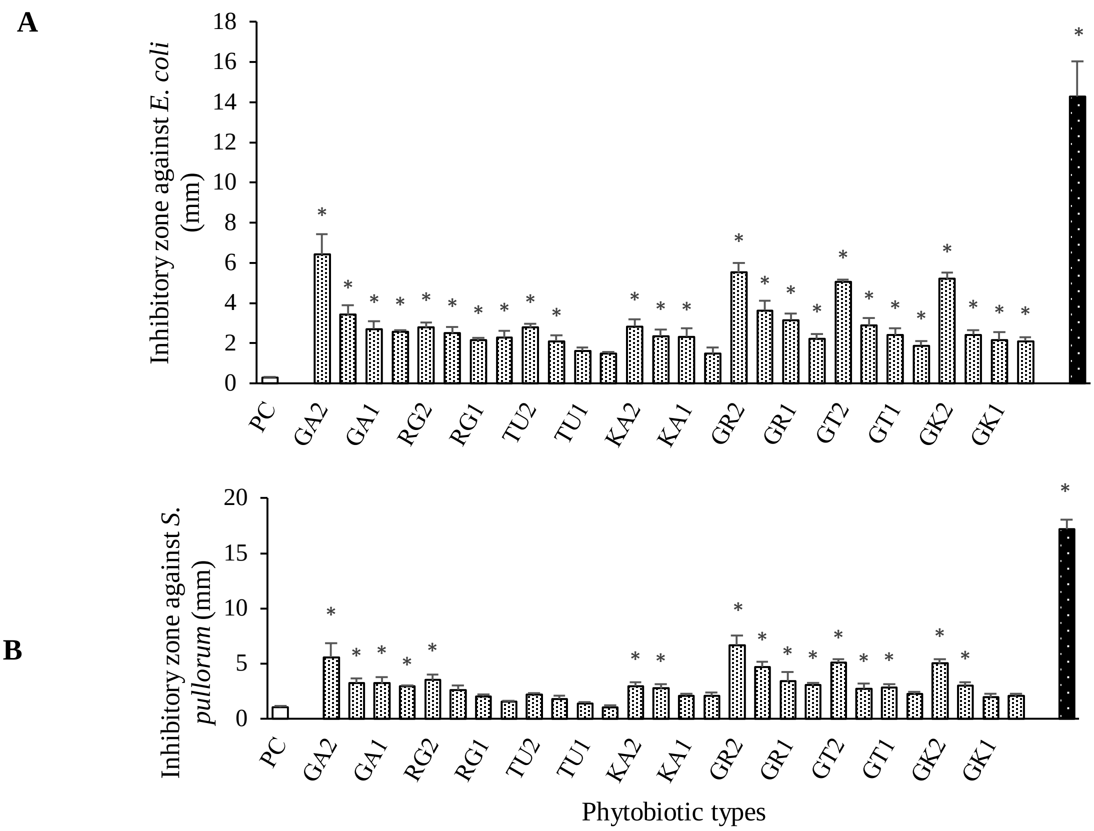

Figure 1. Mean inhibition zones of various phytobiotics aqueous extracts against E. Coli (A) and Salmonella (B) in comparison with standard antibiotics.

PC: positive control (AquaDest); NC: negative control (0.5g/disk Chloramphenicol for Salmonella and 0.5g/disk Ampicillin for E. Coli); GA: garlic 0.5-2%; RG: red ginger 0.5-2%; TU: turmeric 0.5-2%; KA: kaempferia 0.5-2%; GR: garlic + red ginger 0.5-2%; GT: garlic + turmeric 0.5-2%; GK: garlic + kaempferia 0.5-2%. * Mean±SEM differ versus PC (P<0.05).

Table 2. Growth performance and mortality of duck fed with diets supplemented with various phytobiotics from 4-10 weeks of age

Treatments	BW[§] (g, Wk 4)	BW (g, Wk 10)	WG (g)	Feed intake (g)	FCR	Mortality (%)
BD†	555	1254	699^{b}	2099^{a}	3.03^{a}	12.5
GA	677	1348	671^{b}	2120^{a}	3.17^{a}	0
RG	641	1293	652^{b}	2139^{a}	3.29^{a}	6.25
TU	577	1136	558^{a}	2255^{b}	4.06^{b}	0
KA	587	1222	635^{b}	2158^{a}	3.42^{a}	6.25
GARG	581	1233	653^{b}	2141^{a}	3.31^{a}	0
GATU	540	1269	728^{b}	2075^{a}	2.87^{a}	0
GAKA	557	1217	660^{b}	2132^{a}	3.24^{a}	6.25
SEM	11	15	11	12	0.08	1.64
ANOVA	NS[*]	NS	*	*	*	NS[#]

[§] BW: body weight; WG: weight gain; FCR: feed conversion ratio.
[†] BD: basal feed (control); GA: basal feed + garlic 1%; RG: basal feed + red ginger 1%; TU: basal feed + turmeric 1%; KA: basal feed + kaempferia 1%; GARG: basal feed + garlic 0.5% + red ginger 0.5%; GATU: basal feed + garlic 0.5% + turmeric 0.5%; GAKA: basal feed + garlic 0.5% + kaempferia 0.5%.
[*] NS: not significant; *: P <0.05; **: P <0.01.
[a,b]Means within a column with no common letters differ at P< 0.05.
[#]NS: not significant with chi square test

the most potentially active component of garlic that is responsible for its characteristic odor, flavor as well as most of its biological properties (Chowdhury et al., 2002). There was no antibacterial activity in extracts of turmeric and low concentration of red ginger and kencur against *S. pullorum*. On the contrary, earlier work showed that the antibacterial activity of *Zingiberofficinale, Curcuma longa* and *Kaempferia galangal* against Gram-positive and Gram-negative bacteria (Tewtrakul et al., 2005; Niamsa and Sittiwet, 2009; Kochuthressia, et al., 2012; Akintobi et al., 2013). The discrepancies could be associated with the different dosage used, extraction method, the method of antibacterial study, the genetic variation of plant, age of the plant or the environment. It is possible that some of the effective antibacterial active ingredients of these phytobiotics source is not extracted using aqueous extraction method. Cowan (1999) contributed a huge proportion of the plant antibacterial properties to lipid soluble or nonpolar ingredients. It has been reported that plant extracts in organic solvent provided more consistent antimicrobial activity compared to those extracted in water (Parekh et al., 2005). Furthermore, according to Longanga Otshudi et al. (2000) some plants have their full therapeutic effect only if collected at certain time or during a certain season.

It is known that lower number of some gut pathogens such as *E. coli and Salmonella* may improve animal performance (Kim et al., 2011). Overgrowth of some microorganisms in the intestine has been correlated with mucosal layer and villi impairment and therefore reducing the capacity of nutrient absorption (Pelicano et al., 2005). Thus, microbial activity has the potential to alter gut morphology in either positive or negative way (Rebolé et al., 2010). In our study, however, no beneficial effect of feeding phytobiotics was observed on performance parameters. Furthermore, we observed significant negative effect of feeding 1% turmeric on feed intake, weight gain and FCR. Similarly, Durrani et al. (2006) showed that broiler fed by 1% turmeric in their diet had lower FCR than control group. This may arise from overproduction of bile with feeding of turmeric at high levels of inclusion. Al-Sultan and Gameel (2004) associated the positive effect of low dosage of turmeric to its known effect in stimulating bile production in the liver. Garlic was fed to animals in the form of crushed cloves, powder, garlic oil, water and alcohol extracts (Staba et al., 2001). Result on garlic derivative propylpropane thiosulphonate against broiler enteropathogens in vivo reported that suplementation of garlic extract 45 to 135 ml/kg beneficailly reduced the numbers of pathogenic and potentially pathogenic bacteria in the intestine, and improved the morphological structure of the ileal mucosa and the productive parameters of broiler chickens (Peinado et al. 2012). Liquid garlic extract supplementation at the levels of 1.50 and 2.25 ml kg–1 significantly increased body weight compared to the control group (Brzóska et al. 2015).

Regarding the lack of positive effect of garlic, red ginger and kaempferia on performance in our study, no clear explanation could be offered. However, a critical review of the previous studies of medical plants in poultry feeding reveal that there are a huge body of reports on both side indicating beneficial effect or lack of effects. This could be attributed to lack of a standardization in effective dosage, extraction method, species and age of the phytobiotic plant sources among these studies.

Conclusion

Garlic has the most effective antibacterial activity against *S. pullorum and E. coli* compared to turmeric, red ginger and kaempferia. Combining garlic with these three phytobiotic showed no additive antibacterial effects. Supplementation of garlic, red ginger and kaempferia had not improved performance of duck. One percent turmeric addition to diet of growing duck was detrimental to their growth performance.

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