The influence of the mixture of some plant powder with *Anacardium occidentale* shell liquid smoke on total bacteria and *Escherichia coli* in the ileum of broiler

T Pasaribu¹, T Kostaman¹ and A Saenab²

¹Indonesian Research Institute for Animal Production Bogor, West Java, Indonesia  
²Central Research Institute for Animal Science, Indonesia  

Corresponding author e-mail: pasaributurma@yahoo.com

Abstract. Antibiotics/feed additives are commonly used in poultry rations to prevent disease, increase feed efficiency, and improve performance. Plants are the alternatives to replace antibiotics that are safe and without side effects for chicken meat consumers. The aim of this study was to evaluate the effect of administration of a mixture of plant powder and *Anacardium occidentale* shell liquid smoke on the total bacteria and *Escherichia coli* in the broiler ileum. The plant mixture was: *Phyllanthus niruri* L. (PNP) powder: *Anacardium occidentale* shell liquid smoke (AOLS): *Syzygium aromaticum* leaf powder (SAP). The study used a Completely Randomized Design. In total, 80 one-day-old broiler chicks were allotted randomly into eight treatments with two replicates per treatment and 5 birds per replicate. The study with the following treatments: Control, Zn-Bacitracin 0.05%, T1 (1.25%: 0.125%: 0.313%), T2 (1.25%: 0.125%: 0.157%), T3 (0.625%: 0.125%: 0.157%), T4 (0.625%: 0.0625: 0.157%), T5 (0.313%: 0.0313: 0.625%), T6 (0.313%: 0.125%: 0.313%). To find out the total bacteria and *Escherichia coli*, the chicken is then slaughtered 2 hens per replicates to collect feces from the ileum. The results showed that the treatment of the mixture of PNP: AOLS: SAP was not significantly different (P>0.05) with Zn-bacitracin treatment on the total bacteria and *Escherichia coli*. However, the number of bacteria in T1 was lower than that of the Zn-bacitracin treatment, while the number of *Escherichia coli* in T1 was closer to the Zn-bacitracin treatment. It can be concluded that the PNP: AOLS: SAP (1.25%: 0.125%: 0.313%) mixture at T1 treatment has the potential as a natural antibiotic to replace Zn-bacitracin antibiotic in inhibiting total growth of bacteria and *Escherichia coli* in the ileum of broiler.

1. Introduction

Feed additives that are widely used in the livestock industry are antibiotics or commonly known as antibiotic growth promoters (AGP), with the aim of minimizing the number of pathogenic microbes or disturbing microbes in the digestive tract. The administration of AGP such as Virginiamycin and bacitracin methylene disalicylate in general can increase growth, so it is often used in the poultry industry [5] because of the lack of pathogen bacteria. Continuous provision of antibiotics in poultry, especially chickens, can cause residues in meat, so it will impact consumers, residues in meat are around 4.17%, and the liver is around 4.17% - 83.3% [4]. The resistance of *Campylobacter* and *Salmonella* occurring in animals has given fluoroquinolone antibiotics and third-generation cephalosporins [14]. Prohibition on the use of antibiotics as feed additives in the form of products or raw materials of animal medicines mixed in feed has been effective since January 1, 2018, as stipulated in Permentan Number 14/2017 Article 16 [20]. Thus alternatives are sought to replace antibiotics that are safe and without side
effects for chicken meat consumers. Efforts are being made to use a mixture of plants in the form of powder and plant liquid smoke. Plants have bioactive substances that are antibacterial, antifungal, and antiparasitic such as phylanthine, phenol, or eugenol, which play a role in damaging the membrane or cytoplasm [15, 19].

The main products of cashew nuts (Anacardium occidentale) are cashews that have by-products of pseudo fruit and shells. When Anacardium occidentale shells were extracted, it would be obtained a liquid (oil) called Cashew Nut Shell Liquid (CNSL), while the biosmoke and biochar products from cashew shells have not been explored. Biosmoke (liquid smoke) is the result of the decomposition process of a material by heat without using oxygen, which begins with combustion and is followed by total or partial oxidation of the main product [2] which has a low pH, so it is acidic. In addition to bioactive substances, organic acids also have the function of inhibiting the growth of pathogenic microbes through different mechanisms from antibiotics. Organic acids can penetrate microbial cell walls and reduce pH in microbial cells, resulting in denaturation and coagulation of proteins, which consequently interfere with the growth and proliferation of pathogenic microorganisms [9]. Phyllanthus niruri L. (meniran) could cure gastrointestinal disorders and diarrhea because its bioactive substances are antimicrobial [16,21]. The compounds contained in meniran include terpenoids, alkaloids, flavonoids, saponins, and tannins [6,10]. In vitro, at a concentration of 60%, there was no growth of Syzygium dysenteriae bacterial colonies [12].

Clove (Syzygium aromaticum) essential oil or known as an essential oil contains 70-93% eugenol. Eugenol effectively inhibits the growth of the fungus Candida albican (12.1 mm) and Escherichia coli (4.2 mm) [18]. Essential oil has been shown to inhibit the growth of Clostridium perfringens, Escherichia coli, or parasites such as Eimeria in broilers [22], so the absorption of nutrients in the digestive tract can be increased. Information on the provision of plant powder or liquid smoke singly (not mixed) in chickens has been done a lot, but information on the mixture of powder and liquid smoke is still limited. Thus this study was designed by mixing Phyllanthus niruri L powder, Anacardium occidentale shell liquid smoke, and Syzygium aromaticum leaf powder with different concentrations.

The mixture of Phyllanthus niruri L.: liquid smoke of Anacardium occidentale shell: Syzygium aromaticum in extract form showed the same results as Zn-bacitracin on total bacteria and total Escherichia coli [17]. The aim of this study was to evaluate the effect of administration of a mixture of plant powder and Anacardium occidentale shell liquid smoke on the total bacteria and Escherichia coli in the broiler ileum.

2. Material and methods
All experimental procedures followed were approved by the Indonesian Research Institute for Animal Production Ethics Committee.

2.1. Preparation of Phyllanthus niruri L. and Syzygium aromaticum powder

![Figure 1](image_url)

**Figure 1.** Scheme of preparation of Phyllanthus niruri L. and Syzygium aromaticum powders.
2.2. Preparation of *Anacardium occidentale* liquid

The mixture of *Phyllanthus niruri* L. powder: *Anacardium occidentale* shell liquid smoke: *Syzygium aromaticum* leaf powder (PAOLSA) was according to the procedure of [16].

2.3. Treatment

*Phyllanthus niruri* L. powder (PNP) and *Anacardium occidentale* shell liquid smoke (AOLS) in 3 levels, namely High (H) = 1.25%, Medium (M) = 0.625%, and low (L) = 0.313%. While *Syzygium aromaticum* leaf powder (SAP) in 3 levels, namely High (H) = 0.625%, Medium (M) = 0.313%, and low (L) = 0.157%. As treatment, the combination of plant powder and liquid smoke is made with different concentrations that consisted of PNP: AOLS: SAP, as described below:

|       | PNP (%) | AOLS (%) | SAP (%) |
|-------|---------|----------|---------|
| Control | 0       | 0        | 0       |
| Zn-Bacitracin | 0       | 0        | 0       |
| T1     | 1.25    | 0.125    | 0.313   |
| T2     | 1.25    | 0.125    | 0.157   |
| T3     | 0.625   | 0.125    | 0.157   |
| T4     | 0.625   | 0.0625   | 0.157   |
| T5     | 0.313   | 0.0313   | 0.625   |
| T6     | 0.313   | 0.125    | 0.313   |

The method of feeding the broiler is to mix it into the ration. The basal diet consisted of crude protein 22% and metabolizable energy 3050 kcal with the composition of the basal ration consisted of: corn, meat bone meal, soy bean meal, rice bran, crude palm oil, dicalcium phosphate, limestone, sodium bicarbonate, DL-methionine, lysine, threonine, salt, vitamin mix, mineral mix, saligran, choline CL.

2.4. Chickens and management

One hundred and ten chickens aged 1-21 days are reared on a litter husk system, which is divided into 8 treatments, each treatment of which consisted of 2 replications, and each of both replications consisted of 5 chickens. Feed and drinking water are given on an ad-libitum basis. At the end of the study, 2 replications were taken and euthanasia, the contents of the ileum were taken to measure the total bacteria and *Escherichia coli*, so each of the total bacterial count and *Escherichia coli* consisted of 4 replications in each treatment and was carried out according to the APHA [1].

2.5. Statistical analysis

Statistical analysis was performed by analyzing the Complete Random Design variance pattern to be compared between all treatments. When analysis of variance (ANOVA) was conducted, there was a significant difference between treatments at P<0.05, then followed by Duncan's test.

3. Results and discussion

3.1. Number of bacteria

Number of bacteria in the ileum showed that the treatments of T1, T2, T3, T4, T5, and T6 showed no significant difference (P>0.05) with Zn-bacitracin treatments (Figure 2). Although statistically not significantly different, the number of bacteria treated with T1 (4.050 Log10 CFU/g and T2 (4.565 log10 CFU/g was lower than the Zn-bacitracin antibiotic treatment (4858 log10 (CFU/g)). This indicated that the mixture of PNP: AOLS: SAP was more effective in reducing the number of bacteria than the Zn-bacitracin antibiotic. The administration of 2% Moringa oleifera leaf powder could reduce microbial
populations in chicken intestines [3]. Information on the effect of a mixture of plant powder and liquid smoke on total bacteria in chicken ileum is still limited.

![Figure 2. Number of bacteria in broiler ileum that administer the mixture of *Phyllanthus niruri* L. powder, *Anacardium occidentale* liquid smoke, and *Syzygium aromaticum* leaf powder. There was no significant difference (P>0.05) among all treatments.](image)

### 3.2. Number of *Escherichia coli*

Number of *Escherichia coli* in the ileum showed no significant difference (P>0.05) between the mixture of *Phyllanthus niruri* L. powder, *Anacardium occidentale* liquid smoke, and *Syzygium aromaticum* leaf powder with Zn-bacitracin treatments (Figure 3). Although statistically not significantly different, but the number of *Escherichia coli* at T1 (3.157 log_{10} CFU/g) and T5 (3.780 log_{10} CFU/g) treatments showed that the number of *Escherichia coli* was lower than Zn-bacitracin treatment (4.429 log_{10} CFU/g). While the T3 (4.425 log_{10} CFU/g) and T4 (4.425 Log_{10} CFU/g) treatments equaled the Zn-bacitracin antibiotic treatment. Interestingly, the combination with different concentrations, such as T2 to T6, showed that the number of *Escherichia coli* actually increased. This indicates that the formulation of combinations of PNP and AOLS was high concentration, but SAP at the low concentration is less effective to inhibit the growth of *Escherichia coli* than T1 (PNP), and AOLS was high concentration and SAP was medium concentration). Noni powder is given up to 1% in hybrid ducks could reduce the population of *Escherichia coli* in the intestine when compared to non-noni powder treatment. But tetracycline antibiotics (300 mg/kg feed) were still more effective in reducing *Escherichia coli* compared to noni powder [8]. A mixture of garlic and *Phyllanthus niruri* L. with a ratio of 3:1 in encapsulation form and given to chickens up to 1.2% could not reduce the amount of *Escherichia coli* in chicken intestines [13]. It is not certain whether a combination of plants or forms of plant encapsulation causes low inhibition of *Escherichia coli*. In this study, the higher the concentration of PNP and AOLS and medium concentration of SAP, the lower the inhibitory growth of *Escherichia coli* [16]. The combination of *Anacardium occidentale*, *Phyllanthus niruri* L., *Syzygium aromaticum* in high dose also reduces the number of *Escherichia coli* [11]. Therefore, it is possible that the amount of mixture of...
PNP:AOLS:SAP in this study was efficient to inhibit the growth of *Escherichia coli* in the chicken ileum. *Cichorium intybus* L. powder given to chickens up to 0.20% could inhibit the growth of *Escherichia coli* pathogens in the intestine [7]. Information on providing plant leaf powder is still limited, so information support for this study is also limited.

**Figure 3.** Number of *Escherichia coli* in broiler ileum that administer the mixture of *Phyllanthus niruri* L. powder, *Anacardium occidentale* liquid smoke, and *Syzygium aromaticum* leaf powder. There was no significant difference (P>0.05) among all treatments.

### 4. Conclusion

The mixture of *Phyllanthus niruri* L. powder: *Anacardium occidentale* shell liquid smoke: *Syzygium aromaticum* leaf powder was not significantly different from Zn-bacitracin treatment on total bacteria and *Escherichia coli* population. But mixture of PNP:AOLS:SAP at concentration 1.25%:0.125%:0.313% was better than Zn-bacitracin to reduce the number of total bacteria and *Escherichia coli*. It can be concluded that a mixture of *Phyllanthus niruri* L. powder: *Anacardium occidentale* shell liquid: *Syzygium aromaticum* leaf powder at concentration 1.25%:0.125%:0.313% had potential as a natural antibiotic to replace Zn-bacitracin antibiotic.

### References

[1] APHA (American Public Health Association) 2015 *Compendium of Methods for the Microbiological Examination of Foods. 5th*, ed Y Yvonne Salfinger and M L Tortorello (Washington DC (USA): APHA Press) chapter 34 pp 411–421

[2] Bridgewater A V 2004. *Thermal Sci.* 8 21-50

[3] Divya A B, Mandal A, Biswas A S, Yadav and Biswas A K 2014 *Animal Nutrition and Feed Technology*. 14 349-357

[4] Etikaningrum dan Iwantoro S 2017 *J Ilmu Produksi dan Teknologi Hasil Peternakan*. 5 29-33

[5] Gadde U D, Oh S, Lillehoj H S and Lillehoj E P 2018 *Scientific Reports*. 8 3592

[6] Gunawan I G W, Bawa I G A dan Sutrisnayanti 2008 *J Kimia*. 2 31-39

[7] Khoobani M, Hasheminezhad, Javandel F, Nosrati M, Seidavi A, Kadim I T, Laudadio V and Tufarelli 2019 *Antibiotics*. 9 1-9

[8] Kurniawan D, Widodo E and Djunaidi I H 2016 *Buletin Peternakan*. 40 34-39

[9] Lippens M, Huyghebaert G and Cerchiari E 2005 *Arch. Geflügelk*. 69 261-266
[10] Mangunwardoyo W, Cahyaningsih E dan Usia T 2009 *Ilmu Kefarmasan Indonesia*. 7 57-63
[11] Mulyohardjo M 1991 *Jambu Mente dan Teknologi Pengolahannya (Anacardium occidentale L)*. (Yogyakarta: Liberty)
[12] Munfaati P N, Ratnasari E dan Trimulyono G 2015 *Lentera Bio*. 4 64-71
[13] Natsir M H, Hartutik, Sjofjan O and Widodo E 2013 *IJPS*. 12 676-680
[14] Noor S M dan Poeloengan M 2005 Prosiding Lokakarya Nasional Keamanan Pangan Produk Peternakan. Bogor, 14 September 56-64
[15] Pasaribu T, Astuti DA, Wina E, Sumiati and Setiyono A 2014 *IJPS*. 13 347-352
[16] Pasaribu T, Sinurat A P, Wina E, Purwadaria T, Haryati T and Susana I W R 2018 *JITV*. 23 112-122
[17] Pasaribu T, Sinurat A P and Wina E 2019 Prosiding Seminar Nasional Teknologi Peternakan dan Veteriner Jember 15-17 Oktober. 622-628
[18] Pavesi C, Lucy A, Banks LA and Hudaib 2018 *J Pharm Sci Res*. 10 337-339
[19] Pereira A P, Ferreira I C F R, Marcelino F, Valentao P, Andrade P B, Seabra R, Estevinho L, Bento A and Pereira J A 2007 *Molecules*. 12 1153-1162
[20] PerMenTan RI No.14/Permentan/PK.350/5/2017 tentang klasifikasi obat hewan [http://perundangan.pertanian.go.id/admin/file/Permentan%2014-2017%20Klasifikasi%20Obat%20Hewan.pdf](http://perundangan.pertanian.go.id/admin/file/Permentan%2014-2017%20Klasifikasi%20Obat%20Hewan.pdf)
[21] Sinurat A P, Wina E, Rakhmani SIW, Wardhani T, Haryati T, Purwadaria T 2018 *JITV*. 23 18-27
[22] Zeng Z, Zhang S, Wang H and Piao X 2015 *J Anim Sci and Biotech*. 6 1-10