



Clove (*Syzygium aromaticum*): Alternative Antibiotic Growth Promoter for Broiler: A Review

YUSRI SAPSUHA^{1*}, NUR SJAFANI¹, SURYATI TJOKRODININGRAT²

¹Department of Animal Science, Faculty of Agriculture, Universitas Khairun, Ternate, North Maluku, Indonesia;

²Department of Agrotechnology, Faculty of Agriculture, Universitas Khairun, Ternate, North Maluku, Indonesia.

Abstract | Antibiotics Growth Promoter (AGP) is officially banned as a supplement for broiler feed due to an increase in harmful bacteria and causing antibiotic residues in meat which has implications for stunted growth performance and increased health problems in broiler farms. Herbal products have been proposed for use in broiler production in order to maintain health and maximize the growth potential of modern broilers. The performance of broiler chickens is focused on maximizing performance parameters, i.e., fast chicken growth with low feed consumption, and with optimal health status. This study provides an in-depth review of the use of cloves in feed to improve broiler chicken production performance and health. In conclusion, cloves contain bioactive substances that can be used to improve broiler chicken performance and health. Administration of appropriate dose is believed to replace the role of AGP, which is currently prohibited from its use in broiler chicken feed.

Keywords | AGP, Broiler, Cloves, Health status, Performance

Received | January 22, 2023; **Accepted** | February 13, 2023; **Published** | February 23, 2023

***Correspondence** | Yusri Sapsuha, Department of Animal Science, Faculty of Agriculture, Universitas Khairun, Ternate, North Maluku, Indonesia; **Email:** yus_ara01@yahoo.co.id

Citation | Sapsuha Y, Sjafani N, Tjokrodiningrat S (2023). Clove (*Syzygium aromaticum*): Alternative antibiotic growth promoter for broiler: A review. *Adv. Anim. Vet. Sci.* 11(3):459-466.

DOI | <https://dx.doi.org/10.17582/journal.aavs/2023/11.3.459.466>

ISSN (Online) | 2307-8316



Copyright: 2023 by the authors. Licensee ResearchersLinks Ltd, England, UK.

This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (<https://creativecommons.org/licenses/by/4.0/>).

INTRODUCTION

Farmers commonly use antibiotic growth promoter (AGP) to improve feed utility (by chickens) as well as to maximize performance and health of broiler chickens. AGP has been shown to improve growth performance, control diseases caused by pathogenic bacteria, and improve feed conversion (Mehdi et al., 2018). According to recent research findings, the use of AGP can reduce the stress response in broiler chickens (Jeonga et al., 2020). However, the use of AGP in rations causes serious problems for consumers and has been prohibited. This is due to the fact that the use of AGP can result in residues in chicken products, which can be harmful to human health (Stefanello et al., 2020). Based on these circumstances, the poultry industry is expected to discontinue the use of AGP in broiler chickens and instead look for alternative methods

of increasing livestock growth and chicken health, one of which is the use of herbal plants.

The use of herbal plants as natural additives in poultry feed has increased in recent years due to their high content of bioactive substances that can improve production performance, reduce pathogenic bacterial infections, and reduce antibiotic residues in meat and egg products (Reddy et al., 2018). The use of herbal plants in poultry feed has been shown to increase body weight gain, improve nutrient metabolism, and improve meat quality by lowering cholesterol levels and inhibiting peroxidation (Oloruntola et al., 2019; Sapsuha et al., 2021). Among the herbs that are believed to be used as a substitute for AGP is Clove (*Syzygium aromaticum*).

Clove (*Syzygium aromaticum*) is an Indonesian native

plant that is widely used as a culinary spice. Clove is an aromatic tropical plant with a distinct aroma (Bhowmik et al., 2012) that has antioxidant, antimicrobial, analgesic, anti-obesity, and hepatoprotective properties in biological systems (Cortes-Rojas et al., 2014; Adu et al., 2020). The main bioactive substances found in cloves are eugenol, eugenol acetate, and β -caryophyllene (Jimoh et al., 2017). Eugenol is the primary active ingredient in the clove plant, accounting for up to 72-90% of its total content (Al-Shaikh and Perveen, 2017). It is a compound with pharmacological and antioxidant activity that helps to inhibit bacterial growth (Mohammadi et al., 2014). Cloves have traditionally been used to treat digestive disorders, parasitic infestations, and coughs (Bhowmik et al., 2012).

In general, the pharmacological benefits of cloves are associated with various bioactive components that can act as antimicrobial, antifungal, anti-inflammatory, hypoallergenic, and antioxidant (Jimoh et al., 2017; Al-Shaikh and Perveen, 2017; Asimi and Sahu, 2016). Overall, the phenolic compounds, flavonoids, and eugenol found in this clove plant allow it to function as an antibacterial, antifungal, and antioxidant agent, according to their findings. In terms of antibacterial capacity, Hemalatha et al. (2016) also found that the administration of cloves inhibited the growth of *Bacillus subtilis*, *Staphylococcus aureus*, *Klebsiella pneumonia*, and *Vibrio cholera* in their experiments. In addition, Sabdoningrum et al. (2017) showed that herbal plants were effective antibacterial agents against *Mycoplasma gallisepticum*, a pathogen that causes chronic respiratory disease in broilers. In another study, Uddin et al. (2017) assessed several bioactive compounds in clove plants. They discovered that cloves contain high levels of antioxidants such as phenols, flavonoids, saponins, alkaloids, phytosterols, and tannins. In line with this, Asimi et al. (2016) discovered the antioxidant content of clove plants. The clove plant also exhibits immune-boosting properties, which are important for host defense against pathogenic agents. Gandomani et al. (2014) concurred that the clove plant contains triterpenoids, saponins, and their derivatives, as well as mineral salts, all of which are important for increasing body resistance and enhancing the immune system. In addition, Rahman et al. (2017) revealed that the clove plant contains several active compounds that can function as immunomodulatory agents, i.e., deoxyandrographolide, andrographolide, neoandrographolide, 12-didehydroandrographolide, homoandrographolide, diterpenoids, and flavonoids. Many researchers have recently become interested in its diverse pharmacological properties, and many studies have been published with clove as the main subject. This current review focuses on the current state of knowledge regarding the effects of cloves and/or their main constituents on the performance and health of broiler chickens when used as a replacement for AGP.

To prepare for the current review, a literature search was conducted, with a focus on the use of cloves in broilers. Several criteria were used during the literature search, including the publication of peer-reviewed journals in both English and Indonesian, as well as seminar proceedings. During the literature search, the keywords broilers, cloves, and broiler performance and health were used. Scientific websites, including Elsevier, Science-Direct, Springer Link, E-Journal, and Google Scholar were used to select relevant papers.

BOTANICAL COMPOSITION AND PHYTOCHEMICAL CHARACTERISTICS

Syzygium aromaticum, also known as clove, is a medium-sized (8-20 m) tree from the Mirtaceae family native to the Maluku islands in eastern Indonesia. For centuries, the trade in cloves and the pursuit of this valuable spice fueled the Asian region's economic development. Clove trees are commonly grown along coastlines at a maximum elevation of 200 meters above sea level. After 4 years of planting, the production of flower buds, which is the commercial part of this tree, begins. Before flowering, flower buds are collected during the ripening phase. The top clove-producing countries are currently Indonesia, India, Malaysia, Sri Lanka, Madagascar, and Tanzania, particularly the island of Zanzibar (Kamatou et al., 2012). Cloves have been used as a cooking spice as well as a herbal medicine. In addition to the leaves, flowers, and fruit, clove oil is frequently used in aromatherapy and as a topical pain reliever.

This plant is rich in bioactive substances in the form of flavonoids, triterpenoids, phenolics, and tannins which are antibacterial compounds (Jimoh et al., 2017). Clove leaves are also known to contain compounds such as eucalyptol, caryophyllene, α -cardinol, and limonene (Mohammed et al., 2015). According to the results of Uddin et al. (2017), in addition to containing 3-Allyl-6-methoxyphenol-Eugenol compound, cloves also contain other compounds, including caryophyllene, 1, 4, 7-cycloundecatriene, 1, 5, 9, 9-tetramethyl, and eugenol acetate. These compounds are known to have benefits in the field of medicine, including antiseptic, anesthetic, analgesic, antioxidant, anti-inflammatory, and antimicrobial. The advantages of bioactive compounds found in clove plants are shown in Table 1.

In general, the pharmacological benefits of herbal plants are associated with various bioactive components that can function as antimicrobial, antifungal, anti-inflammatory, anti-allergic, and antioxidant agents. Clove essential oil produced from the distillation process contains compounds such as eugenol, tannin, caryophyllene, methyl salicylate, tannin, caryophyllene, methyl salicylate, and other compounds that have many health benefits because they have antiseptic, antimicrobial, and analgesic properties.

Table 1: Benefits of bioactive substances in clove plants (*Syzygium aromaticum*).

| Bioactive substances | Benefits | References |
|--|---|--|
| Phenolic compounds such as eugenol, eugenol acetate and acetic acid | Beneficial in the fields of pharmacy, food, and agriculture | (Cortes-Rojas <i>et al.</i> , 2014) |
| eugenol, eugenol acetate, and β -caryophyllene | Having the ability of pharmacological and antioxidant activities and playing a role in inhibiting bacterial growth | (Jimoh <i>et al.</i> , 2017; Mohammadi <i>et al.</i> , 2014) |
| 3-Allyl-6-methoxyphenol-eugenol, phenol, 2-methoxy-4-(2-propenyl), acetate, caryophyllene, 1, 4, 7,-cycloundecatriene, 1, 5, 9, 9-tetramethyl | Antibacterial agents against <i>Bacillus subtilis</i> , <i>Staphylococcus aureus</i> , <i>Klebsiella pneumonia</i> , <i>Vibrio cholera</i> . Having antioxidant activity | (Hemalatha <i>et al.</i> , 2016) |
| caryophyllene; 1, 4, 7,-cycloundecatriene; 1, 5, 9, 9-tetramethyl; and eugenol acetate | Beneficial in the field of medicine, including as an antiseptic, anesthetic, analgesic, antioxidant, anti-inflammatory, and antimicrobial agents. | (Uddin <i>et al.</i> , 2017) |
| flavonoids, triterpenoids, phenolics, and tannins | Antibacterial agents | (Jimoh <i>et al.</i> , 2017) |
| eucalyptol, caryophyllene, -cardinol, and limonene | Antibacterial agents | (Mohammed <i>et al.</i> , 2015) |
| 3-allyl-6-methoxyphenol-eugenol, caryophyllene 1, 4, 7,-cycloundecatriene, 1, 5, 9, 9-tetramethyl, phenol, 2-methoxy-4-(2-propenyl), and eugenol acetate | Inhibit the growth of <i>Streptococcus mutans</i> bacteria that causes dental caries | (Suhendar and Sogandi, 2019) |
| Essential oil and eugenol | Inhibit the growth of fungi, including: <i>Microsporum canis</i> , <i>Trichophyton mentagrophytes</i> , <i>Trichophyton rubrum</i> , <i>Epidermophyton floccosum</i> , and <i>Microsporum gypseum</i> | (Rana <i>et al.</i> , 2011; Devi <i>et al.</i> , 2010; Park <i>et al.</i> , 2007) |
| phenolic compounds such as flavonoids, hydroxybenzoic acid, hydroxynamic acid, and hydroxyphenyl propens | As an anti-infection agent or food preservative | (Fu <i>et al.</i> , 2007) |
| polyphenols and antioxidant compounds | Free radical scavengers | (Pérez-Jiménez <i>et al.</i> , 2010; Shan <i>et al.</i> , 2005) |
| Eugenol | Inhibit the growth of <i>Escherichia coli</i> (<i>E. coli</i>), <i>Staphylococcus aureus</i> , and <i>Bacillus cereus</i> bacteria | (Sofia <i>et al.</i> , 2007; Pérez-Conesa <i>et al.</i> , 2006; Burt <i>et al.</i> , 2003) |
| eugenol and carvacrol | As antifungal medicines | (Hill <i>et al.</i> , 2013) |
| Eugeniin | Antiviral agent | (Kurokawa <i>et al.</i> , 1998) |
| Flavonoids | Antibacterial agents | (Sjafani <i>et al.</i> , 2022; Neveu <i>et al.</i> , 2010) |
| Essential oil | Relieves toothache and joint pain | (Jirovetz and Buchbauer, 2006) |

The main bioactive compound in cloves is eugenol. Eugenol content in clove ranged from 9381-14650 mg/100 g. Clove flower buds can contain up to 18% essential oil. Approximately 89% of clove essential oil is eugenol, with the remaining 5% to 15% being eugenol acetate and β -cariofileno (Jirovetz and Buchbauer, 2006). It is also claimed that α -humulene is an important compound found in clove essential oil at concentrations of up to 2.1%. β -pinene, limonene, farnesol, benzaldehyde, 2-heptanone, and ethyl hexanoate are other volatile compounds found in low concentrations in clove essential oil.

EFFECT ON BROILER PERFORMANCE

As shown in Table 2, various studies have evaluated the

effect of giving cloves in the form of flour, clove oil or aqueous extract on performance parameters of broiler chickens. Cloves contain natural bioactive compounds that can stimulate appetite, aid in endogenous secretion such as enzymes, and have antimicrobial activity, all of which can help improve livestock performance and health. Cloves can take the place of AGP as a growth promoter. This is due to the high concentration of active ingredients, which include antioxidants, immunostimulants, and antimicrobials (Adu *et al.*, 2020; Wankhede, 2015; Kaur *et al.*, 2019). Several bioactive compounds from plants have previously been identified as potential candidates that can stimulate the growth of beneficial bacteria such as lactobacilli and bifidobacteria while inhibiting the growth of pathogenic

Table 2: Effect of Clove (*Syzygium aromaticum*) and its preparations on performance parameters of broiler chickens.

| Preparation | Optimum dose | Impact on broiler chickens | References |
|------------------------------|---|--|------------------------------------|
| Clove essential oil | 500 mg/kg feed | Eugenol in clove essential oil is very effective in increasing the growth performance of broilers. | (Mohammadi <i>et al.</i> , 2014) |
| Flour | 10 g/kg feed | Increase the growth rate without affecting the health of the liver and intestines of broilers | (Al-Mufarrej <i>et al.</i> , 2019) |
| Clove essential oil | 450 ppm/kg feed | Feed supplementation with 450 ppm clove essential oil increased feed intake (FI), weight gain (BBM), and improved feed conversion ratio (FCR) | (Azadegan <i>et al.</i> , 2014) |
| Clove bud flour | 0.5 g/kg feed | Improve the performance of broiler chickens | (Mahrous <i>et al.</i> , 2017) |
| Clove essential oil | 600 mg/kg feed | Chickens fed with clove oil 600mg/kg recorded more feed intake and body weight compared to the control group and antibiotics and the lowest mortality compared to the control group. | (Mukhtar, 2011) |
| Flour | 2% in feed | Reducing feed costs and reducing the use of antibiotics in broiler rearing | (Sapsuha <i>et al.</i> , 2019) |
| Flour, oil and water extract | 0.8% and 0.4% clove flour in feed, 0.8% clove oil in feed, and 0.4% clove water extract in drinking water | There was an increase in performance due to clove supplementation in the feed and drinking water of broiler chickens exposed to heat stress. | (Salman <i>et al.</i> , 2012) |

bacteria (Fasina *et al.*, 2015) and thus improve the performance of broiler chickens.

Al-Mufarrej *et al.* (2019) discovered that using 10 g of clove flour per kg of ration could increase growth rate without affecting the liver and intestine health of broilers. Another study found that broilers given 450 ppm clove essential oil had a significant increase in feed consumption and weight gain compared to the control group at 0-42 days of rearing (Azadegan *et al.*, 2014). Because of the volatile oil content in cloves, an increase in feed consumption can lead to an increase in weight gain. Plants in the essential oil group have been shown to improve the taste and delicacy of feed, which can lead to increased feed consumption and weight gain (Sugiharto, 2016). Cloves contain approximately 18% essential oil, which is primarily composed of 89% eugenol, 5-15% eugenol acetate and β -cariofileno, 2.1% α -humulene, and trace amounts of β -pinene, limonene, farnesol, benzaldehyde, 2-heptanone, and ethyl hexanoate (Jirovetz and Buchbauer, 2006; Ali *et al.*, 2014).

The mechanisms by which the clove can help improve performance broiler, because cloves contain natural bioactive compounds that can stimulate appetite, modulating the cellular membrane of microbes leading to membrane disruption of the pathogens, stimulating the growth of favourable bacteria such as lactobacilli and bifidobacteria of which can help improve performance broiler (Al-Mufarrej *et al.*, 2019; Sjafani *et al.*, 2022).

EFFECT ON DIGESTIBILITY

Clove supplementation in feed has been associated with increased digestibility of broiler chickens (Agostini *et*

al., 2012), which in turn increases nutrient availability and substrate availability for energy metabolism. Cloves have also been reported to improve the digestive tract (Chowdhury *et al.*, 2018) and improve gut conditions, e.g., pH, digesta, and villi viscosity (Rahman *et al.*, 2017; Ali *et al.*, 2014). These conditions contribute to improved feed digestibility. In addition, the efficacy of clove preparations in restoring intestinal damage and digestive enzyme activity caused by stress and infection (Ghazanfari *et al.*, 2014) may play a role in maintaining optimal digestive function of broiler intestines. Improved histomorphological (i.e., villous height, crypt depth, and villi height to crypt depth ratio) and ecological (e.g., increased lactic acid bacteria and decreased numbers of *Salmonella* sp. and *Escherichia coli*) gut health may also be linked to improved feed nutrient utilization in boiler chickens (Mohammadi *et al.*, 2014; Gandomani *et al.*, 2014; Mahrous *et al.*, 2017; Chowdhury, 2018). Mohammadi *et al.* (2014) also observed a reduction in mortality in broiler chickens fed cloves in their feed. The above-mentioned bioactive compound content can play an important role in improving feed digestibility and improving broiler performance and health.

The mechanism related to clove can to improve the digestive tract in broiler chickens, i.e. the efficacy of clove preparations in restoring intestinal damage and digestive enzyme activity (Rahman *et al.*, 2017) may play a role in maintaining optimal digestive function of broiler intestines and protect intestinal tissue from microbial attack (Suhendar and Sogandi, 2019; Ghazanfari *et al.*, 2014). Eventually, optimal intestinal development can support the development digestive tract in broiler.

EFFECTS ON BROILER HEALTH

Normal physiological conditions are required to ensure that broiler growth capacity is maximized. Herbal plant supplementation has been shown to maintain and improve broiler physiological condition. Previous studies found that cloves were beneficial to broiler health by inhibiting pathogenic bacteria, improving gut health, increasing antioxidant status, improving digestive function, and increasing chicken immunity (Al-Mufarrej et al., 2019; Mahrous et al., 2017). Su et al. (2021) investigated the effect of administering essential oil containing the active ingredients thymol 3.05%, carvacrol 2.3%, and cinnamaldehyde 0.26%, which demonstrated an increase in essential oil levels from 0 to 400 mg/kg and significantly increased superoxide dismutase (SOD) activity in broiler serum at 21 days old. The use of clove leaf flour as a phytobiotic can reduce feed costs and the use of antibiotics in broiler chicken production (Sapsuha et al., 2019).

Several bioactive components found in cloves have been shown to have immunomodulatory and anti-inflammatory properties. Bhowmik et al. (2012) discovered several bioactive compounds in clove leaf flour. They discovered that clove leaf flour contains a high concentration of bioactive compounds such as eugenol, flavonoids, and triterpenoids, all of which have antioxidant activity. In line with this, Adu et al. (2020) discovered that the antioxidant content in clove leaves has immune enhancing properties, which are important for host defense against pathogenic agents. Furthermore, Mohammed et al. (2015) discovered that clove leaves contain several active compounds that can act as immunomodulatory agents, including eucalyptol, karyophyllene, α -cardinol, and limonene, as well as diterpenoids and flavonoids. The presence of bioactive substances such as alkaloids, terpenoids, simple phenolic compounds, polysaccharides, peptides, glycoproteins, and nucleotides in most herbal plants, according to Chabib et al. (2018), may be responsible for the immune-stimulating effects of herbal plants.

Cloves have been shown to improve the health and function of the chicken digestive tract by having a positive impact on the microbial ecosystem in the digestive tract (Chowdhury et al., 2018). According to one recent study, using clove leaves improved gut health and function in broiler chickens. According to Ren et al. (2019), the administration of commercial phytogenics containing the active ingredients carvacrol, cinnamaldehyde, and eugenol may provide more energy for the growth of beneficial bacteria in the gut, such as bifidobacteria and *Lactobacillus* spp. Another study found that administering clove essential oil could reduce the number of pathogenic bacteria in the intestines of broiler chickens (Mohammadi et al., 2014). Meanwhile, Salman and Ibrahim (2012) reported that administration of clove flour, oil, and aqueous extract increased the

concentration of short chain fatty acids (SCFA) in the cecum, promoted better gut development, increased the number of beneficial bacteria, and inhibited pathogenic bacteria in the gut of broiler chickens. Herbal plants have a greater impact because the bioactive substances contained in them can promote the growth of probiotic bacteria while also inhibiting pathogenic bacteria (Prakasita et al., 2019). Furthermore, the carbohydrate content, particularly oligosaccharides from herbal plants, can be a good substrate for the growth of probiotic microbes for improved gut health (Sjafani et al., 2022).

The mechanism related to clove can increase the health in broiler chickens, i.e. can increase the immune response in broiler chickens and can act as immunostimulant substances, and protect intestinal tissue from microbial attack (Ali et al 2014; Adu et al 2020; Sjafani et al., 2022). Eventually, optimal intestinal development can support the development of the immune system in the intestine. The increase in antioxidant status in broiler chickens due to the provision of the clove was due to bioactive substances in clove may act as an antioxidant which synergistically triggers antioxidant activity in the body of broilers (Gandomani et al., 2014; Mahrous et al., 2017).

CONCLUSIONS AND RECOMMENDATIONS

Cloves contain bioactive substances that can be used to improve the performance and health of broiler chickens. The administration of an appropriate dose is thought to replace the role of AGP, which is currently prohibited from use in broiler chicken feed.

ACKNOWLEDGEMENT

Universitas Khairun Research and Community Service Institute (LPPM) provided funding assistance through the Graduate Competitive Research Program (PKUPT).

NOVELTY STATEMENT

Cloves (*Syzygium aromaticum*) contain bioactive substances that can be used to improve broiler chicken performance and health. Administration of appropriate dose is believed to replace the role of Antibiotics Growth Promoter (AGP), which is currently prohibited from its use in broiler chicken feed.

AUTHOR'S CONTRIBUTION

The manuscript was written by Yusri Sapsuha and Nur Sjafani, and edited by Suryati Djokrodiningrat. All authors read and approved the final manuscript.

The authors have declared no conflict of interest.

REFERENCES

- Adu OA, Gbore FA, Oloruntola OD, Falowo AB, Olatimisi OJ (2020). The effects of *Myristica fragrans* seed meal and *Syzygium aromaticum* leaf meal dietary supplementation on growth performance and oxidative status of broiler chicken. *Bull. Natl. Res. Centre* 44: 149. <https://doi.org/10.1186/s42269-020-00396-8>
- Agostini PS, Sola-Oriol D, Nofrarias M, Barroeta, AC, Gasa J, Manzanilla EG (2012). Role of in-feed clove supplementation on growth performance, intestinal microbiology, and morphology in broiler chicken. *Livest. Sci.*, 147: 113-118. <https://doi.org/10.1016/j.livsci.2012.04.010>
- Ali S, Prasad R, Mahmood A, Routray I, Shinkafi TS, Sahin K, Kucuk O (2014). Eugenol-rich fraction of *Syzygium aromaticum* (clove) reverses biochemical and histopathological changes in liver cirrhosis and inhibits hepatic cell proliferation. *J. Cancer Prev.*, 19: 288-300. <https://doi.org/10.15430/JCP.2014.19.4.288>
- Al-Mufarrej SI, Al-Baadani HH, Fazea EH (2019). Effect of level of inclusion of clove (*Syzygium aromaticum*) powder in the diet on growth and histological changes in the intestines and livers of broiler chickens. *S. Afr. J. Anim. Sci.*, 49(1): 166-175. <https://doi.org/10.4314/sajas.v49i1.19>
- Al-Shaikh N, Perveen K (2017). Anti-candidal activity and chemical composition of essential oil of clove (*Syzygium aromaticum*). *J. Essent. Oil Bearing Plants*, 20: 951-958. <https://doi.org/10.1080/0972060X.2017.1375867>
- Asimi OA, Sahu NP (2016). Effect of antioxidant-rich spices, clove and cardamom extracts on the metabolic enzyme activity of *Labeo rohita*. *J. Fish. Livest. Prod.*, 4: 1-6.
- Azadegan MM, Hassanabadi A, Nasiri MH, Kermanshahi H (2014). Supplementation of clove essential oils and probiotic to the broiler's diet on performance, carcass traits and blood components. *Iran. J. Appl. Anim. Sci.*, 4(1): 117-122.
- Bhowmik D, Kumar KPS, Yadav A, Srivastava S, Paswan S, Dutta SS (2012). Recent trends in Indian traditional herb *Syzygium aromaticum* and its health benefits. *J. Pharmacogn. Phytochem.*, 1(1): 1-23.
- Burt SA, Reinders RD (2003). Antibacterial activity of selected plant essential oils against *Escherichia coli* O157:H7. *Lett. Appl. Microbiol.*, 36(3): 162-167. <https://doi.org/10.1046/j.1472-765X.2003.01285.x>
- Chabib L, Muhtadi WK, Rizki MI, Rahman RA, Suhendri MR, Hidayat A (2018). Potential medicinal plants for improve the immune system from Borneo Island and the prospect to be developed as nanomedicine. *MATEC Web. Conf.*, 154: 04006. <https://doi.org/10.1051/mateconf/201815404006>
- Chowdhury S, Mandal GP, Patra AK (2018). Different essential oils in diets of chickens: Growth performance, nutrient utilisation, nitrogen excretion, carcass traits and chemical composition of meat. *Anim. Feed Sci. Technol.*, 236: 86-97. <https://doi.org/10.1016/j.anifeedsci.2017.12.002>
- Cortes-Rojas DF, de Souza CRF, Oliveira WP (2014). Clove (*Syzygium aromaticum*): A precious spice. *Asian Pac. J. Trop. Biomed.*, 4(2): 90-96. [https://doi.org/10.1016/S2221-1691\(14\)60215-X](https://doi.org/10.1016/S2221-1691(14)60215-X)
- Devi KP, Nisha SA, Sakthivel R, Pandian SK (2010). Eugenol (an essential oil of clove) acts as an antibacterial agent against *Salmonella typhi* by disrupting the cellular membrane. *J. Ethnopharmacol.*, 130(1): 107-115. <https://doi.org/10.1016/j.jep.2010.04.025>
- Fasina YO, Newman MM, Stough JM, Liles MR (2015). Effect of *Clostridium perfringens* infection and antibiotic administration on microbiota in the small intestine of broiler chickens. *Poult. Sci.*, 95: 247-260. <https://doi.org/10.3382/ps/pev329>
- Fu Y, Zu Y, Chen L, Shi X, Wang Z, Sun S (2007). Antimicrobial activity of clove and rosemary essential oils alone and in combination. *Phytother. Res.*, 21(10): 989-994. <https://doi.org/10.1002/ptr.2179>
- Gandomani VT, Mahdavi AH, Rahmani HR, Riasi A, Jahanian E (2014). Effects of different levels of clove bud (*Syzygium aromaticum*) on performance, intestinal microbial colonization, jejunal morphology, and immunocompetence of laying hens fed different n-6 to n-3 ratios. *Livest. Sci.*, 167: 236-248. <https://doi.org/10.1016/j.livsci.2014.05.006>
- Ghazanfari S, Mohammadi Z, Adibmoradi M (2014). Effects of clove essential oil on growth performance, carcass characteristics and immune system in broiler chicken. *Vet. J.*, 19: 212-217.
- Hemalatha R, Nivetha P, Mohanapriya C, Sharmila, Muthukumar C (2016). Phytochemical composition, GC-MS analysis, in vitro antioxidant and antibacterial potential of clove flower bud (*Eugenia caryophyllus*) methanolic extract. *J. Food Sci. Technol.*, 53(2): 1189-1198. <https://doi.org/10.1007/s13197-015-2108-5>
- Hill LE, Gomes C, Taylor TM (2013). Characterization of beta-cyclodextrin inclusion complexes containing essential oils (trans-cinnamaldehyde, eugenol, cinnamon bark, and clove bud extracts) for antimicrobial delivery applications. *LWT Food Sci. Technol.*, 51(1): 86-93. <https://doi.org/10.1016/j.lwt.2012.11.011>
- Jeong SB, Kim YB, Jeong WL, Kim DH, Moon BH, Chang HH, Choi YH, Lee KW (2020). Role of dietary gamma-aminobutyric acid in broiler chickens raised under high stocking density. *Anim. Nutr.*, 6: 293-304. <https://doi.org/10.1016/j.aninu.2020.03.008>
- Jimoh SO, Arowolo LA, Alabi KA (2017). Phytochemical screening and antimicrobial evaluation of *Syzygium aromaticum* extract and essential oil. *Int. J. Curr. Microbiol. Appl. Sci.*, 6: 4557-4567. <https://doi.org/10.20546/ijemas.2017.607.476>
- Jirovetz L, Buchbauer G (2006). Chemical composition and antioxidant properties of clove leaf essential oils. *J. Agric. Food Chem.*, 54: 6303-6307. <https://doi.org/10.1021/jf060608c>
- Kamatou GP, Vermaak I, Viljoen AM (2012). Eugenol from the remote Maluku Islands to the international market place: A review of a remarkable and versatile molecule. *Molecules*, 17(6): 6953-6981. <https://doi.org/10.3390/molecules17066953>
- Kaur K, Kaushal S, Rani R (2019). Chemical composition, antioxidant and antifungal potential of clove (*Syzygium aromaticum*) essential oil, its major compound and its derivatives. *J. Essent. Oil Bearing Plants*, 22: 1195-1217. <https://doi.org/10.1080/0972060X.2019.1688689>
- Kurokawa M, Hozumi T, Basnet P, Nakano M, Kadota S, Namba T, Kawana T, Shiraki K (1998). Purification and characterization of eugenin as an anti-herpesvirus compound from *Geum japonicum* and *Syzygium aromaticum*. *J. Pharmacol. Exp. Ther.*, 284(2): 728-735.

- Mahrous HB, El-far AH, Sadek KM, Abdel-Latif MA (2017). Effects of different levels of clove bud (*Syzygium aromaticum*) dietary supplementation on immunity, antioxidant status, and performance in broiler chickens. *Alex. J. Vet. Sci.*, 54(2): 29–39. <https://doi.org/10.5455/ajvs.272231>
- Mehdi Y, Létourneau-Montminy MP, Gaucher ML, Chorfi Y, Suresh G, Rouissi T, Brar SK, Côté C, Ramirez AA (2018). Use of antibiotics in broiler production: Global impacts and alternatives. *Anim. Nutr.*, 4: 170-178. <https://doi.org/10.1016/j.aninu.2018.03.002>
- Mohammadi Z, Ghazanfari S, Moradi MA (2014). Effect of supplementing clove essential oil to the diet on microflora population, intestinal morphology, blood parameters and performance of broilers. *Eur. Poult. Sci.*, 78: 1-11. <https://doi.org/10.1399/eps.2014.51>
- Mohammed NH, Ahmed MH, Hussien MO (2015). Qualitative analysis of the essential oil of *Syzygium aromaticum* (L.) (clove) using gas chromatography-mass spectrometry (GC-MS). *Int. J. Res. Pharm. Chem.*, 5(2): 350-354.
- Mukhtar MA (2011). The effect of dietary clove oil on broiler performance. *Aust. J. Basic Appl. Sci.*, 5: 49–51.
- Neveu V, Perez-Jiménez J, Vos F, Crespy V, du Chaffaut L, Mennen L, Knox C, Eisner R, Cruz J, Wishart D, Scalbert A (2010). Phenol-Explorer: An online comprehensive database on polyphenol contents in foods. Database. <https://doi.org/10.1093/database/bap024>
- Oloruntola OD, Ayodele SO, Adeyeye SA, Jimoh AO, Oloruntola DA, Omoniyi SI (2019). Pawpaw leaf and seed meals composite mix dietary supplementation: Effects on broiler chicken's performance, caecum microflora, and blood analysis. *Agrofor. Syst.*, 94: 555–564. <https://doi.org/10.1007/s10457-019-00424-1>
- Park MJ, Gwak KS, Yang I, Choi WS, Jo HJ, Chang JW (2007). Antifungal activities of the essential oils in *Syzygium aromaticum* (L.) Merr. Et Perry and *Leptospermum petersonii* Bailey and their constituents against various dermatophytes. *J. Microbiol.*, 45(5): 460–465.
- Pérez-Conesa D, McLandsborough L, Weiss J (2006). Inhibition and inactivation of *Listeria monocytogenes* and *Escherichia coli* O157:H7 colony biofilms by micellar-encapsulated eugenol and carvacrol. *J. Food Prot.*, 69(12): 2947–2954. <https://doi.org/10.4315/0362-028X-69.12.2947>
- Pérez-Jiménez J, Neveu V, Vos F, Scalbert A (2010). Identification of the 100 richest dietary sources of polyphenols: an application of the phenol-explorer database. *Eur. J. Clin. Nutr.*, 64(3): S112-S120. <https://doi.org/10.1038/ejcn.2010.221>
- Prakasita VC, Asmara W, Widyarani S, Wahyuni AETH (2019). Combination of herbs and probiotics as an alternative growth promoter: An *in vitro* study. *Vet. World*, 12(4): 614–620. <https://doi.org/10.14202/vetworld.2019.614-620>
- Rahman AM, Mahdavi AH, Rahmani HR, Jahanian E (2017). Clove bud (*Syzygium aromaticum*) improved blood and hepatic antioxidant indices in laying hens receiving low n-6 to n-3 ratios. *J. Anim. Physiol. Anim. Nutr.*, 101: 881–892. <https://doi.org/10.1111/jpn.12502>
- Rana IS, Rana AS, Rajak RC (2011). Evaluation of antifungal activity in essential oil of the *Syzygium aromaticum* (L.) by extraction, purification and analysis of its main component eugenol. *Braz. J. Microbiol.*, 42(4): 1269–1277. <https://doi.org/10.1590/S1517-83822011000400004>
- Reddy DM, Reddy, GV, Mandal PK (2018). Application of natural antioxidants in meat and meat products. A review. *Food Nutr. J.*, pp. FDNJ-173. <https://doi.org/10.29011/2575-7091.100073>
- Ren H, Vahjen W, Dadi T, Saliu EM, Boroojeni FG, Zentek J (2019). Synergistic effects of probiotics and phytobiotics on the intestinal microbiota in young broiler chicken. *Microorganisms*, 7(2): 684. <https://doi.org/10.3390/microorganisms7120684>
- Sabdoningrum EK, Hidanah S, Wahjuni RS, Chusniati S, Arimbi (2017). An *in vitro* antibacterial activity test of *Meniran Herbs'* (*Phyllanthus niruri* L.) ethanol extract against *Mycoplasma gallisepticum* causes chronic respiratory disease (CRD) in broiler chickens. In: *The Veterinary Medicine International Conference*. KnE Life Sciences. pp. 48-61. <https://doi.org/10.18502/kl.v3i6.1106>
- Salman KAA, Ibrahim DK (2012). Test the activity of supplementation clove (*Eugenia caryophyllus*) powder, oil and aqueous extract to diet and drinking water on performance of broiler chickens exposed to heat stress. *Int. J. Poult. Sci.*, 11: 635–640. <https://doi.org/10.3923/ijps.2012.635.640>
- Sapsuha Y, Sjafani N, Syafie Y, Ishak H (2019). Campus intellectual product business development program: Utilization of herbs as phytobiotic in broiler chicken. *Workshop on Engineering, Education, Applied Sciences, and Technology*. IOP Publishing, pp. 1364. <https://doi.org/10.1088/1742-6596/1364/1/012078>
- Sapsuha Y, Suprijatna E, Kismiati S, Sugiharto S (2021). The effect of nutmeg flesh (*Myristica fragrans* Houtt) extract on growth performance, internal organ and carcass of broiler chickens raised at high stocking density. *Livest. Res. Rural Dev.*, 33(6).
- Shan B, Cai YZ, Sun M, Corke H (2005). Antioxidant capacity of 26 spice extracts and characterization of their phenolic constituents. *J. Agric. Food Chem.*, 53(20): 7749–7759. <https://doi.org/10.1021/jf051513y>
- Sjafani N, Hasan S, Sapsuha Y (2022). The influence of clove leaf extract (*Syzygium aromaticum*) on growth performance and bacterial population of broiler chickens raised in stressful conditions of high stocking density. *J. Anim. Behav. Biometeorol.*, 10(2). <https://doi.org/10.31893/jabb.22015>
- Sofia PK, Prasad R, Vijay VK, Srivastava AK (2007). Evaluation of antibacterial activity of Indian spices against common foodborne pathogens. *Int. J. Food Sci. Technol.*, 42(8): 910–915. <https://doi.org/10.1111/j.1365-2621.2006.01308.x>
- Stefanello C, Rosa DP, Dalmoro YK, Segatto AL, Vieira MS, Moraes ML, Santin E (2020). Protected blend of organic acids and essential oils improves growth performance, nutrient digestibility, and intestinal health of broiler chickens undergoing an intestinal challenge. *Front. Vet. Sci.*, 6: 491. <https://doi.org/10.3389/fvets.2019.00491>
- Su G, Wang L, Zhou X, Wu X, Chen D, Yu B, Huang Z, Luo Y, Mao X, Zheng P, Yu J, Luo J, He J (2021). Effects of essential oil on growth performance, digestibility, immunity, and intestinal health in broilers. *Poult. Sci.*, 100(8): 101242. <https://doi.org/10.1016/j.psj.2021.101242>
- Sugiharto S (2016). Role of nutraceuticals in gut health and growth performance of poultry. *J. Saudi Soc. Agric. Sci.*, 15: 99–111. <https://doi.org/10.1016/j.jssas.2014.06.001>
- Suhendar U, Sogandi (2019). Identification of bioactive compounds in clove leaves (*Syzygium aromaticum*) extract as inhibitor *Streptococcus mutans*. *J. Biol.*, 12(2): 229–239. <https://doi.org/10.15408/kauniyah.v12i2.12251>
- Uddin A, Shahinuzzaman, Rana S, Yaakob Z (2017). Study of chemical composition and medical properties of volatile oil

from clove buds (*Eugenia caryophyllus*). Int. J. Pharm. Sci. Res., 8(2): 895-899.

Wankhede T (2015). Evaluation of antioxidant and antimicrobial activity of the Indian clove *Syzygium aromaticum* L. Merr. Perr. Int. Res. J. Sci. Eng., 3(4): 166-172.