The Inclusion of Carrot (*Daucus carota*) Leaves Water Extract in Broiler Drinking Water to Increase Growth and Suppress Pathogenic Bacteria

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Abstract

Phytochemical compounds in herbal leaves are recognized for antibacterial and growth-promoting properties. Therefore, this study aimed to evaluate the impact of including carrot leaves water extract (CLWE) in drinking water on performance, blood lipid profile, and pathogens in broiler intestine. The experiment used 240 day-old-chicks (DOC) broiler which were randomly divided into 4 treatment groups and 6 replications. Each replication had 10 DOC broiler with homogeneous body weights. There were three treatment groups, namely broiler given drinking water containing CLWE of 2% (B), 4% (C), and 6% (D), as well as the control group given drinking water only. CLWE was prepared by squeezing 1 kg of carrot leaves in 1 L of clean water (g/g) at a temperature of 29-30^{0}C, then leaving at room temperature of 20.5±1.5^{0}C and humidity of 60±10% for 24 hours. The results showed that the inclusion of 4% and 6% CLWE in drinking water significantly ($P<0.05$) increased body weight gain and feed efficiency, but significantly ($P<0.05$) reduced LDH, total cholesterol, and abdominal fat in broiler. In addition, the treatment significantly ($P<0.05$) reduced the number of Coliform and E.coli bacteria in the intestine. Based on the results, it was concluded that the inclusion of 4-6% CLWE through broiler drinking water could increase feed efficiency, and reduce blood serum cholesterol, abdominal fat as well as pathogenic bacteria in broiler intestine.

Keywords: *Abdominal fat, carcass, pathogenic bacteria, carrot leaves water extract*

1. Introduction

Broiler at the early stage of life (pre-starter phase) is known to require intensive and careful care, primarily due to high sensitivity to changes in environmental temperature and ease of being infected with disease. One common disease easily infecting broiler is *Colibacillosis* which is caused by pathogenic bacteria *Escherichia coli*.

The use of antibiotics in feed is currently prohibited, due to the tendency to remain in poultry products and have resistance effects on certain bacteria (Babaei et al., 2016; Purbarani et al., 2019). An alternative to antibiotics is the use of phytochemical compounds from herbal leaves extracts. Natural products from plants can stimulate growth and have the potential to be effective antibacterial agents, due to low toxicity to humans and animals (Farha et al., 2020). Rajput et al. (2013) mentioned that supplementation of 0.02% herbs improved growth performance and fat metabolism, as well as increased the absorption area of the small intestinal villi. These changes raised nutrient absorption which had an impact on increasing growth and feed efficiency.

Carrot leaves waste (*Daucus carota*) has great potential as a feed supplement for broiler, with a 50:50 compared to carrot tubers (Bidura et al., 2023). Previous studies have investigated the efficacy of carrot leaves water extract (CLWE) as growth promoter and suppressing pathogenic bacteria. This ability is due to the phytochemical content, such as flavonoids, tannins, saponins, β-carotene (BC), and cryptoxanthin (Çetingül et al., 2020; Hammershoj et al., 2010). According to Goel (2013) and Yuniza & Yuherman (2015), phenolic compounds, such as alkaloids, saponins, flavonoids, and triterpenoids can degrade the cell membranes of pathogenic bacteria in the intestines of poultry (Goel, 2013; Yuniza and Yuherman, 2015), increasing the health status. Phytogenic additives in feed and drinking water have specific effects, namely inhibiting the development of pathogenic microorganisms, regulating the composition and abundance of the gastrointestinal microbiome, promoting regeneration of the intestinal epithelium and villi, as well as exerting antioxidants and immunostimulation (Khasnavis and Pahan, 2012).

Phytogenic feed additives are generally used to improve overall poultry health, increase digestibility, help detoxification in the body, improve blood lipid profile, and reduce cholesterol content in the yolk (Restiayanti et al., 2014; Bidura et al., 2017; Bidura et al., 2020; Sudatri, 2021). Other functions include stimulating growth and controlling pathogenic bacteria in broiler chickens (Bukar et al., 2010; Mattioli et al., 2019). Cholesterol absorption is highly regulated and influenced by certain compounds in the food supply. The feed components that have been identified as inhibiting cholesterol absorption are phytochemical compounds in herbal leaves, including phytosterols, soluble fiber, and phospholipids (Jesch and Carr, 2017). The main mechanism by which saponins in carrot leaves reduce cholesterol in the body is through the transfer of molecules from bile salt micelles, thereby causing the formation of cholesterol deposits unable to pass through the intestinal mucus layer (Vinarova et al., 2015).

Flavonoid compounds obtained from herbal leaves extraction can act as antibacterials by denaturing bacteria cell membrane lipids through hydrogen bonds. Consequently, bacteria cell membrane is damaged and the formation of new cell membranes is not formed, leading to inhibition of growth (Dani et al., 2012). Tannin compounds also act as antibacterial, due to the phenol group which has similar properties to alcohol, namely antiseptic and antimicrobial (Rishika and Sharma, 2012). According to Hadyarrahman et al. (2017), CLWE obtained through cold maceration with a concentration of 2% had quite strong inhibitory power against *Staphylococcus aureus* and *E.coli* bacteria.

Supplementation of 0.02% herbs in feed can improve growth performance and fat metabolism, as well as increase the absorption area of small intestinal villi, thereby increasing nutrient absorption which has an impact on increasing growth and feed efficiency (Rajput et al. (2013). Tannins inhibit growth of pathogenic bacteria through different mechanisms of action including iron chelation, inhibition of cell wall synthesis, cell membrane disruption, and inhibition of the fatty acid biosynthesis pathway (Farha et al., 2020). Therefore, this study aimed to examine the impact of adding CLWE through drinking water from 0-5 weeks of age on growth, feed efficiency (feed consumption: weight gain), carcass, abdominal fat, blood lipid profile, and the population of pathogenic bacteria in the intestine of broiler.

2. Material and Methods

Ethical approval

A total of 240 DOC broiler strains CP 707 used in this study were approved by the Animal Ethics Commission from the Faculty of Veterinary Medicine, Udayana University, Denpasar-Bali, Indonesia.

Experimental design, Animal treatments and diets

This study was carried out at the Research Station, Faculty of Animal Husbandry, Udayana University, Jl. Raya Sesetan, Denpasar, Indonesia. The samples included 240-day-old chickens (DOC) broiler strain CP 707 with homogeneous body weights. The samples were randomly divided into 4 treatment groups and 6 replications. Each replication used 10 DOC male broiler with homogeneous body weights. All animals were kept in battery colony cages with wire floors, hence, droppings fell directly onto the bottom of the cage. All cage plots were placed in one building with a controlled temperature. Moreover, lighting was provided throughout the day, ensuring broiler was free to feed and drink water. Three treatment groups were used namely broiler given drinking water containing CLWE of 2% (B), 4% (C), and 6% (D) as well as control group was given drinking water (A) only. The ration given was a commercial CP 511 produced by PT. Charoen Pokphand, Indonesia. All feed was in crumb form and given *ad libitum* during the study period (0-5 weeks of age). Feeding was given ¾ of the feeder capacity to avoid spills. Additionally, drinking water was given *ad libitum* and replaced every day to keep clean and fit to drink.

Procedure for making CLWE

Carrot leaves used were obtained post-harvest in plantations at Baturiti District, Tabanan Regency, Bali Province, Indonesia. Before use, leaves were first cleaned of dirt, then crushed and soaked in clean water. CLWE was prepared by squeezing 1 kg of carrot leaves in 1L of clean water at a temperature of 29-30^{0}C and a pH ranging from 6.6-6.9, then leaving at room temperature of 20.5±1.5^{0}C and humidity of 60±10%, for 24 hours. Subsequently, filtration was carried out through a double layer of satin cloth followed by storage in the refrigerator. CLWE can only last for two days, hence, it must be administered through drinking water in fresh condition. Based on the results of spectrometry tests at room conditions, CLWE was positive for containing active ingredients such as flavonoids, phenols, tannins, steroids, and glycosides. The flavonoid, tannin, and antioxidant soak content was 40.29mg/100g, 71.98mg/100g, and 48.75% respectively.

Variable measurement

Broiler performance includes final and body weight gain, feed consumption, and conversion ratio (comparison between feed consumption and body weight gain) which were measured every week. At the end of the reserch, 5 ml of blood was taken from the branchial vein with a 3 ml syringe to test blood lipid levels (Liberman and Burchad, 1980). Samples for testing *Coliform* and *Escherichia coli* bacteria were taken from intestinal digestion, following the procedure of Sudatri (1921).

Analysis statistics

The PROC MIXED procedure of SAS 9.4 (SAS Institute Inc., Cary, NC, USA) was used to analyze data. Differences between treatments were compared using Duncan's method and significance was declared at P≤0.05.

3. Results and Discussion

Effect of Clwe on broiler performance

The addition of 2-6% CLWE to broiler drinking water from 0-5 weeks of age had no effect (P>0.05) on feed and drinking water consumption. Meanwhile, the addition of 4% and 6% significantly (P<005) increased the final body weight by 9.96% and 12.19% compared to the control. The weight gain of broiler in groups C and D, namely 10.21% and 12.47% was significantly (P<0.05) higher than the control as presented in Table 1.

The inclusion of 4% and 6% CLWE in broiler drinking water from 0-5 weeks of age, significantly (P<0.05) increased feed efficiency, by 7.10% and 7.69% compared to the control. However, the addition of 2% CLWE had no significant impact.

Although the inclusion of CLWE in drinking water did not affect feed and drinking water consumption, Hammershøj et al. (2010) reported that supplementation of herbal leaves in feed reduced feed consumption. Herbal leaves can function as prebiotics in the digestive tract of poultry, due to the oligosaccharides content that cannot be digested by the host animal. These compounds have a beneficial effect on the host by stimulating growth of beneficial microflora in the digestive tract (Abdurrahman and Yanti, 2018). Similar results were reported by Bidura et al. (2017) stating that the inclusion of 5% water extract of Garlic and *Suropus androgynus* leaves in drinking water of laying hens did not affect feed consumption and efficiency. On the other hand, the treatment reduced the cholesterol content in the yolk. This difference was due to the type of herbal leaves used, the type of broiler, and the time of extract administration.

Table 1. Effect of adding CLWE to broiler drinking water from 0-5 weeks of age on performance.

Variable	CLWE level in drinking water (cc/100 cc)				SE
	0	2	4	6	
Final body weight, g head^{-1}	46.98$^{a3)}$	47.02^{a}	46.79^{a}	47.15^{a}	0.308
Feed consumtion, g head^{-1} 5 weeks^{-1}	3309.68^{a}	3308.89^{a}	3388.63^{a}	3436.17^{a}	70.082
Drinking water, g head^{-1} 5 weeks^{-1}	9870.35 a	9832.91^{a}	9972.57^{a}	9895.27^{a}	148.52
Final body weight, g head^{-1}	2005.37^{a}	2028.39^{a}	2205.15^{b}	2249.82^{b}	42.095
Live weight gains g head^{-1}	1958.39^{a}	1981.37^{a}	2158.36^{b}	2202.67^{b}	41.958
FCR (*feed coversion ratio*)	1.69^{a}	1.67^{a}	1.57^{b}	1.56^{b}	0.032

Note: Values with different letters on the same row are significantly different (P<0.05)

Final body weight and gain of broiler increased with the presence of CLWE in drinking water. The increase in body weight is due to the role of herbs as prebiotics in the digestive tract, which can stimulate optimal growth of intestinal villi. According to Macfarlane et al. (2007), pathogenic bacteria attach to prebiotics, preventing direct infection to the surface of intestinal villi. In general, higher intestinal villi show a more mature epithelium and increased absorptive function. A similar result was reported by Raheem et al. (2012) stating that supplementation of prebiotics, probiotics, and synbiotics in broiler feed improved the histology of intestines, thereby increasing nutrient absorption.

Increased body weight and feed efficiency are caused by the improved surface area of the intestinal villi, which helps to maximize nutrient absorption. Rajput et al. (2013) reported that the inclusion of 0.02% herbal flour (*Curcumin*) in feed improved growth performance and fat metabolism, as well as increased the absorption area of the small intestine villi. These changes improved nutrient absorption which facilitated growth and feed efficiency. Furthermore, tannin compounds can impede growth of pathogenic bacteria by inhibiting cell wall synthesis (Farha et al., 2020). Reducing the population of pathogenic bacteria in the intestine will increase the beneficial population, such as lactic acid bacteria (Zurmiati et al., 2014).

According to a previous study, increasing feed efficiency and digestibility can influence protein synthesis and calcium intake which impacts the high and low levels of protein mass as well as calcium mass of meat (Prabowo et al., 2019). By reducing the number of harmful bacteria in the digestive tract, nutrient absorption becomes more optimal, resulting in increased performance and feed efficiency. Bidura et al. (2017) and Siti & Bidura (2022) reported that administering herbal leaves water extract through drinking water to broiler significantly increased body weight gain, digestibility, and feed efficiency.

Effect of CLWE on blood lipid profile

The addition of 2% CLWE to broiler drinking water from 0-5 weeks of age had no significant effect (P>0.05) on the blood lipid profile and amount of abdominal fat as shown in Table 2. However, at levels of 4% and 6%, the treatment significantly (P<0.05) reduced broiler blood serum Low-Density Lipoprotein (LDL) levels by 20.17% and 18.47% compared to the control. The total cholesterol levels in group C and D broiler namely 13.32% and 11.97%, were significantly (P<0.05) lower than control (A). The inclusion of CLWE in drinking water had no effect (P>0.05) on blood High-Density Lipoprotein (HDL) levels.

Table 2. Effect of inclusion of CLWE in broiler drinking water from 0-5 weeks of age on blood lipid profile and abdominal fat.

Variable	CLWE level in drinking water (cc/100 cc)				SE
	0	2	4	6	
LDL (mg/dl)	98.47[a3)]	95.36[a]	78.61[b]	80.28[b]	3.038
HDL (mg/dl)	65.81[a]	59.72[a]	70.35[a]	69.93[a]	5.916
Triglycerides (mg/dl)	30.82[a]	32.51[a]	29.74[a]	27.95[a]	2.804
Total cholesterol (mg/dl)	161.09[a]	159.25[a]	139.63[b]	141.81[b]	5.025
Abdomen fat (g/100 g body weight)	1.85[a]	1.79[a]	1.41[b]	1.46[b]	0.103

Note: Values with different letters on the same row are significantly different (P<0.05)

Abdominal fat content in the CLWE 2 and 3 broiler groups decreased significantly ($P<0.05$), to 23.78% and 21.08%, which was lower than the control group (CLWE0). Total cholesterol and LDL levels in the blood serum of broiler treated with CLWE decreased significantly. The decrease is attributed to saponin compounds which function as antinutrients, causing decreased fat digestibility (Teteh et al., 2013). In addition, alkaloid compounds can reduce the activity of lipogenic enzymes (Patil et al., 2012). Cholesterol absorption is highly regulated and influenced by certain compounds in the food supply. The feed components that have been identified to inhibit cholesterol absorption include phytosterols, soluble fiber, and phospholipids (Jesch and Carr, 2017). The main mechanism of action by which saponin compounds in carrot leaves reduce cholesterol in the body is through the transfer of molecules from bile salt micelles. This causes the formation of cholesterol deposits that cannot pass through the intestinal mucus layer (Vinarova et al., 2015).

The results showed that administration of CLWE through drinking water during the study period (0-5 weeks of age) improved the blood lipid profile. HDL levels tended to be higher in broiler that received CLWE compared to the control. Krauze et al. (2020) reported that phytobiotics (*Cinnamon* oil) were effective in increasing serum HDL levels in broiler. Supplementation of herbal leaves in feed can improve blood profiles in hens and pigs (Bidura et al., 2017; Bidura et al., 2020; Li et al., 2023). According to Chukwuebuka (2015), the differences in results obtained can be attributed to the variations in dosage, type of extract, and method of administering the herbal extract. The reduction in cholesterol levels may also be caused by the high level of beta-carotene compounds and antioxidant compounds in herbal leaves (Nuarini et al., 2008; Elamin et al., 2019).

The effect of CLWE on pathogenic bacteria

The inclusion of CLWE at a level of 2% in broiler drinking water from 0-5 weeks of age, did not have a significant impact ($P>0.05$) on the population of *Coliform* and *E.coli* bacteria in the intestine (Table 3). However, the 4% and 6% treatments significantly ($P<0.05$) reduced the *Coliform* bacteria population by 22.52% and 31.12% compared to the control (0% CLWE).

The addition of 4% and 6% CLWE to broiler drinking water had a positive impact on reducing the population of *E. coli* bacteria in broiler intestines. The population of *E. coli* bacteria in the intestines of group C and D broiler, was 54.23% and 56.11% respectively, which was significantly ($P<0.05$) lower compared to group A (control).

Table 3. Effect of adding CLWE to drinking water on the population of pathogenic bacteria in broiler intestine.

Variable	CLWE level in drinking water (cc/100 cc)				Normal
	0	2	4	6	
Total *Coliform* (CFU/g)	$1.07 \times 10^6 \pm 0.14 \times 10^6$a	$1.12 \times 10^6 \pm 0.11 \times 10^6$a	$8.29 \times 10^5 \pm 0.15 \times 10^5$b	$7.37 \times 10^5 \pm 0.14 \times 10^5$b	$4.0 \times 10^6 – 9.4 \times 10^6$
Total *E. coli* (CFU/g)	$2.08 \times 10^4 \pm 0.27 \times 10^4$a	$1.94 \times 10^4 \pm 0.19 \times 10^4$a	$9.52 \times 10^3 \pm 0.21 \times 10^3$ b	$9.13 \times 10^3 \pm 0.16 \times 10^3$b	10^4 - 10^5

Note: Values with different letters on the same row are significantly different ($P<0.05$); Cfu = colony forming unit

Carrot leaves extract has relatively strong antibacterial activity (Bukar et al., 2010; Bidura et al., 2023), hence, the application in drinking water can reduce the number of pathogenic bacteria. The presence of *E. coli* in feces is very high, hence, bacteria can be an agent of disease transmission (Roth et al., 2019). Furthermore, *E. coli* is pathogenic and causes colibacilosis in poultry (Delannoy et al., 2021). Kogut and Arsenault (2015) also stated that *Salmonella* infection could reduce growth performance and cause dysbacteriosis, even leading to high mortality. Herbal supplementation in the form of turmeric powder, pennyroyal, and orange peel oil in feed reduces pathogenic bacteria in the intestines of broiler (Sudatri, 2021; (Erhan et al., 2012). A study by Calislar (2019) showed that beta-carotene compounds in herbal plants increased antibody responses in poultry and prevented acute respiratory infections. However, Steenfeldt et al. (2007) reported that the addition of carrot flour to feed did not affect the population of *coliform* bacteria in the intestines of laying hens.

According to Roth et al. (2019), the cell membrane of *E. coli* bacteria consists of a peptidoglycan membrane and polysaccharides which are more susceptible to chemical damage. Tannins can inhibit growth of pathogenic bacteria by inhibiting cell wall synthesis (Farha et al., 2020). As stated by Cowan (1999), these compounds have the ability to deactivate microbial adhesions (cell surfaces) and form complexes with polysaccharides. Furthermore, tannins act as antibacterials, due to the phenol group which has properties similar to alcohol, namely antiseptic and antimicrobial (Rishika and Sharma, 2012). Reflecting on the available evidence, reducing pathogenic bacteria in the intestine will increase the beneficial population, such as lactic acid bacteria (Zurmiati et al., 2014). Flavonoid compounds obtained from herbal leaves extraction can act as antibacterials by denaturing cell membrane lipids through hydrogen bonds. Consequently, bacteria cell membrane is damaged without the formation of new ones, leading to the inhibition of growth (Dani et al., 2012). Cowan (1999) reported that phenolic and terpenoid compounds in herbal leaves can damage the cell wall membrane of pathogenic bacteria. The inhibitory power of herbal compounds against pathogenic bacteria depends on the type and concentration of extracts used (Yuniza and Yuherman, 2015).

4. Conclusion

In conclusion, the addition of CLWE at 4-6 cc/100 cc of drinking water in broiler from 0-5 weeks of age increased feed efficiency and improved blood lipid profiles. On the other hand, the treatment reduced abdominal fat and pathogenic bacteria in the intestines. Broiler breeders may value increased feed efficiency and reduced abdominal fat, as these two variables greatly influence income. A more in-depth study is needed on the effect of CLWE on broiler reared using a litter system. This is because pathogen contamination in the litter is very high. Additionally, the different types of solvent used in extraction including methanol, alcohol, and water, may yield varying results in the phytochemical content.

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Conflict of interest

The authors declare that there are no competing financial interests or personal relationships capable of affecting the work reported in this paper.

Author's Contribution

These authors each contributed equally.

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