

EFFECT OF CARROT LEAF JUICE FOR GROWTH PROMOTION AND BIO-CONTROL OF PATHOGENIC BACTERIA IN DUKS

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ABSTRACT

Background: *Daucus carota* is a herbal plant that is rich in benefits, and has long been used in traditional medicine, because it contains phytochemicals that are antibacterial, antioxidant and growth promoter. Research aims to analyze phytochemical compounds of carrot leaves, as well as the implementation of carrot leaf juice (CLJ) to stimulate growth and suppress pathogenic bacteria in ducks. **Materials and Methods:** Inhibition test of carrot leaves against *E. coli* bacteria was carried out in vitro using the diffusion well method using *E. coli* as the test bacteria. This feeding trial study used 240 male Bali ducks aged two weeks with 4 treatments and 6 replications. The four treatments were a group of ducks that were given drinking water without CLJ as a control (A); supplemented with 1% CLJ (B); 2% CLJ (C); and 3% CLJ (D), respectively. **Results:** The results showed that the phytochemical compounds in *Daucus carota* L. leaves were phenolic compounds, tannins, saponins, flavonoids, steroids, and terpenoids, and beta-carotene was 908.75 mg/100 g leaves. There was a significant increase ($P < 0.05$) in feed consumption, body weight gain, and feed efficiency in groups C and D ducks rather than control. Serum cholesterol in duck groups B, C, and D decreased significantly ($P < 0.05$) rather than control. **Conclusion:** It can be concluded that the implementation of CLJ at the level of 2-3% via drinking water can increase the growth and suppress the number of pathogenic bacteria in the duck intestines.

KEYWORDS: Phytochemicals, carrots, *E. coli*, cholesterol, ducks.

INTRODUCTION

Generally, in large-scale poultry rearing systems, bacterial pathogens are often encountered, such as *Escherichia coli* which causes diarrhea^[1] and can cause *Colibacillosis*.^[2,3] *Colibacillosis* disease that attacks poultry causes a decrease in health which results in a decrease in productivity.^[4] Generally, poultry farmers to cope with *Colibacillosis* disease is to use antibiotics, such as tetracycline, neomycin, and fluoroquinolones. However, the use of antibiotics is currently prohibited, because it causes resistance to bacteria and residues in poultry products.^[5,6]

Therefore, efforts are needed to overcome this, for example by utilizing the efficacy of phytochemical compounds in herbal plants. Utilization of plant herbal efficacy can be used, because it has been shown to suppress the number of *Choloform* and *E. coli* bacteria in the intestine, increase growth and reduce cholesterol levels in serum and yolk.^[7,8,9,10,11,12,13] Not all herbal extracts can function as anti-cholesterol, as reported

by^[14] that supplementation of *Kaempferia galanga* rhizome juice in rats has potential as an antidiabetic drug, but not as an anticholesterol drug. Also reported by^[15] that supplementation of *Turmeric* and *Tamarindus* juice via drinking water did not have a negative effect on kidney performance and serum creatinine levels in broilers.

Several researchers reported that the use of herbal plant leaves has been shown to increase poultry productivity and reduce cholesterol content and abdominal fat in poultry.^[17,10,16,17,18,19,20]

The phytochemical compounds in *Daucus carota* leaves can function as natural antibiotics, because they contain active compounds that function as antibacterial. As reported by^[21,22] that the parts of herbal plants that are usually used as medicine are the rhizome (*Turmeric* or tuber) and the leaves, such as *Moringa*, *Morinda*, *Cardamom*, and so on which can be gastroprotective. Administration of herbal leaf extracts (*Moringa oleifera* and *Allium sativum*) via drinking water significantly

reduced serum cholesterol broilers^[19] and yolk cholesterol.^[16] The content of beta-carotene in carrot leaves, apart from being an antibacterial and antioxidant compound, is also an active substance for carcass color,^[23] so its role is very important in increasing carcass color which is highly favored by consumers.

Based on this, researchers are interested in analyzing the phytochemical compounds in *Daucus carota* leaves, as well as the implementation of carrot leaf juice to stimulate growth and suppress pathogenic bacteria in ducks.

MATERIALS AND METHODS

Feeding trial and experimental design. The research was conducted in several locations. The production of carrot leaf ethanol extract and phytochemical screening and GC-MS testing were carried out at the Integrated Service Laboratory, at the Faculty of Agricultural Technology, and at the Biochemistry Laboratory at the Biology Study Program, Udayana University, Denpasar, Indonesia. After extracting the carrot leaves, phytochemical tests were carried out, GC-MS test, then *in vitro* test of the carrot leaf extract on *E. coli* bacteria to see its inhibition against the growth of *E. coli* bacteria.

Feeding trial experiment with four types of treatment and six replications. Each replication used 10 male *Anas sp.* ducks aged two weeks, so that the total ducks used were $4 \times 6 \times 10 = 240$ tails. The four treatments were (i) ducks given drinking water without using carrot leaf juice (CLJ) as control (A); (ii) given drinking water with 1% CLJ (B); (iii) drinking water with 2% CLJ (C); and (iv) drinking water with 3% CLJ (D). The rations given were commercial rations for growth phase ducks and were given *ad libitum*. Making carrot leaf juice begins with washing carrot leaves with clean water, weighing 100 g, then crushing, adding aquadest until the volume was 100 ml and filtered. The results of the filtering, considered 100% carrot leaf juice (CLJ). Concentrations of 1%, 2%, and 3% CLJ solutions were made by adding aquadest according to the desired concentration.

Carrot Leaves (*Daucus carota* L.). First, carrot leaves were prepared from carrot plantations at Penebel District, Tabanan Regency, Bali Province, Indonesia. The leaves used include the petiole which was separated from the carrot tuber. Carrot leaves were then washed and cut into small pieces. After that, it was dried until the weight was stable, then crushed and sieved, until it became a fine powder. This carrot leaf powder was then used to test the inhibition of bacteria, but first the extraction step must be carried out. Bacterial inhibition test using the diffusion well method.

Phytochemical content test on carrot leaves. Carrot leaf extract was made from 1 kg of carrot leaves (carrot harvest waste), washed with water until clean, then finely chopped and air-dried in a room without direct sunlight, until the weight was constant and becomes simplicia.

Furthermore, the simplicia was blended until smooth, sieved, and macerated in 96.5% ethanol and filtered, so that the filtrate was obtained. The remaining filtered dregs were macerated again with the same process, and the filtrate obtained was combined with the first filtrate. Then the filtrate was evaporated with a rotary evaporator until a more concentrated solution was formed, so that a crude extract was obtained and was ready to be used in testing the content of its phytochemical compounds.^[24]

Phytochemical screening was carried out through a series of tests using certain reagents. This phytochemical screening was carried out in accordance with that carried out by^[25]. Several types of reagents that can be used for phytochemical screening include: Phenols and flavonoids can be detected using 1% FeCl₃ solution in ethanol. The test result was considered positive if it produces green, red, purple, blue or black colors. The Shinode test (concentrated Mg and HCl) can also be used to detect flavonoids. Flavonoids will show a very strong cherry red color when sprayed with this reagent. The saponin test, which was as much as 2 mL of the sample was put into a test tube, then 10 mL of distilled water was added and shaken vigorously for 10 minutes. The results were declared positive if the foam formed was stable for not less than 10 minutes, as high as 1 cm to 10 cm. At the addition of 1 drop of 2N HCL, the foam did not disappear. Terpenoid test: Lieberman-Burchard reagent was a reagent that was often used to test terpenoid compounds. This reagent was prepared from a mixture of acetic anhydride and concentrated H₂SO₄. Most triterpenes and sterols give a blue-green color with this reagent. After phytochemical screening, carrot leaf extract was carried out, then the carrot leaf extract was tested by GC-MS test to identify the types of active compounds in the two ingredients. Furthermore, *in vitro* tests on *E. coli* bacteria were carried out to see the antibacterial ability of the carrot leaf extract. The CLJ were tested on the culture of *E. coli* bacteria using a method similar to that used by.^[26]

Observed Variables. Feed consumption (FI) and live weight gain (LWG): feed consumption and LWG were measured once a week. While feed efficiency was a comparison between FI and LWG (FI:LWG). *In vitro* test of inhibition of carrot leaf aqueous extract against *E. coli* bacteria: The method used to test the inhibition of carrot leaf juice was the diffusion well method according to Retnaningsih et al. (2019). As much as 10 mL of NA media was poured into each petridish and shaken horizontally until well mixed and allowed to solidify. After the media solidified, 2 diffusion wells were made with a cork border (5 mm diameter) on each petridish. Each well was filled with 20 µl with carrot leaf juice to be tested. Furthermore, the NA media and *E. coli* bacteria which had been filled with the tested solution were incubated at 37°C for 18-24 hours. All treatments were repeated 5 times and the inhibition of carrot leaf juice could be seen by measuring the diameter of the clear zone formed with a caliper. Based on the inhibition

zone formed, according to,^[27] the antibacterial activity can be classified into three groups, namely: weak antibacterial activity (inhibition zone <5 mm), moderate antibacterial activity (inhibition zone 5-10 mm), and strong antibacterial activity (zone of inhibition >20 mm).

Statistical Analysis

One-way analysis of variance was used for the analysis of all data. If a significant difference was found (P<0.05) between treatments, it was continued with Duncan's multiple distance test.

RESULTS

The results of the laboratory analysis of the content of phytochemical compounds in *Daucus carota* L. leaves

are presented in Table 1. After phytochemical screening, carrot leaf extract was tested by GC-MS test to identify the types of active compounds in the material. The results of laboratory analysis to identify the content of phytochemical compounds in *Daucus carota* leaves are presented in Table 1. Carrot leaves contain phenolic compounds, tannins, saponins, flavonoids, steroids, and terpenoids. The beta-carotene content of carrot leaves is relatively high, namely: 908.75 mg/100 g of leaves. The results of this analysis are the same as those reported by^[28] that carrot leaves contain phytochemical compounds, such as flavonoids, phenolics, terpenoids, steroids, tannins, and beta carotene as much as 676 mg/100 g leaves.

Table 1: The content of phytochemical compounds in the leaves of *Daucus carota* L.

Types of phytochemical compounds in the leaves of <i>Daucus carota</i> L.						
Phenolics	Tannins	Saponins	Flavonoids	Steroids	Terpenoids	β-carotene (mg/100 g)
++	+++	+	+++	++	+++	908.75

Note: + = weak positive, ++ = positive, dan +++ = strong positive

The results of the in vitro test to test the ability of carrot (*Daucus carota* L.) leaf extract to inhibit *E. coli* bacteria are presented in Figure 1. *Daucus carota* leaf extract turned out to have a strong enough inhibitory power against the growth of *Eschericia coli* bacteria. More detail is presented in Figure 1.

The diameter of the inhibition zone of carrot leaf juice was: 12.93 ± 1.064 mm, while the aquadest inhibition zone (negative control) against *E. coli* bacteria was: 0 mm, and the inhibition zone diameter of 5% tetrachlor

antibiotic (positive control) against *E. coli* bacteria is: 51.58 ± 0.726 mm. The results of ^[29] research reported that the type of extract tested had a very significant effect on the inhibition zone for the growth of *E. coli* bacteria. Likewise with the concentration, the higher the concentration of herbal extracts, the greater the zone of inhibition of growth against *E. coli*. It was also reported that there was an interaction between the type of herbal extract and the concentration of the herbal extract on the size of the inhibition zone formed.

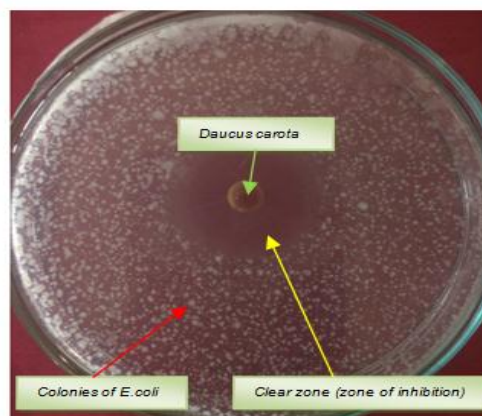


Fig. 1: The diameter of the clear zone (inhibition zone) for the growth of *E. coli* bacterial colonies due to *Daucus carota* leaves extract.

The performance of male bali ducks (*Anas sp.*) aged 2-8 weeks that were given CLJ via drinking water is presented in Table 2. Average FI, BW, LWG, and feed efficiency (FI:LWG) in the group of ducks that received CLJ 1 cc/100 cc of drinking water was not significantly different (P>0.05) compared to the control. However, at a concentration of 2-3 cc/100 cc CLJ in drinking water, it

significantly (P<0.05) increased FI, BW, LWG, and feed efficiency. More details are presented in Table 2.

Table 2: Performance of male bali ducks (*Anas sp.*) aged 2-8 weeks given the juice of *Daucus carota* leaves via drinking water.

Variables	CLJ concentration in drinking water (cc/100 cc)				SEM
	0	1	2	3	
Initial body weight (g)	215.68a	217.31a	215.92a	216.37a	45.186
BW (g)	1150.53b ¹	1165.48b	1458.36a	1481.52a	41.703
LWG (g/bird)	934.85b	948.17b	1242.44a	1265.15a	40.972
FI (g/bird)	3169.14b	3138.44b	3702.47a	3605.68a	98.791
Feed efficiencies (FI:LWG)	3.39a	3.31a	2.98b	2.85b	0.085
Total cholesterol serum (mg/dL)	175.29a	172.71a	151.64b	149.05b	5.072

Note:

1. ^{a,b} Means in rows with different superscripts are different significantly (P<0.05)

The antibacterial activity of a substance is classified as weak if the diameter of the inhibition zone formed is <5 mm; moderate activity if the diameter of the inhibition

zone is between 5-10 mm; strong activity if the diameter of the inhibition zone is 10-20 mm; and very strong if the diameter of the inhibition zone formed is >20 mm^[30]. In this study, 3% carrot leaf juice had the best antibacterial activity against *E. coli* bacteria compared to 1-2% carrot leaf juice, but still lower than tetrachlor antibiotics.

Table 3. Average population of *Coliform* and *E. coli* bacteria in the intestines of ducks that were given CLJ via drinking water.

Variable	CLJ concentration in drinking water (cc/100 cc)				Population Normal
	0	1	2	3	
Total <i>Coliform</i> (CFU/g)	8.85 x 10 ⁶ ± 1.17 x 10 ⁶ a	7.99 x 10 ⁶ ± 0.38 x 10 ⁶ b	2.58 x 10 ⁶ ± 0.26 x 10 ⁶ b	3.05 x 10 ⁶ ± 0.17 x 10 ⁶ b	4,0 x 10 ⁶ – 9,4 x 10 ⁶
Total <i>E. coli</i> (CFU/g)	8.72 x 10 ⁵ ± 1.06 x 10 ⁵ a	8.03 x 10 ⁵ ± 0.49 x 10 ⁵ b	2.94 x 10 ⁵ ± 0.33 x 10 ⁴ b	2.45 x 10 ⁵ ± 0.27 x 10 ⁵ b	10 ⁴ - 10 ⁵

Note:

1. ^{a,b} Means in rows with different superscripts are different significantly (P<0.05)

The mean population of *Choliform* and *E. coli* bacteria in the intestines of ducks that were given CLJ via drinking water is presented in Table 3. The total population of *Choliform* and *E. coli* in the intestines of ducks decreased significantly (P<0.05) in the group of ducks that received 2-3 cc/100 cc CLJ via drinking water. Meanwhile, the concentration of 1 cc/100 cc of CLJ via drinking water was not significantly different (P>0.05) compared to the control.

DISCUSSION

The results showed that CLJ supplementation at the level of 2-3 cc/100 cc of drinking water could increase FI, likewise with BW and LWG ducks. The increase was caused by CLJ containing phytochemical compounds, such as saponins, flavonoids, and tannins (Table 1) and several other phenolic compounds that have antimicrobial activity, such as saponins which have been proven to be effective as antimicrobials^[31]. Contrary to the research of^[19] who reported that giving herbal extracts to broilers did not have an effect on FI, but could significantly increase LWG and feed efficiency. The research of^[32] reported that FI decreased significantly in chickens that were given herbal supplements compared to chickens that were not given herbal supplements.

Reported by^[33] that *Pennyroyal* herbal supplement can reduce FI and increase feed efficiency in broilers.

BW and LWG in the group of ducks that received CLJ were higher than the control group (without CLJ). William and Rosa^[34] reported that the increase in LWG in chickens was due to the nature of plant extracts that could increase crop secretion and form a more balanced intestinal flora with their antimicrobial effect. Cold-pressed carrot seed oil supplementation,^[35] essential oil combination derived from selected herbs and mixture of herbal essential oils^[36,37] in feed significantly increased carcass weight, carcass percentage and characteristics of broiler carcasses, which is contrary to the results of research by^[38] which stated that adding carrot leaf flour to the feed actually reduced the mass of protein meat.

Serum cholesterol of ducks decreased significantly with the supplementation of carrot leaf juice in drinking water. Carrot leaves contain tannins, saponins, flavonoids, steroids, and triterpenoids (Table 1). The administration of herbal leaf extract juice (*Moringa oleifera* and *Allium sativum*) in broiler drinking water significantly reduced abdominal fat and broiler serum cholesterol levels^[19] and yolk cholesterol in hens^[16]. The results of the study of,^[35] that the addition of Carrot seed oil supplementation in the basal ration had no effect on serum biochemical parameters. Furthermore, it was also reported that Carrot seed oil supplementation had resulted in positive changes in body weight gain, carcass yield, number of Lactic acid bacteria in the intestinal.

Escherichia coli bacteria live in the intestines of ducks as normal flora, but there are several strains of *E. coli* bacteria that produce toxins and cause diarrhea and colibacillosis in poultry.^[1,3] The content of phenolic and terfenoid compounds in herbal leaves can damage the cell walls of pathogenic bacteria and inhibit the growth of *S. aureus* bacteria.^[39,40] The decrease in the number of pathogenic bacteria in the intestines of ducks will result in optimal absorption of nutrients, which will have an impact on improving the performance of ducks. Herbal compounds such as: Turmeric powder,^[41] Pennyroyal,^[33] and orange peel oil^[42] in broiler feed, can increase the number of lactic acid bacteria and decrease the number of *E. coli* bacteria in the jejunum. On the other hand,^[43] reported that the number of *Choliform* bacteria and Lactic acid bacteria in the small intestine was not affected by the addition of carrots to the diet in laying hens. Tannins are polyphenolic compounds found in plants, have a bitter taste, can agglomerate proteins, and are antibacterial and antidiarrheal.^[44]

According to^[45] the types of plant herbs and the concentration of herbal extracts used, in fact greatly affect the level of inhibition against *Escherichia coli* and *Salmonella sp.* The bacterial activity of herbal plants can be attributed to the content of phytochemical compounds in their extracts.^[46] The high content of phenolic compounds and supported by the presence of alkaloids, saponins, flavonoids, and triterpenoids, greatly affects its antibacterial activity.^[45] The role of phenol as an antibacterial is by denaturing bacterial proteins through absorption involving hydrogen bonds. At high concentrations, phenolic compounds cause proteins to coagulate, and cell membranes undergo lysis, thereby changing the permeability of bacterial cell membranes.^[47] Calislar^[48] study reported that beta-carotene can boost the immune system by enhancing antibody response and preventing acute respiratory infections in poultry.

CONCLUSION

It can be concluded that supplementation of Carrot leaf juice on drinking water can stimulate the growth of ducks and suppress the number of *Choliform* and *E. coli* bacteria in the intestine. The content of phytochemical compounds in *Daucus carota* L. leaves are phenolic compounds, tannins, saponins, flavonoids, steroids, terpenoids, and the beta-carotene content is 908.75 mg/100 g leaves.

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