



Improving the Health of Weaned Piglets through Supplementation of Carrot Leaf Aqueous Extract via Drinking Water

Eny Puspani ¹, Ni Wayan Siti ¹, Bulkaini ², Desak Putu Mas Ari Candrawati ¹, Ni Made Ari Kusuma Dewi ¹ and I Gusti Nyoman Gde Bidura ^{1*}

¹ Faculty of Animal Husbandry, Udayana University, Denpasar-Bali, Indonesia

² Faculty of Animal Husbandry, Mataram University, West Nusa Tenggara, Indonesia

*Corresponding author: bidura_unud@yahoo.com

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ABSTRACT

This study aims to assess the impact of administering carrot leaf (CL) aqueous extract (AE) via drinking water on the performance, histology, and intestinal pathogens of weaned piglets. Carrot leaf aqueous extract (CLAE) was prepared by squeezing 1kg of carrot leaves with 1L of clean water (g/g), then filtering. The basic feed used was a commercial complete feed, CP 511, for weaned piglets. This study used 120 healthy crossbred female weaned piglets (35 days old), divided into four treatment groups and 6 replications. Piglets in group 1 (n=30) served as controls given drinking water without added CLAE, while piglets in groups 2, 3, and 4 received CLAE at levels of 2, 4, and 6%, respectively. The results of the study showed that the administration of 4 and 6% CLAE in drinking water significantly ($P<0.05$) increased performance, the height of duodenal and jejunal villi, and the number of lactic acid bacteria (LAB) in the intestine. Pathogenic bacteria (*Escherichia coli* and *Coliform*) in the piglet intestine decreased significantly ($P<0.05$) as a result of the addition of CLAE to drinking water. In conclusion, adding 4-6% CLAE to drinking water has the potential to improve performance, villus height, and intestinal lactic acid bacteria. Conversely, this addition can suppress *Coliform* pathogens in the intestines of weaned piglets. To determine the mechanism underlying CLAE's effects, further research is needed.

Keywords: Carrot leaves extract, Performance, Intestinal histology, Pathogen, Weaned piglets

INTRODUCTION

The problem of drug (antibiotic) resistance in bacteria has inspired research on alternative medicine, with a focus on plants containing antimicrobial chemicals (essential sources of biologically active metabolites), which can be used to develop or produce new drugs (Aathira et al. 2021; Babatunde et al. 2023; Bolaji et al. 2024).

Pathogens are becoming resistant to synthetic drugs, so herbal medicines provide an efficient alternative with fewer side effects (Sharma et al. 2011). Plants have been considered among the most sought-after sources for the discovery of antimicrobial agents (Divya-Shree et al. 2022). Phytochemical compounds of herbal plants (flavonoids, tannins, saponins, and cryptoxanthins) are bioactive components that may be responsible for antibacterial activity (Çetingül et al. 2020; Szabó et al. 2023).

The interaction of phenolic compounds with the cell membranes of pathogenic bacteria can damage them, thereby disrupting cation permeability and inhibiting pathogen growth (Alsayeqh and Abbas 2023).

Flavonoids can disrupt lipid bilayers in bacterial cell membranes through hydrogen bonding, thereby damaging the membranes (Dani et al. 2012). Meanwhile, tannin compounds contain phenol groups with properties similar to those of alcohols, namely antiseptic and antimicrobial (Rishika and Sharma 2012). Saponins exhibit an anticoccidial effect against *Eimeria tenella*, although this is still lower than that of the antibiotic salinomycin (Youssef et al. 2021).

Newly weaned piglets are highly susceptible to changes in intestinal morphology, inflammation, and intestinal epithelial permeability caused by several stressors that can disrupt the piglet's intestinal development (Moeser et al. 2017; Szabó et al. 2023). Newly weaned piglets have immature digestive tracts, and their gut microbiota balance and villous structure are also not fully developed, making them highly vulnerable to changes in dietary and environmental factors (Kim and Duarte 2021; Zheng et al. 2021; Shi et al. 2022). Increasing villus height increases the area for nutrient absorption (Kai 2021), thereby improving piglet performance.

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Plant extract additives are beginning to be recognized for their ability to reduce the prevalence of pathogenic microbes, reduce the incidence of severe diarrhea in weaned piglets and show positive effects in changing the colon microbiota which can ultimately treat diarrhea and inhibit the growth of pathogenic bacteria (Wang et al. 2021ab; Xu et al. 2022), improve digestion and absorption (Draskovic et al. 2018). Plants have been considered among the most sought-after sources of antimicrobial compounds. Phytochemical compounds from herbal plants are bioactive components that can exhibit antibacterial activity (Farha et al. 2020; Alsayeqh and Abbas 2023; Jamil et al. 2024; Nasihin 2025). Plant leaf extracts have broad-spectrum activity and the potential to develop useful antibacterial drugs. Carrot (*Daucus carota*) leaf waste has excellent potential as a feed additive, with a 50:50 (g/g) ratio compared to carrot tubers (Siti and Bidura 2022; Wibawa et al. 2024; Puspani et al. 2025; Siti et al. 2025). This study aims to examine the impact of providing CLAE via drinking water on weaned piglet performance, intestinal histology, and pathogenic bacteria.

MATERIALS AND METHODS

Experimental design and feeding trial

This study used 120 healthy, five-week-old female piglets resulting from crossbreeding between sows and Duroc boars. All female piglets were in a healthy condition, and all treatment piglets were given commercial feed CP 511 in pellet form. Piglets in group 1 (n=30) received no CLAE supplementation, while piglets in groups 2, 3, and 4 had CLAE levels in their drinking water of 2, 4, and 6%, respectively. During the experimental period, all piglets were fed and watered ad libitum. All piglets were housed in concrete floor pens. Lighting was provided 24 hours a day. The basic feed used was a complete commercial feed for weaned piglets, CP 511, in pellet form, produced by Limited Liability Company Charoen Phokphan Indonesia Tbk. Highway Surabaya, Sidoarjo, East Java. Product Registration Number: PD.6.2-224090648. The nutrient content in the CP 511 ration for weaned piglets is presented in Table 1.

Table 1: Nutrient content of commercial feed CP 511 for weaned piglets aged 35-66 days

Nutrients	Unit %
Water content, max	13
Crude protein, min	17.5
Crude fat, min	3.0
Crude fiber, max	5.5
Ash, max	7.5
Calcium (Ca)	0.7-1.2
Total phosphorus (P), min	0.50
Urea	0
Total aflatoxin, max	40.0 µg/kg
Antibiotics	0
Methionine, min	0.34
Lysine, min	1.13

CLAE and its phytochemical content

CLAE was prepared by squeezing 100g of carrot leaves in 100mL of clean water (g/g), then cold-macerating at 24.5±1.9°C and 65±15% humidity for 24 hours, and filtering through double-satin cloth.

The aqueous extract of carrot leaves was analyzed at the Agricultural Technology Laboratory, Udayana University, Denpasar, and found to contain active substances, including flavonoids, phenols, tannins, steroids, and glycosides. The flavonoid, tannin, and antioxidant contents were 41.86mg/100g, 74.25mg/100g, and 50.39%, respectively.

Experimental variables and analytical procedures

Body weight gain (LWG) and feed intake (FI) were calculated weekly. Feed efficiency was calculated from FI/LWG (g/g). Twenty-four piglets were slaughtered at the end of the study (66 days of age), and the duodenum and jejunum were collected for intestinal histological analysis according to the procedures described by Ermayanti et al. (2021). Piglets' intestines (duodenum, jejunum, and ileum) were collected using sterile media, placed in plastic bags, and stored in a refrigerator. *Escherichia coli* and *Coliform* bacteria were counted using the Most Probable Number (MPN) method, following the procedure adopted by Chaniago (2017).

Statistical Analysis

All data were analyzed using one-way analysis of variance to determine the significance of treatment effects. If there was a significant difference ($P \leq 0.05$), treatment means were compared using Duncan's multiple range test (Steel and Torrie 1980).

RESULTS

Table 2 shows the impact of providing CLAE in drinking water on the performance of piglets from 35 to 66 days of age. Piglet growth was significant ($P < 0.05$) in groups 3 and 4, from 49-66 days of age after CLAE administration (Table 2). Overall, CLAE administration through drinking water had no significant effect ($P > 0.05$) on FI. Groups 3 and 4 had final body weights that were 18.11 and 13.16% higher ($P < 0.05$) than those of control pigs. Feed efficiency in pig groups 3 and 4, namely: 15.56 and 11.11% higher ($P < 0.05$) than that in control pig groups. More details are presented in Table 2.

The addition of CLAE at concentrations of 2, 4, and 6% increased the length of the jejunum, namely: 24.24, 24.02, and 23.62% higher ($P < 0.05$) than the control (without CLAE). Similarly, the length of the jejunum organ in pig groups 3 and 4, namely, 2.91% and 1.96% longer ($P < 0.05$) than group 1 (Table 3). The total length of the intestine of piglets at the inclusion of 4 and 6% CLAE, namely: 1.61% and 1.16% longer ($P < 0.05$) than group 1 pigs (without CLAE).

Table 4 shows that the addition of 4% and 6% CLAE to the drinking water of 35-66-day-old piglets increased duodenal villus height by 13.19% and 14.59%, respectively ($P < 0.05$) compared to group 1 (control). Similarly, the height of jejunal villi in piglets in groups 3 and 4 was 12.98% and 7.94% higher ($P < 0.05$), respectively, compared to the control (drinking water without CLAE).

The impact of adding CLAE in the drinking water of 35-66 day old piglets on the number of pathogens in the intestines of piglet groups 3 and 4 decreased ($P < 0.05$) compared to piglet groups 1. Conversely, the number of LAB in the intestines of piglet groups 3 and 4 decreased

Table 2: Performance of weaned piglets given CLAE through drinking water at 35-66 days of age

Variable	Experimental group carrot leaf aqueous extract level in drinking water (cc/100 cc)				SEM
	0	2	4	6	
Average weight of piglets (kg)					
Day 35 of age (weaning of age)	8.01 ^a	7.95 ^a	7.99 ^a	7.98 ^a	0.021
Day 42 of age	10.52 ^a	10.39 ^a	10.04 ^a	10.61 ^a	0.028
Day 49 of age	13.50 ^b	12.81 ^a	12.80 ^a	14.00 ^b	0.127
Day 56 of age	15.51 ^a	15.20 ^a	18.08 ^b	18.31 ^b	0.653
Day 63 of age	18.90 ^a	18.45 ^a	22.21 ^b	21.80 ^b	0.135
Day 66 of age	19.60 ^a	20.14 ^a	23.15 ^b	22.88 ^b	0.087
Feed intake (g/day)					
Days 35-42 of age	0.53 ^a	0.52 ^a	0.53 ^a	0.54 ^a	0.006
Days 42-49 of age	0.69 ^a	0.68 ^a	0.68 ^a	0.69 ^a	0.005
Days 49-56 of age	0.80 ^a	0.79 ^a	0.80 ^a	0.80 ^a	0.005
Days 56-63 of age	0.91 ^a	0.91 ^a	0.92 ^a	0.92 ^a	0.004
Days 63-66 of age	1.27 ^a	1.26 ^a	1.27 ^a	1.27 ^a	0.004
Days 35-66 of age	4.20 ^a	4.16 ^a	4.20 ^a	4.22 ^a	0.006
Average feed utilization per 1kg of body weight (FCR=kg/kg)					
Days 35-42 of age	1.47 ^a	1.49 ^a	1.81 ^a	1.44 ^a	0.137
Days 42-49 of age	1.62 ^a	1.97 ^a	1.73 ^a	1.43 ^a	0.182
Days 49-56 of age	2.79 ^a	2.32 ^a	1.06 ^b	1.30 ^b	0.051
Days 56-63 of age	1.88 ^a	1.96 ^a	1.56 ^b	1.844 ^a	0.036
Days 63-66 of age	5.45 ^a	2.24 ^b	4.06 ^a	3.53 ^b	0.192
Average FCR from 35-66 days of age (21 days)	0.90 ^a	0.91 ^a	0.76 ^b	0.80 ^b	0.027

CLAE = Carrot leaf aqueous extract, SEM = Standard error of means, LWG = live weight gain, FI = feed intake, FCR= Feed Conversion Ratio, Means in the same row with different superscripts differ significantly (P<0.05).

Table 3: Intestinal length of 66-day-old piglets

Variable	Experimental group carrot leaf aqueous extract level in drinking water (cc/100 cc)				SEM
	0	2	4	6	
Live weight, kg	19.51 ^a	20.34 ^a	23.18 ^b	22.83 ^b	0.381
Age at slaughter, days	66	66	66	66	-
Intestine length (cm)					
Duodenum length, cm	27.35 ^a	33.98 ^b	33.92 ^b	33.81 ^b	1.298
Length of the jejunum, cm	1064.51 ^a	1068.72 ^a	1095.49 ^b	1085.38 ^b	6.039
Length of the ilium, cm	496.03 ^a	485.17 ^a	483.91 ^a	487.16 ^a	4.916
Total intestinal length, cm	1587.89 ^a	1587.87 ^a	1613.42 ^b	1606.35 ^b	5.179

SEM = Standard error of means. Means in the same row with different superscripts differ significantly (P<0.05).

Table 4: Jejunal mucosal epithelial structure in 66-day-old piglets given carrot leaf water extract in drinking water

Variable	Experimental group carrot leaf aqueous extract level in drinking water (cc/100 cc)				SEM
	0	2	4	6	
<i>Duodenum</i>					
Villus height (µm)	460.35 ^a	449.82 ^a	521.05 ^b	527.53 ^b	9.725
Villus width (µm)	55.81 ^a	57.49 ^a	69.17 ^a	64.39 ^a	3.109
Crypt depth (µm)	102.17 ^a	109.35 ^a	115.36 ^a	121.93 ^a	6.972
Villus height/crypt depth	4.51 ^a	4.11 ^a	4.52 ^a	4.33 ^a	0.514
<i>Jejunum</i>					
Villus height (µm)	506.71 ^a	500.39 ^a	572.47 ^b	546.93 ^b	8.152
Villus width (µm)	65.31 ^a	62.27 ^a	69.25 ^a	71.93 ^a	3.075
Crypt depth (µm)	82.61 ^a	79.48 ^a	92.01 ^a	88.72 ^a	4.904
Villus height/crypt depth	6.13 ^a	6.30 ^a	6.22 ^a	6.16 ^a	0.591

SEM = Standard error of means. Means in the same row with different superscripts differ significantly (P<0.05).

Table 5: Number of microbes in the small intestine digesta (CFU/g)

Variable	Experimental group carrot leaf aqueous extract level in drinking water (cc/100 cc)			
	0	2	4	6
<i>Escherichia coli</i> enumeration	8.81x10 ⁶ ±0.06x10 ^{6a}	8.69x10 ⁶ ±0.08x10 ^{6a}	9.92x10 ⁵ ±0.12x10 ^{5b}	9.73x10 ⁵ ±0.15x10 ^{5b}
<i>Coliform</i> enumeration	8.37x10 ⁶ ±0.13x10 ^{6a}	8.41x10 ⁶ ±0.12x10 ^{6a}	7.69x10 ⁵ ±0.16x10 ^{5b}	7.81x10 ⁵ ±0.14x10 ^{5b}
Lactic acid bacteria	8.87x10 ⁷ ±0.15x10 ^{7a}	8.98x10 ⁷ ±0.16x10 ^{7a}	9.92x10 ⁸ ±0.17x10 ^{8b}	9.87x10 ⁸ ±0.16x10 ^{8b}
Intestinal pH	6.91 ^a	6.85 ^a	6.73 ^a	6.62 ^a

Means in the same row with different superscripts differ significantly (P<0.05).

(P<0.05) as a result of adding CLAE in their drinking water. Meanwhile, the degree of acidity (pH) in the intestinal digesta had no effect (P>0.05). More details are presented in Table 5.

DISCUSSION

The growth of weaned piglets aged 35-66 days in group 4 increased following administration of CLAE via

their drinking water. CLAE contains flavonoid compounds that can protect the intestinal epithelium from pathogenic bacterial adhesion, thereby optimizing nutrient absorption. As reported by Oso et al. (2019), herbal medicines contain phytochemical compounds, including flavonoids, which have antimicrobial and anti-inflammatory effects.

The detailed mechanism by which herbal leaf extract supplementation improves growth performance in growing pigs remains unclear. However, it may explain the study by Hoque and Kim (2023) that found that herbal extract supplementation can increase crude protein digestibility and modulate intestinal LAB populations in broilers. Similarly, Liu et al. (2021) demonstrated that supplementation with herbal leaf extract (*Achyranthes japonica*) can improve growth performance, likely due to increased digestibility. Administration of fermented *Gliricidia* leaf extract through drinking water can increase the amount of duck carcass meat (Sedana et al. 2025).

Phytochemical additives can improve growth, digestibility, and immune response in piglets (Huang et al. 2011). In line with Hanczakowska and Swiatkiewicz (2012), herbal plant extracts can improve piglet performance, primarily by altering small intestinal morphology. Furthermore, herbal leaves can increase nitrogen digestibility, which impacts piglet growth and feed efficiency (Hoque and Kim 2023; Ozung et al. 2025). Although the exact mechanism of the relationship between carrot leaf aqueous extract supplementation and improved growth performance in growing pigs remains unclear, it is known that herbal leaves contain many phytochemical compounds. Herbal leaves contain antioxidants and other active ingredients, such as saponins and triterpenoids, which are pretty high (Liu et al. 2008; Lee et al. 2012).

Pigs in groups 2, 3, and 4 had higher jejunal length and villus height than those in group 1 (control). Intestinal histology of piglets compromised by weaning is characterized by reduced small intestine length and villus height (Moeser et al. 2017), and the internal surface area of the intestinal wall is largely determined by the high villi for nutrient absorption (Hanczakowska and Swiatkiewicz 2012; Kai 2021).

The inclusion of CLAE in drinking water significantly increased intestinal villus height. Research by Oso et al. (2019) reported that supplementing broiler diets with a phytochemical mixture can increase jejunal length and villus height compared with broilers fed only a basal diet. Phytochemical additives in feed and drinking water have specific effects, namely balancing the gastrointestinal microbiota and encouraging the regeneration of intestinal epithelium and villi (Hanczakowska and Swiatkiewicz 2012; Khasnavis and Pahan 2012).

According to Fang et al. (2009), villus height in the duodenum, jejunum, and ileum increased, and conversely, crypt depth decreased in response to herbal extract supplementation. Piglets fed phytochemical additives experienced an 11.1% increase in the VH/CD ratio compared to piglets fed antibiotics (Matoso et al. 2024). This may be the reason for the increased dry matter digestibility and energy digestibility (Hoque and Kim 2023). Increased intestinal villus height in piglets may contribute to improved digestibility (Hanczakowska and Swiatkiewicz 2012). In contrast, Firman et al. (2025) reported that administering fermented mangosteen peel

water extract using Effective Microorganism-4 in drinking water did not improve intestinal histology in broilers.

The addition of 4 and 6% CLAE to the drinking water of piglets from 35-66 days of age significantly reduced the concentration of pathogenic bacteria, while increasing the concentration of LAB in the intestine. Herbal plant phytochemicals are bioactive components responsible for antibacterial activity, and this ability is due to the presence of tannin, flavonoid, saponin, and cryptoxanthin compounds (Çetingül et al. 2020; Szabó et al. 2023). Inhibition of peptidoglycan synthesis occurs due to membrane damage and changes in the hydrophobicity of bacterial cell surfaces by herbal leaf phytochemicals (Rasooli et al. 2008).

The tannin, flavonoid, and saponin compounds in carrot leaves can inhibit the growth of *E. coli* bacteria in the intestines of piglets (Hadyarrahman et al. 2017). The reduction in bacterial growth in the intestines of poultry is caused by saponins and flavonoids, which can degrade the cell membranes of pathogenic bacteria (Yuniza and Yuherman 2015). A similar opinion was reported by Youssef et al. (2021), who found that saponins have an anticoccidial effect against *Eimeria tenella*, although still lower than that of salinomycin. Meanwhile, tannins act as antibacterials due to the presence of phenol groups, which have properties similar to those of alcohols, namely antiseptic and antimicrobial (Rishika and Sharma 2012).

The active compounds in herbal leaves can prevent the adhesion of pathogenic bacteria and increase LAB in the intestine (Liu et al. 2021). A report by Hanczakowska and Swiatkiewicz (2012) found no difference in bacterial populations in the ileal chyme after treatment with herbs. According to Ajayi and Ogunjobi (2024), these differences in results are due to the type of phytochemicals and their activity.

Conclusion

In conclusion, the addition of 4-6% CLAE to the drinking water of weaned piglets from 35-66 days of age can increase final body weight, feed efficiency, total intestinal length (duodenum, jejunum), villus height, and lactic acid bacteria in the intestine. Conversely, the addition of CLAE significantly reduced pathogens in the intestinal tract of weaned piglets. Further research is needed to determine the different types of herbal leaf extracts and the mechanisms underlying the effects of CLAE.

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