The effect of giving fermented *Daucus carota* leaf flour by yeast culture in feed on performance, jejunal villi and *Escherichia coli* bacteria in duckling digesta

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ABSTRACT
The combination of phytobiotic effects of herbal leaves and probiotics on the duck digestive tract effectively increases nutrient absorption and intestinal health. The purpose of the research is to investigate the effect of a giving fermented carrot (*Daucus carota*) leaf flour in diet on performance, intestinal histology and Eschericia coli bacteria in duckling digesta. The research mechanism was carried out by taking carrot leaves from carrot harvest waste, then making it into flour. Then fermented with probiotics (*Saccharomyces* spp.) facultatively anaerobically at a temperature of 28^0C for five days. After five days of fermentation, it was then dried in the sun and mixed into commercial feed at levels 2, 4, and 6% as treatments B, C, and D. As a control (A) is commercial feed without the addition of fermented carrot leaf flour (FCLF). The repetition was carried out 6 times using 10 healthy male ducklings (*Anas sp*), so that the total ducklings used were 240 male ducklings. The results of the study found that supplementation of 4-6% FCLF in commercial rations significantly different ($P<0.05$) improving performance,, villus height, number of lactic acid bacteria, ratio of villus height and crypt depth. Conversely, it significantly ($P<0.05$) reduced the number of *Eschericia coli* bacteria. It was concluded that the addition of FCLF at a level of 4-6% in commercial feeds can improve growth and feed efficiency. There was an increase in villi height and lactic acid bacteria in the jejunal of ducklings as an effect of the addition of FCLF to the ration, while the number of *Eschericia coli* bacteria decreased.
Keywords: Daucus carota; probiotics; intestinal histology; pathogenic bacteria; ducks

Introduction

Herbal leaves and their phytochemical compounds are interesting to study for their efficacy on poultry performance and health. Bioactive compounds in herbal leaves are expected to improve poultry health [1,2]. Herbal leaves contain various bioactive substances, such as alkaloids, flavonoids, saponins, tannins, phenolics, polyphenols, terpenoids, and antioxidants. The effectiveness of compounds contained in herbal leaves varies greatly, is largely determined by the source of the herbal plant, the age of the herbal plant, and the solvent used in extraction [3].

Intestinal morphology plays an important role in nutrient absorption and growth performance in animals. Significant changes in intestinal morphology, such as villous atrophy and crypt depth hyperplasia, can directly result in malabsorption, diarrhea, and growth inhibition in livestock [4]. In addition, villus height and crypt depth serve as important indicators to assess the impact of small intestinal morphology, reflecting the digestive capacity and nutrient absorption [5].

Carrot leaves (*Daucus carota*) are very potential as a source of phytobiotics and prebiotics, because they are abundantly available and contain phytochemical compounds. Carrot plants are known worldwide as vegetables rich in minerals, fiber, antioxidants, flavonoids, and beta-carotene [6]. Carrot production is quite high, while post-harvest waste in the form of carrot leaves can reach 50% of the carrot harvest [7]. Its use in laying hen feed can increase digestibility, increase yolk color and lower blood cholesterol levels [8].

Yeast culture (*Saccharomyces spp*.) can act as a source of probiotics [9]. Research by [10] reported that the addition of 0.20% *Saccharomyces* spp. to feed can stimulate growth and suppress pathogenic bacteria (*Eschericia coli* and *Coliform*) in the intestine. Probiotic microbes in the digestive tract of poultry can increase the growth of lactic acid bacteria, thereby creating an acidic environment that can suppress the growth of pathogenic bacteria [11], reduce the incidence of diarrhea and increase growth and feed efficiency [12].

The combination of herbal leaf flour and probiotics used as a nutritional supplement for poultry feed and can be synbiotic. The combination of the phytobiotic effects and probiotics on the digestive tract of broilers effectively increases nutrient absorption and intestinal health of broilers. The combination of herbal leaf flour and probiotics is used as a nutritional supplement for poultry feed and can be synbiotic. The combination of phytobiotic effects of herbal leaves and probiotics on the digestive tract of broilers effectively increases nutrient absorption and intestinal health of broilers. The addition of herbal leaf flour and probiotics to feed can digest nutrients and health of poultry and swine [13].

The purpose of the research is to investigate the effect of a giving fermented carrot (*Daucus carota*) leaf flour in feed on performance, intestinal histology and pathogenic bacteria in duck intestines.

Materials and Methods

Experimental design

The research mechanism was carried out by taking carrot leaves from carrot harvest waste, then making it into flour. Then fermented with probiotics (*Saccharomyces* spp.) facultatively anaerobically at a temperature of 28^{0}C for three days. After three days of fermentation, it was then dried in the sun and mixed into commercial feed at levels 2, 4, and 6% as treatments B, C, and D. As a control (A) is commercial feed without the addition of fermented carrot leaf flour (FCLF). The repetition was carried out 6 times using 10 healthy male ducklings (Anas sp), so that the total ducklings used were 240 male ducklings. FCLF was then added to commercial feed for ducks, namely CP 512, an antibiotic-free product produced by PT. Charoen Phokphan Tbk, Sidoarjo, East Java, Indonesia. Furthermore, FCLF was tested through feeding trials on male Bali ducks aged 2-10 weeks, to determine the optimal level of provision in rations seen from the aspects of performance, villi length and crypt depth of the jejunum intestine. The health aspect of ducklings is seen from the number of beneficial bacteria (lactic acid bacteria) and harmful bacteria in the duckling's intestines.

Fermentation of carrot leaf flour (*Daucus carota*) with probiotic *Saccharomyces* spp.

The carrot leaves used were carrot leaves (*Daucus carota* Lam) after the carrot tuber harvest in Baturiti, Tabanan, Bali, Indonesia. As much as 1 kg of carrot leaf flour Carrot leaf flour was mixed evenly with 10% molasses solution. Then add 1% Yeast culture flour and stir evenly. Then incubate in a plastic bag, and incubated at a temperature of 28^{0}C for five days [14]. After five days, FCLF was ready to be added into commercial feed (2, 4 and 6%) in commercial feed.

Observed Variables.

Measurement of body weight gain (LWG), and feed intake (FI) is done every week. While feed efficiency is calculated based on the comparison between FI and LWG in the same time unit. At the end of the study, one duck was taken from each experimental unit to be slaughtered and its jejunum was taken to measure the height of the villus and crypt depth according to the procedure [15].

The total number of microbes in the intestinal digesta sample using PCA (Plate Count Agar) media. While the method used to obtain the total *Coliform* and *E. coli* bacteria was the spread method using EMBA media. Analysis of the number of plates in intestinal digesta was carried out as done by [16]. Total Escherichia coli and Coliform bacteria in intestinal digesta using the distribution method in EMBA media [17].

Statistical analysis

Data analysis using one-way ANOVA. If there is a significant difference ($P<0.05$), then continue with Duncan's test to determine the difference between treatments [18].

Results and Discussion

Performance

Supplementation of 2% fermented carrot leaf flour (FCLF) by probiotic *Saccharomices cerevisiea* (Sc) in male Bali duck feed from 2-8 weeks of age, had no effect ($P>0.05$) on BW, LWGs, FI and feed efficiency (Table 1). However, BW showed a significant increase ($P<0.05$) with the presence of 4 and 6% FCLF, namely: 7.27% and 6.77% higher than the control. Likewise, the LWG during six weeks of observation experienced a significant increase ($P<0.05$), namely: 9.57% and 9.03% higher than the control. The addition of FCLF at levels of 4 and 6% to commercial feed for ducklings aged 2-8 weeks, can increase feed efficiency, namely: 6.93% and 6.40% ($P<0.05$) more efficient than feed without FCLF (Table 1). The addition of FCLF in commercial duck ratios at a level of 4-6% from 2-8 weeks of age significantly improved duck performance (BW, LWG and feed efficiency). Fermented herbal products contain probiotics (which help maintain the health of the duck's digestive tract [19]. According to [20], fermented feed products can improve feed palatability and broiler performance.

Table 1. Performance of ducklings fed with additional fermented carrot leaf flour.

Variable	FCLF level in commercial duck feed (%)				SE
	0	2	4	6	
Initial body weight, g	361.67^a	360.05^a	361.24^a	359.85^a	2.592
Final body weight, g/head	1525.81^a	1521.94^a	1636.74^b	1629.17^b	19.271
Live wiight gains, g/head/6 weeks	1164.14^a	1161.89^a	1275.50^b	1269.32^b	18.593
Feed consumption, g/head/6 weeks	4365.53^a	4391.94^a	4515.49^a	4455.31^a	75.926
Feed efficiency (FI/LWG)	3.75^a	3.78^a	3.49^b	3.51^b	0.063

Note: Values with different superscript letters in the same row indicate significantly different ($P<0.05$).

Puspani et al. [10] reported that an alternative to stimulate growth and suppress pathogenic bacteria is to utilize a combination of herbal leaf flour (Moringa) and yeast which has been proven in native chickens, that supplementation of Moringa-Yeast as much as 2-6% in feed can increase BW, LWG, and nutrient absorption..

The same thing was reported by [21] that providing fermented feed as much as 25-50% can increase growth and immunity in free-range chickens raised in the wild. In line with [22], that herbal leaves in feed significantly increased growth, LWG and feed efficiency in growing pigs. Similarly, findings by [23] reported that supplementation of Achyranthes japonica extract significantly increased growth and improved protein digestibility in broilers. Research by [24] reported that phytogenic additives caused increased growth and nutrient absorption.

Intestinal Histology

The inclusion of FCLF in commercial feed for ducklings had no effect (P>0.05) on duodenal length and Cript depth. However, at the level of 4 and 6% FCLF in duck feed significantly (P<0.05) increased villus height, namely: 12.47% and 13.69% higher than the control. Likewise, the ratio between VH and CD at FCLF levels of 4% and 6%, namely: 15.21% and 23.45% higher (P<0.05) compared to feed without FCLF (Table 2).

Diets containing 4-6% FCLF can increase the height of the duodenal villi of ducklings. Although the mechanism is not yet clear, phytogenic compounds in herbal leaves will facilitate an increase of *Lactobacillus* in the broiler digestive system, so that the increase in the number of *Lactobacillus* bacteria can limit the number of harmful bacteria in the intestine [25]. It has been proven by [26] that the inclusion of rice bran and corn fermented by Sc in feed significantly increases feed digestibility and duodenal villi height. In line with [27], there was an increase in the height and width of the villi in the duodenum and jejunum as a result of supplementation of fermented feed with probiotics.

Supplementation of fermented herbal products with Sc. has great potential to improve host intestinal health. Jejunal villi height increased significantly with the administration of probiotics [28]. In line with [29] that supplementation of Sc. probiotics (625 g/ton) in feed can improve broiler intestinal health, as measured by an increase in the length of intestinal villi. Hong et al.[30] also reported the same thing that fermented feed with Sc. probiotics significantly increased the height of villi in the duodenum.

Table 2. Histology of the duodenum of male Bali ducks fed with feed containing FCLF from 2-8 weeks of age.

Variable	FCLF level in commercial duck feed (%)				SE
	0	2	4	6	
Duodenum length, cm)	26.49[a]	27.08[a]	26.75[a]	26.94[a]	0.391
Villi height, µm	698.25[a]	683.97[a]	785.29[b]	793.82[b]	31.072
Cript depth, µm)	179.85[a]	168.93[a]	175.72[a]	165.84[a]	11.029
Ratio VH/CD	3.88 [a]	4.05 [a]	4.47 [b]	4.79 [b]	0.863

Note: Values with different superscript letters in the same row indicate significantly different (P<0.05).

Herbal leaves also contain phytochemical compounds that have the potential to affect intestinal health. As reported by[31] that supplementation of herbal extracts (*Acanthopanax senticosus*) can increase the height of the duodenal, jejunal, and ileal villi, while the cript depth decreases. In contrast, [32] reported that supplementation of fermented yeast culture in feed had no effect on the morphology of the jejunum of laying hens.

Pathogenic Bacteria

There was a significant decrease (P<0.05) in the number of Eschericia coli bacteria in the intestines of children with the presence of FCLF in the feed. In contrast, lactic acid bacteria increased significantly (P<0.05) with the supplementation of 2-6% FCLF in duck feed from 2-8 weeks of age (Table 3). Inclusion of FCLF in the feed had no effect on the number of *Coliform* bacteria and intestinal pH.

The decrease in pathogenic bacteria and the increase in the number of lactic acid bacteria in the duck intestine as a result of the inclusion of phytogenic products in the feed that can stimulate the production of intestinal mucus, which can further contribute to reducing pathogen pressure through inhibition of mucosal adhesion [13]. In addition, the ability of saponins to form complex compounds with soluble and extracellular proteins, as well as with bacterial cell walls. Flavonoid compounds and their derivatives are strong antibacterial agents against various microorganisms. Both are antibacterial due to their lipophilic properties, which can break down microbial membranes [33].

Flavonoids, tannins, and alkaloids are responsible for the antibacterial properties of therapeutic plants [34]. However, the effectiveness of these substances depends on the type, concentration, and proper interaction with other components. Tannins are also used as antidotes to alkaloids, indicating that plants high in tannins can suppress the bacteria that cause these diseases [35].

Table 3. The effect of FCLF inclusion in male Bali duck feed from 2-8 weeks of age on pH and intestinal microbes.

Variable	FCLF level in commercial duck feed (%)				SE
	0	2	4	6	

Lactic acid bacteria, cfu/g	$7.03x10^{5a}$	$9.16x10^{5b}$	$1.08x10^{6b}$	$1.12x10^{6b}$	$0.17x10^{5}$
Eschericia coli, cfu/g	$5.17x10^{5a}$	$4.39x10^{4b}$	$3.82x10^{4b}$	$4.16x10^{4b}$	$0.45x10^{4}$
Coliform, cfu/g	$8.15x10^{4}$	$6.93x10^{4}$	$7.23x10^{4}$	$6.21x10^{4}$	$0.69x10^{4}$
pH	6.69	6.52	6.51	6.47	0.079

Note: Values with different superscript letters in the same row indicate significantly different ($P<0.05$); Cfu = colony forming units.

Herbal feed fermented with probiotics can increase beneficial bacteria (lactic acid bacteria) and maintain the normal morphology of the intestinal mucosa of pigs [31]. In line with [22] who reported that supplementation of feed with herbal leaf extracts can increase beneficial bacteria and reduce harmful bacteria. In contrast, [32] reported that supplementation of fermented yeast culture in feed had no effect on the morphology of the jejunum of laying hens. The type of phytochemicals contained in herbal leaves determines its activity, and therefore, differences in results obtained may be due to the number of types of phytochemicals present.

Conclusion

It was concluded that the addition of fermented carrot leaf flour by *Saccharomyces* sp. as much as 4-6% in commercial rations can improve duckling performance. There was an increase in the number of lactic acid bacteria and the height of the duodenal villus of ducklings due to the influence of the addition of FCLF to the ration, while the number of Eschericia coli bacteria decreased.

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Conflict of Interest.

None

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