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The Effect of Carrot Leaf Flour in a Concentrated Diet on Carcasses, Blood Lipid Profiles, and Pathogens in the Intestines of Rabbits

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ABSTRACT

The aim of this research was to examine the effect of the inclusion of carrot leaf flour in concentrate in grass-based rations on carcass, cholesterol and intestinal pathogenic bacteria. This study used 120 local male rabbits aged 8 weeks, divided into four treatment groups and 6 replications. The four treatments were: rabbits fed a grass-based diet supplemented with concentrate without carrot leaf meal as a control (Group CLF0), concentrate with 10% carrot leaf meal (CLF1); concentrate with 20% carrot leaf flour (CLF2); and concentrate with 30% carrot leaf flour (CLF3). Concentrate was given as much as 40g/head/day, while grass was *ad libitum*. The results showed that slaughter weight, carcass, carcass percentage, carcass meat, carcass bone, and meat bone ratio (MBR) in the CLF2 and CLF3 rabbit groups were higher ($P<0.05$) than those in CLF0 group. Likewise, the villus height and crypt depth of the jejunum in Groups CLF2 and CLF3 were significantly ($P<0.05$) higher than the control group (CLF0). Giving 20 and 30% carrot leaf flour in concentrate significantly ($P<0.05$) reduced total blood cholesterol, total *Escherichia coli* and Coliform bacteria in the intestines of rabbits. It was concluded that the inclusion of 20-30% carrot leaf flour (*Daucus carota*) in the concentrate could increase the carcass percentage, the amount of carcass meat, and the meat bone ratio (MBR). On the other hand, it can reduce cholesterol and the number of pathogenic bacteria in the rabbit's intestines.

Key words: Carcass, Carrot leaves, Cholesterol, Concentrate, Rabbit.

INTRODUCTION

The advantage of rabbit farming as a meat producer is that it has a high protein content with low fat content, and is better than other livestock meat, such as beef and pork. Rabbits are one of the alternative livestock that have great potential to be bred to meet the animal protein needs of the Indonesian people as well as to improve the welfare of breeders.

In an intensive rabbit rearing pattern, the largest production costs come from feed, which is around 60-70%. Therefore, farmers must make efforts to improve feed efficiency. Rabbit farming is an option for cultivation, because the feed does not compete with human needs. Rabbits can be kept by providing forage combined with agricultural waste (Nuriyasa et al. 2017).

One of the agricultural wastes that can be utilized is carrot leaves (*Daucus carota*). According to data from the Central Statistics Agency (BPS), throughout 2021 Indonesia will be able to produce 720.09 thousand tons of carrots. The ratio between carrot production and carrot leaves produced is 50:50 and beta-carotene content of carrot leaves is 908.75mg/100g leaves (Wibawa 2022), so carrot leaves have great potential as animal feed. Carrot leaves can act as an alternative to antibiotics, because of their phytochemical content. Carrot leaves contain phytochemical compounds, such as flavonoids, tannins, saponins, beta-carotene and cryptoxanthin (Hammershoj et al. 2010; Çetingül et al. 2020).

Carrot leaves have good nutrient content, so they can be a good alternative feed for rabbits (Muzaki et al. 2017). Adding carrot leaf flour to concentrate is the best strategy to increase carcass productivity and biocontrol pathogenic bacteria in rabbits. According to Bukar et al. (2010), phenolic compounds in herbal leaves can act as antimicrobials. According to Alsayeqh and Abbas (2023), the interaction of phenolic compounds with cell membranes causes disruption of the membrane's

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permeability to cations, which will result in the loss or reduction of membrane potential. This process causes the outflow of cellular constituents to be disrupted, so that ATP and protein synthesis stops, resulting in pathogenic cell death. It was also reported that phenolic compounds helped reduce damage caused by oxidative stress in poultry with coccidiosis.

Reported by Mattioli et al. (2019), that early administration of dried alfalfa is a promising solution to improve livestock health status by prioritizing beneficial digestive microbiota. The inclusion of carrot leaf flour in the concentrate will limit exposure to mycotoxins which harm rabbits' health. As reported by Jamil et al. (2024) that mycotoxins are secondary fungal metabolites produced by various species of toxigenic fungi and are found in several feed ingredients, especially in plants during pre- and post-harvest conditions, such as processing, transportation and storage. Mycotoxins are of great concern to food safety and security, due to their negative impact on health and the economy. Saponins have been proven to have antimicrobial properties and reduce blood cholesterol levels.

The inclusion of herbal leaf extracts in broiler drinking water significantly reduces abdominal fat and cholesterol levels in broiler blood (Bidura et al. 2017). According to Jamil et al. (2022), the mechanism of action of herbal compounds is through the inhibition of pro-oxidant enzymes (protein, kinase C, xanthine oxidase, and membrane-related NAD(P)H oxidase), which has an impact on reducing stress and increasing immunity, increasing folate and vitamin B, increasing calcium and other minerals have an effect on increasing productivity. It is also reported that herbal leaves can increase high density lipoprotein (HDL) and reduce total cholesterol and low density lipoprotein (LDL) in poultry.

The fiber component in carrot leaves can influence the height of the villi and the depth of the crypts of the duodenum and ileum (Gidenne 2015). The higher the villi, the wider the space for nutrient absorption, so that feed efficiency can increase.

The aim of this research was to examine the effect of giving carrot leaf flour in concentrate to grass-based rations on carcasses, cholesterol levels, intestinal histology, and the population of pathogenic bacteria in the intestines of rabbits.

MATERIALS AND METHODS

Ethical approval

A total of 120 rabbits as experimental animals had been approved by the Animal Ethics Commission from the Faculty of Veterinary Medicine, Udayana University, Denpasar.

Experimental design and Feeding trial

This study used 120 local male rabbits aged 8 weeks, divided into four treatment groups and 6 replications. The four treatments were: rabbits fed a grass-based diet supplemented with concentrate without carrot leaf meal as a control (Group CLF0), concentrate with 10% carrot leaf meal (CLF1); concentrate with 20% carrot leaf flour (CLF2); and concentrate with 30% carrot leaf flour (CLF3), respectively. Concentrate was given as much as 40g/head/day, while grass was provided *ad libitum*.

The concentrate used in this study was calculated based on the table of food substance composition according to Scott et al. (1982) using ingredients such as: yellow corn, rice bran, coconut male, soybean meal, fish meal, coconut oil, and oysters shell grit. Carrot leaf flour contains 9.27% crude protein; 19.64% crude fiber; 1.20% ether extract; 0.65% Calcium and 0.51% phosphorus (Muzaki et al. 2017). The concentrate was composed of isocaloric (ME: 2900 kcal/kg) and isoprotein (CP: 18%). Concentrate was given as much as 40g/head/day, while grass was *ad libitum*. This was based on preliminary research that the average feed consumption for rabbits aged 8-16 weeks was 80-90g/head/day.

Cages and Rabbits

Research was carried out at the Research Station, Faculty of Animal Husbandry, in Sesetan, Denpasar, while laboratory analysis was carried out at the Animal Products Technology Laboratory, Faculty of Animal Husbandry, Udayana University, Denpasar, Indonesia. The rabbits used were obtained from local rabbit farmers in the Baturiti area, Tabanan, which were eight weeks old with a body weight ranging between 335.62±18.29g. All rabbits were placed in 24 battery colony cages made of iron wire. Each cage plot measures: 1.2m long, 1.0m wide and 0.40m high. All cage plots were equipped with feed and drinking water.

Carrot Leaf Flour (*Daucus carota*)

Carrot leaves were obtained from post-harvest local carrots in carrot plantations owned by farmers in the Baturiti area, District Tabanan, Bali, Indonesia. The carrot leaves were separated from the carrot tubers, then cleaned and dried in the sun for two days until dry, then finely ground and filtered. Carrot leaf flour was ready to be mixed into concentrate feed. Feed composition and CLF levels in concentrate feed are presented in Table 1.

Table 1: Feed composition and nutrients in concentrates for rabbits from 8-16 weeks of age

Compositions	Carrot leaf flour (CLF) levels in concentrate diets (%)			
	0	10	10	30
Ingredients (%):				
Yellow corn	48.50	42.00	33.80	28.50
Rice bran	14.50	9.80	9.45	4.30
Coconut meal	15.00	15.70	12.90	12.50
Soybean meal	13.60	13.80	14.05	15.50
Fish meal (CP, 58%)	6.50	6.50	7.00	6.60
Coconut oil	1.50	1.80	2.40	2.20
Carrot leaf flour	0.00	10.00	20.00	30.00
Oysters shell grit	0.40	0.40	0.40	0.40
Total	100	100	100	100
Chemical composition:				
Metabolizable energy, (kcal/kg)	2901	2900	2900	2901
Crude protein, %	18	18	18	18
Ether Extract, %	8.58	8.18	8.58	7.84
Crude fiber, %	5.71	7.09	8.45	9.69
Calcium, %	0.85	0.81	0.91	0.88
Phosphorous, %	0.48	0.51	0.57	0.51

Parameters of body weight (BW), feed consumption (FC), rabbit body weight gain (LWGs), and feed conversion ratio (FCR) were recorded every week. Feed Conversion Ratio compares FC and LWG in the same unit of time (g:g). Determination of *Escherichia coli* and *Coliform* populations followed the Sudatri (2021) procedure. Cholesterol levels were analyzed according to the Lieberman-Burchard method (Lieberman and Burchard 1980). Rabbit slaughter procedures and carcass acquisition, as well as carcass percentages followed the method of Alhaidary et al. (2010). *Coliform* and *Escherichia coli* bacteria testing followed the procedure of Sudatri (2021). Measurement of villus height and crypt depth followed the procedure (Ermayanti et al. 2021).

Statistical analysis

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. Significance was declared at P≤0.05.

RESULTS

The effect of giving carrot leaf flour (CLF) in concentrate in grass-based feed on the carcass and blood lipid profile of rabbits is presented in Table 2. At the level of 10% carrot leaf flour (CLF1) in concentrate, it did not have a significant effect (P>0.05) on slaughter weight, carcass weight, carcass percentage, carcass meat, carcass bone, and carcass meat-to-bone ratio. However, at levels of 20% (CLF2) and 30% (CLF3) carrot leaf flour in concentrate, significantly (P<0.05) increased cutting weight, namely 14.17 and 17.33% higher than the control (concentrate without carrot leaf flour).

Using 20 and 30% carrot leaf flour in concentrate significantly (P<0.05) increased carcass weight, namely 20.44 and 24.05% higher than the control. The same increase occurred in the percentage of rabbit carcasses, increasing significantly (P<0.05) with administration of 20 and 30% carrot leaf flour in concentrate, namely 5.49 and 5.72% higher than the control.

The amount of carcass meat in the CLF2 and CLF3 rabbit groups, namely 20.81 and 22.20% was significantly (P<0.05) higher than CLF0. The number of carcass bones in the CLF2 and CLF3 rabbit groups, namely 5.40 and 3.33% was significantly (P<0.05) higher than Group CLF0. The meat bone ratio in the CLF 2 and CLF3 rabbit groups, namely 14.61 and 18.26% was significantly (P<0.05) higher than the CLF0 rabbit group. More details are presented in Table 2.

The inclusion of CLF in the concentrate apparently had no significant effect (P<0.05) on triglyceride, LDH and HDL levels in rabbit blood. However, the blood cholesterol levels of rabbits given CLF showed a significant difference (P<0.05). Total blood cholesterol levels in the CLF1, CLF2, and CLF3 rabbit groups were 10.01; 10.53; and 8.88%, significantly (P<0.05) lower than the control rabbit group (CLF0). More details are presented in Table 2.

Table 3 presents the impact of the inclusion of CLF in concentrating on the number of *Escherichia coli* and *Coliform* bacteria in the intestine, as well as the height of the villi and crypt depth of the rabbit jejunal. The total *Escherichia coli* bacteria in the intestines of the CLF1, CLF2, and CLF3 rabbit groups significantly (P<0.05) decreased, namely 45.47; 45.14; and 49.89% lower than control (CLF0). The average number of *Coliforms* in the rabbit intestine decreased significantly (P<0.05) with increasing inclusion of CLF in the concentrate. Total Coliform bacteria in the intestines of CLF1, CLF2, and CLF3 rabbit groups, respectively, were 14.59; 8.98; and 18.06% significantly (P<0.05) lower than the group of rabbits fed concentrate without CLF0. More details are presented in Table 3.

Table 2: Effect of administering carrot leaf flour in concentrate on the carcass and blood lipid profile of rabbits

Variable	Carrot leaf flour levels in concentrate diets (%)				SE
	0	10	20	30	
Slaughter weight (g/head)	1525.71a	1568.35a	1741.84b	1790.17b	31.207
Carcass weight (g/head)	727.61a	753.59a	876.32b	902.60b	25.412
Carcass percentage (%)	47.69a	48.05a	50.31b	50.42b	0.704
Meat (g)	471.32a	495.27a	569.38b	575.94	14.295
Bone (g)	215.21a	217.22a	226.84a	222.37a	5.983
Meat bone ratio (meat:bone)	2.19a	2.28a	2.51b	2.59b	0.0531
Profil lipida darah (mg/dL)					
• Total cholesterol	89.07a	80.15b	79.69b	81.16b	2.082
• Triglycerides	58.81a	54.19a	56.28a	52.73a	2.614
• LDL	46.17a	47.38a	45.72a	45.05a	1.019
• HDL	32.79a	34.51a	35.26a	35.91a	0.895

Note: [ab] values with different letters on the same row are significantly different (P<0.05).

Table 3: Effect of carrot leaf flour (CLF) in concentrating on jejunum histology and the population of pathogenic bacteria in the rabbit intestine.

Variable	Carrot level in concentrate based diets (%)			
	0	10	20	30
Escherichia coli bacteria (colony forming unit/g)	$1.79.10^{5}$ ±$0.15x10^{5}$a	$9.76.10^{4}$ ±$0.31x10^{4}$b	$9.82.10^{4}$ ±$0.22x10^{4}$b	$8.97.10^{4}$±$0.39x10^{4}$b
Coliform bacteria (colony forming unit/g)	$0.98x10^{6\pm}0.14x10^{6}$a	$8.37x10^{5\pm}0.19x10^{5}$b	$8.92x10^{5\pm}0.28x10^{5}$b	$8.03x10^{5\pm}0.25x10^{5}$b
Villus height (µm)	2831.52±23.79a	3079.35±39.62b	3115.82±42.47b	3126.74$^{\pm}$39.46b
Jejunal crypt depth (µm)	979.31±20.58a	1085.14±25.93b	1063.28$^{\pm}$22.75b	1095.59$^{\pm}$29.62b

Note: [ab] values with different letters on the same row are significantly different (P<0.05).

In Table 3, the effect of including CLF in the concentrate on jejunal histology (villus height and crypt depth) is presented, which turns out to have a significant effect (P<0.05). Jejunal villus height in the CLF1, CLF2, and CLF3 rabbit groups was 8.75; 10.04; and 10.43% significantly (P<0.05) higher than in the CLF0 rabbit group. The average jejunal crypt depth in rabbit groups CLF1, CLF2, and CLF3, respectively, was 10.81; 8.57; and 11.87% significantly (P<0.05) increased compared to the group of rabbits given concentrate without carrot leaf flour (CLF0).

DISCUSSION

Increasing the level of carrot leaf flour in the concentrate significantly increased slaughter weight, carcass weight, and rabbit carcass percentage. Providing concentrate can increase the availability of nutrients for rabbits, so that growth and rabbit carcass weight can be optimal (Nuriyasa et al. 2018). Calislar (2019) research shows that the high beta-carotene content in CLF can increase the body's immunity and prevent acute respiratory infections in poultry. In contrast, Qisthon (2012) reported that giving concentrate to rabbits had no effect on the rabbit's body weight.

Carcass percentage is an important variable in determining the performance of the rabbit carcass produced. The higher the carcass percentage value, the more economic value of rabbit farming. Rabbit carcass and meat characteristics are associated with different categories of rabbit body size when slaughtered (Nuriyasa et al. 2018).

Carrot leaves contain quite high levels of phytochemical compounds, such as flavonoids and beta carotene (Wibawa 2022). Phytochemical compounds in herbal leaves can inhibit pathogenic microbes and endotoxins in the gastrointestinal tract and increase pancreatic activity in broilers (Grashorn 2010). Several studies have found that to obtain optimal and efficient rabbit growth, 60-80% of the forage given is given, while concentrate is 20-40% of the total amount of feed given. The presence of CLF in the concentrate can improve the quality of the concentrate. Supplementation with cold-press carrot seed oil resulted in positive changes in weight gain, carcass percentage, and shelf life of chicken breast meat (Ürüşan et al. 2018).

The inclusion of CLF in concentrates can increase the amount of carcass meat and meat bone ratio. Carrot leaf flour contains phytochemical compounds which can reduce the number of pathogenic bacteria in the intestine, so that nutrient absorption is optimal. Concentrate supplementation combined with carrot leaf herbs will improve the morphology of the small intestine using different mechanisms (Karukarach et al. 2016), so that nutrient absorption can be optimal. Besides that, the age of the rabbit when slaughtered includes the age of the growing rabbit, so that meat synthesis is optimal.

The meat to bone ratio of rabbits receiving CLF increased significantly. According to Wahyono et al. (2021), rabbits should be slaughtered at a body weight greater than 2kg, because a slaughter weight smaller than 2kg results in a low meat bone ratio (MBR). It was also reported that the MBR ratio of New Zealand White rabbits was in the range of 2.17-2.55. In the research of Wibowo et al. (2014), the MBR ratio produced by New Zealand White rabbits ranges from 2.30-2.88. At a more mature age (slaughter weight >3 kg), New Zealand White rabbits can produce MBR in the range 5.82-5.96 (Brahmantiyo et al. 2017). There were no differences in MBR between several breeds of rabbits selected randomly at 63 days of age (Zotte et al. 2015). Royadi et al. (2016) reported that the higher the MBR value, the higher the quality of the rabbit carcass, because the amount of meat produced is higher. The MBR value of rabbits slaughtered at 12 months of age was apparently higher than those slaughtered at 6 months of age. The supply of nutrients from the fetus to the suckling child apparently influences carcass characteristics and meat quality (Metzger et al. 2011).

The blood lipid profile (triglycerides, LDL, and HDL) in this study did not show any differences, except for total cholesterol which significantly decreased with the presence of CLF in the concentrate. The saponin compound in CLF acts as an antinutrient which can reduce digestion and lipids, thereby reducing the fat and cholesterol content in yolk (Teteh et al. 2013). Apart from the phytochemical compounds in herbal leaves, cholesterol levels can also be reduced by the crude fiber content in the forage plants eaten by rabbits. According to Mandey et al. (2017), increasing crude fiber levels in feed can reduce feed consumption, percentage of abdominal fat, and LDL-blood cholesterol, but does not affect final body weight. The high phenolic content and the presence of alkaloids, saponins, flavonoids and triterpenoids have anti-bacterial properties (Yuniza and Yuherman 2015).

Ermayanti et al. (2021) found that the length of rabbit duodenal villi ranged from 3079.0-3668.0µm, while the depth of duodenal crypts ranged from 1041.0-1530.8µm. Meanwhile, according to Makovicky et al. (2014), the average height of intestinal villi in rabbits aged 49 days ranged from: 460.29-461.38µm; 56 days old ranged between 511.58-643.14µm; aged 63 days ranged between 545.17-574.03µm; and 70 days old ranged between 578.29-609.42µm. Meanwhile, crypt depth at age 49, 56, 63, and 70 days ranged between 116.28-128.36µm; 129.68-155.56µm; 109.97-137.18µm and 116.64-145.26µm. Rajput et al. (2013) stated that the depth of the crypts shows the speed of tissue repair in the villi when peeling occurs. A similar thing was reported by Sherwood et al. (2013) that new cells that are continuously formed at the base of the crypts migrate up the villi to replace old cells that are sloughed off. New cells are formed due to high mitotic activity in the crypts. Thus, the histomorphometric increase in the rabbit duodenum in this study was probably caused by increased mitotic activity in the crypts. Makovicky et al. (2014) reported that villi height, crypt depth, and small intestine length in rabbits changed with the intensity of food restriction and age. Different fiber components influence the height of the villi and the thickness of the muscle layers of the jejunum and colon, as well as influencing the depth of the crypts of the duodenum and ileum. Significant damage to the surface of the villi in the duodenum and jejunum due to lignin supplementation indicates significant damage to the cecum mucosa due to cellulose, pectin and alfalfa supplementation. Flattened colon villi were seen in the low dietary fiber group, and increasing fiber content did not show a significant effect (Gidenne 2015). According to Adibmoradi et al. (2006), supplementation of herbal leaf flour (garlic) in feed significantly increased the height of the villi and the depth of the underground space and reduced the thickness of the epithelium and the number of goblet cells in the broiler duodenum.

Conclusion

It can be concluded that the addition of 20-30% carrot leaf flour (*Daucus carota*) to the concentrate can increase the carcass percentage, the amount of carcass meat, and the meat bone ratio (MBR). On the other hand, it can reduce serum cholesterol and pathogenic bacteria in the intestines of rabbits.

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Authors' Contributions

All authors (NWS, IGNGB, NWS and NND) contributed equally to the research and writing of this article.

Conflict of Interest

All manuscript authors have no conflicts of interest.

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