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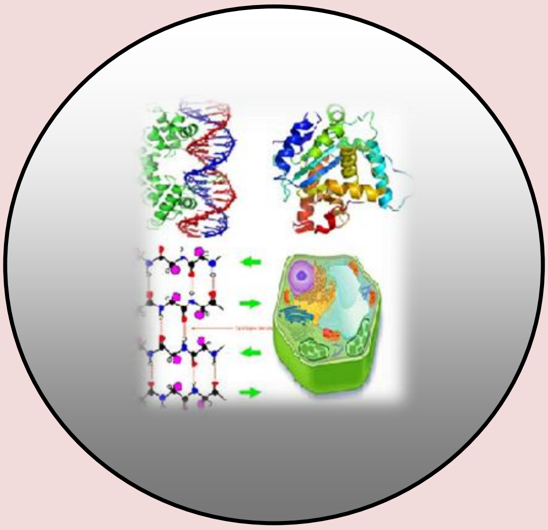

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# Growth Performance, Meat Cholesterol and β-Carotene Content in Rabbit Fed With Carrot Leaves, Grass, and Concentrates

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## ABSTRACT
*The present study was conductive the effect of giving carrot leaves to increase performans and the content of β-carotene in meat of local rabbits up to 5 weeks of age. This study used a feeding trial using 120 local 5-week old rabbits in a completely randomized design with four treatments and 6 replications. The four treatments were rabbits fed: A: 100% Carrot leaves; B: 70% Carrot leaves+30% Concentrate; C: 100% Local-grass; and D: 70% Lokac-grass+30% Concentrate, respectively. The results showed that cholesterol levels in rabbit meat and feed consumption among treatment groups were not significantly different (P>0.05). Final body weight and weight gain in group A were significantly different (P <0.05) higher than group C. Also feed efficiency in treatment group A was significantly different (P<0.05) higher than group group C. Rabbit meat treatment B had the highest beta carotene content (P<0.05), followed by treatment A, D, and C, respectively. It was concluded that rabbits fed with carrot leaves and supplemented with concentrate could improve performance and increased beta-carotene content in rabbit meat compared with local-grass feed.*
*Key words: β-karoten, vitamin A, carrot leaves, Grass and Rabbits.*

## INTRODUCTION
Increasing attention has been paid recently to issues related to the presence of natural biologically active substances in animals feed mixture, which would enrich products of animal origin and at the sometime improve animals' health (Lee *et al*., 2017). In the past few decades, much attention has been paid to rabbit farming to increase production without negatively affecting meat quality and animal health. In recent years, research has been carried out on the addition of natural antioxidants (carrot leaves) as nutritional supplements in animal feed to improve performance, health, meat quality, and shelf life of raw meat products (Marliyati *et al*., 2012). In the pattern of intensive livestock raising, the largest production costs come from feed, which is equal to 60-70%. Therefore, efforts need to be made to improve feed efficiency or decrease feed costs. Rabbits are an option to be cultivated, because the feed does not compete with human needs. The provision of high forage in rabbit livestock can improve the efficiency of feed use rabbit can be maintained by providing forage feed combined with agricultural waste. One of the agricultural wastes that can be utilized, namely carrot leaves (*Daucus carota*).

Carrot leaves contain crude protein 18.71%; crude fiber 15.69%; crude fat 3.19%; and ash 33.58% (Sartika *et al*., 2013). The data shows that carrot leaves have a higher protein content and low crude fiber compared to field grass, so carrot leaves can be a good alternative feed as rabbit feed.

Carrot leaves can be a potential feed in the rabbit's diet to improve the performance and quality of rabbit meat, because carrot leaves contain high antioxidants and beta carotene. Carrots (*Daucus carota L.*) is a type of vegetable that is well-known as a source of provitamin A (carotenoids). Carotenoid content is very high and much higher than tomatoes. In addition to the high carotenoid content, the production of carrots in Indonesia is quite abundant. It was reported by Mancini *et al*. (2018), that antioxidants high in Ginger turned out to improve the performance and quality of rabbit meat. The results of β-carotene analysis using the HPLC method showed that the content of β-carotene in carrot cells was 20550 µg/100 g. This value is classified as lower when compared to the content of β-carotene in carrot chips, which is in the range of 25196-43804 µg β-carotene/100 g carrot chips (Sulaeman *et al*., 2001). If converted into vitamin A, carrot powder contains vitamin A of 3425 RE or 1712.5 RAE (Marliyati *et al*., 2012). Beta-carotene is a pigment found in plants that gives yellow and orange fruits, and vegetables with similar colors. Beta-carotene is converted in the body into vitamin A, a powerful antioxidant that plays an important role in maintaining eye health, skin, and neurological functions.

Shete dan Quadro (2013) reported that β-carotene is the most abundant provitamin A carotenoid in human diet and tissues. It exerts a number of beneficial functions in mammals, including humans, owing to its ability to generate vitamin A as well as to emerging crucial signaling functions of its metabolites. Carotenoids are C40 tetraterpenoid pigments that are found in plants, fungi and bacteria. Mammals obtain carotenoids predominantly through foods of plant origin (Stahl dan Sies, 2012). In plants, these compounds accumulate in the plastids giving the characteristic bright yellow, red and orange color to many fruits and vegetables. Even though β-carotene is generally considered a safer form of vitamin A due to its highly regulated intestinal absorption, detrimental effects have also been ascribed to its intake, at least under specific circumstances. A better understanding of the metabolism of β-carotene is still needed to unequivocally discriminate the conditions under which it may exert beneficial or detrimental effects on human health and thus to enable the formulation of dietary recommendations adequate for different groups of individuals and populations worldwide. Here we provide a general overview of the metabolism of this vitamin A precursor in mammals with the aim of identifying the gaps in knowledge that call for immediate attention. Beta-carotene as a carcass color active ingredient (Ayssiwede *et al.,* 2011), so it is very important that its role in increasing the color of rabbit carcass from white to red is very preferred by consumers.

Herbal feed additives have attracted increasing interest as alternative dietary supplementation in animal production because they have attained more acceptability among consumers as natural feed additives. The supplementation of alfalfa flavonoids in rabbit diets improved muscle oxidation stability of the frozen meat in a dose-related manner without affecting growth performance. Alfalfa extract is a suitable herbal additive for rabbit feeds with positive impact on qualitative characteristics of rabbit meat (Lee *et al*., 2017). The aim of this study was to study the use of carrot leaves as a source of natural β-carotene in diets based concentrates on performance, and rabbit meat quality.

## MATERIALS AND METHODS

**Animals and experimental design.** One hundrid and twenty lokal rabbits of 35 days old were randomly allotted into four groups and housed in cages. Groups were fed *ad libitum*, and the rabbits were randomly divided into four groups A: 100% Carrot leaves; B: 70% Carrot leaves+30% Concentrate; C: 100% Lokal grass; and D: 70% Lokal grass+30% Concentrate, respectively. Water was available *ad libitum* from nipple drinkers. The ingredients and chemical compositions of experimental diets are shown in Table 1. Each treatment consisted of six replicate pens with five rabbits per pen at 100 cm×50 cm×40 cm height. Each experimental diet was in pellet form and the rabbits had free access to feed and water throughout the experiment. Body weights and feed intake were registered weekly.

During the experiment, the live weight and feed intake were recorded individually on a fortnightly basis. Performance data were recorded over 84 days. Mortality was recorded daily throughout the experimental period. The average daily feed intake, average daily gain, and feed conversion ratio were calculated. Data pertaining to any animal that died were excluded from the calculations of the growth performance parameters.

**Live performance.** Continuous lighting and access to feed and water was provided throughout the experiment. Body weight, live weight gains, feed intake and feed conversion ratio for rabbits were recorded separately from week 1 until week 8. Feed consumption (gram per rabbit) was recorded weekly at each replication by weighing the remaining diet. The total feed intake for each replicate was measured during the test experiment period. The FCR was calculated as gram of feed consumed of per gram body weight gained. Ingredients of the experimental diet were identical as in our previous trial and chemical composition is shown in Table 1.

**Table 1. Ingredients and calculated nutrient content of the feed of growing rabbits up to five weeks old.**

| Ingredients | Groups[1] | | | |
|---|---|---|---|---|
| | A | B | C | D |
| Concentrate (%) | - | 30 | - | 30 |
| Carrot (*Daucus carota*) (%) | 100 | 70 | - | |
| Local-grass (%) | - | - | 100 | 70 |
| *Total* | *100* | *100* | *100* | *100* |
| *Chemical composition* * | | | | |
| DM (%) | 92,08 | 91,56 | 93,58 | 93,13 |
| Gross energy (Kcal/g) | 3,5954 | 3,69284 | 3,7142 | 3,7760 |
| Crude protein (%) | 19,51 | 22,50 | 11,04 | 16,57 |
| Ether extract (EE) (%) | 16,23 | 16,48 | 12,03 | 13,54 |
| Crude fibre (CP) (%) | 14,46 | 11,58 | 25,71 | 19,46 |

*) Laboratorium analisis

1 A: 100% Carrot leaves; B: 70% Carrot leaves+30% Concentrate; C: 100% Local-grass; and D: 70% Local-grass+30% Concentrate

At the end of the feeding experiment, rabbits were weighed and feed consumption was recorded by replicate to calculate average daily gain (ADG), average daily feed intake (ADFI) on the basis of DM, and feed conversion ratio (FCR, feed DM intake/weight gain). Rabbits were deprived from feed for 12 h (water was provided) before weighing to ensure the emptying of the digestive tract of the bird. Mortality was recorded on a daily basis as it occurred. Any rabbit that died or was removed was weighed and used to correct FCR.

**Slaughter procedures**

At 84 days of age, 12 rabbits per group with a weight close to the average of the group (mean weight 330.83±5.67g) were selected and slaughtered, in a commercial slaughterhouse. The slaughtered rabbits were bled and the skin, genitals, urinary bladder, gastrointestinal tract and distal part of the legs were removed. The carcass was weighed, while the skin and full gastrointestinal tract weights were recorded and expressed as a percentage of slaughter weight . The carcasses (with head, thoracic cage organs, liver, and kidneys) were chilled at 4°C for 24 h in a refrigerated room. The chilled carcass weight was recorded, and the dressing out percentage was calculated as the ratio between chilled carcass weight and slaughter weight. Head and liver weight were expressed as percentages of chilled carcass weight. The head, thymus, trachea, esophagus, heart, lungs, liver and kidney weights were removed from the chilled carcass weight in order to obtain the reference carcass weight.

At 1 day post mortem, left and right *Longissimus thoracis* et lumborum muscles were dissected and used for meat quality assessments. Longissimus thoracis et lumborum muscle of each animal was individually packaged in Styrofoam trays overwrapped with polyethylene film and stored at $4^0$C up to seven days (namely eight post mortem days) after been divided in sub-samples. Meat was analysed at days 2 post mortem days for β-carotene.

**Statistical analysis**
Data collected was subjected to Analysis of Variance and if significant different (P<0.05) among the treatment group was noted, they were then underwent further statistical analysis following Duncan's Multiple Range Test (Steel and Torrie, 1989).

## RESULTS
No significant differences (P>0.05) were recorded for the feed consumption and meat cholesterol traits, except for final body weight, live weight gains, feed efficiency, and β-caroten concentration in meat between teratment groups (Table 2). Dietary carrot leaves supplementation significantly (p<0.05) affected the body weight gains, feed efficiencies, and levels of the β-caroten in the meat of rabbits recorded rather than grass feed groups (group C and D).

**Table 2. Effect of carrot leaves and local-grass supplemented with concentrations on performance and meat quality in local rabbits.**

| Variables | Groups[1] | | | | SEM[2] |
|---|---|---|---|---|---|
| | A | B | C | D | |
| Initial body weight (g/head) | 331a | 329a | 328a | 332a | 1,424 |
| Final body weigt (g/head) | 1677,0b[3] | 1779,5a | 1438,25c | 1651,5b | 12,891 |
| Body weight gains (g/head) | 1346,0b | 1450,5a | 1155,3c | 1319,5b | 12,354 |
| Feed consumption (g/head) | 5087,00a | 5094,25a | 5145,50a | 5043,00a | 52,526 |
| Feed conversion ratio (feed consumpsion : live weight gains) | 3,78bc | 3,51c | 4,45a | 3,82b | 0,062 |
| Meat cholesterol (mg/dg) | 48,07a | 52,11a | 49,22a | 51,86a | 2,949 |
| Beta-carotene (mg/100 g) | 36,03b | 40,98a | 25,86c | 27,77c | 1,55 |

Note:
[1] A: 100% Carrot leaves; B: 70% Carrot leaves+30% Concentrate; C: 100% Local-grass; and D: 70% Local-grass+30% Concentrate
[2] SEM: standard error of treatment means
[3] Means with different superscripts within raw values are significantly different (*P*<0.05)

Based on Table 2. The final body weight of rabbits in group C was 14.24% significantly different (P<0.05) lower than group A, and the final weight of rabbits group D did not show significant difference (P>0.05) with group A. Supplementation of concentrates in carrot leaf feed (group B) increased rabbit end weight 6.11% significantly different (P<0.05) higher than group A. Live weight gains inrabbit group C was 14.17% significantly different (P<0.05) lower than group A, while group D did not show significant difference (P>0.05) with group A.
Efficiency of feed use (ration consumption/weight gain) of rabbits in group B was 7.14% significantly different (P<0.05) higher than group A and treatment C was 17.72% significantly different (P<0.05 ) lower than rabbits in group A.
The content of β-carotene in rabbit meat in group B was 13.74% significantly different (P<0.05) higher than group A, and the content of β-carotene in rabbit meat in group C was 28.23% significantly different (P<0.05) lower than group A.

## DISCUSSION
The growth of rabbits fed with carrot leaves and real concentrates was higher compared to field grass. This is because carrot leaves contain phytochemical compounds, such as flavonoids and beta carotene which are quite high compared to grass. As reported by Xiong *et al*. (2012) and Zhu *et al.* (2009) that alfafa flavonoids with estrogenic effect, was shown to significantly promote growth performance, improve carcass quality, remove free radicals and increase body's antioxidant and enhance immunity in a certain range of additive on livestock. The meat color parameters in the present study were not affected significantly by alfalfa flavonoids supplementation except for lightness that resulted lower in the control group (Lee *et al*., 2017).

However, the results that were not significantly different were reported by Ouyang *et al*. (2016) who showed that Alfalfa flavonoids supplemental diet did not affect the meat color of the breast meat of broiler chickens. Carotenes (such as β-carotene, α-carotene, and β-cryptoxanthin) are non-oxygen carotenes which may be linear or have cyclic hydrocarbons at one or both ends of the molecule. Xanthophils (such as lutein, zeaxanthin, meso-zeaxanthin, astaxanthin and canthaxanthin) are carotene oxygen derivatives (Von-Lintig, 2013). Beta-carotene and other carotenoids, have antioxidant activity and the ability to prevent chronic diseases. Beta-carotene protects the body from damaging free radicals, which are a major cause of aging and degeneration. Research has shown an inverse relationship between the presence of various cancers and carotenoid diets or blood carotenoid levels (Bohm *et al*., 2012). Dabbou *et al.* (2018) repoted that the dietary inclusion of alfalfa flavonoids in rabbit diets improved muscle oxidation stability with no adverse effects on the growth performance of the animals even if a slight impact on meat lightness color parameter was recorded. Absorption of β-carotene by the body will increase with increasing fat intake, because β-carotene dissolves in fat. βeta-carotene is widely used as a supplement because it is one of the antioxidants that has benefits to prevent various diseases, increase endurance, and provide analgesic and anti-inflammatory effects. The properties of insoluble and easily oxidized β-carotene in water are a problem in β-carotene formulations for oral applications related to dissolution, absorption, and bioavailability in the body (Dixon *et al*., 2013). Patil *et al*. (2010) reported that cholesterol and triglyceride reduction by alkaloids was partly due to reduced lipogenic enzyme activity and increased excretion of bile acids in feces. The presence of beta-carotene in herbal extracts can lower cholesterol levels in the blood because it inhibits the action of HMG-CoA reductase enzyme that plays a role in the formation of mevalonate in cholesterol biosynthesis in the liver. The results of laboratory analysis showed that carrot leaves contain phytochemical compounds, such as flavonoids, fenolik, terpenoid, steroid, tannins, dan beta carotens (676 mg/100 g). Phytochemical compounds of flavonoids, saponins, tannins, estrogen-like flavonoids were able to slow down the reduction of bone mass (osteomalacia), reduce blood cholesterol levels, and increase HDL levels, while saponins proved efficacious as anticancer, antimicrobial, and reduce blood cholesterol levels (Santoso *et al*., 2002; Bidura *et al.,* 2017). Hestera (2008), Cervantes-Valencia (2015) reported that the use of phytochemical properties in *Moringa oleifera* and Curcumin leaves in feed can reduce cholesterol content of chicken meat. Xanthophils (such as lutein, zeaxanthin, meso-zeaxanthin, astaxanthin and canthaxanthin) are carotene oxygen derivatives (Von-Lintig, 2013). The results of Chelry *et al.* (2015) showed that the supply of carrot leaves in the ration gave the best results in terms of consumption of nutrients, weight gain and feed conversion in rabbits. Firmansyah *et al*. (2015) reported that local rabbits given additional feed soursop leaf could increase the intensity of color of rabbit meat compared to without the addition of soursop leaf flour. Supplementation of concentrates in forage feed can improve rabbit performance (group B and D). According to Priyatna (2011), in order to increase the performans of rabbits, feeding must be arranged so that it is balanced between forage and concentrate. Study found that forage as much as 60-80%, while concentrations as much as 20-40% of the total amount of feed given, can provide the best results. Weight gain and feed efficiency in group C rabbits were the lowest compared to other group rabbits. This is due to the high crude fiber content in the grass which can reduce feed digestibility. Mandey *et al.* (2017), states that an increase in crude fiber content in feed can reduce feed consumption, percentage of abdominal fat and LDL-blood cholesterol, but does not affect final weight. Inclusion of dietary fiber to pig diets increases fiber fermentation and the availability of desirable microbiota in the gut (Nahm, 2003). Increased crude fiber content in group C (100% grass) reduces rabbit performance. As reported by Mpendulo *et al.* (2018), as the level of fiber inclusion increased, fecal consistency and nitrogen content increased linearly. Urea nitrogen decreased as the inclusion level increased across all the fibers. As dietary fiber content increased, fecal nitrogen content also increased. At high fiber inclusion levels, pigs are expected to consume more feed to compensate for the reduction in nutrient density and for them to meet nutrient satiety. The gut capacity of the rabbit, however, prevents them from continuing to increase voluntary feed intake. Consequently, growth performance starts to drop. The roles of such common fiber sources on excreta characteristics, however, still remain unclear (Mpendulo *et al*., 2018). One week of intensive feed restriction in early weaned rabbits affects some morphological muscle fibre characteristics but has no effect on performance, carcase traits, and meat quality (Chodová *et al*., 2019).

Adibmoradi *et al*. (2016) reported that type of fibre influenced the performance and digestive traits of broilers with effects varying in accordance with the level of fibre. Rice hulls inclusion consistently improved growth performance and CP digestibility in broilers regardless of dietary inclusion level. However, barley hulls at 1.5% level improved crypt depth: villous height ratio and feed conversion ratio had an opposite effect on villous height.
The beneficial effects of hulls were more obvious with the 1.5% dietary inclusion level than the 0.75% level. The useful effects of rice hulls inclusion on broiler efficiency was mainly because of its effect on CP digestibility while barley hulls at 1.5% level positively changed jejunum morphology. The data suggest that broilers may have a nominal requirement for insoluble fibre, however, the source and dietary inclusion rate of fibre might be important factors to satisfy dietary fibre requirements in birds (Adibmoradi *et al*., 2016).

## CONCLUSIONS
The authors concluded that rabbits fed with carrot leaves and supplemented with concentrate could improve performance and increased β-carotene content in rabbit meat compared with Local-grass feed.


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