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Original Research

Feeding of Culled Carrots to Growing Rabbits: Effect on Growth, Nutrient Utilization, and Blood Biochemical Profile

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Abstract

The effect of feeding culled carrots on the growth, nutrient utilization, and blood biochemical profile of rabbits was analyzed. In the 90 d of the feeding trial, 21 New Zealand White rabbits (Oryctolagus cuniculus) were divided into three groups of 7 rabbits each. Rabbits in group I were only fed with concentrate mixture, whereas, those in groups II and III were fed with concentrate mixture mixed with culled carrots @ 10% and 20% (on DM basis) respectively. Green berseem was offered ad-lib to fulfill the remaining nutritional requirements. The proximate composition, total phenolic contents, and DPPH assay of the culled carrots were estimated before feeding. The body weight gain and nutrient digestibility were estimated at the middle of the feeding trial. Blood biochemical profile and antioxidant activity in rabbit blood were measured at the start and end of the feeding trial. There was no difference in feed intake, body weight gain, or digestibility of nutrients among all groups. The serum cholesterol level was found to be significantly lower ($p<0.05$) in culled carrot-fed groups (II and III) whereas glutathione peroxidase and catalase activity showed a significant increase ($p<0.05$) in treatment groups than the control (CON) group. However, serum levels of ALT, AST, and ALP were found comparable among all three groups. In conclusion, the feeding of culled carrots at 10 and 20% levels in the concentrate feed to the rabbits resulted in reduced serum cholesterol levels and improved antioxidant status without adverse effects on growth and nutrient utilization.

Keywords: Antioxidant, Culled Carrot, Rabbits, Serum Cholesterol.

Introduction

Globally, the livestock population has been increasing consistently over the years (Kaasschieter *et al.,* 1992; FAO, 2006). However, this increase in livestock population has not always been paralleled by an improved and cost-effective availability of livestock feed resources. Due to huge population pressure on both humans and livestock, there is a severe shortage of feed and fodder in the country, and animals suffer from malnutrition which results in lower production. Furthermore, farmers are reluctant to purchase concentrate feed because of the continuous increasing the price of concentrate feed ingredients (maize, wheat, soybean meal, and other cakes, etc.). So, under such conditions, there is a need to search for alternative feed to reduce the cost of livestock feeds and ultimately reduce the cost of livestock production.

Carrot is an important biennial or annual edible herb with thick fleshy taproot cultivated during winter and summer or spring in hot (tropical) and cold (temperate) regions, respectively. The carrots during harvesting, processing, and marketing produced 10-15% waste which is suitable for livestock feeding and contains 10% crude protein and 17.1 MJ /kg DM of Gross energy (Bakshi *et al.*, 2016). Salisu *et al.* (2012) reported that the 15% inclusion of carrot leaf meal in the diet of growing rabbit meal had good performance in terms of average daily gain, feed intake, and carcass weight. Similarly, the inclusion of carrots in the diet of rabbits shows improvement in feed intake and average daily gain (Goby and Gidinne, 2008; Owoleke *et al.*, 2016). So, carrots could be considered a good source of nutrients for the growing rabbit.

Carrot is a good source of dietary fibre and has the second-highest level of β-carotenoids among vegetables. The fibre represents 3-4% of carrots by weight, of which 48% is soluble and 52% is insoluble fibre (Nicolle *et al.,* 2003). Carotenoid content ranges from 5.33 – 16.4 mg/100g in which α-carotene accounts for 3.2 – 3.9 mg/100g and β-carotene for 6.5 – 7.1 mg/100g. Carotenoids prevent the undesirable oxidation of LDL (low-density lipoprotein) and thus help to maintain normal blood cholesterol levels and prevent arterial blockages. Several studies have shown that binding of the viscous water-soluble fraction of fibre with cholesterol induces a lowering of plasma cholesterol level (Fernandez *et al.,* 1995 Mazur *et al.*, 1990). It is rich in antioxidant vitamins such as vitamin E (513 ug/100g), vitamin C (7 mg/100g), thiamine, riboflavin, niacin, and folic acid (Alasalvar *et al.,* 2001). These antioxidant micronutrients reduce the risk of cardiovascular ailments, probably by protecting lipoproteins from peroxidation, quenching free radicals, and arresting cellular damage.

Experiments on lipid metabolism in rats that were fed with carrots @ 15 percent on a dry matter basis revealed a reduction of 44% in cholesterol and 40% in triglyceride level in the liver, with a concomitant 30% increase in the total steroid excretion in faeces. Reduction in apparent cholesterol absorption in the CON group was also noticed (Nicolle *et al.,* 2003). In the rat, with experimental liver damage induced by carbon tetrachloride where oral administration of carrot juice (25 ml/ kg BW) resulted in the reduction of the concentration of serum concentration of hepatic enzymes *viz.* alanine aminotransferase, aspartate aminotransferase, alkaline phosphatase and gamma-glutamyl transferase (Abo-Golayel and Al-Khayat, 2014). In addition, feeding of carrots to rats experimentally challenged with DMH (1, 2-dimethylhydrazine hydrochloride) showed an increase in the activity of glutathione peroxidase and catalase in the liver (Anilakumar *et al.,* 2000). The culled carrots which are not suitable for human consumption are cheaply available in the vegetable markets and no deleterious effect has been reported so far due to their feeding in animals. Therefore, the present study was proposed to study the effect of feeding culled carrots (*Daucus carota*) on growth, nutrient utilization, and blood biochemical profile in NZW rabbits.

Material and Method

Animal and Diet

Twenty-one male New Zealand White (NZW) rabbits (average age 45±3 d and average body weight 711±33 g) were divided into three groups of 7 animals each. Group I was served as the CON group wherein rabbits were fed with the concentrate mixture without carrot, whereas, rabbits in groups II and III were fed with carrot @ 10% and 20% level (DM basis) respectively, along with the concentrate mixture. Green berseem was offered *ad-lib* to rabbits in all study groups. An iso-nitrogenous experimental diet (concentrate mixture) was prepared (Table 1) to provide 18% crude protein and 12% crude fiber to all experimental rabbits as per ICAR 2013 requirements. Every day, fresh carrots were purchased from the vegetable market, chopped into small pieces, and fed to rabbits. The rabbits were individually offered weighed quantity of diet at 9.00h and the residue was weighed the next morning to record daily feed intake. Rabbits were weighed at fortnightly interval during experiment. A digestion trial of 4 days was conducted after an adaptation period of 2 days at the middle of the feeding trial.

Table 1: Composition of concentrate mixture

Ingredients	Concentrate mixture (%)
Maize	43
Soybean meal	18
Wheat bran	36
Mineral mix.	2
Salt	1
Total	100

Table 2: Nutrient composition of feeds (DM basis) used in the experiment.

Components (%)	Concentrate mix.	Carrot	Berseem (green)
DM	95.98	8.01	14.03
OM	95.67	89.62	88.53
CP	17.98	9.53	17.03
EE	3.25	2.02	4.18
CF	8.43	10.40	21.17
Total ash	4.33	10.38	11.47
NDF	25.50	12.18	46.88
ADF	9.48	10.69	22.44

Housing Management

The experimental rabbits were randomly distributed to different dietary treatments by placing them in individual metallic cages to facilitate the exact record of feed intake and faeces output of each rabbit. The experimental rabbits were provided 12 hours of light and 12 hours of dark throughout the experimental period of February 2020 to April 2020. During this period average weather temperatures were 18-31°C so the ceiling fan was put on during the trial.

Chemical Analyses

Representative samples of feed were analyzed for its dry matter (DM), organic matter (OM), crude protein (CP), ether extract (EE), crude fibre (CF), and total ash contents as per AOAC (2005) and neutral detergent fibre (NDF) and acid detergent fibre (ADF) by Goering and Van Soest's method (Goering and Van Soest, 1970) and the estimates obtained are listed in Table 2).

Estimation of Total Phenolics

An extract of carrots was prepared using 70% aqueous acetone. The total phenols (TP) in carrot extract were estimated by the Folin-Ciocalteau method in combination with polyvinyl polypyrrolidone (PVPP), with tannic acid (gallic acid) as a reference standard (Makkar, 2003). A calibration curve of gallic acid was prepared, and the results were expressed as mg GAE (gallic acid equivalents/g DW).

DPPH Assay

Methanolic extract of carrot was prepared to determine its antioxidant activity against DPPH radicals using the previously described method (Sharma and Bhat, 2009). The antioxidant activity was expressed as % inhibition of DPPH radical.

The total phenolic content in the carrots was 106.74 mg gallic acid equivalent (GAE)/ 100 g DM, while antioxidant activity was 68.2% inhibition as per the DPPH assay.

Blood Biochemical Parameters

At the end of the trial period (90 d), rabbits were physically restrained on a table without using any sedatives to aseptically collect the blood from the ear vein, into the 'EDTA', 'sodium fluoride', and 'serum plus' blood collection tubes (BD Vacutainer, Franklin Lake, NJ, USA), for haematology and blood glucose estimation and serum separation, respectively. After 4 hours, serum plus vials were centrifuged at 1008 g for 10 min to harvest the serum. Until further study, the harvested serum samples were stored at -20 °C.

Clinical Chemistry Indices

Serum Cholesterol

The total cholesterol concentration in serum samples was estimated using a diagnostic kit (RCHO1040, Coral Clinical system, Pantnagar, Uttarakhand, India) following manufacturer guidelines.

Serum Enzymes

The serum concentration of hepatic transaminases *viz.* AST (Aspartate transaminase) and ALT (Alanine aminotransferase) were estimated by using a Coral diagnostic kit (RGOT1016/RGPT1016, Coral clinical system, Pantnagar, Uttarakhand, India) following manufacturers guidelines.

Antioxidant Profile

Antioxidant enzyme (glutathione peroxidase and catalase) activity of the blood samples was estimated by the method of Paglia and Valentine (1967) and Bergmayer (1983), respectively within an hour of blood collection to obtain realistic estimates. The reagent containing hemolysate was added with 100 µl H_2O_2 and the absorbance of the mixture was measured spectrophotometrically at 340 nm. Catalase activity of the RBC was estimated by measuring the absorbance of the solution containing hemolysate and 1000 µl H_2O_2 spectrophotometrically at 240 nm.

Statistical Analysis

Data generated from the study were subjected to the analysis of variance (ANOVA) as per the standard statistical procedure Snedecor and Cochran (2004) using SPSS (version 20.0) software. The means of different treatment groups were compared by using Tukey's posthoc test (1953).

Result and Discussion

Effect of Culled Carrot Feeding on Growth Performance

The study revealed that irrespective of the level of incorporation of culled carrots in the feed, the growth rate of rabbits among Control (group I) and experimental groups were statistically similar ($p>0.01$) as indicated by the comparable average daily gain (ADG) of 13.42 ± 0.07, 13.41 ± 0.16 and 13.44 ± 0.07 g/day among control and treatments groups, respectively (Fig.1). Salisu *et al.* (2012) reported the lower ADG (8.03 to 12.20 g/d) in the growing rabbits fed carrot leaf meal. Goby and Gidenne (2008) reported higher ADG (37.7 to 39.9 g/d) in the rabbits fed dried carrots. This shows that culled carrots incorporation as such had no unfavourable effect on the growth. The lower growth rates in the present study than the others reported on different diets thus can be attributed to poor genetic makeup along with tropical environmental conditions as reported by Lukefahr *et al.* (1982) and Cheeke (1986), who observed 10-20 g ADG in tropics as against 35-40 g under temperate climate due to heat stress, Moreover, lower performance could be expected while using mash form of diet instead of pelleted form with the similar composition (Cheeke, 1987).

Effect of Feeding Culled Carrot on Nutrient Intake and Digestibility

In the present study, the nutrient intake DM, OM, CP, CF, EE, NFE, NDF, and ADF, and their digestibility were found statistically similar among all three groups. Non-significant nutrient intake and digestibility could be due to the equal nutrient density of the diet, poor fibre digesting ability of an experimental animal, and saponification of fat in the gut of rabbits (Parigi-Bini *et al.*, 1974; Fekete *et al.*, 1990; Sayed, 2009; Abonyi *et al.*, 2012 and Abd El-

Latif *et al.,* 2012). Like any other species, the nutrient digestibility in rabbits is also influenced by dietary composition, forage quality, level of intake (Miller *et al.*, 1954), energy, protein and fibre density, rate of passage, and caecal retention (Aguilera *et al.,* 1970). In addition to all these factors, the age of the animal and climatic conditions also affects nutrient digestibility. Average daily nutrient intake and their digestibility are presented in Table 3.

Table 3: Effect of feeding culled carrot on intake and digestibility of nutrients

Sr no.	Parameter	Group I	Group II	Group III	SEM	p value
1	DM intake (g)	75.91±1.41	74.72±0.90	73.82±1.89	0.79	0.59
	DMD (%)	77.51±1.08	78.30±1.32	78.47±0.86	0.62	0.81
2	OM intake (g)	72.09±1.34	71.95±0.88	70.74±0.63	0.58	0.99
	OMD (%)	91.92±0.23	92.73±0.50	92.14±0.34	0.22	0.31
3	CP intake (g)	13.23±0.06	13.04±0.19	12.89±0.16	0.08	0.16
	CPD (%)	79.24±0.52	79.10±1.26	78.60±0.77	0.50	0.88
4	CF intake (g)	10.68±0.11	10.61±0.07	10.59±0.06	0.05	0.76
	CFD (%)	46.07±1.37	47.37±4.63	47.25±2.25	1.73	0.94
5	EE intake (g)	2.81±0.05	2.79±0.03	2.78±0.03	0.02	0.83
	EED (%)	84.72±0.92	86.34±1.32	84.23±0.29	0.54	0.93
6	NFE intake (g)	46.21±1.11	45.47±0.71	44.25±0.60	0.50	0.31
	NFED (%)	87.63±0.23	88.35±0.81	87.70±0.70	0.35	0.66
7	NDF intake (g)	22.67±0.75	22.93±0.47	22.28±0.45	0.32	0.89
	NDFD (%)	47.88±1.35	48.04±3.59	47.86±2.95	1.52	0.99
8	ADF intake (g)	10.91±0.16	10.88±0.13	10.86±0.13	0.08	0.98
	ADFD (%)	35.28±2.00	35.33±2.75	35.15±3.33	1.52	0.99

Effect of Culled Carrot Feeding on Serum Cholesterol

In the present study, the mean serum cholesterol concentrations (mmol/l) were found significantly ($p<0.05$) lower in the study groups fed with carrots, as compared to the control group at the end of the trial period. The concentration of serum cholesterol, serum enzymes, and blood antioxidants are presented in Table 4. Significant ($p<0.05$) interactions were seen between treatments and different periods. In this study, the rabbits fed with a diet containing 10 and 20 % of carrots exhibited a significant cholesterol-lowering effect (-16% and -22% decrease, respectively) compared to the control group. The result was found in agreement with the findings of Nicolle *et al.* (2003), where the rats fed with carrot (@15% DM) resulted in a reduction in serum cholesterol concentration. Similarly, the supplementation of carrots caused a significant reduction in serum cholesterol concentration in animals fed with a high-cholesterol diet (Wehr *et al.*, 1988). As per the available literature, the studies available at present about the effect of feeding carrots on the cholesterol level of rabbits are scanty. However, it is reported that feeding of fibre fraction (5.69 g/100 g of diet) derived from carrot pomace resulted in a 33.5% reduction of serum cholesterol level in hamsters (Hsu *et al.,* 2006). The reduction in serum cholesterol level observed in this study may be attributed to the viscous water-soluble fraction of fibre in carrots, which prevents cholesterol absorption from the gut epithelium (Fernandez *et al.,* 1995 and Mazur *et al.*, 1990)

Effect of Carrot Feeding on Serum Enzymes

In the present experiment, the mean serum concentration (U/l) of alanine aminotransferase, aspartate aminotransferase, and alkaline phosphatase were found comparable ($p>0.05$) among study and control groups. The interaction between treatment and period was also found to be non-significant ($p>0.05$). The higher serum levels of transaminases such as ALT, AST, and other enzymes like ALP enzymes indicate liver damage, bone disorder, and muscle damage respectively (Robertson *et al.*,1979 and Limdi and Hyde *et al.*, 2003). The serum concentration of ALT, AST, and ALP (U/l) in this experiment was well within the normal physiological range (Hewitt *et al.*, 1989; Giannini *et al.*, 2005; Shousha *et al.*, 2017 and Özkan *et al.*, 2012) indicating normal liver functioning in all the study groups. Further, the effect of carrot feeding on ALT, AST, and ALP levels in rabbits could not be compared with other studies, due to the paucity of literature, though similar findings were reported in mice (Bishayee *et al.*, 1995).

Table 4: Effect of culled carrot feeding on blood parameters

Parameters	Days	Group I	Group II	Group III	Mean	p value		
						T	P	T*P
Cholesterol (mmol/l)	0	1.94	1.83	1.78	1.85	0.00	0.017	0.012
	90	2.10	1.54	1.28	1.64			
	Mean	2.02^{a}	1.69^{b}	1.53^{b}				
ALT (U/l)	0	9.53	9.12	9.11	9.25	0.338	0.077	0.980
	90	9.04	8.74	8.69	8.82			
	Mean	9.29	8.93	8.90				
AST (U/l)	0	16.62	16.52	16.44	16.52	0.967	0.197	0.906
	90	15.94	15.45	15.38	15.59			
	Mean	16.28	15.98	15.91				
ALP (U/l)	0	18.10	17.53	17.29	17.64	0.377	0.384	0.978
	90	17.77	16.92	16.79	17.16			
	Mean	17.93	17.22	17.04				
GPx (U/mg Hb)	0	0.358	0.362	0.366	0.362	0.02	0.27	0.04
	90	0.292	0.439	0.441	0.391			
	Mean	0.325^{b}	0.401^{a}	0.403^{a}				
Catalase (U/mg Hb)	0	0.295	0.287	0.289	0.290	0.05	0.88	0.02
	90	0.199	0.341	0.342	0.294			
	Mean	0.247^{b}	0.314^{a}	0.315^{a}				

ab Within row overall means with different superscript letter differ significantly (P< 0.05); T –Treatment; P-Period

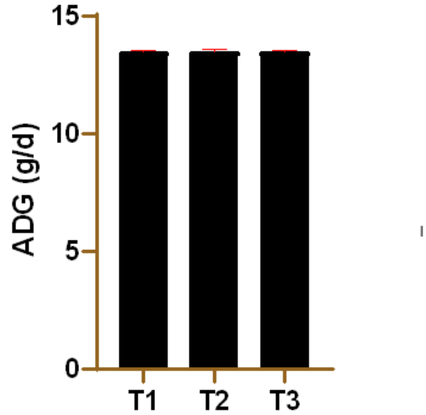

Fig 1: Average daily gain in rabbits

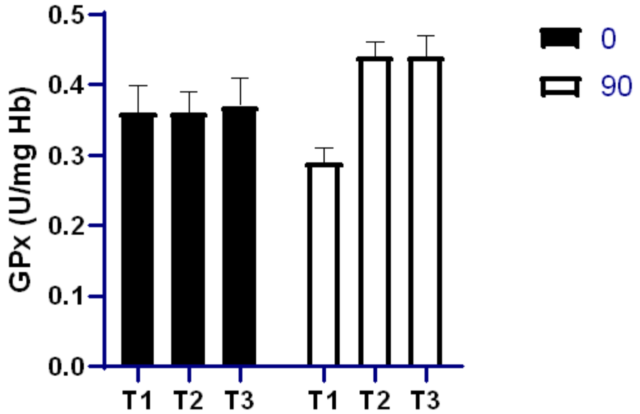

Fig 2; Glutathione peroxidase concentration (U/mg Hb) in rabbits

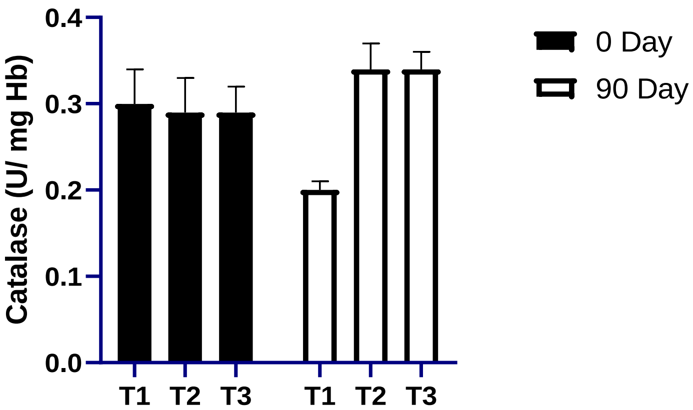

Fig 3: Catalase concentration (U/mg Hb) in rabbits

Effect of Carrot Feeding on Blood Antioxidant Level

It is worth noting that, the blood level (U mg/Hb) of glutathione peroxidase enzyme differed significantly ($p<0.05$) among the control and study groups. The higher glutathione peroxidase activity was recorded among the study group fed with the carrots (both II and III) compared to the CON group (group I) (Fig.2). Similar results were observed with regard to the activity of catalase enzyme also (Fig.3). The interaction between treatment and period for both glutathione peroxidase and catalase were found significant ($p<0.05$). Oxidative stress induced by free radicals affects the integrity of the cell membrane, leading to tissue damage and reduced performance of animals. Erythrocyte antioxidant enzymes are the major circulating antioxidant enzymes in the oxidative stress defense system and measuring the activity of these antioxidant enzymes is a non-invasive method to assess the antioxidant defense status (Yang *et al.*, 2014). In the present study, there was significant improvement ($p<0.05$) in the antioxidant status of rabbits experimentally fed with carrots (@10 and 20%. The current findings are in agreement with the previous study that reported an increase in GPx and catalase levels in the liver of DMH (1,2-dimethylhydrazine hydrochloride) challenged rats, fed with carrots (Anilakumar *et al.*, 2000). A similar antioxidant effect was also reported due to β-carotene in paracetamol-induced hepatic damage in rats (Kumar *et al.*, 2005). This suggests that β-carotene, ascorbic acid, and dietary fibre play an important role in scavenging free radicals and nullifying any toxicological effect (Anilakumar *et al.*, 2000). Similarly, higher values obtained for total phenolics and % inhibition against DPPH radicals in our assessment confirm the antioxidant properties of carrots. It was concluded that the inclusion of culled carrot could significantly reduce the serum cholesterol level and improve the antioxidant status in the growing rabbits. Therefore, culled carrots can be included up to 20% level with comparable overall performance of rabbits. The present research findings can further be verified by conducting long feeding trials for their reproducibility in livestock.

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Clinical trial registry

Permission for the experimental protocol (including the number of rabbits, research design, and duration of the study) was approved by the Institutional Animal Ethics Committee (IAEC), ICAR-IVRI, Izatnagar, Bareilly, UP

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Contribution by Authors

Rathod Akash, Saha S.K and Choudhary L.C conceptualized and designed the research programme; Rathod Akash conducted research; Rathod Akash, Saha S.K analysed the data; Rathod Akash, Saha S.K wrote the manuscript; Rathod Akash, Saha S.K had responsibility for the final content. All authors contributed to the manuscript revision, and read, and approved the submitted version..

Conflict of Interests

There is no conflict of interest.

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