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Effects of Carrot (*Daucus carota*) Leaves Powder on Reproductive and Growth Characteristics of Rabbit (*Oryctolagus cuniculus*) Does

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Abstract

The present study was conducted to evaluate the effects of carrot leaf powder in the diet on reproductive and growth characteristics in rabbits. Thirty sexually matured rabbits does of 8 months old, 3.00 to 3.20 kg, were divided into three groups of 10 rabbits each, which were comparable in terms of body weight. Throughout the experiment, the rabbits of group 1 (control group) were fed *ad libitum* with a feed not containing carrot leaf powder (control ration), while those of groups 2 and 3 received the control ration supplemented with carrot leaf powder in concentrations of 0.5% and 1% respectively. Data was collected on feed intake, weight gain, receptivity, fertility, gestation length of does, litter size, weight, viability and sex ratio of the young rabbits till 5 weeks *post-partum*. There was no significant ($P > 0.05$) difference in the fertility rate, gestation duration and sex ratio of the kits with reference to the control. Litter size at birth was not significantly ($P > 0.05$) influenced in does fed diets containing carrot leaf powder compared to the control. However, the reversed effects were observed at five weeks *post-partum*. The live weight of weaned kits and weight gain of rabbit does at birth were significantly ($P < 0.05$) increased in treated rabbits compared to the control. The supplementation of carrot leaf powder in feed can improve production performance in female rabbits, through their antioxidant activities thanks to their various components, thus fighting against oxidative stress effects to which animals are exposed both from endogenous and environmental sources.

Keywords

Antioxidant, Carrot Leaves, Growth, Rabbit Doe, Reproduction

1. Introduction

One of the essential functions for the existence of all living organisms is reproduction, which ensures the continuity of species and is considered an elementary faculty of living things that produce similar offspring [1]. Reproduction is of capital importance in animal breeding, since it contributes to improving productivity and the perpetuation of animal species. A dysfunction in reproduction would bring about a decline in performance in terms of growth as well as reproduction in farms, leading to very high losses in the economy. The malfunctioning of reproduction may be caused by many factors like food insufficiency, pollution of air, climate change, some drugs [2] physiological aging, stress (oxidative, feed, heat), and disease [3].

To remedy this situation, a variety of synthetic feed additives including antibiotic growth promoters (AGPs) have been used by farmers to maximize production efficiency and quality of products and to control diseases. Despite the advantages, antibiotics bring about many fatal hazards which endanger human and animal health [4].

The administration of antibiotics in animal feed leads to the spread of antimicrobial-resistant bacteria, which is a cause of worldwide concern [5]. It has been suggested by scientific evidence that the unregulated massive use of antibiotics has resulted in more antibiotic resistance problems [6] which lead to the spread of resistant microbes and the presence of antibiotic residues in feed and environment [7] [8], which led to the banning of certain AGPs in farms by governments. The removal of AGP's authorization led to the substantial rise in infection in animal production [9] [10].

Breeders then turned towards the search for alternatives to AGPs in order to reduce the rates of infection. The requirements for these alternatives are to be environmentally friendly and safe for animals as well as for humans who consume products from animals [11], for example, through the use of the efficacy of phytochemical compounds in herbal plants [12] as sources of natural products. Thus, various plants which possess antioxidant, anti-carcinogenic, anti-inflammatory, antibacterial, antiprotozoal, antiviral hepato-protective, growth and fertility-stimulating properties [13] [14] have been studied. In the same manner, research has been carried out on the effects on animal reproduction of green tea extracts [15] aqueous extracts of garlic (*Allium sativum*) [16] leaves of *Tribulus terrestris* [17] leaves of *Momordica charantia* [18] as well as essential oils of *Syzygium aromaticum* [19] and guava leaves [20].

Daucus carota (carrot) leaves have been regarded as waste products, but today

they are the focus of many debates and studies, including those on their antioxidant effects in the food industry and nutritive values [21], anti-carcinogenic and immune-boosting properties [22] as well as free radical scavenging activities [23].

Carrot leaves are very rich in nutrients such as vitamin A, C, K, and β-carotene, fibers and several minerals such as Na, P, K, Ca, Mg, Mn, Zn, and Fe [24]; phenolic compounds, tannins, saponins, flavonoids, steroids, and terpenoids [12] [25].

Phenolic compounds act as antioxidants that enter oxidation processes by breaking down the chain of active reactions, "primary oxidation" or by removing the free radicals "secondary oxidation", as reported by Ndhlala, *et al.* [26]; Augspole *et al.* [27].

Not all herbal extracts can function as antioxidants; so based on the various research [21] [24], it is then suspected that carrot leaves may be one of the natural sources of antioxidants readily available and not in competition with human consumption as well as environmentally friendly, less harmful to animals and humans who consume animal products. However, to the best of our knowledge, there are few studies on the use of carrot leaves in the growth and reproduction of farm animals especially as natural sources of antioxidants. So, the main goal of this work was to contribute to the improvement of animal production through the use of medicinal plants.

2. Materials and Methods

2.1. Animal Material

Thirty rabbits (*Oryctolagus cuniculus*) does of the New Zealand breed, 8 months of age with average weights of 3.00 to 3.20 kg produced at the Agro-Sylvo-Pastoral Animal Farm of Dschang were used.

2.2. Housing

The animals were housed individually at ambient temperature and at a natural rhythm, in wooden cages measuring 100 cm × 45 cm × 25 cm. These cages were suspended from a device forming batteries of cages (Californian type). Each cage was equipped with a metal feeder, a cylindrical drinker (about 800 ml capacity) and a nest box measuring 40 cm × 30 cm × 10 cm, placed in the cages 4 days before the expected date of parturition.

2.3. Feeding

Throughout the experimental period, rabbits were given a formulated feed and drinking water *ad libitum* and the estimated composition and calculated chemical characteristics of the feed are summarized in Table 1.

2.4. Sanitary Protection

Prior to the start of the experiment, the selected rabbits were physically treated against internal and external parasites by the use of Ivermectine (subcutaneous)

injection (0.2 ml/Kg live body weight). A broad spectrum antibiotic (Oxytetracyline L.A) was also administered subcutaneously at the rate of 0.2 ml/kg live body weight of rabbit. The rabbits were also given a prophylactic treatment against Coccidioses with Vetacox according to the manufacturer's prescription. TTS (Tylosin, Trimenthoprim, Sulfadiazine sodium) was given against respiratory diseases. The entire rabbitry, feces trays, feeders and drinkers were washed every after every 3 days and portable water provided *ad-libitum* throughout the experimental period.

Table 1. Percentage composition and chemical characteristics of the feed.

Ingredients	Quantities (%)
Maize	27.00
Wheat brand	14.00
Palm kernel meal	18.00
Soya bean meal	5.00
Cotton seed meal	4.00
*CMAV*10%	5.00
Fish meal	3.00
Palm oil	2.00
Shell fish	1.50
Salt	0.50
Rice pulp	20.00
Total	**100.00**
Calculated bromatological characteristics of the ration	
Crude protein (% DM)	16.47
ME (kcal/kg DM)	2435.23
Crude fiber (% DM)	13.65
Calcium (% DM)	1.26
Phosphorus (% DM)	0.55
Soduim (% DM)	0.28
Lysine (% DM)	0.83
Methionine (% DM)	0.36

CMAV: Nitrogenous and vitaminized mineral complex, EM: Metabolizable Energy DM: Dry Matter.

2.5. Plant Material

Mature carrot leaves (*Daucus carota*) from the same farm were used. The leaves were washed, dried in a shade and crushed to coarse particles then milled into a homogenous powder that was stored in opaque containers.

A phytochemical test was carried out on the powder in the Microbiology and Antimicrobial Substance Laboratory of the Faculty of Science at the University of Dschang to determine the presence or absence of phytochemical components. Table 2 presents the results of the phytochemical components present or absent in carrot leaves.

Table 2. Phytochemical components present in carrot leaf powder.

Phytochemical components	Results
Alkaloids	+
Phenols	+
Flavonoids	+
Sterols	–
Triterpenoids	+
Tannins	–
Saponins	–
Saponins	+

+: Presence; –: Absence.

2.6. Experimental Design and Procedure

At the start of the experiment, the 30 rabbits with the characteristics mentioned above were weighed, identified and divided into three groups of 10 animals each, comparable in terms of body weight, corresponding to three treatments (T0, T1 and T2) in a completely randomized manner. Carrot leaf powder was incorporated into their diet at the following rates:

- Group 1 (control): Experimental feed without carrot leaf powder (T0);
- Group 2: experimental feed + 0.5% carrot leaf powder in diet (T1);
- Group 3: experimental feed +1% carrot leaf powder in diet (T2).

The animals were adapted to the different experimental diets for 2 weeks before the experiment. Feed was distributed daily every morning between 7:00 am and 8:00 am until parturition and later weaning at 35 days. Water was distributed *ad lubitum.* The weight of the females was taken before introducing them to the cages of sexually matured untreated males in the ratio 1:2. Pregnancy was checked by palpation, 14 days after mating. In case of successful mating, nest boxes were placed 5 days before the presumed date of parturition, allowing the female to prepare her nest.

2.7. Data Collection and Study Parameters

2.7.1. Food Consumption and Weight Evolution of the Kits

Feed consumption was obtained by taking the difference between the quantity served in the week considered and the refusals of the same week. The following formula was used:

$$Qc = Qs - Qr$$

with:

Qc = quantity consumed;

Qs = quantity served;

Qr = remaining quantity.

For changes in body weight, the animals were weighed on an empty stomach at the start of the experiment and every 7 days until the end of the test.

2.7.2. Reproductive Characteristics

The reproductive traits assessed were:

- Responsiveness was expressed as time and responsiveness rate. The time of receptivity was obtained by simply counting the number of days taken by the doe to accept the male. The receptivity rate was evaluated using the following formula:

$$\text{Receptivity rate} = \frac{\text{number of females mated}}{\text{number of females presented to the male}} \times 100$$

- **Gestation period**

The gestation period was obtained by calculating the time in days between the date of mating and the date of birth.

- **Fertility of reproducers:**

$$\text{Rate of fertility} = \frac{\text{number of females having delivered}}{\text{number of mated females}} \times 100$$

$$\text{Fertility (\%)} = \frac{\text{Number of females put to birth}}{\text{Number of females put in reproduction}} \times 100$$

- **Litter size:** litter size was obtained by counting the number of kits at farrowing and at weaning.
- **Viability rate of kits:** Rate of viability = (number of live kids/litter size) × 100.
- Changes in the live weight of the rabbits: the rabbits were weighed at parturition and every 7 days up to 5 weeks postpartum.
- **Sex ratio:** the sex ratio in animals refers to the proportion of males and females within a population. The formula for calculating the sex ratio is:

$$\text{Sex Ratio} = \text{Number of Males/Number of Females}$$

2.8. Statistical Analysis

The data were subjected to one-way analysis of variance (ANOVA) to test the effect of the carrot leaf powder supplementation rate on the parameters studied. Duncan's test was used to separate means when there were significant differences.

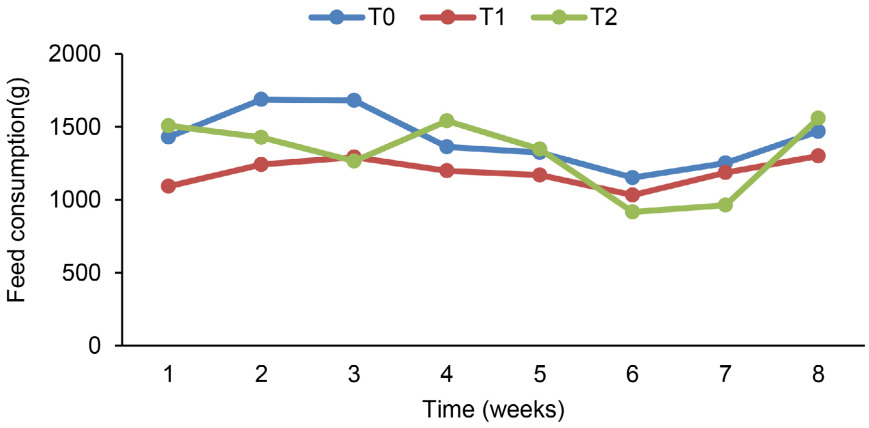
The results were expressed as mean ± standard deviation. The significance limit was 5% and SPSS 23 software was used for the analysis.

3. Results

3.1. Effects of Carrot Leaf Powder on Growth Characteristics

3.1.1. Effects of Carrot Leaf Powder on Evolution of Feed Consumption

The effects of carrot leaf powder supplementation in feed on the evolution of feed consumption are demonstrated in Figure 1. It appears that feed intake in general varied in an irregular manner from the first to the last week of the experiment.

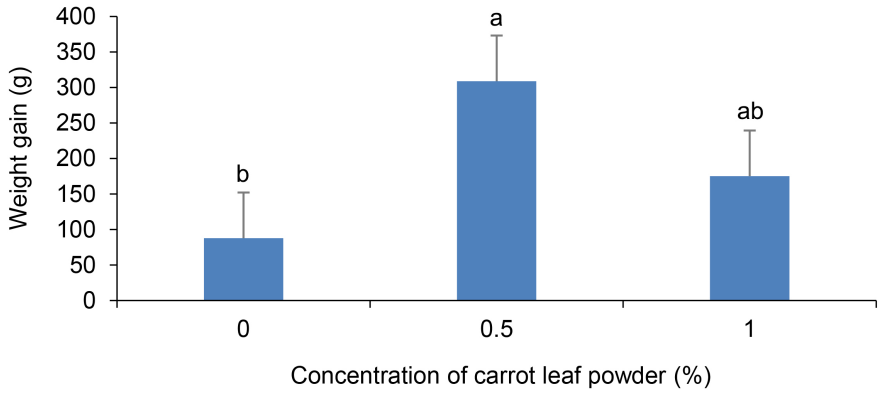


T0: control ration without carrot leaf powder; T1: ration supplemented with 0.5% carrot leaf powder; T2: ration supplemented with 1% carrot leaf powder.

Figure 1. Effects of carrot leaf powder supplementation in feed on the weekly evolution of feed consumption in rabbit does.

3.1.2. Effects of Carrot Leaf Powder on Weight Gain of Reproducers

The weight gain of rabbit does according to supplementation rate of carrot leaf powder is presented in Figure 2. The weight gain was significantly higher ($P < 0.05$) for the groups that received carrot leaf powder supplemented-feed with the highest value recorded in animals given feed supplemented at 0.5%.

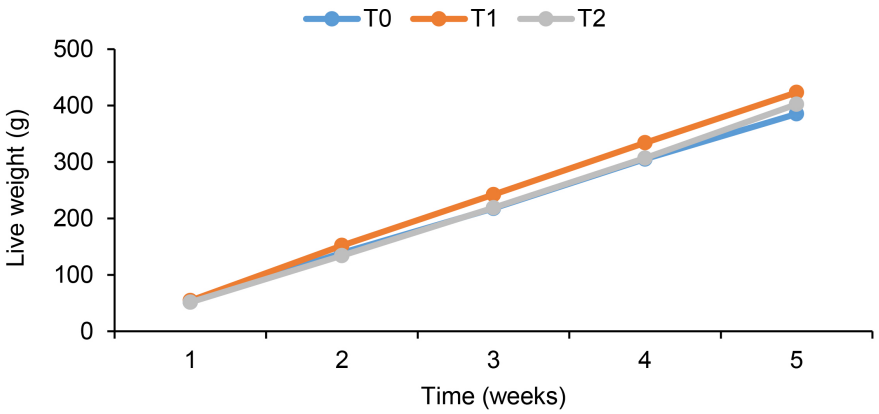


a, b: Values affected with the same letter are not significantly different ($p > 0.05$).

Figure 2. Effects of carrot leaf powder on weight gain of rabbit does.

3.1.3. Effects of Carrot Leaf Powder on the Evolution of the Live Weight of Kits

Figure 3 presents evidence of the change in the live weight of kits. It resulted that, live weight increased regularly from birth to weaning whatever the treatment considered. The live weight curve recorded in the T1 treatment remained superior to the other treatments. Nevertheless, there was no significant difference ($P > 0.05$) recorded between the groups.

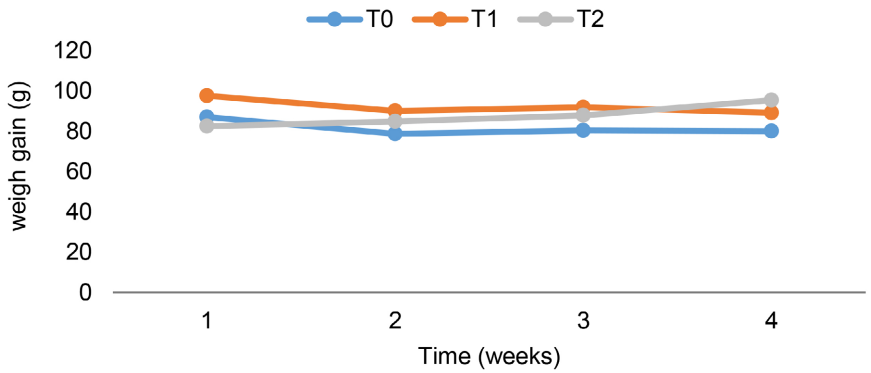


T0: control ration without carrot leaf powder; T1: ration supplemented with 0.5% carrot leaf powder; T2: ration supplemented with 1% carrot leaf powder.

Figure 3. Effect of carrot leaf powder supplementation in feed on the evolution of the live weight of kits.

3.1.4. Effects of Carrot Leaf Powder Supplementation in Diet on the Evolution of Weight Gain in Kits

The weekly evolution of weight gain according to the concentrations of carrot leaf powder supplementation is presented in Figure 4. It demonstrates that weight gain decreased slightly from week 1 to week 2 in T0 and T1 and remained almost constant from the 2nd to the 4th week. Meanwhile, in T2, there was an increase from the first to the 4th week, which was highest compared to the other groups.



T0: control ration without carrot leaf powder; T1: ration supplemented with 0.5% carrot leaf powder; T2: ration supplemented with 1% carrot leaf powder.

Figure 4. Effects of carrot leaf powder supplementation on the evolution of weekly weight gain in kits.

3.2. Effects of Carrot Leaf Powder on Reproductive Characteristics

3.2.1. Effects of Carrot Leaf Powder on Receptivity

Figure 5 illustrates the effects of carrot leaf powder on receptivity in rabbit does. It appears that there was a reduction in receptivity time in carrot leaf powder treated-groups compared to the rest of the groups and was lowest in the group fed with carrot leaf powder supplemented feed at 1%.

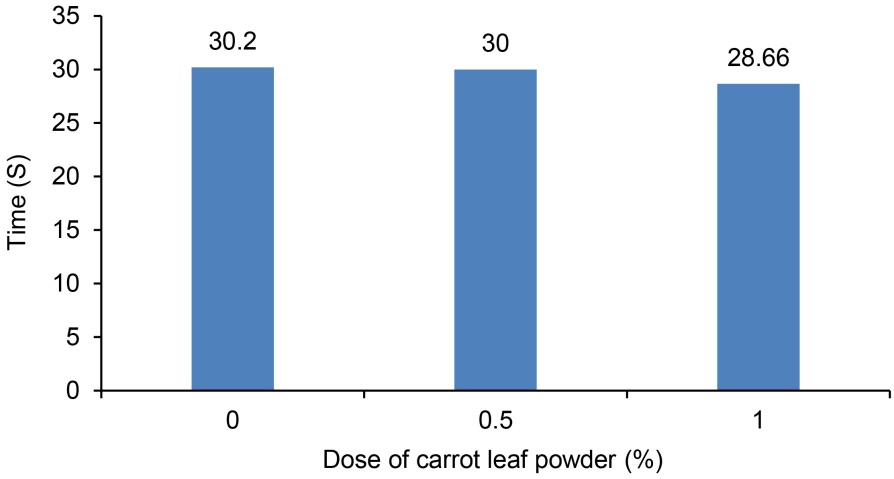


Figure 5. Effect of carrot leaf powder supplementation in feed on receptivity.

3.2.2. Effects of Carrot Leaf Powder on Fertility

Fertility rate (Figure 6) was comparable ($P > 0.05$) in all the groups considered.

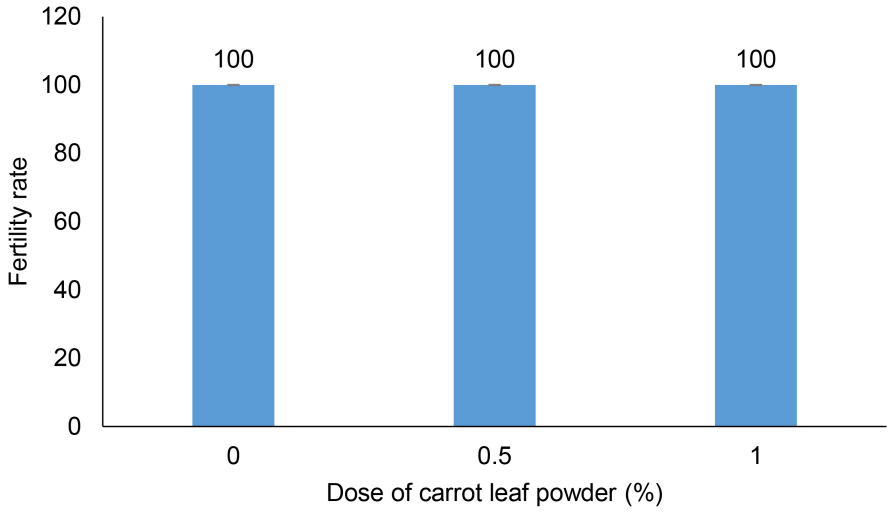


Figure 6. Effects of carrot leaf powder supplemented feed on fertility rate.

3.2.3. Effects of Carrot Leaf Powder on Gestation Duration

The variation in the length of gestation according to the concentrations of carrot leaf powder in rabbits is highlighted in Figure 7. It emerges from this figure that, the length of gestation was higher in the group that received 1% compared to the rest of the groups, although this difference was not significant ($P > 0.05$).

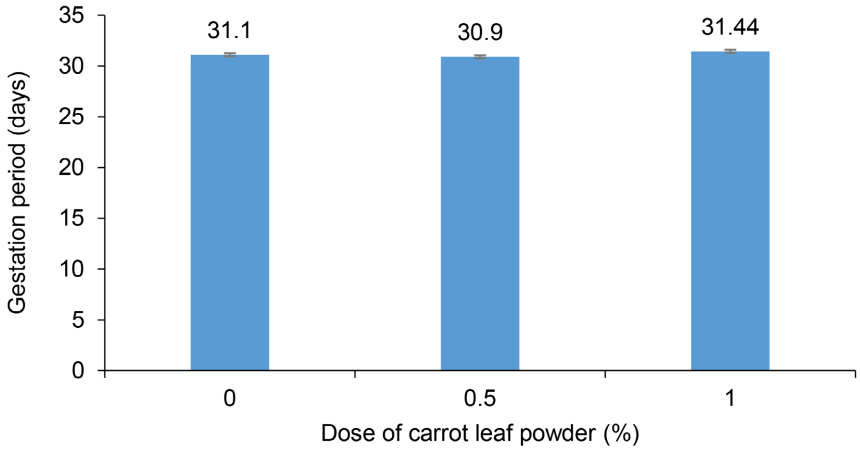


Figure 7. Effects of carrot leaf powder supplementation in feed on gestation length in rabbits.

3.2.4. Effects of Carrot Leaf Powder on Litter Size

Figure 8 shows litter sizes at different concentrations of carrot leaf powder supplementation in does. These results demonstrate that, there was a dose dependent increase in the litter size of rabbits that received 0.5% and 1% carrot leaf powder compared to the control group, which had the lowest litter size, though this increase was not significant ($P > 0.05$).

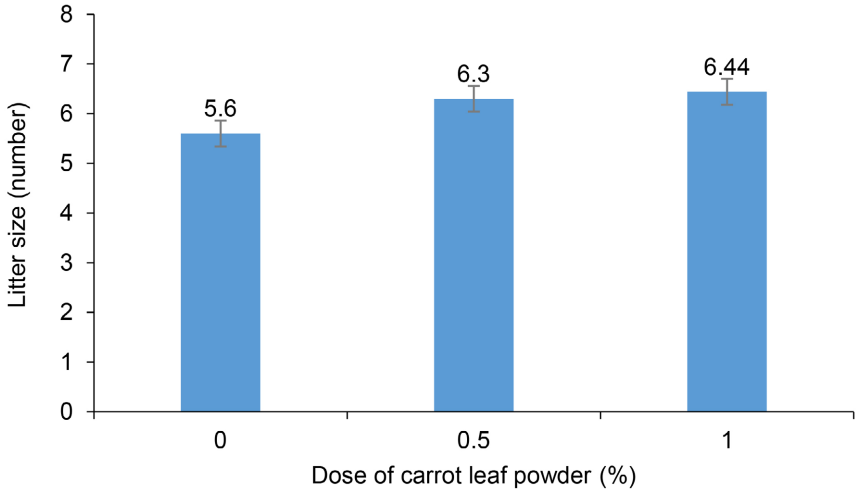


Figure 8. Effects of carrot leaf powder supplementation in diet on the litter size of rabbit does.

3.2.5. Effects of Carrot Leaf Powder in Feed on Viability Rate at Weaning

The viability of the kits (**Figure 9**) was comparable for all the treatments. However, there was a dose dependent increase in viability in the groups that received 0.5% and 1% supplementation of carrot leaf powder compared to the control group which had the lowest viability.

3.2.6. Effects of Carrot Leaf Powder on Sex Ratio of Kits

Figure 10 demonstrates the sex ratio of rabbit kits according to the concentrations

of carrot leaf powder supplementation in feed. The number of males in the control group was higher compared to that of carrot leaf powder supplemented-feed groups. Meanwhile, the group treated with 0.5% and 1% carrot leaf powder showed an increase in the number of females in comparison to the control animals.

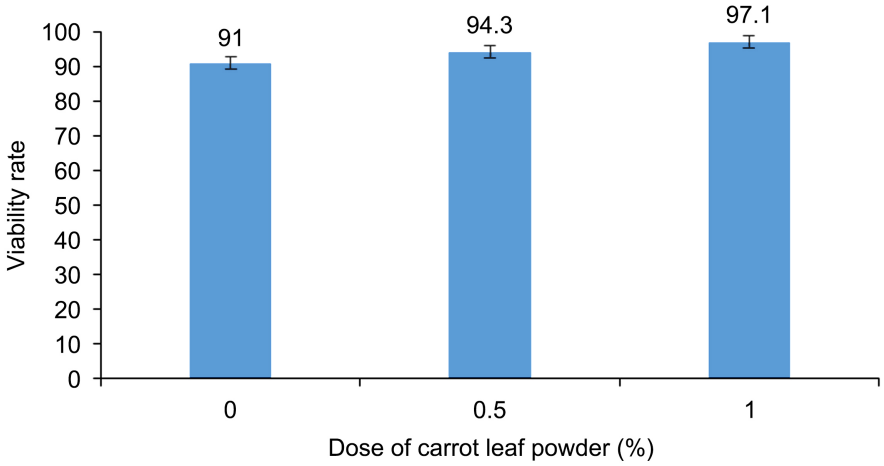


Figure 9. Effects of carrot leaf powder in feed on viability rate at weaning.

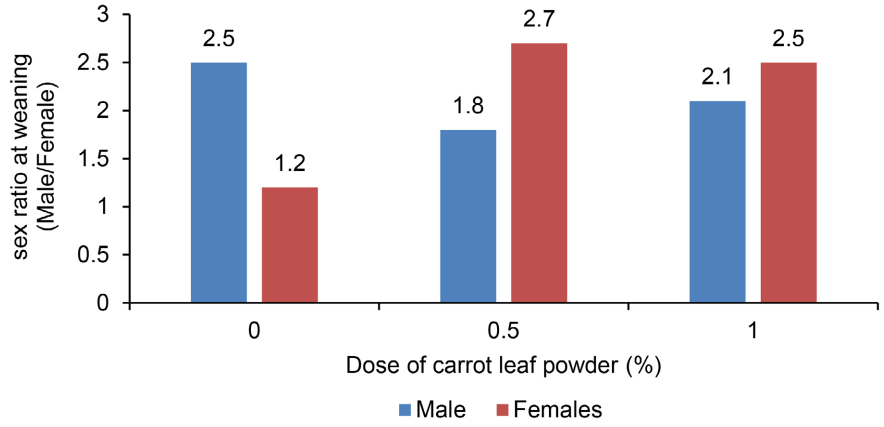


Figure 10. Effects of carrot leaf supplementation in feed on sex ratio.

3.2.7. Effects of Carrot Leaf Powder Supplementation in Feed on Live Birth

Figure 11 illustrates that the live birth of kits in the different groups of rabbit does given feed supplemented with carrot leaf powder were comparable ($P > 0.05$), although it was higher for the group treated at 1% supplement.

3.2.8. Effects of Carrot Leaf Powder Supplementation in Feed on Litter Weight

The effects of carrot leaves powder in feed on litter weight are illustrated in Figure 12. No matter the dose of carrot leaf powder incorporation in feed, litter weight remained comparable ($P > 0.05$) in all the groups. However, the group that received 0.5% supplement registered a higher litter weight compared to the other groups.

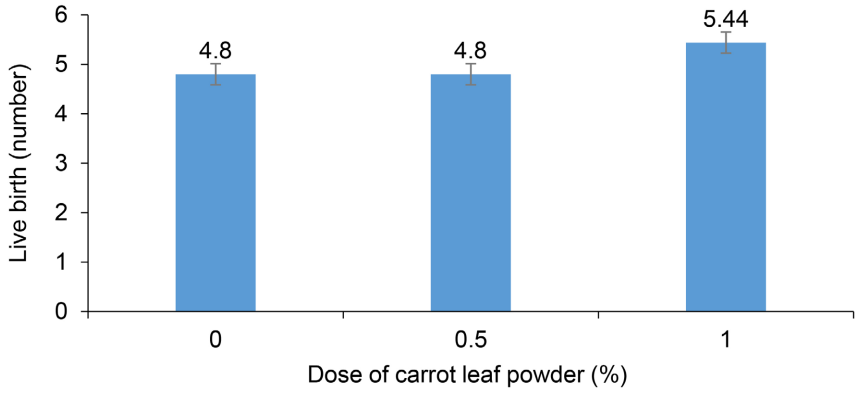


Figure 11. Effects of carrot leaf powder supplemented-feed on live birth.

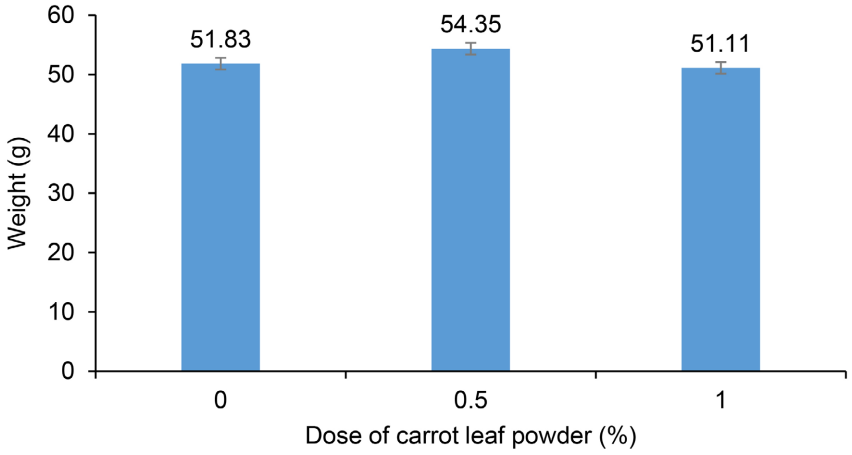


Figure 12. Effects of carrot leaf powder supplementation in feed on litter weight.

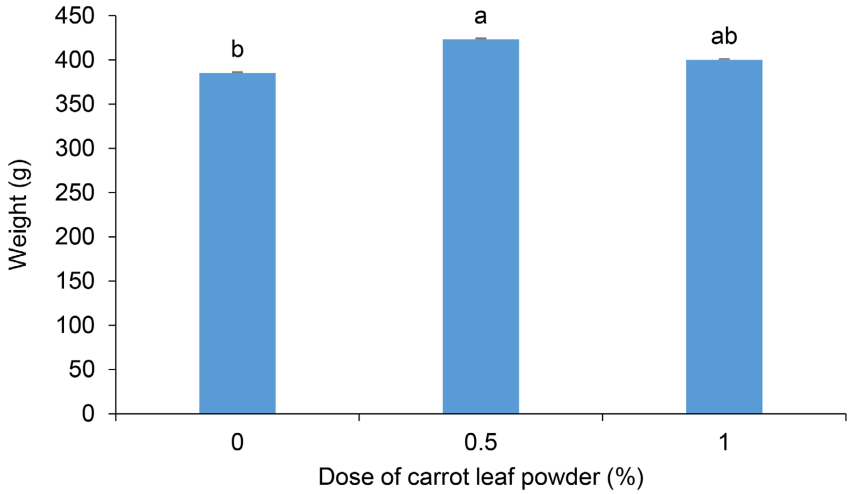


Figure 13. Effects of carrot leaf powder supplementation in feed on the live weight of weaned rabbits.

3.2.9. Effects of Carrot Leaf Powder Supplemented-Feed on the Live Weight of Weaned Rabbits

Figure 13 represents the live weight of weaned kits according to different

concentrations of carrot leaf powder. The results show that there was a significant increment ($P < 0.05$) in the group fed with 0.5% dose of carrot leaf powder in comparison to the other groups.

4. Discussion

Animals are exposed daily to both environmental and internal factors that bring about undesirable effects like the consequences of oxidative stress to their growth and reproductive performances. Due to the ban on synthetic antioxidants used by farmers, for many negative secondary effects; efforts are now on natural and readily available sources of antioxidants.

Some characteristics like feed intake and weight gain are often used to evaluate the effects of substances on animal growth while receptivity, gestation length and some characteristics of the young animals give results on their reproduction. The results of this study showed that the supplementation of carrot leaf powder in feed induced a significant increase in the body weight of female adult rabbits treated with different concentrations of carrot leaf powder (0.5% and 1%). These results are similar to those reported by Chongsi *et al.* [28] in rabbits treated with spirulina powder at concentrations of 0.6% and 1.2%; Djuissi *et al.* [29] in primiparous adult rabbits treated with avocado pits powder at rates of 0.5%, 1%, and 1.5%; Sorelle *et al.* [30] and Dorice *et al.* [31] in rabbits treated respectively with the aqueous extract of spirulina at concentrations of 5, 10 and 20 mg/kg and the aqueous extract of guava leaves at concentration 10, 20 and 30 mg/kg; Ousmane *et al.* [32] in rabbits treated with methanol extract of spirulina at concentration 5, 15 and 25 mg/kg; Razafindrajaona *et al.* [33] in male rats treated with aqueous extracts of spirulina at concentrations of 2 and 8 mg/kg. Anak *et al.* [12] equally reported similar results in ducks given carrot leaf juice in drinking water at 1%, 2% and 3%. Also, Ngoshe *et al.* [34] reported that by feeding carrot leaf meal to growing rabbits, the live weight was numerically superior to the controls. Abdu *et al.* [35] recorded that carrot meal inclusion in the diets of rabbits significantly influenced their live weight. Carrot-fed hens had a higher final body weight [36] suggesting that large amounts of easily fermented components like sugars and soluble non-starch polysaccharides contributed energy to the hens.

This increase in body weight in their studies could be the consequence of the increase in feed consumption observed in those animals. This subsequently increased the cell membrane thickness and animal weight. In a study on the utilization of carrot juice wastes as corn replacement in broiler chicken diet, Rizal *et al.* [37] stated that the feed consumption of broilers was improved by the treatments. They attributed this to the increase in the palatability of diets. Contrary to these, the results of Esam [38], on carrot powder at doses of 0.5%, 1% and 1.5% showed that dietary supplementation of carrot powder had no significant difference in feed consumption, body weight gain and final live weight of broiler compared to control group. This agrees with feed consumption in the present work, and the lack of variation can be attributed to the form, dose and part of carrot used unlike

the other authors.

The same effects as in the does were observed in body weight of kits. That is, the supplementation of carrot leaf powder in the diet significantly increased the body weight of treated rabbits kits compared to the control. This result is similar to that reported by Djuissi *et al.* [29] in rabbits kits treated with avocado pits powder at rates of 0.5%, 1%, and 1.5%. The increase in body weight at 5 weeks postpartum can equally be attributed to the presence of phytochemical components with antioxidant properties found in the carrot leaf powder in the does.

Supplementation of carrot leaf powder in the diet had no significant effect on the time of receptivity in rabbits. This is in line with the results recorded by Djuissi *et al.* [29] in rabbits treated with avocado pits powder at rates of 0.5%, 1%, and 1.5%. However, this observation made on the receptivity time is contradictory to that reported by Dorice *et al.* [31] in rabbits treated with the aqueous extract of guava leaves at doses of 10, 20 and 30 mg/kg. Receptivity is dependent on estrogens, in particular estradiol. The presence of phytoestrogen compounds such as steroids; saponins in plants would stimulate heat. Indeed, phytoestrogens have a biological activity that stimulates the hypothalamic-pituitary complex and therefore the secretion of follicle stimulating hormone (FSH) and luteinizing hormone (LH) [39], the latter stimulating the production of oestradiol. This is how saponins would act at the level of LH receptors on the internal theca of ovarian follicles via cAMP which stimulates the conversion of cholesterol into androstenedione, which will in turn be converted into estrogen that will enter into blood circulation, thus inducing estrus in female animals [40]. The absence of a significant effect recorded in this study could be explained not only by the relatively short duration of treatment before mating (2 weeks), which would not have been sufficient for the carrot leaf powder to react sufficiently. Furthermore, the relatively low concentration of bioactive compounds in the powder compared to the extracts would explain this contradictory result.

In the present experiment, viability rate and litter size at five weeks *postpartum* increased but non-significantly in does fed with rations containing carrot leaf powder, compared to those in the control group. These results corroborate those obtained by Akinola *et al.* [41], Uboh *et al.* [42] and Dorice *et al.* [31], respectively in mice treated with aqueous extracts of *Tribulus terrestris* at doses of 100 and 200 mg/kg bw; rats treated with aqueous extracts of *Ficus platyphylla* at doses of 100; 200 and 400 mg/kg and in rabbits treated with the aqueous extract of guava leaves at doses of 10, 20 and 30 mg/kg. This increase in the viability rate of rabbits could be explained by the stimulation and the transfer of maternal immunity (obtained from the components in carrot leaf powder thanks to their immune-boosting properties reported by Tanaka *et al.* [22]; to the rabbits via placenta and colostrum.

In addition, it should be noted that at birth, the organism of rabbits is very rich in lipids and they are then potentially susceptible to free radicals. Certain compounds present in carrot leaf powder such as phenolic compounds, tannins,

saponins, flavonoids, steroids, and terpenoids, and betacarotene with antioxidant activity [22] and free radicals scavenging capacities [23] could have played a role in this increase in the viability of young rabbits, in protecting them against free radicals.

The sex ratio in this study decreased *i.e.* more females than males in rabbits treated with carrot leaf powder compared to control group. This agrees with the findings of Chongsi *et al.* [28] in rabbits treated with spirulina powder in concentrations of 0.6% and 1.2%. Nevertheless, they are in contradiction with those reported by Sorelle *et al.* [30] and Dorice [31] in rabbits treated respectively with aqueous extract of spirulina at doses 5, 10 and 20 mg/kg and with aqueous extract of guava leaves at doses of 10, 20 and 30 mg/kg. In mammals, the number of males born relative to the total number of pups born is generally constant in a given population [43] [44]. Thus, the decrease in the sex ratio observed would be due to the inhibition of the synthesis and masculinizing action of androgens produced by the fetal testis during sexual differentiation.

5. Conclusion

From the results of this work, it can be concluded that supplementing rabbit feed with carrot leaf powder as a natural source of antioxidants, improves some reproductive and growth characteristics of female rabbits which are generally exposed to both environmental and internal factors that bring about dangerous effects of oxidative stress. Nevertheless, the improvement in some characteristics of carrot leaf powder was not significant.

Contribution of Authors

Chongsi MMM: Data collection and interpretation, manuscript final writing, editing and approval;

Deutcheu SN: Data collection and literature review;

Noumbissi MNB: data collection and literature review;

Bend EFM: Data collection and literature review;

Tchoffo H: Data collection, data interpretation, designed research methodology;

Nguatem K: Drafting of the article, Data collection and literature review;

Vemo BN: Data analysis and literature review;

Mahamat TMA: Data collection and literature review;

Ngoula F: Conceptualization and supervision of the study.

Ethical Consideration

The protocols used in this work in accordance with the Ethical committee of the Department of Animal Science of the University of Dschang (ECDAS-UDs 23/02/2015/UDs/FASA/DSAES) and in conform with the internationally accepted standard ethical guide lines for laboratory animal use and care according to the description of the European Community guidelines; EEC Directive86/609/EEC,

of the 24th November 1986.

Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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