Special Issue:
Artificial Intelligence in Veterinary Medicine: Current Status and Future Prospects

Impact of Dietary Supplementation of Goji Berry on Reproductive Performance of Rabbit Does

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Abstract | Goji berry, Lycium barbarum is a widely consumed fruit with a rich nutritional profile and recognized medicinal properties in human health. However, few studies have evaluated its effects on livestock. This study assessed the effects of dietary goji berry addition on reproductive performance in rabbit does. Twenty-four New Zealand White does were divided into four groups: a control group and three treatment groups receiving diets supplemented with 0.25%, 0.5%, and 1% goji berries for three months, respectively. Reproductive traits, hormonal profiles, antioxidant status, milk yield, litter traits, and histopathological changes were assessed. Results showed that goji berry supplementation reduced gestation length in groups G3 and G4, while it was increased in G2. It enhanced milk production, and improved litter size, body weight, and daily weight gain, particularly at 0.5% and 1% inclusion. Hormonal analysis revealed increased luteinizing hormone and decreased estrogen levels in non-pregnant groups, and reduced progesterone in pregnant does, while antioxidant capacity showed no consistent improvement. Histopathology indicated enhanced follicular development and uterine receptivity in supplemented groups. Overall, goji berry supplementation, at 1%, improved reproductive traits and offspring growth, although its effects on fertility parameters were limited. These findings highlight the potential uses of goji berries as a natural nutraceutical to enhance rabbit production and welfare.

Keywords | Reproductive performance, Milk production, Rabbits, Goji berry

Received | October 12, 2025; **Accepted** | November 23, 2025; **Published** | December 05, 2025
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Citation | Khafagy AHE-DR, Hashem NMA, El-Desouky AE-SM, El-Sead YYA (2025). Impact of dietary supplementation of goji berry on reproductive performance of rabbit does. Adv. Anim. Vet. Sci., 13(s1):32-43.
DOI | https://dx.doi.org/10.17582/journal.aavs/2025/13.s1.32.43
ISSN (Online) | 2307-8316

INTRODUCTION

Good nutrition has an important role in reproductive performance in animals (Ding *et al.*, 2024). Rabbits serve as an excellent animal model for studying reproduction (Lopez-Tello *et al.*, 2019). Natural dietary supplements play a crucial role in improving overall health and enhancing reproductive efficiency (Singh *et al.*, 2021). Among these supplements, Goji Berry has gained increasing scientific attention due to its high nutritional value and numerous health benefits (Ma *et al.*, 2023). Goji Berry is a small red fruit native to regions in Asia. It has been used for centuries in traditional Chinese medicine for its health-promoting properties (Wenli *et al.*, 2021). Goji Berry is a rich source of antioxidants, including carotenoids such as beta-carotene and lycopene, along with essential vitamins such as vitamin C and vitamin A, and minerals like iron, zinc, and selenium (Jagdale *et al.*, 2021). Studies by Skenderidis *et al.* (2022) have shown that, the polysaccharides present in Goji Berry exhibit strong immunomodulatory and antioxidant properties that contribute to overall health and disease prevention.

Goji berry may play a role in reproductive health by reducing oxidative stress and supporting hormonal balance (Brecchia *et al*., 2023). Similarly, it protects oocytes from oxidative damage, thereby enhancing their quality and increasing fertility rates (Agradi *et al*., 2022). Goji berry supplementation promotes the secretion of reproductive hormones such as estrogen, which enhances fertility (Kayode *et al*., 2023). Furthermore, it supports blood circulation, ensuring an optimal supply of nutrients to reproductive tissues (Li, 2022). Goji berries are rich in powerful nutrients that may help boost the immune system and improve fertility in rabbits (Brecchia *et al*., 2021). Therefore, incorporating Goji berries as an alternative approach to antibiotics and hormonal treatments offers a potential strategy for improving reproductive performance safely and sustainably (Agradi *et al*., 2022).

Goji berry supplementation improved the growth rates and viability of offspring, which they linked to better maternal milk production (Andoni *et al*., 2021). Goji berry supplementation had been shown to influence metabolic performance in various animal models (Menchetti *et al*., 2020). Goji berries could improve food conversion efficiency, reduce insulin resistance, and enhance energy utilization (Vidović *et al*., 2022). Furthermore, Sun *et al*. (2019) demonstrated that, goji berry extract had been found to help regulate blood sugar levels, reduce the risk of metabolic disorders, and improve overall metabolic health. In female rabbits, Goji berry supplementation had been associated with improvements in metabolic performance (Andoni *et al*., 2021).

Few investigations have examined the impact of goji berry in rabbits. Most available studies have focused on growth performance and immunity, while its impact on reproductive and productive traits remains largely unexplored. Therefore, this study aimed to evaluate the effects of dietary goji berry supplementation on productive and reproductive performance in rabbit does. It was hypothesized that goji berry supplementation would enhance reproduction and productivity through modulation of hormonal balance, liver function, and antioxidant activity.

MATERIALS AND METHODS

Animals and experimental design

The experimental work was conducted on twenty-four mature New Zealand White does aged 5–6 months, with an average weight between 2.75 and 3.00 kg. The experimental animals were obtained from a private rabbit production farm at Dekkrns District, Dakahlia Governorate, Egypt having a total number of 180 rabbits does. They were individually kept in wire cages measuring (50 x 60 x 35 cm), The does were divided into four groups (six does per group). Does were housed in controlled environmental conditions where the temperature ranged from +18 to +21°C, the relative humidity from 55% to 65%, and an artificial ventilation rate of 0.3 m^3/s, and the lighting was scheduled 16 L:8 D at 40 lux. All rabbits completely consumed their daily rations.

Diet and additives

The system provided animals with fresh drinking water and diets which were offered ad libitum all times over the experimental period. The experimental diets were supplied in pelleted form, which were prepared in the farm using the available ingredients to meet the nutrient requirements of rabbits following the recommendations of NRC (1978), as presented in Table 1.

Table 1: The composition and calculated chemical analysis of the diet Ingredients (% of diet) fed to doe rabbits during the experimental period.

Ingredients	**Control**	**G1**	**G2**	**G3**
Barley	28	28	28	28
Wheat bran	17	17	17	17
Soybean meal (44%)	14	14	14	14
Alfalfa hay	30	30	30	30
Mint straw	7.5	7.25	7.0	6.5
Di-calcium phosphate	1.3	1.3	1.3	1.3
Lime stone	1.2	1.2	1.2	1.2
Salt	0.4	0.4	0.4	0.4
Premix[1]	0.3	0.3	0.3	0.3
Yeast	0.1	0.1	0.1	0.1
Antitoxin	0.1	0.1	0.1	0.1
Methionine	0.1	0.1	0.1	0.1
Goji berries (wolf berry)	0.00	0.25	0.50	1.00
Total	100	100	100	100
Calculated chemical analysis				
Digestible energy (DE)	2696.00	2696.00	2696.00	2695.00
CP	18.12	18.18	18.18	18.19
CF	13.57	13.56	13.56	13.55
EE	2.53	2.55	2.56	2.59
Calcium	1.14	1.15	1.15	1.17
Available phosphorus	0.56	0.56	0.56	0.56
Lysine	0.98	0.94	0.91	0.91
Methionine	0.31	0.32	0.32	0.32

[1]Each 1 Kg of the Premix contains: Vitamin A 2 MIU, Vit. D_3 150000 IU, Vit. E 8.33 g, Vit. K 0.33 g, Vit. B_1 1 g, Vit.B_2 1.09 g, Vit.B_6 0.33 g, Vit.B_5 8.33 g, Vit. B_{12} 1.7 mg, Pantothenic acid 3.33 g, Folic acid 0.83 g, Biotin 33 mg, Choline chloride 20 g, Mg 66.79 g, Zn 11.79 g, Fe 12.5 g, Cu 0.5 g, I 0.3 g, Se 16.6 mg, Co 1.33 mg and carrier $CaCO_3$ up to 1000 g. [2] According to NRC (1977).

Plant extract

Goji berry (*Lycium barbarum*)

Goji berry was obtained from China (from Shandong Golden Central Agricultural Limited Company) in dried form. They were ground into smaller pieces, mixed with the rest of the diet ingredients, and, finally, pelleted (Yu *et al*., 2023). Experimental groups were provided with diets supplemented with goji berries at concentrations of 0.25%, 0.50%, and 1% of diet for three months (Agradi *et al*., 2022).

Experimental design and groups

After a period of one month of adaptation to doe rabbits, the does were submitted to mating at 6 months of age. All rabbits were maintained under the same management conditions. Cages and nest boxes were routinely cleaned and disinfected prior to each kindling. A lighting program of 12–14 hours per day was applied during the period of the study, and urine and feces were cleaned from the cages every day in the morning. All twenty-four mature does were divided into four groups (six does in each group) as shown in Table 2. The mating program was performed according to the directions of Aiello *et al*. (2016); a female doe in estrus was transferred individually to the fertile male cage for mating and then returned to its original cage. Pregnancy was confirmed through abdominal palpation, 12 days post-mating twice, by a highly skilled person.

Table 2: Groups of the experiment divided into.

Group	Description
Group 1 (G1)	control group take only standard diet without any additives in the form of pellets.
Group 2 (G2)	provided with a diet supplemented with 0.25% goji berry in the form of pellets, one month before mating and continue for two months.
Group 3 (G3)	provided with diet Supplemented with .5% goji berry, one month before mating and continue for two months.
Group 4 (G4)	provided with diet Supplemented with 1% goji berry, one month before mating and continue for two months.

Sampling

Blood samples

Blood samples were collected at the following time points: At one month, just before mating, and at 20^{th} day of pregnancy. The rabbits were sedated with diethyl ether and blood samples were collected from the ear vein, left to coagulate, and then serum was separated by centrifugation at 3000 rpm for 15 minutes, that stored frozen at -20°c until biochemical and hormonal assays.

Tissue sampling

At the end of the first month of the supplementation, and before mating, one rabbit does from each group was deeply anaesthetized and euthanasia. Tissue specimens were taken from the uterus and ovaries and were fixed immediately in 10% neutral buffered formalin solution to be stained with H and E stain for histopathological examination based on the methods and procedures of Agradi *et al*. (2022).

Reproductive performance traits

Twenty-four does were chosen for natural mating; the reproductive performance of the does, including the number of services per conception (NSC), days open (DO), gestation period length (GPL), and kindling interval (KI) was calculated (Menchetti *et al*., 2020; Brecchia *et al*., 2021).

Litter traits

Daily weight gain (DWG) of progeny was calculated for the periods of (from birth till 21 days, from 21 till 35 days, and for the whole period from birth till weaning (at 35 days)). Litter size (LS), as one of the sensitive parameters related to the economic return of production, was also recorded in the present investigation at birth, 21 days (from birth up to 21 days of age), and 35 days (from birth up to 35 days of age). Litter weights (LW) were recorded at 21 days and 35 days of age. Mean litter weight (Mean bunny weight) (MBW) was measured at birth, 21 days, and 35 days of age (Andoni *et al*., 2021). Also, Total feed intake of both does and progeny at 0- 21 days, Total litter weight at 0- 21 days, milk conversion rate, and feed conversion rate (FCR) were measured.

Milk production calculation

Milk production was calculated weekly from birth until weaning by the methods previously adapted by Palka *et al*. (2020).

Biochemical and hormonal assay

Biochemical parameters, such as Total antioxidant capacity, were determined colorimetrically by kits supplied by Biodiagnostic, Egypt, following the directions of the producer according to Koracevic (2001). Hormonal assays, including f LH, were determined using Elecsys LH Kits (which is based on Electrochemiluminescence immunoassay (ECLIA) technology) according to Howell *et al*. (2001). While estrogen and progesterone were measured using Elecsys Estrogen III Kits according to Johnson *et al*. (1993).

Statistical analysis

All the obtained data were represented as mean ± standard error (Mean±SEM). The difference between means was considered significant when ($P < 0.05$). One-way analysis of variance (ANOVA) test was applied, followed by Tukey's multiple comparisons to compare between different groups. Statistical calculations and graphics were carried out using GraphPad Prism 8.0 by GraphPad Software, according to the method described by Snedecor and Cochran (1989).

RESULTS

Effect of goji berry (0.25%, 0.5% and 1%) on reproductive performance traits (number of services per conception (NSC), gestation length (GL), days open (DO) and kindling interval (KI): As shown in Figure 1, dietary goji berry supplementation resulted in a significant reduction in GL in groups G3 and G4 compared to the control, while GL in G2 was significantly increased compared to other groups. Other reproductive data including NSC, DO, or KI showed no significant variations between groups.

Effect of goji berry supplementation on milk production (g): As shown in Figure 2, dietary goji berry supplementation significantly increased (p < 0.05) milk production during the five-week lactation period in groups G2, G3, and G4 compared to the control (G1). The highest yield was observed in G4 (1% goji berry), followed by G3 and then G2.

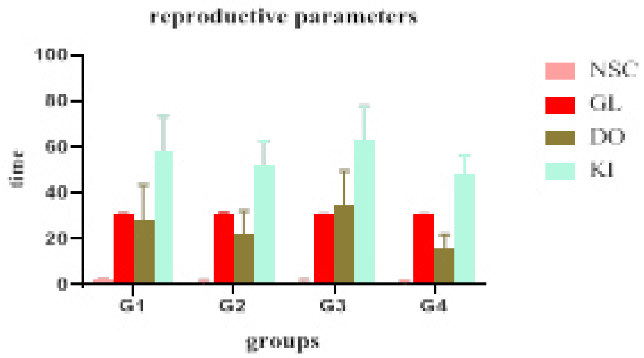

Figure 1: Effect of goji berry (0.25%,0.5% and 1%) on reproductive performance traits (number of services per conception (NSC), gestation length (GL), days open (DO), and kindling interval (KI).

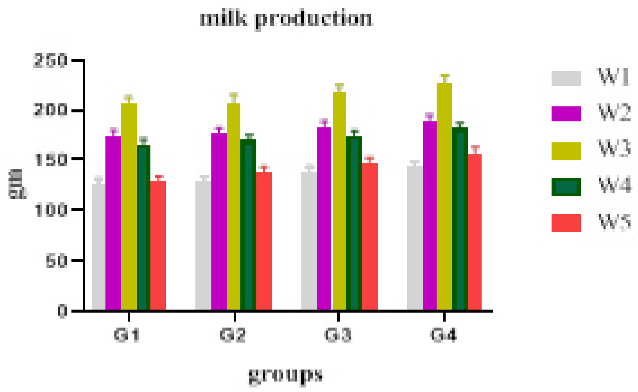

Figure 2: Effect of goji berry (0.25%, 0.5% and 1%) supplementation on milk production (gm).

Effect of goji berry (0.25%, 0.50% and 1%) on rabbit does Total anti-oxidant (mmol /L) for two months: As shown in Figure 3, TAO levels were not significantly affected (P > 0.05) by goji berry supplementation in either non-pregnant or pregnant rabbits. But also revealed that, TAO were generally higher in pregnant groups when compared to non-pregnant groups. Notably, the control pregnant group exhibited the highest antioxidant capacity among the pregnant groups followed closely by G2 and G4. The lowest TAO levels were recorded in the G4 non-pregnant rabbits. suggesting a possible interaction between supplementation level and physiological status in modulating antioxidant responses.

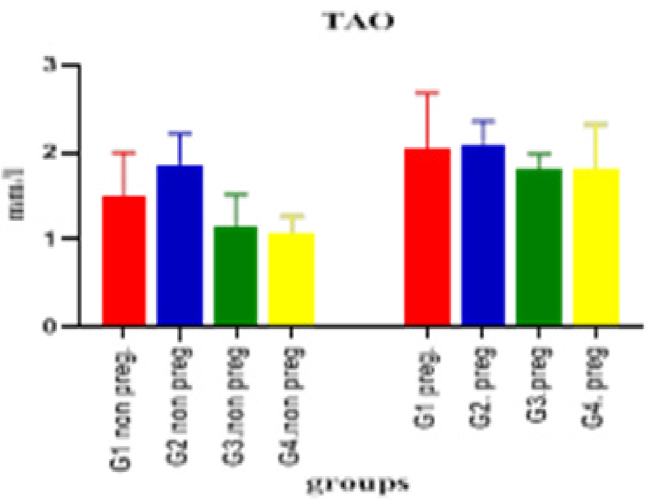

Figure 3: Effect of goji berry (0.25%, 0.50% and 1%) on rabbit does total antioxidant capacity (TAC) (mmol/L).

Effect of goji berry (0.25%, 0.50% and 1%) on rabbit does plasma hormones for two months: Effect of goji berry (0.25%, 0.50% and 1%) on rabbit does LH (IU/ml): As shown in Figure 4A, the result revealed that dietary goji berry supplementation in non-pregnant rabbits in G2, G3 and G4 exhibited a significant (p<0.05) increase in LH level when compared to non-pregnant rabbits in the control group. In the same respect, the highest value was observed in G4 and the lowest value in G2. LH concentrations did not significantly differ among pregnant groups. These results suggest that goji berry supplementation, particularly at higher doses, may enhance LH secretion in both physiological states.

Effect of goji berry (0.25%, 0.50% and 1%) on rabbit does estrogen(pg/ml): According to Figure 4B, dietary goji berry supplementation showed that, non pregnant rabbits in G2,G3 and G4 showed a significant (p<0.05) decrease in estrogen hormone level when compared to non pregnant rabbits in control group (G1), a marked decline in estrogen were recorded in G3 and G4 with goji berry supplementation. Pregnant rabbits in G2, G3 and G4 showed non significant difference in estrogen hormone level between groups.

Effect of goji berry (0.25%, 0.50% and 1%) on rabbit does

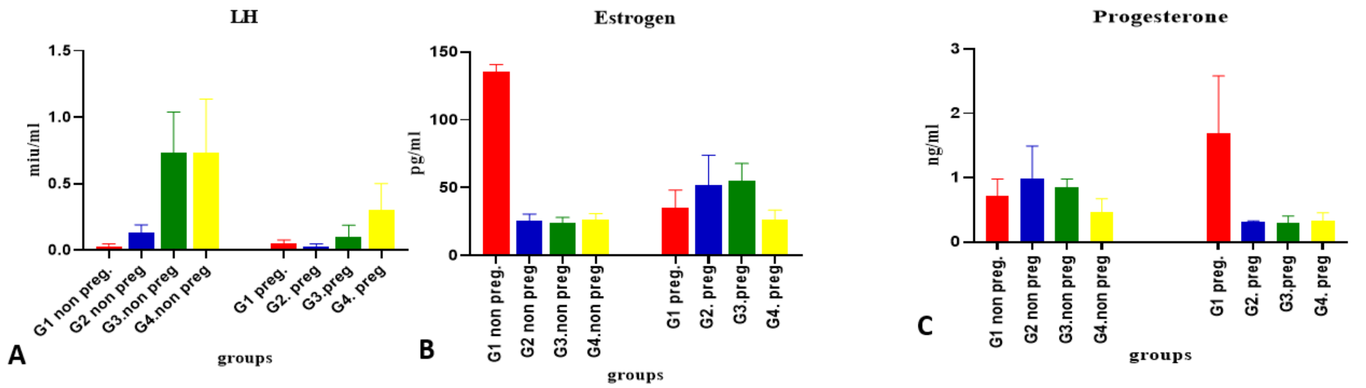

Figure 4: Effect of goji berry (0.25%, 0.50% and 1%) on rabbit does LH (A) (miu/ml), estrogen(pg/ml) (B) and progesterone(ng/ml) (C).

progesterone(ng/ml): As presented in Figure 4C, pregnant rabbits in G2, G3 and G4 showed a significant ($p<0.05$) decrease in progesterone hormone level when compared to pregnant rabbits in the control group (G1). Non-pregnant rabbits in showed non significant difference in progesterone hormone between groups.

Effect of goji berry on Litter size (number of kits): According to the results presented in Table 3, dietary goji berry supplementation showed a significant ($p<0.05$) increase in Litter size of rabbits in(G2, G3 and G4) at 21days of age and weaning when compared to control group (G1), with non- significant variations between groups at birth.

Table 3: Effect of goji berry (0.25%, 0.5% and 1%) on Litter size (number of kits).

Variable	Productive parameter		
	Litter size (by number) at		
	Birth	21days	weaning
G1	6.90 ± 1.19	5.90 ± 0.87^{b}	5.40 ± 0.96^{b}
G2	7.20 ± 1.05	7.00 ± 0.05^{ab}	6.70 ± 0.82^{a}
G3	7.60 ± 1.07	7.10 ± 0.73^{a}	7.00 ± 0.66^{a}
G4	7.10 ± 1.28	6.80 ± 0.03^{ab}	6.80 ± 1.03^{a}
P value	N.S.	0.0397	0.0022

N.S.: non-significant. Means carry different superscript letters in the same column are significant at $p< 0.05$

Effect of goji berry on litter body weight(gm): Results presented in Table 4 indicated that, dietary goji berry supplementation showed a significant ($p<0.05$) increase in litter body weight in (G2, G3 and G4) at 21days and at weaning when compared to the control group (G1), with non-significant variations between groups at birth.

Effect of goji berry supplementation on Mean bunny weight (gm): Results presented in Table 5 showed that, dietary goji berry supplementation significantly ($p<0.05$) increase in mean bunny weight from birth till weaning when compared to control group(G1).

Table 4: Effect of goji berry (0.25%, 0.5% and 1%) on litter body weight(gm).

Varia-ble	Productive parameter		
	Litter weight by gram at		
	Birth	21days	weaning
Effect of treatment			
G1	333.00 ± 66.96^{a}	1746.00 ± 235.14^{b}	3814.00 ± 709.07^{b}
G2	409.50 ± 59.27^{a}	2091.00 ± 275.73^{a}	5112.00 ± 594.97^{a}
G3	436.00 ± 56.45^{a}	2172.00 ± 210.29^{a}	5607.00 ± 576.36^{a}
G4	422.00 ± 56.02^{a}	2103.00 ± 295.02^{a}	5422.00 ± 623.71^{a}
P value	N.S.	0.0052	<.0001

N.S.: non-significant, Means carry different superscript letters in the same column are significant at $p< 0.05$

Table 5: Effect of goji berry (0.25%, 0.5% and 1%) on Mean body weight(gm).

Variable	Productive parameter		
	Mean bunny weight at		
	Birth	21 days	Weaning
G1	55.55 ± 3.37^{b}	296.65 ± 8.43^{c}	706.42 ± 27.50^{c}
G2	56.13 ± 2.26^{b}	299.52 ± 10.28^{bc}	763.79 ± 22.70^{b}
G3	57.45 ± 1.32^{ab}	306.37 ± 4.62^{ab}	801.35 ± 35.75^{a}
G4	59.94 ± 3.68^{a}	309.77 ± 5.66^{a}	797.59 ± 30.38^{ab}
P value	0.0028	0.0024	<.0001

Means carry different superscript letters in the same column are significant at $p< 0.05$

Effect of goji berry supplementation on total Weight gain (gm): The results presented in Table 6, dietary goji berry supplementation showed a significant ($p<0.05$) increase in weight gain of rabbits in (G2, G3 and G4) during the periods of 21-35 days and 0-35 days when compared to the control group(G1). rabbits in G3 showed the highest weight gain value.

Table 6: Effect of goji berry (0.25%, 0.5% and 1%) supplementation on total Weight gain (gm).

Variable	Productive parameter		
	Total Weight gain by gram at		
	0-21days	21-35days	0-35days
G1	241.09 ± 8.70^{a}	409.76 ± 26.32^{b}	650.86 ± 27.17^{c}
G2	243.38 ± 9.71^{a}	464.26 ± 19.05^{a}	707.65 ± 21.24^{b}
G3	248.85 ± 5.52^{a}	495.04 ± 32.09^{a}	743.90 ± 36.47^{a}
G4	249.82 ± 4.28^{a}	487.82 ± 28.14^{a}	737.65 ± 29.85^{ab}
P value	N.S.	<.0001	<.0001

N.S.: non-significant. Means carry different superscript letters in the same column are significant at p< 0.05

Effect of goji berry (0.25%, 0.5% and 1%) supplementation on daily weight gain(gm) for progeny

The results presented in Table 7 revealed that, dietary goji berry supplementation showed a significant increase (p<0.05) on daily weight gain (DWG) during both 21–35 days and 0–35 days with non-significant variations between groups at 0–21 days. Rabbit does in G3 recorded the highest DWG, followed by G4 and G2, while the control group (G1) showed the lowest values. These results indicate that goji berry supplementation had a positive effect on postnatal growth, particularly during the later stages of the pre-weaning period.

Table 7: Effect of goji berry (0.25%, 0.5% and 1%) supplementation on daily weight gain(gm) for progeny.

Variable	Productive parameter		
	Daly weight gain by gram at		
	0-21days	21-35days	0-35days
G1	11.48 ± 0.41	29.27 ± 1.88^{c}	20.18 ± 0.78^{c}
G2	11.59 ± 0.46	33.16 ± 1.36^{a}	21.82 ± 0.65^{b}
G3	11.85 ± 0.26	35.36 ± 2.29^{a}	22.89 ± 1.02^{a}
G4	11.89 ± 0.20	34.84 ± 2.01^{a}	22.78 ± 0.86^{ab}
P. Value	N.S.	<.0001	<.0001

N.S.: non-significant; Means carry different superscript letters in the same column are significant at p< 0.05

Effect of goji berry (0.25%, 0.5% and 1%) on total litter weight at age (0-21) days, total feed intake of both does and progeny at age (0-21) days and feed conversion rate: Results presented in (Table 8) showed that, dietary goji berry supplementation significantly (p<0.05) increase total litter weight of rabbits in (G2, G3 and G4) when compared to control group(G1). with non-significant variations between groups in total feed between groups. Results showed a significant (p < 0.05) decrease in the feed conversion rate (FCR). Rabbits in G3 showed a lower FCR than those in G2 and G4, and G4 showed a lower FCR than G2.

Table 8: Effect of goji berry (0.25%, 0.5% and 1%) on total litter weight at age (0-21) days, total feed intake of both does and progeny at age (0-21) days and feed conversion rate.

Variable	Total feed intake (gm)	Total litter weight (gm)	F.C. R
G1	17709.00 ± 1558.30	3814.00 ± 709.07^{b}	4.74 ± 0.70^{a}
G2	17339.50 ± 1742.09	5112.00 ± 594.97^{a}	3.44 ± 0.60^{b}
G3	17149.00 ± 1241.34	5607.00 ± 576.36^{a}	3.08 ± 0.33^{b}
G4	16451.00± 1420.73	5422.00 ± 823.71^{a}	3.08 ± 0.45^{b}
P. value	N.S.	<.0001	<.0001

N.S.: non-significant; Means carry different superscript letters in the same column are significant at p< 0.05

Table 9: Effect of goji berry (0.25%, 0.50% and 1%) supplementation on average milk production, litter weight gain, and milk conversion ratio during the first 21 days.

Variable	Productive parameter		
	Milk production (gm)	Weight gain (gm)	Milk conversion rate%
	0-21 days	0-21 days	
G1	3554.60±94.76^{c}	1363.00±198.48^{b}	2.66 ± 0.45^{a}
G2	3594.50±109.50^{c}	1681.50±232.54^{a}	2.17 ± 0.28^{b}
G3	3762.50±131.21^{b}	1737.00±168.17^{a}	2.18 ± 0.19^{b}
G4	3934.00±114.54^{a}	1680.50±245.29^{a}	2.38 ± 0.34^{ab}
	P. Value < 0.0001	0.0027	0.0113

Means carry different superscript letters in the same column are significant at p< 0.05.

Effect of goji berry (0.25%,0.50% and 1%) supplementation on milk conversion rate (%): Results presented in (Table 9) showed that, dietary goji berry supplementation resulted in a significant (p < 0.05) decrease in the milk conversion ratio during the five weeks lactation period of rabbits in (G2, G3 and G4) when compared to control group (G1).The most efficient milk conversion was recorded in rabbits in (G2) followed by (G3) then (G4) respectively,while control group (G1) show the highest value(the least efficient conversion ratio, indicating the lowest productivity relative to milk output).

Effect of goji berry (0.25%,0.50% and 1%) concentration on histopathological examination of non-pregnant does: Ovarian tissues from the control group displayed normal ovarian structures with follicles at different stages of development, including corpora lutea. G2 showed a slight increase in developing (black arrows) and mature(yellow arrows) follicles, while G3 exhibited moderate follicular enhancement, with more mature follicles compared to the control group. G4 rabbits presented a marked increase in both developing and mature follicles, indicating dose-dependent stimulation of ovarian function (Figure 5).

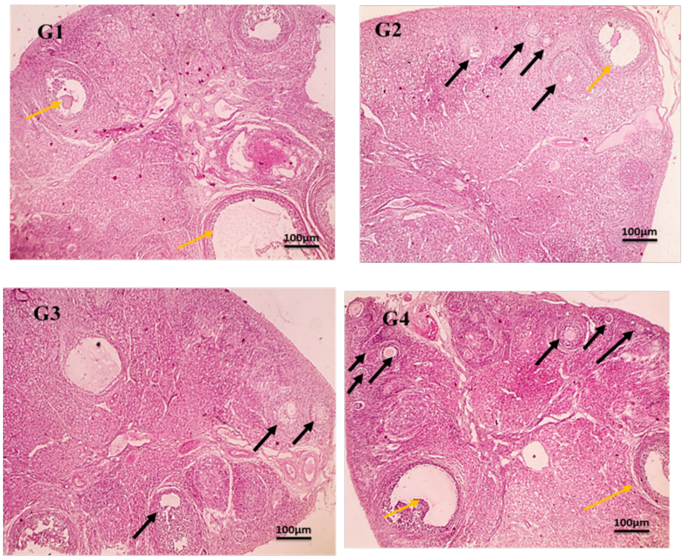

Figure 5: Microscopic picture showed ovarian tissues of control and treated does (H and E). Magnification. X: 100

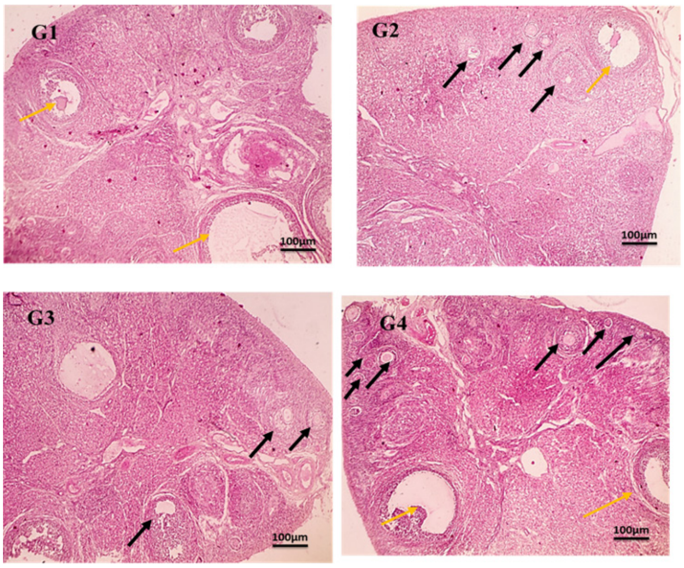

Figure 6: Microscopic picture showed uterine tissues of control and treated does (H and E). Magnification (X: 100).

The endometrial structure(arrowheads) in G1 appeared normal with intact glands (arrows) and stroma (S). G2 showed Slight thickening of the endometrial lining was observed, with well-organized glands and stroma, while G3 demonstrated increased glandular activity and endometrial thickness. G4 uterine tissues revealed Marked improvement in uterine structure with enhanced glandular development and stromal organization, indicating better uterine receptivity (Figure 6).

DISCUSSION

Goji berry, *Lycium barbarum*, is known for its rich profile of bioactive compounds (Ma *et al*., 2023), including polysaccharides, flavonoids, and essential nutrients (Vidović *et al*., 2022), with documented antioxidant, hypolipidemic, and immunomodulatory effects (Zhou *et al*., 2021).

The present study showed that dietary supplementation with goji berry improved reproductive performance in rabbit does, mainly through the reduction of gestation length. The highest improvement was observed at 1% supplementation, whereas no significant effects were detected on number of services per conception, days open, or kindling interval. These findings agree with previous reports (Menchetti *et al*., 2020; Andoni *et al*., 2021), which attributed the positive effects of goji berry to hormonal modulation, improved folliculogenesis, and antioxidant protection of reproductive tissues, largely mediated by Lycium barbarum polysaccharides (LBPs). The slight variation in response among intermediate doses suggests that the effect may not be strictly dose-dependent and could be influenced by parity, body condition, or mating timing. this is in line with Seetharaman *et al*. (2024), who observed that excessive phytochemical intake may sometimes impair reproduction. In the present study, 1% goji berry supplementation proved most effective, likely through combined effects of hormonal regulation, antioxidant activity.

The present study showed that goji berry supplementation significantly enhanced milk production, with the highest yield recorded at 1% (G4), which was reflected in improved growth of suckling bunnies. This aligns with Garcia-Garcia *et al*. (2024), who linked goji berry to improved metabolic hormones such as insulin and prolactin. However, Menchetti *et al*. (2019) reported that while 1% improved milk yield, higher doses (3%) induced metabolic stress without further benefit. Similarly, Agradi *et al*. (2022) observed no improvement at very low doses (0.1–0.3%), indicating that both dose and breed may modulate goji's effect on lactation.

The results of the present investigation showed that goji berry supplementation did not significantly affect total antioxidant capacity (TAO) in either pregnant or non-pregnant does. Overall, TAO was higher in pregnant rabbits, reflecting the physiological upregulation of antioxidant defenses during gestation. Within pregnant groups, moderate increases in TAO were observed, consistent with reports that goji berry enhances systemic antioxidant status (Andoni *et al*., 2021). In contrast, the lowest TAO was recorded in non-pregnant G4 does, suggesting that antioxidant responses may vary with reproductive status and metabolic demand (Menchetti *et al*., 2020). This aligns with evidence that while low to moderate doses of plant antioxidants enhance redox balance, higher doses may induce feedback inhibition of antioxidant enzymes (Chen and Tsim, 2020).

The present study demonstrated that LH levels were significantly affected by both pregnancy status and goji berry supplementation. In non-pregnant does, supplementation at 0.25–1% increased LH concentrations compared to controls, with the highest level in G4, while G2 (0.25%) showed the lowest. In contrast, no significant differences were observed among pregnant groups, indicating that gestational endocrine regulation may override dietary influences. These findings agree with Menchetti *et al.* (2020) and Andoni *et al.* (2021), who reported that Lycium barbarum polysaccharides (LBPs) stimulate reproductive hormone secretion via modulation of the hypothalamic–pituitary–gonadal axis, thereby supporting ovulation and corpus luteum function. Similarly, Hassan *et al.* (2025) observed significant elevations in LH, FSH, and testosterone following oral LBP administration in rats, even under testicular toxicity, confirming its endocrine-stimulating and protective roles. The collective evidence suggests that goji berry enhances LH secretion primarily through antioxidant mechanisms and GnRH-mediated pituitary stimulation (Shi *et al.*, 2017). The lack of conflicting evidence in rabbits further supports the novelty and consistency of these results.

The present study revealed that dietary goji berry supplementation significantly ($p < 0.05$) reduced estrogen levels in non-pregnant rabbits of groups G2, G3, and G4 compared with the control (G1). In contrast, pregnant rabbits showed no significant differences ($p > 0.05$) among groups, suggesting that pregnancy-associated hormonal regulation masked goji berry's influence. These findings indicated that goji berry bioactive compounds may affect estrogen synthesis through pathways such as aromatase modulation or hepatic metabolism. Similar effects were described by Yuan *et al.* (2025), who reported that LBPs influenced steroidogenesis depending on physiological context. This is consistent with Hashem *et al.* (2020), who reported that reduced estrogen at high supplementation levels (G4) may reflect polyphenol-induced suppression of biosynthesis. Additionally, Telang *et al.* (2014) demonstrated that *Lycium barbarum* extracts modulated estrogen metabolism *in vitro*. Overall, literature remains limited, and the present results provide novel insights into estrogen regulation by goji berry supplementation.

The present study revealed that progesterone levels in non-pregnant rabbits did not differ among groups ($p > 0.05$), indicating no effect of goji berry on luteal activity outside pregnancy. However, pregnant rabbits in the control group (G1) had significantly higher progesterone than all supplemented groups ($p < 0.05$). Similar reductions were reported by Liu *et al.* (2020) in pregnant mice and by Hu *et al.* (2023), who linked flavonoids to reduced steroidogenic enzyme activity. Conversely, Agradi *et al.* (2022) observed progesterone elevation in goats at moderate doses, highlighting a possible biphasic effect. Kumar (2016), also reported no change in rabbits with herbal antioxidants. Collectively, these findings suggest that goji berry bioactives may exert dose- and species-dependent effects on luteal physiology, with pregnancy representing a critical window of sensitivity.

The results of the current study showed that goji berry supplementation significantly increased litter size at 21 days, and weaning. The highest improvement was observed in the 0.50% group (G3). These findings agree with those reported by Menchetti *et al.* (2020) and Andoni *et al.* (2021), who found that Lycium barbarum enhanced reproductive efficiency in rabbit does, including litter size and survival. The antioxidant and anti-inflammatory properties of goji berry have been shown to reduce oxidative stress in reproductive tissues, thus supporting fetal development (Jiang *et al.*, 2024). But these findings disagree with results of Andoni *et al.* (2021) who found that, while 1% goji significantly increased litter size at weaning, the 3% group showed no significant improvement compared to controls in both litter size and weight.

The results of the present investigation revealed that dietary supplementation with goji berry at G3 and G4 significantly enhanced daily weight gain in growing rabbits, particularly during the early growth phase (0–21 days), The enhanced daily weight gain is in agreement with the findings of Shi *et al.* (2024), who reported that goji berry (*Lycium barbarum*) contains bioactive compounds such as polysaccharides, flavonoids, and amino acids that enhance nutrient absorption and promote muscle growth through antioxidant and metabolic support. Similarly, Yin *et al.* (2022) observed improved body weight gain and feed efficiency in weaned piglets supplemented with Lycium barbarum polysaccharides, attributing the effect to better intestinal health and immune modulation. However, not all studies agree with the growth-promoting effects of goji berry. Duru and Barıt (2023) reported no significant difference in daily weight gain in broilers supplemented with goji berry extract, suggesting that the response may depend on species, formulation, or environmental factors. Moreover, Liang *et al.* (2022) found that in heat-stressed rabbits, goji berry had limited effects on growth parameters, possibly due to altered metabolic priorities under thermal stress.

The results obtained from this study demonstrated that dietary supplementation with goji berry, particularly at 1% (G4), significantly increased the mean body weight of bunnies from birth to 21 days of age. These improvements suggest a positive dose-dependent effect of goji berry on early postnatal growth and lactation-related performance in rabbit. These findings are in agreement with the results of Andoni *et al*. (2021), who reported that goji berry extract improved neonatal growth and survival rates by enhancing maternal milk composition and antioxidant capacity. Similarly, Agradi *et al*. (2022) found that supplementation with herbal extracts, including Lycium barbarum, improved milk quality and led to higher body weight gain in suckling kits as well as the potential transfer of antioxidants and immunoactive compounds through the milk. Ma *et al*. (2023) noted that bioactive polysaccharides in goji berries could enhance maternal physiology, indirectly benefiting offspring development. However, these results of present investigation disagree with some studies, which reported limited or non-significant effects of goji supplementation on neonatal body weight (Rocha *et al*., 2023). For instance, Menchetti *et al*. (2019) found that while maternal supplementation with goji berry improved reproductive hormone levels, the effect on offspring body weight was minimal, possibly due to differences in parity or environmental conditions.

The results of the present investigation indicated that, litter body weight at 21 days and weaning significantly increased in all goji berry-supplemented groups These findings are consistent with those of Menchetti *et al*. (2020), who reported that dietary inclusion of *Lycium barbarum* enhanced litter performance, body development, and survival in rabbit kits, likely through improved maternal milk production and antioxidant transfer via lactation. Similarly, Ludwiczak *et al*. (2021) highlighted that better maternal nutrition correlates positively with increased kit weight and reduced mortality during lactation. Ma *et al*. (2023) suggested that high levels of certain phytochemicals could reach a biological threshold with diminishing returns. Conversely, other studies such as Menchetti *et al*. (2020) found no significant effect of goji berry supplementation on offspring weight in rodents, possibly due to differences in dosage form, bioavailability, or species-specific responses.

The findings of the present investigation revealed that dietary supplementation with goji berry at 0.25%, 0.50%, and 1% significantly improved overall weight gain during all growth periods. These results align with the findings of Antonelli and Donelli (2024) demonstrated that goji berry polysaccharides enhanced feed utilization and weight gain in weaned piglets. Chen and Tsim (2020) explained that, goji's polysaccharides and flavonoids stimulate metabolic activity, reduce oxidative damage, and support gastrointestinal health. The slightly lower gain observed in G4 (1% goji berry) explain that, that excessive supplementation may not lead to additional benefits, This concept is consistent with the dose-dependent response theory proposed by Deng *et al*. (2020), where high concentrations of bioactive compounds may reach a threshold beyond which biological systems show diminished response a characteristic feature of non-monotonic dose response relationships or hormetic effects. But these findings differed from those reported by Placha *et al*. (2022), who observed no significant effect of herbal supplementation on long-term weight gain in rabbits. This discrepancy may be attributed to differences in environmental conditions, rabbit breed, or the specific formulation of the herbal additive used.

The findings from the current study showed that supplementation with goji berry at levels of 0.25%, 0.50%, and 1% significantly increased total litter weight while simultaneously reducing feed conversion rate, This aligns with the findings of (Menchetti *et al*., 2020), who reported that *Lycium barbarum* supplementation in rabbit does improved nutrient utilization and feed conversion, likely due to its digestive-enhancing properties. Cremonesi *et al*. (2022) showed that, the reduction in feed intake accompanied by increased litter output may reflect improved digestive enzyme activity and enhanced nutrient absorption. High concentrations of bioactive compounds might influence feed palatability or metabolic regulation, which was previously demonstrated by Chen and Tsim (2020) who emphasized the potential downsides of exceeding optimal phytochemical levels in animal diets.

Moreover, the reduced milk conversion ratio (i.e., improved efficiency) in treated groups, especially G2 and G3, suggests better nutrient partitioning and utilization during milk synthesis. Similar improvements were described by Ludwiczak *et al*. (2021) in studies assessing milk output and feed efficiency in does under herbal supplementation. However, Leparmarai (2018) cautioned that high doses of certain phytocompounds might affect milk flavor or energy balance, potentially reducing intake in suckling kits, which might explain the relatively lower efficiency in G4 compared to G3 despite higher milk volume.

CONCLUSION AND RECOMMENDATIONS

This study concluded that goji berry supplementation had beneficial effects on rabbit does, mainly through improved milk yield, better offspring growth, and modulation of reproductive hormones. The 1% supplementation level proved most effective in enhancing productivity, while 0.5% also showed positive impacts on growth and litter performance. However, reproductive parameters such as number of services per conception and kindling interval were not significantly affected. The findings suggest that goji berry can be used as a safe dietary supplement to improve production efficiency and reduce the reliance on synthetic treatments in rabbit production. Further research is recommended to explore long-term effects, optimal doses, and economic feasibility.

ACKNOWLEDGEMENTS

Authors appreciated Dr. Walaa Fikry Awadin, Department of Pathology, for her valuable help in conducting the histopathological examinations in the current study.

NOVELTY STATEMENT

This study provides the first comprehensive evaluation of goji berry supplementation on both reproductive and productive performance in rabbit does, highlighting its dual role in hormonal modulation and offspring growth.

AUTHOR'S CONTRIBUTION

The research strategy did by Prof. Dr. Youssef Yahia Awad El-Seady. While conduct the experimental conditions by Prof. Adel El-Sayed Mohamed El-Desouky and Aya Hossam El-Dein Roshdy Khafagy, composed the article and the statistics done and language revision by Aya Hossam El-Dein Roshdy Khafagy, the finished manuscript was examined and revised by Dr. Prof. Dr. Youssef Yahia Awad El-Seady and Dr. Nada Mohamed Ali Hashem.

Ethical statement

All the experimental animals and procedures are under the "Guide for the Care and Use of Laboratory Animals," which was approved by the Ethics Committee of Mansoura University-Animal Care and Use Committee (MU-ACUC), registration number (MU-ACUC; M/146).

Generative AI and AI-assisted technology statement

The authors declare that generative AI technologies including large language models as (chat GPT) or AI tools not utilized in any capacity during the preparation, writing, analyze, draw data or editing of this research.

Conflict of interest

The authors have declared no conflict of interest.

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