Scientific Research Publishing

Food and Nutrition Sciences, 2016, 7, 1276-1289
http://www.scirp.org/journal/fns
ISSN Online: 2157-9458
ISSN Print: 2157-944X

Physiological Studies on the Effect of Fenugreek on Productive Performance of White New-Zealand Rabbit Does

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How to cite this paper: Abdel-Rahman, H., Fathalla, S.I., Assayed, M.E., Masoad, S.R. and Nafeaa, A.A. (2016) Physiological Studies on the Effect of Fenugreek on Productive Performance of White New-Zealand Rabbit Does. *Food and Nutrition Sciences*, **7**, 1276-1289.
http://dx.doi.org/10.4236/fns.2016.713117

Received: October 8, 2016
Accepted: November 15, 2016
Published: November 18, 2016

Abstract

The objective of the current work is to investigate the possible beneficial effects of forced feeding of fenugreek to rabbit does on their milk composition, milk yield, anemia screening parameters, immunity and the growth performance of bunnies. Twenty white New Zealand female rabbits were randomly assigned into 5 groups: normal control and 4 treated groups (vehicle (molasses), fenugreek powdered seeds + molasses, sprouted fenugreek + molasses, and fenugreek oil + molasses). All treated groups took the given doses orally for two days before parturition and 13 days after parturition for 2 successive pregnancies. Blood samples were collected from each doe on the 13th day of lactation for determination of hematological, biochemical and immunological parameters. Milk samples were taken for evaluation of milk composition. All rabbit does and their bunnies were weighed for calculation of the milk yield. The fenugreek seeds and oil administered groups (G_3 & G_5) recorded the highest percent values of fat, protein, lactose, total solids and solid not fat contents of the rabbits' milk in both 1st and 2nd lactation periods relative to other tested groups. At the beginning and mid of lactation periods, the milk yields recorded non-significant differences either between tested groups or between 1st and 2nd pregnancy in the same group. Whereas, at the end of lactation, germinated seeds (G_4) and oil (G_5) treated groups recorded significantly higher rates of milk yield relative to control and fenugreek seeds groups. Bunnies' body weights suckling from dams of group III (G_3) recorded the highest weights relative to other tested groups in both 1st and 2nd pregnancy. During the 1st lactation period, all experimental groups showed significant reduction of white blood cells, while, G_3 and G_4 recorded significant elevation during 2nd lactation period. Rabbits treated with fenugreek seeds powder (G_3) showed a signifi-

cant raise of phagocytic activity and phagocytic index. Fenugreek seeds group (G_3) and germinated one (G_4) showed significant red blood corpuscles and packed cell volume raise relative to other groups during both 1st and 2nd lactation periods.

Keywords

Fenugreek, Rabbits, Milk Yield, Milk Composition, Phagocytic Activity, Antioxidants

1. Introduction

Fenugreek (*Trigonella foenum graecum*) is an annual herb characterized by white flowers and hard, yellowish brown, angular seeds, known for its nutritional value beside its medicinal effects [1]. It is widely cultivated in Asia and the Mediterranean countries where its dried seeds have been traditionally used for their beneficial health effects such as galactagogue, anti-inflammatory, antibacterial and insulinotropic properties [2]. The medicinal and beneficial values of fenugreek are attributed to its chemical compositions (20% - 25% protein, 45% - 50% dietary fiber, 20% - 25% mucilaginous soluble fiber, 6% - 8% fixed fatty acids and essential oils, and 2% - 5% steroidal saponins). Furthermore, some minor components such as alkaloids (trigonelline, cholin, gentianine, carpaine, etc.), free unnatural amino acids (4-hydroxyisoleucine), and individual spirostanols and furastanols like diosgenin, gitogenin and yamogenin have also been identified and considered as main components for their biological effects [3].

Fenugreek can increase the erythrocyte insulin receptors and peripheral glucose utilization showing improved pancreatic functions [4]. Also, fenugreek exhibits important pharmacological properties such as antitumor, antiviral, antimicrobial, anti-inflammatory, hypotensive and antioxidant properties [5] [6].

Fenugreek seeds and leaves have been used extensively to prepare powders and extracts for medicinal uses [7]. The powder or germinated form of fenugreek seeds exhibited anti-diabetic or insulinotropic properties [8] [9], hypocholesterolemic effect [10] [11] [12], anticancer effect [13], thyroxin-induced hyperglycaemic effect [14] and protective effect on ethanol toxicity [15] [16]. Furthermore, Fenugreek oil is a waxy liquid pressed or distilled from the seeds, which acts as a phytoestrogen that regulates ovulation, lactation and overall female sexual development [17].

Since ancient times, medicinal herbs have been traditionally used as galactagogues to improve breast milk production. The most frequently used herb was fenugreek [18]. It has also been reported that, fenugreek has estrogenic activity that is effective on breast milk production [19]. Moreover, [20] demonstrated that, fenugreek seeds contain estrogen-like compounds, which stimulate pS2 expression in MCF-7 cell lines. Furthermore, phytoestrogens and diosgenin of fenugreek increase the milk flow [21].

The objective of the current work is to investigate the possible beneficial effects of forced feeding of fenugreek to rabbit does on their milk composition, milk yield, ane-

mia screening parameters (PCV, Hb, RBCs count and blood indices), immunity (WBCs count, differential leukocytic count, phagocytic count and phagocytic index) and the growth performance of bunnies.

2. Material and Methods

2.1. Animals

The present study was carried out on 20 white New Zealand female rabbits of 150 - 160 days old and weighing 3.100 ± 0.100 Kg**.** All animals were subjected to acclimatization for two weeks. During the experimental period, the rabbit does were maintained separately in individual galvanized wire cages provided with galvanized nest box hanged outside on the cage with 12 hours' light and dark cycle. Fresh water was given *ad libitum*. Food quantities started at 180 g firstly, increased to 200 - 220 g during pregnancy then reached 300 g after parturition.

2.2. Medical Plants

a) Fenugreek powder was suspended in molasses (3.0 ml) and given to animal orally two days before parturition and 13 days after parturition at a dose of 1.5 g/does according to [22].

b) Sprouted fenugreek. The seeds were soaked in water overnight**.** Water was poured off on the next day and seeds were rinsed with clear water, placed on a windowsill with the lid slightly ajar. The seeds were rinsed with water daily [23]. The seeds sprouted in around five days. Rabbit does were fed sprouted seeds two days before parturition and 13 days after parturition at a dose of 60-66.

c) Fenugreek oil (El-Captain Company, CAP. PHARM., Egypt) was mixed with molasses (3.0 ml) and given to animal orally two days before parturition and 13 days after parturition at a dose of 0.3378 ml /kg of body weight according to [24].

2.3. The Diet

The rabbit does were fed on a pelleted diet according to [25] [26] as shown in **Table 1.**

Table 1. The chemical composition of the pelleted diet.

Ingredients	
Crude protein %	18
Digestible energy Kcal/kg of diet	2600
Crude fiber %	10 - 12
Calcium %	1.2
Phosphorus %	0.8
Lysin %	0.75
Methionine and cysteine %	0.65

2.4. Acute Toxicity Study of Aqueous Extract of Fenugreek

Thirty white New Zealand female rabbits were randomly divided into five equal groups, each of six rats. The first four groups received the following doses of fenugreek seed aqueous extract: 500, 1000 1500 or 2000 mg/kg body weight respectively, while the fifth group served as control group that only received distilled water (10 ml/kg body weight, orally). The rabbits in all groups were returned to their home cages, and provided with food and water *ad libitum.* Animals were observed for signs of toxicity for 72 hours. Clinical signs of toxicity sickness and/or mortality data were recorded.

2.5. Animal Grouping

The animals were randomly assigned into 5 groups of 4 rabbits in each group. Group I (control): rabbits did not take any medical plant or vehicle. Group II: each rabbit was supplied with 3.0 ml molasses. Group III: each rabbit was supplied with 1.5 g fenugreek powdered seeds suspended in molasses. Group IV: each rabbit was fed on sprouted fenugreek (60 - 66 sprouted seed) and molasses. Group V: each rabbit was supplied with 0.34 ml fenugreek oil mixed with molasses. All treated groups took the given doses orally for two days before parturition and 13 days after parturition for 2 successive pregnancies.

2.6. Blood Samples

Blood samples were collected from each doe on the 13th day of lactation from ear vein using heparinized vacuum tubes at 9 am. Each blood sample was divided in to 2 parts, the first part for hemogram including estimation of red blood cell count (RBCs), hemoglobin concentration (Hb), packed cell volume (PCV), total white blood cell count (WBCs) and differential leucocytic count which were determined according to routine methods adopted by [27] and blood indices according to [28]. While, the 2nd blood sample was used for estimation of biochemical parameters in plasma.

2.7. Milk Collection and Analysis

Milk samples were obtained from all does of each group. Litters were removed from their dams on the evening before the sample was taken. The following morning, each doe was given 2.5 i.u. oxytocin by injection into the ear vein in order to stimulate milk let down then milk was expressed by gentle massaging the of mammary tissue. Milk flow usually began about 3 min after the injection and continued for about 15 min [29]. The samples were collected on the 13th day of lactation. The concentration of milk fat, protein, lactose and total solids (TS) were determined by infra-red spectroscopy using milko-scan 133BN (Foss Electric Denmark, according to manufacturer's manual).

2.8. Determination of Milk Yield by Litter Sucking Technique

It is known that, rabbit pups are nursed only for about 3 minutes once every 24 hours [30]. Hands covered by gloves were used to separate the litters from their dams for 24 hours. Both litters and dams were weighted before and after suckling. Milk yield was

calculated by taking the mean of the differences in body weight of litters and their dams before and after sucking using the following equation:

$$\text{Daily milk yield} = \frac{D_1 + D_2}{2}$$

D_1 = Weight of litter after sucking-weight before sucking.

D_2 = Weight of dam before sucking-weight after sucking.

2.9. Immunological Parameters (Phagocytic Activity of Neutrophils)

Phagocytosis of polymorphnuclear cells using heat-killed *Candida albicans* (Obtained from Dept. Bact., Fac. Vet. Med., Sadat City Univ.-Egypt) was performed according to the method described by [31]. One hundred neutrophils were examined and the number of neutrophils ingesting *Candida* was counted and expressed as percentage.

$$\text{Phagocytic activity \%} = \frac{\text{No.neutrophils ingesting Candida}}{\text{Total No.nutrophils}} \times 100$$

$$\text{Phagocytic index} = \frac{\text{Total No.ingested Candida}}{\text{Phagocytic activity \%}}$$

2.10. Statistical Analysis

The data were statistically analyzed for variance (ANOVA) with confidence limits set as 95% (significance at $p < 0.05$ probability level) described by [32]. Results were reported as Mean ± Standard error "SE", multiple range tests should be performed to compare among different groups or different periods of the experiment. Statistical analysis was performed using SPSS student version 10.0.7, June 2000.

3. Results

The used doses of seeds (powdered or germinated) and oil of fenugreek in the present study did not show any symptoms of toxicity or mortality throughout the experimental periods either for rabbit does or their bunnies.

3.1. Chemical Composition (%) of Milk and Milk Yield of Lactating Rabbits

In general, the fenugreek powdered seeds and oil administered groups (G_3 & G_5) recorded the highest percent values ($p < 0.05$) of fat, protein, lactose, total solids and solid not fat contents of the rabbits' milk in both the 1st and 2nd lactation periods relative to other tested groups (Table 2). However, at the beginning and mid of lactation periods, the milk yields recorded non-significant differences ($p > 0.05$) either between tested groups or between 1st and 2nd pregnancies in the same group. Whereas, at the end of lactation, germinated seeds (G_4) and oil (G_5) of fenugreek recorded significantly higher rates ($p < 0.05$) of milk yield relative to the control groups (Table 3).

3.2. Bunnies Growth Performance

The data presented in Table 4 revealed significant differences ($p < 0.05$) between fenu-

Table 2. Effect of seeds (powder), germinated and oil of fenugreek administration on rabbits' milk chemical composition (%).

		G1	G2	G3	G4	G5
Fat	1st pregnancy	8.57 ± 1.83$^{b.1}$	10.57 ± 1.61$^{ab.1}$	9.95 ± 2.35$^{ab.1}$	14.55 ± 1.32$^{a.1}$	13.05 ± 0.11$^{ab.2}$
	2nd pregnancy	9.12 ± 1.50$^{ab.1}$	12.12 ± 1.90$^{a.1}$	12.32 ± 2.73$^{a.1}$	4.32 ± 0.74$^{b.2}$	13.85 ± 0.74$^{a.1}$
Protein	1st pregnancy	9.82 ± 0.51$^{c.1}$	12.17 ± 0.71$^{b.1}$	14.12 ± 0.89$^{a.1}$	11.87 ± 0.30$^{b.1}$	13.32 ± 0.16$^{ab.1}$
	2nd pregnancy	9.75 ± 0.34$^{b.1}$	11.35 ± 0.47$^{b.1}$	13.32 ± 0.50$^{a.1}$	11.32 ± 0.95$^{b.1}$	13.70 ± 0.14$^{a.1}$
Lactose	1st pregnancy	3.10 ± 0.28$^{b.1}$	3.45 ± 0.29$^{ab.1}$	3.90 ± 0.14$^{a.1}$	3.02 ± 0.16$^{b.2}$	2.27 ± 0.04$^{c.2}$
	2nd pregnancy	3.07 ± 0.24$^{b.1}$	3.20 ± 0.10$^{b.1}$	3.92 ± 0.27$^{a.1}$	3.82 ± 0.13$^{a.1}$	2.47 ± 0.06$^{c.1}$
Total solid	1st pregnancy	24.50 ± 1.15$^{b.1}$	29.02 ± 1.69$^{ab.1}$	30.95 ± 3.03$^{a.1}$	32.42 ± 0.98$^{a.1}$	31.45 ± 0.12$^{a.2}$
	2nd pregnancy	25.07 ± 1.14$^{bc.1}$	29.50 ± 2.27$^{ab.1}$	32.62 ± 3.37$^{a.1}$	22.45 ± 1.14$^{c.2}$	32.65 ± 0.21$^{a.1}$
Solids not fat	1st pregnancy	15.92 ± 0.79$^{c.1}$	18.47 ± 0.95$^{b.1}$	21.05 ± 1.02$^{a.1}$	17.90 ± 0.46$^{bc.1}$	18.62 ± 0.42$^{b.1}$
	2nd pregnancy	15.87 ± 0.55$^{c.1}$	17.32 ± 0.47b$^{c.1}$	20.35 ± 0.76$^{a.1}$	18.15 ± 1.09$^{b.1}$	18.80 ± 0.12$^{ab.1}$

In the same column, means ± SE with different superscripts litters are significantly different ($p < 0.05$). In the same raw, means ± SE with different superscripts numbers between 1st pregnancy and 2nd pregnancy in same substance and group are significantly different ($p < 0.05$).

Table 3. Effect of seeds (powder), germinated and oil of fenugreek administration on rabbit's milk yield (g).

		G1	G2	G3	G4	G5
At the beginning of lactation	1st pregnancy	61.00 ± 1.73$^{a.1}$	66.87 ± 2.57$^{a.1}$	66.50 ± 3.22$^{a.1}$	66.25 ± 3.30$^{a.1}$	62.12 ± 1.97$^{a.1}$
	2nd pregnancy	61.00 ± 1.77$^{a.1}$	66.62 ± 2.48$^{a.1}$	66.87 ± 3.08$^{a.1}$	66.00 ± 2.73$^{a.1}$	62.12 ± 1.89$^{a.1}$
Mid of lactation	1st pregnancy	70.62 ± 0.62$^{a.1}$	61.25 ± 2.16$^{a.1}$	66.87 ± 2.30$^{a.1}$	75.62 ± 10.32$^{a.1}$	73.75 ± 2.39$^{a.1}$
	2nd pregnancy	70.37 ± 0.74$^{a.1}$	61.62 ± 1.97$^{a.1}$	69.37 ± 3.02$^{a.1}$	76.50 ± 8.92$^{a.1}$	74.25 ± 2.83$^{a.1}$
End of lactation	1st pregnancy	74.00 ± 0.88$^{ab.1}$	67.50 ± 1.44$^{c.1}$	66.62 ± 2.10$^{c.1}$	82.50 ± 6.84$^{a.1}$	79.37 ± 2.77$^{a.1}$
	2nd pregnancy	73.50 ± 1.19$^{ab.1}$	68.00 ± 1.47$^{c.1}$	71.62 ± 2.82$^{ab.1}$	82.50 ± 6.66$^{a.1}$	79.8 ± 2.83$^{a.1}$

In the same column, means ± SE with different superscripts litters are significantly different ($p < 0.05$). In the same raw, means ± SE with different superscripts numbers between 1st pregnancy and 2nd pregnancy in the same group are significantly different ($p < 0.05$).

Table 4. Effect of seeds (powder), germinated and oil of fenugreek administration on bunnies' body weight (g) of lactating rabbits.

		G1	G2	G3	G4	G5
1st week	1st pregnancy	100.75 ± 17.10$^{a.1}$	106.25 ± 4.13$^{a.1}$	94.50 ± 8.89$^{a.1}$	113.75 ± 5.93$^{a.1}$	124.75 ± 15.59$^{a.1}$
	2nd pregnancy	98.25 ± 17.28$^{b.1}$	85.50 ± 8.65$^{b.2}$	108.75 ± 4.51$^{b.1}$	82.00 ± 9.68$^{b.2}$	150.25 ± 17.85$^{a.1}$
2nd week	1st pregnancy	160.50 ± 19.70b$^{b.1}$	200.50 ± 8.46$^{b.1}$	264.25 ± 25.31$^{a.1}$	190.25 ± 14.66$^{b.1}$	177.00 ± 23.67$^{b.1}$
	2nd pregnancy	163.25 ± 20.32$^{ab.1}$	124.50 ± 8.61$^{c.2}$	207.75 ± 24.20$^{a.1}$	141.00 ± 31.37$^{ab.1}$	201.00 ± 23.87$^{a.1}$
3rd week	1st pregnancy	205.75 ± 26.19$^{b.1}$	237.25 ± 7.00b$^{b.1}$	355.50 ± 25.89$^{a.1}$	256.00 ± 19.46$^{b.1}$	235.25 ± 27.97$^{b.1}$
	2nd pregnancy	208.50 ± 26.01$^{b.1}$	198.50 ± 11.80$^{b.2}$	306.25 ± 26.05$^{a.1}$	202.50 ± 33.52$^{b.1}$	256.25 ± 24.70$^{ab.1}$
4th week	1st pregnancy	277.50 ± 15.06$^{c.1}$	306.00 ± 31.53$^{c.1}$	433.25 ± 31.73$^{a.1}$	382.00 ± 35.87$^{ab.1}$	359.00 ± 34.75$^{ab.1}$
	2nd pregnancy	282.75 ± 12.02$^{b.1}$	312.50 ± 22.45$^{b.1}$	424.50 ± 53.31$^{a.1}$	278.00 ± 36.00$^{b.2}$	374.00 ± 31.24$^{ab.1}$

In the same column, means ± SE with different superscripts litters are significantly different ($p < 0.05$). In the same raw, means ± SE with different superscripts numbers between 1st pregnancy and 2nd pregnancy in same substance and group are significantly different ($p < 0.05$).

greek treated groups G_3 and G_5 (seed and oil) related to G_1 and G_2 (control negative and positive) in growth performance of bunnies with the highest body weights were in bunnies suckling from dams of seeds treated group (G_3).

3.3. Immunological Parameters

Data represented in Table 5 revealed that, during the 1st lactation period, all experimental groups showed significant reduction ($p < 0.05$) of WBCS related to G_1. While, rabbits administered powdered seeds (G_3) and germinated seeds (G_4) of fenugreek were recorded significant elevation ($p < 0.05$) of WBCS related to control (G_1) group during 2nd lactation period (Table 5).

Although, significantly ($p < 0.05$) higher values of neutrophils recorded in fenugreek treated groups mainly during 1st lactation period, noticeable ($p < 0.05$) lesser values of lymphocyte were recorded in the same groups (G_3, G_4 & G_5) relative to both control groups (G_1 & G_2) during the 1st and 2nd lactation periods. Fenugreek seeds ingested group (G_3) showed a marked rise ($p < 0.05$) in monocytes and eosinophils percentages as compared to the other groups during both 1st and 2nd lactation periods. Moreover, rabbits treated with different forms of fenugreek (G_3, G_4 & G_5) recorded significantly marked values ($p < 0.05$) of phagocytic activity and phagocytic index in both 1st and 2nd lactation periods in comparing with both control groups (G_1 & G_2).

3.4. Anemia Screening Parameters

The results of the present study are shown in Table 6. Generally, fenugreek powdered seeds (G_3) and germinated one (G_4) treated groups showed significant ($p < 0.05$) RBCs and PCV raise relative to other groups during both 1st and 2nd lactation periods. Moreover, fenugreek seeds (G_3) and germinated one (G_4) groups were recorded significant ($p < 0.05$) higher values of Hb concentration particularly in 1st lactation period in comparison with other groups.

Mean corpuscular volume recorded non-significant differences between the tested groups either in 1st lactation period or 2nd lactation one. Although Mean corpuscular hemoglobin recorded significant increases ($p < 0.05$) in fenugreek treated groups (G_3, G_4 & G_5), the mean corpuscular hemoglobin concentration recorded non-significant increases in the same groups relative to both control groups (G_1 & G_2) mainly in 1st lactation period.

3.5. Acute Toxicity Study of Aqueous Extract of Fenugreek

Table 7 shows the mortality data in New Zealand female rabbit does exposed to progressive dosage levels of fenugreek seed aqueous extract. These data were recorded within a period of 72 hours. The animals in all groups did not display any signs of post-administration sickness and recorded zero% mortality among the treated rabbit does.

Table 5. Effect of seeds (powder), germinated and oil of fenugreek administration on total and differential leukocytic count, phagocytic activity and phagocytic index of lactating rabbits.

		G_1	G_2	G_3	G_4	G_5
WBCs	1st pregnancy	$11.70 \pm 0.44^{a.1}$	$7.77 \pm 0.86^{b.1}$	$7.250 \pm 0.75^{b.2}$	$8.92 \pm 1.3^{.b.2}$	$8.17 \pm 0.38^{b.2}$
	2nd pregnancy	$9.50 \pm 0.50^{bc.2}$	$7.65 \pm 0.61^{c.1}$	$11.42 \pm 0.93^{ab.1}$	$12.50 \pm 1.04^{.a.1}$	$9.35 \pm 0.23^{.bc.1}$
Phagocytic activity (%)	1stpregnancy	$64.80 \pm 1.32^{d,1}$	$71.75 \pm 1.31^{c,1}$	$88.87 \pm 2.29^{b,1}$	$94.50 \pm 1.55^{a,1}$	$91.00 \pm 1.41^{ab,1}$
	2nd pregnancy	$64.70 \pm 1.13^{d,1}$	$70.25 \pm 1.03^{c,1}$	$92.75 \pm 2.01^{b,1}$	$93.00 \pm 1.87^{a,1}$	$91.40 \pm 1.58^{b,1}$
Phagocytic index	1st pregnancy	$2.02 \pm 0.06^{a.1}$	$0.95 \pm 0.15^{b.1}$	$1.80 \pm 0.27^{a.1}$	$1.82 \pm 0.08^{a.1}$	$2.30 \pm 0.07^{a.1}$
	2nd pregnancy	$1.77 \pm 0.04^{b.2}$	$1.25 \pm 0.08^{c.1}$	$1.97 \pm 0.12^{b.1}$	$1.92 \pm 0.04^{b.1}$	$2.30 \pm 0.07^{a.1}$
Neutrophils (%)	1st pregnancy	$33.50 \pm 0.86^{b.1}$	$34.50 \pm 0.64^{b.1}$	$36.50 \pm 0.28^{a.1}$	$36.75 \pm 0.25^{a.2}$	$37.00 \pm 0.57^{a.1}$
	2nd pregnancy	$35.00 \pm 0.40^{c.1}$	$35.50 \pm 0.64^{bc.1}$	$36.50 \pm 0.28^{ab.1}$	$39.00 \pm 0.40^{a.1}$	$37.00 \pm 0.40^{b.1}$
Lymphocyte (%)	1st pregnancy	$65.25 \pm 1.03^{a.1}$	$64.25 \pm 0.75^{a.1}$	$59.75 \pm 0.47^{b.1}$	$61.25 \pm 0.57^{b.1}$	$62.30 \pm 0.75^{b.1}$
	2nd pregnancy	64.25 ± 0.47^{a1}	63.75 ± 0.47^{ab1}	59.75 ± 0.25^{c1}	59.75 ± 0.62^{c1}	62.25 ± 0.62^{b1}
Monocytes (%)	1st pregnancy	$1.25 \pm 0.25^{c.1}$	$1.25 \pm 0.25^{c.1}$	$2.75 \pm 0.25^{a.1}$	$2.25 \pm 0.47^{ab.1}$	$1.25 \pm 0.25^{c.1}$
	2nd pregnancy	$0.75 \pm 0.25^{b.1}$	$0.75 \pm 0.25^{b.1}0$	$2.75 \pm 0.25^{a.1}$	$1.25 \pm 0.25^{b.2}$	$0.75 \pm 0.25^{b.1}$
Esinophils (%)	1st pregnancy	$0.00 \pm 0.00^{b.1}$	$0.00 \pm 0.00^{b.1}$	$1.00 \pm 0.57^{a.1}$	$0.00 \pm 0.00^{b.1}$	0.50 ± 0.28^{ab1}
	2nd pregnancy	$0.00 \pm 0.0^{b.1}$	0.00 ± 0.00^{b1}	$1.00 \pm 0.00^{a.1}$	$0.00 \pm 0.00^{b.1}$	0.00 ± 0.00^{b1}

In the same raw, means ± SE with different superscripts litters are significantly different ($p < 0.05$). In the same column, means ± SE with different superscripts numbers between 1st pregnancy and 2nd pregnancy in the same group are significantly different ($p < 0.05$).

Table 6. Effect of seeds (powder), germinated and oil of fenugreek administration on anemia screening parameters in lactating rabbits.

		G_1	G_2	G_3	G_4	G_5
RBCs 10^6/mm^3	1st pregnancy	$6.65 \pm 0.43^{a.1}$	$5.47 \pm 0.26^{bc.1}$	$6.57 \pm 0.23^{a.1}$	$6.35 \pm 3.50^{ab.1}$	$5.10 \pm 0.15^{c.1}$
	2nd pregnancy	$5.55 \pm 0.25^{ab.2}$	$4.35 \pm 0.73^{c.1}$	$6.47 \pm 0.18^{a.1}$	$6.57 \pm 0.55^{a.1}$	$4.90 \pm 0.15^{c.1}$
PCV (%)	1st pregnancy	$37.25 \pm 1.1^{a,1}$	$36.50 \pm 1.19^{ab,1}$	$37.50 \pm 1.44^{a,1}$	$37.50 \pm 0.95^{a,1}$	$34.75 \pm 1.70^{ab,1}$
	2nd pregnancy	$35.00 \pm 0.40^{ab,2}$	$34.50 \pm 3.32^{ab,1}$	$37.50 \pm 1.19^{a,1}$	$37.75 \pm 1.54^{a,1}$	$34.00 \pm 1.35^{ab,1}$
Hb (g/dl)	1st pregnancy	$12.25 \pm 0.10^{b.1}$	$10.47 \pm 0.31^{c.1}$	$13.77 \pm 0.20^{a.1}$	$14.05 \pm 0.66^{a.1}$	$12.67 \pm 0.47^{b.1}$
	2nd pregnancy	$10.94 \pm 0.40^{a.2}$	$11.22 \pm 1.41^{a.1}$	$11.50 \pm 0.38^{a.2}$	$12.00 \pm 0.71^{a.2}$	$11.65 \pm 0.29^{a.1}$
MCV (Fl)	1st pregnancy	$56.95 \pm 5.072^{a.1}$	$66.80 \pm 1.059^{a.2}$	$57.07 \pm 5.17^{a.1}$	$59.37 \pm 1.87^{a.1}$	$68.05 \pm 1.87^{a.1}$
	2nd pregnancy	$63.47 \pm 3.18^{bc.1}$	$82.82 \pm 7.00^{a.1}$	$57.95 \pm 5.43^{c.1}$	$58.05 \pm 2.63^{c.1}$	$69.32 \pm 0.98^{abc.1}$
MCH (pg)	1st pregnancy	$18.60 \pm 1.450^{c.1}$	$19.27 \pm 1.21^{bc.2}$	$22.05 \pm 0.66^{ab.1}$	$22.20 \pm 0.84^{ab.1}$	$24.85 \pm 0.82^{a.1}$
	2nd pregnancy	$19.00 \pm 1.16^{c.1}$	$26.57 \pm 1.77^{a.1}$	$17.90 \pm 0.72^{c.1}$	$18.40 \pm 0.68^{c.2}$	$23.30 \pm 0.48^{b.1}$
MCHC (%)	1st pregnancy	32.97 ± 0.84^{ab1}	$28.82 \pm 1.40^{b.1}$	$36.60 \pm 2.07^{a.1}$	$37.45 \pm 1.49^{a.1}$	36.57 ± 0.98^{a1}
	2nd pregnancy	$29.95 \pm 1.38^{a.2}$	$32.35 \pm 1.56^{a.1}$	$30.10 \pm 15.23^{a.1}$	$31.77 \pm 1.25^{a.2}$	$31.35 \pm 2.49^{a.2}$

In the same raw, means ± SE with different superscripts litters are significantly different ($p < 0.05$). In the same column, means ± SE with different superscripts numbers between 1st pregnancy and 2nd pregnancy in the same group are significantly different ($p < 0.05$).

Table 7. Acute toxicity study of fenugreek seed aqueous extract in rabbits.

Group	No. of Rabbits/group	Dosage level (mg/Kg b. wt.)	Dead rabbits	Live rabbits	Mortality %
I	6	500	0	6	0
II	6	1000	0	6	0
III	6	1500	0	6	0
IV	6	2000	0	6	0
Control	6	Distilled water	0	6	0

4. Discussion

The animals in all groups did not display any signs of sickness and recorded zero % mortality after treatment with fenugreek seed aqueous extract. Hence, according to the convenient tables and the arithmetic formula adopted by [33], the LD50 or fenugreek aqueous extract could be anticipated to be higher than the highest dose tested of fenugreek (2000 mg/Kg b. wt.).

4.1. Chemical Composition (%) of Milk and Milk Yield of Lactating Rabbits

The fenugreek powdered seeds and oil administered groups recorded the highest percent values of fat, protein, lactose, total solids and solid not fat contents of the rabbits' milk in both the 1st and 2nd lactation periods. Whereas, at the end of lactation, germinated seeds and oil of fenugreek recorded significantly higher rates of milk yield relative to the control groups. These results may agree with the results obtained by [21] who stated that, phytoestrogens and diosgenin of fenugreek appear to account for increasing the milk flow. Also, the results are consistent with the results of [19] who demonstrated that, fenugreek has estrogenic activity that is effective on breast milk production.

The obtained results may be attributed to that, fenugreek seeds are high in phytoestrogens [34] which are plant-derived xenoestrogens functioning as the primary female-sex hormone not generated within the endocrine system but consumed by eating phytoestrogonic plants. Their structural similarity with estradiol (17-β-estradiol), gives them the ability to cause estrogenic effects [35]. The most potent component extracted from fenugreek is diosgenin which caused significant increases in estrogen, progesterone and prolactin [36]. Moreover, fenugreek seeds contain estrogen-like compounds, which stimulate pS2 expression in MCF-7 cell lines [20].

4.2. Bunnies Growth Performance

Fenugreek treatment of rabbit doses improved growth performance of bunnies with the highest body weights were in bunnies suckling from dams of powdered seeds treated group. These findings were matched with [37] who stated that the dietary supplementation of fenugreek seeds improved the 1 - 21 days' bunnies weight gain and reduced the

pre-weaning mortality in New Zealand rabbits. However, the findings of the current study did not agree with [22] who reported, that the body weight of bunnies at the 17 days did not differ significantly between fenugreek and control bunnies.

Since, growth of suckling depends on the nursing capacity of their mothers with improving does milk yield resulted in an appropriate milk supply of the bunnies [22]. It was suggested that, the administration of seed, germinated and oil of fenugreek into rabbit does resulted in high protein, fat, lactose and total solid content of their milk which improved growth performance of suckling bunnies.

4.3. Immunological Parameters

During the 1st lactation period, all experimental groups showed significant reduction of WBCS. While, rabbits administered powdered seeds and germinated seeds of fenugreek were recorded significant elevation of WBCS related to control group during 2nd lactation period. Moreover, rabbits treated with different forms of fenugreek (G_3, G_4 & G_5) recorded significantly marked values of phagocytic activity and phagocytic index in both 1st and 2nd lactation periods in comparing with both control groups.

The findings of the current study were similar with [38] who concluded that, Fenugreek extract in albino Swiss mice showed immune modulatory property through various mechanisms such as increase in relative organ weight of thymus, delayed type of hypersensitivity response, humoral immunity, increase in phagocytic index and phagocytic capacity of macrophages. Also, data obtained by [39] showed significant differences ($p < 0.05$) in lymphocyte, neutrophil, eosinophil, monocyte and highly significant difference ($p < 0.05$) in basophil ratios in response to fenugreek saponin ingestion. On the other hand, the findings of the current study were not matched with [40] whose result indicated that addition of fenugreek flour in the diet of alcoholic animals had an opposite effect: it strongly increased the cells of unspecific immunity and restored to the control value the cells of specific immunity.

It was suggested that the administration of seed, germinated and oil of fenugreek were responsible for improvement of Immunological profile of lactating female rabbits through increase humeral immunity, phagocytic index and phagocytic capacity of macrophages. Moreover, the antimicrobial activity of fenugreek can be attributed to the presence of flavonoids; which exert their anti-ulcer lesions induced by various necrotic agents. Fenugreek extract derived via solid state bioconversion using *Rhizopus oligosporus* has shown its defensive effect on gastric mucosa by its antimicrobial activity on *Helicobacter pylori* [41].

It may be suggested that, the possible mechanisms responsible for gastro protective action may include the production of acidic environment by altering the urease activity of the bacteria, disruption of membrane proton motive force; attributed to phenolic extract of fenugreek due to which bacterial cell lysis may occur; presence of scopoletin, a coumarin derivative of fenugreek reported to inhibit electron transport chain in prokaryotes [41]. Also, *T. foenum-graceum* extract was one of the strongest antibacterial extracts [42].

T. foenum-graceum seeds [43] and leaves [44] are used as an ingredient in traditional medicine and have been reported to exhibit pharmacological properties which have different therapeutic effects, for example saponins and fenugreekine of its seeds introduced as anti-inflammatory, antiviral and antimicrobial elements of the plant [45]. Moreover, [46] concluded that, fenugreek infusion having antimicrobial and antibiotics like properties in broiler chicks.

4.4. Anemia Screening Parameters

Generally, fenugreek powdered and germinated seeds treated groups showed significant increase in RBCs count, PCV%, Hb concentration and MCH value. These findings were identical with those of [46] who reported that there was significant increase in the levels of Hb, RBCs and PCV after 7 days of fenugreek extract treatment. Also, [47] recorded significant differences ($p < 0.05$) in hemoglobin concentration and red blood cells count. On the other hand, the obtained results in the present study disagree with those obtained by [48] who reported that administration of fenugreek did not cause any significant differences in RBCs and PCV. However, increasing fenugreek level to 10% decrease Hb concentration in blood.

The findings of the current study were matched with [49] whose result indicated thatin animals which received fenugreek RBCs count remained elevated, due to the antioxidant activity of flavonoids. Moreover, the findings of the current study were identical with [50]. Who concluded that the high iron content of fenugreek seed flour stimulated hemoglobin synthesis.

It was suggested that the administration of seed and germinated fenugreek that contain high iron content stimulated hemoglobin synthesis beside the antioxidant activity of flavonoids were responsible for improvement of complete blood count of lactating female rabbits. Also, fenugreek bears potential of a powerful antioxidant activity been evaluated in human erythrocytes by the exposure to fenugreek seeds extract which showed protective effects against hydrogen peroxide-induced oxidation by protecting the erythrocytes from hemolysis and lipid per oxidation due to the presence of flavonoids and poly phenols [51].

5. Conclusion

Administration of fenugreek (powdered seeds, germinated seeds or oil) to rabbit does improved quality and quantity of milk, bunnies body weight, immunological profiles (WBCs count, differential leukocytic count, phagocytic activity and phagocytic index) and erythrogram (RBCs, Hb, PCV and blood indices).

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