Effect of Sprouted *Trigonella foenum graecum* L. Incorporation into the Diet on Milk Production of Rabbit Does and Growth of Young Rabbits in the Northeast of Algeria

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RESEARCH ARTICLE

Abstract

The aim of this study was to assess the effect of sprouted fenugreek incorporation into the diet of rabbits on their dairy and growth performances. Data from 24 lactations performed by 8 Californian rabbit does were studied. They were divided into 2 homogeneous groups, a control (batch C) having received an ordinary ration and an experimental (batch E) having received the same ration supplemented with sprouted fenugreek. Growth rates of suckling pups were also recorded. Then, Growth performance of 152 young rabbits' issue from these females was monitored. They were divided into 4 homogeneous groups, one control having received an ordinary diet (batch C) and 3 experimental having received the same diet with substitution of 12%, 15% and 25% (batch E1, E3 and E3) of the concentrate by sprouted fenugreek. The results showed an improvement in the dairy performance of the rabbit does of batch E, with an average peak lactation of 287 g/d vs 236 g/d in batch C ($p < 0.01$). The daily weight gain of suckling pups was better in batch E (13 g/d vs 11.7 g/d; $p < 0.01$). Likewise, the weaned young rabbits of the experimental batches recorded higher growth rates than the control batch ($p < 0.01$). Sprouted fenugreek incorporation into the rabbit's ration allowed to improve their performances.

Keywords: Algeria; growth; sprouted fenugreek; rabbit; milk production.

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INTRODUCTION

These last years, modern consumers have become increasingly aware of the link between diet and health. They see it as a valuable way to improve the quality of their lives. As a result, their interest in healthier meats and meat products has increased worldwide (Tărnăuceanu et al., 2016).

Rabbit meat is an ideal meat, with its high protein content (around 21%) (Rekik and Bergaoui, 2016a), vitamin B12 (the richest among the most common meats), Phosphorus, Potassium, Sodium, Zinc and Selenium, as well as its favorably low fat (8.5%) (Tărnăuceanu et al., 2016).

This meat, increasingly prized for its nutritional profile which can be improved by feeding rabbits, has stimulated the interest of many researchers who have tried to improve the performance of these animals by incorporating different raw materials in their diet. Fenugreek (*Trigonella foenum-graecum* L.) is an annual herbaceous plant characterized by its nutritional value in addition to its medicinal effects (Khorshidian

et al., 2016; Hilles and Mahmood, 2016). Its dried seeds and leaves are traditionally used as galactogogues and appetite stimulants (Basch et al., 2003; Danditiya et al., 2013).

The use of fenugreek seeds in the diet has been reported to improve the milk production of rabbit does and the weight gain of young rabbits (Rekik and Bergaoui, 2016).

The objective of this experiment is to evaluate the effect of the incorporation of this raw material in its germinated form into the diet on the dairy performance of rabbit does and the growth of young rabbits before and after weaning.

MATERIALS AND METHODS

Study area

The experiments as well as the forage analysis were conducted in a laboratory at the ISV of the University of MENTOURI in Constantine, in the period from November 2018 to May 2019.

Biological material

Biological material consisted of a total of 161 rabbits of Californian breed, including:

- One adult male and 8 adult female rabbits, weighing between 2715 and 2980 g.
- 152 young rabbits issued from these rabbit breeders.

The rabbits were housed in individual cages of 75 cm in length, 46 cm in width and 30 cm height which are mounted in Flat-Deck and are equipped each with a feeder and a drinker bottle (Figure 1), placed in a closed laboratory, clean, warm enough, calm, well ventilated and lighted by natural and artificial light (in case of a night visit). For female's wooden nest boxes, clean, disinfected and lined with wood shavings and straw were placed 3 days before the intended date of parturition.

Figure 1. Rabbits housed in a flat deck individual cages.

Technical material

The weighings were carried out using a DAHONGYING brand electronic balance. The animals were identified by tattooing the numbers on their left ears. The pregnancy diagnosis was carried out using a CHISON D600VET ultrasound system equipped with an adjustable frequency probe (5 MHz). During artificial insemination, the semen was collected using an artificial vagina maintained at a temperature of 39 °C, and a teaser doe (Figure 2). Then, a semen extender (DJo) was prepared with sodium citrate and egg yolk (Akpo et al., 2018).

Food

The concentrate pellets used is intended for feeding rabbit does and growing young rabbits. It comes from an industrial unit. According to the manufacturer's indications, it is composed of: Alfalfa, Milling by-products, Wheat, Sunflower meal, Molasses, Poly-Vitamins, Trace elements, calcium carbonate, Amino acids, Anticoccidial and Salt. Fenugreek seeds were germinated by hydroponics (rapid germination in 5 days). The only conditions necessary for good germination are: humidity, air and light.

Methods

Study of sprouted fenugreek effect on the milk production of rabbit does

In total, 24 lactations performed by 8 rabbits were studied (3 reproduction cycles). In order to breed, the does were inseminated with 0.5 ml of extended semen, followed by the injection of 0.2 ml of GnRH intramuscularly to induce ovulation (Figure 2).

Figure 2. Stages of rabbit reproduction: semen collect (a,b), semen deposit (c), intramuscular injection of GnRH (d).

After a positive pregnancy diagnosis, the rabbit does were divided into two batches: a control batch (batch C) having received an ordinary diet (concentrate pellets and wheat straw) and an experimental batch (batch E) having received the same diet supplemented with sprouted fenugreek (Table 1). Food was given once a day during the last third of gestation (10 days) and the entire lactation period (33 days) with water ad libitum.

In order to eliminate the genetic factor, the 2 batches were reversed at each new cycle (control batch having become experimental and vice versa).

Table 1. Daily rations distributed to rabbit does.

Period	Batch C		Batch E		
	CP	WS	CP	WS	SF
Gestation	200	10	200	10	85
Lactation	250 à 400	10	240 à 390	10	85

CP : Concentrate Pellets ; WS=Wheal Straw ; SF=Sprouted Fenugreek (expressed in g/d)

From the kindling, the litters were counted and weighed. The number of rabbit pups suckled by a female was equilibrate by adoption.

The quantities of milk produced were recorded through an indirect measurement by the variations in the doe weights during the suckling. Mothers and litters were weighed before and after each suckling every 4 days for 5 weeks. Doe weight loss and pups weight gain provided an estimate of the amount of milk produced (Lebas and Sardi, 1968).

The young rabbit's growth before weaning was also an indicator of the mothers' milk performances. their weights were recorded regularly every 4 days.

Study of sprouted fenugreek effect on young rabbit's growth

152 weaned and dewormed young rabbits were divided into four homogeneous groups (according to weight and sex) of 38 subjects each, receiving four different diets (Table 2) wich were calculated according to the results of germinated fenugreek and concentrate pellets analysis to meet their nutritional needs (Drogoul et al., 2004).

- Batch C: control ;
- Batch E1, E2 and E3: with substitution of 10%, 15% and 25% of concentrate by sprouted fenugreek (Figure 3).

Table 2. Daily rations of the four batches of weaned rabbits.

Age (weeks post-weaning)	Batch C		Batch E1			Batch E2			Batch E3		
	CP	WS	CP	SF	WS	CP	SF	WS	CP	SF	WS
1	60	10	60	10	10	60	15	10	60	20	10
2	80	10	75	12,5	10	70	17,5	10	65	22,5	10
3	100	10	90	12,5	10	85	17,5	10	75	22,5	10
4	120	10	105	15	10	95	20	10	85	25	10
5	140	10	120	15	10	115	20	10	105	25	10
6	160	10	140	17,5	10	125	25	10	115	30	10
7	180	10	170	20	10	160	23	10	145	40	10

CP: Concentrate Pellets ; WS=Wheal Straw ; SF=Sprouted Fenugreek (expressed in g/d)

Figure 3. Rabbits eating sprouted fenugreek.

The changes in body weight of young rabbits were recorded once a week until the seventh week post-weaning.

Data processing

Several parameters were calculated: Reproduction parameters: fertility, prolificacy, birth and pre-weaning mortality rates. Milk production parameters: total milk production (TMP) and peak lactation. Growth parameters: daily weight gain (DWG).

Statistical analysis

Statistical analysis was performed by R software (version 3.6.2). A verification of the normality of the samples (by Shapiro-Wilk test) was followed by the application of an adequate comparison test (Student, ANOVA, Wilcoxon-Man-Whitney, Kruskal-Wallis) and correlation (Pearson), in order to assess the influence of certain variation factors.

To study the effect of certain qualitative factors, analysis of variance was performed using the following model:

$$Y_{ij} = \mu + P_i + R_j + e_{ij}$$

μ = general mean;

P_i = effect of the lactation rank ($i = 1$ to 3);

R_j = effect of the ration ($j = 1$ to 2);

e_{ij} = residual error.

RESULTS AND DISCUSSIONS

Reproduction parameters

Reproduction parameters (Table 3) were calculated globally for all rabbit does, as they were fed the same diet (ordinary) at that time. The majority of the recorded parameters were better than those appearing in various works, in particular the fertility rate (85.7%) which is higher than those reported by Rekik and Bergaoui (2016b) in Tunisia (63.19; 75.2 and 67.66 %), and by Bonnes et al. (2005) in France (78; 77.8 and 79%). However, the prolificacy rate (729%) is lower than those obtained by Rekik and Bergaoui (2016b) (922 ; 857 and 633%), as well as Bonnes et al. (2005) (986; 994 and 893%). The mortality rate of young rabbits before weaning (13.1%) is comparable to those reported by Bonnes et al. (2005) (15.1; 15.2 and 14.6%) and lower than those reported by Rekik and Bergaoui (2016b) (18.61 and 17.06%).

These observed differences are mainly due to the used rabbit breeds and diets, as well as the rhythm of reproduction.

Table 3. Reproduction performance of rabbit does.

Fertility rate	85.7 %
Prolificacy rate	729 %
Total mortality rate	13.1 %
Mortality rate (0-7 days)	5.71%
Mortality rate (8 to 33 days)	7.43%
Mortality rate (34 to 91 days)	0%

Effect of sprouted fenugreek on the milk production of rabbit does

All the obtained lactation curves have the same general shape as the average curve (Figure 4), as well as the regular shape of the lactation curve in the rabbit, with an ascending phase followed by a descending phase after a lactation peak which is reached around 21 days after parturition (Lebas and Sardi, 1968; Bonnes et al., 2005; McNitt et al., 2013).

The results showed a very significant difference ($p < 0.01$) between the milk parameters recorded in the control batch and those of the experimental batch in favor of the latter (Table 4).

Figure 4. Average lactation curves of doses of the two batches.

The daily quantities of milk produced were greater in the rabbit does of the experimental batch with an average peak of 287 g/d. This value is significantly higher compared to the control batch (236 g/d; $p < 0.01$).

The average lactation peak for batch C was 236 g/d, corresponding to the species standard reported by Drogoul et al. (2004) and Lebas (1971) who consider that the production reaches at the peak of lactation 200 to 250 g/d. however, it is higher than that reported by Rekik and Bergaoui (2016b) in Tunisia in a similar batch (164 g/d).

As for the peak of batch E (287 g/d), it was higher than the norm (Drogoul et al., 2004; Lebas, 1971), and comparable to that reported by Rekik and Bergaoui (2016b) in a batch of crossbred does fed with fenugreek seeds (273 g/d).

From the last 2 weeks, there was a drop in milk production which was slightly remarkable in the doses of group C ($p > 0.05$). this drop is explained by the change in the feeding behavior of the young rabbits who start consuming solid food in addition to milk (Bonnes et al., 2005; McNitt et al., 2013).

The average total milk production of the rabbits in batch E was significantly higher by 1569 g than that of the rabbits in batch C ($p < 0.01$; Table 4).

All of the variation factors such as lactation rank, litter size and the number of suckling pups did not have a statistically significant influence on all the milk parameters of the two batches ($p > 0.05$). Drogoul et al. (2004) reported that rabbit milk production does not increase in proportion to litter size.

Only the diet had a significant effect where an improvement in milk yield in batch E could be attributed to the incorporation of sprouted fenugreek into the ration. The positive effect of fenugreek on milk production has been demonstrated and observed in numerous studies in many species (Wani and Komar, 2016; Rekik and Bergaoui, 2016b). Al-Shaikh et al. (1999) had observed better results in goats supplemented with 25% of fenugreek seeds compared to a control group.

Indeed, fenugreek seeds are rich in phytoestrogens and diosgenin (Abdel-Rahman, 2016) which are xenoestrogens characterized by a structure similar to oestradiol giving them the ability to cause estrogenic effects (Yildiz, 2005).

It should be noted that a strong correlation was recorded between the amount of milk produced by rabbit does and that consumed by rabbit pups ($R^2 = 0.95 \pm 0.15$).

Effect of sprouted fenugreek on the pre-weaning growth of the rabbit pups

Up to 3 weeks, the rabbit pups consume only breast milk and their growth depends on the nursing capacity of their mothers. Therefore, growth is a reliable indicator of the amount of milk they have received (Lebas and Sardi, 1968; Eiben et al., 2004).

The daily weight gains of the kits suckled by the does in batch E (13 ± 0.36 g/d) was better compared to that of the kits of the batch C ($p < 0.01$; Figure 5; Table 5).

The mothers' diet had no significant effect on the birth weight of the rabbit pups ($p > 0.05$). It was from the 6th day after birth that the difference in growth between the pups in Batch C and Batch E became significant ($p < 0.01$).

It has been shown in several studies that the addition of fenugreek in the diet of rabbits resulted in an increase in the content of the milk in protein, fat and lactose, etc. which allowed to improve the growth performance of suckling rabbits (Eiben et al., 2004; RASHWAN, 1998).

There was no significant effect of birth weight, litter size or number of suckling kits per female on weaning weight and DWG of kits ($p > 0.05$).

Table 4. Results of milk recording

Batch	Lactation rank	Litter size	Suckling pups	Amount of produced milk (g/day)									TMP
				J2	J6	J9	J13	J17	J21	J25	J29	J33	
C	2 ± 0.85	7.17±1.53	6.00±0.95	105±5.85 ^a	122±6.47 ^a	144±16.6 ^a	165±17.4 ^a	198±15.9 ^a	234±15.2 ^a	211±23.2 ^a	192±18.3 ^a	176±16.3 ^a	5707±354 ^a
E	2 ± 0.85	7.42±1.93	6.67±1.23	119±5.62 ^b	144±10.3 ^b	202±10.2 ^b	226±12.7 ^b	258±14.71 ^b	287±16.4 ^b	265±14.2 ^b	247±11.9 ^b	224±12.7 ^b	7276±234 ^b
<i>p</i> value	NS	NS	NS	***	***	***	***	***	***	***	***	***	***

The results are presented as Means ± Standard Deviation (g/day).

Significance levels: NS non-significant ; ***P < 0.001.

a,b Means with different superscripts in the same column are significantly different (P < 0.05).

Table 5. Evolution of the average weights of the rabbit pups during the lactation phase.

Batch	Age (day)										DWG (g/d)
	J0	J2	J6	J9	J13	J17	J21	J25	J29	J33	
C	43.2±4.60 ^a	63±4.56 ^a	102.5±4.73 ^a	145.9±4.21 ^a	186.5±6.48 ^a	232.6±5.89 ^a	276.4±4.48 ^a	309.6±41.5 ^a	367±6.78 ^a	429.5±15.9 ^a	11.7±0.48 ^a
E	46.1±8.28 ^a	66.2±8.11 ^a	111.5±8.40 ^b	162.1±8.22 ^b	223.9±9.11 ^b	285.2±8.96 ^b	337.4±10 ^b	391.2±9.13 ^b	400.6±11.2 ^b	476.5±15.2 ^b	13±0.36 ^b
<i>p</i> value	NS	NS	**	***	***	***	***	***	***	***	***

The results are presented as Means ± Standard Deviation (gram).

Significance levels: NS non significant; **P < 0.01 ; ***P < 0.001.

a,b Means with different superscripts in the same column are significantly different (P < 0.05).

Table 6. Evolution of the average weights of the weaned young rabbits.

Batch	Age (day)							DWG (g/d)	
	J33	J40	J47	J54	J61	J68	J75	J81	
C	453 ± 25 ^a	662 ± 21.8 ^a	1003 ± 20.3 ^a	1385 ± 20.1 ^a	1667 ± 24.3 ^a	1873 ± 20.3 ^a	2046 ± 50.2 ^a	2392 ± 47.3 ^a	39.6 ± 0.75 ^a
E1	454 ± 42.4 ^a	699 ± 42 ^b	959 ± 38.4 ^b	1396 ± 39.8 ^a	1617 ± 53.2 ^b	1938 ± 42 ^b	2010 ± 48.4 ^a	2421 ± 45.9 ^{ac}	40.1 ± 0.45 ^a
E2	452 ± 28.3 ^a	746 ± 25.9 ^c	1035 ± 24.1 ^c	1445 ± 95.5 ^a	667 ± 22.6 ^a	2048 ± 46.6 ^c	2149 ± 24.3 ^b	2506 ± 51.5 ^b	41.9 ± 0.59 ^b
E3	453 ± 22.3 ^a	669 ± 20.9 ^b	949 ± 34.1 ^b	1417 ± 44.5 ^a	1639 ± 35.2 ^{ab}	2068 ± 102 ^c	2170 ± 63 ^b	2459 ± 29.2 ^c	34 ± 0.53 ^c
<i>p</i> value	NS	***	***	NS	NS	***	***	**	***

The results are presented as Means ± Standard Deviation (gram).

Significance levels: NS non significant ; **P < 0.01 ; ***P < 0.001

a,b,c Means with different superscripts in the same column are significantly different (P < 0.05).

Figure 5. Average growth curves of the rabbit pups of the two batches.

Effect of sprouted fenugreek on the post-weaning growth of young rabbits

Overall, the growth performance of the young rabbits improved after incorporating sprouted fenugreek into their diet (Figure 6).

The young rabbits of the experimental batches recorded the best performance with an average final weight of between 2421 ± 45.9 g (batch E1) and 2506 ± 51.5 g (batch E2).

At the age of 81 days, the weights of our young rabbits correspond to the norm in rabbits which is between 2.3 to 2.6 kg at 10 weeks of age (Drogoul et al., 2004).

Our results are comparable with those obtained by Mabrouki et al. (2016) in young rabbits supplemented with 5% and 10% of pre-germinated fenugreek (weights between 2410 g and 2430 g). This author did not find a significant effect on weight gain.

On the other hand, they are lower than the results published by Rekik and Bergaoui (2016c) in batches supplemented with fenugreek seeds (2788 to 2923 g in 84 days). This can be attributed to the difference in ration nutrient density. The growth rate of young rabbits is mainly affected by the diet energy and protein content (Mohsen et al., 2015).

The best DWGs were recorded in the experimental batches, in particular those of Batch E2 (41.9 ± 0.71 g/d) corresponding to the standards in rabbit which are of the order of 40 to 50 g/d between weaning and slaughter (Drogoul et al., 2004).

Figure 6. Average growth curves of the young rabbits of the four batches.

Statistical analysis showed that there was no significant influence of birth and weaning weights on the final weights and DWGs of the young rabbits ($p > 0.05$) and that only the diet had a impact on these performances which were better in

the experimental batches compared to the control batch ($p < 0.01$; Table 6). This can be attributed to the presence of fenugreek in the experimental rations.

The beneficial effect of fenugreek on growth has been demonstrated in several species: fish (Abbas et al., 2019), broilers (Park and Kim, 2015) and rabbits (Abd El-Rahman et al., 2011). According to Kalpana and Srinivasan (2001), the saponins contained in fenugreek stimulate growth.

However, it was observed that the incorporation of fenugreek beyond a certain rate (batch E3) negatively influences performance. This has been observed in numerous studies in many species (Al-Shaikh et al., 1999). Mabrouki et al. (2016) found a negative significant effect of fenugreek on the daily food intake (with incorporation of 10%). Supplementation tends to reduce food consumption (Eiben et al., 2004).

The decrease in intake is linked to the fraction of soluble fiber which forms a gelatinous structure creating a feeling of satiety in the abdomen and thus decreases appetite (Akbari et al., 2012; Mabrouki et al., 2015). In the present study, reduction in food intake was not observed, possibly because food was not given ad libitum.

CONCLUSION

The incorporation of sprouted fenugreek in the diet of rabbits showed an improvement in their performance:

- The milk yield was amplified in females with an average lactation peak of 287 g/d obtained in 21 days.
- The weight gain of the rabbit pups suckled by these females was improved (DWG = 13 g/d) reflecting a better suckling capacity.
- The weight gain of weaned young rabbits was better with the substitution of 15% of concentrate by sprouted fenugreek (DWG = 41.9 g/d).

The optimal form and rate of incorporation of fenugreek should be determined for best performance.

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Conflicts of Interest

The authors would like to declare that there is no conflict of interest related to the publication of this paper.

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