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The Effect of Anethum Graveolens Seeds Administration on the Concentrations of Some Hormones and Histological Changes in the Ovaries, Uterus, and Lactational System During Estrus and Pregnancy Stages in Arabi Ewes

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ABSTRACT

Objective: *This study aimed to assess the reproductive performance of Arabi ewes by examining the effects of administering Anethum graveolens seeds (dill seeds) on hormonal profiles (estrogen, progesterone, and prolactin) and histological changes in ovarian, uterine, and mammary tissues during estrus and pregnancy.* ***Methods:*** *Sixteen Arabi ewes (aged 1.5–2 years; weighing 45–53 kg) were randomly divided into four groups: T1 (5 g dill seeds/day orally for 25 days), T2 (10 g dill seeds/day orally for 25 days and again one month postpartum), T3 (intravaginal sponges with Medroxyprogesterone acetate (MAP) for 14 days followed by PMSG injection), and T4 (control group with no treatment). Hormone levels were measured monthly via blood serum, and histological analyses of ovaries, uterus, and mammary glands were performed.* ***Results:*** *Administration of dill seeds significantly influenced hormone concentrations. Estrogen levels increased notably in T2 across pregnancy stages ($p<0.05$, $p<0.01$). Progesterone levels also significantly rose in T2 during early pregnancy months ($p<0.01$). Additionally, prolactin concentrations were significantly elevated in T2 throughout pregnancy and postpartum periods ($p<0.01$). Histological examinations showed no pathological changes in the uterine tissues.* ***Novelty:*** *Anethum graveolens seeds supported corpus luteum function, promoted increased progesterone and prolactin secretion, and mildly stimulated mammary gland development. Dill seeds could therefore be considered beneficial in enhancing milk production in Arabi ewes.*

INTRODUCTION

Livestock constitutes a vital component of national economies and global food security. Its role in sustaining the growing human population through the provision of meat, milk, and fibers has driven extensive scientific interest in improving animal growth, health, and reproductive performance [1]. In recent years, advanced reproductive technologies such as ultrasound imaging, embryo transfer, artificial insemination, and sex determination have been adopted to enhance reproductive efficiency and genetic improvement in farm animals [2]. Among these, optimizing the reproductive potential of ewes, particularly ovulation rates, hormone regulation, and maternal care, is crucial for improving productivity in regions where agriculture is a key contributor to the GDP [3].

Anethum graveolens (commonly known as dill) is a widely cultivated medicinal herb traditionally used to manage reproductive health issues and stimulate lactation. Its seeds are rich in biologically active compounds such as flavonoids, phenols, saponins, tannins, and terpenes [4]. Previous studies have indicated that dill seeds can modulate

reproductive hormones, particularly by promoting progesterone secretion and influencing estrus behavior [5]. Among its key constituents, the flavonoids kaempferol, vicenin, and myristicin exhibit phytoestrogenic activity, mimicking the action of endogenous estrogens like 17β-estradiol. These compounds bind to estrogen receptors and influence gene expression, thereby affecting reproductive tissue development, ovulation, and hormonal balance [6]. In addition to estrogenic effects, dill components have been reported to stimulate uterine contractions, enhance blood circulation, and facilitate placental expulsion postpartum [7], [8]. Given these potential effects, the present study aims to evaluate the influence of Anethum graveolens seed supplementation on hormonal profiles (estrogen, progesterone, and prolactin) and histological changes in the ovaries, uterus, and mammary glands of Arabi ewes during estrus and pregnancy stages.

RESEARCH METHOD

Animals

This study was conducted on sixteen healthy Arabi ewes aged between 1.5 and 2 years and weighing 45 to 53 kilograms. The animals were randomly assigned to four equal groups (n = 4 per group). Additionally, three fertile rams, aged 2 to 3 years and weighing 65 to 75 kilograms, were used exclusively during the mating period. All animals were housed in semi-enclosed pens under standard management conditions. They were fed a balanced ration consisting of green fodder and a concentrate mixture, calculated at 3% of each ewe's body weight per day. The concentrate was composed of 37% barley, 35% wheat bran, 20% yellow corn, 5% soybean meal, 2% limestone, and 1% table salt. Straw was offered as a source of roughage, while clean water and salt blocks were available ad libitum. To minimize stress and digestive disturbances, particularly bloat, a two-week acclimatization period preceded the experiment.

Animal management

The animals were treated as follows: Group 1 (T1) received 5 grams of *Anethum graveolens* (dill) seed powder daily via gelatin capsules for 25 consecutive days. Group 2 (T2) was administered 10 grams of dill seed powder daily for the same period and again for an additional 30 days starting one month postpartum. Group 3 (T3) was treated with intravaginal sponges impregnated with 20 mg of Medroxyprogesterone acetate (MAP), which were coated with antiseptic cream and inserted using a sterile glass rod and speculum. After 14 days, the ewes in this group received an intramuscular injection of 500 IU of pregnant mare serum gonadotropin (PMSG). Group 4 (T4) served as the untreated control. All oral treatments were administered early in the morning before feeding.

Collecting blood samples and measuring hormones

Blood samples were collected from all ewes via the jugular vein using sterile 10 mL syringes. Sampling occurred before pregnancy, monthly throughout gestation, and one month after parturition. The collected blood was transferred into gel-containing tubes and centrifuged at 3,000 revolutions per minute to separate the serum. The serum was then stored at −20°C until hormonal analysis. Serum concentrations of estrogen,

progesterone, and prolactin were measured using enzyme-linked immunosorbent assay (ELISA) kits, following the manufacturer's instructions.

Histological study

Following Slaughter at the conclusion of the study, tissue samples from the ovaries, uterus, and mammary glands were collected for histological evaluation. The samples were trimmed into appropriate sizes, fixed in 10% buffered formalin for 24 hours, and processed for paraffin embedding. Sections were prepared using a microtome (China) and stained with hematoxylin and eosin (H&E), according to the protocol described by Luna [9]. Microscopic examination was conducted to identify any histological alterations attributable to the treatments.

Statistical analysis

Statistical analysis was performed using one-way analysis of variance (ANOVA) in SPSS software version 20. Differences among treatment means were tested using Tukey's post hoc multiple comparison method. All data were presented as mean ± standard deviation (SD), with statistical significance considered at *p*-values less than 0.05 and 0.01.

RESULTS AND DISCUSSION

Results

Hormonal Analysis

Estrogen Levels

The estrogen hormone concentrations varied significantly among treatments throughout the pregnancy stages (Table 1). Treatment 2 (10 g dill seeds) showed consistently higher estrogen levels, particularly at the first and fourth months of pregnancy and after 21 days of seed administration ($p<0.01$). Treatment 1 (5 g dill seeds) exhibited significantly elevated estrogen levels during the second and fourth months of pregnancy ($p<0.05$). Treatment 3 (intravaginal sponges) had notably increased estrogen concentrations only during the third month ($p<0.01$). However, no significant differences were observed before dill administration, in the fifth month of pregnancy, or postpartum among all treatments.

Table 1. The effect of administering Anethum graveolens seed powder on the average concentration of estrogen in Arabi ewes (pg./ml) during different physiological stages. (Mean± SD).

Treatments	Without the administration of dill seeds	After 21 days of administration of dill seeds	1st month of pregnancy	2nd month of pregnancy	3rd month of pregnancy	4th month of pregnancy	5th month of pregnancy	After parturition
T1	42.41 ±1.35	73.87 b ±1.35	43.11 b ±5.74	38.39 A ±1.09	27.38 c ±0.91	47.01 A ±1.06	78.82 ±1.45	38.82 ±1.53

T2	42.80 ±2.16	75.11 A ±2.33	48.37 A ±0.81	37.55 b ±0.75	26.42 c ±2.03	47.77 A ±1.95	78.93 ±1.52	38.87 ±1.42
T3	41.74 ±0.81	42.94 c ±1.95	40.52 c ±0.24	37.59 b ±1.31	43.22 A ±0.72	43.77 b ±0.65	78.80 ±2.17	38.07 ±1.97
T4	41.26 ±0.58	43.58 c ±0.81	42.11 b ±0.65	39.68 A ±0.85	37.58 b ±1.68	42.29 b ±1.19	74.84 ±3.40	38.25 ±1.90
Significant level	N. S	*	*	**	**	**	N. S	N. S

Different letters within the same column mean significant differences at the level of *($p \leq 0.05$), ** ($p \leq 0.01$), N.S. There are no significant differences between the experimental treatments.

Progesterone Levels

Progesterone concentrations increased significantly with dill seed supplementation (Table 2). Treatment 2 significantly surpassed other treatments during the first four months of pregnancy ($p<0.01$). In contrast, Treatment 1 exhibited higher progesterone concentrations specifically after the initial 21 days of dill seed administration compared to other treatments ($p<0.01$). No significant differences in progesterone levels were detected in the fifth month of pregnancy or postpartum period among treatments.

Table 2. The effect of administering *Anethum graveolens* seed powder on the average concentration of progesterone hormone in Arabi ewes (ng/ml) during different physiological stages. (Mean ±SD).

Treatments	Without the administration of dill seeds	After 21 days of administration of dill seeds	1st month of pregnancy	2nd month of pregnancy	3rd month of pregnancy	4th month of pregnancy	5th month of pregnancy	After parturition
T1	1.82 A ± 0.11	2.56 A ± 0.22	3.38 b ± 0.16	3.89 b ± 0.09	4.52 b ± 0.27	5.50 b ± 0.18	4.26 ± 0.20	1.57 ± 0.03
T2	1.57 d ± 0.09	2.40 b ± 0.25	3.43 A ± 0.11	4.40 A ± 0.21	5.26 A ± 0.10	6.24 A ± 0.10	4.26 ± 0.21	1.62 ± 0.03
T3	1.72 c ± 0.04	1.82 c ± 0.11	1.92 d ± 0.06	3.34 d ± 0.15	4.43 c ± 0.18	4.00 c ± 0.58	4.17 ± 0.06	1.53 ± 0.06
T4	1.77 b ± 0.05	1.73 d ±.0.57	2.29 c ± 0.28	3.53 c ± 0.29	4.45 c ± 0.29	3.94 d ± 0.36	4.34 ± 0.16	1.75 ± 0.20

Significant level	**	**	**	**	**	**	N. S	N. S

Prolactin Levels

Prolactin hormone levels significantly varied across treatments (Table 3). Treatment 2 consistently demonstrated the highest prolactin concentrations during pregnancy and postpartum stages ($p<0.01$), notably during the first, second, third, fifth months, and postpartum period. Conversely, Treatment 1 showed a significant elevation in prolactin levels after the first 21 days of dill administration and during the fourth month of pregnancy ($p<0.01$). Treatments 3 and 4 showed relatively lower prolactin concentrations throughout the experimental period.

Table 3. The effect of administering Anethum graveolens seed powder on the average concentration of prolactin hormone in Arabi ewes (ng/ml) during different physiological stages. (Mean ±SD).

Treatments	Without the administration of dill seeds	After 21 days of administration of dill seeds	1st month of pregnancy	2nd month of pregnancy	3rd month of pregnancy	4th month of pregnancy	5th month of pregnancy	After parturition
T1	4.13 ±0.76	5.64 A ±0.48	8.24 b ±0.11	10.39 b ±0.25	15.33 b ±0.73	52.75 A ±1.28	166.89 b ±9.35	127.94 b ±1.41
T2	4.07 ±0.21	4.86 d ±0.48	10.17 A ±0.54	18.56 A ±0.49	25.68 b ±1.38	45.09 b ±3.28	226.88 A ±2.73	189.52 A ±48.22
T3	3.62 ±0.47	5.05 c ±0.24	7.38 c ±0.20	7.62 C ±0.19	8.57 c ±0.66	10.60 c ±0.20	106.18 d ±5.80	125.56 b ±2.19
T4	4.37 ±0.34	5.20 b ±0.05	7.33 d ±0.20	7.7 3 c ±0.16	8.35 c ±0.14	10.34 c ±0.24	144.36 c ±4.86	122.43 b ±6.43
Significant level	N. S	*	**	**	**	**	**	**

Different letters within the same column mean significant differences at the level of * ($p\leq 0.05$), ** ($p\leq 0.01$), N.S. There are no significant differences between the experimental treatments

Histopathologic Examinations

Ovarian Histology

Histological examination of ovarian tissues indicated that dill seed treatments (5 g and 10 g) and intravaginal sponge administration increased the number of healthy follicles compared to controls. The ovaries in Treatment 2 showed advanced follicular development, including mature antral follicles characterized by well-developed cavities and pronounced cumulus oophorus layers surrounding isolated oocytes. No pathological alterations were observed across treatment groups (Fig. 1).

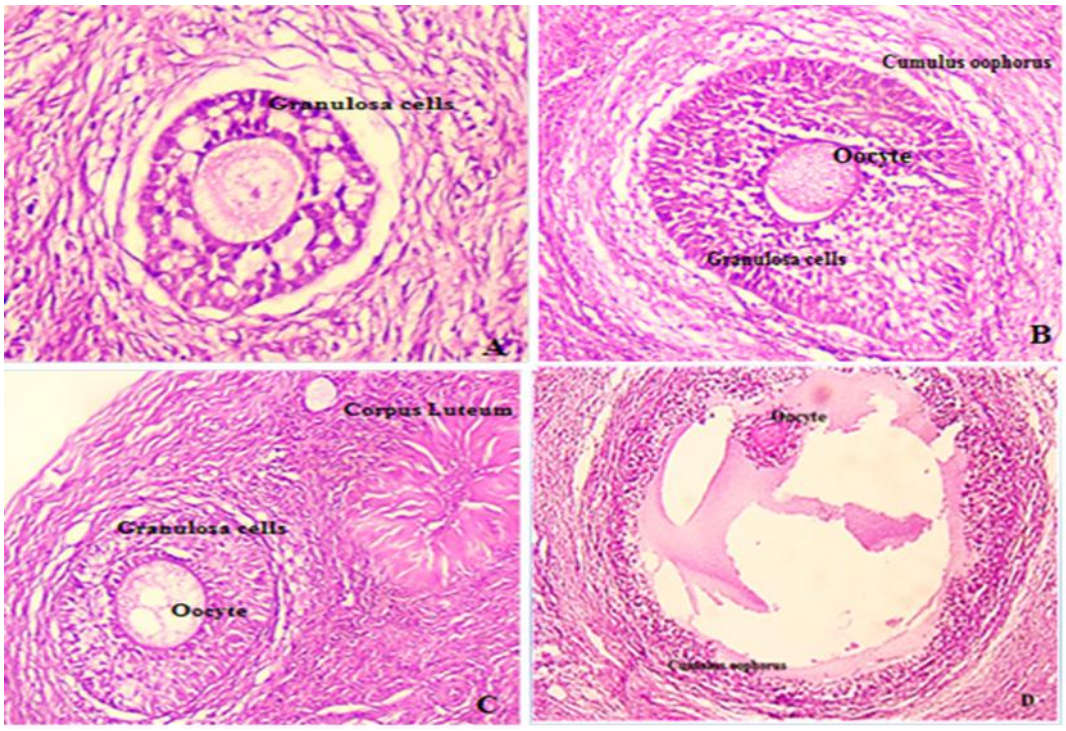

Figure 1. Histological section of the ovary (A). Ovarian sections from the control group showing the absence of secondary follicles in the ovary and the absence of oocyte accumulation (H and E 400X).B vaginal sponge group's ovaries. The oocyte and zona pellucida, which are encircled by varying numbers of concentric layers, are visible in the secondary follicles. The ovarian lumen (B) contains two or more distinct cuboidal granulosa cells. (H and E 400X). (C) Sections of ovaries from ewes that received 5 g of dill seeds. The corpus luteum, zona pellucida, oocyte, and developing microcosm are all visible in the secondary follicles. It was noted that the follicles were early ovarian follicles.(H and E 400X). (D) The lateral ovarian follicles of treated lambs given 10 g of dill seeds display a clearly developed ovarian lumen with an almost solitary oocyte encircled by oocyte accumulation (D) (H and E 400 X).

Uterine Histology

Uterine tissue analysis revealed no pathological changes in the endometrium, myometrium, or perimetrium across treatments. However, slight uterine enlargement was noted in groups treated with dill seeds (5 g and 10 g) and intravaginal sponges. Treatments led to significantly elongated uterine glands compared to the control group,

without adversely affecting epithelial differentiation or gland proliferation. Blood vessels appeared normal across all groups (Fig. 2).

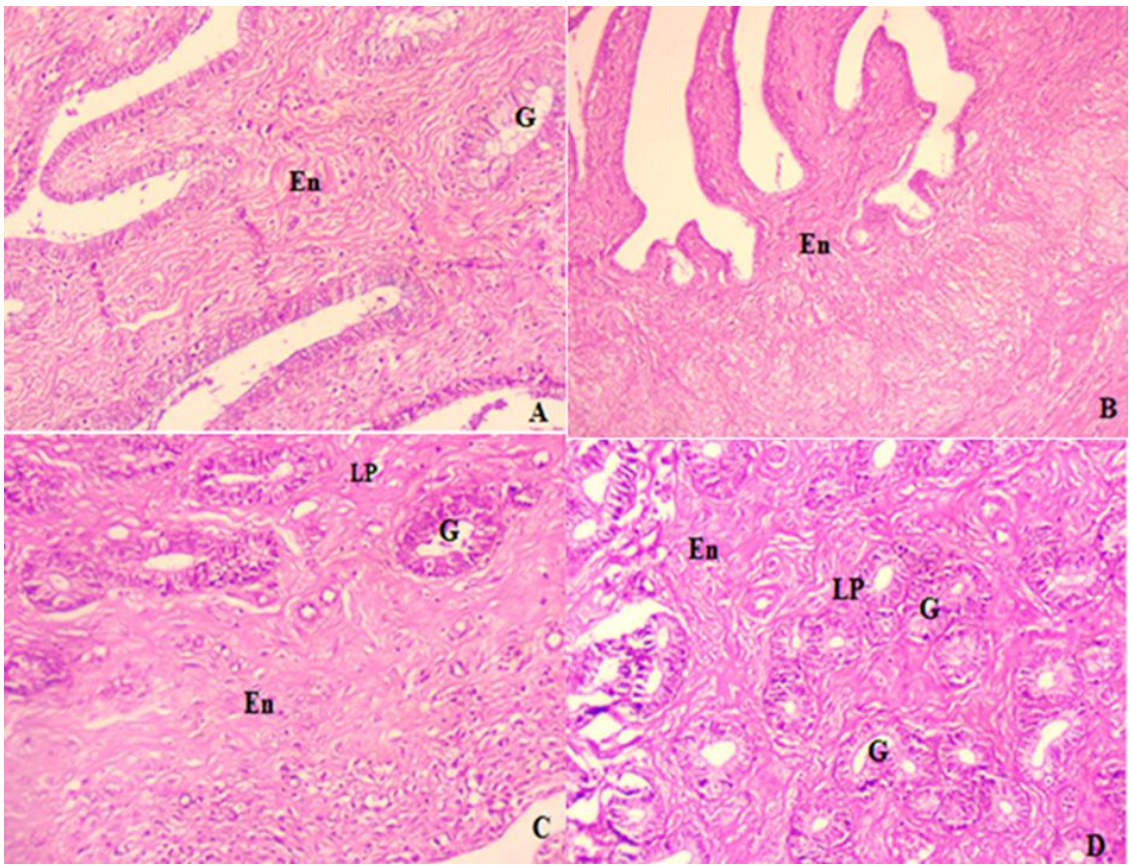

Figure 2. Uterine histology. (A) The control group shows the broad endometrium (En) and the dominant glands (G),200x. (B)Vaginal sponge group Uterus shows the broad endometrium (En) 200x. (C) Section of the Uterus from ewes that received 5 g of Dill seeds. The broad endometrium (En) with its mucosal surface and the increased lamina propria (LP),200x. (D) Section of the Uterus from ewes that received 10 g of Dill seeds. Note the broad endometrium (En) with its mucosal surface facing the uterine cavity (L) and lying in longitudinal folds. Also note the relatively thin myometrium (My). Note the increased lamina propria (LP) bearing the dominant glands (G), 200x.

Mammary Gland Histology

Mammary gland samples from dill seed-treated ewes demonstrated moderate glandular development, including mild enlargement of alveoli and increased lobular differentiation. Histological sections showed prominent lobular structures containing alveoli, ducts, and interlobular connective tissue. Intravaginal sponge treatment showed similar mammary gland development without significant differences from dill seed treatments (Fig. 3).

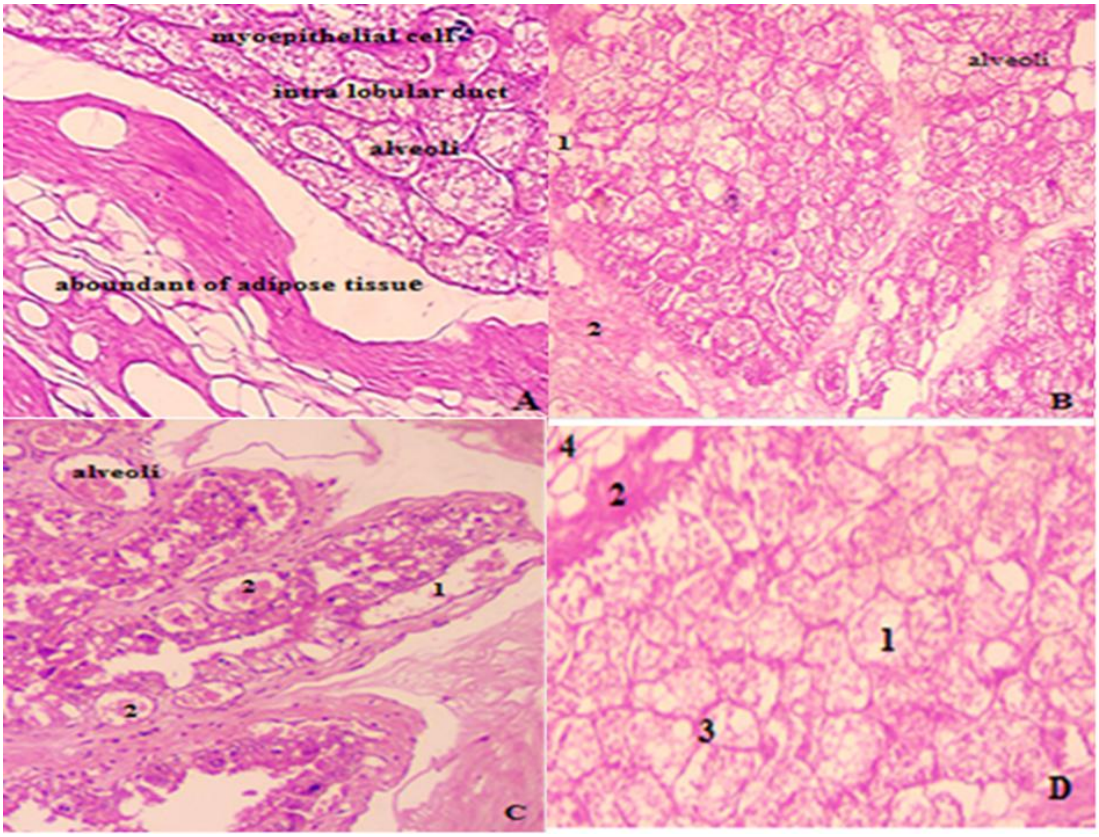

Fig. 3. Histological sections of mammary tissue. (A) The control group shows alveoli containing milk secretions, intralobular ducts, and myoepithelial cells.200x. (B) the intra vaginal sponges group show 1. Small glandular lobules, 2. Abundant connective tissue, alveoli containing milk secretions, H&E X200. (C) Ewe mammary gland that reserved 5 g of Dill seeds showing: 1. Lactose duct (collecting duct), 2. Alveoli (H&E, 200X). (D) Ewe mammary gland that reserved 10 g of Dill seeds showing: 1. Alveoli, 2. Interlobular connective tissue, 3. Myoepithelial cells, 4. Abundant adipose tissue (H&E X200).

Discussion

Acute The present study explored the physiological impact of dill seeds (*Anethum graveolens*) on Arabi ewes, specifically focusing on hormone concentrations and histological changes in reproductive and lactational organs during estrus and pregnancy stages. Significant variations in estrogen, progesterone, and prolactin hormone levels were observed, consistent with earlier findings reported in similar contexts. The significant increase in estrogen concentrations observed in Treatment 2 aligns with the findings of Alwan *et al*., and Shweta *et al*., who reported elevated estradiol levels during ewe pregnancy progression [10], [11]. Additionally, the estrogenic effects observed may be attributed to phytoestrogens in dill seeds, such as kaempferol and vicenin, which mimic natural estrogens and influence enzyme activities involved in steroidogenesis [6], [12].

Progesterone concentrations were notably higher in treatment 2 throughout the early to mid-pregnancy months, corroborating Monsefi *et al.,* findings on the supportive

role of dill seeds in corpus luteum viability and function [5]. The observed increase in progesterone levels could result from dill seeds enhancing granulosa lutein cell proliferation and smooth endoplasmic reticulum development, crucial for progesterone biosynthesis. Furthermore, dill seed constituents, particularly phytoestrogens, could mediate endocrine feedback mechanisms, augmenting progesterone secretion by stimulating the hypothalamic-pituitary-gonadal axis [13]. Prolactin concentrations significantly increased, especially in treatment 2, throughout the pregnancy and postpartum stages. These results align with previous studies [13], [14], attributing heightened prolactin levels towards the end of pregnancy and postpartum periods to diminished progesterone concentrations. Moreover, dill seeds' nutrient composition may directly stimulate prolactin synthesis, thereby positively influencing mammary gland function and milk production [15].

Histological findings reinforced the safety and beneficial effects of dill seeds. No pathological changes were detected in ovarian, uterine, or mammary tissues. Instead, improvements such as enhanced follicular development, uterine gland elongation, and moderate mammary gland proliferation were evident. These histological alterations are likely due to dill seed constituents stimulating estrogen and progesterone receptors in reproductive tissues. Specifically, kaempferol, a major dill seed flavonoid, might enhance hormonal receptor expression, thus supporting reproductive tissue growth and differentiation without adverse effects [16].

The mammary gland findings, particularly the mild glandular enlargement and increased alveolar development, indicate dill seeds' potential galactagogue effects, aligning with previous herbal medicinal studies [17], [18]. The physiological enhancement in mammary tissues strongly supports dill seeds as natural agents to improve lactational performance and milk yield. Overall, the study substantiates dill seeds' potential benefits in improving reproductive efficiency and lactational performance in Arabi ewes. Given the absence of adverse histopathological changes and the observed hormonal and physiological enhancements, incorporating dill seeds into ewe feeding strategies appears promising for enhancing livestock productivity.

CONCLUSION

Fundamental Finding : This study demonstrated that the administration of Anethum graveolens (dill) seeds had a beneficial impact on the reproductive and lactational physiology of Arabi ewes. Ewes treated with dill seeds, particularly at the 10 g dosage, exhibited significant increases in the concentrations of key reproductive hormones—estrogen, progesterone, and prolactin—across various stages of pregnancy and postpartum. These hormonal changes are indicative of enhanced reproductive function and improved mammary gland activity. Dill seed supplementation was associated with favorable tissue changes, such as increased follicular development in the ovaries, elongation of uterine glands, and mild proliferation of alveolar structures in the mammary glands. These findings suggest that dill seeds may promote corpus luteum survival, stimulate progesterone and prolactin synthesis, and modestly enhance

mammary tissue development. **Implication :** Taken together, the results support the potential of Anethum graveolens as a natural phytogenic supplement to improve reproductive efficiency and milk production in ewes. Based on these findings, dietary inclusion of dill seeds—particularly during critical physiological stages—may be recommended as a supportive strategy to enhance productivity in sheep farming systems. **Limitation :** This study was conducted on a relatively small sample size of Arabi ewes, which may limit the generalizability of the findings to other breeds, management systems, or environmental conditions. In addition, the study focused on short-term hormonal and histological responses and did not evaluate long-term reproductive performance, milk yield, or economic outcomes associated with dill seed supplementation. **Future Research :** Future studies should involve larger sample sizes and include different sheep breeds to validate the broader applicability of these findings. Further research is also recommended to assess the long-term effects of Anethum graveolens supplementation on milk production, reproductive efficiency, offspring performance, and overall farm profitability. In addition, investigations into optimal dosage levels, duration of supplementation, and the underlying molecular mechanisms may provide deeper insights into the role of dill seeds as a phytogenic feed additive.

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