

Milk Inducting in Non-Lactating Ewes (OvisAries) in Off-Breeding Season

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ABSTRACT

Turkish ewes (*Ovis aries*) milk production is limited by seasonal breeding patterns, resulting in reduced milk availability during the off-breeding season when ewes are neither pregnant nor lactating. This study aimed to evaluate a hormonal protocol for inducing lactation and assess mammary gland development, milk production and composition, and physiological responses. The study was conducted at the Faculty of Agriculture, Kabul University, Afghanistan, from 23 August–14 September 2025. Eight dry, non-pregnant Turkish ewes received estradiol benzoate and progesterone during Days 1–7, dexamethasone, and udder massage during Days 1–10. Udder length, rectal temperature, milk yield, milk composition, and behavioral responses were evaluated. We analyzed data using IBM SPSS Statistics version 20. Data were summarized as mean $\pm$ standard deviation, while one-way ANOVA and repeated-measures ANOVA assessed differences, with LSD tests for multiple comparisons. Udder length increased from 2.87 ± 1.72 cm on Day 1 to 6.85 ± 2.19 cm on Day 23 ($P < 0.001$). Milk yield ranged from 10 to 130 mL per 24 h, and protein differed significantly from the reference value ($P = 0.002$). Overall, the findings support increased milk production and improved farm economics.

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INTRODUCTION

Livestock production, particularly sheep farming, is an important component of rural livelihoods and food security in Afghanistan. Sheep are among the major livestock resources of Afghan pastoral and rural communities; however, environmental, nutritional, and management challenges continue to constrain livestock production. Afghanistan also continues to struggle to achieve self-sufficiency in livestock products, with substantial quantities of animal products imported (Berryhill et al., 2016). Improving the productivity and utilization of existing sheep resources is therefore important for strengthening local dairy production and rural livelihoods. Sheep are strongly seasonal breeders, and photoperiodic

and neuroendocrine mechanisms, including melatonin and gonadotropin-releasing hormone pathways, influence reproductive activity (Confuorti et al., 2024).

Induced lactation represents a potential strategy for stimulating mammary development and milk secretion in non-pregnant animals. Lactation is regulated by coordinated endocrine interactions involving estrogen, progesterone, prolactin, glucocorticoids, and other hormonal pathways that control mammary growth, differentiation, and milk synthesis. In particular, progesterone withdrawal, prolactin activity, and glucocorticoid signaling are important components of the transition toward active milk secretion (Aguirre-Flores et al., 2018). Mammary tissue responses to endocrine stimulation depend on the expression and activity of several hormone receptors. In sheep, estrogen receptor 1 (ESR1), prolactin receptor (PRLR), growth hormone receptor (GHR), and insulin-like growth factor 1 receptor (IGF1R) are expressed in mammary tissue and are associated with mammary development and lactational status (Choudhary et al., 2018).

Recent transcriptomic studies have also demonstrated substantial differences in gene expression between lactating and non-lactating ovine mammary glands, indicating that mammary development and milk synthesis involve extensive molecular changes (Salmon-Legagneur et al., 2026; Vailati-Riboni et al., 2016). Furthermore, estrogen and progesterone receptor signaling plays an important role in reproductive and mammary physiology (Choudhary et al., 2018). These findings provide a physiological and molecular basis for investigating hormonal stimulation to initiate lactation in non-pregnant ewes.

Mammary development is closely related to the capacity of the gland to synthesize and secrete milk. Udder morphology has been associated with milk yield and milkability, suggesting that changes in udder size can indicate mammary development during lactation (Berryhill et al., 2016). However, the extent of mammary development and subsequent milk secretion after hormonal induction may vary by breed, reproductive status, season, nutrition, and management conditions. Several animal and management factors likewise influence milk yield and composition. Lactation stage can significantly affect milk yield and major milk components (Radavelli et al., 2016), while dietary interventions may modify milk production and fatty-acid composition (Salmon-Legagneur et al., 2026). Production systems can also influence the chemical and nutritional characteristics of sheep milk (Lu et al., 2016). In addition, breed, parity, lactation stage, and milking frequency may contribute to variation in milk characteristics (Pires et al., 2016). Therefore, evaluation of induced lactation should consider not only milk yield but also milk composition and relevant physiological responses.

Despite advances in mammary endocrinology and sheep milk production, no published research or other published document was identified specifically addressing practical lactation induction in non-pregnant Turkish ewes during the off-breeding season under Afghan climate conditions. Therefore, this study investigated the response of non-lactating Turkish ewes (*Ovis aries*) to hormonal stimulation combined with udder stimulation, focusing on mammary development, induced milk production and composition, and selected physiological responses. Turkish sheep contribute to small-scale and commercial dairy

production, but seasonal breeding and limited lactation persistency can leave many non-pregnant ewes dry for extended periods. This reduces their productive use of feed resources and may increase the need for artificial rearing of orphan or low-milk lambs, potentially increasing production costs and lamb mortality. The present study addressed four main research questions.

1. Does the hormonal protocol combined with udder massage effectively induce mammary gland development in dry, non-pregnant Turkish ewes during the off-breeding season?
2. Can the induced-lactation protocol produce measurable milk with acceptable yield and composition in non-pregnant Turkish ewes?

MATERIALS AND METHODS

Experimental Animals

The experiment was conducted on eight mature indigenous Turkish ewes (*Ovis aries*) between 23 August and 14 September 2025. At the start of the experiment, all animals were dry and confirmed non-pregnant, with no history of lambing in the previous six months. Before the study, all ewes underwent comprehensive clinical examination and screening for general health status, absence of mammary gland disorders, previous lactation history, age, and body condition. The animals had a mean age of 5 ± 2 years and an average body weight of 60 ± 10 kg (Luz et al., 2020; Ramgattie et al., 2014).

The animals were housed in the livestock facilities of the Faculty of Agriculture, Kabul University, under identical management conditions and natural photoperiod. They were fed a standard farm maintenance ration of alfalfa hay and natural grass hay, and clean drinking water was provided ad libitum throughout the experimental period.

Three pens housed the animals. The first and second pens each accommodated three ewes, whereas the third pen housed the remaining two ewes. Throughout the experiment, all animals were maintained under identical environmental and management conditions.

Experimental Design

The study employed a single-group longitudinal experimental design with repeated measurements to evaluate the feasibility of inducing lactation in dry, non-pregnant Turkish ewes (*Ovis aries*) during the non-breeding season under field conditions in Afghanistan. Eight clinically healthy ewes were enrolled, and all animals received the same hormonal induction protocol combined with mechanical udder stimulation. No untreated control group was included. Therefore, we evaluated responses longitudinally within the same animals at predetermined time points. The primary outcomes included mammary gland development, milk production and composition, rectal temperature, and behavioral responses.

Hormonal Induction Protocol

The hormonal treatment protocol consisted of progesterone, estradiol benzoate, and dexamethasone. The induction protocol was adapted from the methods described by

(Sawyer et al., 1986; Skliarov et al., 2021), with modifications appropriate for sheep. From Day 1 through Day 7 of the experiment, each ewe received daily intramuscular injections of estradiol benzoate at 0.5 mg/kg body weight together with progesterone at 1.25 mg/kg body weight.

To mimic the endocrine changes that normally occur immediately before parturition, we administered dexamethasone intramuscularly at 16 mg per animal on Days 17, 19, and 20 of the experiment. Figure 1 illustrates the chronological sequence of the hormonal induction protocol.

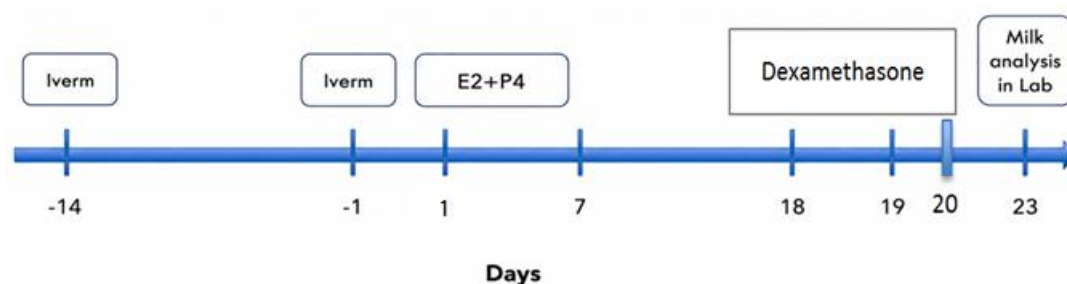


Figure 1. Illustrates the experimental timeline from the initiation of hormonal treatment to the onset of milk secretion.

In addition to hormonal treatment, we performed mechanical stimulation of the udder to enhance mammary gland activity and facilitate the initiation of lactation. From Day 1 to Day 10 of the experiment, each ewe received udder massage twice daily (morning and evening) for approximately 5 minutes per session. Udder massage was performed using gentle circular movements with the palm over the mammary tissue to stimulate cutaneous sensory receptors and promote prolactin secretion (Lacasse et al., 2016; Toledo et al., 2020; Yart et al., 2014). Previous studies have demonstrated that mechanical stimulation of the mammary gland enhances milk production and improves lactation persistency in domestic animals (Paiano et al., 2018). The animals were monitored daily for mammary gland development and the onset of milk secretion. Once milk leakage or secretion was detected, the animals entered a regular milking schedule. In most ewes, milk secretion commenced approximately 22 ± 2 days after the initiation of the hormonal induction protocol.

To evaluate mammary gland development in non-pregnant ewes, udder length was measured on Days 1, 17, 20, and 23 of the experiment. Measurements were obtained using a calibrated ruler under standardized conditions for all animals. Udder length was recorded from the point of attachment to the abdominal wall to the distal end of the mammary gland and expressed in centimeters (cm). To assess the physiological status of the animals, rectal body temperature was measured on Days 1, 17, 20, and 23 using a digital veterinary thermometer and recorded in degrees Celsius ($^{\circ}\text{C}$). All measurements were performed at the same time of day and under identical environmental conditions to minimize experimental variation.

Behavioral responses to the lactation induction protocol were evaluated by observing the ewes during and immediately after udder massage on Days 1, 10, and 17. Based on their response to udder handling, animals were classified into two behavioral categories: normal

and mildly aggressive. Milk samples were collected at predetermined intervals following udder stimulation to evaluate milk secretion. Researchers assessed milk physical characteristics, including color and consistency, by direct visual examination and classified them as either watery or white. Milk yield from each ewe was measured using a graduated cylinder and recorded in milliliters (mL).

Data Collection

Before the experiment, all ewes were selected based on their Body Condition Score (BCS), overall health status, and absence of clinical disease. The animals were then randomly assigned to the experimental treatment and subjected to the lactation induction protocol, which included administering ivermectin, progesterone, estradiol, and dexamethasone. Experimental data were collected systematically at predetermined time points throughout the study. Measurements included mammary gland development, milk secretion, and milk yield. Milk composition, including fat, protein, lactose, and other chemical constituents, was analyzed using an advanced Lactoscan® milk analyzer. In addition, we recorded physiological parameters, including rectal body temperature, and behavioral changes throughout the experimental period.

Statistical Analysis

Following data collection and organization, statistical analyses were performed using IBM SPSS Statistics version 20. Descriptive statistics, including the mean $\pm$ standard deviation (Mean $\pm$ SD), were calculated to summarize the experimental data. We analyzed differences among the measured variables, including mammary gland growth, milk yield, and milk composition (fat, protein, and lactose), using one-way analysis of variance (ANOVA). Whenever significant differences among treatment means were detected, comparisons were performed using the Least Significant Difference (LSD) multiple comparison test at 5% ($P < 0.05$) and 1% ($P < 0.01$) levels of significance.

FINDING

Mammary Gland Growth

The results showed a marked increase in mammary gland growth in non-pregnant ewes throughout the experimental period. As shown in Figure 2, mean udder length was evaluated at four time points (Days 1, 17, 20, and 23), showing a progressive, continuous increase over time.

The mean udder length increased from 2.87 cm on Day 1 to 3.65 cm on Day 17, indicating the initial stage of mammary tissue development following hormonal treatment. By Day 20, the mean udder length had further increased to 5.10 cm, representing a substantial increase compared with Day 17. The steeper slope of the growth curve during this period suggests an accelerated rate of mammary development relative to the earlier stage of the experiment.

The greatest increase in mammary growth occurred between Days 20 and 23, during which the mean udder length increased from 5.10 cm to 6.85 cm. This segment of the growth

curve exhibited the steepest slope, indicating the highest rate of mammary tissue development observed during the study. Overall, Figure 2 demonstrates a consistent upward trend in udder growth, with continuous enlargement of the mammary gland throughout the experimental period.

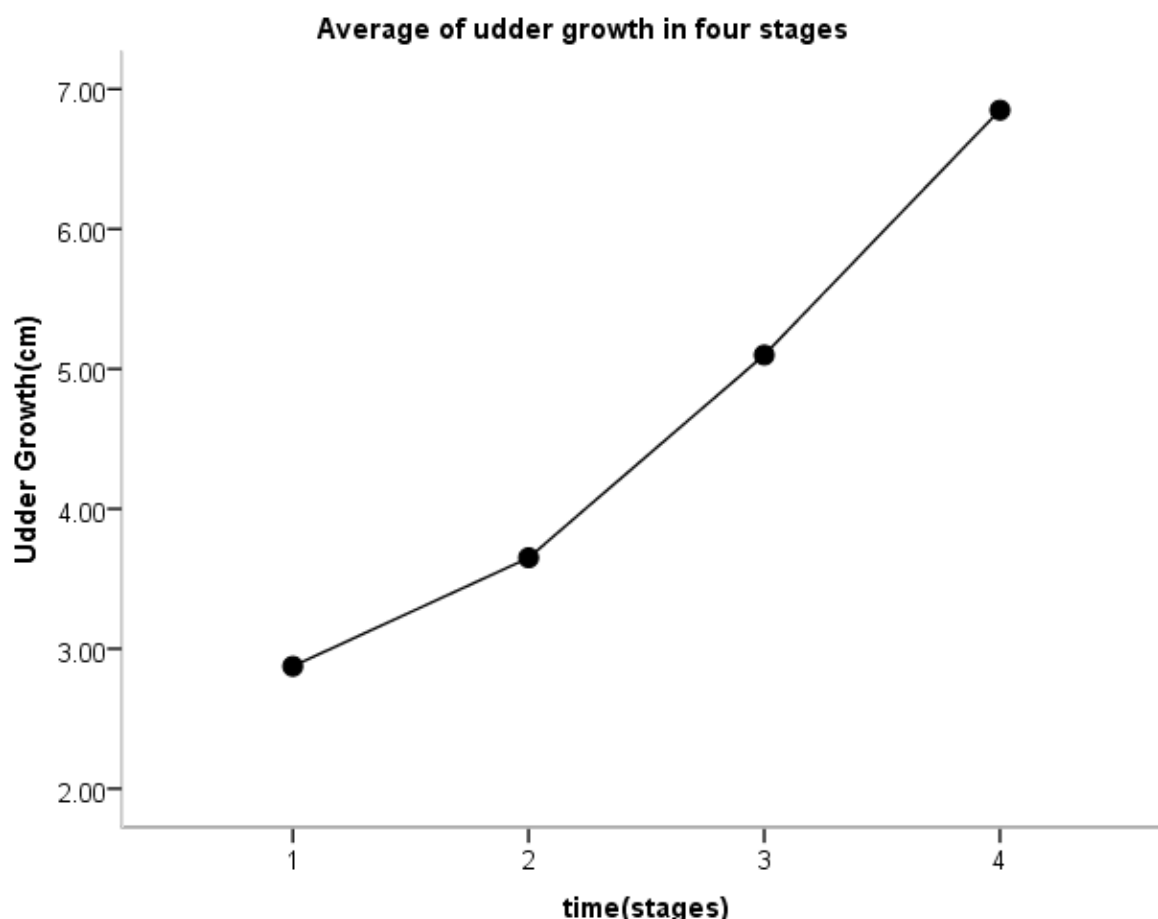


Figure 2. Progressive increase in mean udder length from Day 1 to Day 23, demonstrating continuous mammary gland development following the lactation induction protocol.

Table 1. Mean ($\pm$ SD) body temperature and mammary at four time points

Days	Udder growth (Mean $\pm$ SD, cm)	Temp (Mean $\pm$ SD, °C)
1	2.87 $\pm$ 1.72	39.12 $\pm$ 0.93
17	3.65 $\pm$ 2.41	38.85 $\pm$ 0.75
20	5.10 $\pm$ 1.97	38.95 $\pm$ 0.25
23	6.85 $\pm$ 2.19	38.94 $\pm$ 0.28

Body temperature remained relatively stable throughout the study, showing only minor physiological fluctuations. The mean rectal temperature decreased slightly from $39.12 \pm 0.93^{\circ}\text{C}$ on Day 1 to $38.94 \pm 0.28^{\circ}\text{C}$ on Day 23, indicating that the hormonal induction protocol did not produce clinically significant alterations in body temperature. In contrast, mammary gland growth increased steadily during the experimental period. Mean udder length increased from 2.87 ± 1.72 cm on Day 1 to 3.65 ± 2.41 cm, 5.10 ± 1.97 cm, and 6.85 ± 2.19 cm on Days 17, 20, and 23, respectively. These findings demonstrate continuous mammary development following hormonal treatment.

Comparing these two physiological parameters indicates that mammary gland growth progressed independently of changes in body temperature. Therefore, the observed increase in mammary development cannot be attributed to alterations in thermoregulatory status but rather reflects the physiological response to the lactation induction protocol. The repeated-measures analysis presented in Table 2 demonstrated a highly significant effect of time on mammary gland growth. The ANOVA yielded an F-value of 40.246 with $P < 0.001$, indicating statistically significant differences among the measurement periods. Furthermore, the large effect size ($\eta^2 = 0.852$) suggests that time accounted for a substantial proportion of the observed variation in mammary growth.

Table 2. Measures of time effect on mammary gland growth

Time	Udder growth (cm), Mean $\pm$ SD	F	df	p-value	Partial η^2
Day 1	2.87 $\pm$ 1.72				
Day 17	3.65 $\pm$ 2.41				
Day 20	5.10 $\pm$ 1.97				
Day 23	6.85 $\pm$ 2.19	40.246	2.054, 14.375	<0.001	0.852

Effects of Hormonal Treatment

The box-and-whisker plot in Figure 3 shows the distribution and progression of mammary gland growth across the four experimental stages (E2 + P₄, Post_Hor, Post_Dex, and Final). The median udder length increased progressively from approximately 2.5 cm at E2 + P₄ to higher values at Post_Hor and Post_Dex, reaching approximately 7 cm at the Final stage. The upward shift in the median and overall distribution indicates continuous mammary gland development following hormonal stimulation.

During E2 + P₄, values ranged from approximately 1 to 6 cm, whereas wider distributions were observed during Post_Hor and Post_Dex, with values reaching approximately 9 cm at Post_Dex. At the Final stage, both the median and interquartile range were higher than at earlier stages, with increased minimum and maximum values. Overall, the figure shows a progressive, sustained increase in mammary gland growth throughout the hormonal induction protocol.

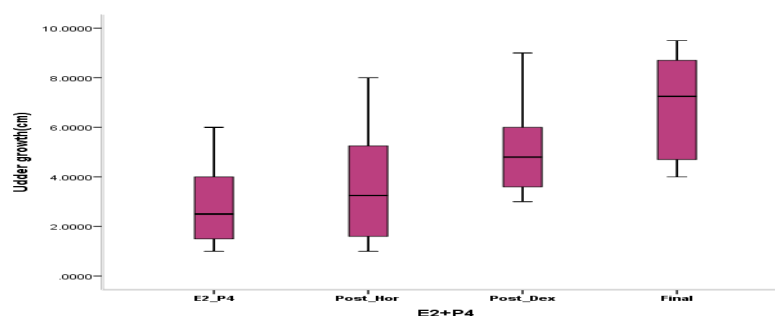


Fig 3. Box plot illustrating progressive mammary gland growth during successive stages of the hormonal induction protocol (E2 + P₄, Post_Hor, Post_Dex, and Final).

Table 3 summarizes changes in animal behavior during udder massage, milk appearance, and milk production at different stages of the experiment. Behavioral observations indicated that most ewes exhibited normal responses throughout the study. On Days 1 and 10, all animals displayed normal behavior during udder massage, with no noticeable behavioral abnormalities. On Day 17, only ewes A and B exhibited mildly aggressive behavior, whereas the remaining animals continued to respond normally. These findings suggest that the hormonal induction protocol had minimal influence on most animals' behavioral characteristics.

Evaluation of milk appearance showed that milk was either absent or watery during the early stages of the experiment. On Day 10, all milk samples were classified as watery. As the experiment progressed, milk color gradually changed from watery to white. By Day 17, most ewes produced white milk, except ewe G, whose milk remained watery. On Day 23, milk from all animals was uniformly white, indicating complete maturation of milk secretion by the end of the experimental period.

Milk production varied considerably among individual animals. The highest milk yield was recorded in ewe G (130 mL), whereas ewe F produced the lowest yield (10 mL). The remaining animals produced between 12 and 42 mL of milk. Overall, despite the observed variation in milk yield among ewes, milk appearance became uniformly white by the end of the study, and normal behavioral responses were maintained in the majority of animals.

Table 3. *Changes in behavioral response, milk appearance, and milk yield during the study*

Group	Behavior in days			milk color in day's				volume
	B. D1	B. D10	B. D17	C.D1	C.D10	C.D17	C.D23	Milk (ml)
A	N	N	MR	WA	WA	WH	WH	12
B	N	N	MR	-	WA	WH	WH	42
D	N	N	N	-	WA	WH	WH	25
E	N	N	N	-	WA	WH	WH	28
F	N	N	N	-	WA	WH	WH	10
G	N	N	N	-	WA	WA	WH	130
H	N	N	N	-	WA	WH	WH	12

N: Normal; **B:** Behave; **C:** Color; **MR:** Mild rough; **WA:** Watery; **WH:** White.

Milk Composition

Figure 4 illustrates the lactation induction process in seven of the eight experimental ewes at two sampling periods. The first stage presents milk samples collected immediately after completion of the hormonal induction protocol. Differences in milk color, viscosity, and secretion volume among samples indicate that individual ewes' physiological responses to hormonal treatment varied. Samples obtained from ewes A and B appeared more homogeneous and exhibited a whiter color, suggesting a more successful initiation of lactation. In contrast, samples from ewes C and D appeared less uniform, indicating incomplete milk synthesis or the production of immature mammary secretions during the early stage of lactogenesis.

The second stage presents milk samples collected on the second and third days after the onset of regular milking. At this stage, most samples showed greater viscosity and a more homogeneous

appearance, reflecting the mammary gland's gradual physiological adaptation to sustained milk secretion.

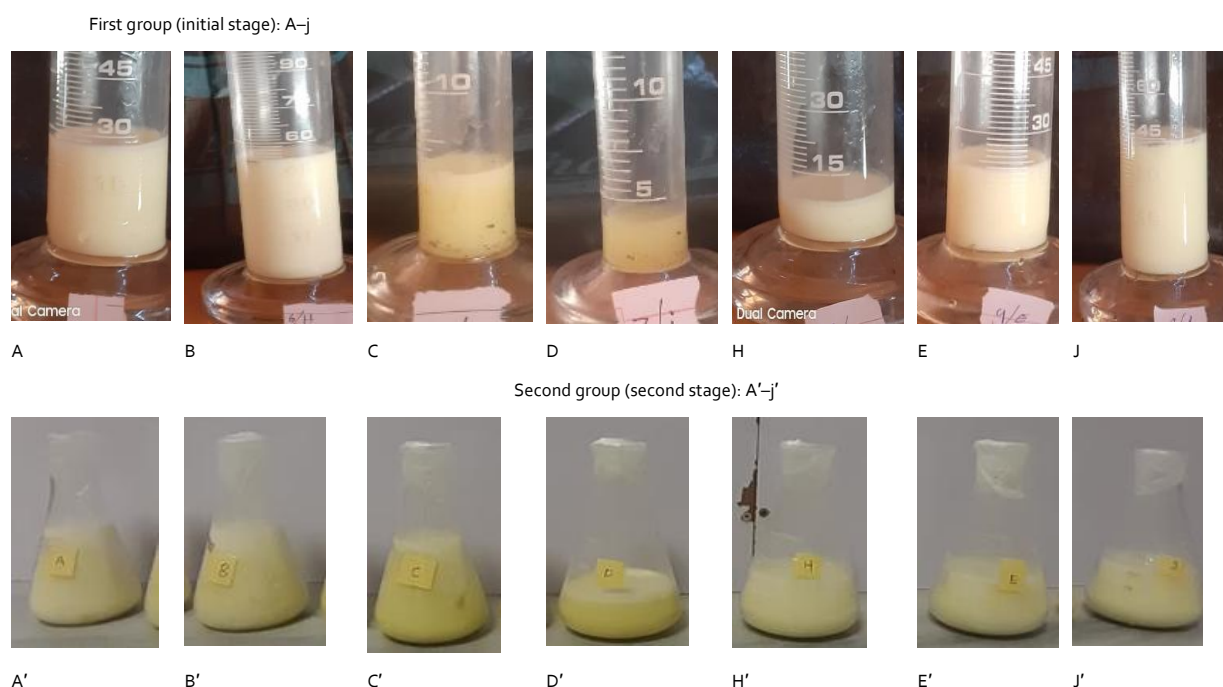


Figure 4. Initial and second stages of lactation in non-lactating, non-pregnant Turkish ewes one day after dexamethasone treatment. The upper panels (A-j) show milk obtained during the initial stage of lactation, whereas the lower panels (A'-j') show milk obtained during the second stage of lactation.

Milk composition was analyzed at the Animal Health Laboratory, Darulaman, Kabul, Afghanistan. The concentrations of fat, protein, lactose, and solids-not-fat (SNF) were determined using a Lactoscan® milk analyzer. The results of the one-sample t-tests and comparison of the observed mean values with the established reference values are presented in Table 4. Among all milk quality parameters evaluated, protein was the only constituent that differed significantly from its reference value. The protein concentration was significantly higher than the reference value of 4.15%, with a t-value of 5.05 and $P = 0.002$. The mean difference was 1.05%, and the 95% confidence interval (0.54–1.56) was entirely above zero, confirming a significant increase in protein concentration. In contrast, milk fat did not differ significantly from its reference value of 6.53% ($t = -0.56$, $P = 0.594$), with a mean difference of -0.43% and a 95% confidence interval of -2.32 to 1.45% .

The other milk quality parameters showed no statistically significant differences from their respective reference values. Lactose, with a reference value of 5.15%, showed a mean difference of -0.20% ($P = 0.351$; 95% CI: -0.68 to 0.28), indicating that the observed lactose concentration was statistically comparable with the reference value. Similarly, SNF did not differ significantly from its reference value of 10.15% ($t = 1.90$, $P = 0.106$), despite a mean difference of 0.84% . Total solids, freezing point, and pH also remained statistically comparable to their respective reference values ($P = 0.724$, 0.215 , and 0.658 , respectively). Because all of these P-values were greater than 0.05, the results indicate that the hormonal induction protocol did not produce significant changes in these milk composition parameters.

Table 4. One-sample t-test of milk quality parameters against reference values. One-sample t-test of milk quality parameters against reference values

Variable	Std Value	t (df=6)	p-value	Mean Difference	Confidence Interval(95%)
Fat	6.53	-0.56	0.594	-0.43	[-2.32, 1.45]
Protein	4.15	5.05	0.002**	1.05	[0.54, 1.56]
Lactose	5.15	-1.01	0.351	-0.20	[-0.68, 0.28]
SNF	10.15	1.90	0.106	0.84	[-0.24, 1.92]
Total Solids	17.44	-0.37	0.724	-0.35	[-2.70, 1.99]
Freezing Point	-0.691	1.39	0.215	0.04	[-0.03, 0.12]
PH	6.60	-0.47	0.658	-0.04	[-0.27, 0.18]

Overall, the findings presented in Table 4 demonstrate that the lactation induction protocol produced milk with a chemical composition largely comparable to the established composition of normal sheep milk. Milk protein was the only parameter that differed significantly, with a higher concentration than the reference value, whereas fat, lactose, SNF, total solids, freezing point, and pH remained statistically similar to their respective reference values. These results suggest that hormonal induction of lactation did not substantially alter most of the measured chemical characteristics of the resulting milk. Therefore, except for the elevated protein concentration, the induced milk exhibited a generally comparable compositional profile to the reference sheep milk.

DISCUSSION

Table 1 shows that the mean body temperature of the experimental ewes remained within the normal physiological range throughout the study period. The average body temperature decreased only slightly from 39.12°C on Day 1 to 38.95°C on Day 17 and 38.94°C on Day 23. This minor reduction suggests that no substantial physiological changes occurred during the experimental period and that body temperature remained relatively stable. Furthermore, the relatively low standard deviations, particularly on Days 17 and 23, indicate limited variability among animals, reflecting high consistency and uniformity in the recorded measurements. Overall, these findings suggest that time had no appreciable effect on body temperature, and the observed variations were biologically negligible. The significant increase in udder length observed in the present study indicates that mammary gland development can be stimulated in dry, non-pregnant ewes without pregnancy. The progressive increase in udder size may reflect hormonally stimulated proliferation and functional differentiation of mammary epithelial cells. This interpretation is supported by Wang et al. (2020), who demonstrated substantial differences in mammary gene expression between lactating and non-lactating Small-Tailed Han sheep, particularly in genes associated with mammary function and milk synthesis. Similarly, Wang et al. (2021) reported differential microRNA expression in ovine mammary tissue between peak lactation and the non-lactating period, indicating molecular regulation of mammary development and lactational activity. Thus, the increase in udder

length observed in the present study may represent a morphological response to hormonal stimulation and increased functional activity of the mammary gland.

These findings also agree with previous studies in sheep. (Khan et al., 2022) reported that administration of estrogen, progesterone, and dexamethasone successfully induced lactation in approximately 73% of dry ewes. Although milk yield was not the primary outcome of the present investigation, the significant increase in mammary gland length may be an important physiological indicator of mammary development and readiness for lactation, supporting findings from previous studies. From a metabolic perspective, these results are consistent with those reported by Luz et al. (2020), who showed that increased glucose availability and lipid mobilization play essential roles in preparing the mammary gland for milk synthesis. Furthermore, the absence of significant changes in body temperature indicates that the hormonal induction protocol did not induce severe physiological or metabolic stress, unlike some intensive induction protocols reported previously (Baumgard et al., 2017). Overall, the hormonal protocol significantly stimulated mammary gland development, as demonstrated by the progressive increase in udder length in dry, non-pregnant ewes. This increase likely reflects hormone-induced proliferation and functional differentiation of mammary epithelial cells, leading to enhanced mammary gland development.

During the initial stage (E2 + P₄), when estradiol and progesterone were administered, the mean mammary gland length remained relatively low (approximately 2–4 cm). This stage represents the onset of hormonal stimulation and indicates that, despite exposure to the reproductive hormones, substantial mammary tissue development had not yet occurred. Nevertheless, noticeable individual variation among the ewes was evident, suggesting differences in physiological responsiveness to hormonal treatment. During the second stage (Post_Hor), after continued hormonal administration, mammary gland length increased markedly, with a broader range of observed values. These findings suggest that the cumulative effects of estradiol and progesterone progressively stimulated mammary tissue development. The increased variability among animals at this stage may reflect differences in hormone receptor sensitivity or individual physiological status.

In the third stage (Post_Dex), following dexamethasone administration, mammary gland growth continued to increase. This stage is particularly important because dexamethasone, a synthetic glucocorticoid, promotes cellular metabolism and enhances the physiological processes involved in mammary tissue development. Compared with the previous stage, the variation among animals became relatively more stable, indicating greater physiological uniformity in the response to treatment. The Final stage exhibited the greatest mammary gland development, with udder length reaching approximately 7–9 cm. This substantial increase reflects the cumulative and synergistic effects of the hormonal induction protocol throughout the experimental period.

The marked increase in mammary growth observed during the Post_Hor stage is comparable to the findings of (Freitas et al., 2010), who reported that the duration of

hormonal treatment influences the magnitude of mammary development. However, it does not necessarily result in proportional increases in milk production. Similarly, the present study demonstrates that the combined effects of estradiol and progesterone promoted mammary growth; however, the response was not uniform among all animals. Such variation may be attributed to differences in hormone receptor sensitivity or individual physiological characteristics, as previously suggested by other investigators.

The further increase in mammary development following dexamethasone administration (Post_Dex) agrees closely with the findings of (Luz et al., 2020), who demonstrated that glucocorticoids play a pivotal role in initiating the second stage of lactogenesis. In the present study, dexamethasone appears to have enhanced mammary tissue development by stimulating mammary cellular metabolism and promoting lactose synthesis. These findings suggest that the combined administration of reproductive steroids and glucocorticoids exerts a synergistic effect on mammary gland development.

During the Final stage, when the greatest mammary growth was observed, the present findings were consistent with those reported by (Itana & Duguma, 2021), who documented a high success rate for hormonally induced lactation in ewes. Although the primary objective of the present study was to evaluate mammary gland growth rather than milk yield, the increase in udder length to 7–9 cm indicates substantial physiological preparation for milk production. This stage likely corresponds to the advanced phase of lactogenesis described in previous studies, during which the mammary gland approaches its maximum functional capacity. the hormonal treatment had a positive effect on mammary gland development. Mammary gland length increased progressively from approximately 2–4 cm during the E2 + P4 stage to 7–9 cm at the Final stage, indicating that the combined effects of estradiol, progesterone, and dexamethasone successfully stimulated mammary tissue development and prepared the gland for milk production.

The findings of the present study showed that the chemical composition of milk remained relatively stable across most quality parameters, with milk protein as the only component that changed significantly. For milk fat, the statistical analysis indicated that the observed difference was not statistically significant. Furthermore, the mean difference (–0.43%) and the 95% confidence interval, which included zero, confirm that the variation in milk fat content occurred randomly and could not be attributed to the experimental treatment. Therefore, milk fat, a principal indicator of milk quality, was not significantly affected by the hormonal induction protocol.

The most notable finding was a significant increase in milk protein concentration compared with the reference value ($t = 5.05$, $P = 0.002$). The mean difference of 1.05 percentage points, with a 95% confidence interval of 0.54–1.56, indicates a consistent increase in protein concentration in the induced milk. This response may reflect the hormonal protocol's effects on mammary epithelial development and the initiation of lactational activity (Yao et al., 2021). Estradiol and progesterone are involved in mammary tissue development, while glucocorticoids contribute to the functional differentiation of mammary

epithelial cells during lactogenesis. Similar responses have been reported in hormonally induced lactation studies (Radavelli et al., 2016). A hormonal protocol involving estradiol, progesterone, and dexamethasone in Lacaune ewes demonstrated that hormonal induction could successfully initiate lactation, although the biochemical characteristics of induced milk were not identical to those of naturally produced milk (Berryhill et al., 2016; Do et al., 2017). In addition, Hooper et al. (2020) reported that estradiol treatment altered mammary endocrine and molecular responses, including increased STAT5 expression and changes in prolactin-related activity, demonstrating that reproductive hormones can influence mammary cellular function. Therefore, the increased protein concentration observed in the present study may reflect enhanced mammary epithelial activity during hormonally induced lactogenesis. However, because protein synthesis was not measured directly at the cellular or molecular level, this interpretation should be considered a possible physiological explanation rather than direct evidence of increased protein synthesis.

The absence of significant changes in milk fat, lactose, SNF, total solids, freezing point, and pH suggests that the milk composition response to hormonal induction was selective rather than generalized. Milk fat did not differ significantly from the reference value ($t = -0.56$, $P = 0.594$), indicating that the hormonal protocol did not produce a detectable alteration in fat secretion under the conditions of the present study. This contrasts with some observations in other hormonally treated animals and may be related to differences in species, hormone dosage, treatment duration, and stage of lactation. Importantly, (Hooper et al., 2020; Li et al., 2020) found that estradiol treatment in dairy cows increased milk protein and lactose while producing only limited effects on other milk components, illustrating that individual milk constituents may respond differently to hormonal manipulation. Lactose in the present study also remained statistically unchanged ($t = -1.01$, $P = 0.351$), while SNF showed a small but non-significant increase ($P = 0.106$). The stability of these components may indicate that the basic osmotic and secretory functions of the mammary gland remained relatively stable during induced lactation. Similarly, the absence of significant differences in total solids ($t = -0.37$, $P = 0.724$), freezing point, and pH suggests that the hormonal treatment did not cause a substantial alteration in the overall physicochemical characteristics of the milk. (Radavelli et al., 2016; Ramirez Ramirez et al., 2016) also emphasized that milk composition and biochemical responses following induced lactation can differ from those observed under natural lactation, supporting the importance of considering the specific induction protocol and physiological condition of the animals.

Overall, the findings indicate that the hormonal induction protocol produced milk with generally stable physicochemical characteristics, while milk protein showed the clearest response. The significant elevation in protein concentration may reflect changes in mammary epithelial activity associated with the transition toward lactational function. In contrast, the lack of significant changes in fat, lactose, SNF, total solids, freezing point, and pH suggests that the treatment did not broadly disrupt milk composition. However, interpret the results cautiously because the study involved a relatively small number of ewes and used established

reference values rather than a contemporaneous untreated control group. In addition, the study did not measure circulating or milk concentrations of estradiol, progesterone, prolactin, or dexamethasone, nor did it assess molecular markers of mammary protein synthesis. These limitations prevent a direct determination of which component of the hormonal protocol drove the observed protein increase.

Furthermore, evidence from other species demonstrates that hormonal effects on milk production and mammary function can vary considerably. For example, Toledo et al. (2020) found that estradiol altered prolactin-related signaling and mammary STAT5 expression while moderately reducing milk production in dairy cows, highlighting the complexity of endocrine regulation of lactation. Similarly, (Confuorti et al., 2024) demonstrated that dexamethasone administration can modify responses of milk components in goats, although their study focused primarily on antimicrobial components rather than milk protein or fat. Thus, these findings support a selective effect of the induction protocol on milk protein, but further studies with larger sample sizes, untreated controls, repeated milk sampling, and direct hormonal and molecular measurements are needed to establish the underlying mechanism. Thus, the hormonal protocol significantly increased milk protein concentration ($t = 5.05$, $P = 0.002$), whereas fat, lactose, SNF, total solids, freezing point, and pH did not differ significantly from their reference values. Therefore, the results suggest that hormonal induction primarily influenced protein secretion while maintaining the overall physicochemical stability of the induced milk.

A major limitation of this study was the small sample size and the absence of a concurrent untreated control group, which limited statistical power and the ability to distinguish the effects of hormonal treatment from normal individual variation among ewes. In addition, the study was conducted under the practical constraints of laboratory facilities in Afghanistan, and advanced analyses of circulating and milk hormone concentrations, such as estradiol, progesterone, prolactin, and dexamethasone, were not available. Consequently, hormonal or molecular measurements could not directly confirm the physiological mechanisms behind the observed increase in milk protein. The milk samples were also evaluated at a limited number of time points, which may not fully represent changes in milk composition throughout the entire induced lactation period. Therefore, the findings should be considered preliminary, and future studies using larger groups, an untreated control group, repeated milk sampling, and direct measurement of relevant hormones and mammary biomarkers are recommended to confirm these observations.

CONCLUSION

The results of the present study demonstrate that the hormonal induction protocol stimulated mammary gland development and initiated lactation in non-pregnant dry ewes without the need for pregnancy. Moreover, the chemical composition of the induced milk remained largely within normal physiological limits, indicating that hormonally induced lactation can produce milk of acceptable quality. From a practical perspective, these findings

suggest that hormonal induction of lactation may represent a valuable management strategy for improving productivity and farm income, particularly in production systems where reproductive limitations reduce milk availability. This approach may also improve the use of non-pregnant or infertile ewes, enhancing the overall profitability and sustainability of sheep production systems. Nevertheless, additional research is required to optimize hormone dosages, refine nutritional management, and evaluate the long-term effects of lactation induction on animal health, reproductive performance, milk quality, and economic efficiency. Future studies involving larger sample sizes and different sheep breeds are recommended to further validate the applicability and sustainability of this technique under diverse production conditions.

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Author Contribution

Sayeed Qadir Danishiar conceived and designed the study, conducted the experiment, collected and analyzed the data, and prepared the manuscript. Jahid Zabuli contributed to supervision, study design, and critical review of the manuscript. Habibullah Rahamani, Mohammadullah Arghandiwal, and Samiullah Subhan contributed to animal management, experimental procedures, data collection, and laboratory-related activities. All authors reviewed and approved the final version of the manuscript.

Conflict of Interest

The authors declare that they have no known financial, personal, or professional conflicts of interest that could have influenced the design, conduct, analysis, or reporting of this study.

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Data Availability Statement

The data generated and analyzed during the current study are available from the corresponding author upon reasonable request. The authors are willing to provide the relevant datasets and supporting information for purposes of verification or further scientific research, subject to reasonable institutional considerations.

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