Science Journal of University of Zakho
DOI: https://doi.org/10.25271/sjuoz.2026.14.3.1920

Original Article

DIETARY ENERGY DENSITY REGULATES GROWTH PERFORMANCE, LEPTIN CONCENTRATION, AND PUBERTY ATTAINMENT IN AWASSI EWE LAMBS

Yousif Hussein Mohammed [1,*], and Araz Jafar Pedawy [1]

[1]Department of Animal Production, College of Agricultural Engineering Sciences, University of Duhok, Duhok, Kurdistan Region, Iraq.

[*]*Corresponding author, E-mail:*araz.jafar@uod.ac (Tel: +964-7504575421)

Received
April 19, 2026

Accepted
June 21, 2026

Published
July 15, 2026

ABSTRACT

This study evaluated the effects of graded dietary energy density on growth performance, serum leptin concentration, and puberty attainment in Awassi ewe lambs. After a 30-day acclimation period, 27 Awassi ewe lambs aged approximately 195 $\pm$ 5 days were randomly allocated to three dietary treatments providing 9.5, 10.5, or 11.5 MJ metabolizable energy/kg dry matter (n = 9/group). The 120-day experiment comprised 30 days of acclimation, 60 days of controlled individual feeding, 15 days of exposure to two rams, and 15 days of post-exposure reproductive monitoring. Puberty was assessed from standing estrus or external estrus signs, followed 10 days later by serum progesterone measurement; concentrations $\geq$1.0 ng/mL supported luteal activity consistent with ovulation. Average daily gain was 69.7 $\pm$ 2.4, 112.5 $\pm$ 1.7, and 146.4 $\pm$ 1.4 g/day in the low-, medium-, and high-energy groups, respectively ($p < 0.001$). Dietary energy, time, and their interaction affected serum leptin ($p < 0.001$), with Day-75 concentrations of 0.79 $\pm$ 0.02, 1.58 $\pm$ 0.02, and 1.93 $\pm$ 0.02 ng/mL. Puberty attainment was 22.2%, 44.4%, and 66.7%, and time-to-event distributions differed among groups (log-rank $\chi^2 = 6.42$, df = 2, $p = 0.041$). Higher dietary energy improved growth, leptin response, and earlier puberty attainment.

Keywords: Awassi ewe lambs; dietary energy density; leptin; behavioral estrus; luteal activity; right-censoring.

1. INTRODUCTION

Awassi sheep are an important fat-tailed breed in Iraq and neighboring semi-arid regions. Their ability to remain productive under variable feeding conditions makes them valuable for local production, but delayed puberty can prolong the non-productive rearing period of replacement females and reduce the opportunity for first breeding at the desired age (Alkass *et al*., 2021). Evidence on how graded dietary energy affects puberty in Awassi ewe lambs under local conditions remains limited.

Puberty in ewe lambs is the transition from prepubertal growth to functional reproductive competence. It requires coordinated somatic growth, endocrine maturation, ovarian activity, ovulation, luteal function, and estrous behavior within a seasonally suitable environment. Chronological age contributes, but nutritional status, body reserves, breed, and environmental cues also influence the timing of reproductive activation (Rosales-Nieto *et al*., 2018; Martin, 2022; Duittoz & Kenny, 2023; Neimaur *et al*., 2024). Earlier puberty can shorten the unproductive rearing interval and improve the likelihood that ewe lambs enter the flock at their first breeding opportunity (Kenyon & Corner-Thomas, 2022).

Nutrition is one of the most practical factors that can be modified to influence puberty in young ruminants. Adequate dietary energy supports skeletal and muscle growth, rumen development, tissue deposition, and body-reserve formation. Studies in growing lambs show that energy supply and the dietary protein-to-energy balance affect average daily gain, nutrient utilization, rumen function, and serum biochemical responses (Herath *et al*., 2021; Wang *et al*., 2024). Recent work also shows effects of dietary energy or nutrient supply on growth, digestibility, nitrogen metabolism, and energy utilization in sheep (Çelik & Muruz, 2024; Feng *et al*., 2025; Jiao *et al*., 2025; Mahgoub *et al*., 2025). These responses are relevant to puberty because insufficient energy can restrict growth and delay the metabolic conditions required for reproductive activation (Duittoz & Kenny, 2023).

Live weight and body condition score are practical indicators of somatic development and energy-reserve status. Heavier ewe lambs at mating generally show better fertility and later productive outcomes (Haslin *et al*., 2021; Haslin *et al*., 2022; Haslin *et al*., 2023). Live weight and body condition can have additive relationships with reproductive performance, although neither alone confirms functional puberty (Thompson *et al*., 2021; Thompson *et al*., 2024). Associations of age, live weight, and body condition with fertility have also been reported in synchronized ewes (Nageye & Koyuncu, 2024). Because body condition reflects tissue-reserve deposition and relates to metabolic and ovarian processes, evaluating it together with body weight provides a stronger description of reproductive readiness (Chaves *et al*., 2024).

Leptin is an adipose-derived hormone linking body reserves with reproductive function. In ruminants, circulating leptin changes with nutritional status and body fatness and is best interpreted as a permissive signal of energy sufficiency rather than a single trigger of puberty (Delavaud *et al*., 2000; Chilliard *et al*., 2005). Leptin has been evaluated as an indicator of body-fat reserves in Awassi lambs (Orman *et al*., 2018), and positive relationships among leptin, body weight, progesterone, and puberty have been reported in ewe lambs (Al-Ali & Rahawy, 2022). Female Arabi sheep also show increasing leptin and

Access this article online

DOI:https://doi.org/10.25271/sjuoz.2026.14.3.1920

Science Journal of University of Zakho
Vol. 14, No. 03, pp.552-561, July -2026

Printed ISSN 2663-628X;
Electronic ISSN 2663-6298

insulin-like growth factor 1 across heavier body-weight classes (Ali *et al*., 2025). Leptin interacts with neuroendocrine pathways that regulate the hypothalamic-pituitary-gonadal axis (Xie *et al*., 2022; Herdis *et al*., 2025).

Exposure to males is a practical method for stimulating reproductive activity in maiden ewes and can improve early reproductive responses under managed production conditions (Ben Salem *et al*., 2024; Rosales-Nieto *et al*., 2024).

The novelty of this study is the evaluation of graded dietary energy-density regimens as practical modifiers of growth, body condition, circulating leptin, and early attainment of a progesterone-supported behavioral estrus criterion in Awassi ewe lambs under semi-arid conditions. Individual feeding and similar crude-protein concentrations were used to reduce major differences in protein supply, although ingredient composition and neutral detergent fiber changed as energy density increased. The endpoint was observed estrus followed by serum progesterone $\geq$1.0 ng/mL; it was intended to identify early progesterone-supported reproductive activation after ram introduction, not to prove the exact date of first ovulation in every lamb. The hypothesis was that increasing dietary energy density would improve growth and body reserves, increase serum leptin, and shorten the observed time to criterion attainment within the predefined early breeding window.

2. MATERIALS AND METHODS

Ethical approval and study site

All procedures involving animals followed the institutional animal-care requirements of the College of Agricultural Engineering Sciences, University of Duhok, Iraq. Ethical approval was issued by the Animal Ethics Committee (Authority No. AEC 7062026; approval date: 6 June 2026). The 120-day experiment was conducted at the institutional animal farm in the Kurdistan Region of Iraq from 21 March to 18 July 2025.

Animals, housing, and experimental design

Twenty-seven clinically healthy Awassi ewe lambs born within a similar lambing period were enrolled after veterinary examination. At the beginning of the feeding phase, the lambs were approximately 195 $\pm$ 5 days old. The 27-animal SPSS analysis dataset gave an overall baseline body weight of 28.00 $\pm$ 0.17 kg (mean $\pm$ SD), with group-specific values of approximately 28.00 $\pm$ 0.18 kg. Each lamb was identified by ear tag and housed in an individual 1.5 $\times$ 2.0 m pen during controlled feeding. Visual and auditory contact among the animals was maintained, and fresh water was available ad libitum.

Treatment allocation and experimental timeline

The study used a completely randomized design and lasted 120 days: 30 days of acclimation, 60 days of controlled individual feeding, 15 days of direct ram exposure, and 15 days of post-exposure reproductive monitoring. After the 30-day acclimation period (study Day 30; feeding Day 0), the lambs were weighed and randomly allocated to low-, medium-, or high-energy treatments (n = 9 per treatment). The diets supplied 9.5, 10.5, or 11.5 MJ metabolizable energy (ME)/kg dry matter (DM), respectively, while crude-protein concentration was maintained at approximately 13.0%.

Individual feeding ended on study Day 90. The lambs were then removed from the individual pens. At feeding times, they were separated into three dietary-treatment groups and offered their assigned low-, medium-, or high-energy diets in designated group-feeding areas outside the pens to prevent mixing of diets. Immediately after feeding, all 27 lambs were recombined and maintained together as one flock. During the 15-day ram-exposure phase (study Days 91–105), the combined flock was exposed simultaneously to two rams, which were not assigned to any dietary treatment. The rams were removed after Day 105. External reproductive signs continued to be monitored for a further 15 days (study Days 106–120), completing the 30-day reproductive-monitoring period on 18 July 2025. For reproductive signs detected near the end of this period, progesterone blood collection continued after 18 July and was completed by 28 July 2025. This confirmatory sampling did not extend the dietary-treatment or reproductive-monitoring period. The assigned diets continued throughout the 30-day group-managed phase, but individual feed intake was not recorded.

Experimental diets and feeding management

The three diets were formulated to establish a graded energy-density range while maintaining similar crude-protein supply. Estimated ME concentrations were 9.5, 10.5, and 11.5 MJ/kg DM for the low-, medium-, and high-energy diets, respectively. PowerMin vitamin–mineral premix was included at 1.0% in all diets. During the 60-day individual-feeding phase, feed was offered daily at approximately 3% of body weight on a DM basis, and feed offered and refusals were weighed for routine feeding control. After the lambs left the individual pens, the three treatment groups were separated only during feeding and were offered their assigned diets in designated group-feeding areas. They were then recombined as one flock for the remainder of each day. During the 15-day ram-exposure phase, the combined flock had simultaneous contact with two rams.

Table 1: Ingredient composition and estimated nutrient values of the experimental diets (% as-fed basis).

Ingredient / parameter	Low energy 9.5 MJ	Medium energy 10.5 MJ	High energy 11.5 MJ
Corn stalks	24.00	20.00	15.00
Corn grain	29.00	34.00	39.00
Molasses	4.00	4.00	4.00
Cottonseed meal	7.50	6.50	5.00
Soybean meal	10.00	8.50	7.50
Extruded soybean	0.00	2.00	5.00
Rumen-protected fat	0.00	0.50	1.50
Corn husk	12.60	11.60	11.10
Corn germ meal	10.00	10.00	10.00
Limestone	1.20	1.20	1.20
Salt	0.70	0.70	0.70
Vitamin-mineral premix	1.00	1.00	1.00
Total	100.00	100.00	100.00
Metabolizable energy (MJ/kg DM)	9.5	10.5	11.5
Crude protein (%)	13.0	13.0	13.0
Neutral detergent fiber (%)	38.5	35.2	31.8

Note: PowerMin vitamin-mineral premix was included at 1.0% of each diet. According to the manufacturer's label, each kilogram contained vitamin A, 150,000 IU; vitamin D_3, 50,000 IU; vitamin E, 200 mg; vitamin B_{12}, 50 mg; vitamin B_1, 50 mg; niacin, 20 mg; manganese, 1,200 mg; zinc, 1,500 mg; copper, 200 mg; iron, 1,000 mg; cobalt, 20 mg; iodine, 30 mg; magnesium, 10,000 mg; sodium bicarbonate, 150,000 mg; and antioxidant, 1,000 mg.

Growth and body condition measurements

Body weight (BW) and body condition score (BCS) were recorded at feeding Days 0, 14, 28, 42, and 56. In addition, when a lamb showed standing estrus or clear external estrus signs, its body weight was recorded on the same day. For lambs subsequently confirmed with serum progesterone $\geq$1.0 ng/mL, this measurement was reported as body weight attainment of the progesterone-supported estrus criterion. The same trained observer assessed BCS by palpation of the lumbar region using the standard 1–5 scale for sheep (Russel *et al*., 1969). Average daily gain (ADG) for each 14-day interval was calculated as the difference between consecutive BW measurements divided by 14 and expressed as g/day.

Blood sampling and serum preparation

Routine blood samples for serum biochemical and protein variables were collected once every 30 days on study Days 0, 30, 60, 90, and 120 and were designated M1, M2, M3, M4, and M5, respectively. Thus, the M4 sample used for glucose, cholesterol, and triglycerides in the exploratory correlation analysis was collected on study Day 90. Leptin followed a separate three-point schedule: L1 at feeding Day 0 (baseline, immediately after acclimation), L2 at feeding Day 45, and L3 at feeding Day 75. Progesterone was not included in the monthly sampling program. A progesterone blood sample was collected 10 days after a lamb showed standing estrus or clear external estrus signs. For signs detected near the end of the reproductive-monitoring period, progesterone sampling continued after 18 July and was completed by 28 July 2025. All samples were obtained by jugular venipuncture before the morning feeding. Blood was placed in plain tubes, allowed to clot for 30 min at room temperature, and centrifuged at 1,500 × g for 15 min. Serum was transferred to labelled microtubes and stored at $-80\,^{\circ}$C until analysis.

Biochemical and hormonal analyses

Serum glucose, cholesterol, triglycerides, total protein, and albumin were measured on a Roche cobas automated chemistry analyzer using manufacturer-supplied reagents and procedures. Globulin was calculated as total protein minus albumin. These variables were evaluated as supportive indicators of energy, lipid, and protein metabolism. Serum leptin was measured in duplicate using a sheep-specific enzyme-linked immunosorbent assay (FineTest®, Sheep LEP ELISA Kit, catalogue No. ESH0050). Blank-corrected optical-density values were converted to concentrations by a four-parameter logistic model in GraphPad Prism version 10.6.1. The calibration range was 31.25–2,000 pg/mL. An operational lower limit of quantification of 62.5 pg/mL was applied; values below this limit were assigned 31.25 pg/mL before conversion to ng/mL. Duplicate pairs were accepted when their coefficient of variation was ≤10%; pairs exceeding this limit were repeated. The intra- and inter-assay coefficients of variation were <10%. Samples above the upper calibration standard were to be diluted with assay diluent and reassayed, and no concentration was reported by extrapolation beyond 2,000 pg/mL.
Serum progesterone was quantified using the Elecsys® Progesterone III assay on a Roche cobas automated immunoassay analyzer by electrochemiluminescence immunoassay. Values below the measuring range were reported as <0.05 ng/mL. A concentration ≥1.0 ng/mL, measured 10 days after an observed reproductive sign, was used operationally as evidence of luteal activity consistent with ovulation. The 120-day reproductive-monitoring period ended on 18 July 2025. For signs detected near the end of this period, confirmatory progesterone collection continued after 18 July and was completed by 28 July 2025. Because a separate ovine-matrix validation was not performed, progesterone results were interpreted as supportive endocrine evidence rather than as an independent diagnosis of the exact ovulation date.

Reproductive monitoring and operational puberty criterion

During the 30-day reproductive-monitoring period, each lamb was observed for standing estrus and external estrus signs, including vulvar redness or swelling and vaginal mucus discharge. Standing response was assessed during the first 15 days while the two rams were present, and external estrus signs continued to be monitored during the subsequent 15-day follow-up period after ram removal. When a lamb showed standing estrus or clear external estrus signs, a blood sample for progesterone determination was collected 10 days after the observed sign. Therefore, for signs detected near the end of the monitoring period on 18 July 2025, progesterone sampling continued after the animal-monitoring endpoint and was completed by 28 July 2025. The reproductive event date was recorded as the date on which the behavioral or external estrus sign was first observed, rather than the later progesterone-sampling date. A lamb met the operational puberty criterion when the observed sign was followed by serum progesterone ≥1.0 ng/mL (Fitzgerald & Butler, 1982). Lambs with progesterone <1.0 ng/mL did not meet the criterion. Lambs showing no reproductive sign by 18 July 2025 were right-censored on that date. Completely silent ovulations without behavioral or external signs could not be detected by this protocol.

Statistical analysis

Analyses were performed in IBM SPSS Statistics (version 26.0). Baseline age, BW, and BCS were compared among treatments by one-way analysis of variance. Repeated-measures general linear models were fitted for BW, interval ADG, BCS, serum protein variables, and leptin, with time as the within-subject factor and dietary energy as the between-subject factor. Greenhouse–Geisser-adjusted results were used when the sphericity assumption was violated, and significant effects were followed by Bonferroni-adjusted comparisons of estimated marginal means. Criterion attainment was summarized as n (%). Exploratory Pearson correlations were calculated among selected continuous variables; correlations involving the binary criterion variable are point-biserial equivalents. Time from ram introduction to the observed reproductive event or censoring was analyzed by Kaplan–Meier curves and the log-rank test. Statistical significance was set at $p < 0.05$.

3. RESULTS

Growth performance and body condition

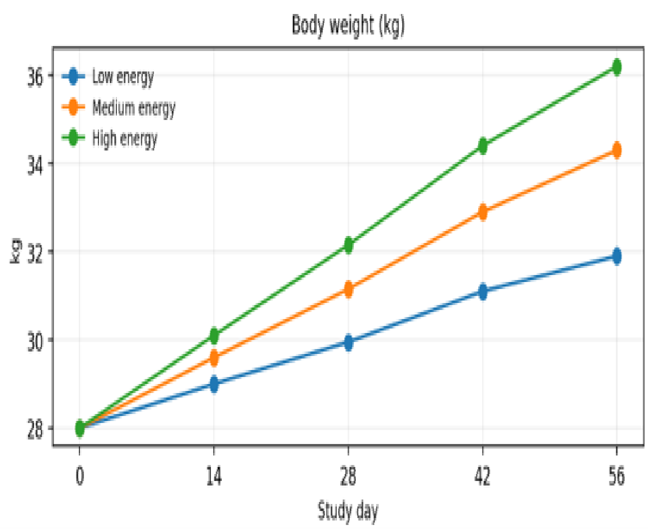
Baseline body weight (BW), body condition score (BCS), and age did not differ significantly among treatment groups, confirming comparability at the start of feeding. Values taken directly from the 27-animal SPSS analysis dataset showed a baseline BW of 28.00 ± approximately 0.18 kg (mean ± SD) in each group and 28.00 ± 0.17 kg overall; the baseline one-way ANOVA was $F(2, 24) = 0.000$, $p = 1.000$. Body weight increased over time in all groups, and the energy × time interaction was significant. On Day 56, body weight was 31.90 ± 0.19, 34.30 ± 0.18, and 36.20 ± 0.19 kg in the low-, medium-, and high-energy groups, respectively.
Average daily gain, calculated from four consecutive 14-day intervals, increased from 69.7 ± 2.4 g/day in the low-energy group to 112.5 ± 1.7 and 146.4 ± 1.4 g/day in the medium- and high-energy groups. Body condition scores also increased progressively with dietary energy. These results are summarized in Table 2 and Figure 1.

Table 2: Effect of dietary energy level on body weight, average daily gain, and body condition score in Awassi ewe lambs (estimated marginal mean ± SE).

Item	Low energy (9.5 MJ)	Medium energy (10.5 MJ)	High energy (11.5 MJ)	Overall mean	SE
BW Day 0 (kg)	28.00 ± 0.06	28.00 ± 0.06	28.00 ± 0.06	28.00	0.04
BW Day 14 (kg)	29.00 ± 0.17	29.60 ± 0.16	30.10 ± 0.11	29.57	0.12
BW Day 28 (kg)	29.95 ± 0.17	31.15 ± 0.19	32.15 ± 0.19	31.08	0.20
BW Day 42 (kg)	31.10 ± 0.20	32.90 ± 0.12	34.40 ± 0.19	32.80	0.28
BW Day 56 (kg)	31.90 ± 0.19	34.30 ± 0.18	36.20 ± 0.19	34.13	0.36
Overall mean BW	29.99 ± 0.15	31.19 ± 0.15	32.17 ± 0.15	—	—
p energy < 0.001; p energy × time < 0.001; p (time) < 0.001					
ADG Days 0–14 (g/day)	71.4 ± 9.9	114.1 ± 9.6	150.1 ± 12.5	111.9	8.7
ADG Days 14–28 (g/day)	67.8 ± 8.6	110.9 ± 6.8	146.4 ± 11.0	108.3	8.0
ADG Days 28–42 (g/day)	82.2 ± 7.1	124.9 ± 7.6	160.8 ± 14.8	122.6	8.6
ADG Days 42–56 (g/day)	57.2 ± 9.8	99.9 ± 12.3	128.5 ± 14.1	95.2	8.9
Overall mean ADG	69.7 ± 2.4	112.5 ± 1.7	146.4 ± 1.4	—	—
p energy < 0.001; p energy × time = 0.397; p (time) < 0.001					

BCS Day 0	2.20 ± 0.01	2.20 ± 0.02	2.20 ± 0.02	2.20	0.01
BCS Day 14	2.25 ± 0.02	2.35 ± 0.02	2.45 ± 0.02	2.35	0.02
BCS Day 28	2.30 ± 0.02	2.50 ± 0.03	2.65 ± 0.03	2.48	0.03
BCS Day 42	2.38 ± 0.02	2.68 ± 0.02	2.88 ± 0.02	2.65	0.04
BCS Day 56	2.45 ± 0.02	2.82 ± 0.02	3.02 ± 0.03	2.76	0.05
Overall mean BCS	2.32 ± 0.02	2.51 ± 0.02	2.64 ± 0.02	—	—
p energy < 0.001; *p* energy × time < 0.001					

Note: The SPSS descriptive output showed baseline BW of 28.00 ± 0.18 kg (mean ± SD) in each dietary group and 28.00 ± 0.17 kg overall; the baseline one-way ANOVA was $F(2, 24) = 0.000$, $p = 1.000$. Table values are estimated marginal means ± SE from the repeated-measures model. *p* (time) is the overall within-subject effect across recording times; *p* (energy) is the overall between-group effect; and *p* (energy × time) tests whether the pattern of change over time differed among dietary groups.



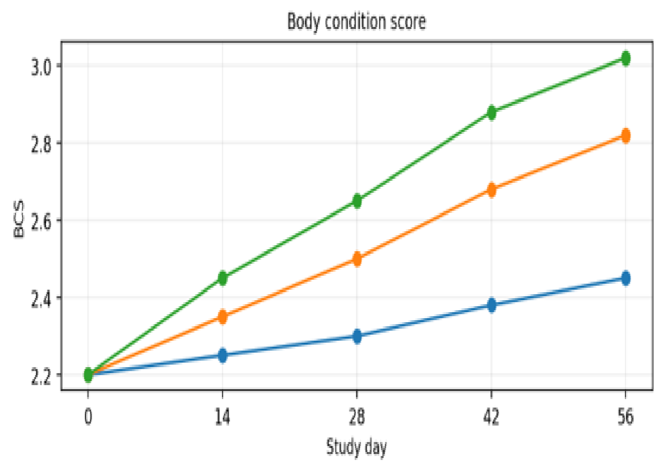
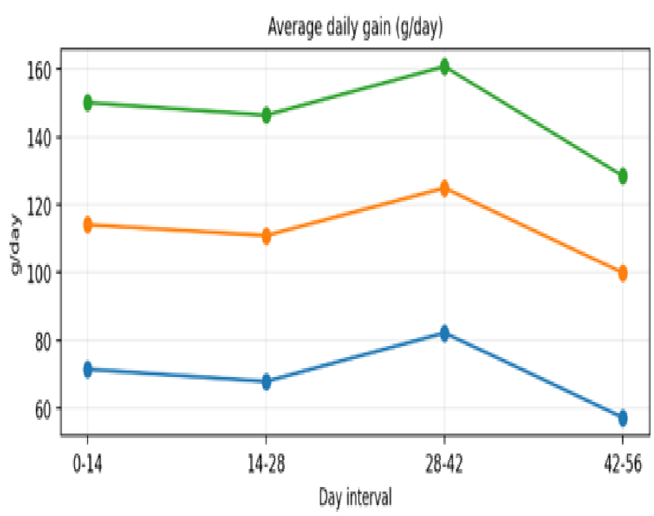


Figure 1: Growth patterns of body weight, average daily gain, and body condition score in Awassi ewe lambs by dietary energy level.



Serum protein profile and biochemical variables

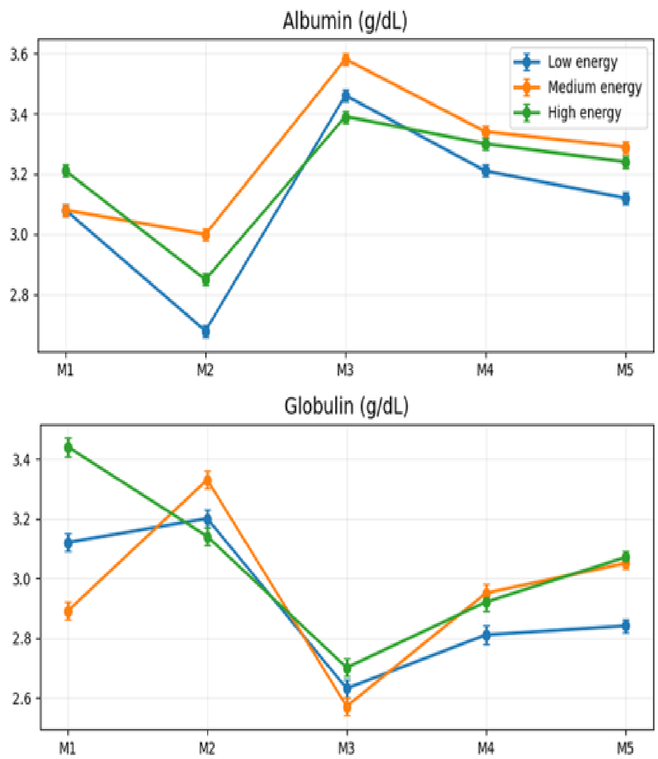
Dietary energy level, time, and their interaction affected albumin, globulin, and total protein concentrations. Albumin and total protein values generally indicated better nutritional status in lambs receiving the medium- and high-energy diets than in lambs receiving the low-energy diet. Globulin also differed among groups and time points, indicating that the dietary energy gradient was accompanied by broader physiological changes in the serum protein profile. These serum protein trajectories are shown in Figure 2.

Glucose, cholesterol, and triglycerides were retained as supportive metabolic variables rather than primary puberty endpoints. Their patterns were interpreted cautiously because circulating metabolites can be influenced by homeostatic regulation, lipid partitioning in fat-tailed sheep, and sampling time. In the correlation analysis, glucose, cholesterol, and triglycerides measured at the routine monthly M4 sample (study Day 90) were not significantly associated with criterion status.

Table 3: Table 5. Summary of selected serum protein responses to dietary energy level in Awassi ewe lambs (mean ± SE). Routine biochemical sampling was performed monthly at study Days 0, 30, 60, 90, and 120 (M1–M5); only M1 and M5 values are displayed for compact presentation. P values are from the repeated-measures model across all monthly sampling points.

Variable	Time	Low energy	Medium energy	High energy	Overall mean	SE
Albumin (g/dL)	M1	3.08 ± 0.02	3.08 ± 0.02	3.21 ± 0.02	3.12	0.015
	M5	3.12 ± 0.02	3.29 ± 0.02	3.24 ± 0.02	3.22	0.017
Globulin (g/dL)	M1	3.12 ± 0.05	2.89 ± 0.05	3.44 ± 0.05	3.15	0.045
	M5	2.84 ± 0.02	3.05 ± 0.02	3.07 ± 0.02	2.99	0.022
Total protein (g/dL)	M1	6.20 ± 0.06	5.97 ± 0.06	6.65 ± 0.06	6.27	0.058
	M5	5.96 ± 0.04	6.34 ± 0.04	6.31 ± 0.04	6.20	0.038

Note: p energy < 0.001; p energy × time < 0.001 (all three variables). p (time) is the overall within-subject effect across sampling points; p (energy) is the overall between-group effect; and p (energy × time) tests whether temporal changes differed among dietary groups.



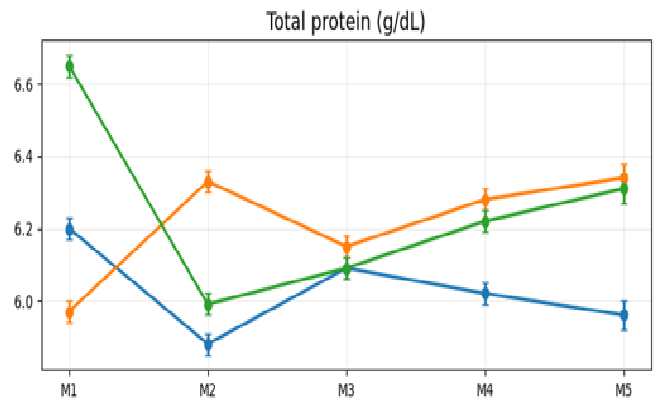


Figure 2: Trajectories of albumin, globulin, and total protein across dietary energy groups.

Serum leptin concentration

Baseline leptin concentrations were similar among groups. Leptin then increased in a graded pattern across the energy-density regimens, and the significant regimen × time interaction showed progressive divergence among groups ($p < 0.001$). At leptin L3 (Day 75), mean concentrations were 0.79 ± 0.02, 1.58 ± 0.02, and 1.93 ± 0.02 ng/mL in the low-, medium-, and high-energy groups, respectively (Table 4 and Figure 3).

Table 4: Effects of dietary energy level on serum leptin concentration in Awassi ewe lambs (ng/mL; mean ± SE).

Time	Low energy	Medium energy	High energy	Overall mean	SE	p (time)
L1 baseline (Day 0)	0.62 ± 0.01	0.61 ± 0.01	0.62 ± 0.01	0.62	0.005	
L2 during feeding (Day 45)	0.67 ± 0.01	0.80 ± 0.01	1.09 ± 0.02	0.85	0.037	
L3 post-feeding (Day 75)	0.79 ± 0.02	1.58 ± 0.02	1.93 ± 0.02	1.43	0.094	<0.001
Overall mean	0.69 ± 0.01	1.00 ± 0.01	1.21 ± 0.02			

p (energy) < 0.001; p (energy × time) < 0.001

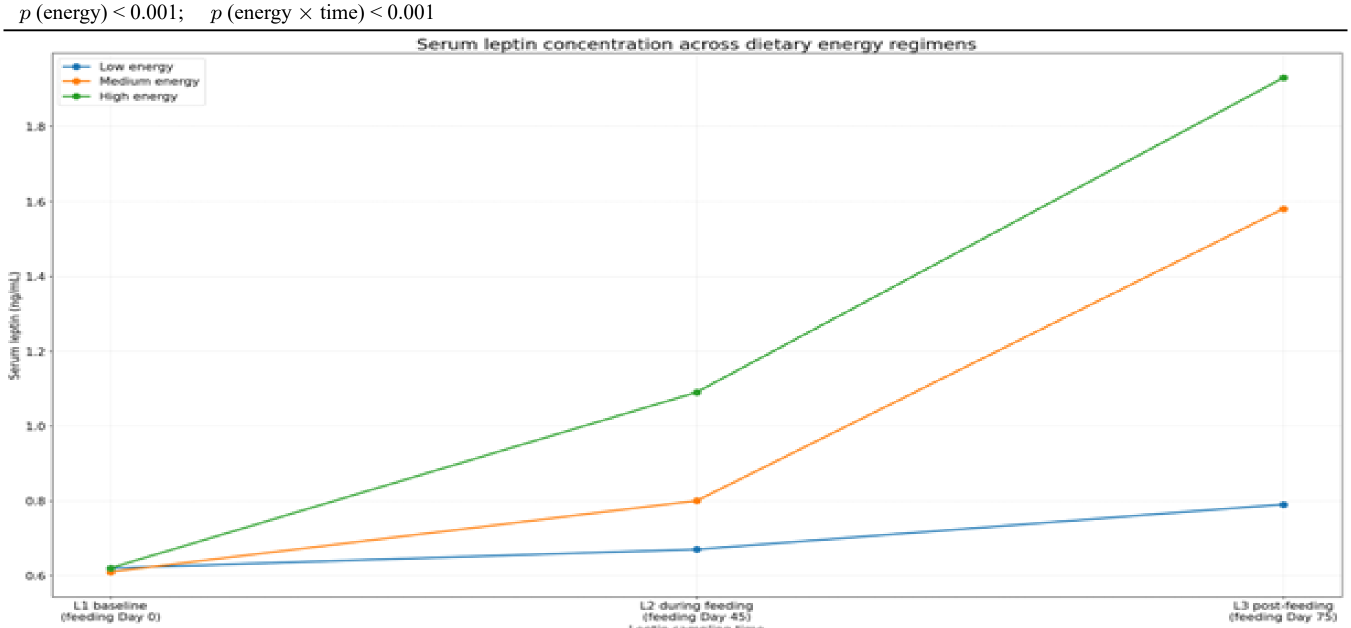


Figure 3: Serum leptin concentration at L1 (baseline, feeding Day 0), L2 (feeding Day 45), and L3 (feeding Day 75) across dietary energy groups. L3 was obtained 30 days after L2 and 15 days after completion of individual feeding. Error bars represent SE.

Correlations and criterion response

Final BW, leptin L2, and leptin L3 were positively intercorrelated. Criterion status was positively correlated with final BW ($r = 0.611$, $p = 0.0007$), leptin L2 ($r = 0.604$, $p = 0.0008$), and leptin L3 ($r = 0.492$, $p = 0.009$). Criterion status was not significantly correlated with baseline leptin L1 ($r = 0.000$, $p = 1.000$), glucose at monthly M4 (study Day 90; $r = 0.012$, $p = 0.953$), cholesterol at monthly M4 ($r = 0.320$, $p = 0.104$), or triglyceride at monthly M4 ($r = 0.338$, $p = 0.085$). The key criterion-related relationships are summarized in Figure 4.

Table 5: Pearson correlation matrix among selected study variables and operational criterion status.

Variable	Final BW	Leptin L1	Leptin L2	Leptin L3	Glucose M4	Chol. M4	TG M4
Final BW	—						
Leptin L1	0.019	—					
Leptin L2	0.963**	0.114	—				
Leptin L3	0.989**	0.003	0.944**	—			
Glucose M4	0.370	-0.268	0.299	0.413*	—		
Chol. M4	-0.304	-0.163	-0.295	-0.390*	0.331	—	
TG M4	-0.116	-0.311	-0.246	-0.176	0.488**	0.857**	—
Criterion status	0.611**	0.000	0.604**	0.492**	0.012	0.320	0.338

Note: Values are Pearson r coefficients; lower triangle only. Correlations involving binary criterion status are point-biserial equivalents. Final BW = BW at Day 56; Chol. = cholesterol; TG = triglyceride. Asterisks indicate significance levels based on exact two-tailed p-values (*$p < 0.05$, **$p < 0.01$).

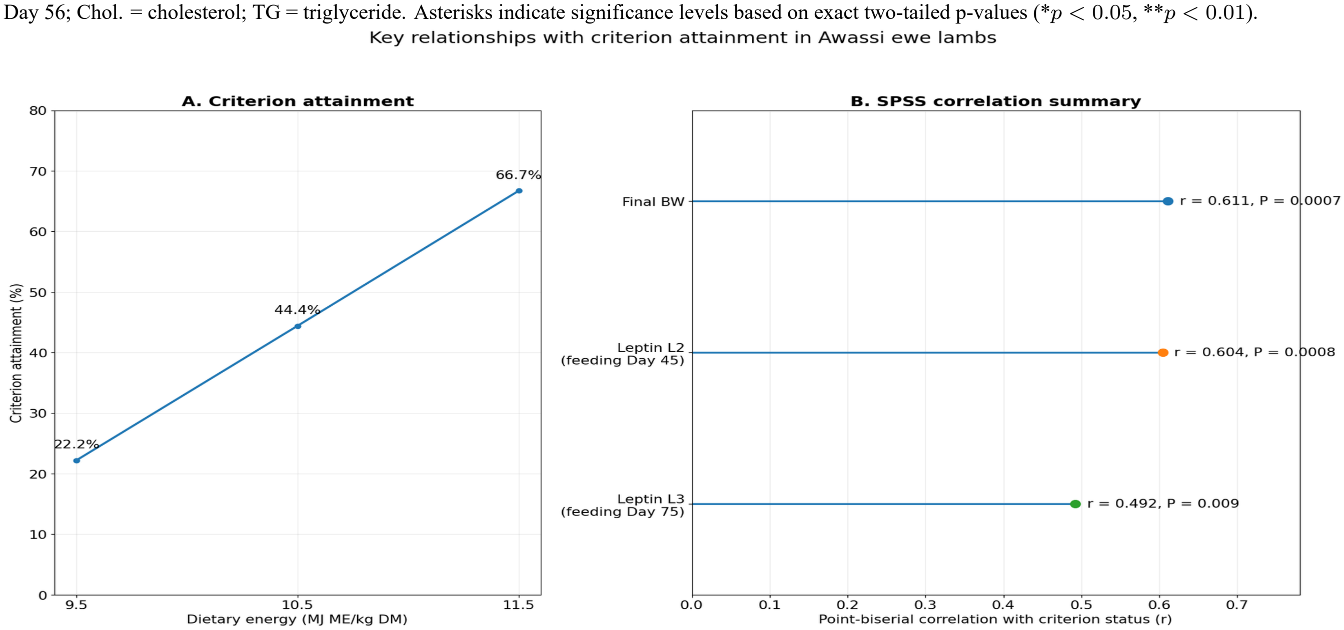


Figure 4: Key relationships with attainment of the progesterone-supported behavioral estrus criterion in Awassi ewe lambs. Panel A shows the observed increase in criterion attainment across dietary energy levels. Panel B summarizes the SPSS Pearson/point-biserial correlations of criterion status with final BW, leptin L2 (feeding Day 45), and leptin L3 (feeding Day 75). Exact two-tailed p-values were taken from the SPSS output. Final BW = BW at Day 56.

The criterion was met by 2 of 9 low-energy lambs (22.2%), 4 of 9 medium-energy lambs (44.4%), and 6 of 9 high-energy lambs (66.7%). These percentages describe attainment within the predefined 30-day reproductive-monitoring period and do not estimate the final proportion that would eventually reach puberty at older ages. For each lamb meeting the criterion, the event was assigned to the observed estrus date and was confirmed by serum progesterone ≥ 1.0 ng/mL measured 10 days later. Mean age at criterion attainment was 277.5 ± 2.5, 269.5 ± 2.8, and 264.8 ± 1.0 days, respectively. The corresponding body weights measured on the estrus date were 36.4 ± 0.2, 39.1 ± 0.3, and 41.3 ± 0.2 kg. Using the exact event and censoring times retained in the analysis dataset, the exploratory time-to-criterion distributions differed among groups (log-rank $\chi^2 = 6.42$, df = 2, $p = 0.041$). The descriptive Kaplan–Meier curves are shown in Figure 5. For signs detected near the administrative endpoint, confirmatory progesterone sampling continued after 18 July and was completed by 28 July 2025; event times remained assigned to the observed-sign dates within the 120-day monitoring period.

Table 6: Attainment of the progesterone-supported behavioral estrus criterion, age at estrus, and body weight at estrus by dietary energy group.

Energy group	n	Criterion met n (%)	Age at estrus event (days)	BW at estrus event (kg)
Low energy (9.5 MJ)	9	2 (22.2%)	277.5 ± 2.5	36.4 ± 0.2
Medium energy (10.5 MJ)	9	4 (44.4%)	269.5 ± 2.8	39.1 ± 0.3
High energy (11.5 MJ)	9	6 (66.7%)	264.8 ± 1.0	41.3 ± 0.2

Note: Age and BW are shown only for lambs whose behavioral estrus or external estrus signs were followed 10 days later by serum progesterone ≥ 1.0 ng/mL (mean $\pm$ SE). The event date and BW correspond to the observed-sign date, not the progesterone-sampling date. Lambs showing no reproductive sign by 18 July 2025 were right-censored on that date; right-censoring means that the criterion was not observed by the cutoff, not that the lamb would remain permanently prepubertal. The exploratory log-rank analysis used the exact, unrounded event and censoring times from the analysis dataset and showed an overall difference among groups ($\chi^2 = 6.42$, df = 2, $p = 0.041$). For signs detected near the endpoint, confirmatory progesterone collection continued after 18 July and was completed by 28 July 2025, without changing the event or censoring dates used in the survival analysis.

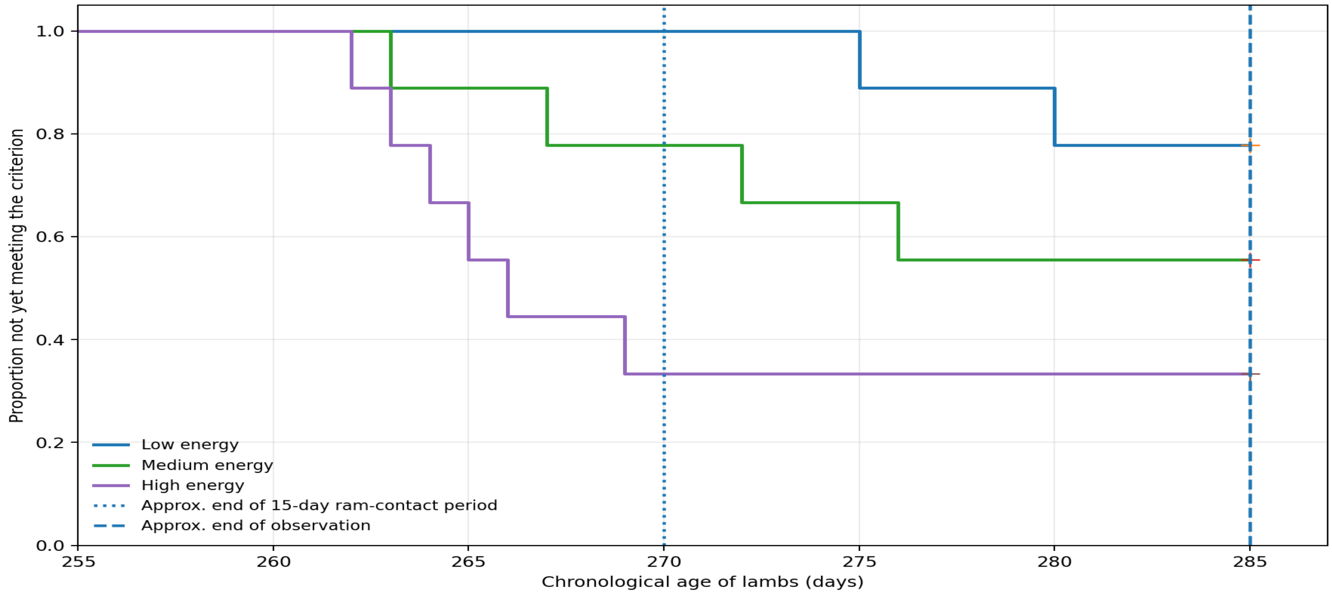


Figure 5. Descriptive Kaplan–Meier curves showing the proportion of Awassi ewe lambs not yet meeting the progesterone-supported behavioral estrus criterion according to rounded chronological age. Events were assigned to the observed estrus date when serum progesterone measured 10 days later was $\geq$1.0 ng/mL. The inferential log-rank test used the exact, unrounded number of days from ram introduction to each event or censoring time in the analysis dataset; chronological age is displayed only for biological interpretation. Plus signs indicate right-censoring at the approximate administrative end of observation. The dotted line marks the approximate end of the 15-day ram-contact period at 270 days, and the dashed line marks the administrative end of the 30-day reproductive observation window at 285 days, based on the mean age at feeding Day 0. Time-to-criterion distributions differed among energy groups (log-rank $\chi^2 = 6.42$, df = 2, $p = 0.041$).

4. DISCUSSION

The growth response was consistent with the dietary design. BW at feeding Day 56 increased from 31.90 kg in the low-energy group to 34.30 and 36.20 kg in the medium- and high-energy groups, and ADG increased in parallel. Similar effects of dietary energy on lamb growth, digestibility, and nutrient utilization have been reported (Zhang *et al*., 2023; Wang *et al*., 2024; Feng *et al*., 2025). Similar crude-protein concentrations reduced a major nutritional source of variation, but the regimens also differed in ingredients and NDF; therefore, the response should be attributed to the complete energy-density treatments rather than to ME alone. This pattern is biologically relevant because adequate prepubertal growth supports the somatic and endocrine conditions required for ovarian activity (Duittoz & Kenny, 2023).

Body condition score increased with dietary energy, indicating greater reserve deposition in the medium- and high-energy groups. Live weight and body condition at breeding are associated with ewe-lamb reproductive performance (Haslin *et al*., 2023; Thompson *et al*., 2024). Body condition also provides information beyond live weight because animals of similar weight can differ in reserve status and ovarian readiness (Chaves *et al*., 2024). The parallel increases in body condition and leptin were biologically coherent, and Awassi-specific evidence supports leptin as an indicator of body-fat reserves (Orman *et al*., 2018).

Serum leptin was similar among groups at baseline but diverged progressively during individual feeding and the early post-feeding period, reaching 0.79, 1.58, and 1.93 ng/mL at leptin L3 (Day 75) in the low-, medium-, and high-energy groups. This graded response agrees with Awassi and ewe-lamb evidence linking leptin with body reserves, body weight, progesterone, and reproductive maturation (Orman *et al*., 2018; Al-Ali & Rahawy, 2022). Nutritional management has also been shown to alter circulating leptin in local sheep breeds (Pedawy, 2025). Nevertheless, baseline leptin was unrelated to later criterion attainment, not every lamb with higher later leptin met the criterion, and T3 was obtained after some reproductive events. Leptin is therefore best interpreted as a permissive metabolic signal within the hypothalamic–pituitary–gonadal pathway rather than as an independent trigger of puberty (Herdis *et al*., 2025).

Progesterone measurement strengthened the reproductive endpoint because external estrus signs alone do not establish luteal activity. One progesterone sample was collected 10 days after a lamb showed standing estrus or external signs such as vulvar redness or swelling and vaginal mucus discharge. This included lambs with external signs but without a standing response. Lambs with progesterone $<$1.0 ng/mL did not meet the criterion, whereas concentrations $\geq$1.0 ng/mL were interpreted as evidence of luteal activity consistent with ovulation. The event was assigned retrospectively to the first date of observed behavior or external signs; therefore, age and BW represented that date rather than the later blood-sampling day. For signs detected near the end of monitoring, progesterone sampling continued after 18 July and was completed by 28 July 2025. This use of progesterone is consistent with its established role as an endocrine marker of luteal activity, although one event-triggered sample cannot exclude a completely silent ovulation without any observable sign (Fitzgerald & Butler, 1982). Recent Iraqi ewe-lamb studies also use progesterone as a central marker of pubertal responses (Alrawy & Hussain, 2025a, 2025b). Serial estradiol, gonadotropins, and ovarian ultrasonography were not measured, but these processes remain biologically relevant to coordinated neuroendocrine and ovarian maturation (Aerts *et al*., 2021; Xie *et al*., 2022).

The correlation results were biologically coherent but require cautious interpretation. Criterion status was positively associated with BW at Day 56 and with leptin at T2 and T3, whereas baseline leptin and the routine monthly M4 metabolites collected on study Day 90 were not significantly associated with the criterion. This pattern supports an association of accumulated growth and later metabolic signaling with reproductive readiness (Rosales-Nieto *et al*., 2018; Duittoz & Kenny, 2023). However, the analyses were unadjusted, not corrected for multiple testing and may partly reflect dietary-group separation. They should therefore be interpreted as nominal exploratory associations rather than independent causal effects.

Albumin, globulin, and total protein changed with treatment and time, supporting a systemic nutritional response. Dietary protein-to-energy balance and controlled changes in nutrient intake can alter lamb growth, nitrogen metabolism, and serum biochemical indices (Herath *et al*., 2021; Feng *et al*., 2025; Jiao *et al*., 2025). In contrast, glucose, cholesterol, and triglycerides measured at the routine monthly M4 sample on study Day 90 were not associated with criterion status. These metabolites are influenced by short-term homeostasis, fermentation, hepatic metabolism, and sampling conditions and may not change in direct proportion to energy intake (National Research Council, 2007; Çelik & Muruz, 2024). Within this dataset, BW at Day 56 and pre-event leptin L2 (feeding Day 45) were more informative correlations of early criterion attainment than a single monthly metabolite measurement.

Breed background also affects interpretation. Awassi sheep are fat-tailed, and their reproductive efficiency is influenced by body development, season, and management (Alkass *et al*., 2021). Fat-tail reserve partitioning may modify relationships among live weight, body condition, adiposity, and leptin; therefore, Awassi leptin values should be interpreted together with growth and progesterone-supported classification (Orman *et al*., 2018; Al-Ali & Rahawy, 2022). The high-energy group did not reach 100% criterion attainment within the observation window, confirming that body weight and body condition are not absolute guarantees. Neuroendocrine maturation, ovarian competence, season, and response to male exposure also contribute. Breed comparisons show variation in reproductive maturation, and enhanced early nutrition can influence ovarian development in fat-tailed ewe lambs (Neimaur *et al*., 2024; Souri *et al*., 2024).

The findings are consistent with ewe-lamb management studies in which greater live weight at mating improved fertility and later production (Haslin *et al*., 2023; Thompson *et al*., 2024). The present endpoint was earlier and narrower: behavioral estrus after ram introduction supported by one progesterone measurement. The results therefore suggest that appropriately formulated higher-energy regimens may prepare replacement lambs for earlier reproductive activation, but they do not demonstrate the precise timing of first spontaneous ovulation. Practical feeding should target balanced development rather than weight gain alone and should match energy supply to age, feed quality, and production stage without unnecessary overfeeding (Kenyon & Corner-Thomas, 2022; Duittoz & Kenny, 2023).

The study is limited by the small treatment groups, only 12 events, the short reproductive observation period, and the use of one event-triggered progesterone measurement. Progesterone was measured after standing estrus or detectable external estrus signs, including vulvar redness or swelling and vaginal mucus discharge; consequently, lambs with external signs but no standing response could be evaluated. However, completely silent ovulations without any detectable sign may still have been missed, and the progesterone assay was not separately validated in an ovine matrix. The diets differed in ingredients and NDF as well as ME density, so the treatment effect cannot be attributed to ME alone. During the final 30-day group-managed phase, the lambs were separated by dietary treatment only during feeding and were then recombined as one flock; therefore, individual feed intake was unavailable during this phase, but dietary treatment was not continuously confounded with separate group enclosures during ram exposure. Routine biochemical and serum protein variables were sampled at M1–M5, whereas leptin followed the separate L1–L3 schedule. Progesterone was collected after reproductive signs; for signs detected near the end of monitoring, confirmation sampling extended after 18 July and was completed by 28 July 2025 without extending treatment or behavioral observation. Serial insulin, insulin-like growth factor 1, non-esterified fatty acids, gonadotropins, estradiol, and ovarian ultrasonography were not available. The high-energy leptin L3 (feeding Day 75) mean remained within but close to the upper assay standard, so values near this limit require caution. Criterion-attainment percentages are descriptive, and the log-rank result should be viewed as exploratory despite the reported overall difference ($\chi^2 = 6.42$, df = 2, $p = 0.041$). Figure 5 displays rounded chronological ages, whereas the analysis used exact days from ram introduction. Direct ram contact lasted 15 days; after ram removal, external-sign monitoring continued for another 15 days to complete the 30-day reproductive observation window. Censored lambs could have matured later. Larger cohorts with independently replicated feeding groups, longer follow-up, and repeated endocrine and ovarian measurements are needed to separate the contributions of dietary regimen, growth, body reserves, leptin, ram exposure, and ovarian maturation.

Taken together, the graded energy-density regimens were associated with growth, body-reserve development, circulating leptin, and earlier attainment of the progesterone-supported behavioral estrus criterion in these Awassi ewe lambs. The exploratory time-to-event comparison indicated different time-to-criterion distributions among groups within the predefined early observation window. The pattern supports the view that reproductive maturation integrates nutrition, somatic growth, metabolic signaling, ovarian activity, male stimulation, and seasonal suitability rather than chronological age or one circulating hormone alone (Rosales-Nieto *et al*., 2018; Duittoz & Kenny, 2023; Herdis *et al*., 2025).

5. CONCLUSION

Within the predefined early reproductive window, increasing dietary energy density improved body weight, average daily gain, body condition score, and serum leptin and was associated with a greater proportion and shorter observed time to attainment of the progesterone-supported behavioral estrus criterion. For lambs meeting the criterion, the event was assigned to the observed estrus date, and BW measured on that date showed that criterion attainment occurred across a range of live weights, with higher mean values in the higher-energy groups. Progesterone measured 10 days after estrus provided supportive evidence of luteal activity and excluded behavioral responders with progesterone <1.0 ng/mL. The Kaplan–Meier comparison showed an exploratory overall difference among groups (log-rank $\chi^2 = 6.42$, df = 2, $p = 0.041$). This finding should not be interpreted as direct measurement of first ovulation in every lamb or as evidence that censored lambs would remain prepubertal after the observation cutoff.

Leptin was most informative as a dynamic marker of nutritional and developmental status rather than as an independent trigger. The exploratory SPSS correlations linked criterion status with BW at Day 56 and with leptin at T2 and T3, while baseline leptin and the selected routine monthly M4 metabolites collected on study Day 90 were not associated. Strategic improvement of prepubertal dietary energy may help prepare Awassi replacement ewe lambs for earlier breeding, but confirmation requires larger cohorts, independently replicated feeding groups, longer behavioral follow-up, serial progesterone or ovarian assessment, and complete animal-level validation of analytical outcomes.

ACKNOWLEDGEMENTS

The authors would like to thank the College of Agriculture and the Animal Production Project at the University of Duhok for providing the appropriate facilities.

ETHICAL STATEMENT

Animal procedures were conducted in accordance with the institutional animal-care practices applied at the College of Agricultural Engineering Sciences, University of Duhok, Iraq. Following completion of the experiment, the Animal Ethics Committee issued an official institutional certificate documenting the study (Authority No. AEC 7062026; certificate date: 6 June 2026). The certificate date reflects administrative issuance after completion of the animal work and is reported transparently.

Funding:

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

Author Contributions:

Yousif Hussein Mohammed: Validation, Formal analysis, Investigation, Data curation, Visualization, Project administration, Funding acquisition (self-funded). Araz Jafar Pedawy: Conceptualization, Methodology, Supervision, Writing – Original Draft, Writing – Review & Editing.

Conflict of Interest:

The authors declare no conflicts of interest.

Data Availability:

The de-identified data supporting the findings of this study are available from the corresponding author on reasonable request.

6. REFERENCES

Aerts, E. G., Harlow, K., Griesgraber, M. J., Bowdridge, E. C., Hardy, S. L., Nestor, C. C., & Hileman, S. M. (2021). Kisspeptin, neurokinin B, and dynorphin expression during pubertal development in female sheep. *Biology*, 10(10), 988. DOI:https://doi.org/10.3390/biology10100988

Al-Ali, M. Q., & Rahawy, M. A. (2022). Relationship between the leptin, progesterone, body weight, and onset of puberty in ewe lambs. *Iraqi Journal of Veterinary Sciences*, 36(4), 833–837. DOI:https://doi.org/10.33899/ijvs.2022.131232.1932

Ali, N. M. J., Kassem, W. Y., Eissa, A. H., & Hassan, A. F. (2025). Relationship of different body weight on some biochemical parameters, gender, metabolic hormones of blood serum in female Arabi sheep. *Tikrit Journal for Agricultural Sciences*, 25(2), 88–93. DOI:https://doi.org/10.25130/tjas.25.2.8

Alkass, J. E., Hermiz, H. N., & Baper, M. I. (2021). Some aspects of reproductive efficiency in Awassi ewes: A review. *Iraqi Journal of Agricultural Sciences*, 52(1), 20–27. DOI:https://doi.org/10.36103/ijas.v52i1.1232

Alrawy, I. Y., & Hussain, S. O. (2025a). Efficacy of gonadotropin releasing hormone on puberty in Iraqi Awassi cross breed ewe lambs. *Egyptian Journal of Veterinary Sciences*, 56(1), 61–68. DOI:https://doi.org/10.21608/ejvs.2024.262822.1782

Alrawy, I. Y., & Hussain, S. O. (2025b). Impact of both growth hormone and gonadotropin releasing hormone on puberty based on serum progesterone and insulin-like growth factor-1 level in Iraqi local breed ewe lambs. *Egyptian Journal of Veterinary Sciences*, 56(4), 739–746. DOI:https://doi.org/10.21608/ejvs.2024.275211.1897

Ben Salem, I., Rouatbi, M., Mlika, M., Getachew, T., Govind, A., Haile, A., Raggem, M., Lahsoumi, R., & Rekik, M. (2024). Response of maiden ewes to the ram effect is a robust management practice and a candidate selection trait for enhanced reproductive performance in drylands. *Animal*, 18(11), 101332. DOI:https://doi.org/10.1016/j.animal.2024.101332

Çelik, S., & Muruz, H. (2024). Growth performance, blood metabolites, carcass characteristics and meat quality of lambs fed diets containing different energy levels supplemented with rumen-protected choline. *Animals*, 14(11), 1682. DOI:https://doi.org/10.3390/ani14111682

Chaves, A. S., Silva, F., Valentim, R., & Quintas, H. (2024). Body condition in small ruminants—Effects of nutrition on the hypothalamic–pituitary–gonad axis and ovarian activity that controls reproduction. *Physiologia*, 4(2), 213–225. DOI:https://doi.org/10.3390/physiologia4020012

Chilliard, Y., Delavaud, C., & Bonnet, M. (2005). Leptin expression in ruminants: Nutritional and physiological regulations in relation with energy metabolism. *Domestic Animal Endocrinology*, 29(1), 3–22. DOI:https://doi.org/10.1016/j.domaniend.2005.02.026

Delavaud, C., Bocquier, F., Chilliard, Y., Keisler, D. H., Gertler, A., & Kann, G. (2000). Plasma leptin determination in ruminants: Effect of nutritional status and body fatness on plasma leptin concentration assessed by a specific RIA in sheep. *Journal of Endocrinology*, 165(2), 519-526. DOI:https://doi.org/10.1677/joe.0.1650519

Duittoz, A. H., & Kenny, D. A. (2023). Review: Early and late determinants of puberty in ruminants and the role of nutrition. *Animal*, 17(Suppl. 1), 100812. DOI:https://doi.org/10.1016/j.animal.2023.100812

Feng, W., Zheng, J., Jiao, N., Ma, C., Li, H., Zhang, J., Yang, J., Xu, H., Cai, Y., Gao, D., Cao, X., Feng, X., & Guo, P. (2025). Effects of energy intake on nutrient digestibility, nitrogen metabolism, energy utilization, serum biochemical indices, and rumen microbiota in Lanzhou fat-tailed sheep. *Agriculture*, 15(7), 698. DOI:https://doi.org/10.3390/agriculture15070698

Fitzgerald, J., & Butler, W. R. (1982). Seasonal effects and hormonal patterns related to puberty in ewe lambs. *Biology of Reproduction*, 27(4), 853–863. DOI:https://doi.org/10.1095/biolreprod27.4.853

Haslin, E., Corner-Thomas, R. A., Kenyon, P. R., Pettigrew, E. J., Hickson, R. E., Morris, S. T., & Blair, H. T. (2021). Effect of breeding heavier Romney ewe lambs at seven months of age on lamb production and efficiency over their first three breeding seasons. *Animals*, 11(12), 3486. DOI:https://doi.org/10.3390/ani11123486

Haslin, E., Corner-Thomas, R. A., Kenyon, P. R., Pettigrew, E. J., Hickson, R. E., Morris, S. T., & Blair, H. T. (2022). Effects of heavier live weight of ewe lambs at mating on fertility, lambing percentage, subsequent live weight and the performance of their progeny. *New Zealand Journal of Agricultural Research*, 65(2–3), 114–128. DOI:https://doi.org/10.1080/00288233.2020.1840399

Haslin, E., Pettigrew, E. J., Hickson, R. E., Kenyon, P. R., Gedye, K. R., Lopez-Villalobos, N., Jayawardana, J. M. D. R., Morris, S. T., & Blair, H. T. (2023). Genome-wide association studies of live weight at first breeding at eight months of age and pregnancy status of ewe lambs. *Genes*, 14(4), 805. DOI:https://doi.org/10.3390/genes14040805

Herath, H. M. G. P., Pain, S. J., Kenyon, P. R., Blair, H. T., & Morel, P. C. H. (2021). Effect of dietary protein to energy ratio on growth performance of pre- and post-weaned lambs. *Animal Feed Science and Technology*, 272, 114787. DOI:https://doi.org/10.1016/j.anifeedsci.2020.114787

Herdis, H., Inounu, I., Santoso, S., Anwar, R. I., Hayanti, S. Y., Hudaya, M. F., Mahari, D. A., Lupitasari, F. B. I., Hafid, A., & da Costa, M. A. (2025). Reproductive integration of leptin and kisspeptin in small ruminants: Mechanisms, biomarker potential, and prospects for precision breeding. *Veterinary World*, 18(6), 1614–1633. DOI:https://doi.org/10.14202/vetworld.2025.1614-1633

Jiao, N., Feng, W., Ma, C., Li, H., Zhang, J., Zheng, J., & Guo, P. (2025). Effects of dietary protein levels on digestion, metabolism, serum biochemical indexes, and rumen microflora of Lanzhou fat-tailed sheep. *Animals*, 15(1), 25. DOI:https://doi.org/10.3390/ani15010025

Kenyon, P. R., & Corner-Thomas, R. A. (2022). Breeding ewe lambs: An Australasian perspective. *Animals*, 12(22), 3207. DOI:https://doi.org/10.3390/ani12223207

Mahgoub, O., Osman, N. E. H. I. E., & Lu, C. D. (2025). Nutrient utilization and requirements in sheep and goats raised under different systems and fed low nutritional novel feeds for meat production. *Animals*, 15(18), 2658. DOI:https://doi.org/10.3390/ani15182658

Martin, G. B. (2022). Frontiers in sheep reproduction—Making use of natural responses to environmental challenges to manage productivity. *Animal Reproduction*, 19(4), e20220088.

DOI:https://doi.org/10.1590/1984-3143-AR2022-0088

Nageye, F. I., & Koyuncu, M. (2024). Effect of age, live weight and body condition score on fertility in estrous synchronization of Kıvırcık sheep. *Journal of Agricultural Faculty of Bursa Uludag University*, 38(1), 67–87. DOI:https://doi.org/10.20479/bursauludagziraat.1418005

National Research Council. (2007). *Nutrient requirements of small ruminants: Sheep, goats, cervids, and New World camelids*. National Academies Press. DOI:https://doi.org/10.17226/11654

Neimaur, K., Perdigón, F., Negrín, F., Cal-Pereyra, L., Capucho, M. C., Minteguiaga, M., & Kremer, R. (2024). Characterizing differences in the time to onset of puberty and reproductive performance between East Friesian and Corriedale ewe lambs. *Revista Brasileira de Zootecnia*, 53, e20230119. DOI:https://doi.org/10.37496/rbz5320230119

Orman, A., Çalışkan, G. Ü., Temizel, E. M., Gençoğlu, H., Kara, Ç., & Ünal, C. (2018). The usefulness of leptin measurements and ultrasound fat thickness for assessment of body fat reserves of Awassi lambs. *Italian Journal of Animal Science*, 17(3), 706–713. DOI:https://doi.org/10.1080/1828051X.2018.1426393

Pedawy, A. J. (2025). Effects of hydroponic barley and concentrate diets on leptin, ghrelin, prolactin, and serum biochemical parameters related to lipid and energy metabolism in lactating Karadi ewe. *Tropical Animal Health and Production*, 57(8), 468. DOI:https://doi.org/10.1007/s11250-025-04714-y

Rosales-Nieto, C. A., Ferguson, M. B., Thompson, A. N., & Martin, G. B. (2018). A new perspective on managing the onset of puberty and early reproduction in ewe lambs. *Animal Production Science*, 58(11), 1967–1975. DOI:https://doi.org/10.1071/AN17787

Rosales-Nieto, C. A., Thompson, A. N., Cuevas-Reyes, V., Hernández-Arteaga, L. E. S., Greeff, J. C., Ehrhardt, R., Veiga-Lopez, A., & Martin, G. B. (2024). Utilising male stimulus to improve the reproductive efficiency of 8-month-old nulliparous ewes and adult parous ewes. *Theriogenology*, 217, 143–150. DOI:https://doi.org/10.1016/j.theriogenology.2024.01.023

Russel, A. J. F., Doney, J. M., & Gunn, R. G. (1969). Subjective assessment of body fat in live sheep. *The Journal of Agricultural Science*, 72(3), 451–454. DOI:https://doi.org/10.1017/S0021859600024874

Souri, M., Moghaddam, A. A., & Panah, M. (2024). The impact of enhanced early postnatal nutrition on the histomorphological features of the ovaries in fat-tailed Sanjabi ewe lambs. *Tropical Animal Health and Production*, 56, 385. DOI:https://doi.org/10.1007/s11250-024-04218-1

Thompson, A. N., Bowen, E., Keiller, J., Pegler, D., Kearney, G., & Rosales-Nieto, C. A. (2021). The number of offspring weaned from ewe lambs is affected differently by liveweight and age at breeding. *Animals*, 11(9), 2733. DOI:https://doi.org/10.3390/ani11092733

Thompson, A. N., Ferguson, M. B., Kearney, G. A., Kennedy, A. J., Kubeil, L. J., Macleay, C. A., Rosales-Nieto, C. A., Paganoni, B. L., & Trompf, J. P. (2024). Additive impacts of liveweight and body condition score at breeding on the reproductive performance of Merino and non-Merino ewe lambs. *Animals*, 14(6), 867. DOI:https://doi.org/10.3390/ani14060867

Wang, X., Zhou, J., Lu, M., Zhao, S., Li, W., Quan, G., & Xue, B. (2024). Effects of dietary energy levels on growth performance, nutrient digestibility, rumen barrier and microflora in sheep. *Animals*, 14(17), 2525. DOI:https://doi.org/10.3390/ani14172525

Xie, Q., Kang, Y., Zhang, C., Xie, Y., Wang, C., Liu, J., Yu, C., Zhao, H., & Huang, D. (2022). The role of kisspeptin in the control of the hypothalamic-pituitary-gonadal axis and reproduction. *Frontiers in Endocrinology*, 13, 925206. DOI:https://doi.org/10.3389/fendo.2022.925206

Zhang, D., Yuan, C., Guo, T., Liu, J., & Lu, Z. (2023). Effects of different dietary energy levels on development, quality of carcass and meat, and fatty acid profile in male lambs. *Animals*, 13(18), 2870. DOI:https://doi.org/10.3390/ani13182870