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Effects of graviola oil extract on fattening performance and circadian rhythms of adipokine, cardiac and mitochondrial function markers in lambs

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Abstract

Background This study investigated the effects of dietary graviola (*Annona muricata* L.) oil extract (GOE) (supplemented at 200, 400, and 600 mg/kg) on the growth performance and circadian rhythm profiles of adipokine, cardiac, and mitochondrial function biomarkers in 48 male Anatolian Merino lambs (2.5 months old).

Methods Lambs were assigned to a control group (G0) or supplementation groups (G200, G400, G600 mg/kg) for a 60-day trial (70 days total). The study was conducted under a 12-h light/12-h dark cycle to ensure controlled environmental conditions. To analyze circadian profiles, blood samples were collected on days 0, 15, 30, 45, and 60 at four time points (07:00, 13:00, 19:00, and 01:00). Serum levels of Apelin, cTnI, BMAL1, MOTS-c, and BMCP1 were measured by ELISA. Data were analyzed using GLM Multivariate analysis ($p < 0.05$).

Results Key findings showed the G400 dose resulted in the most effective linear increase in live weight and live weight gain by day 60, and had a significant overall effect on live weight ($p < 0.05$). The G200 dose significantly improved the feed conversion ratio ($p < 0.05$), while feed consumption was unaffected ($p > 0.05$). Regarding biomarkers on day 60, the G400 dose effectively modulated BMAL1, MOTS-c, and BMCP1 (peak at 19:00). The G600 dose yielded the best results for Apelin (peak at 19:00) and cTnI (peaks at 13:00 and 07:00).

Conclusions In conclusion, GOE at a dose of 400 mg/kg (G400) not only optimizes fattening performance in Anatolian Merino lambs but also supports metabolic homeostasis by modulating adipokine levels, cardiac function, and mitochondrial biogenesis processes in a manner consistent with the circadian rhythm. These findings demonstrate that the G400 dose is a safe and effective phyto-genic strategy for improving animal health and production efficiency.

Highlights

- 400 mg/kg GOE added to lamb diets has been shown to affect fattening performance through circadian changes in the levels of the circadian rhythm protein BMAL1, as well as apelin, cTnI, MOTS-c, and BMCP1.

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- This study demonstrates that GOE at a dose of G400 had the best effect on live weight gain in lamb fattening at day 60.
- Low BMAL1 levels in the non-GOE group and increased BMAL1 levels due to GOE use, suggest that the bioactive compounds in GOE regulate circadian rhythm by increasing BMAL1.
- MOTS-c and BMCP1 play critical roles in mitochondrial function and maintenance of mitochondrial health.

Keywords Circadian Rhythm, Apelin, Cardiac troponin I (cTnI), Brain and muscle ARNT-like protein 1 (BMAL1) and mitochondrial function, Mitochondrial-derived peptide (MOTS-c), Brain Mitochondrial Carrier Protein-1 (BMCP1), Graviola, Lamb

Introduction

Circadian rhythms are cycles consisting of the repetition of physiological, biochemical and behavioral rhythms in living things during the 24-hour movement of the Earth around its axis [1, 2]. The circadian system consists of the central clock in the brain known as the suprachiasmatic nucleus (SCN) and peripheral clocks. BMAL1 regulates the 24-hour rhythm of the body by forming the basis of the circadian clock through the cyclical feedback mechanism formed by CLOCK (Circadian locomotor output cycles kaput), cryptochrome (Cry) and Period (PER) genes and proteins [3] in organs such as the liver, pancreas, skeletal muscle and adipose tissue [4].

Circadian rhythms regulate many physiological processes, including energy metabolism, sleep-wake cycles, and body temperature [6]. They are vital for the growth, development, reproduction, and digestive systems of animals. Factors such as light cycle disruptions, inadequate lighting, stress, and disease can disrupt circadian rhythms, leading to growth retardation, decreased appetite and reproductive performance, behavioral disorders, and weakened immune systems [6]. Apelin is an adipokine secreted by adipose tissue that controls feeding behaviors by affecting melatonin secretion [7, 8]. Apelin receptors are widely distributed in the suprachiasmatic nucleus (SCN), the brain's main center for regulating circadian rhythms [9].

Cardiac troponin I (cTnI), a cardiac muscle-specific protein, is released into the bloodstream when cardiac muscle cells are damaged and is therefore used as one of the most sensitive and specific indicators of cardiac damage [10, 11]. Examining cTnI levels in lambs is critical to identify cardiac damage caused by factors such as nutritional disorders (conditions such as white muscle disease caused by vitamin E and selenium deficiency), systemic diseases (viral infections such as foot and mouth disease or metabolic disorders such as acute ruminal lactic acidosis), and environmental stress (stress factors such as inadequate lighting, high temperature, or strenuous exercise) [12]. Mitochondrial-Derived Peptide (MOTS-c) is a peptide produced by mitochondria that regulates

cellular energy metabolism and homeostasis [13, 14]. Since both circadian rhythm and MOTS-c have a critical effect on energy metabolism, an indirect relationship between them is thought to exist [15]. Brain Mitochondrial Carrier Protein-1 (BMCP1) is a protein that carries molecules that are vital for the maintenance of metabolic processes and energy production in the mitochondria of the brain and for the production of energy in the mitochondria of brain cells [13]. Since circadian rhythm regulates energy metabolism and mitochondrial functions [6, 16] disruptions in circadian rhythm are thought to negatively affect the energy metabolism of brain cells by affecting the activity of Brain Mitochondrial Carrier Protein-1 (BMCP1).

Medicinal and aromatic plants have a regulating effect on circadian rhythms through hormone release via the endocrine system [6]. Graviola, also known as *Annona muricata* L. and considered a miraculous food, is widely used as a reliable product in traditional complementary medicine due to its numerous biological effects, including antioxidant [17], antimicrobial [18], and anti-inflammatory [19, 20]. The Anatolian Merino is a sheep breed native to Türkiye, developed by crossing the Akkaraman and German Meat Merinos. It combines high meat and fleece yield with resistance to regional conditions. No study was found examining the effects of GOE added to lamb rations at different rates on fattening performance and circadian changes in adipokine (Apelin), cardiac (cTnI), BMAL1, and mitochondrial function (MOTS-c and BMCP1) responses. In this context, the aim of this study is to investigate the fattening performance of GOE added to Anatolian Merino lamb rations at different rates and the effects of adipokine (Apelin), cardiac (cTnI), BMAL1, and mitochondrial function (MOTS-c and BMCP1) responses on circadian changes.

Materials and methods

This study was carried out in a 500-head sheep farm operating within the borders of Alaca district of Çorum Province in the Eastern Black Sea region of Türkiye (40°10'36.3"N; 35°01'46.9"E).

Table 1 Ration Nutrient Content and Analysis (g/kg)

Content	Quantity
Crude Protein (%)	12.31
Crude Fiber (%)	5.63
Crude Fat (%)	3.60
Crude Ash (%)	7.02
Starch (%)	39.34
Moisture (%)	8.90
Energy (kcal)	2696

*Grained Yearling Sheep Fattening Feed contains barley, corn, cottonseed meal, molasses, marble dust, and vitamin-mineral premix

** Vitamin and mineral values per kilogram of the ration are as follows: Vitamin A 2,500.00 IU, vitamin D3 225.00 IU, iron 60.00 mg, iodine 0.80 mg, cobalt 0.30 mg, copper 3.00 mg, manganese 30.00 mg, zinc 50.00 mg, and selenium 0.30 mg

Material

The animal material of the study consists of 48 male Anatolian Merino lambs, approximately 2.5 months old, weaned, clinically healthy, raised under intensive conditions and whose records are regularly followed. The study lasted a total of 70 days, including a 10-day adaptation and a 60-day fattening trial, between March and May 2023. Lambs were treated with internal parasite medication at the beginning of adaptation, and they were vaccinated twice: before the trial and 15 days later [21]. In the study, animals were randomly selected and 4 groups were formed, each consisting of 1 control (G0) and 3 experimental groups (G 200, G 400 and G600), each consisting of 12 male lambs, so that the live weight averages of the groups were close to each other. GOE was added to the lamb fattening rations at 0 (control), 200, 400 and 600 mg/kg levels, respectively.

Lambs were housed at 25 ± 1 °C, with 50–60% humidity and a 12-h light/12-h dark cycle, with ad libitum access to feed and water. Feed consumption was calculated by weighing the remaining feed at 15-day intervals. All groups were housed and fed under the same conditions, with licking stones provided to meet their mineral and salt requirements. To assess circadian rhythm, apelin, cTnI, BMAL1, MOTS-c, and BMCP1 levels were determined by ELISA in blood serum samples taken from the jugular vein of lambs at specific hours (07:00, 13:00, 19:00, and 01:00) on days 0, 15, 30, 45, and 60 of the study. In order to determine the circadian rhythm of physiological parameters in lambs, pulse and respiratory rates were measured using a stethoscope (Kruuse Stethoscope/DENMARK) and rectal body temperature was measured using a digital thermometer with a sensitivity of 0.1 °C (Kruuse Veterinary Thermometer/DENMARK) on the same day and at the same time. To assess circadian physiological parameters, body temperature, pulse, and respiratory rate were measured on the same day and at the same time as the study. Pulse and respiratory rate were measured using

Table 2 Mean values of graviola oil extract content analyses (%)

Phytochemical Components	%
Acetogenins	25.69
Alkaloids	15.23
Flavonoids	13.08
Terpenoids	0.64
Phenols	1.37
Tannins	0.92
Reducing Sugars	0.26
Other Components	44.18

a stethoscope at the junction of the left foreleg with the body. Rectal temperature was determined using a digital thermometer with a sensitivity of 0.1 °C.

Feed material

The feed materials used in the study were supplied by a private company, and the analyses of the raw materials for dry matter, crude ash, crude protein, and crude fat were performed using the analysis methods reported in the AOAC (2016) while crude cellulose was performed according to Crampton and Maynard [22]. Neutral detergent fiber (NDF) and acid detergent fiber (ADF) analyses were performed according to Van Soest et al. and Goering and Van Soest, respectively [23]. The nutrient composition and chemical analyses of the lamb diets are presented in Table 1.

Graviola oil extract

Phytochemical analyses of graviola fruit oil extract were performed in a private laboratory using the HPLC-UV/FLD method, ASU 64 LFGB L 07.00.40, and the average values obtained are shown in Table 2 [24].

Method

Live weight gain calculation

Throughout the trial, live weight gains were measured by individual weighing at the beginning of fattening and then every 15 days before morning feeding. ADG (Average Daily Gain) was calculated by dividing the difference between two weighings, using a 50-gram weighbridge, by the number of days [21].

$$ADG, g = \frac{\text{Live weight at the end of period (kg)} - \text{Live weight at the beginning of period}}{\text{Number of days}}$$

Determining feed consumption

Each day, before morning feeding, the remaining feed in the feeders was weighed to calculate daily consumption. Since twelve animals in each group were fed together, the daily feed intake was divided by twelve to determine individual feed consumption [21].

Table 3 Changes in live weight values of the study groups (Mean $\pm$ SD)

Days	G0	G200	G400	G600	SEM
Start of Fattening (Day 0)	23,08 $\pm$ 1,15	23,64 $\pm$ 0,57	23,55 $\pm$ 0,42	23,27 $\pm$ 0,39	0,21
Day 15	27,10 $\pm$ 1,10	27,70 $\pm$ 0,53	27,70 $\pm$ 0,46	27,60 $\pm$ 0,36	0,11
Day 30	31,80 $\pm$ 1,10c	32,40 $\pm$ 0,58b	33,40 $\pm$ 0,40a	33,10 $\pm$ 0,36a	0,00
Day 45	37,70 $\pm$ 0,89d	38,40 $\pm$ 0,42c	40,10 $\pm$ 0,23a	39,60 $\pm$ 0,22b	0,00
End of Fattening (Day 60)	45,70 $\pm$ 0,66d	46,10 $\pm$ 0,35c	48,40 $\pm$ 0,20a	47,60 $\pm$ 0,24b	0,00

Table 4 Changes in feed consumption values of the study groups (Mean $\pm$ SD)

Days	G0	G200	G400	G600	SEM
Day 15	790 $\pm$ 15,39b	810 $\pm$ 21,17a	820 $\pm$ 17,75a	805 $\pm$ 10,48ab	0,02
Day 30	1007 $\pm$ 15,30	1017 $\pm$ 31,40	1026 $\pm$ 26,84	1034 $\pm$ 13,98	0,17
Day 45	1194 $\pm$ 22,03b	1182 $\pm$ 19,26b	1237 $\pm$ 15,81a	1218 $\pm$ 21,73a	0,00
Day 60	1403 $\pm$ 13,85b	1405 $\pm$ 15,88b	1434 $\pm$ 19,24a	1419 $\pm$ 18,50ab	0,01
Fattening Beginning - Fattening End	0,72 $\pm$ 0,02	0,71 $\pm$ 0,03	0,72 $\pm$ 0,03	0,71 $\pm$ 0,03	0,98

Feed conversion ratios

In this study, the feed conversion ratio was determined by calculating the amount of feed required for 1 kg of live weight gain, taking into account the 60-day average of the animals' daily feed consumption and live weight gain [21].

$$\text{Feed Conversion Ratio (FCR), g} = \frac{\text{Daily Feed Consumption, g}}{\text{Daily live weight gain, g}}$$

Pulse, respiratory rate and body temperature measurement

For the circadian evaluation of physiological parameters, body temperature, pulse, and respiratory rates were measured on days 0, 15, 30, 45, and 60 of the study at 07:00, 13:00, 19:00, 01:00, and 07:00. Pulse and respiratory rates were measured using a stethoscope (Kruuse Stethoscope/DENMARK) at the junction of the left foreleg with the body. Rectal temperature was determined using a digital thermometer (Kruuse Veterinary Thermometer/DENMARK) to a precision of 0.1.

Collection of serum samples

Serum was obtained by centrifuging 5 ml blood samples taken from the jugular vein of lambs for 10 min at 3000 rpm at +4 °C. The serum samples were transferred to sterile tubes and stored at -80 °C.

Data analysis

Study data were analyzed using Repeated Measures and One-Way ANOVA tests in SPSS 22. Duncan's

multiple comparison test was used to determine differences between groups, and statistical significance levels were accepted as $p < 0.05$ and $p < 0.01$.

$$Y_{ijk} = \mu + R_i + C_e + R_e(R \times C \times R)_{ej} + e_{ijk}$$

Where Y_{ijk} is an observation, μ is the overall mean, R_i is the ration effect, R_e is the day effect, C_e is the hour effect, $(R \times C \times R)_{ej}$ is the interaction effect, and e_{ijk} is the experimental error.

Results

Evaluation of live weight changes in the study groups: The average live weight changes of the lambs in the G0, G200, G400 and G600 groups on the 0th, 15th, 30th, 45th and 60th days are shown in Table 3.

^{a,b,c,d} Means within the same row with different superscripts differ significantly ($P < 0.05$). While there was no statistical difference in live weight between the groups at the beginning of fattening (day 0) and on the 15th day ($p > 0.05$), the live weight of the G400 group to which 400 mg/kg GOE was added from the 30th day onwards was significantly higher than the other groups ($p < 0.05$), and this situation continued on the 45th and 60th days (Table 3), ($p < 0.05$).

Evaluation of feed consumption rates of the study groups

The average feed consumption rates of the lambs in the G0, G200, G400 and G600 groups on the 0th, 15th, 30th, 45th and 60th days of the study are presented in Table 4.

^{a-b} Means within the same row with different superscripts differ significantly ($P < 0.05$). While no statistically

Table 6 Changes in feed conversion ratio values of the study groups (Mean $\pm$ SD)

Days	G0	G200	G400	G600	SEM
Day 15	2,58 $\pm$ 0,26a	2,62 $\pm$ 0,28a	2,53 $\pm$ 0,17a	2,25 $\pm$ 0,12b	0,02
Day 30	3,18 $\pm$ 0,16a	3,10 $\pm$ 0,30a	2,6 $\pm$ 0,13b	2,67 $\pm$ 0,13b	0,00
Day 45	2,6 $\pm$ 0,14a	2,61 $\pm$ 0,15a	2,45 $\pm$ 0,07b	2,44 $\pm$ 0,11b	0,02
Day 60	2,35 $\pm$ 0,08	2,34 $\pm$ 0,05	2,28 $\pm$ 0,10	2,37 $\pm$ 0,08	0,23
Fattening Beginning - Fattening End	2,13 $\pm$ 0,20	2,15 $\pm$ 0,08	1,98 $\pm$ 0,19	2,10 $\pm$ 0,17	0,25

Table 5 Changes in live weight gain values of the study groups (Mean $\pm$ SD)

Days	G0	G200	G400	G600	SEM
Day 15	0,31 $\pm$ 0,03b	0,31 $\pm$ 0,03b	0,33 $\pm$ 0,02b	0,36 $\pm$ 0,02a	0,00
Day 30	0,33 $\pm$ 0,03b	0,33 $\pm$ 0,02b	0,40 $\pm$ 0,02a	0,39 $\pm$ 0,02a	0,00
Day 45	0,46 $\pm$ 0,02b	0,46 $\pm$ 0,02b	0,50 $\pm$ 0,01a	0,50 $\pm$ 0,02a	0,00
Day 60	0,60 $\pm$ 0,02b	0,60 $\pm$ 0,01b	0,63 $\pm$ 0,02a	0,60 $\pm$ 0,01b	0,00
Fattening Beginning - Fattening End	0,34 $\pm$ 0,03b	0,33 $\pm$ 0,01b	0,36 $\pm$ 0,02a	0,34 $\pm$ 0,02b	0,04

significant difference was found between the groups in terms of feed consumption on the 30th day of the study ($p > 0.05$), the feed consumption of the G200 and G400 groups on the 15th day and only the G400 group on the 45th and 60th days showed a statistically significant increase compared to the control group (G0) (Table 4), ($p < 0.05$).

Evaluation of live weight gains of the study groups

The average live weight gain rates of the lambs in the G0, G200, G400 and G600 groups within the scope of the

study on the 0th, 15th, 30th, 45th and 60th days are presented in Table 5.

^{a,b} Means within the same row with different superscripts differ significantly ($P < 0.05$). Live weight gain was higher in the G400 and G600 groups on the 15th, 30th and 45th days of the study ($p < 0.05$), and only the G400 group on the 60th day ($p < 0.05$) showed a higher average live weight gain than the G0 and G200 groups, while there was no statistically significant difference between the G0 and G200 groups (Table 5), ($p > 0.05$).

Table 7 Changes in mean serum apelin (ng/ml) values of lambs in the study groups at different days and times of blood collection periods (Mean $\pm$ SD)

DAYS	HOURS	G0	G200	G400	G600	Source of variation (P values)	
Day 0	07:00	0,711 L	0,737k	0,77hj	0,802 fg	Days	0
	13:00	0,798fgh	0,815ef	0,846d	0,88c	Hours	0
	19:00	0,842de	0,885c	0,927b	0,957a	G	0
	01:00	0,751jk	0,778ghj	0,812f	0,826def	Days*Hours	0
	07:00	0,753jk	0,778ghj	0,805 fg	0,825def	Days* G	0
Day 15	07:00	0,737 lm	0,772jk	0,805gh	0,845def	Hours* G	0
	13:00	0,828 fg	0,862cde	0,879c	0,932b	Days*Hours* G	0
	19:00	0,874 cd	0,921b	0,946b	1,007a	Main effect means diet	
	01:00	0,681n	0,713 m	0,752kl	0,777hjk	G0	0,872d
	07:00	0,782hjk	0,795hj	0,841ef	0,884c	G200	0,907c
Day 30	07:00	0,811 m	0,85kl	0,893gj	0,928ef	G400	0,946b
	13:00	0,873jkl	0,9fgj	0,931ef	0,963d	G600	1,003a
	19:00	0,906fgj	0,941de	0,962d	1,022c	SEM	0,002
	01:00	0,779 m	0,799 m	0,853kl	1,057b	Hours	
	07:00	0,842 L	0,882gjk	0,913efg	1,13a	07:00	0,873d
Day 45	07:00	0,868 lm	0,916j	0,96 h	0,982gh	13:00	0,981b
	13:00	1,015f	1,056e	1,091d	1,147c	19:00	1,057a
	19:00	1,133c	1,16c	1,19b	1,241a	01:00	0,829e
	01:00	0,814n	0,842mn	0,884kl	0,918j	07:00	0,921c
	07:00	0,903jk	0,952 h	1,01 fg	1,052e	SEM	0,002
Day 60	07:00	0,92 lm	0,967k	1,044j	1,134 g	Days	
	13:00	1,148 fg	1,177f	1,223e	1,263 cd	Day 0	0,815e
	19:00	1,237de	1,279c	1,334b	1,372a	Day 15	0,832d
	01:00	0,855n	0,868n	0,899 m	0,928 lm	Day 30	0,912c
	07:00	0,948kl	1,037j	1,087 h	1,207e	Day 45	1,007b
SEM	0,01					Day 60	1,096a
						SEM	0,002

^{a,b-n} Means within the same row with different superscripts differ significantly ($p < 0.05$). G: Graviola oil extract added to the ration; G0: Group without graviola oil extract; G200: Group with 200 mg/kg of graviola oil extract added to the lambs' ration; G400: Group with 400 mg/kg of graviola oil extract added to the lambs' ration; G600: Group with 600 mg/kg of graviola oil extract added to the lambs' ration; Each day and each team in the table were analyzed within themselves and evaluated at a significance level of $p < 0.05$

Evaluation of feed conversion ratio of the study groups

The average feed conversion ratios of the lambs in the G0, G200, G400 and G600 groups on the 0th, 15th, 30th, 45th and 60th days are given in Table 6.

^{a,b} Means within the same row with different superscripts differ significantly ($P < 0.05$). Changes in feed conversion ratio values were found to be statistically higher in the G0 and G200 groups than in the GOE supplemented groups (G400 and G600) on days 15, 30 and 45 of the study ($p < 0.05$). However, no statistically significant difference was found between these ratios on day 60 and during the total fattening period (Table 6), ($p > 0.05$).

Evaluation of serum apelin levels of the study groups

The average serum apelin levels of the lambs in the G0, G200, G400 and G600 groups within the scope of the study on the 0th, 15th, 30th, 45th and 60th days and at 07:00; 13:00; 19:00; 01:00; 07:00 are given in Table 7.

According to the research results, Day, Hours, G, Day*Hours, Day*G, Hours*G and Day*Hours*G interactions

were found to be statistically significant ($p < 0.05$). When Day*Group*Hours interactions were examined in the study, the highest values were determined at 19:00 in the G600 mg/kg dose groups on days 0, 15, 45 and 60 of the study and similarly in the groups at 07:00, the completion of the day period, on day 30. The lowest mean serum apelin values were determined in the lamb groups measured at 07:00 and 01:00, the beginning of the day period, on days 0, 15 and 60 in the G0 dose groups and at 01:00 on days 30, 45 and 60 ($p < 0.05$). The highest mean apelin levels of the lambs included in the study in terms of group, day and hour were determined in the G600 groups on the 60th day and at 19:00, while the lowest level was determined in the G0 groups on the 0th day and at 01:00 (Table 7) ($p < 0.05$).

Evaluation of serum cTnI levels of the study groups

The mean serum cTnI levels of the lambs in the G0, G200, G400 and G600 groups within the scope of the study on the 0th, 15th, 30th, 45th and 60th days and at 07:00; 13:00; 19:00; 01:00; 07:00 are given in Table 8.

Table 8 Changes in mean serum cTnI (ng/ml) values of lambs in the study groups at different days and hours of blood collection periods (Mean $\pm$ SD)

DAYS	HOURS	G0	G200	G400	G600	Source of variation (P values)	
Day 0	07:00	0,233	0,228	0,249	0,256	Days	0
	13:00	0,246	0,257	0,261	0,263	Hours	0
	19:00	0,24	0,283	0,288	0,266	G	0
	01:00	0,21	0,223	0,232	0,242	Days*Hours	0,029
	07:00	0,253	0,253	0,272	0,276	Days* G	0,129
Day 15	07:00	0,241	0,24	0,252	0,268	Hours* G	0,727
	13:00	0,26	0,246	0,27	0,282	Days*Hours* G	0,91
	19:00	0,254	0,233	0,263	0,247	Main effect means diet	
	01:00	0,223	0,212	0,231	0,215	G0	0,254d
	07:00	0,251	0,255	0,269	0,278	G200	0,262c
Day 30	07:00	0,25	0,264	0,269	0,276	G400	0,276b
	13:00	0,264	0,283	0,292	0,3	G600	0,283a
	19:00	0,242	0,251	0,261	0,291	SEM	0,002
	01:00	0,218	0,238	0,248	0,258	Hours	
	07:00	0,272	0,268	0,282	0,296	07:00	0,266b
Day 45	07:00	0,258	0,27	0,284	0,295	13:00	0,284a
	13:00	0,279	0,288	0,295	0,32	19:00	0,271b
	19:00	0,268	0,278	0,294	0,285	01:00	0,24c
	01:00	0,228	0,234	0,264	0,273	07:00	0,282a
	07:00	0,268	0,283	0,293	0,307	SEM	0,002
Day 60	07:00	0,278	0,288	0,296	0,321	Days	
	13:00	0,295	0,317	0,326	0,336	Day 0	0,251d
	19:00	0,288	0,284	0,31	0,299	Day 15	0,249d
	01:00	0,245	0,261	0,274	0,278	Day 30	0,266c
	07:00	0,293	0,312	0,315	0,338	Day 45	0,278b
SEM	0,009					Day 60	0,298a
						SEM	0,002

^{a,b,c,d} Means within the same row with different superscripts differ significantly ($p < 0.05$). G: Graviola oil extract added to the ration; GO: Group without graviola oil extract; G200: Group with 200 mg/kg of graviola oil extract added to the lambs' ration; G400: Group with 400 mg/kg of graviola oil extract added to the lambs' ration; G600: Group with 600 mg/kg of graviola oil extract added to the lambs' ration; Each day and each team in the table were analyzed within themselves and evaluated at a significance level of $p < 0.05$

According to the research results, Day, Hours, G interactions were found to be statistically significant ($p < 0.05$); while Day*G, Hours*G, Day*Hour, Day*Hour* G interactions were not found to be statistically significant (Table 8) ($p > 0.05$). When the interactions of the study day, hours and G dose groups were examined, no interaction was detected on the mean serum cTnI levels of the lambs in the G0, G200, G400 and G600 mg/kg dose groups on the 0th, 15th, 30th, 45th and 60th days and at 07:00; 13:00; 19:00; 01:00; 07:00 h ($p > 0.05$).

The highest level of mean serum cTnI levels of the lambs included in the study in terms of group, day and hour was determined in the G600 groups on the 60th day at 13:00 and at 07:00 in the morning at the end of the day period, while the lowest level was determined in the G0 groups on the 0th and 15th day at 01:00; the results were found to be statistically significant (Table 8), ($p < 0.05$).

Evaluation of serum BMAL1 levels of the study groups

The average serum BMAL1 levels of the lambs in the G0, G200, G400 and G600 groups within the scope of the

study on the 0th, 15th, 30th, 45th and 60th days and at 07:00; 13:00; 19:00; 01:00; 07:00 are given in Table 9.

According to the research results, Day, Hours, D, Day*G, Day*Hour, Hours*G interactions were found to be statistically significant ($p < 0.05$); while Day*Hour* G interactions were not found to be statistically significant (Table 9) ($p > 0.05$). When the interactions of the study day, hours and G dose groups were examined, no interaction was detected on the mean serum BMAL1 levels of the lambs in the G0, G200, G400 and G600 mg/kg dose groups on the 0th, 15th, 30th, 45th and 60th days and at 07:00; 13:00; 19:00; 01:00; 07:00 h (Table 9), ($p > 0.05$).

The highest level of mean serum BMAL1 levels of the lambs included in the study in terms of group, day and hour was determined in the G400 groups on the 60th day and at 13:00, while the lowest level was determined in the G0 groups on the 15th day and at 07:00 in the morning at the beginning of the day period. The results were found to be statistically significant (Table 9), ($p < 0.05$).

Table 9 Changes in mean serum BMAL1 (ng/ml) values of lambs in the study groups at different days and hours of blood collection periods (Mean $\pm$ SD)

DAYS	HOURS	G0	G200	G400	G600	Source of variation (P values)	
Day 0	07:00	0,9	1,217	1,8	1,4	Days	0
	13:00	1,408	1,475	2,083	1,7	Hours	0
	19:00	1,117	1,383	1,933	1,583	G	0
	01:00	0,683	1,133	1,342	1,217	Days*Hours	0
	07:00	1,292	1,383	1,808	1,692	Days* G	0
Day 15	07:00	1,192	1,292	1,883	1,475	Hours* G	0
	13:00	1,533	1,575	2,108	1,817	Days*Hours* G	0,42
	19:00	1,092	1,2	1,467	1,4	Main effect means diet	
	01:00	0,792	0,917	1,2	1,108	G0	1,523d
	07:00	1,308	1,4	1,992	1,733	G200	1,704c
Day 30	07:00	1,483	1,592	1,967	1,758	G400	2,153a
	13:00	1,8	1,883	2,317	2,1	G600	1,912b
	19:00	1,208	1,517	1,883	1,708	SEM	0,013
	01:00	1,092	1,183	1,492	1,292	Hours	
	07:00	1,717	1,825	2,192	1,933	07:00	1,785d
Day 45	07:00	1,758	1,908	2,383	2,117	13:00	2,182a
	13:00	2,2	2,517	2,9	2,592	19:00	1,83c
	19:00	1,592	2,083	2,492	2,283	01:00	1,351e
	01:00	1,217	1,6	1,775	1,683	07:00	1,966b
	07:00	1,883	2,1	2,492	2,283	SEM	0,015
Day 60	07:00	2,125	2,192	2,8	2,458	Days	
	13:00	2,483	2,675	3,358	3,108	Day 0	1,427d
	19:00	2,308	2,458	3,075	2,817	Day 15	1,424d
	01:00	1,608	1,717	2,1	1,867	Day 30	1,697c
	07:00	2,275	2,367	2,975	2,675	Day 45	2,093b
SEM		0,065				Day 60	2,472a
						SEM	0,015

* G: Graviola oil extract added to the ration; G0: Group without graviola oil extract; G200: Group with 200 mg/kg of graviola oil extract added to the lambs' ration; G400: Group with 400 mg/kg of graviola oil extract added to the lambs' ration; G600: Group with 600 mg/kg of graviola oil extract added to the lambs' ration; Each day and each team in the table were analyzed within themselves and evaluated at a significance level of $p < 0.05$

Evaluation of serum MOTS-C levels of the study groups

According to the study, the interactions of Day, Hours, D, Day*G and Hours*G on the mean serum MOTS-c levels of lambs were found to be statistically significant ($p < 0.05$), while the interactions of Day*Hours and Day*Hours*G were not found to be significant ($p > 0.05$). In addition, the highest MOTS-c level was measured at 19:00 on day 60 in the G400 group, and the lowest level was measured at 07:00 on day 0 in the G0 group (Table 10), ($p < 0.05$).

Evaluation of serum BMCP1 levels of the study groups

While Day, Hours, D, Day*G and Hours*G interactions on the mean serum BMCP1 levels of lambs were found to be statistically significant ($p < 0.05$), Day*Hours and Day*Hours*G interactions were not found to be significant (Table 11), ($p > 0.05$).

The highest mean serum BMCP1 levels of the lambs included in the study were determined in terms of group, day and hour in the G400 groups on the 60th day and at

19:00, while the lowest levels were determined in the G0 groups on the 0th day and at 07:00 in the morning at the beginning of the day period. The results were found to be statistically significant (Table 11) ($p < 0.05$).

Evaluation of pulse data of the study groups

While Day, Hours, Day, and various combinations of these had statistically significant effects on the average pulse data of the lambs ($p < 0.05$), the Hours*Day interaction was not found to be significant ($p > 0.05$). It was determined that pulse values generally reached their highest levels at noon (13:00), with the highest pulse data measured at 13:00 on day 0 in the G600 group, and the lowest pulse data measured at 01:00 on day 60 in the G0 group (Table 12), ($p < 0.05$).

Evaluation of respiratory rate data for the study groups

While Day, Hours, G, and various combinations of these had statistically significant effects on the mean respiratory rate of lambs ($p < 0.05$), the Hours*G interaction was

Table 10 Changes in mean serum MOTS-c (ng/ml) values of lambs in the study groups at different days and hours of blood collection periods (Mean $\pm$ SD)

DAYS	HOURS	G0	G200	G400	G600	Source of variation (P values)	
Day 0	07:00	0,683	0,733	0,811	0,749	Days	0
	13:00	0,709	0,746	0,865	0,805	Hours	0
	19:00	0,732	0,777	0,919	0,837	G	0
	01:00	0,651	0,683	0,788	0,718	Days*Hours	0,014
	07:00	0,7	0,748	0,845	0,772	Days* G	0,001
Day 15	07:00	0,702	0,719	0,782	0,73	Hours* G	0,004
	13:00	0,724	0,747	0,861	0,807	Days*Hours* G	0,174
	19:00	0,746	0,776	0,898	0,843	Main effect means diet	
	01:00	0,683	0,705	0,827	0,775	G0	0,735d
	07:00	0,721	0,745	0,848	0,805	G200	0,763c
Day 30	07:00	0,721	0,743	0,848	0,798	G400	0,872a
	13:00	0,748	0,783	0,889	0,827	G600	0,809b
	19:00	0,773	0,807	0,933	0,862	SEM	0,002
	01:00	0,711	0,743	0,816	0,753	HOURS	
	07:00	0,735	0,757	0,868	0,818	07:00	0,770d
Day 45	07:00	0,725	0,754	0,879	0,825	13:00	0,810b
	13:00	0,765	0,787	0,916	0,848	19:00	0,842a
	19:00	0,791	0,816	0,973	0,883	01:00	0,755e
	01:00	0,733	0,748	0,85	0,786	07:00	0,798c
	07:00	0,746	0,784	0,908	0,85	SEM	0,002
Day 60	07:00	0,749	0,774	0,864	0,805	Days	
	13:00	0,794	0,811	0,914	0,849	Day 0	0,763d
	19:00	0,828	0,853	0,941	0,856	Day 15	0,772c
	01:00	0,724	0,754	0,848	0,794	Day 30	0,797b
	07:00	0,774	0,789	0,903	0,837	Day 45	0,818a
SEM	0,01					Day 60	0,823a
						SEM	0,002

^{a,b,c,d} Means within the same row with different superscripts differ significantly ($p < 0.05$). G: Graviola oil extract added to the ration; G0: Group without graviola oil extract; G200: Group with 200 mg/kg of graviola oil extract added to the lambs' ration; G400: Group with 400 mg/kg of graviola oil extract added to the lambs' ration; G600: Group with 600 mg/kg of graviola oil extract added to the lambs' ration; Each day and each team in the table were analyzed within themselves and evaluated at a significance level of $p < 0.05$

Table 11 Changes in mean serum BMCP1 (ng/ml) values of lambs in the study groups at different days and hours of blood collection (Mean $\pm$ SD)

DAYS	HOURS	G0	G200	G400	G600	Source of variation (P values)	
Day 0	07:00	0,54	0,602	0,712	0,638	Days	0
	13:00	0,608	0,659	0,764	0,691	Hours	0
	19:00	0,65	0,705	0,787	0,769	G	0
	01:00	0,515	0,543	0,649	0,595	Days*Hours	0,023
	07:00	0,583	0,627	0,737	0,687	Days* G	0
Day 15	07:00	0,592	0,636	0,727	0,681	Hours* G	0
	13:00	0,639	0,688	0,768	0,713	Days*Hours* G	0,214
	19:00	0,673	0,737	0,817	0,815	Main effect means diet	
	01:00	0,56	0,592	0,681	0,663	G0	0,64d
	07:00	0,641	0,672	0,752	0,71	G200	0,684c
Day 30	07:00	0,617	0,667	0,768	0,708	G400	0,771a
	13:00	0,66	0,729	0,809	0,752	G600	0,729b
	19:00	0,711	0,767	0,862	0,843	SEM	0,002
	01:00	0,609	0,662	0,712	0,694	Hours	
	07:00	0,647	0,72	0,788	0,752	07:00	0,676d
Day 45	07:00	0,643	0,669	0,77	0,711	13:00	0,725b
	13:00	0,694	0,725	0,808	0,749	19:00	0,778a
	19:00	0,717	0,768	0,856	0,842	01:00	0,643e
	01:00	0,587	0,657	0,712	0,692	07:00	0,708c
	07:00	0,666	0,715	0,793	0,725	SEM	0,002
Day 60	07:00	0,66	0,689	0,768	0,727	Days	
	13:00	0,714	0,732	0,818	0,773	Day 0	0,653d
	19:00	0,738	0,765	0,891	0,842	Day 15	0,688c
	01:00	0,641	0,659	0,731	0,713	Day 30	0,724b
	07:00	0,686	0,724	0,787	0,752	Day 45	0,725b
SEM	0,009					Day 60	0,74a
						SEM	0,002

*a,b,c,d Means within the same row with different superscripts differ significantly ($p < 0.05$). G: Graviola oil extract added to the ration; G0: Group without graviola oil extract; G200: Group with 200 mg/kg of graviola oil extract added to the lambs' ration; G400: Group with 400 mg/kg of graviola oil extract added to the lambs' ration; G600: Group with 600 mg/kg of graviola oil extract added to the lambs' ration; Each day and each team in the table were analyzed within themselves and evaluated at a significance level of $p < 0.05$

not found to be significant ($p > 0.05$). The highest respiratory rate was generally measured at 1:00 PM, with the highest value being found at 1:00 PM on day 0 in the G600 group, and the lowest at 1:00 AM on day 60 in the G0 group (Table 13), ($p < 0.05$).

Evaluation of body temperature data of the study groups

Day, Hours, G, and all their two-way and three-way interactions on the average body temperature data of lambs were found to be statistically significant ($p < 0.05$). The highest body temperature values were measured at 13:00 on day 60 in the G600 group, while the lowest values were determined at 07:00 on day 30 in the G0 group (Table 14), ($p < 0.05$).

Discussion

Circadian rhythm is a system consisting of approximately 24-hour biological cycles, effective at every stage of life, and vital for the healthy growth and development of animals [25]. The circadian system plays a key role in regulating physiological and behavioral systems such as

sleep-wake cycles, body temperature, energy metabolism, and locomotor activity. It also influences the gastrointestinal tract, cardiovascular activity, endocrine system, and renal activity [26–29].

Apelin, an adipokine secreted from adipose tissue, is a ligand for the G-protein-coupled receptor. Apelin plays a role in the control and regulation of feeding, as well as endocrine, immune, cardiovascular, and neuroendocrine functions [30]. Although the literature review on our research topic was limited, the results obtained in our study are consistent with other existing studies [30–33]. This may be due to increased lipid mobilization to meet the energy requirements for body weight gain and to fluctuating levels of energy storage and utilization during the evening and night. The increase in serum apelin levels during the G400 dose administration period, measured at 7:00 PM on day 60 of our current study, is thought to be due to increased fatty acid oxidation due to increased insulin levels in the evening, stimulating apelin release to maintain glucose metabolism and energy regulation. Furthermore, we hypothesize that GOE, through

Table 12 Changes in pulse values of lambs in the study groups (Mean $\pm$ SD)

DAYS	HOURS	G0	G200	G400	G600	Source of variation (P values)	
Day 0	07:00	82,5 h	89,75ef	82,833 h	82,083 h	Days	0
	13:00	95ab	95,417ab	95,917a	95,667ab	Hours	0
	19:00	92,667 cd	91,25de	93,583bc	94,25bc	G	0
	01:00	75,5k	76,083k	74,5k	78,667j	Days*Hours	0
	07:00	87,583 g	88 fg	89,25efg	88,75 fg	Days* G	0
Day 15	07:00	86,833 fg	84,5jk	87,75ef	88,833e	Hours* G	0,353
	13:00	91,833 cd	94,417a	93,833ab	92,833abc	Days*Hours* G	0
	19:00	91,083d	92,5bcd	92,333bcd	93,25abc	Main effect means diet	
	01:00	73,583 m	75,167 L	76,667 L	76,25 L	G0	85,023d
	07:00	85,417gjk	84,25k	86,167fgj	85,917gjk	G200	85,83c
Day 30	07:00	87,333f	86,583 fg	89,25e	87,917f	G400	86,893b
	13:00	92,917 cd	93,583c	96,417a	95,333ab	G600	87,483a
	19:00	92d	90,417e	94,917b	95,083ab	SEM	0,107
	01:00	72,833n	73,083n	77,583 L	75,833 m	Hours	
	07:00	84,083k	84,333jk	84,917jk	85,667gj	07:00	86,117c
Day 45	07:00	86,25 fg	83,25kl	86,667f	88,833e	13:00	94,042a
	13:00	92,583c	92,917bc	94,083b	95,75a	19:00	92,267b
	19:00	90,5d	93,583bc	92,417c	92,667c	01:00	73,983e
	01:00	71,167p	69,667r	73,917 m	72,5n	07:00	85,129d
	07:00	82,25 L	84,083hk	85,083gh	86,417f	SEM	0,119
Day 60	07:00	81,75j	85,417f	84,333 fg	89,667de	Days	
	13:00	90,667 cd	91,583c	93,667b	96,417a	Day 0	87,462a
	19:00	88,917e	91,75c	90,75 cd	91,417c	Day 15	86,671c
	01:00	70,583 m	71,583 lm	71,833 lm	72,667 L	Day 30	87,004b
	07:00	79,75k	82,583hj	83,667gh	84,417 fg	Day 45	85,729d
SEM	0,533					Day 60	84,671e
						SEM	0,119

^{a-n} Means within the same row with different superscripts differ significantly ($p < 0.05$). G: Graviola oil extract added to the ration; G0: Group without graviola oil extract; G200: Group with 200 mg/kg of graviola oil extract added to the lambs' ration; G400: Group with 400 mg/kg of graviola oil extract added to the lambs' ration; G600: Group with 600 mg/kg of graviola oil extract added to the lambs' ration; Each day and each team in the table were analyzed within themselves and evaluated at a significance level of $p < 0.05$

its antioxidant, hepatic glucose and lipid metabolism, and protective role in pancreatic β -cells, as reported in the literature, influences the circadian rhythm in serum apelin release.

Cardiac troponin (cTnI) is a cardiospecific protein that plays a role in myocardial contraction by controlling actin-myosin interactions via calcium. It is released into the plasma after cardiac injury and is used in the assessment of myocardial damage and disease [11, 34, 35]. Inadequate nutrition or electrolyte imbalance (e.g., Na, K, Ca) in animals can lead to cardiac problems [36]. Therefore, it is important to assess cardiac troponin (cTnI) levels in lamb fattening practices to prevent cardiac problems and monitor animal health. Although research on the cardioprotective effects of different doses of GOE and serum cTnI levels in fattening lambs is limited, the results of our current study are consistent with reference values in the literature [37, 38]. Although cTnI concentrations increased slightly in fattened lambs due to growth and muscle gain, it was concluded that there was no significant cardiac damage because these values were within the reference range.

The circadian clock coordinates internal behavioral and physiological rhythms by acting at the genomic level through the CLOCK-BMAL1 transcriptional heterodimer [39]. BMAL1 serves as a core pacemaker gene, crucial for maintaining metabolic homeostasis, growth, and skeletal development [40]. In our study, the significantly lower serum BMAL1 levels observed in the control (G0) group suggest a potential 'circadian blunting' or a weakened molecular oscillation in the absence of phytochemical support. Conversely, the G400 dose significantly elevated BMAL1 levels at 1:00 PM, suggesting that the bioactive compounds in Graviola (such as acetogenins and flavonoids) may act as exogenous zeitgebers (time-givers). We hypothesize that GOE facilitates the entrainment of peripheral clocks, enhancing the amplitude of BMAL1 expression. This molecular synchronization likely optimizes nutrient utilization and metabolic efficiency, directly correlating with the improved live weight gain observed in the G400 group. These findings are consistent with recent evidence highlighting the role of natural polyphenols in reinforcing circadian amplitude [41, 42].

Table 13 Changes in Respiratory Rate of Lambs in the Study Groups (Mean $\pm$ SD)

DAYS	HOURS	G0	G200	G400	G600	Source of variation (P values)	
Day 0	07:00	67,75def	68,583de	69,083 cd	70,667c	Days	0
	13:00	94,167a	94,583a	96,167a	96,083a	Hours	0
	19:00	83,083b	83,5b	83,417b	83,167b	G	0
	01:00	64,417 g	65,5 fg	66,083 fg	66,167 fg	Days*Hours	0
	07:00	66,833ef	67,083def	66,917def	66,75ef	Days* G	0
Day 15	07:00	69,167de	69,917d	68,583def	69,417d	Hours* G	0,023
	13:00	91,25ab	91,167ab	89,5b	92,583a	Days*Hours* G	0
	19:00	81,583c	82,333c	83,083c	82,833c	Main effect means diet	
	01:00	64,167k	65,083jk	65,917gjk	68,583def	G0	72,873d
	07:00	66,083gjk	65,167jk	67,333efg	66,583fgj	G200	73,597c
Day 30	07:00	65,5kl	68,25gj	70,083 g	69,667 g	G400	74,397b
	13:00	88,917bc	88,167c	91,417a	90,75ab	G600	75,46a
	19:00	80,083ef	79,75f	82,583d	81,917de	SEM	0,136
	01:00	63,333 m	64,583klm	64,333 lm	69,75 g	Hours	
	07:00	65,417kl	65klm	66,25kl	66,667jk	07:00	68,621c
Day 45	07:00	65,833gh	67,25 fg	70,417de	71,083d	13:00	89,433a
	13:00	86,417a	87,75a	88,083a	88,25a	19:00	80,996b
	19:00	78,667c	78,417c	78,917c	82,5b	01:00	65,283e
	01:00	64,5 h	65,583gh	65,75gh	68,917ef	07:00	66,075d
	07:00	64,583 h	64,417 h	65,333gh	67,25 fg	SEM	0,152
Day 60	07:00	67,25f	67,417f	68,417f	68,083f	Days	
	13:00	81,583c	83,167abc	84,583a	84,083ab	Day 0	76,00a
	19:00	76,25e	77,083de	78,333d	82,417bc	Day 15	75,017b
	01:00	61,083 h	63,417 g	64,75 g	63,75 g	Day 30	74,121c
	07:00	63,917 g	66,75f	64,583 g	68,583f	Day 45	73,496d
SEM	0,678					Day 60	71,775e
						SEM	0,152

*a,b,c,d Means within the same row with different superscripts differ significantly ($p < 0.05$). G: Graviola oil extract added to the ration; G0: Group without graviola oil extract; G200: Group with 200 mg/kg of graviola oil extract added to the lambs' ration; G400: Group with 400 mg/kg of graviola oil extract added to the lambs' ration; G600: Group with 600 mg/kg of graviola oil extract added to the lambs' ration; Each day and each team in the table were analyzed within themselves and evaluated at a significance level of $p < 0.05$

Mitochondria are a unique and important metabolic organelle called the powerhouse of cells [13]. MOTS-c, a peptide consisting of 16 amino acids, has a therapeutic effect on metabolic syndrome by regulating cellular energy balance as well as muscle and fat physiology [43]. BMCP1 is an important protein that controls vital functions of mitochondria to maintain cell energy balance and health [13]. MOTS-c supports glucose utilization, insulin sensitivity, and metabolic balance by inhibiting the folate cycle and de novo purine biosynthesis, activating AMPK [44]. Serum MOTSc and BMCP1 levels obtained in our study are parallel to the results of similar studies in the literature [13]. We believe that the increase in live weight obtained by lamb fattening is due to the increase in mitochondrial functions to provide the energy required for metabolic and physiological processes. Taken together, the significant enhancement in weight gain observed in the G400 group is likely driven by the synergistic upregulation of mitochondrial peptides (MOTS-c and BMCP1) and the synchronization of the BMAL1-mediated circadian clock, which collectively optimize metabolic efficiency and energy expenditure.

Live weight gain and feed conversion ratio, used to evaluate lamb fattening performance, are important measures that indicate how much efficiency the animals obtain from the feed they consume. Chukwunonso Jiwuba et al. [45] reported that graviola should be included in the goat feeding program to improve live weight gain and health status of the animals. Our results regarding live weight gain and feed conversion ratio are consistent with the results of similar studies in the limited literature [45, 46]. It was determined that the best results of graviola supplementation on live weight gain at the end of day 60 were achieved at the G400 dose. This is thought to be due to the bioactive components in the oil extract promoting live weight gain by increasing appetite.

Circadian rhythm affects physiological parameters such as heart rate, respiratory rate, and body temperature. Pulse provides information about heart rate, rhythm, and regularity and is generally an important parameter in assessing health status [47]. Circadian rhythm causes heart rate to decrease in the morning and increase in the evening and at night, depending on the body's rest and energy expenditure cycle [48]. Respiratory rate is the

Table 14 Body temperature changes of lambs in the study groups (Mean $\pm$ SD)

Days	Hours	G0	G200	G400	G600	Source of variation (P values)	
Day 0	07:00	38,6jk	38,5kl	38,8gh	38,9 g	Days	0
	13:00	39,6d	39,9c	40,1b	40,3a	Hours	0
	19:00	39,1f	39,3e	39,4e	39,6d	G	0
	01:00	38,1p	38,2np	38,4 lm	38,6jk	Days*Hours	0
	07:00	38,3mn	38,5kl	38,7hj	38,8gh	Days* G	0
Day 15	07:00	38,3 lm	38,6gj	38,7 g	38,9f	Hours* G	0
	13:00	39,2e	39,7b	40a	40,1a	Days*Hours* G	0
	19:00	38,6gj	39,4d	39,6bc	39,5 cd	Main effect means diet	
	01:00	38,2 m	38,2 m	38,5jk	38,4kl	G0	38,784d
	07:00	38,6gj	38,4kl	38,6gj	38,9f	G200	38,9c
Day 30	07:00	38,5 fg	38,3 h	38,4gh	38,8e	G400	39,039b
	13:00	39,7b	39,7b	39,683b	39,9a	G600	39,116a
	19:00	39,3d	39,3d	39,3d	39,5c	SEM	0,011
	01:00	38,3 h	38,4gh	38,5 fg	38,5 fg	Hours	
	07:00	38,4gh	38,6f	38,6f	38,5 fg	07:00	38,615c
Day 45	07:00	38,4 g	38,7de	38,6ef	38,4 g	13:00	39,844a
	13:00	39,7b	39,7b	40,2a	39,8b	19:00	39,355b
	19:00	39,4c	39,3c	39,4c	39,4c	01:00	38,405e
	01:00	38,4 g	38,4 g	38,7de	38,8d	07:00	38,58d
	07:00	38,5 fg	38,7de	38,7de	38,6ef	SEM	0,012
Day 60	07:00	38,6gh	38,7 fg	38,9e	38,7 fg	Days	
	13:00	39,8b	39,8b	39,9b	40,1a	Day 0	38,985a
	19:00	39,3d	39,5c	39,3d	39,6c	Day 15	38,92b
	01:00	38,3kl	38,2 L	38,5hj	38,5hj	Day 30	38,909b
	07:00	38,4jk	38,5hj	38,5hj	38,8ef	Day 45	38,99a
SEM	0,055					Day 60	38,995a
						SEM	0,012

*a,b,c,d Means within the same row with different superscripts differ significantly ($p < 0.05$). G: Graviola oil extract added to the ration; G0: Group without graviola oil extract; G200: Group with 200 mg/kg of graviola oil extract added to the lambs' ration; G400: Group with 400 mg/kg of graviola oil extract added to the lambs' ration; G600: Group with 600 mg/kg of graviola oil extract added to the lambs' ration; Each day and each team in the table were analyzed within themselves and evaluated at a significance level of $p < 0.05$

number of breaths the body takes per minute to meet its oxygen needs and eliminate carbon dioxide [49]. Circadian rhythm increases respiratory rate during wakefulness and decreases it during sleep, according to metabolic needs [50]. Body temperature varies throughout the day depending on metabolism and energy expenditure; it is lowest in the morning and highest in the evening [51]. The data on pulse, respiratory rate, and body temperature obtained in our study are consistent with the results of similar studies in the literature [52–55]. We predict that graviola may indirectly affect circadian rhythm, pulse, respiratory rate, and body temperature through its antioxidant and anti-inflammatory effects.

A limitation of this study is its relatively short duration of 60 days, which restricts the evaluation of long-term effects of graviola oil extract (GOE) on wool quality, reproductive performance, and sustained circadian rhythm stability. While serum biomarkers like cTnI, Apelin, and mitochondrial peptides provide

significant physiological insights, they reflect protein-level responses. Future studies should incorporate gene expression analysis via RT-PCR (e.g., *BMAL1*, *CLOCK*, and *PER* mRNA levels) in tissues such as the liver, hypothalamus, and adipose tissue to further elucidate the molecular mechanisms underlying GOE's influence on the core molecular clock. Additionally, larger cohorts and different age groups or breeds are necessary to validate the generalizability of these preliminary findings.

Conclusion

The study concluded that 400 mg/kg GOE added to the lamb diet improved growth performance and overall health by influencing circadian changes in the levels of the circadian rhythm protein BMAL1, as well as apelin, cTnI, MOTS-c, and BMCP1. These parameters are expected to contribute to the development of feeding programs in the sheep sector and the assessment of lamb health status.

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Disclosure statement

No potential conflict of interest was reported by the author(s).

Authors' contributions

G.C.K. and B.B. designed the study. G.C.K. and B.B. collected data. G.C.K. analyzed the data. B.B. prepared the draft plan. All authors contributed to writing the manuscript. All authors read and approved the final manuscript.

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Data availability

The corresponding author upon reasonable request will provide data supporting the findings of this study.

Declarations

Ethics approval and consent to participate

The study was approved by the Etlik Veterinary Control Central Research Institute Animal Experiments Local Ethics Committee with the decision numbered 2022/27 dated 26.10.2022, and the study process was carried out in accordance with ethical principles and rules, protecting animal welfare and rights. Furthermore, written informed consent was obtained from all animal owners for the inclusion of their animals in this study, and official permission was received from the management of the sheep farm where the research was conducted, confirming the voluntary nature of participation.

Consent for publication

Not applicable.

Competing interests

The authors declare no competing interests.

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