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Comparison of pre- and postpartum serum leptin, ghrelin, and lipid levels in sheep

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Abstract: Serum leptin and ghrelin play important roles in energy metabolism and inducing appetite during pregnancy and lactation. We investigated the serum levels of leptin and ghrelin and their relationship with the duration of lactation and serum biochemical responses during the prepartum (last week before lambing) and postpartum periods in sheep. They were also evaluated between pregnant and nonpregnant (control ewes) ewes. For these purposes, pregnant (n = 15) and control Awassi ewes (n = 15) that were 2 years of age were used in this study. Blood samples were collected 1 week before the expected date of lambing (baseline) and for 12 weeks after parturitions. Serum leptin, ghrelin, cholesterol, triglyceride, VLDL, LDL, HDL, NEFA, and phospholipid levels were assayed. Leptin and ghrelin levels of the prepartum period in pregnant and control ewes were 4.5 ng/mL and 3.4 ng/mL (P < 0.05) and 75 pg/mL and 166 pg/mL (P < 0.01), respectively. The baseline value of ghrelin (81 pg/mL) decreased to 43 pg/mL within 1 week after parturition (P < 0.01). Serum ghrelin levels increased after 1 week. There was also a significant difference in serum ghrelin levels between control ewes and those a week before parturition in pregnant ewes (P < 0.001). The NEFA level was higher in pregnant than in control sheep (P < 0.001). In conclusion, leptin levels tended to decrease during the lactation period, which could be associated with a negative energy balance. Additionally, ghrelin levels tended to gradually increase during lactation to adapt to a negative energy balance. We think that variations in serum leptin and ghrelin might be used to monitor metabolic adaptation during lactation.

Key words: Leptin, ghrelin, parturition, energy balance, sheep

1. Introduction

Awassi sheep are primarily found in southeastern Turkey as well as the Middle East. Awassi sheep are a breed of dairy sheep bred for multiple lambing and high milk yield in Turkey (1). Pregnancy is the most important metabolic burden in ewes, which have multiple offspring (2,3). In this period, energy and protein intake usually do not cover the energy and protein requirements in many sheep herds (3,4).

Leptin, a product of the *ob* gene, is a protein mainly secreted by white adipose tissue (5). This hormone is responsible for the long-term regulation of energy balance and depressing appetite (6). Leptin plays an important role in energy metabolism during pregnancy and lactation (7,8). Many studies showed that plasma leptin level increased during pregnancy and declined during the transition to lactation (9–12). Carcangiu et al. (13) reported that body condition scores were positively correlated with serum leptin concentration but were negatively correlated with milk yield after delivery in ewes with production levels. Additionally, leptin was negatively correlated with growth hormone (GH) and nonesterified fatty acid (NEFA) in the lactation periods (13).

Otherwise, ghrelin mainly arises from the abomasum in ruminants. Ghrelin induces GH release and stimulates appetite in the brain (14–16). Ghrelin concentration increases prior to meals, but it is suppressed after feeding. Ghrelin also decreases fat oxidation and increases insulin concentration (17–19).

The objective of the present study was to evaluate fluctuations and possible interactions of the leptin, ghrelin, and lipid profile of ewes in the pre- and postparturient periods and control sheep.

2. Materials and methods

2.1. Animals and nutrition

The present study was carried out in the town of Nilüfer in Bursa Province, Turkey. Pregnant Awassi ewes (last week before lambing) that were (n = 15) or were not pregnant (control group) (n = 15), weighing 68.4 ± 10.4 kg (53.4–80.6 kg), and aged 2 years were used in the study. All ewes were selected from among those that were to have twin lambs. During this study period, all sheep were fed a controlled diet and water was provided ad libitum. All animals were housed under the same conditions. The herd consisted of a

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total of 150 Awassi sheep. In different compartments of the same pen, all animals were fed alfalfa and foraged wheat bran, bran, sunflower meal, and soybean meal, as well as a concentrated commercial mixture for lambs, which consisted of ground corn, barley, calcium carbonate, and vitamin premix. Blood sampling was performed under the control of the farm health coordinator in accordance with animal welfare guidelines. The study was approved by the Committee of the Animal Experiments of Uludağ University (1.06.2004/2).

2.2. Collection of samples

The study was carried out between February and May. Blood samples were collected from the jugular vein between 1130 and 1430 a week before the expected date of lambing parturition, at baseline, and at 0.5 (day 4 just after the parturition), 1, 2, 3, 4, 6, 8, and 12 weeks after parturitions. Serum was then extracted following centrifugation, frozen, and stored at -80°C until analyzed.

2.3. Biochemical analysis

Serum leptin and ghrelin levels were measured by radioimmunoassay (RIA) using a commercially available kit (Multispecies leptin RIA kit and active ghrelin RIA kit, Linco Research, St. Charles, MO, USA). Since active ghrelin is too unstable to be measured in stored samples (20) and the acidification of plasma prevents the rapid deacylation of ghrelin (21), 1 N hydrogen chloride was added to serum samples before freezing (22).

Serum total cholesterol, high-density lipoprotein cholesterol (HDL-C), very low-density lipoprotein cholesterol (VLDL-C), and triglyceride levels were measured by an automated clinical chemistry analyzer (Architect ci8200; Abbott GmbH Co KG, Wiesbaden, Germany) using commercially available assay kits (Abbott GmbH Co KG). Low-density lipoprotein cholesterol (LDL-C) concentrations were calculated using the formula:

$\text{LDL-C (in milligrams per deciliter)} = \text{total cholesterol} - (\text{HDL} - \text{C} + \text{triglyceride}/5)$. Serum nonesterified fatty acid (NEFA) concentration was measured using a commercially available enzymatic colorimetric assay kit (Wako Chemicals, Neuss, Germany). Serum phospholipids were measured by an enzymatic colorimetric method using a commercially available assay kit (Wako Chemicals) and were reported as choline-containing phospholipids (in milligrams per deciliter).

2.4. Statistical analysis

Data sets were analyzed by analysis of variance (ANOVA), and Tukey's test was chosen as the post hoc multiple comparison test. Bivariate correlation was applied and Pearson's correlation was chosen. Differences were considered significant at a probability level of $P < 0.05$, $P < 0.01$, and $P < 0.001$ in all analyses. All statistical analyses were performed with SigmaStat (SigmaStat GmbH, Erkrath, Germany).

3. Results

There were 7 singletons and 8 twins in the pregnant ewes. Leptin levels tended to decrease during lactation (Figure 1). Baseline values of ghrelin (81 pg/mL) decreased to 43 pg/mL within 1 week of parturition ($P < 0.01$). Serum ghrelin levels tended to increase after a 1-week period (Figure 1). Parturition leptin and ghrelin levels in pregnant and control ewes were 4.5 ng/mL and 3.4 ng/mL ($P < 0.05$) and 75 pg/mL and 166 pg/mL ($P < 0.001$), respectively (Figure 2; Table).

Repeated measurements of cholesterol levels surged during lactation. Cholesterol levels tended to decrease up to week 3 compared to the baseline; thereafter, they returned to a baseline value at week 12. Triglyceride levels were low up to week 12 compared to the baseline level. LDL, HDL, and VLDL levels had a surge during the

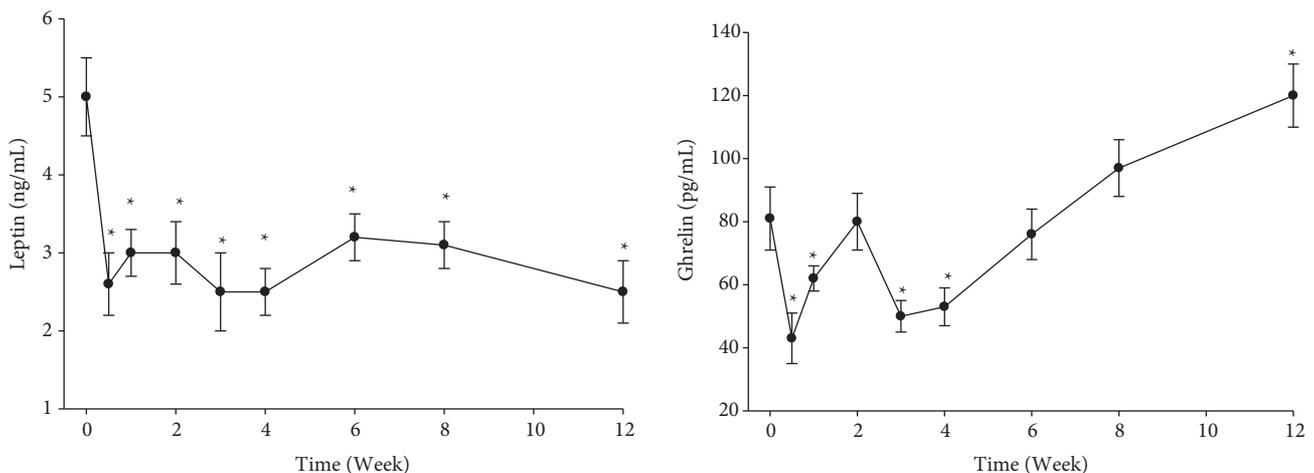


Figure 1. Serum leptin and ghrelin levels profile in baseline (birth) and postparturient period.

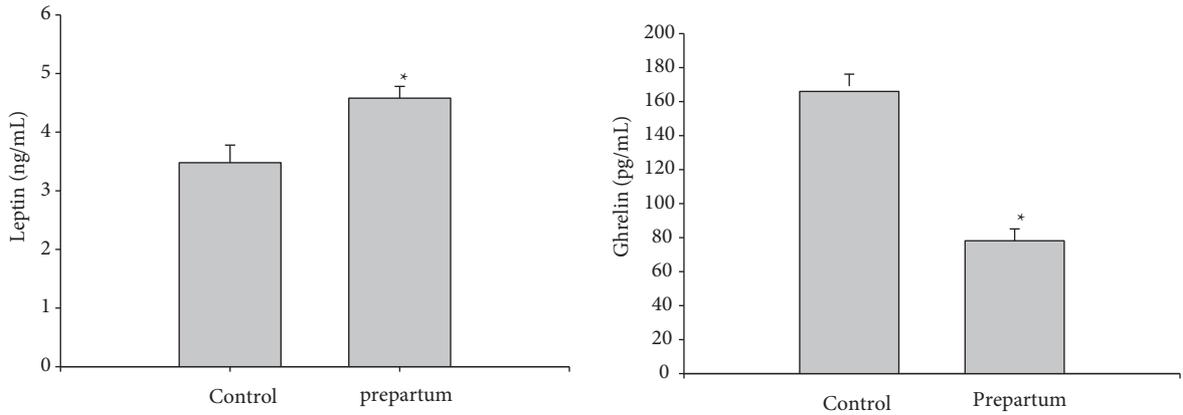


Figure 2. Serum leptin and ghrelin levels in control (nonpregnant ewes) and preparturient ewes.

Table. Serum leptin, ghrelin and lipid profile in preparturient, postparturient, and control ewes.

	Leptin (nmol/L)	Ghrelin (pmol/L)	Total cholesterol (mg/dL)	Triglyceride (mg/dL)	LDL (mg/dL)	HDL (mg/dL)	VLDL (mg/dL)	Phospholipid (mg/dL)	NEFA (mmol/L)
Control	3.48 ± 0.30 ^a	166.00 ± 7.00 ^a	75.00 ± 7.00	26.00 ± 0.80 ^c	31.00 ± 0.70	38.00 ± 1.40 ^a	5.00 ± 0.20 ^a	112.00 ± 3.00 ^a	0.32 ± 0.03 ^a
Prepartum	4.58 ± 0.20 ^b	75.00 ± 7.00 ^b	70.00 ± 7.00 ^a	40.00 ± 0.80 ^d	32.00 ± 0.70	31.00 ± 1.00 ^b	8.00 ± 0.20 ^b	105.00 ± 2.00 ^b	0.35 ± 0.03 ^b
Baseline	5.00 ± 0.50 ^a	81.00 ± 10.00 ^c	69.00 ± 0.70 ^a	40.00 ± 0.90 ^c	32.00 ± 0.70	31.00 ± 0.30 ^a	8.00 ± 0.20 ^a	105.00 ± 2.00 ^a	0.36 ± 0.02 ^a
0.5 week	2.60 ± 0.40 ^b	43.00 ± 8.00 ^d	57.00 ± 0.70 ^b	16.00 ± 0.50 ^d	32.00 ± 0.70	27.00 ± 0.30 ^b	3.20 ± 0.10 ^b	83.00 ± 4.00 ^b	0.63 ± 0.04 ^b
Week 1	3.00 ± 0.30 ^b	62.00 ± 4.00 ^d	50.00 ± 0.80 ^b	15.00 ± 0.60 ^d	36.00 ± 0.30 ^a	22.00 ± 0.30 ^b	3.00 ± 0.10 ^b	79.00 ± 3.00 ^b	0.38 ± 0.03
Week 2	3.00 ± 0.40 ^b	80.00 ± 9.00	55.00 ± 0.90 ^b	19.00 ± 0.50 ^d	25.00 ± 0.30 ^b	26.00 ± 0.30 ^b	3.30 ± 0.10 ^b	87.00 ± 4.00 ^b	0.36 ± 0.02 ^a
Week 3	2.50 ± 0.50 ^b	50.00 ± 5.00 ^d	51.00 ± 0.70 ^b	14.00 ± 0.40 ^d	27.00 ± 0.90 ^b	25.00 ± 0.70 ^b	3.00 ± 0.10 ^b	91.00 ± 4.00 ^b	0.42 ± 0.04 ^a
Week 4	2.50 ± 0.30 ^b	53.00 ± 6.00 ^d	55.00 ± 0.60 ^b	17.00 ± 0.30 ^d	26.00 ± 0.70 ^b	27.00 ± 0.70 ^b	3.10 ± 0.10 ^b	96.00 ± 0.30 ^b	0.46 ± 0.03 ^a
Week 6	3.20 ± 0.30 ^b	76.00 ± 8.00	62.00 ± 0.80 ^b	20.00 ± 0.50 ^d	25.00 ± 0.30 ^b	32.00 ± 0.70	3.00 ± 0.10 ^b	105.00 ± 4.00	0.30 ± 0.03 ^a
Week 8	3.10 ± 0.30 ^b	97.00 ± 9.00	65.00 ± 0.70 ^b	24.00 ± 0.40 ^d	26.00 ± 0.30 ^b	34.00 ± 0.30 ^a	4.00 ± 0.20 ^b	101.00 ± 3.00	0.33 ± 0.02 ^a
Week 12	2.50 ± 0.40 ^b	120.00 ± 10.00 ^d	68.00 ± 0.70	13.00 ± 0.30 ^d	28.00 ± 0.30 ^b	36.00 ± 0.70 ^a	3.00 ± 0.10 ^b	104.00 ± 4.00	0.41 ± 0.03 ^a

^{ab} indicate statistically significance among column P < 0.001
^{AB} indicate statistically significance among column P < 0.05
^{c,d and cd} indicate statistically significance among column P < 0.01

postparturient period, but these parameters were lower than baseline levels, except at week 1 for LDL and weeks 6, 8, and 12 for HDL (Figure 3; Table)

Serum leptin, triglyceride, VLDL, and NEFA levels were higher in pregnant sheep (before parturient) than in control ewes (P < 0.001). Serum levels of ghrelin, cholesterol, HDL, and phospholipid were lower in pregnant ewes compared to the controls (P < 0.001) (Table).

4. Discussion

In the present study, leptin concentration was high in the prepartum and baseline periods, while it was at the lowest level in week 3, week 4, and week 12 of lactation (P < 0.001). The fluctuating decline in leptin concentration continued until week 12 compared to the baseline.

Observed decreases in serum leptin levels in this study might be related to changes in adipose tissue density in response to parturition in sheep. This low level of leptin may also be associated with the negative energy balance that resulted from increasing metabolic requirements just after parturition, consistent with increasing serum NEFA at the same time point (day 4 after parturition), as reported previously (13,23). Reduced dry matter intake in response to low leptin concentration may be thought to increase the negative energy balance in the sheep studied. Despite the fluctuating course of the NEFA level in the later periods after day 4 of the lactation period, the higher level compared to the initial value may be related to the lower concentration of leptin in this period. However, this may be associated with multifactorial effects, such as nutritional status,

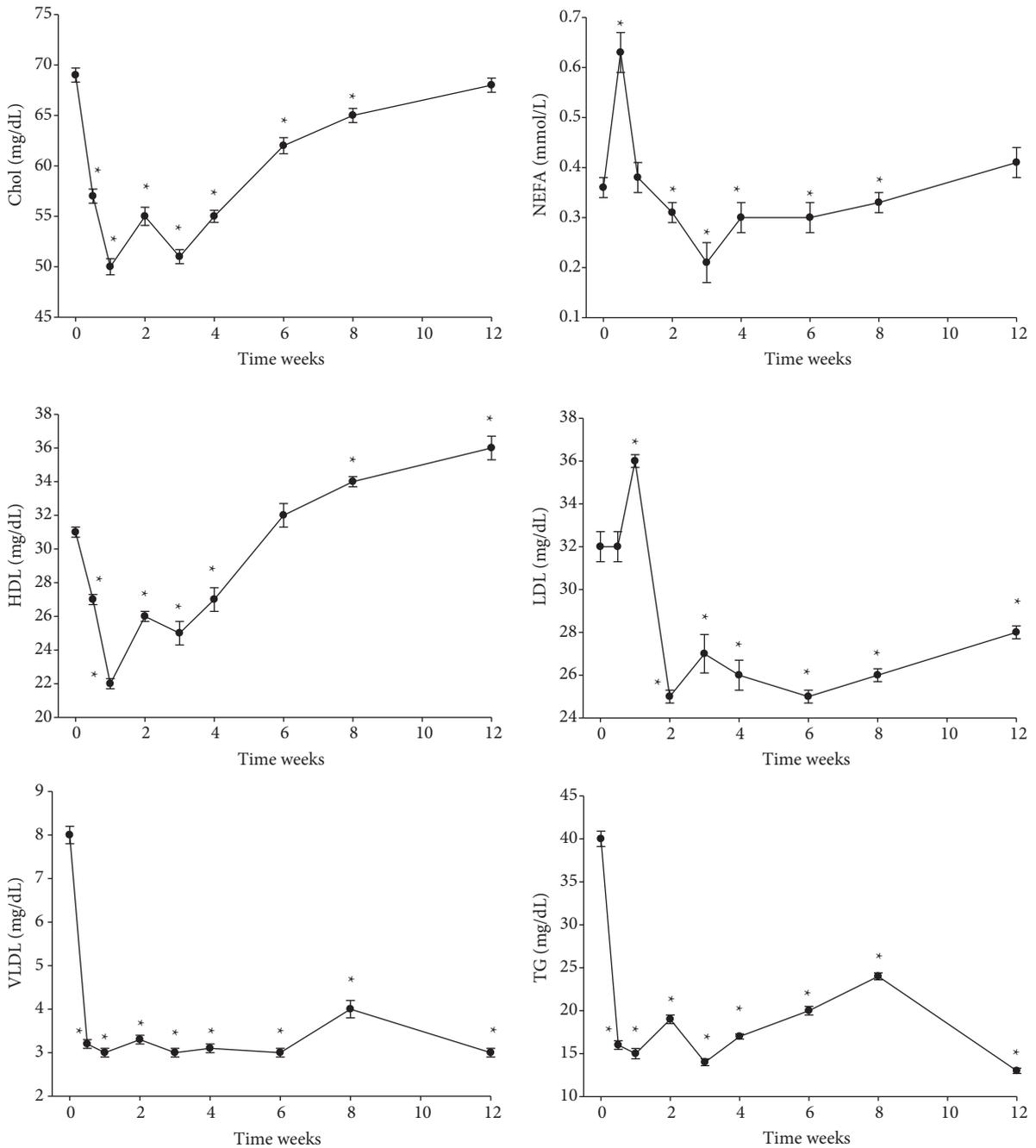


Figure 3. Lipid profile in preparturient and postparturient periods.

breed differences, and other hormonal factors (2,24). The onset of lactation is characterized by a decrease in dry matter consumption versus increased energy demand due to milk production. This leads to the formation of a negative energy balance and a decrease in the body score (2). Leptin concentration is closely related to nutritional status, adipose tissue density, and other hormonal levels (5,25,26). Leptin concentration, which varies according to adipose tissue density, results in decreased dry matter

intake due to the effects on the central nervous system (26–28). Studies in cattle and sheep have indicated a decrease in the postpartum plasma leptin concentration (13,29). However, there is an increase in blood NEFA concentration and a decrease in body score, especially as a sign of negative energy balance in high milk-producing animals (30,31). Taken together, our observations of low serum leptin and an increase in metabolic requirements (meaning negative energy balance) agreed well with the

study by Carcangiu et al. (13) that reported that the high milk yield group had low plasma leptin concentration compared to the low milk yield group.

In the present study, the ghrelin level was low compared to the initial level, but a fluctuating course was seen. At week 2, ghrelin reached the baseline value, but it was accompanied by decreased serum leptin levels at weeks 3 and 4. In the present study, observed increases in the level of NEFA during this period suggest that the decline in both leptin and ghrelin levels may be considered a physiological hormonal response to the negative energy balance. It has been determined that the ghrelin level reached baseline levels at 6 weeks. The fact that the level of NEFA in these periods is close to the values in the baseline and control sheep can be considered a sign of appetite stimulation. Serum leptin and ghrelin significantly affect fetal development during pregnancy (2). As in other mammalian species, ghrelin also stimulates GH hormone from the pituitary gland in ruminants (16). Thus, blood ghrelin concentration may vary during the development and lactation periods. There is also an increase in plasma ghrelin with a decrease in the rumen fold and as a response to hunger (14,15). Kurose et al. (32) reported that the level of ghrelin measured after fasting was higher in animals with more adipose tissue than in weaker sheep.

Ghrelin is known to have important roles in the energy metabolism necessary for the stimulation of appetite, glucose release, cell proliferation, and reproductive activity. The level of maternal ghrelin has been shown to have a great effect on fetal development, especially in the late period of pregnancy (33,34). Fuglsang et al. (35) reported that the ghrelin level was higher in the early and middle periods of pregnancy compared to the baseline level, whereas it decreased significantly in the late gestation period. In accordance with these observations (33–35), in the present study, the ghrelin level was significantly higher in the control sheep than in the late gestation sheep. The difference in the ghrelin level in the sheep in the control and prepartum periods in the present study suggests that ghrelin may be associated with an increase in the ghrelin mRNA level versus the lower blood level of ghrelin in the late period of pregnancy (35).

Leptin plays an important role in regulating fat metabolism (36). Leptin has a role in the regulation of acute triglyceride metabolism, HDL-cholesterol clearance, lipid oxidation, and the lipogenic effects of insulin at the cellular level (37,38). Various studies have suggested that leptin is positively associated with cholesterol and HDL. Otherwise, a weak positive correlation with triglyceride concentration has been reported (36,38,39). In a study by Comba et al. (24), the relationship between leptin level and lipid profile was evaluated in different sheep breeds that were not pregnant and 2–3 years old. In that study

(24), there was a positive correlation between leptin concentration and VLDL, LDL, total cholesterol, and triglyceride concentrations. Cohen et al. (40) reported that triglyceride levels were elevated in animals that received exogenous leptin. In the present study, there were no statistically significant differences between postpartum leptin and triglycerides, VLDL, and total cholesterol levels, but a positive relationship was found between them. The level of triglycerides was low until week 12 after birth ($P < 0.01$), but the level of LDL was low from week 2 to week 12 based on the baseline value ($P < 0.05$). Total cholesterol level was low until week 8 compared to the baseline ($P < 0.05$). These findings suggest that low postpartum leptin levels may be associated with negative energy balance, suggesting that the low level of leptin could also induce changes in the lipid profile in the postparturient period, in agreement with a previous study (41).

Total cholesterol and phospholipids play an important role in the membrane and basic substance of the cell. For this reason, various studies reported that the levels of both have not changed (42,43). However, it was noted by Caldeira and Portugal (31) that total cholesterol and phospholipid levels were elevated in sheep with high body condition scores. Another study, by Nielsen and Jakobsent (44), noted that total cholesterol, triglyceride, and phospholipid levels were high in goats with high feed consumption. In the present study, phospholipid and total cholesterol levels decreased between day 4 and week 4, and the leptin level was also lower than the baseline levels in this period. The increasing level of NEFA in this period compared to the initial level suggests the presence of a negative energy balance. The present study findings suggest that low levels of leptin may be associated with a decrease in total cholesterol and phospholipid levels, particularly in the early stages of lactation, which agreed with the findings reported by Bitman and Wood (45). In the present study, the total cholesterol level was low in ewes in the prepartum period compared to control ewes, whereas various authors reported that total cholesterol levels were reduced throughout the late pregnancy period due to rising steriogenesis (46–48).

In conclusion, this study reveals the changes in serum leptin and ghrelin levels in the pre- and postnatal periods in sheep and the relationship between these changes and the serum lipid profile. Our findings show that serum leptin and ghrelin levels may be effective in clarifying the changing physiological conditions in the postpartum period. Our findings in this study provide important data for studies in which negative energy balance and hormonal relationships are assessed in ewes.

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