Received: 16 July 2023 | Revised: 11 September 2023 | Accepted: 6 October 2023

DOI: 10.1111/asj.13886

RESEARCH ARTICLE

Animal Science Journal WILEY

Effects of propylene glycol used at different doses in Akkaraman lambs rations on metabolism-related parameters and liver gene and protein expression during different feeding periods

Akın Yakan[1] | Hüseyin Özkan[1] | Ufuk Kaya[2] | Hasan Hüseyin Keçeli[1] | Sevda Dalkiran[3] | Irem Karaaslan[4] | Necmettin Ünal[5] | Aytaç Akçay[6] | Korhan Arslan[7] | Bilal Akyüz[7] | Güven Güngör[8] | Baran Çamdeviren[3] | Mehmet Küçükoflaz[9] | Savaş Sariözkan[10] | Ceyhan Özbeyaz[5]

[1]Faculty of Veterinary Medicine, Department of Genetics, Hatay Mustafa Kemal University, Antakya, Hatay, Turkiye

[2]Faculty of Veterinary Medicine, Department of Biostatistics, Hatay Mustafa Kemal University, Antakya, Hatay, Turkiye

[3]Institute of Health Sciences, Department of Molecular Biochemistry and Genetics, Hatay Mustafa Kemal University, Antakya, Hatay, Turkiye

[4]Technology and Research & Development Center (MARGEM), Hatay Mustafa Kemal University, Antakya, Hatay, Turkiye

[5]Faculty of Veterinary Medicine, Department of Animal Science, Ankara University, Ankara, Turkiye

[6]Faculty of Veterinary Medicine, Department of Biostatistics, Ankara University, Ankara, Turkiye

[7]Faculty of Veterinary Medicine, Department of Genetics, Erciyes University, Kayseri, Turkiye

[8]Faculty of Veterinary Medicine, Department of Biostatistics, Erciyes University, Kayseri, Turkiye

[9]Faculty of Veterinary Medicine, Department of Animal Health Economics and Management, Kafkas University, Kars, Turkiye

[10]Faculty of Veterinary Medicine, Department of Animal Health Economics and Management, Erciyes University, Kayseri, Turkiye

Correspondence
Akın Yakan, Faculty of Veterinary Medicine, Department of Genetics, Hatay Mustafa Kemal University, Antakya, 31060 Hatay, Turkiye.
Email: yakanakin@hotmail.com

Abstract

This study aimed to investigate the metabolic effects of propylene glycol (PG) over 60, 90, and 120 days in lambs. Seventy-two weaned male lambs were allocated into three groups: control (Con), PG1.5 (1.5 mL/kg live weight$^{0.75}$), and PG3 (3 mL/kg live weight$^{0.75}$). Blood samples were collected at the beginning and slaughter days. Biochemical parameters (glucose, triglycerides, ALT, AST, LDH, BUN, and insulin) and gene and protein levels of peroxisome proliferator activated receptor gamma (PPARγ), diacylglycerol o-acyltransferase 1 (DGAT1), carbohydrate responsive element binding protein (ChREBP), and sterol regulatory element binding transcription factor 1c (SREBP-1c) in the liver were determined. Glucose in PG1.5 was increased on Day 60, while significant differences were observed in biochemical parameters except for insulin on the 60, 90, and 120 days. Biochemical parameters such as ALT, AST, LDH, and BUN increased over time, while triglycerides decreased. DGAT1 gene and protein levels were lower, while SREBP-1c and PPARγ were higher in PG groups on Day 60. While *SREBP-1c* was lower in PG1.5, ChREBP was higher in PG3 on Day 90. *PPARγ*, *DGAT1*, and *ChREBP* were upregulated in PG3 on Day 120. Positive correlations were found between proteins. The long-term use of PG in lambs did not have detrimental effects on metabolism. The study provides valuable insights into the molecular mechanisms underlying the metabolic effects of PG in lambs, shedding light on its potential applications in lamb production.

KEYWORDS
gene expression, lamb ration, metabolism, propylene glycol, protein expression

Funding information
Scientific and Technological Research Council of Turkiye (TUBİTAK), Grant/Award Number: 119R076

1 | INTRODUCTION

Lamb meat is a nutrient-dense food that has been consumed by humans for centuries. In addition to vitamins B6 and B12, it is a good source of protein and minerals such as iron, zinc, and selenium. Lamb meat also contains conjugated linoleic acid (CLA), a type of fatty acid that provides health benefits such as reducing the risk of heart disease and cancer. In recent years, there has been a growing interest concerning the health benefits of lamb. In an increasingly conscious world of consumption, lamb meat has emerged as a delicious and high-quality animal product that stands out for healthy living (Benjamin et al., 2015; Gonzales-Barron et al., 2021).

In order to meet the growing demand for lamb meat, it is important to develop production targets that are both sustainable and profitable. The production of lamb meat is a complex process that involves several factors, including genetics, management, and nutrition. Nutrition is one of the major crucial factors for lamb breeding, and studies are being conducted on lamb fattening using different rations and feed additives such as propylene glycol (PG) (Kenyon & Corner-Thomas, 2022).

PG is a clear, colorless, and odorless liquid that is widely used in a variety of industrial and consumer products. It is also a synthetic compound that is used as a food additive and a livestock feed supplement. In ruminant feeding, PG is used as a glucogenic precursor, and it can be converted into glucose in the liver. This makes PG a potential treatment for ketosis, a metabolic disorder that can occur in ruminants during periods of negative energy balance. By converting PG to glucose, blood glucose levels are maintained, and the production of ketone bodies is prevented (Ayoub et al., 2015; Zhang et al., 2020). PG has also been shown to be effective in reducing the risk of fatty liver in ruminants (Rukkwamsuk et al., 2005).

In addition to protective effects for ketosis and fatty liver disease, PG is also used in livestock farming for profitability purposes, such as improving meat yield and fattening performance. However, limited information is available regarding the metabolic effects of PG on lambs. This study aims to investigate the metabolic effects of PG at different doses over a period of 60, 90, and 120 days using biochemical and molecular parameters.

2 | MATERIALS AND METHODS

The animal experiment of the study was carried out at the Small Ruminant Experimental Unit of the Agricultural Research and Application Center of Erciyes University and approved by the Erciyes University Local Animal Experiments Ethics Committee (decision no.: 19/044). The study was conducted using 72 weaned male Akkaraman breed lambs weighing between 15 and 20 kg in a 3 × 3 experimental design with two dependent variables. The dependent variables were the PG dosage (PG1.5: 1.5 mL/kg live weight$^{0.75}$ and PG3: 3 mL/kg live weight$^{0.75}$) and the feeding period (60, 90, and 120 days), along with a control group (Con).

The animals were provided with lamb fattening feed (2600 kcal/kg Metabolic Energy and 16% Crude Protein) and clean water ad libitum. Additionally, each animal received 100 g of dry hay daily. Before the study began, the animals underwent a 1-week acclimation period to the feed. Feed intake was monitored daily, and the PG dosage was calculated based on the weekly average live weights of the animals. The PG was administered orally to the lambs in the morning at 8:00 a.m. While group feeding was applied for lamb fattening feed, PG was individually presented.

2.1 | Slaughtering, blood, and tissue collection

To measure the levels of glucose, triglycerides, ALT, AST, LDH, BUN, and insulin, blood samples were collected at the first day of feeding and slaughtered days (60, 90, and 120 days). Lambs in each group were slaughtered after the 60th, 90th, and 120th days of the feeding period. On each slaughter day, eight lambs were slaughtered from each group (Con, PG1.5, and PG3). On the evening before slaughter, the lambs were not fed but had unrestricted access to water. Following slaughter, liver tissues were collected under nuclease-free conditions and quickly frozen with liquid nitrogen. The collected liver tissues were kept at −80°C until gene and protein analysis.

2.2 | Determination of biochemical parameters

Collected blood samples were centrifuged at 1500 ×g for 10 min at +4°C, and the obtained plasma samples were used for biochemical analysis. While glucose, triglycerides, ALT, AST, LDH, and BUN levels of the lambs were analyzed using an autoanalyzer (Siemens Adcia 1800, Japan), insulin was measured according to the manufacturer's instructions by commercial ELISA kit (BT Lab, South Korea, Cat No: E0036Sh) with the microplate ELISA reader (Allsheng AMR-100, China) at 450 nm.

2.3 | RNA isolation and cDNA synthesis

RNA isolation was performed from liver tissues according to the modified TRIzol method (Rio et al., 2010). Approximately 50 mg of tissue exposed to 1 mL TRIzol Reagent (Thermo Fisher Scientific, USA, Cat No: 15596018) was used. The obtained RNA pellets were air-dried for 10 min. Subsequently, 30–100 μL of nuclease-free water was added to RNA pellets and homogenized.

The concentration and purity of isolated RNA were measured using a nucleic acid analyzer (Merinton-SMA 1000, China). The

integrities of RNA samples were checked by 1% agarose gel electrophoresis. To eliminate potential genomic DNA, a DNA digestion protocol was applied using DNase I (Thermo Fisher Scientific, USA, Cat No: EN0521). Subsequently, cDNA synthesis was performed using a commercial kit (High Capacity Kit, Thermo Fisher Scientific, USA, Cat No: 4368814) in a thermal cycler (Bio-Rad T100, USA). The protocol was set as follows: 10 min at 25°C, 120 min at 37°C, and 5 min at 85°C. After the reaction, the samples were brought up to 200 µL and stored at −20°C until further molecular analysis.

2.4 | RT-qPCR application

Even if many genes are responsible for the metabolic regulation in the organism, some of them, particularly *ChREBP*, *SREBP-1c*, and *PPARγ*, have major effects on metabolism (Özkan & Yakan, 2019). *DGAT1* is also known as a critical gene for fatty acid metabolism (Altwaty et al., 2020; Pirzad et al., 2014). *ChREBP*, *SREBP-1c*, *PPARγ*, and *DGAT1* gene expression levels were determined in the liver. Peptidyl-prolyl isomerase A (*PPIA*) and ribosomal protein lateral stalk subunit P0 (*RPLP0*) housekeeping genes were used as reference genes. The forward and reverse sequences of the primers used for the amplification of the studied genes were designed by the authors using Primer-Blast (National Center for Biotechnology Information [NCBI]) and Primer3Plus (Table 1). The amplifications of the genes were determined using the SYBR Green I dye-containing kit (Power SYBR Green PCR Master Mix; Thermo Fisher Scientific, USA, Cat No: 4368702) in qPCR (Rotor-Gene Q MDx 5plex HRM; Qiagen, USA). Each sample was run in triplicate, and the qPCR protocol consisted of an initial denaturation at 95°C for 10 min, followed by 40 cycles of denaturation at 95°C for 15 s and annealing/extension at 60°C for 60 s.

2.5 | Western blot analysis

Targeted protein levels encoded by *ChREBP*, *SREBP-1c*, *PPARγ*, and *DGAT1* genes in the liver were determined at the 60th, 90th, and 120th days of feeding. Liver samples were homogenized by tissue homogenizer (Biopreb, Allsheng, China) in phenylmethylsulphonyl fluoride (PMSF), sodium orthovanadate, and protease inhibitor cocktail containing radioimmunoprecipitation assay (RIPA) buffer (sc-24948; Santa Cruz, CA, USA). Homogenates were incubated at 4°C for 30 min, then centrifuged at 14,000 ×*g* for 15 min at 4°C. Following centrifugation, the total protein concentration of the homogenized samples supernatant was determined at 562 nm with ELISA Reader (AMR-100; Allsheng, China) using Pierce BCA Protein assay kit (23227, Thermo Fisher Scientific, USA).

Ten micrograms of total protein from each sample was used for separation on 4%–12% Tris-Glycine gels. Following separation, the proteins were transferred onto a nitrocellulose membrane (Invitrogen, MA, USA). Following the transfer, non-fat milk powder (5%) in TBST (10 mM Tris-HCl pH 7.4, 150 mM NaCl, 0.1% Tween-20) was added with gentle shaking at room temperature and incubated for 1 h for blocking. Then, the blots were incubated overnight at +4°C with primary antibodies diluted in TBST containing 5% non-fat dry milk. The membranes were washed three times in TBST and incubated for 1 h at room temperature with goat anti-rabbit IgG (for ChREBP, SREBP-1c, PPARγ, and DGAT1, 1:2500, 31460, Thermo Fisher Scientific, USA) and goat anti-mouse secondary antibodies (for GAPDH, 1:10.000, 31430; Thermo Fisher Scientific, USA). Then, the membranes were washed six times using TBST, and band images were collected with ECL dye in the dark using the I-Bright 1500 (Invitrogen, USA). The signal intensities of proteins were converted to quantitative values using Image J (version 1.53k14). Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) was used as a housekeeping protein.

2.6 | Statistical analysis

Statistical analyses were performed using Stata version 15.1 (StataCorp, College Station, TX, USA). The variables were examined as parametric test assumptions. The Spearman correlation coefficient was performed to assess the correlation between biochemical parameters and targeted protein levels of genes. To test the differences in each biochemical parameter between sampling time (the first day of feeding and slaughter

TABLE 1 Forward and reverse sequences of primers.

Genes	Forward and reverse sequences	Product length (bp)
ChREBP	F-5′-CCTTCAACGGGATGGTGTCT-3′ R-5′-AGAACAGTTGGTCGGAGAGC-3′	106
SREBP-1c	F-5′-ATCTGTTGGAGCGAGCACTG-3′ R-5′-ATCCGAGGGCATCTGAGAAC′-3′	93
PPARγ	F-5′-ACTTTGGGATCAGCTCCGTG-3′ R-5′-AACCATCGGGTCAGCTCTTG-3′	146
DGAT1	F-5′-GGACACAGACAAGGACGGAG-3′ R-5′-CAGAACTGAACAGGGAGTCCT-3′	89
RPLP0	F-5′-TCCTTCTTCCAGGCTTTAGGC-3′ R-5′-AATCAGCTGCACATCGCTCA-3′	78
PPIA	F-5′-GCAAAGTGAAAGAGGGCATGAA-3′ R-5′-CAATGGTGATCTTCTTGCTGGT-3′	87

Abbreviation: bp, base pair.

day) in groups (Con, PG1.5, and PG3), two-way mixed ANOVA was used. Sampling time, groups, and their interaction were included as a fixed factor in statistical models. Pairwise comparisons were performed using a Bonferroni adjustment. While geometric means were determined for housekeeping genes, expression levels of studied genes were calculated with the $2^{-\Delta\Delta Ct}$ method and presented as fold change (Livak & Schmittgen, 2001). Moreover, the levels of proteins encoded by target genes were determined with ANOVA in matched samples. To assess the significance of differences in targeted protein levels of genes between groups on each slaughtered day, Tukey's test was performed for multiple comparisons. Differences with $p < 0.05$ were considered statistically significant. Results were calculated as "mean ± standard error of mean" and presented in figures.

3 | RESULTS

On Day 60, an increase in blood glucose levels of the PG1.5 group was detected ($p < 0.01$), while variations were observed in all parameters except for insulin on the slaughter day compared with the beginning of the study (Figure 1).

As indicated, there were no significant changes in the biochemical parameters of the experimental groups in the 90th-day samples, but significant differences occurred in all parameters except insulin and glucose compared with the beginning of the study on the slaughter day ($p < 0.05$). Triglyceride levels were decreased while ALT, AST, BUN, and LDH levels were increased (Figure 2).

At the 120th day, it has been found that significant changes occurred in the biochemical parameters studied, except for glucose and insulin, in line with the results obtained from the 90th-day samples ($p < 0.01$) (Figure 3).

PPARγ protein levels were higher in the PG groups, while gene expression levels of *PPARγ* were similar in the 60th-day samples. In addition, *DGAT1* was downregulated in both PG1.5 and PG3 ($p < 0.05$). Similar to gene expression results, DGAT1 protein levels were lower in PG3. While *ChREBP* and *SREBP-1c* gene expression levels were similar in all groups, SREBP-1c protein levels were found to be higher in PG1.5 ($p < 0.05$) (Figure 4).

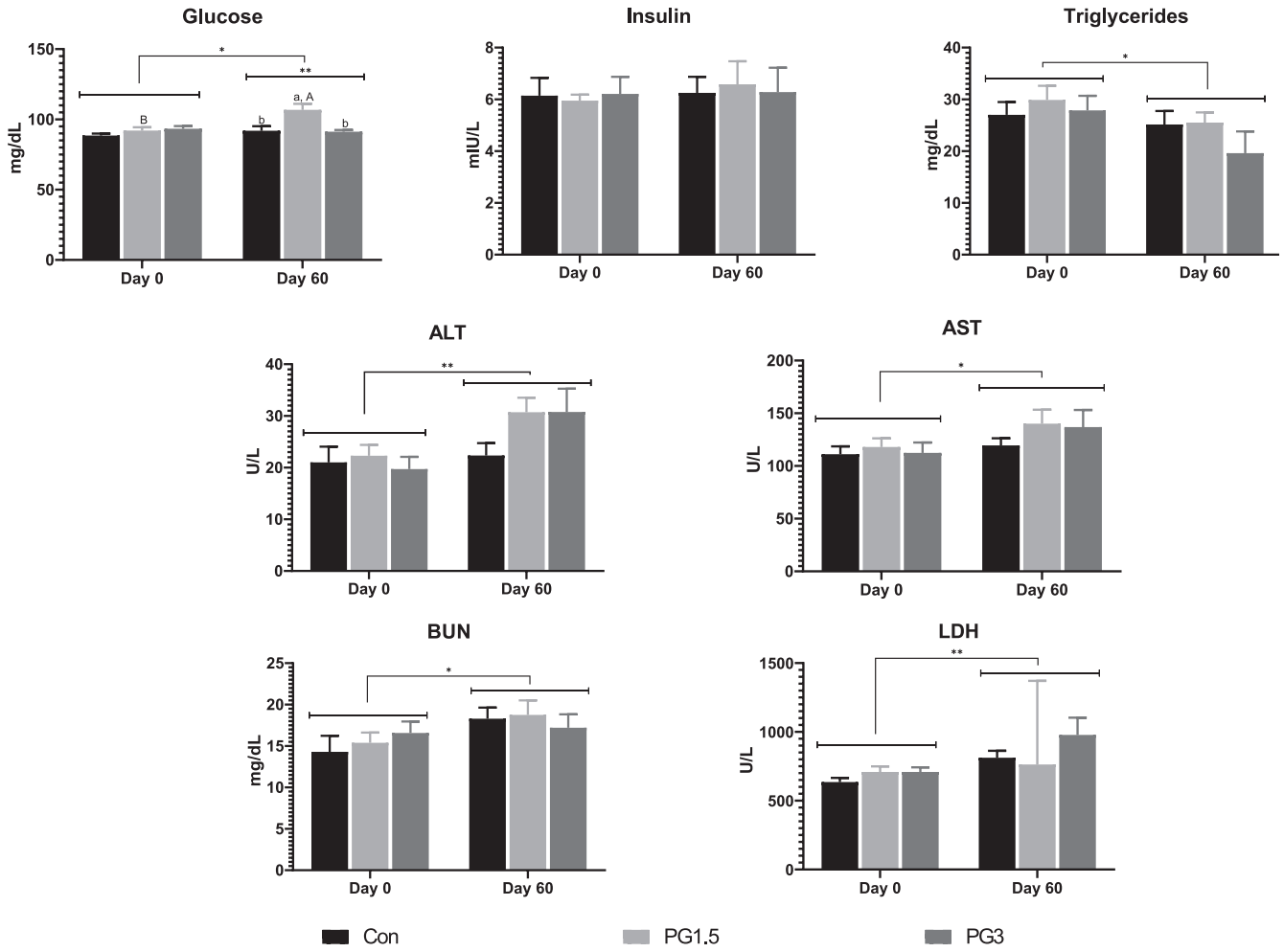

FIGURE 1 Biochemical parameters of groups (Day 0 to Day 60). a, b, c, shows the difference between groups on the same days; A, B, shows the difference between groups on the different days. *$p < 0.05$; **$p < 0.01$.

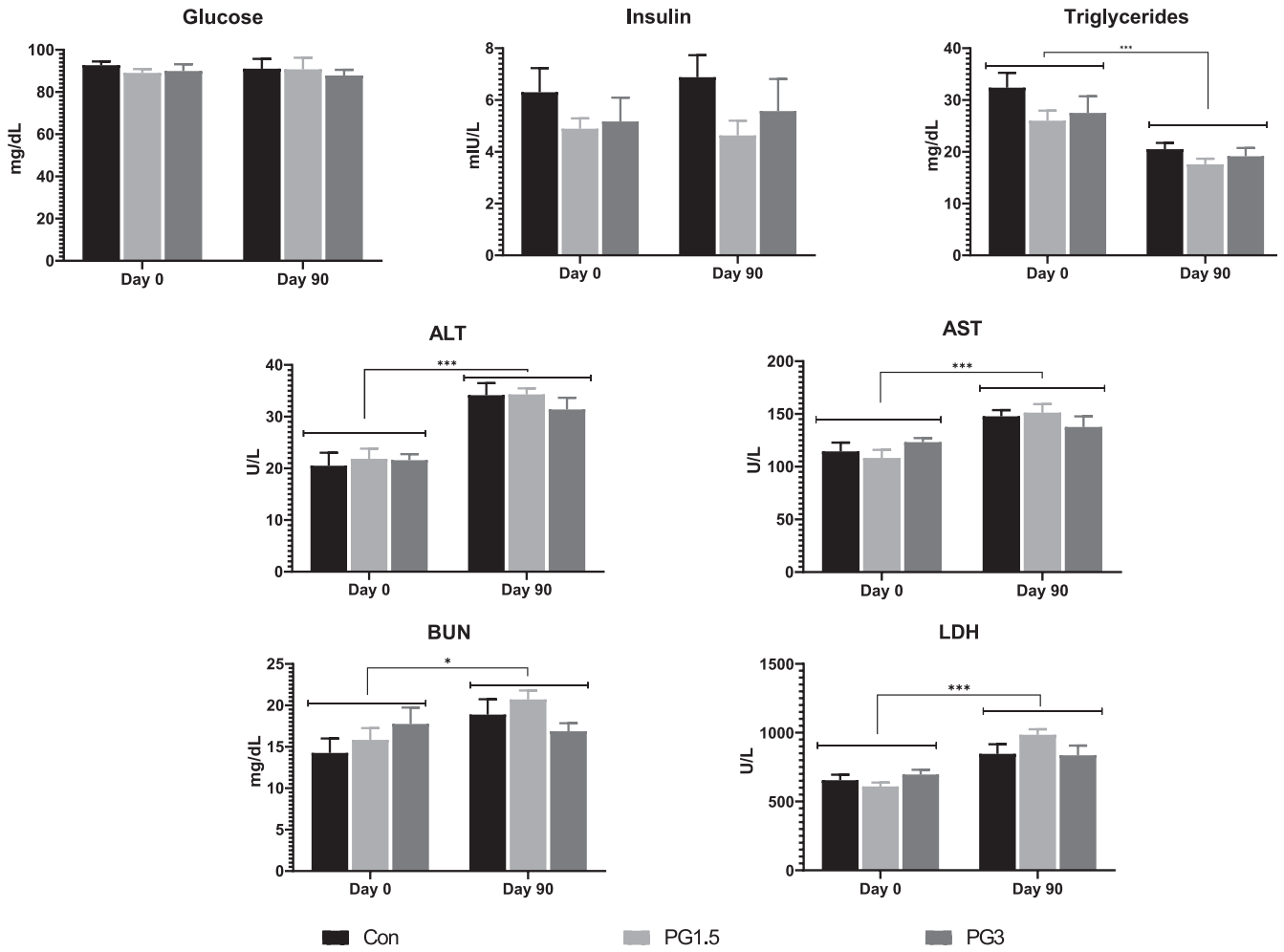

FIGURE 2 Biochemical parameters of groups (Day 0 to Day 90). *$p < 0.05$; **$p < 0.01$; ***$p < 0.001$.

PPARγ and SREBP-1c protein levels were similar in the 90th-day samples. On the other hand, DGAT1 and ChREBP protein levels significantly changed in groups. While the DGAT1 protein levels of the PG1.5 group were significantly lower than PG3 ($p < 0.05$), PG groups were similar to Con in terms of DGAT1 levels. In addition, ChREBP protein levels were similar in the Con and PG1.5 groups, while they were higher in the PG3 group ($p < 0.05$). *SREBP-1c* was downregulated in PG1.5 (Figure 5).

Compared with Con, expression levels of *PPARγ*, *DGAT1*, and *ChREBP* were upregulated in PG3 in the 120th-day samples ($p < 0.05$). Moreover, *DGAT1* and *SREBP-1c* gene expression levels were higher in PG1.5 ($p < 0.05$). Protein levels were found to be statistically similar between the groups (Figure 6).

In addition to biochemical and molecular parameters, the relationship between the studied parameters was also evaluated. According to the results, a positive correlation was found between DGAT1 and ChREBP proteins ($p < 0.01$). SREBP-1c and PPARγ were also positively correlated ($p < 0.05$). As expected, ALT and AST had a strong positive correlation ($p < 0.001$). Moreover, LDH and BUN were positively correlated with ALT and AST ($p < 0.001$). While a weak but negative correlation was found between triglycerides and ChREBP ($p < 0.05$), BUN was positively correlated with ALT, AST, and LDH ($p < 0.05$). In addition, there was a negative correlation between BUN and triglycerides ($p < 0.01$) (Table 2).

4 | DISCUSSION

PG has long been used as a glycogenic source in farm animals, especially in metabolic diseases such as ketosis (Kalyesubula et al., 2019). However, studies on the long-term use of PG are limited. In this study, it was determined that oral administration of different doses of PG to lambs for 60, 90, and 120 days caused significant changes in biochemical parameters. While PG is known to have glycogenic effects, it has been found that plasma glucose levels increased in lambs fed with PG for 60 days in addition to the normal ration, with a dosage of 1.5 mL/kg$^{\text{Live Weight}}$. However, plasma glucose levels in the PG3 were similar to those in the Con. Furthermore, no significant difference in glucose levels was observed among the PG groups during the 90- and 120-day feeding periods. This was considered to be related to the duration of feeding and the dosage of PG used (Chiofalo et al., 2005; Kalyesubula et al., 2019). In fact, in a study conducted with sheep

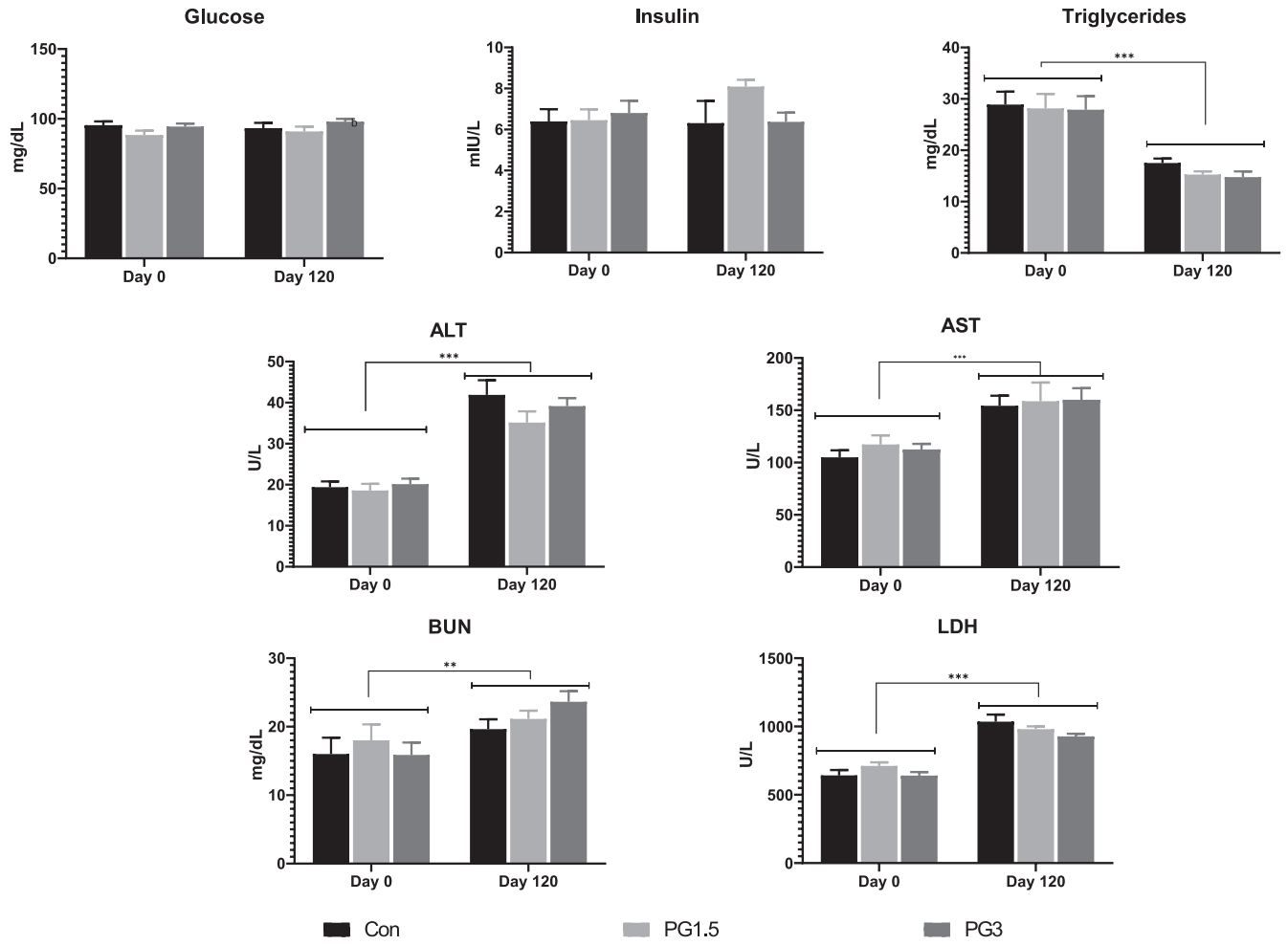

FIGURE 3 Biochemical parameters of groups (Day 0 to Day 120). $^{**}p < 0.01$; $^{***}p < 0.001$.

treated with high doses of PG for 2 months, plasma glucose levels were reported to be higher compared with the control group (Chiofalo et al., 2005). The similar insulin levels among the groups, together with the plasma glucose levels, were interpreted as an expected result. Moreover, it can be stated that the use of PG at the doses used in the study does not have a negative effect on energy metabolism through glucose and insulin.

ALT and AST parameters are important biochemical parameters that provide insights into liver physiology. In this study, similar to the findings of dos Santos et al. (2015), no significant differences were observed between the groups on different days. However, a significant increase in both parameters was determined on the 60th, 90th, and 120th days. The increase in these parameters is known to be related to the energy content of the ration, diseases, and various chemicals (Giráldez et al., 2021; Murray et al., 2009). The increase in ALT and AST parameters over time, depending on liver physiology, is considered physiologically normal (dos Santos et al., 2015; Udum et al., 2013).

Similar to ALT and AST parameters, it was determined that there was no negative effect of PG on BUN and LDH, and these two parameters increased significantly over time. The results obtained are consistent with similar studies and are associated with growth physiology, similar to other parameters (Assunçao, 2012; dos Santos et al., 2015; Lopez-Vargas et al., 2022; Udum et al., 2013).

Metabolic processes in the organism are under the control of complex molecular mechanisms. Major influential genes involved in physiological processes provide important insights at the mRNA and protein levels for the evaluation of various physiological and pathological conditions. PPARγ is a transcription factor with important roles in energy metabolism. It belongs to the nuclear hormone receptor superfamily and has been mainly studied in muscle and adipose tissues, and its activity in adipose tissue has been reported to increase depending on the energy content of the ration (Deng et al., 2020; Su et al., 2019; Wang et al., 2018). PPARγ, which is largely responsible for the balance between lipogenesis and lipolysis, was increased at the protein level in both PG groups, although the mRNA levels were similar between the PG groups after 60 days of feeding. Considering that anabolic pathways are more active in the early stages of nutrition, this finding was attributed to the feeding period (Lehrke & Lazar, 2005).

DGAT1, which is involved in lipid accumulation and regulation of fatty acid synthesis in mammals, is significantly associated with the

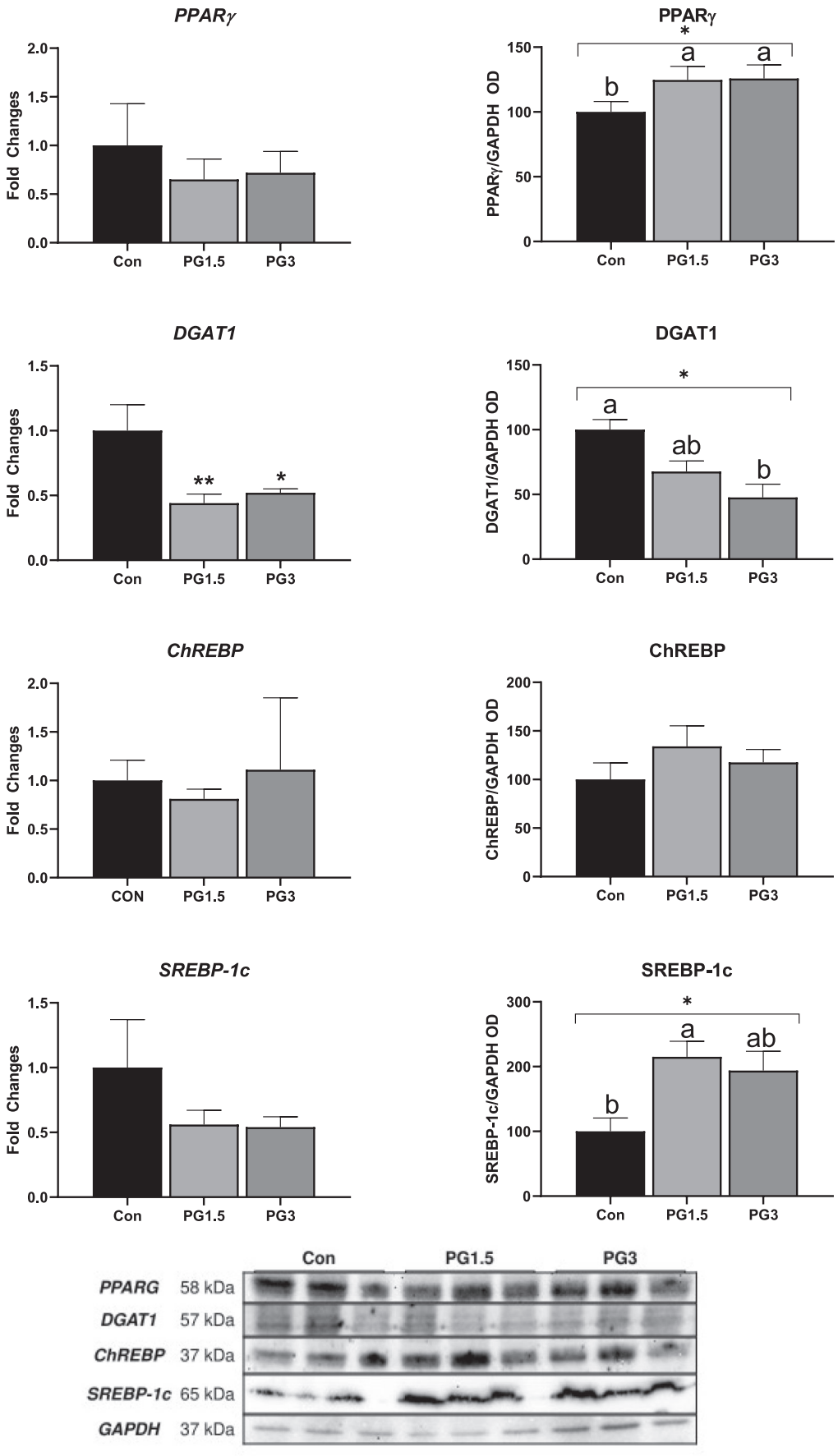

FIGURE 4 Gene and protein expression levels of slaughtered Day 60. a, b, shows the difference between groups. *$p < 0.05$.

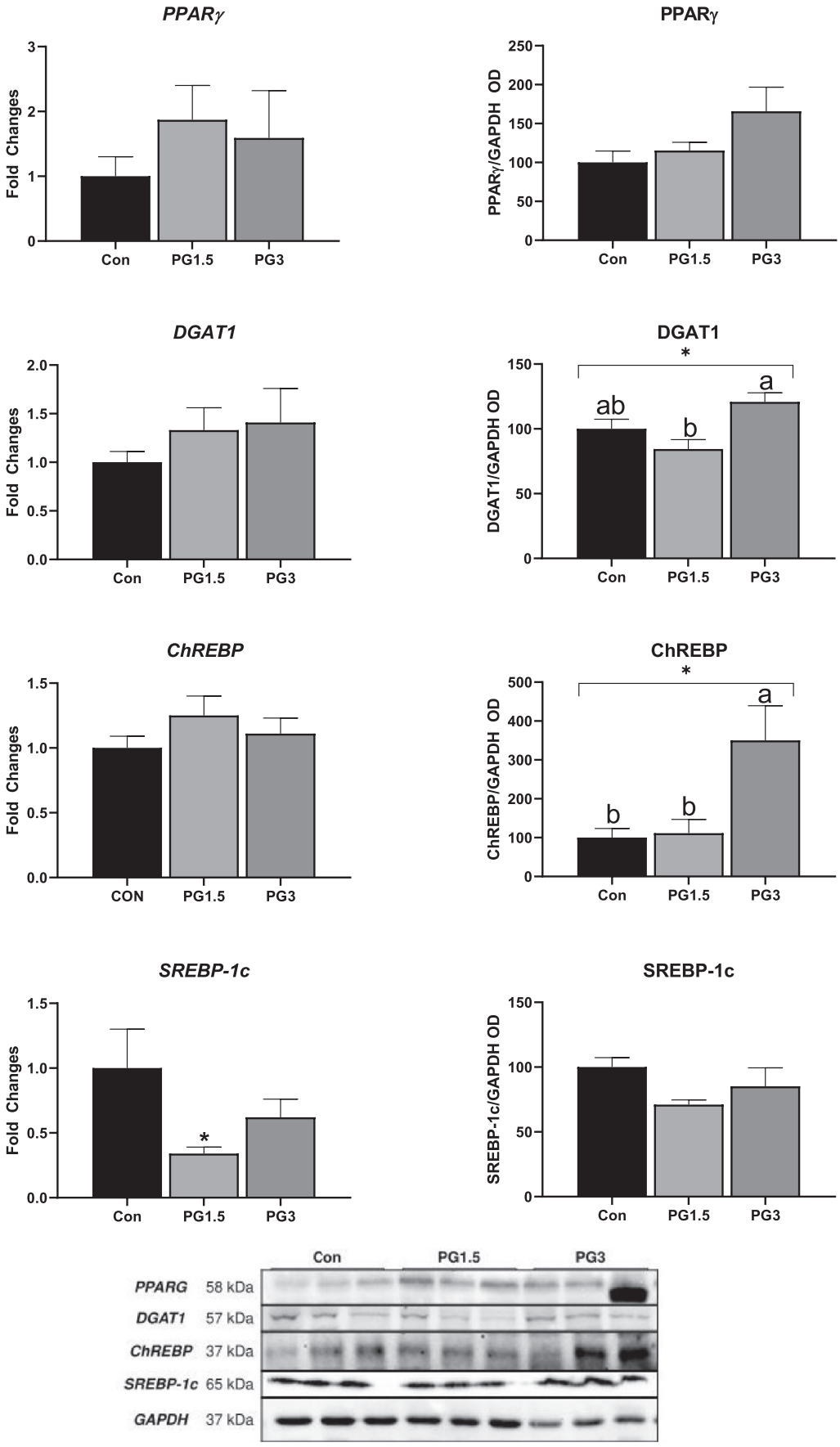

FIGURE 5 Gene and protein expression levels of slaughtered Day 90. a, b, shows the difference between groups. *$p < 0.05$.

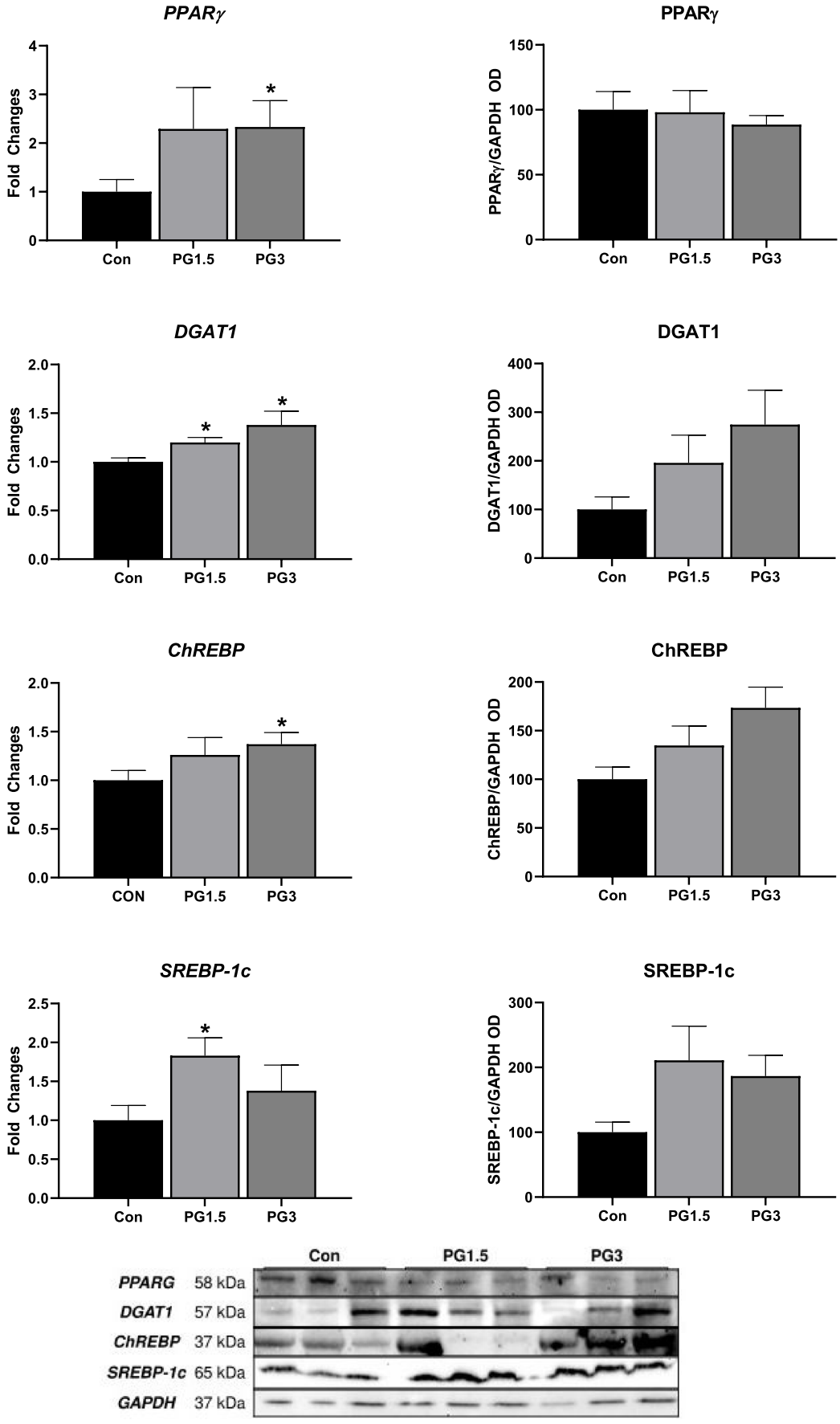

FIGURE 6 Gene and protein expression levels of slaughtered Day 120. *$p < 0.05$.

TABLE 2 Correlations between studied parameters.

	DGAT1	PPARγ	SREBP-1c	ChREBP	ALT	AST	Glucose	LDH	Triglycerides	BUN
DGAT1	1	−0.055	−0.213	0.390**	−0.044	−0.011	0.142	0.082	−0.178	0.139
PPARγ		1	0.260*	0.047	−0.107	−0.031	0.089	0.145	−0.105	0.078
SREBP-1c			1	0.046	0.150	0.048	0.047	0.002	−0.047	0.045
ChREBP				1	0.216	0.202	0.095	0.158	−0.282*	0.058
ALT					1	0.732***	−0.121	0.514***	−0.029	0.260*
AST						1	0.067	0.624***	0.027	0.297*
Glucose							1	−0.147	0.14	−0.085
LDH								1	−0.187	0.378**
Triglycerides									1	−0.263*
BUN										1

Abbreviations: ALT, alanine transaminase; AST, aspartate aminotransferase; BUN, blood urea nitrogen; LDH, lactate dehydrogenase.
*$p < 0.05$.
**$p < 0.01$.
***$p < 0.001$.

activation of energy metabolism due to its relation to ration energy content (Cui et al., 2019). Upregulation of the *DGAT1* gene is reported to increase the rate of triglyceride storage in various tissues, including skeletal muscle, liver, and adipose tissue (Altwaty et al., 2020; Pirzad et al., 2014). While there was a tendency for decreased DGAT1 protein levels in the liver of lambs in the early stage of growth due to PG supplementation, an increasing trend was observed in the PG3 with the prolongation of the feeding period. This finding was considered to be related to the physiological regulation during the feeding and growth processes.

ChREBP, a glucose-sensitive transcription factor, is of vital importance for lipid metabolism and glucose homeostasis (Herman et al., 2012). Limited studies have focused on this transcription factor in sheep, mainly on muscle and adipose tissues (Hoffman et al., 2014; Yan et al., 2023). While ChREBP mRNA and protein levels were found to be similar between the groups on the 60th day, there was a significant increase in protein levels in the PG3 group at the 90th day. At the 120th day, there was an increase in ChREBP mRNA levels in the PG3, but it was not statistically significant at the protein level. The activity of ChREBP may increase in the liver depending on the prolongation of the feeding period, continued growth, and the dosage of PG used. It was concluded that PG could increase anabolic activity in the liver through ChREBP and affect carbohydrate metabolism-related elements regulated by ChREBP.

SREBP-1c regulates the necessary genes for lipid metabolism, adipocyte differentiation, and regulation of fatty acid synthesis, playing an important role in the control of anabolic events in the liver (Özkan & Yakan, 2019; Urrutia et al., 2016). Similar to ChREBP, this transcription factor may have shown different activities in the liver depending on the growth stage. In a study conducted with cattle where different diet applications were offered, it was reported that the SREBP-1c gene was not affected by ration changes (Hiller et al., 2012). Gallardo et al. (2015) reported that there was no change in the mRNA activity of SREBP-1c in different tissues of Chilota lambs under different grazing conditions. In another study, in which genes in the lipogenesis pathway were investigated in different cattle breeds, it was reported that *SREBP-1c* was downregulated in some breeds depending on ration ingredients (Zhou et al., 2022). The activity of SREBP-1c, which increases with PG consumption in the early stage of feeding, was similar to the Con in the liver as growth continued.

While the relations between biochemical parameters were as expected, notable correlations were determined between proteins and biochemical parameters. SREBP-1c and PPARγ are both transcription factors that play crucial roles in lipid metabolism (Özkan et al., 2022; Özkan & Yakan, 2019). In mammals, upregulation of SREBP-1c leads to increased expression of genes involved in lipogenesis, such as PPARγ. According to the correlation results of this study, it was determined that there were weak but significant positive relationships between these transcription factors in growing lambs, similar to the relationship in other mammals. In addition, a significant correlation was found between DGAT1 and ChREBP. ChREBP can bind to the promoter region of DGAT1 and lead to the synthesis of triglycerides in the liver (Benhamed et al., 2012). Although there are physiologically significant differences between ruminants and other mammals, it has been found that physiological activity in the liver is similar in determining the relationships between the main regulators of metabolism in this study.

In conclusion, based on the results of glucose and insulin parameters, as well as other parameters such as ALT and AST, it has been determined that long-term use of PG in lamb rations does not have adverse effects on the liver and metabolism. It was shown that PG affects metabolism at the molecular level in the liver in a dose-dependent manner (3 mL/kg live weight$^{0.75}$). As far as we know, this is the first study to investigate the metabolic effects of long-term PG use in lambs at the mRNA and protein levels in the liver.

ACKNOWLEDGMENTS

This study was financially supported by the Scientific and Technological Research Council of Turkiye (TUBİTAK) with a 119R076 project number.

CONFLICT OF INTEREST STATEMENT

The authors declare no conflict of interest for this article.

ORCID

Akın Yakan https://orcid.org/0000-0002-9248-828X
Baran Çamdeviren https://orcid.org/0000-0003-1508-7869

REFERENCES

Altwaty, N. H., Salem, L. M., & Mahrous, K. F. (2020). Single nucleotide polymorphisms in the growth hormone receptor gene and Alu1 polymorphisms in the diacylglycerol acyltransferase 1 gene as related to meat production in sheep. *Veterinary World*, *13*, 884–889. https://doi.org/10.14202/vetworld.2020.884-889

Assunçao, J. M. P. (2012). *Influence of slaughter season, muscle type and gluconeogenesis precursors on intramuscular fat quality of ruminant meats*. Ph.D. Thesis (Vol. 105). Univeridade Tecnica de Lisboa, Faculdade de Medicina Veterinaria.

Ayoub, A. G., Sabah, M. M., & El-Shorbagi, A. I. (2015). Effect of propylene glycol supplementation to feed of dairy cows on some biochemical measurements. *Egyptian Journal of Chemistry and Environmental Health*, *1*, 899–913. https://doi.org/10.21608/EJCEH.2015.253917

Benhamed, F., Denechaud, P. D., Lemoine, M., Robichon, C., Moldes, M., Bertrand-Michel, J., Ratziu, V., Serfaty, L., Housset, C., Capeau, J., Girard, J., Guillou, H., & Postic, C. (2012). The lipogenic transcription factor ChREBP dissociates hepatic steatosis from insulin resistance in mice and humans. *The Journal of Clinical Investigation*, *122*, 2176–2194. https://doi.org/10.1172/JCI41636

Benjamin, S., Prakasan, P., Sreedharan, S., Wright, A. D. G., & Spener, F. (2015). Pros and cons of CLA consumption: An insight from clinical evidences. *Nutrition and Metabolism*, *12*, 1–21, 4. https://doi.org/10.1186/1743-7075-12-4

Chiofalo, V., Todaro, M., Liotta, L., Margiotta, S., Manzo, T., & Leto, G. (2005). Effect of propylene glycol on pre-and postpartum performance by dairy ewes. *Small Ruminant Research*, *58*, 107–114. https://doi.org/10.1016/j.smallrumres.2004.09.001

Cui, J. X., Zeng, Q. F., Chen, W., Zhang, H., & Zeng, Y. Q. (2019). Analysis and preliminary validation of the molecular mechanism of fat deposition in fatty and lean pigs by high-throughput sequencing. *Mammalian Genome*, *30*, 71–80. https://doi.org/10.1007/s00335-019-09795-3

Deng, K., Ren, C., Fan, Y., Liu, Z., Zhang, G., Zhang, Y., You, P., & Wang, F. (2020). miR-27a is an important adipogenesis regulator associated with differential lipid accumulation between intramuscular and subcutaneous adipose tissues of sheep. *Domestic Animal Endocrinology*, *71*, 106393. https://doi.org/10.1016/j.domaniend.2019.106393

dos Santos, R. P., Sousa, L. F., de Sousa, J. T. L., Andrade, M. E. B., Macedo Júnior, G. D. L., & da Silva, S. P. (2015). Blood parameters of growing lambs sons of supplemented sheep with increasing levels of propylene glycol. *Revista Brasileira de Ciências Agrárias (Agrária)*, *10*(3), 473–478. https://doi.org/10.5039/agraria.v10i3a4924

Gallardo, M. A., Cárcamo, J. G., Hiller, B., Nuernberg, G., Nuernberg, K., & Dannenberger, D. (2015). Expression of lipid metabolism related genes in subcutaneous adipose tissue from Chilota lambs grazing on two different pasture types. *European Journal of Lipid Science and Technology*, *117*, 23–30. https://doi.org/10.1002/ejlt.201400033

Giráldez, F. J., Santos, N., Santos, A., Valdés, C., López, S., & Andrés, S. (2021). Fattening lambs with divergent residual feed intakes and weight gains: Unravelling mechanisms driving feed efficiency. *Animal Feed Science and Technology*, *273*, 114821. https://doi.org/10.1016/j.anifeedsci.2021.114821

Gonzales-Barron, U., Popova, T., Piedra, R. B., Tolsdorf, A., Geß, A., Pires, J., Domínguez, R., Chiesa, F., Brugiapaglia, A., Viola, I., Battaglini, L. M., Baratta, M., Lorenzo, J. M., & Cadavez, V. A. P. (2021). Fatty acid composition of lamb meat from Italian and German local breeds. *Small Ruminant Research*, *200*, 106384. https://doi.org/10.1016/j.smallrumres.2021.106384

Herman, M. A., Peroni, O. D., Villoria, J., Schön, M. R., Abumrad, N. A., Blüher, M., Klein, S., & Kahn, B. B. (2012). A novel ChREBP isoform in adipose tissue regulates systemic glucose metabolism. *Nature*, *484*, 333–338. https://doi.org/10.1038/nature10986

Hiller, B., Hocquette, J. F., Cassar-Malek, I., Nuernberg, G., & Nuernberg, K. (2012). Dietary n-3 PUFA affect lipid metabolism and tissue function-related genes in bovine muscle. *British Journal of Nutrition*, *108*, 858–863. https://doi.org/10.1017/S0007114511006179

Hoffman, M. L., Rokosa, M. A., Zinn, S. A., Hoagland, T. A., & Govoni, K. E. (2014). Poor maternal nutrition during gestation in sheep reduces circulating concentrations of insulin-like growth factor-I and insulin-like growth factor binding protein-3 in offspring. *Domestic Animal Endocrinology*, *49*, 39–48. https://doi.org/10.1016/j.domaniend.2014.05.002

Kalyesubula, M., Rosov, A., Alon, T., Moallem, U., & Dvir, H. (2019). Intravenous infusions of glycerol versus propylene glycol for the regulation of negative energy balance in sheep: A randomized trial. *Animals*, *9*(10), 731. https://doi.org/10.3390/ani9100731

Kenyon, P. R., & Corner-Thomas, R. A. (2022). Breeding ewe lambs: An Australasian perspective. *Animals*, *12*, 3207. https://doi.org/10.3390/ani12223207ayou

Lehrke, M., & Lazar, M. A. (2005). The many faces of PPARγ. *Cell*, *123*, 993–999. https://doi.org/10.1016/j.cell.2005.11.026

Livak, K. J., & Schmittgen, T. D. (2001). Analysis of relative gene expression data using real-time quantitative PCR and the 2− ΔΔCT method. *Methods*, *25*, 402–408. https://doi.org/10.1006/meth.2001.1262

Lopez-Vargas, A., Tristán, T. Q., Segura, C. U. H., Barragán, R. M., Flores, A. G. V., Martínez, R. O., & Millán, C. L. H. (2022). Effect of propylene glycol on blood metabolıtes, ruminal and productıve parameters of growıng-fınıshıng lambs. *Tropical and Subtropical Agroecosystems*, *25*, 80. https://doi.org/10.56369/tsaes.4137

Murray, R. K., Bender, D. A., Botham, K. M., Kenelly, P. J., Rodwell, V. W., & Weil, P. A. (2009). *Harper's illustrated biochemistry, lipids of physiologic significance* (28th ed.) (pp. 121–131). The McGraw- Hill Company.

Özkan, H., Kutlu, T., Yakan, A., & Özsoy, Ş. Y. (2022). Molecular, biochemical, and histopathological effects of long-term low and high-percentage fructose consumption on the liver in rats. *Ankara Üniversitesi Veteriner Fakültesi Dergisi*, *69*, 409–417. https://doi.org/10.33988/auvfd.855124

Özkan, H., & Yakan, A. (2019). Dietary high calories from sunflower oil, sucrose and fructose sources alters lipogenic genes expression levels in liver and skeletal muscle in rats. *Annals of Hepatology*, *18*, 715–724. https://doi.org/10.1016/j.aohep.2019.03.013

Pirzad, M., Ansarı, M. S., & Edrıss, M. A. (2014). Influence of the bovine acyl-CoA: Diacylglycerol acyltransferase1 (DGAT1) K232A on milk production and somatic cell score Holstein cows. *International Journal of Advanced Biological and Biomedical Research*, *2*, 1300–1306.

Rio, D. C., Ares, M., Hannon, G. J., & Nilsen, T. W. (2010). Purification of RNA using TRIzol (TRI reagent). *Cold Spring Harbor Protocols*, *2010*, pdb-prot5439. https://doi.org/10.1101/pdb.prot5439

Rukkwamsuk, T., Rungruang, S., Choothesa, A., & Wensing, T. (2005). Effect of propylene glycol on fatty liver development and hepatic fructose 1, 6 bisphosphatase activity in periparturient dairy cows. *Livestock Production Science*, *95*, 95–102. https://doi.org/10.1016/j.livprodsci.2004.12.006

Su, R., Luo, Y., Wang, B., Hou, Y., Zhao, L., Su, L., Yao, D., Qian, Y., & Jin, Y. (2019). Effects of physical exercise on meat quality characteristics of Sunit sheep. *Small Ruminant Research*, *173*, 54–58. https://doi.org/10.1016/j.smallrumres.2019.02.002

Udum, D., Üstüner, H., Belenli, D., & Uzabacı, E. (2013). Kıvırcık Irkı Koyunlara Ait Bazı Biyokimyasal Değerlerin Belirlenmesi Üzerine Bir

Araştırma. *Uludağ Üniversitesi Veteriner Fakültesi Dergisi*, *32*, 7–10. https://doi.org/10.30782/uluvfd.163479

Urrutia, O., Mendizabal, J. A., Insausti, K., Soret, B., Purroy, A., & Arana, A. (2016). Effects of addition of linseed and marine algae to the diet on adipose tissue development, fatty acid profile, lipogenic gene expression, and meat quality in lambs. *PLoS ONE*, *11*, e0156765. https://doi.org/10.1371/journal.pone.0156765

Wang, B., Yang, L., Luo, Y., Su, R., Su, L., Zhao, L., & Jin, Y. E. (2018). Effects of feeding regimens on meat quality, fatty acid composition and metabolism as related to gene expression in Chinese Sunit sheep. *Small Ruminant Research*, *169*, 127–133. https://doi.org/10.1016/j.smallrumres.2018.08.006

Yan, W., Kan, X., Wang, Y., & Zhang, Y. (2023). Expression of key genes involved in lipid deposition in intramuscular adipocytes of sheep under high glucose conditions. *Journal of Animal Physiology and Animal Nutrition*, *107*, 444–452. https://doi.org/10.1111/jpn.13750

Zhang, F., Nan, X., Wang, H., Zhao, Y., Guo, Y., & Xiong, B. (2020). Effects of propylene glycol on negative energy balance of postpartum dairy cows. *Animals*, *10*, 1526. https://doi.org/10.3390/ani10091526

Zhou, M., Zhu, Z., Sun, H. Z., Zhao, K., Dugan, M. E., Bruce, H., Fitzsimmons, C., Li, C., & Guan, L. L. (2022). Breed dependent regulatory mechanisms of beneficial and non-beneficial fatty acid profiles in subcutaneous adipose tissue in cattle with divergent feed efficiency. *Scientific Reports*, *12*, 4612. https://doi.org/10.1038/s41598-022-08572-8

How to cite this article: Yakan, A., Özkan, H., Kaya, U., Keçeli, H. H., Dalkiran, S., Karaaslan, I., Ünal, N., Akçay, A., Arslan, K., Akyüz, B., Güngör, G., Çamdeviren, B., Küçükoflaz, M., Sariözkan, S., & Özbeyaz, C. (2023). Effects of propylene glycol used at different doses in Akkaraman lambs rations on metabolism-related parameters and liver gene and protein expression during different feeding periods. *Animal Science Journal*, *94*(1), e13886. https://doi.org/10.1111/asj.13886