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Gene expression levels in some candidate genes for mastitis resistance, milk yield, and milk quality of goats reared under different feeding systems

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Abstract: This study aimed to identify the expression levels of some candidate genes for mastitis resistance (*LTF* and *BRCA1*), milk yield (*POU1F1* and *IGF-1*), and milk quality (*PPARγ* and *CSN2*) in different feeding systems (pen- and pasture-based) used for Damascus goats. Milk samples were taken from all goats in both pen and pasture groups at 4 times, at the 1st, 3rd, 5th, and 7th months of the study. It was found that, compared to the pen group, the *LTF* and *CSN2* genes in the pasture group had significant levels of upregulation in the 7th month of lactation (3.538- and 5.436-fold, respectively), the *PPARγ* gene had downregulation at the beginning of lactation (0.399-fold), and the *IGF-1* gene had upregulation in the 3rd month of lactation (2.313-fold) but had significant levels of downregulation (2.332-fold) in the 5th month of lactation. It was determined that *LTF* gene expression levels in goats might be used as a criterion in identifying mastitis resistance, *IGF-1* for lactation milk yield, and *PPARγ* for milk fat yield. This study is the first report that presents *LTF*, *BRCA1*, *IGF-1*, and *PPARγ* gene expression levels in goat milk and udder epithelial cells and the effect of *BRCA1*, *POU1F1*, and *PPARγ* genes on milk protein yield.

Key words: Goat, gene expression, *LTF* and *BRCA1*, *POU1F1* and *IGF-1*, *PPARγ* and *CSN2*

1. Introduction

Since goats have the organic ability to make good use of pastures, they are generally bred semiintensively throughout the world, especially in Asia and Africa (1). However, they can also be bred intensively in regions with no pastures. Goats are suitable for semiintensive production systems, which reduce costs and increase pasture quality for the next vegetation period as a result of the urine and excrement left by goats (2). Goats bred in semiintensive conditions receive higher amounts of cellulose compared to goats bred in intensive conditions.

The fact that there is a significant relationship between feeding and gene expression levels has been proven by studies conducted on many animal species (3,4). Goat breeding is done throughout the world in arid and warm climates with extensive or semiintensive systems, but in both cases goat breeding is based on pasture use. Pens are sometimes intensively used in regions where milk production is significant. It is known that fodder consumption and content can affect milk yield and milk quality. Similarly, starvation and ad libitum feeding situations are also known to make changes in gene expression profiles. In their study on Alpine goats, Ollier et al. (5) found that the expression levels of 161 genes in the udder tissue that affect milk yield and quality criteria directly changed with feeding.

In goat breeding, it is important to know the candidate gene expression levels that can affect mastitis resistance, milk yield, and milk quality in breeding via pasture or pen. This study aimed to examine the following in milk somatic cells: for mastitis resistance, the expression levels of *LTF* (lactoferrin), which inhibits proinflammatory cytokines such as TNF-α, IL-1β, and IL-6 (6), and the expression levels of *BRCA1* (breast cancer type 1), which has a proliferative impact on udder epithelium alveoli (7,8); for milk yield, the expression levels of *POU1F1* (POU-domain class 1 transcription factor 1), which acts as a positive regulator of prolactin (PRL), growth hormone (growth hormone), and thyroid-stimulating hormone (TSH) (9), and the expression levels of *IGF-1* (insulin-like growth hormone), which has the impact of increasing arterial infusion in mammary glands as well as decreasing apoptotic losses of udder epithelial cells (10); for milk fat levels as milk quality, the expression levels of *PPARγ* (peroxisome proliferator-activated receptor gamma), which triggers lipogenesis and adipogenesis (11); and for

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milk protein ratio, the expression levels of *CSN2* (β-casein predominance), which acts as a molecular marker in terms of casein production in udder alveolar cells (12).

2. Materials and methods

2.1. Animal materials and research design

This study was approved by the Mustafa Kemal University Animal Experiments Local Ethics Committee (approval number: 2014-07/10). The study material comprised 24 Damascus goats. Goats were randomly assigned to pasture (n = 12) or pen (n = 12) based on their ages and birth types homogeneously. Ages of pasture and pen groups were 4.60 ± 0.37 and 4.36 ± 0.36 years, respectively, and there was no difference between the groups in terms of ages. Along the same lines, each group included goats that had 2 single and 10 twin births so as not to generate any differences based on birth type. Prior to the start of the study, goats were vetted for general health and udder health with the California mastitis test; thus, the study started with healthy goats whose general health and udder health were controlled throughout the study.

2.1.1. Pen group

Goats in this group were kept in a pen with at least 4 m^2/head area. The kids were kept with their mothers until they were weaned, but they were separated from their mothers on milk yield test days. Until they were weaned, the kids were separated from their mothers at 0600 hours the day before milk yield test day and milking was completed. Milking was repeated at 0600 hours the next morning and yield for 24 h was identified. Throughout the study, the goats were given 1.2 kg/head concentrated feed and 1 kg/head wheat straw on a daily basis.

2.1.2. Pasture group

Throughout lactation, goats in this group were taken to pasture at 0700 hours if there was no downpour, and they were brought back to the pen before it was dark. Until the kids were weaned, they were left with their mothers when the goats were returned to the pen and were separated again in the morning when their mothers were taken out to the pasture. On milk control days, the kids were not allowed to nurse when their mothers were back in the pen and milking was done at 0600 hours, before they were let out to the pasture, to control milk yield. Hence, milk yield for 24 h was identified. Goats were given 0.6 kg/head concentrated feed in the pen on a daily basis and were penned in an area of at least 4 m^2/head.

2.2. Collecting milk samples and milk quality characteristics

Milk control days were standardized based on the interpolation method. Lactation milk yield was calculated according to International Committee for Animal Recording. Sample collection was undertaken 4 times with 2-month intervals in the 1st, 3rd, 5th, and 7th months of lactation. Since the goats in the study groups dried up at 7–7.5 months of lactation, the last samples were collected in the 7th month of lactation. During sample collection days, single milking was undertaken by hand, the routine practice of the enterprise, at 0600 hours. Before milking, nipples were washed with warm antiseptic water, wiped with single-use sterile wipes, and dried. Milk samples were collected as 150 mL (3 × 50 mL) in 50-mL sterile Falcon tubes after the first few squeezes at the nipples were poured. Milk samples were collected in approximately 15–20 min and all samples were immediately transferred (within approximately 10–15 min) to the laboratory in an icebox. Approximately 100 mL of each sample (2 × 50 mL) was used for RNA isolation, and 50 mL (1 × 50 mL) was used to determine milk quality characteristics.

The milk that was brought to the lab was tested for pH by using a portable pH meter (Mettler-Toledo, InLab). Milk samples, which were supplemented with chemical preservative tablets (Microtab II), were sent to İstanbul University, Veterinary Faculty, Department of Animal Breeding Labs, with 4 °C cold chain for fat, protein, and somatic cell count (SCC) analyses. The milk samples that arrived at the laboratory were heated in a 40 °C water bath and milk fat, protein, and SCC values were identified. The analyses were done using a Combi 150 (Bentley) device formed by integrating a SCC device (Somacount 150) and the milk-component measurement device (Bentley 150), which worked with the flow cytometry analysis method.

2.3. RNA isolation, cDNA synthesis, and RT-qPCR application

Each sample was brought to the lab as 100 mL (2 × 50 mL) and was later centrifuged at 1800 × *g* for 15 min at 4 °C. The cream that formed at the top of the tubes was removed with the help of a spatula, and the remaining milk at the top was removed until 5 mL was left at the bottom of the tube. Later, the two 50-mL samples were combined in a single tube, into which phosphate-buffered saline (PBS) (pH 7.2) at 4 °C was added. This sample was again centrifuged at 1800 × *g* for 10 min at 4 °C and supernatant was removed. PBS containing EDTA with a final concentration of 0.5 mM was added to the cell pellet that remained at the bottom of the tube, and it was centrifuged at the same speed for the same duration to remove casein micelles on the cell. In order to obtain the final cell pellet, a final PBS flushing was done by applying the centrifuge process at the same speed for the same duration.

RNA was isolated from the obtained cell pellet according to the TRI-Reagent protocol (Protocol No.: T9424, Sigma-Aldrich, USA). DEPC water (20 µL) was added to the obtained total RNA pellet, and concentration and purity measurements were done with the help of NanoDrop (Merinton SMA 1000). Later, the suitable

samples were run in gel electrophoresis to control RNA integrity. Samples that were deemed usable were frozen in liquid nitrogen and kept at –85 °C until analyses. Unusable samples were thrown away, and new samples were collected to obtain appropriate ones to be kept for analyses.

Before starting cDNA synthesis, samples were treated with DNase (DNase I, RNase-free, Thermo Fisher Scientific, USA) against possible DNA contamination. Later, cDNA synthesis was done from the total RNA according to the cDNA synthesis kit protocol (RevertAid First Strand cDNA Synthesis Kit, Thermo Fisher Scientific), and it was covered with DEPC water until the final volume was 100 µL.

The *RPLP0* gene was used as an internal control (13) in order to determine the expression levels of the *LTF*, *BRCA1*, *POU1F1*, *IGF-1*, *PPARγ*, and *CSN2* genes. Each sample was amplified in triplicate on RT-qPCR based on SYBR Green (Bio-Rad, USA). The RT-qPCR protocol was adjusted to 10 min at 95 °C, 15 s at 95 °C, 60 s at 60 °C (40 cycles), and 30 s at 72 °C. Table 1 presents the sizes and sequences of the primers that were used. While internal control gene primers were designed by Jarczak et al. (13), primers of target genes were designed by the present authors. Melting curves of all the primers used for the amplified genes had single peaks; they were used later.

2.4. ELISA application

Specific ELISA kits were used in the study based on goat type (CUSOBIO for CSN2, China; SunRed for other proteins, China). The protocols of the kits were duplicated. The samples were skimmed milk.

2.5. Evaluation of data

The study utilized analysis of variance for repeated measures for the data obtained to study daily milk yield in different periods of lactation (1st, 3rd, 5th, and 7th months) and some milk quality parameters, as well as the levels of LTF, BRCA1, POU1F1, IGF-1, PPARγ, and CSN2 proteins in milk. The statistical model is as follows:

$Y_{ij} = \mu + \Pi_i + T_j + e_{ij}$ (i = 1, …., N; j = 1, ….., n);

μ = constant;

Π_i = experimental groups (feeding system);

T_j = lactation periods time (1st, 3rd, 5th, and 7th lactation months);

e_{ij} = error for subject i and time j.

The differences between pasture and pen groups for milk yield, some milk quality parameters, lactation period, and LTF, BRCA1, POU1F1, IGF-1, PPARγ, and CSN2 proteins in milk were analyzed with a t-test in SPSS 14.0. Expression levels of the *LTF*, *BRCA1*, *POU1F1*, *IGF-1*, *PPARγ*, and *CSN2* genes were determined according to the $2^{\Delta\Delta Ct}$ method using the t-test procedure (14). Level of significance between groups was accepted as $P < 0.05$ in all analyses.

3. Results

Average values for milk yield throughout lactation and average values for some quality characteristics (Table 2) were identified after lactation and the start of the collection of milk samples. While there were significant differences between pasture and pen groups in terms of lactation milk yield (283.73 kg and 184.37 kg; $P < 0.001$), no significant

Table 1. Primer sequence and amplicon size.

Primers	Sequence	Amplicon size (bp)
LTF	Forward: CAA GTG TGT GCC CAA CTC TA Reverse: GCT CTC TCC ATT CGT GTT CTC	130
BRCA1	Forward: CCA GCC AGC CGC ATA TAT TA Reverse: GGC TGT GGA AGT ACT GAA GAG	114
POU1F1	Forward: CTG GAG AGA CAC TTT GGA GAA C Reverse: CCA AAC CCT CAC CAC TTC TT	99
IGF-1	Forward: TCC TCC TCG CAT CTC TTC TAT Reverse: GAG AGC ATC CAC CAA CTC AG	105
PPARγ	Forward: GTT CAA CGC GCT GGA ATT AG Reverse: GGG CTT CAC ATT CAG CAA AC	97
CSN2	Forward: TCC TTC ACT TCT TCT CCT CTA CT Reverse: TTG AGT TCT TCC TGC TCT CTT	111
RPLP0	Forward: CAA CCC TGA AGT GCT TGA CAT Reverse: AGG CAG ATG GAT CAG CCA	227

Table 2. Milk yield and milk traits (mean ± standard deviation).

Traits	Feeding systems	Lactation periods				P
		1st month	3rd month	5th month	7th month	
Daily milk yield (g)	Pasture (n = 12)	1001.41 ± 113.36^{c}	2100.40 ± 154.66^{a}	1320.29 ± 92.58^{b}	549.66 ± 67.86^{d}	***
	Pen (n = 12)	809.13 ± 68.77^{b}	1206.83 ± 156.33^{a}	936.00 ± 62.24^{b}	364.33 ± 34.47^{c}	***
	P	-	**	**	*	
Milk fat ratio (%)	Pasture (n = 12)	3.50 ± 0.08^{a}	2.80 ± 0.20^{b}	3.36 ± 0.21^{a}	3.42 ± 0.29^{a}	*
	Pen (n = 12)	3.18 ± 0.14	3.02 ± 0.14	3.61 ± 0.15	3.68 ± 0.39	-
	P	-	-	-	-	
Milk total protein ratio (%)	Pasture (n = 12)	2.74 ± 0.08^{b}	3.20 ± 0.06^{a}	2.99 ± 0.16^{b}	3.34 ± 0.10^{a}	**
	Pen (n = 12)	2.62 ± 0.07^{c}	2.94 ± 0.08^{b}	3.08 ± 0.09^{b}	3.34 ± 0.08^{a}	***
	P	-	*	-	-	
Milk pH	Pasture (n = 12)	6.62 ± 0.02^{ac}	6.56 ± 0.01^{a}	6.71 ± 0.03^{b}	6.66 ± 0.03^{bc}	*
	Pen (n = 12)	6.66 ± 0.03^{ab}	6.62 ± 0.01^{b}	6.68 ± 0.01^{a}	6.71 ± 0.01^{a}	*
	P	-	*	-	-	
SCC (×1000/mL)	Pasture (n = 12)	762.12 ± 137.99	624.20 ± 216.58	833.41 ± 98.74	1111.16 ± 124.85	-
	Pen (n = 12)	488.04 ± 107.86^{b}	730.08 ± 189.23^{b}	699.25 ± 104.84^{b}	1468.66 ± 202.95^{a}	**
	P	-	-	-	-	
Lactation milk yield (kg)	Pasture (n = 12)	283.73 ± 16.19				
	Pen (n = 12)	184.37 ± 13.83				
	P	***				
Lactation length (day)	Pasture (n = 12)	224.41 ± 11.25				
	Pen (n = 12)	210.58 ± 2.16				
	P	-				

$^{a, b, c, d}$: Means with different letters in rows differ significantly ($P < 0.05$).
-: $P > 0.05$; *: $P < 0.05$; **: $P < 0.01$; ***: $P < 0.001$.

differences were detected between the groups in terms of lactation period (224.41 days and 210.58 days). Milk fat content was similar in pasture and pen groups, and the lowest milk fat ratio was identified in the 3rd month of lactation in both groups. Milk pH was generally similar between the groups (apart from the 3rd month of lactation); it was found to become higher in both groups after the 3rd month of lactation, but presented significant differences ($P < 0.05$). Milk SCC was found to be similar throughout lactation for the pasture group, but it presented significant changes in the pen group at the end of the lactation period compared to the beginning ($P < 0.01$).

Tables 3–5 display the expression changes calculated from Ct values obtained with the help of RT-qPCR reaction. Since the environmental effects were more controlled in the pen group compared to the pasture group, the pen group was regarded as the control group, while the pasture group was the experimental group in this study (Table 3). At the same time, the pen and pasture groups were taken as controls separately for previous and subsequent samples in different periods of lactation, and fold changes in gene expressions were calculated (Tables 4 and 5).

Significant upregulation was detected for the *LTF* and *PPARγ* genes at the end of lactation in fold change situations where the pen group was regarded as the control and the pasture group as the experimental group ($P < 0.001$). While the *PPARγ* gene (0.399) had downregulation in the 1st month of lactation and the *BRCA1* gene (0.379) in the 3rd month of lactation, this process was not found to be significant.

Table 3. Fold changes of genes in the pasture group according to pen group (n = 12).

Genes	1st month	3rd month	5th month	7th month
LTF	1.013	0.718	0.961	3.538***
BRCA1	0.738	0.379	1.303	0.796
POU1F1	0.580	1.121	1.429	0.850
IGF-1	0.733	2.313	2.332*	1.276
PPARγ	0.399	0.880	1.036	0.793
CSN2	1.685	1.392	1.383	5.436**

Red color is downregulation; blue color is upregulation.
*: P < 0.05; **: P < 0.01; ***: P < 0.001.

Table 4. Fold changes of genes in different lactation periods in the pen group.

Groups		*LTF*	*BRCA1*	*POU1F1*	*IGF-1*	*PPARγ*	*CSN2*
Control (n = 12)	Test (n = 12)						
1st month of lactation	3rd month of lactation	1.382	0.330	0.046	0.083	0.114	6.631**
1st month of lactation	5th month of lactation	1.475	0.807	0.016	0.099	0.278*	6.399***
1st month of lactation	7th month of lactation	1.273	0.708	0.085	0.146	0.262*	1.968
3rd month of lactation	5th month of lactation	1.067	2.443*	0.352**	1.183	2.444*	0.965
3rd month of lactation	7th month of lactation	0.921	2.144	1.868	1.754	2.302*	0.297
5th month of lactation	7th month of lactation	0.864	0.877	5.299***	1.482	0.942	0.308

Red color is downregulation; blue color is upregulation.
*: P < 0.05; **: P < 0.01; ***: P < 0.001.

Table 5. Fold changes of genes in different lactation periods in the pasture group.

Groups		*LTF*	*BRCA1*	*POU1F1*	*IGF-1*	*PPARγ*	*CSN2*
Control (n = 12)	Test (n = 12)						
1st month of lactation	3rd month of lactation	0.707	0.190	0.098	0.211	0.260	2.554
1st month of lactation	5th month of lactation	1.224	1.090	0.047*	0.274*	0.666	3.656*
1st month of lactation	7th month of lactation	2.954**	0.945	0.112	0.259*	0.747	2.565
3rd month of lactation	5th month of lactation	1.733	5.732*	0.478*	1.299	2.561	1.432
3rd month of lactation	7th month of lactation	4.182***	4.967*	1.138	1.226	2.870	1.005
5th month of lactation	7th month of lactation	2.413	0.866	2.384**	0.944	1.121	0.702

Red color is downregulation; blue color is upregulation.
*: P < 0.05; **: P < 0.01; ***: P < 0.001.

Examination of the effects of different periods of lactation compared with one another showed that the *CSN2* gene had significant upregulation in the 3rd month of lactation compared to the 1st month (P < 0.001), whereas *POU1F1*, *IGF-1*, and *BRCA1* had downregulation. As in the 3rd month of lactation, the *CSN2* gene had significant upregulation in the 5th month compared to the 1st month (P < 0.001).

In the pasture group, the *LTF* gene had continuous upregulation during later periods of lactation compared to earlier periods. However, the *CSN2* gene had upregulation in the later periods of lactation compared to the 1st month of lactation. It was found that *POU1F1* and *IGF-1* had downregulation in the later periods of lactation compared to the 1st month of lactation.

Table 6 presents the levels of milk protein determined by genes whose expression levels were identified.

4. Discussion

4.1. *LTF*

While the *LTF* gene did not change in the 1st, 3rd, and 5th months of lactation in the pasture group compared to the pen group, it was expressed 3.538-fold during the 7th month of lactation ($P < 0.001$). This increase was confirmed with the *LTF* levels in milk (263.5 µg/mL compared to 168.93; $P < 0.05$). While there is no information in the literature related to *LTF* gene expression levels in goat milk, it was observed that *LTF* milk level is similar to the values reported for German fawn and German white goats. The regulation mechanism of *LTF* uses more than one pathway, such as steroid hormone, growth factor, and kinase cascade. Estrogen synthesis plays an especially important role in the steroid hormone pathway (15). Therefore, the differences in *LTF* gene expression level in the 7th month of lactation for pasture and pen groups can be interpreted in several ways. First of all, pastures get dry in the 7th month of lactation (July, August), and coarse fodder with high cellulose content was consumed. Since these goats do not stay in the pen, the difference may be based on food intake, as was also reported by Boutinaud et al. (16) and Ollier et al. (5). It was also reported that the months of July and August, which corresponded to the 7th month of lactation, were extremely hot, and increased water consumption due to heat may affect goat physiology (17). In this context, it is thought that the immune response generated by the physiological change based on exposure of the pasture group goats to different environmental impacts may cause changes in *LTF* gene expression. Secondly, the

Table 6. The protein yields determined in milk by *LTF*, *BRCA1*, *POU1F1*, *IGF-1*, *PPARγ*, and *CSN2* genes (mean ± standard deviation).

Protein	Feeding systems	Lactation period				P
		1st month	3rd month	5th month	7th month	
LTF (µg/mL)	Pasture (n = 10)	120.92 ± 33.57^{a}	227.72 ± 48.73^{ab}	136.60 ± 26.45^{a}	263.50 ± 27.22^{b}	*
	Pen (n = 10)	179.70 ± 51.83	104.70 ± 15.56	130.12 ± 23.32	168.93 ± 28.76	-
	P	-	*	-	*	
BRCA1 (ng/L)	Pasture (n = 10)	167.21 ± 25.81^{a}	117.35 ± 11.40^{a}	87.28 ± 4.77^{b}	91.38 ± 10.87^{ab}	*
	Pen (n = 10)	113.00 ± 16.16	95.55 ± 11.03	105.14 ± 12.28	90.00 ± 12.23	-
	P	-	-	-	-	
POU1F1 (ng/L)	Pasture (n = 10)	167.62 ± 18.50^{a}	93.47 ± 12.19^{b}	107.03 ± 14.80^{b}	110.26 ± 8.83^{b}	**
	Pen (n = 10)	188.35 ± 36.81^{ac}	91.73 ± 8.27^{b}	117.47 ± 12.91^{bc}	209.24 ± 29.17^{a}	*
	P	-	-	-	**	
IGF-1 (ng/mL)	Pasture (n = 10)	91.08 ± 8.61^{a}	55.89 ± 8.68^{ab}	42.13 ± 4.89^{b}	66.50 ± 11.30^{a}	*
	Pen (n = 10)	41.05 ± 6.78	30.78 ± 5.12	52.42 ± 11.18	51.24 ± 11.67	-
	P	***	*	-	-	
PPARγ (ng/mL)	Pasture (n = 10)	0.49 ± 0.09	0.29 ± 0.06	0.28 ± 0.05	0.44 ± 0.05	-
	Pen (n = 10)	0.50 ± 0.09^{a}	0.30 ± 0.03^{b}	0.33 ± 0.04^{b}	0.23 ± 0.04^{b}	*
	P	-	-	-	**	
CSN2 (mg/mL)	Pasture (n = 10)	8.76 ± 2.29^{a}	22.50 ± 0.43^{b}	20.77 ± 1.20^{b}	17.03 ± 1.44^{c}	**
	Pen (n = 10)	18.82 ± 1.56^{a}	21.01 ± 1.26^{a}	19.30 ± 0.90^{a}	11.21 ± 1.04^{b}	**
	P	**	-	-	**	

*: $P < 0.05$; **: $P < 0.01$; ***: $P < 0.001$. Means with different letters differ significantly ($P < 0.05$).

LTF gene expression level may have increased to stimulate LTF synthesis due to increases in parasite load in the pasture in this period. Finally, estrus in goats may point to activated hormone mechanisms during this period due to the relationship between *LTF* regulation and estrogen (15). Breeders report that, compared to goats bred in pens, goats bred in pastures display early estrus and have more distinct estrous findings.

In terms of mastitis resistance, the *LTF* gene and LTF protein are associated with milk SCC in cows (6,18,19). However, in goats, milk SCC is affected by many variables such as lactation milk yield, lactation number, birth season, milk protein and fat contents, and breed (20,21). The US Food and Drug Administration reports the legal limit for SCC for goats as 1000×10^3 cells/mL (22). SCC, which can be used in general as a mastitis determinant in cattle (23,24), cannot be used in goats. Although it is reported that there may be no relationship between SCC and mastitis, *LTF* gene expression levels in goats might be an important indicator for mastitis resistance, since the majority of somatic cells in goats are composed of epithelial cells due to milk synthesis mechanisms and *LTF* gene expression in epithelial cells is 20 times more than in leukocytes (23).

When the pen and pasture groups were followed separately based on lactation periods, no changes in expression were observed in the pen group, but the pasture group displayed more upregulation in the 7th month of lactation compared to the 1st, 3rd, and 5th months (2.954, $P < 0.01$; 4.182, $P < 0.001$; and 2.413, $P > 0.05$, respectively). Milk level of LTF was found to be highest in the pasture group in the 7th month of lactation at 263.5 µg/mL, which was significantly higher than during the other periods of lactation ($P < 0.05$). In this sense, a consistent trend was identified for the pasture group in terms of *LTF* gene expression and LTF protein level in milk. Hence, it is thought that milk somatic cell *LTF* gene expression may be a criterion for mastitis resistance for the pasture group. It was identified that LTF milk somatic cell gene expression levels were affected by different feeding systems.

4.2. *BRCA1*

The *BRCA1* gene is known to be responsible for controlling pituitary gland- and ovarian-originated hormones such as growth hormone, PRL, progesterone, and estrogen in normal breast channels and gland cell proliferation (7,25). Studies conducted on mice (26,27) showed that *BRCA1* upregulation occurred during puberty and pregnancy. Breast alveoli cell proliferation that starts with this upregulation may provide information on lactation performance and breast health. It is known that there is a high positive correlation between milk yield and mastitis (28). Extreme milk secretions from breast alveoli epithelial cells will reduce resistance against diseases and therefore mastitis can occur as the first step of infection.

When the *BRCA1* gene expression level was assessed in this study based on feeding systems, it was determined that the pasture group did not undergo significant changes throughout lactation and only experienced an insignificant level of downregulation in the 3rd month of lactation. When the level of BRCA1 protein in milk was examined, no differences were found between pasture and pen groups, and this result was found to be confirmatory for gene expression level.

When pasture and pen groups were evaluated independently in different periods of lactation, a significant upregulation was detected for both groups between the 3rd and 5th months of lactation (for pasture group, 5.732; for pen group, 2.443) and between the 3rd and 7th months of lactation (for pasture group, 4.967; for pen group, 2.144). It was not possible to form any relationship between the data when these differences were evaluated in terms of both mastitis and milk yield. Clinical mastitis was not observed in goats during this period, and it was found that milk yield had decreased in the later months of lactation. MacLachlan et al. (29) and Yuan et al. (8) reported that *BRCA1* may be effective on cells through other mechanisms in addition to cell proliferation and a continuation of genomic stability. These data point to the fact that other mechanisms may be effective on the milk expression level and phenotypic image of *BRCA1*.

The level of BRCA1 protein in milk was found to be higher in the 1st month of lactation compared to other months in both pasture and pen groups. This difference was significant in the pasture group ($P < 0.05$). It is thought that BRCA1 milk level can be higher at the beginning of lactation due to proliferation of udder epithelial cells. Therefore, the somatic cell expression level of *BRCA1* in goats is important since it can provide information about udder alveoli epithelial cell proliferation at the start of lactation. The higher the epithelial proliferation, the higher will be the formation of epithelial cells that can produce milk.

4.3. *POU1F1*

POU1F1 is a gene with lifelong somatotropic, lactotropic, and thyroidropic influences due to embryonic differentiation. It is known to be related to milk yield based on its lactotropic influence (30,31). Ollier et al. (5) reported that leaving goats hungry for 48 h generated significant downregulation in *POU1F1* gene expression level. While this study identified significant differences in lactation of the milk yield of pasture- and pen-based goats (Table 2), similar *POU1F1* gene expression levels (multifold change between 0.580 and 1.429; $P < 0.05$) were found for both groups. On the other hand, the milk yield level for different lactation periods for both feeding groups displayed significant differences ($P < 0.001$), and the *POU1F1* gene had downregulation for months 1–3, 1–5, 1–7, and 3–5 of lactation for both groups (Table 4 for pen group, Table 5 for pasture group), while significant

upregulation was identified for months 5–7 of lactation ($P < 0.001$ for pen group; $P < 0.01$ for pasture group). Just as no relationship could be established between lactation milk yield and *POU1F1* gene expression levels, no relationships could be established among POU1F1 protein milk level, gene expression level, and lactation milk yield. The *POU1F1* gene has exon region variation, and this gene has gene interactions such as penetrative influence and expressivity. Based on these influences, it can generate variations in characteristics such as growth, carcass, milk, and fleece (32). The main reasons why this study could not make connections between *POU1F1* gene expression levels and milk yield may be related to its effects on thyroid metabolism along with GH and PRL, its variation in the exon region, the penetrative impact of the gene, and its expressivity. On the other hand, studies on pigs, cattle, and goats (9,32) showed that the polymorphic structure of the *POU1F1* gene had a significant impact on lactation. In their study on sheep, Bastos et al. (33,34) reported that *POU1F1* had variants called β, γ, and δ; *POU1F1-β* among these variants promoted PRL, and *POU1F1-γ* and *POU1F1-δ* had no promoter activity. The fact that no relationships were established in the present study between milk yield in goats and PRL of *POU1F1*, which is directly related to milk yield, may be related to existence of variants such as *POU1F1-β*, *-γ*, and *-δ*, similar to studies on sheep. Zhao et al. (31) stated that milk yield in goats is controlled by many polygenes with small impacts. Another reason why direct relationships between *POU1F1* gene expression level and lactation milk yield were not established may be related to the fact that a great number of genes may have an impact on milk yield.

4.4. *IGF-1*

Secretor activity of mammary glands throughout lactation is a complex process that is regulated via many hormonal and paracrine interactions (35). In this process, *IGF-1* plays an important role in almost all tissue and organ cell proliferation with its metabolic impact on growth and development pathways (36). While it was reported that *IGF-1* had no relationship in pigs with lactation milk yield (37), the studies on goats identified relationships between both blood and milk plasma IGF-1 levels and milk yield (35,38). In this study, the IGF-1 level (between 42.13 and 91.08 ng/mL) measured in the pasture group with higher milk yield compared to the pen group (pasture group 283.73 kg, pen group 184.37 kg; $P < 0.001$) was higher than the milk IGF-1 level in the pen group (between 30.78 and 52.42 ng/mL). The finding that milk IGF-1 level changed based on feeding systems is consistent with the findings of Magistrelli et al. (38). In addition, the blood IGF-1 level (41.7 ng/mL) identified in Saanen goats (35) was found to be similar to the milk IGF-1 level (between 30.78 and 52.42 ng/mL) of the pen group, and it was similar to or higher than that of the pasture group (between 42.13 and 91.08 ng/mL). This finding shows that milk and blood IGF-1 levels in goats are similar.

Examination of *IGF-1* gene expression levels based on feeding systems shows that daily amounts of milk identified on milk test days in different periods of lactation and *IGF-1* gene expression are similar (other than during month 7 of lactation). The *IGF-1* gene expression level was similar in both pasture and pen groups in the first month of lactation (0.733), while daily milk yields during the same milk control were also similar (1001.41 g and 809.13 g). Daily milk yield in the pasture group during months 3 and 5 of lactation was significantly higher than that of the pen group; compared to the pen group, the *IGF-1* gene in the pasture group had 2.313- and 2.332-fold upregulation ($P < 0.05$). During month 7 of lactation, the lactation milk yield in the pasture group was found to be significantly higher compared to the pen group (549.66 g and 364.33 g; $P < 0.05$); gene expression level in the pasture group was found to be somewhat higher (1.276), which was regarded as similar.

IGF-1 milk levels and *IGF-1* upregulation identified in the first 5 months of lactation, which provided the majority of lactation milk yield, were confirmed by daily milk yield in phenotypic terms. Findings obtained in this framework helped form the opinion that *IGF-1* gene expression level and IGF-1 protein milk level may be used as criteria for lactation milk yield in goats bred under different feeding systems.

4.5. *PPARγ*

The *PPARγ* gene has a role in adipocyte differentiation and is characterized by transcription of adipocytes. In their study on biological pathways that are effective on milk fat formation in cows during lactation, Bionaz and Loor (39) expressed that adipocytes abounded in the mammary glands at the beginning of lactation but would decrease during the subsequent periods, and therefore *PPARγ* expression level would decrease (be downregulated) during the subsequent periods of lactation compared to the level found at the beginning of lactation. Examination of *PPARγ* expression levels in both pasture and pen groups in this study also showed downregulation (0.114-, 0.278-, and 0.262-fold, $P < 0.05$ in the pen group; 0.260-fold in the pasture group) as lactation progressed, similar to the mechanism reported for cows (39). On the other hand, it was identified that *PPARγ* had upregulation in both pasture and pen groups from the 3rd month of lactation until the end. This finding can be explained by the process of upregulation compared to the normal state throughout lactation to ensure that milk fat secretion of adipocytes decreased in the middle of the lactation period.

Examination of *PPARγ* expression levels in different feeding systems shows similar expression levels for pasture and pen groups throughout lactation other than the 1st month. Changes in milk secretion as a result of feeding during lactation occur via the PRL pathway (33); however, this influence does not generate changes in adipocytes, and therefore *PPARγ* expression stays constant.

Examination of milk fat ratios identified throughout lactation and *PPARγ* expression level together point to the fact that milk fat ratio was at the lowest during the 3rd month of lactation when daily milk yield was the highest in both pasture and pen groups. It was found that, compared to the 1st month of lactation, *PPARγ* had downregulation in the 3rd month of lactation in the pasture group (0.260-fold), and compared to the 3rd month, *PPARγ* had upregulation (2.561- and 2.870-fold) during months 5 and 7 of lactation, respectively. These changes in expression were found to be consistent with milk fat ratios identified for the same days. Similarly, it was found that compared to the 1st month of lactation, *PPARγ* had downregulation in the 3rd, 5th, and 7th months of lactation in the pen group (0.114-, 0.278-, and 0.262-fold; $P < 0.05$) and compared to the 3rd month, *PPARγ* had upregulation (2.444- and 2.302-fold; $P < 0.05$) during the 5th and 7th months of lactation. Based on these findings, it might be possible to claim that *PPARγ* controls milk fat secretions from mammary glands in goats, similar to cows (11).

4.6. CSN2

CSN2 is the gene that predetermines β-casein, which is an important fraction of casein in goats. In their study on Damascus goats, Guney et al. (40) found that milk protein level identified in the 9th month of lactation (4.31%) was higher than the milk protein level identified in the 2nd month of lactation (3.85%). Consistent with Guney et al.'s (40) report, this study also determined that milk protein level identified in Damascus goats in the 7th month of lactation (3.34%) in pasture and pen groups was higher than the values identified for the 1st month of lactation (2.74% for pasture group and 2.62% for pen group; $P < 0.01$ for pasture group and $P < 0.001$ for pen group). Milk CSN2 (β-casein) amounts show that daily milk yield amounts were significantly higher in the 3rd and 5th months of lactation for both groups compared to other months ($P < 0.01$). While β-casein gene transcription is adjusted in the cell as a protein–protein synergistic influence of PRL and hydrocortisone on the one hand (41,42), it is directly repressed by progesterone receptors on the other hand (43). Since PRL is known as a hormone that directly controls milk secretion and progesterone is known as a hormone that inhibits prolactin synthesis, this mechanism explains the high levels of CSN2 during periods when daily milk yield is high. In their study on Alpine goats, Ollier et al. (5) reported that *CSN2* had downregulation when goats were hungry for 48 h. This finding may be related to the fact that downregulation occurs via the PRL mechanism as a result of malnutrition. Nonexistence of downregulation findings in this study is regarded as normal since goats in both the pasture and pen groups were fed ad libitum.

While examination of gene expression level of *CSN2* shows similar expression levels in the pasture and pen groups in the 1st, 3rd, and 5th months of lactation, it was found that the pasture group had 5.436-fold ($P < 0.01$) upregulation in the 7th month of lactation compared to the pen group. As a reflection of this finding, the milk CSN2 level in the pasture group was found to be higher than that of the pen group (11.21 and 17.03 mg/mL; $P < 0.01$). While *CSN2* gene expression had significant upregulation in the pen group from the start of lactation until the 5th month of lactation (6.631- and 6.399-fold; $P < 0.01$ and $P < 0.001$, respectively); downregulation was identified in months 3–5 and 3–7 of lactation (0.297- and 0.308-fold). These data show that the PRL–CSN2 synergistic influence continued throughout lactation (12). Similar to the pen group, it was also found in the pasture group that *CSN2* had upregulation compared to the start of lactation (2.554-, 3.656-, and 2.565-fold); however, the upregulation was nonexistent in months 3–5 and 5–7 (1.005- and 0.702-fold).

With these findings, it is suggested that the *CSN2* gene expression level may be a genetic criterion for β-casein, a milk protein. However, since total milk protein is composed of both other fractions of casein and other proteins (44), it is thought that *CSN2* does not have the potential to be a criterion for total milk protein.

4.7. Conclusions

It was observed that the expression levels of the genes studied in this research did not present marginal changes when differences in feeding systems were kept within physiological boundaries. However, it was identified that the feeding differences generated changes in the expression levels of some genes during different periods of lactation (the end of lactation for *LTF* and *CSN2*; the middle of lactation for *IGF-1*).

LTF milk somatic cell gene expression level might be used as a selection criterion for mastitis resistance in pasture-based feeding systems. However, it was observed that *BRCA1* cannot be used for this purpose. While it was determined that *IGF-1* milk somatic cell gene expression level may be used as a selection criterion for lactation milk yield, the same could not be said for *POU1F1*. It was found that *PPARγ* somatic cell gene expression level can be used for a genotypic selection criterion for identifying milk fat; *CSN2* milk somatic cell gene expression level can provide information only about β-casein among milk proteins; and *CSN2* did not have a determinant quality on total milk proteins.

This study is the first to establish the gene expression levels of the *LTF*, *BRCA1*, *IGF-1*, and *PPARγ* genes in goat milk and mammary alveolar epithelial cells, and it is the first report on the effects of *BRCA1*, *POU1F1*, and *PPARγ* genes on milk yields.

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