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RESEARCH ARTICLE

Effects of Oxidative Stress-Related Major Molecular Parameters on Milk Composition in Weaning Period of Damascus Goats [1]

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Abstract: The weaning process in goat breeding is applied in various methods depending on the breeding administration. In this study, blood and milk samples have been collected from Damascus goats at the weaning day (postpartum 105th day-weaned day) and a week later (post-weaned day). In addition to determining cortisol and MDA in plasma, COX-2 and NFE2L2 activities have been investigated both mRNA and protein levels from leucocytes and plasma. Compositional parameters of milk have also been analyzed and the possible relations between studied parameters have been investigated. While expression levels of COX-2 and SCC of milk decreased, pH of milk was increased in post-weaned day samples. The MDA, FFDM, protein, lactose and freezing point were decreased in the post-weaned day. Milk fat was negatively correlated with NFE2L2, and milk protein had positively correlated with SCC and FFDM. On the other hand, lactose was positively correlated with FFDM and protein. In addition, most of the compositional parameters positively correlated with a freezing point; they were negatively correlated with electrical conductivity. According to the results obtained from the study, it is thought that the decrease in milk fat in goats may be an indicator of increased oxidative stress in lactating goats due to the increase in NFE2L2 protein, which has a central role in the antioxidant response.

Keywords: *COX-2, Damascus goat, Gene expression, NFE2L2, Weaning in goats*

Sütten Kesim Dönemindeki Damascus Keçilerinde Oksidatif Stresle İlişkili Majör Moleküler Parametrelerin Süt Bileşimi Üzerine Etkileri

Öz: Keçi yetiştiriciliğinde sütten kesme işlemi, yetiştirme yönetimine bağlı olarak çeşitli yöntemlerle uygulanmaktadır. Bu çalışmada Damascus keçilerinden sütten kesim günü (postpartum 105. gün-Sütten kesim günü) ve bir hafta sonra (sütten kesim sonrası) kan ve süt örnekleri alınmıştır. Plazmada kortizol ve MDA'nın belirlenmesine ek olarak, COX-2 ve NFE2L2 aktiviteleri lökosit ve plazmada hem mRNA hem de protein düzeyinde araştırılmıştır. Süt kompozisyon parametreleri de analiz edilmiş ve çalışılan parametreler arasındaki olası ilişkiler araştırılmıştır. Sütten kesim sonrası alınan örneklerde COX-2 ekspresyon seviyesi ve SCC azalırken, süt pH'sı artmıştır. Sütten kesim sonrası MDA, FFDM, protein, laktoz ve donma noktası seviyeleri düşmüştür. Süt yağı, NFE2L2 ile negatif korelasyon gösterirken süt proteini, SCC ve FFDM ile pozitif korelasyona sahip olmuştur. Bununla birlikte laktoz, FFDM ve protein ile pozitif korelasyon göstermiştir. Ek olarak, kompozisyon parametrelerinin çoğu donma noktası ile pozitif, elektriksel iletkenlik ile negatif korelasyon göstermiştir. Çalışmadan elde edilen sonuçlara göre, keçilerde süt yağındaki azalmanın, antioksidan yanıtta merkezi rolü olan NFE2L2 proteinindeki artışa bağlı olarak laktasyondaki keçilerde artan oksidatif stresin bir göstergesi olabileceği düşünülmektedir.

Anahtar sözcükler: *COX-2, Damascus keçisi, Gen ekspresyonu, Keçilerde sütten kesim, NFE2L2*

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Introduction

Goat is one of the precious farm animals in the supply of quality milk and dairy products [1]. It is reported that there are more than 1000 goat breeds and more than 1 billion goats worldwide [2,3]. Breeding of goats is mainly preferred for milk, and dairy products. Goat milk is an exceedingly valuable animal product, especially for children, the elderly and those with food allergies. As in other mammals, milk is an essential requirement for the nutritional necessities and maintenance of health in goat kids [4,5]. Although there are various changes between species, the milk sucking period, which begins with colostrum in ruminants after birth, ends with the transition to roughage and concentrated feeds. Weaning is an important stress factor affecting the health and growth performance of kids [5,6]. However, physiological changes related to stress and oxidative stress that may occur due to weaning in goats are known to a limited extent [7]. On the other hand, the possible weaning-related changes in milk composition are directly related to milk quality are potentially crucial for goat breeding.

The weaning process in goat breeding is applied in various methods and periods depending on the breeding administration [8,9]. Milk production is also maintained in varying periods after weaning. In this process, oxidative stress may occur in mammals. It is known that oxidative status crucially effects the physiology of the organism and composition of milk [10]. Although there are studies on physiological changes in kids after weaning, there are few studies investigating the remarkable changes in goats [5,6]. The study aimed to determine plasma cortisol and MDA (Malondialdehyde) levels in the weaning process in Damascus goats. COX-2 ((Cyclooxygenase 2) and NFE2L2 (Nuclear Factor Erythroid 2-Related Factor 2) activities were also investigated both mRNA and protein levels in leucocytes and plasma. The milk composition was analyzed in this process, and the possible relations between studied parameters were investigated.

Material and Methods

Ethical Statement

The study was approved by Hatay Mustafa Kemal University Animal Experiments Local Ethics Committee (Approval no: 2021/02-08).

Animals and Sample Collection

The study was conducted on a private farm in Hatay Province in Türkiye in May 2021 (36°18'65.6"N and 36°18'63.9"E). Healthy and lactating Damascus goats were used in the study (n=24). The health status of goats was evaluated by veterinarians. Animals were selected by random sampling method from a herd of approximately 100 goats on similar ages. The animals were at the 3th-4th lactation period and gave singleton birth (44-50 months old). Approximately 2 m^2/goat area was arranged for each animal. Animals were fed with 1 kg/goat concentrate feed (88.91% dry matter, 16.51% crude protein, and 2650 kcal/kg total Metabolic Energy) and 1 kg/goat dry alfalfa.

Animals were weaned at postpartum 105th day (105±5). Blood and milk samples were collected from goats at the weaning day (Weaned) and a week later (post-weaned) at the morning milking time. While blood samples were collected from the left jugular vein into EDTA-containing tubes with a volume of 10 mL, approximately 150 mL milk samples from each animal were collected into falcon tubes with 50 mL (three tubes were used). Sterile conditions were complied with during sampling stages and nuclease-free falcons were used. Before collecting milk samples, goat's udders were washed with alcohol-based disinfectant and cleaned with water and sterile cotton gauze swabs. The samples were transferred to the laboratory of Genetics in a cold chain within about 30 min.

Determination of Composition, pH, and Somatic Cell Count of Milk

Milk samples were manually homogenized before analysis and 10 mL of each sample was stored at -80°C for MDA analysis. While compositional parameters were measured with a milk analyzer (Milkotester Master Classic LM2-P1, Bulgaria), the pH of samples was determined with a pH meter (Hanna pH meter, HI83141, USA). At the same time, somatic cell counts of samples were determined via somatic cell counter (Lactoscan SCC 6010, Bulgaria). Validated devices were used to measure the composition, pH, and somatic cell count of the samples.

Plasma and Leucocyte Collection from Blood Samples

Blood samples were centrifuged at 3000 xg for 10 min at +4°C for the plasma and leucocyte collection. While obtained plasma was portioned to 1.5 mL tubes, the leucocyte layer of samples was transferred to new tubes and treated with Red Blood Cell Lysis Buffer [11]. Then the samples were centrifuged at 3000 xg for 10 min at +4°C and aqua phases were discarded. Approximately 1 mL of TRIzol Reagent (Thermo Fisher Scientific, USA, Cat No: 15596018) was added to leucocytes pellets and samples were homogenized by pipetting and stored at –80°C until molecular analysis.

RNA Isolation and cDNA Synthesis

According to the modified Trizol method, total RNA isolation was performed from leucocytes kept in Trizol Reagent [12]. Following the chloroform-isopropyl alcohol-ethyl alcohol steps, the total RNA pellets were obtained. According to the pellet sizes, samples were diluted with 30-50 µL nuclease-free water. Purity (A260/280) and RNA concentration were measured by the nucleic acid meter

(SMA-1000 Spectrophotometer, Merinton, China). In addition, the integrity of RNA was evaluated with 1% agarose gel electrophoresis.

Following the elimination of possible genomic DNA contamination via DNA digestion kit (DNase I, RNase free, Thermo Fisher Scientific, USA, Cat no: EN0521), cDNA was synthesized using RevertAid First Strand cDNA synthesis kit (Thermo Fischer Scientific, USA, Cat no: K1621). Thermal cycler (BioRad T100, USA) protocol was as follows: 10 min at 25°C, 120 min at 37°C and 5 min at 85°C, respectively. Then, all samples were completed to 200 µL with nuclease-free water and kept at –20°C until the gene expression analysis.

Real-Time PCR Application

Amplifications of *COX-2*, *NFE2L2,* and *ACTB* genes were performed in Real-Time PCR (Rotor-gene Q MDx 5plex HRM, Qiagen, USA). SYBR Green dye-containing kit (Power SYBR Green PCR Master Mix, Thermo Fisher Scientific, USA, Cat no: 4367659) was used for the amplification. *ACTB* was selected as the housekeeping gene. Each sample was studied in duplicate and a 10 µL volume sample was used for the reaction. The Real-Time PCR protocol was as follows: 10 min at 95°C, followed by 15 s at 95°C, 60 s at 60°C, and 40 cycles. Forward and Reverse sequences of primers were presented in *Table 1*.

Determination of MDA Levels and ELISA Application

The levels of MDA from plasma and milk samples were measured spectrophotometrically according to the method reported by Esterbauer and Cheeseman [15]. Cortisol, COX-2, and NFE2L2 levels of plasma samples were determined by goat-specific ELISA kits (E0021Go, E1375Go, E1376Go, respectively, Bioassay Technology Laboratory, USA) at 450 nm with ELISA reader (AMR-100, Allsheng, China).

Statistical Analysis

Statistical analyses were performed using IBM SPSS Statistics Software Version 23.0. The sample size was calculated with G*Power software (Version 3.1.9.2). Result of the sample size calculation showed that the minimum number of Damascus goats was 24, considering an effect size of 0.30, an alpha value of 0.05, and a power of 0.80. The variables were examined as parametric test assumptions. Spearman correlation coefficient was performed to assess the correlation between SCC, MDA, compositional parameters of milk, plasma concentration levels of cortisol, MDA, COX-2, NFE2L2. A mixed model for repeated measures was used to test the differences in each variable between sampling periods (Weaned, Post-weaned). Animals were included as a random factor in all models, while sampling time was included as a fixed factor. Pairwise comparisons were made using a Bonferroni adjustment. Gene expression levels were calculated by the $2^{-\Delta\Delta Ct}$ method. The results were determined by comparing to "Weaned day" and presented as fold changes [16]. Differences with $P<0.05$ were considered statistically significant. Results were calculated as "Mean ± Standard Error of Mean" and presented as a figure.

Results

The SCC of the Post-Weaned day samples were found to be significantly lower ($P<0.001$). On the other hand, pH values of milk increased on post-weaned days ($P<0.05$). While the fat percentage and electrical conductivity were found to be similar between sampled days, MDA levels, FFDM (Fat-Free Dry Matter), protein, lactose, and freezing point increased in the post-weaned day *(Fig. 1)*.

The purity (A260/280=1.85±0.01) and concentration (384.51±23.80 ng/µL) values of samples were appropriate for conversion to cDNA and gene expression analysis. Compared to Weaned day samples, the *COX-2* gene was downregulated almost 2-fold in samples of post-weaned day ($P<0.05$). As with *NFE2L2* gene expression results, COX-2 and NFE2L2 protein levels were found to be similar in plasma on both days *(Fig. 2)*.

According to the correlation analysis, variable and significant correlations were found between studied parameters *(Table 2)*. Milk fat levels were negatively correlated with NFE2L2 protein levels; milk protein was positively correlated with SCC and FFDM ($P<0.05$). On the other hand, lactose was positively correlated with FFDM and milk protein. In addition, while most of the composition parameters were positively correlated with the freezing point, a negative correlation was found between electrical conductivity and these parameters at varying significance levels ($P<0.05$) *(Table 2)*.

Table 1. *Forward and reverse sequences of primers used in the study*

Genes	Forward and Reverse Sequences	Product Length	Reference
COX-2	F: 5'-GTAGGCCAGGAGGTCTTTGG-3' R: 5'-GCCTGCTTGTCTGGAACAAC-3'	142 bp	[10]
NFE2L2	F: 5'-CCAACTACTCCCAGGTAGCCC-3' R: 5'-AGCAGTGGCAACCTGAACG-3'	227 bp	[13]
ACTB	F: 5'-CTTCCAGCCGTCCTTCCT-3' R: 5'-TGTTGGCATACAGGTCCTTTC-3'	105 bp	[14]

Bp: *Base-pair*

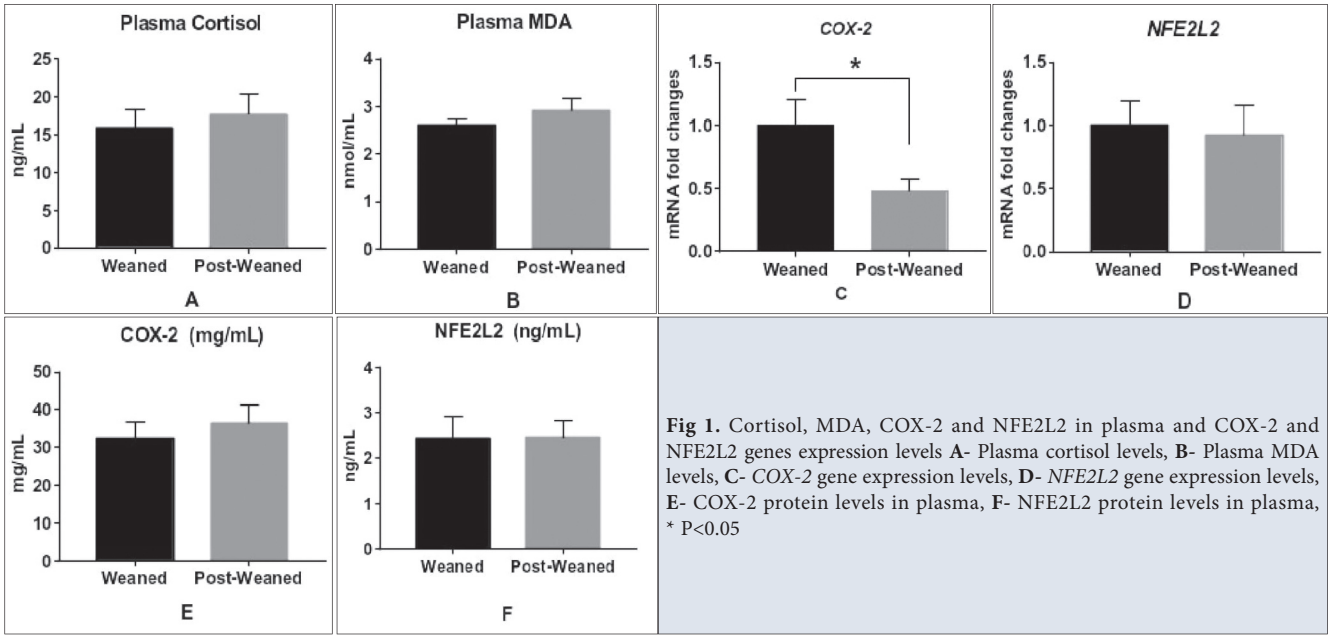

Fig 1. Cortisol, MDA, COX-2 and NFE2L2 in plasma and COX-2 and NFE2L2 genes expression levels **A**- Plasma cortisol levels, **B**- Plasma MDA levels, **C**- *COX-2* gene expression levels, **D**- *NFE2L2* gene expression levels, **E**- COX-2 protein levels in plasma, **F**- NFE2L2 protein levels in plasma, * P<0.05

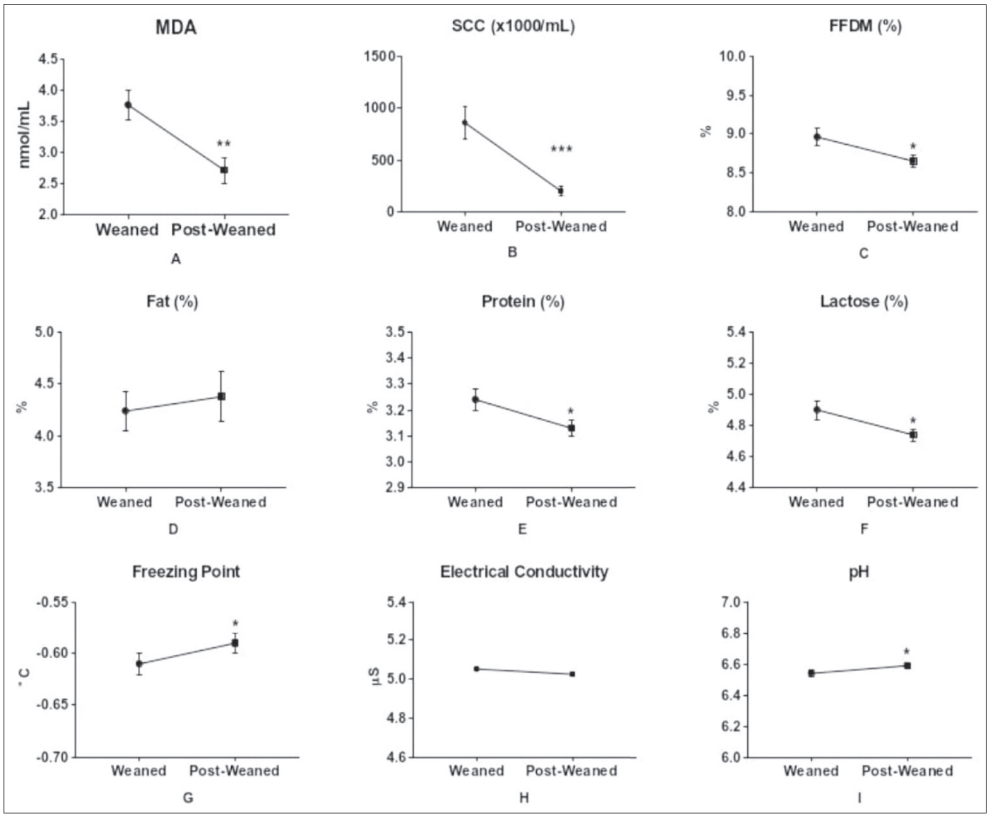

Fig 2. Changes in compositional parameters of milk in weaning process, * P<0.05, ** P<0.01, P<0.001

Discussion

Goat milk has recently become a valuable animal product that attracts tremendous attention due to the realization of its positive effects on health. Therefore, goat breeding is mainly done for milk production [17,18]. Even if the milk yield is a major factor affecting profitable production, sucking the kids is an inevitable application for developing their immunity and sustainable health. Weaning is applied at different periods among breeders depending on the type of breeding, breed of animal, and environmental factors [17,19]. After weaning, researchers mainly focus on the health status and developmental changes of kids [20]. However, there is limited knowledge at the molecular

Table 2. *Correlations between studied parameters*

Item	Cortisol	COX-2	NFE2L2	MDA	Milk MDA	SCC	Fat	FFDM	Protein	Lactose	Freezing Point	Electrical Conductivity	pH
Cortisol	1.000	-0.178	-0.015	0.089	0.091	-0.056	0.133	0.239	0.219	0.270	0.264	-0.230	-0.099
COX-2		1.000	-0.102	0.084	0.130	-0.084	0.049	-0.116	-0.051	-0.108	-0.076	-0.144	-0.212
NFE2L2			1.000	-0.169	-0.142	-0.225	-0.320*	-0.093	-0.097	-0.114	-0.159	0.210	-0.109
MDA				1.000	-0.041	-0.169	-0.025	-0.037	-0.091	-0.029	-0.056	0.129	0.014
Milk MDA					1.000	0.251	0.115	0.153	0.129	0.137	0.193	-0.232	-0.153
SCC						1.000	0.163	0.245	0.283*	0.230	0.296*	-0.074	-0.190
Fat							1.000	0.058	0.049	0.042	0.231	-0.472**	0.030
FFDM								1.000	0.967***	0.991***	0.975***	-0.532***	0.031
Protein									1.000	0.965***	0.956***	-0.579***	0.028
Lactose										1,000	0.969***	-0.532***	0.051
Freezing P.											1.000	-0.619***	0.020
Electrical C.												1.000	-0.133
pH													1.000

** P<0.05; ** P< 0.01; *** P<0.001,* ***FFDM:*** *Fat-Free Dry Matter,* ***Freezing P:*** *Freezing Point,* ***Electrical C:*** *Electrical Conductivity*

levels about stress and oxidative stress status of goats after weaning application.

During the weaning process, cortisol levels in plasma have been found similar in goats in this study. While plasma cortisol levels have been widely used as a potential biomarker for the determination of stress, it has been reported in several studies that the cortisol levels of plasma might vary depending on the endogenous and non-specific environmental factors such as circadian rhythm and season [6]. In addition, the hypothalamic-pituitary-adrenal (HPA) axis is also responsible for maternal stress in response to lactation in mammals [21]. However, considering the postpartum weaning time, the stable cortisol levels in plasma are thought to be due to physiological reasons. Indeed, the stress-related mechanisms become sensitive to various stress factors starting from late pregnancy [21]. It is believed that during the different lactation stages, molecular activities in physiological and biological pathways might affect these parameters [10,22].

MDA is the main product of oxidative stress, and it is widely used as a biomarker in tissues and biological liquids to detect oxidative stress status [23]. Although it has been reported that plasma MDA levels may change even depending on the differences of ratio ingredients, it has been found similar in both sampled days [24]. COX-2 and NFE2L2 are highly active transcription factors for maintaining oxidative balance. While COX-2 activity increases on the oxidative stress state, NFE2L2 has a significant role in response to oxidative stress [10,25]. NFE2L2 is also an antioxidant transcription factor in the organism [26]. It has been known that *COX-2* and *NFE2L2* are the most related genes with the oxidative status of the tissues and biological liquids [10]. While *COX-2* gene expression has been found almost 2-fold downregulated on the post-weaned day, there are no statistical differences between two different sampled days regarding *NFE2L2* gene expression levels. It has been thought that the response to weaning might be the main reason for the downregulation of *COX-2* gene expression levels.

On the other hand, post-transcriptional mediators such as miRNAs might be the reason for the similar levels of the proteins encoded by the *COX-2* gene [27]. As it is well known, miRNAs regulate the levels of proteins in several pathways. On the other hand, there are few studies on circulating and milk miRNAs in goats. Also, there are still newly identified miRNAs in ruminants [28,29].

MDA levels, which were at similar levels in plasma, were significantly reduced in post-weaned milk as somatic cells. SCC has known as an effective indicator of mammary gland health. Higher SCC in goat milk is generally considered physiological because of the apocrine type of secretion. However, it is reported that milk SCC should be below one million for the mammary glands to be considered healthy. In small ruminants, SCC shows a wide range [10,18]. Although SCC is within the physiological limits, it is thought that the number of somatic cells decreased with weaning may have occurred depending on the suckling behavior of the offspring [30,31]. In contrast to our findings, it has been found in a study conducted Murciano-Granadina goats that weaning does not affect SCC in milk [32]. Even if it has been reported that MDA and SCC are related parameters in milk, no correlation has been found between these two parameters [10,33]. The obtained results suggest that the oxidative balance in the mammary gland has a more complex molecular network

than anticipated. Furthermore, the pH value of milk was significantly increased. While it is known that the pH value of milk is strongly related to the composition and SCC of milk, it has been thought that other parameters such as minerals in milk are thought to be the reason for obtained results [34-36].

Although the contents of ration and the lactation period were similar, the compositional parameters significantly changed in post-weaned milk. While FFDM, protein, and lactose content of milk decreased, the freezing point of milk increased concerning these findings. During suckling, udder stimulation and evacuation affect milk composition in the mammary gland [37]. In addition, hormonal regulation and weaning period have also been thought to lead to variable changes in milk composition during the weaning process [38,39].

Even if variable correlations were detected between studied parameters, one of the most remarkable findings was the negative correlation between plasma *NFE2L2* protein levels and milk fat. *NFE2L2* has an influential role in cellular defense against elevated oxidative damage [40]. In addition to a crucial mission in response to oxidative stress, it has been reported that the *NFE2L2* is a potential regulator of fatty acid metabolism [41,42]. The study pointed out that deletion of *NFE2L2* gene has led to obesity in mice fed with high-fat diets [43]. Therefore, the negative correlation between *NFE2L2* and milk fat has been considered reasonable. While most researchers focus on the relation between *NFE2L2* and oxidative status, energy metabolism has gained importance with this transcription factor. According to the results obtained from the study, it is thought that the decrease in milk fat of goats may be an indicator of increased oxidative stress in lactating goats due to the increase in *NFE2L2* protein.

In conclusion, it has been thought that notable findings on several parameters on milk and plasma of goats after the weaning process have been obtained with this study. The mechanism of suckling-related stress and molecular changes in farm animals, particularly in small ruminants, is still largely unknown. According to the obtained results about the relation between *NFE2L2* and milk fat, it is thought that possible regulators of *NFE2L2* should be investigated at the molecular levels in ruminants. Due to the particular importance of goat milk, it is thought that further studies are required at the molecular level, particularly in the weaning process.

Availability of Data and Materials

Datasets analyzed during the study are available from the corresponding author (H. Özkan) on reasonable request.

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Conflict of Interest

The authors declared that there is no conflict of interest.

Ethical Statement

The study was approved by Hatay Mustafa Kemal University Animal Experiments Local Ethics Committee (Approval no: 2021/02-08).

Author Contributions

HÖ, MY, and AY conceived and investigated the study. HÖ, UK, and MY collected samples. HÖ, SD, and İK determined the milk parameters. HÖ and BÇ performed RNA isolation, gene expression and ELISA applications. HÖ, AY, and UK analyzed the results. All authors read and approved the final manuscript.

References

1. Yurchenko S, Sats A, Tatar V, Kaart T, Mootse H, Jõudu I: Fatty acid profile of milk from Saanen and Swedish Landrace goats. *Food Chem*, 254, 326-332, 2018. DOI: 10.1016/j.foodchem.2018.02.041

2. Crisà A, Ferrè F, Chillemi G, Moioli B: RNA-sequencing for profiling goat milk transcriptome in colostrum and mature milk. *BMC Vet Res,* 12 (264): 1-21, 2016. DOI: 10.1186/s12917-016-0881-7

3. FAO: Food and Agriculture Organization Corporate Statistical Database. http://www.fao.org/faostat/en/#data; *Accessed:* 12.12.2021.

4. Jasper J, Weary DM: Effects of ad libitum milk intake on dairy calves. *J Dairy Sci*, 85 (11): 3054-3058, 2002. DOI: 10.3168/jds.S0022-0302(02)74391-9

5. Izuddin WI, Loh TC, Samsudin AA, Foo HL, Humam AM, Shazali N: Effects of postbiotic supplementation on growth performance, ruminal fermentation and microbial profile, blood metabolite and GHR, IGF-1 and MCT-1 gene expression in post-weaning lambs. *BMC Vet Res,* 15 (1): 1-10, 2019. DOI: 10.1186/s12917-019-2064-9

6. Magistrelli D, Aufy AA, Pinotti L, Rosi F: Analysis of weaning-induced stress in Saanen goat kids. *J Anim Physiol Anim Nutr,* 97 (4): 732-739, 2013. DOI: 10.1111/j.1439-0396.2012.01315.x

7. Redondo E, Franco A, Garcia A, Masot AJ: Changes in concentrations of cortisol and melatonin in plasma, expression of synaptophysin, and ultrastructural properties of pinealocytes in goat kids in situations of stress due to early weaning: The effect of melatonin. *N Z Vet J*, 58 (3): 160-167, 2010. DOI: 10.1080/00480169.2010.67518

8. Ugur F, Atasoglu C, Tolu C, Diken F, Pala A: Effects of different weaning programs on growth of Saanen kids. *Anim Sci J,* 78 (3): 281-285, 2007. DOI: 10.1111/j.1740-0929.2007.00436.x

9. Zobel G, Freeman H, Watson T, Cameron C, Sutherland M: Effect of different milk-removal strategies at weaning on feed intake and behavior of goat kids. *J Vet Behav*, 35, 62-68, 2020. DOI: 10.1016/j.jveb.2019.10.004

10. Yakan A, Özkan H, Çamdeviren B, Kaya U, Karaaslan İ, Dalkiran S: Expression patterns of major genes in fatty acid synthesis, inflammation, oxidative stress pathways from colostrum to milk in Damascus goats. *Sci Rep*, 11 (1): 1-10, 2021. DOI: 10.1038/s41598-021-88976-0

11. Lim JH, Goh LPW, Chong ETJ, Lee PC: High integrity total RNA isolation from human peripheral blood that is as competitive to commercialize kits. *Res J Pharm Biol Chem Sci*, 7 (4): 1937-1942, 2016.

12. Rio DC, Ares M, Hannon GJ, Nilsen TW: Purification of RNA using TRIzol (TRI reagent). *Cold Spring Harb Protoc,* 2010 (6):pdb-prot5439, 2010. DOI: 10.1101/pdb.prot5439

13. Deng S, Yu K, Jiang W, Li Y, Wang S, Deng Z, Yao Y, Zhang B, Liu G, Liu Y, Lian Z: Over-expression of Toll-like receptor 2 up-regulates heme

oxygenase-1 expression and decreases oxidative injury in dairy goats. *J Anim Sci Biotechno*, 8 (1): 1-10, 2017. DOI: 10.1186/s40104-016-0136-2

14. Modesto P, Peletto S, Pisoni, G, Cremonesi P, Castiglioni B, Colussi S, Caramelli M, Bronzo V, Moroni P, Acutis PL: Evaluation of internal reference genes for quantitative expression analysis by real-time reverse transcription-PCR in somatic cells from goat milk. *J Dairy Sci,* 96 (12): 7932-7944, 2013. DOI: 10.3168/jds.2012-6383

15. Esterbauer H, Cheeseman KH: Determination of aldehydic lipid peroxidation products: Malonaldehyde and 4-hydroxynonenal. *Methods Enzymol,* 186, 407-421, 1990. DOI: 10.1016/0076-6879(90)86134-h

16. Livak KJ, Schmittgen TD: Analysis of relative gene expression data using real-time quantitative PCR and the 2−ΔΔCT method. *Methods*, 25 (4): 402-408, 2001. DOI: 10.1006/meth.2001.1262

17. Belanche A, Palma-Hidalgo JM, Nejjam I, Jiménez E, Martín-García AI, Yáñez-Ruiz DR: Inoculation with rumen fluid in early life as a strategy to optimize the weaning process in intensive dairy goat systems. *J Dairy Sci*, 103 (6): 5047-5060, 2020. DOI: 10.3168/jds.2019-18002

18. Yakan A, Özkan H, Eraslan Şakar A, Ateş CT, Ünal N, Koçak Ö, Doğruer G, Özbeyaz C: Milk yield and quality traits in different lactation stages of Damascus goats: Concentrate and pasture based feeding systems. *Ankara Üniv Vet Fak Derg*, 66, 117-129, 2019. DOI: 10.33988/auvfd.547470

19. Khan MA, Bach A, Weary DM, Von Keyserlingk MAG: Invited review: Transitioning from milk to solid feed in dairy heifers. *J Dairy Sci*, 99 (2): 885-902, 2016. DOI: 10.3168/jds.2015-9975

20. Liao R, Lv Y, Zhu L, Lin Y: Altered expression of miRNAs and mRNAs reveals the potential regulatory role of miRNAs in the developmental process of early weaned goats. *PloS One*, 14 (8):e0220907, 2019. DOI: 10.1371/journal.pone.0220907

21. Ralph CR, Tilbrook AJ: The hypothalamo-pituitary-adrenal (HPA) axis in sheep is attenuated during lactation in response to psychosocial and predator stress. *Domest Anim Endocrinol*, 55, 66-73, 2016. DOI: 10.1016/j.domaniend.2015.11.003

22. Kapusta A, Kuczyńska B. Puppel K: Relationship between the degree of antioxidant protection and the level of malondialdehyde in high-performance Polish Holstein-Friesian cows in peak of lactation. *PLoS One,* 13:e0193512, 2018. DOI: 10.1371/journal.pone.0193512

23. Özkan H, Kutlu T: The relationship of fructose consumption with MDA levels in rat liver and its effect on the expression levels of COX-2 and NRF-2 genes. *Ankara Üniv Vet Fak Derg*, 67 (4): 387-392, 2020. DOI: 10.33988/auvfd.645713

24. Yonny ME, García EM, Lopez A, Arroquy JI, Nazareno MA: Measurement of malondialdehyde as oxidative stress biomarker in goat plasma by HPLC-DAD. *Microchem J*, 129, 281-285, 2016. DOI: 10.1016/j.microc.2016.07.010

25. Pérez-Rodríguez L, Romero-Haro AA, Sternalski A, Muriel J, Mougeot F, Gil D, Alonso-Alvarez C: Measuring oxidative stress: The confounding effect of lipid concentration in measures of lipid peroxidation. *Physiol Biochem Zool*, 88 (3): 345-351, 2015. DOI: 10.1086/680688

26. Moretti DB, Santos CB, Alencar SM, Machado-Neto R: Colostrum from primiparous Holstein cows shows higher antioxidant activity than colostrum of multiparous ones. *J Dairy Res,* 87, 356-359, 2020. DOI: 10.1017/S0022029920000813

27. Harper KA, Tyson-Capper AJ: Complexity of COX-2 gene regulation. *Biochem Soc Trans*, 36 (3): 543-545, 2008. DOI: 10.1042/BST0360543

28. Do DN, Dudemaine PL, Mathur M, Suravajhala P, Zhao X, Ibeagha-Awemu EM: MiRNA regulatory functions in farm animal diseases, and biomarker potentials for effective therapies. *Int J Mol Sci*, 22 (6):3080, 2021. DOI: 10.3390/ijms22063080

29. Bourdon C, Boussaha M, Bardou P, Sanchez MP, Le Guillou S, Tribout T, Larroque H, Boichard D, Rupp R, Le Provost F, Tosser-Klopp G: In silico identification of variations in microRNAs with a potential impact on dairy traits using whole ruminant genome SNP datasets. *Sci Rep*, 11 (1): 1-13, 2021. DOI: 10.1038/s41598-021-98639-9

30. Antonič J, Jackuliaková L, Uhrinčať M, Mačuhová L, Oravcová M, Tančin V: Changes in milk yield and composition after lamb weaning and start of machine milking in dairy ewes. *Slovak J Anim Sci*, 46 (3): 93-99, 2013.

31. Bergonier D, Berthelot X: New advances in epizootiology and control of ewe mastitis. *Livest Prod Sci,* 79 (1):1-16, 2003. DOI: 10.1016/S0301-6226(02)00145-8

32. Fernández N, Palomares JL, Pérez-Baena I, Rodríguez M, Peris C: Effect of the rearing system on financial returns from Murciano-Granadina breed goats. *Animal*, 13 (8): 1730-1735, 2019. DOI: 10.1017/S1751731118003336

33. Andrei S, Matei S, Ruginǎ D, Bogdan L, Ştefănuţ **C:** Interrelationships between the content of oxidative markers, antioxidative status, and somatic cell count in cow's milk. *Czech J Anim Sci*, 61 (9): 407-413, 2016. DOI: 10.17221/70/2015-CJAS

34. Nian Y, Chen BY, Aikman P, Grandison A, Lewis M: Naturally occurring variations in milk pH and ionic calcium and their effects on some properties and processing characteristics of milk. *Int J Dairy Technol*, 65 (4): 490-497, 2012. DOI: 10.1111/j.1471-0307.2012.00861.x

35. Stocco G, Pazzola M, Dettori ML, Paschino P, Summer A, Cipolat-Gotet C, Vacca GM: Effects of indirect indicators of udder health on nutrient recovery and cheese yield traits in goat milk. *J Dairy Sci*, 102 (10): 8648-8657, 2019. DOI: 10.3168/jds.2019-16369

36. Singh M, Yadav P, Garg VK, Sharma A, Singh B, Sharma H: Quantification of minerals and trace elements in raw caprine milk using flame atomic absorption spectrophotometry and flame photometry. *J Food Sci Technol,* 52 (8): 5299-5304, 2015. DOI: 10.1007/s13197-014-1538-9

37. McKusick BC, Thomas DL, Romero JE, Marnet PG: Effect of weaning system on milk composition and distribution of milk fat within the udder of East Friesian dairy ewes. *J Dairy Sci*, 85 (10): 2521-2528, 2002. DOI: 10.3168/jds.S0022-0302(02)74335-X

38. Panzuti C, Mandrile G, Duvaux-Ponter C, Dessauge F: Early weaning and high feeding level in post-weaning period did not impact milk production in Alpine dairy goats. *J Dairy Res,* 85 (3): 277-280, 2018. DOI: 10.1017/S0022029918000377

39. Lamb GC, Miller BL, Lynch JM, Grieger DM, Stevenson JS, Lucy MC: Suckling reinitiated milk secretion in beef cows after an early postpartum hiatus of milking or suckling. *J Dairy Sci*, 82 (7): 1489-1496, 1999. DOI: 10.3168/jds.S0022-0302(99)75376-2

40. Vomhof-DeKrey EE, Picklo Sr MJ: The Nrf2-antioxidant response element pathway: A target for regulating energy metabolism. *J Nutr Biochem,* 23 (10): 1201-1206, 2012. DOI: 10.1016/j.jnutbio.2012.03.005

41. Ludtmann MHR, Angelova PR, Zhang Y, Abramov AY, Dinkova-Kostova AT: Nrf2 affects the efficiency of mitochondrial fatty acid oxidation. *Biochem J*, 457 (3): 415-424, 2014. DOI: 10.1042/BJ20130863

42. da Costa RM, Rodrigues D, Pereira CA, Silva JF, Alves JV, Lobato NS, Tostes RC: Nrf2 as a potential mediator of cardiovascular risk in metabolic diseases. *Front Pharmacol*, 10 (382): 1-12, 2019. DOI: 10.3389/fphar.2019.00382

43. Chartoumpekis DV, Ziros PG, Psyrogiannis AI, Papavassiliou AG, Kyriazopoulou VE, Sykiotis GP, Habeos IG: Nrf2 represses FGF21 during long-term high-fat diet–induced obesity in mice. *Diabetes*, 60 (10): 2465-2473, 2011. DOI: 10.2337/db11-0112