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# **RESEARCH ARTICLE**

# Effect of Parity and Pasture Period on Milk Lipid Composition in Awassi Sheep

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# ABSTRACT

This study aimed to explore how pasture periods and parity impact the lipid profiles of 38 Awassi sheep raised in a traditional grazing system on natural pastures. Milk samples were gathered for lipid profile analysis at the commencement, midpoint, and conclusion of the pasture period. Data analysis employed repeated measurements through two-way ANOVA within the General Linear Model (GLM) framework. The statistical model encompassed the effects of pasture periods, parity, and their interactions. Although parity did not affect lipid profiles, the pasture period had a significant effect (p<0.01) on triacylglycerol (TAG), free fatty acid (FFA), and monoacylglycerol (MAN). A significant effect (p<0.05) on cholesterol (COL) was also observed. Furthermore, neither pasture period nor parity had any effect on phospholipids. As a result of the study, it was determined that parity had no effect on the lipid profile in milk, while pasture period had a very significant effect on triacylglycerol, free fatty acids, monoacylglycerol and a significant effect on cholesterol.

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## 1. Introduction

Sheep farming holds a significant place in livestock organization. The selection of sheep breeds varies according to countries' climate conditions, consumption preferences, and cultural structures (Kaymakçı & Sönmez, 1996; Türkyılmaz, 2014; Dagdelen & Esenbuga, 2022). Türkiye ranks very high in the world in terms of the number of sheep. However, Türkiye numerical superiority is not valid in terms of productivity per animal (Epstein, 1985; Özbey & Akcan, 2000; Biçer et al., 2019). Sheep farming activity, which is an important part of animal production activities worldwide, is of great importance both economically and in terms of human health in Türkiye as well. Especially in the Central and Eastern Anatolia Region,

sheep farming is quite common, and the climate and pasture structure in this region make sheep farming an important source of income (Keskin et al., 2007; Özyürek, 2017; Türkyılmaz et al., 2021). Throughout history, sheep have played an important role in the economic and social life of Turkish communities both in nomadic migrations from Central Asia to Anatolia and in settled life (Özbey & Akcan, 2000; Biçer et al., 2019). It is of the utmost importance to ensure the nutritional needs of sheep are met at all times (Şahin et al., 2003). The performance of sheep in terms of fattening is influenced by both genetic and environmental factors. Among these environmental factors, the feeding system plays a significant role. Sheep are unable to fully express their genetic potential for fattening when raised under inadequate feeding systems (Keskin et al., 2010).

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Additional feeding or pasture periods are employed to ascertain the genetic potential of the subject. The pasture structures in Türkiye and the ongoing consumption habits of the people emphasize the importance of sheep products for healthy nutrition. Furthermore, the potential to create job opportunities with little capital further highlights the socio-economic importance of sheep farming.

The Awassi breed is a fat-tailed sheep breed raised in the Southeast Anatolia region. This breed adapts excellently to the hot climate conditions of the region and has the highest milk yield among native breeds. Awassi sheep, known for their flocking instinct and excellent walking ability, are recognized worldwide as a valuable sheep breed (Konar et al., 1991; Macit & Aksoy, 1996; Özyürek, 2017).

The components of sheep milk vary depending on various environmental factors such as genotype, parity, season, regional climate conditions, lambing interval and time, type of birth, care and feeding conditions, and udder structure lead to changes in the composition of sheep milk (Kesava Rao et al., 2003). However, generally, sheep milk contains 18.8% dry matter, 7.5% fat, 5.6% protein, and 4.6% lactose. These differences are typically most pronounced proportionally in milk fat (Park et al., 2007; Yılmaz et al., 2011).

Triglycerides are known as fat molecules that meet the body's energy needs and help maintain body temperature balance. They also support intracellular nutrient intake and body nourishment by participating in the structure of the cell membrane. Triglyceride levels can be measured through blood tests (Bitman & Wood, 1990). Normally, triglyceride levels are considered 150 mg/dl, with values between 150-199 mg/dl being borderline, values above 200 mg/dl considered high, and 500 mg/dl or above considered very high. Triglycerides are formed by the esterification of nutrients absorbed from the intestines in the liver.

Cholesterol, with its soft, fat-like structure, is one of the milk lipids found in all cells of the body. Cholesterol plays an important role in the production of cell membranes and some hormones necessary for a healthy body (Costa et al., 2009; Contarini & Povolo, 2013). However, high cholesterol levels are considered a key risk factor for coronary heart diseases such as heart attacks and strokes. The body obtains cholesterol from two sources: synthesis within the body and intake through food. While the liver and other cells produce most of the blood cholesterol, the remainder comes from food. Cholesterol, which is one of the building blocks of nerve cells, is also required for the synthesis of vitamin D and sex hormones (Kaynar et al., 2013). Cholesterol obtained from foods is hydrolyzed and absorbed by pancreatic cholesterol enzymes in the small intestine. It constitutes approximately 0.25-0.45% of total lipids in milk and is mostly present in esterified form with lipids. Cholesterol is transported in the blood by fat-protein complexes; low-density lipoproteins (LDL) carry cholesterol to tissues, while high-density lipoproteins (HDL) facilitate its return transport (Manlongat et al., 1998). LDL cholesterol, when present in excessive amounts in the bloodstream, can increase the risk of heart attacks and strokes and is often associated with the consumption of foods containing saturated fat, trans fat, and cholesterol. It can lead to the formation of thick and hard plaque known as atherosclerosis (Güldür et al., 2007).

The amounts of cholesterol obtained from animal-derived foods vary. It is 110 mg/dl in beef, 160 mg/dl in sheep meat, 1602 mg/dl in egg yolks, 3100 mg/dl in the brain, 375 mg/dl in kidneys, 88 mg/dl in cheese, and 19 mg/dl in sheep milk. Although phospholipids found in milk fat are present in small amounts, they perform important functions in the body. Phospholipids, especially those found in high concentrations in the brain, are known as molecules containing amino acids and fatty acids. Phospholipids are naturally present in all cells of the body, especially in the brain (Wat et al., 2009). Additionally, phospholipids have many functions related to tissues and organs, but the most important is their role in forming the building blocks of the brain, playing a key role for billions of cells (Turkyilmaz & Esenbuga, 2022).

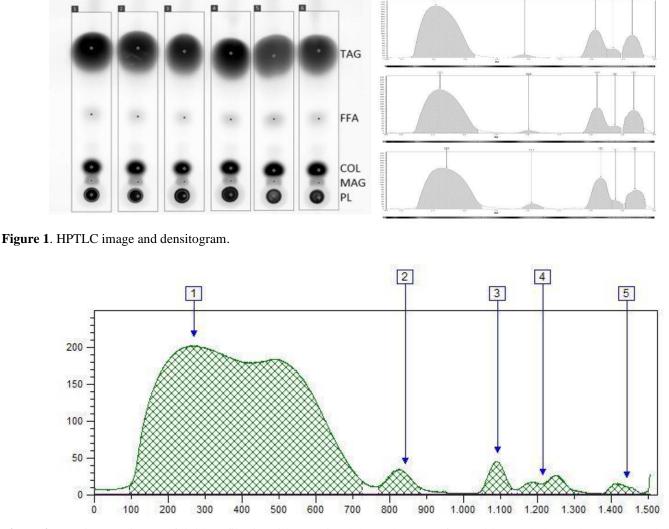
## 2. Materials and Methods

The study was conducted at the Food and Livestock Application and Research Center of Atatürk University. In the study, 38 sheep of Awassi breeds were used. The sheep were divided into two groups, comprising those in the 3<sup>th</sup> (n=18) and  $4^{\text{th}}$  (n=20) parity. The sheep were grazed on the pasture in from May to September. Milk samples were collected on May (beginning of pasture), July (middle of pasture) and September (end of the pasture) for understanding the variation in milk lipid profile within each period is crucial for herd management and for guiding future research endeavors. During the pasture period, dry meadow grass obtained from the Plant Production Application and Research Center was also given to the animals ad libitum along with drinking water. In addition to pasture, the sheep were fed concentrate feed (13% crude protein, 8.70% crude cellulose, 2% crude fat, 9% crude ash, 0.35% sodium, 0.20% magnesium, Vitamin A 15 000 IU, Vitamin D3 3 000 IU and Vitamin E 30 mg/IU) at 1.5% of their live weight.

Control milking was conducted on the sheep used to determine to lipid profiles. These milking were initiated after the lambs were weaned and continued until the end of the pasture season. Control milking was started between 19:00 and 19:30 after the sheep returned from pasture and continued for approximately one hour. Before milking, the sheep's udders were cleaned with a moist cloth. Samples were taken at the beginning, middle, and end of the pasture season during the control milking to determine milk components and lipid profiles. These samples were taken to the Animal Science Department's Milk Analysis Laboratory at Atatürk University.

#### 2.1. Determination of Lipid Profile

Lipid profiles, including triacylglycerol (TAG), phospholipid (PL), free fatty acid (FFA), monoacylglycerol (MAG), and cholesterol (COL), were analyzed by highperformance thin-layer chromatography (HPTLC) HPTLC plates were scanned at high resolution, and the Rf values were automatically calculated using TotalLab 1D (TL120) software, and the classification of lipids in milk samples was made (Figure 1). The area covered by each lipid spot was determined using a densitogram in TotalLab 1D (TL120) program (Figure 2), and the lipid class in total milk fat was expressed as a percentage (Kaynar et al., 2013).



**Figure 2.** Densitogram image of lipid profiles in milk (1: Triacylglycerol, 2: Free fatty acids; 3: Cholesterol; 4: Monoacylglycerol; 5: Phospholipid).

#### 2.2. Statistical Analysis

The data underwent analysis as repeated measurements via two-way ANOVA within the General Linear Model (GLM) procedure (SPSS Inc., Chicago, IL, USA). The mathematical model incorporated the effects of pasture periods and age, as well as all interactions.

## 3. Results and Discussion

The lipid profiles of milk from Awassi sheep are outlined in Table 1. Research on the lipid composition of sheep milk remains limited, but it's noted that lipids in sheep and goat milk are predominantly found in globules smaller than  $3.5 \mu m$ ,

which is beneficial for digestibility and efficient lipid metabolism compared to cow's milk fat (Miller et al., 2006; Park et al., 2007; Kaynar et al., 2013; Merlin Junior et al., 2015).

The major lipid components in sheep milk include triglycerides (TAG), free fatty acids (FFA), cholesterol (COL), monoacylglycerols (MON), and phospholipids (PL). TAG, with a complex composition, primarily resides in the core of milk globules, constituting nearly 90% of the lipids observed in this study. It was unexpected that parity did not appear to affect the content of sheep milk in terms of TAG, FFA and MON. However, the pasture period was found to exert a highly significant effect (p<0.01) on these ratios. It's noteworthy that the TAG content observed in this study was lower than what was reported by Miller et al. (2006) for sheep milk.

It was observed that the cholesterol (COL) content in Awassi sheep milk was significantly higher, with a notable difference (p<0.05) noted. Furthermore, the pasture period was found to have a significant effect (p<0.05) on the COL levels. Specifically, the COL ratio was initially high at the beginning of the pasture period, gradually decreasing significantly as the period progressed towards the midpoint and end stages.

Phospholipids (PL) constitute a portion of the milk fat globule membrane, comprising approximately 0.2-1% of all milk lipids. They play a pivotal role in this membrane due to their highly surface-active properties. Some studies propose that PL may possess anti-cancer and anticholesterolemic functions. PL has been associated with tumor suppression by influencing cell proliferation and is acknowledged as a highly bioactive compound with bacteriostatic and cholesterollowering properties. Notably, the phospholipid (PL) levels in milk from both age groups were similar. The observed differences were not attributed to age but rather to the influence of varying pasture periods. It is noteworthy that the phospholipid (PL) levels in milk from different age groups exhibited similarity. The observed discrepancies were not attributable to age but rather to the impact of different pasture periods. Phospholipids (PL) are integral to the milk fat globule membrane, comprising 0.2-1% of all milk lipids and playing a crucial role due to their highly surface-active properties. Some

**Table 1.** Lipid profiles of Awassi sheep milk (± standard error).

studies suggest PL may possess anti-cancer and anticholesterolemic functions, with reported tumoursuppressing properties by influencing cell proliferation and exhibiting bacteriostatic and cholesterol-lowering effects.

The PL level in milk from both age groups was similar, with differences not attributed to age but rather to various pasture periods. However, the effect of the pasture period on PL was not observed in this study. Additionally, the PL levels obtained were lower than those reported by some researchers (Boersma et al., 1991).

While no effect of age on free fatty acids (FFA) and monoacylglycerols (MON) was observed, a highly significant effect (p<0.01) of the pasture period on these variables was noted. The FFA and COL values were higher than those reported by Miller et al. (2006) but TAG and PL values were lower than those reported by Bitman and Wood (1990).

Lipids, along with other milk components, exhibit variability due to genetics, physiology, and environmental factors such as season. In this study, the pasture period emerged as crucial due to seasonal and climatic changes affecting the nutritional composition of animal feed and, consequently, animal physiology, thereby influencing milk quality. Seasonal variations in the lipid profile of milk during pasture periods have been documented in numerous studies. It was observed that the age and pasture period were not significant on lipid profiles (Lu et al., 2018).

	Ν	TAG	FFA	COL	MON	PL
General Average		89.67±0.571	2.79±0.278	2.85±0.155	3.61±0.187	$1.06 \pm 0.055$
Parity		ns	ns	ns	ns	ns
3	18	$89.35 {\pm} 0.676$	2.99±0.357	2.98±0.219	3.64±0.186	$1.04{\pm}0.084$
4	20	90.67±0.624	2.33±0.329	$2.64 \pm 0.202$	3.28±0.172	$1.07{\pm}0.078$
Period of Pasture		**	**	*	**	ns
Beginning	16	87.22 <sup>b</sup> ±0.699	3.81ª±0.369	3.25ª±0.227	4.58ª±0.193	$1.12{\pm}0.087$
Middle	10	91.52ª±0.886	2.06 <sup>b</sup> ±0.467	2.66 <sup>b</sup> ±0.287	2.75 <sup>b</sup> ±0.244	$1.01{\pm}0.111$
End	12	91.29ª±0.808	2.10 <sup>b</sup> ±0.426	2.50 <sup>b</sup> ±0.262	3.06 <sup>b</sup> ±0.223	$1.04{\pm}0.101$

ns: Non-significant; \*\*: P<0.01, \*: P<0.05; TAG: Triacylglycerol; FFA: Free Fatty Acids; COL: Cholesterol; MON: Monoacylglycerol; PL: Phospholipid.

# 4. Conclusion

Most parameters exhibited higher values at the end of the pasture compared to the beginning, indicating a notable change over time. The reason why the pasture period has a significant effect on triacylglycerol, free fatty acids, cholesterol, monoacylglycerol and phospholipid is thought to be due to the fatty acid conformation in the vegetative process of the plants in the pasture. Conversely, there were no discernible differences observed in milk composition and lipid profiles across different parities of sheep. This study addresses this gap by highlighting the significant impact of pasture on lipid profiles rather than parity. Thus, to potentially manipulate milk composition and lipid profile, which are crucial for human nutrition, further research is recommended.

# **Compliance with Ethical Standards**

The study protocol was approved in advance by Atatürk University Local Ethics Committee of Animal Trials with the decision number 49 dated 27.02.2015.

## **Conflict of Interest**

The authors declare that they have no conflict of interest.

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