Acta fytotechn zootechn, 29, 2026(1): 1–12
http://www.acta.fapz.uniag.sk

Original Paper

Profiles of Blood Amino, Fatty and Volatile Acids in Awassi Ewes Across Different Reproductive Stages and Their Association with Litter Size and Offspring Sex

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Article Details: Received: 2025-06-14 | Accepted: 2025-07-16 | Available online: 2026-03-31

https://doi.org/10.15414/afz.2026.29.01.1-12

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This study aimed to investigate the dynamic changes in the blood profiles of amino acids, fatty acids, and volatile acids in Iraqi Awassi ewes across different reproductive stages, and to evaluate their associations with litter size and offspring sex. A total of thirty mature ewes were selected. Blood samples were collected via jugular venipuncture at five key reproductive stages: day 0 (pre-mating), 14 days post-mating, and at 45, 75, and 135 days of gestation. Amino acid concentrations were determined using High-Performance Liquid Chromatography (HPLC), whereas fatty acids and volatile acids were analyzed by Gas Chromatography (GC) at each reproductive stage. The concentrations of both essential and non-essential amino acids declined significantly ($P \leq 0.0001$) as pregnancy progressed, with the lowest levels observed on day 75 of gestation. Furthermore, saturated fatty acid levels were significantly higher ($P \leq 0.0001$) during mid-gestation (day 75) compared to the other reproductive stages. In contrast, unsaturated fatty acid levels showed a declining trend toward the end of gestation. Additionally, volatile acid levels increased significantly ($P \leq 0.0001$) up to mid-gestation (day 75). However, no significant associations were observed between amino acid concentrations and either litter size or offspring sex, except for asparagine, which was significantly higher ($P \leq 0.003$) in ewes carrying male fetuses compared to those carrying females. The concentrations of fatty acids and volatile acids showed no significant association with either litter size or offspring sex. In conclusion, the blood profiles of amino acids, fatty acids, and volatile acids may serve as valuable biomarkers for pregnancy detection and for evaluating the nutritional requirements of the developing embryo across different reproductive stages. These findings have the potential to enhance reproductive management strategies and contribute to the improvement of sheep breeding programs.

Keywords: amino acids, fatty acids, pregnancy, sheep

1 Introduction

Reproductive efficiency is a fundamental determinant of productivity in livestock breeding stations, as it ensures a sustainable supply of offspring for meat, milk, and wool production (Ali et al., 2024). During gestation, ewes experience heightened nutritional demands driven by rapid fetal growth and mammary gland development, placing considerable strain on their metabolic systems. These metabolic adaptations required to support fetal development can also influence the levels of various blood biomarkers (Mohammadi et al., 2016). Metabolites, which function as physiological biomarkers, play critical roles in the reproductive functions of both males and females (Al-Saedi and Abdulkareem, 2022; Eidan and Khudhir, 2023; Abdulkareem et al., 2023, 2024; Ali, 2024; Eidan et al., 2024; Al-Gebouri and Eidan, 2024a,b; Musa and Abdulkareem, 2024). Consequently, metabolite profiling is widely utilized to evaluate reproductive performance, as it offers valuable insights into the physiological status and health of reproductive tissues (Habeeb et al., 2023).

Metabolite levels are influenced by various maternal factors, including age, body weight, and reproductive stage, as maternal tissues demand greater energy throughout gestation to support fetal development (Bahr et al., 2022). These physiological demands result in alterations in the mother's biochemical profile. Therefore,

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monitoring these biochemical indicators can aid in the early detection of metabolic imbalances that may negatively affect fetal development (Ahmadzadeh et al., 2016). Amino acids play a critical role in maintaining a conducive uterine environment and enhancing embryonic survival rates (La et al., 2020). Luther et al. (2009) reported that dietary supplementation with arginine in pregnant ewes improves ovarian function and increases embryo survival, with a significant positive correlation identified between arginine levels and progesterone concentration. This highlights arginine's essential role in sustaining pregnancy and supporting early embryonic development. Methionine functions as a major methyl group donor for DNA methylation processes, particularly in late gestation, helping to preserve genomic stability in developing embryos, especially male offspring, when maternal methionine intake is adequate (Wooldridge et al., 2018). Additionally, valine, a branched-chain amino acid, promotes protein synthesis in fetal muscle tissue, contributing to fetal growth (Regnault et al., 2005).

Reproductive traits in sheep involve complex endocrine signaling among the pituitary gland, ovaries, and adipose tissues (Mohammed and Al-Thuwaini, 2024). Adipose tissue plays a crucial role in various aspects of reproduction (Al-Thuwaini, 2022). Furthermore, Fatty acids play significant roles in embryonic development, regulating gene expression associated with lipid metabolism and overall energy homeostasis in mammals. An imbalance in fatty acid composition during fetal development induce structural and functional changes in metabolic pathways (Roque-Jiménez et al., 2021). During the prenatal period, fatty acids and their metabolites are critical for supporting cellular growth and differentiation and coordinating metabolic and neuroendocrine interactions (Innis, 2007). The fetus adapts to maternal fatty acid intake by modulating the production of fetal and placental hormones that influence metabolic regulation, blood flow redistribution, and growth processes (Nickles et al., 2019). Volatile acids are generated as key intermediates in various metabolic pathways, including the catabolism of amino acids and fatty acids, serving as essential substrates for the trivolatile acid (TCA) cycle, the mitochondrial respiratory chain, and neurotransmitter biosynthesis. Volatile acids also play a vital role in the synthesis of various cellular components by supplying carbon atoms that serve as fundamental backbones for numerous metabolic and biosynthetic pathways, including cholesterol biosynthesis (Sauer et al., 2008). Furthermore, Su et al. (2017) demonstrated the potential utility of blood amino acid and fatty acid profiles as biomarkers for pregnancy detection and for evaluating the nutritional and metabolic status of Chinese Hu ewes at days 50, 70, 90, and 110 of gestation. Litter size refers to the number of offspring born per birthing event in animals such as sheep and is influenced by hormonal regulation of ovarian function (Ibrahim et al., 2024). Goldansaz et al. (2022) identified significant associations between specific blood biomarkers including amino acids, fatty acids, and volatile acids, and both pregnancy status and litter size in American Suffolk × Dorset ewes at days 0, 35, 50, and 70 of gestation. However, the serum profiles of these metabolites at 14 days post-mating, and at 75 and 135 days of gestation, as well as their associations with litter size and offspring sex in Awassi ewes, have not been previously investigated. Therefore, the present study aimed to characterize the serum profiles of amino acids, fatty acids, and volatile acids in Awassi ewes across different reproductive stages and to examine their relationships with litter size and offspring sex.

2 Material and Methods

2.1 Animals and Design

This study was conducted from November 2023 to March 2024 at the Khairat Al-Ittihad private livestock station, located in the Shumali region of the Wassit Governorate, in collaboration with the laboratories of the Ministry of Science and Technology. A total of 30 Iraqi Awassi ewes, aged between 3.5 and 4.5 years and weighing 45–50 kg, were selected for the study. All animals were clinically healthy and remained under continuous veterinary supervision throughout the experimental period. Throughout the first three months of gestation, ewes were fed a concentrate diet equivalent to 2%.

their live body weight. The concentrate consisted of 30% barley, 12% wheat bran, 10% maize, 10% soybean meal, 1% limestone, 1% salt, and 1% vitamin and mineral premix. In addition, each ewe was provided with 20 kg of alfalfa hay and 15 kg of wheat straw. This diet provided a total crude protein content of 14.3% and a gross energy value of 3416 Mcal/kg. During the final two months of pregnancy, the dietary allowance was increased to 3% of the ewe's live body weight. The reformulated concentrate included 25% barley, 25% wheat bran, 22% maize, 10% soybean meal, 1% limestone, 1% salt, and 1% vitamin and mineral premix, along with 10 kg of alfalfa hay and 5 kg of wheat straw. The adjusted diet contained 15.6% crude protein and a gross energy content of 3777 Mcal/kg. Blood samples were collected from all ewes at five distinct reproductive stages: day 0 (pre-mating), 14 days post-mating (PM), 45 days PM, and at 75 and 135 days of gestation. At each stage, serum concentrations of amino acids, fatty acids, and volatile acids were analyzed. Additionally, the potential effects of litter size (singleton or multiple) and offspring sex on these biochemical

parameters were evaluated. Estrus synchronization was performed using a Controlled Internal Drug Release (CIDR) vaginal device. Estrous behavior was monitored three times daily with the assistance of teaser rams to detect the onset of estrus. The onset of estrus in cyclic ewes was designated as day 0. Pregnancy was confirmed using three complementary methods:

1. observation of non-return to estrus, with monitoring by professional veterinarians on days 17 to 18 post-mating (PM);
2. transabdominal ultrasonography conducted on day 45 PM;
3. measurement of serum progesterone concentrations on days 14 and 45 PM.

Reproductive performance indicators including fertility rate, conception rate, lambing rate, twinning rate, litter size, and barrenness, were evaluated in accordance with the procedures described by Vlahek et al. (2023). Blood samples were collected from each ewe at five reproductive stages: day 0 (pre-mating), 14 days post-mating (PM), 45 days PM, 75 days of gestation, and 135 days of gestation. Samples were obtained via jugular venipuncture using sterile vacutainer needles and plain tubes without anticoagulant (i.e., free of ethylenediaminetetraacetic acid [EDTA]). Following collection, blood samples were allowed to clot at room temperature, and serum was separated by centrifugation at 3,000 rpm for 5 minutes. The serum was then aliquoted and stored under appropriate conditions for subsequent biochemical analysis.

2.2 Amino Acids, Fatty Acids and Volatile Acids Assay

Serum amino acid concentrations were determined following the method described by Abadi et al. (2017). Quantification was conducted using an amino acid analyzer (Model YL9100, Korea), calibrated with standard curves. The mobile phase consisted of acetonitrile, buffer, and distilled water in a volumetric ratio of 60:10:30. Chromatographic separation was performed using a C18-NH_2 column (25 cm × 4.6 mm) at a flow rate of 1.0 mL/min. Detection of amino acids was carried out via fluorescence, with excitation and emission wavelengths set at 365 nm and 445 nm, respectively. Serum fatty acid concentrations were determined using the method described by Majnooni et al. (2016), with slight modifications. Derivatization was performed by adding 250 µL of 9-fluorenylmethyl chloroformate (FMOC-Cl) solution to 1 mL of oil, followed by the addition of 25 µL of sodium phosphate buffer (0.05 M, pH 9.3). After brief mixing, the samples were incubated at 40°C for 10 minutes. Subsequently, 100 µL of the reaction mixture was injected into the high-performance liquid chromatography (HPLC) system. The HPLC setup included a dual-pump solvent delivery system (SYKAM, Germany) and a spectrofluorometric detector, operated at excitation and emission wavelengths of 265 nm and 315 nm, respectively. The analytical separation was carried out using a Shim-pack C18-ODS column (250 mm × 4.6 mm). The mobile phase consisted of a gradient elution of acetonitrile and water, with the following conditions: 85 : 15 (v/v) from 0 to 4 minutes (Phase A), 87 : 13 from 5 to 8 minutes (Phase B), and 97 : 3 from 9 to 14 minutes (Phase C). The column oven temperature was maintained at 50 °C. The mobile phase was filtered, degassed, and delivered at a flow rate of 1.5 mL/min. The separation of volatile acids; acetic acid, butyric acid, and propionic acid, were performed using a high-performance liquid chromatography (HPLC) system (SYKAM, Germany). The mobile phase consisted of distilled water containing 2% phosphoric acid (H_3PO_4) and methanol in a 30 : 70 (v/v) ratio. An ODS-C18 column (4.6 mm × 25 cm) was used for the analysis. The injection volume was 100 µL, with a flow rate of 1.0 mL/min. Detection was carried out at a wavelength of 210 nm.

2.3 Statistical analysis

Statistical analysis was performed using the Statistical Analysis System (SAS, 2018), employing a completely randomized design (CRD) with a one-way classification model. Differences among means were evaluated using Duncan's multiple range test (Duncan, 1955) to determine statistical significance.

3 Results and Discussion

3.1 Reproductive Performances of the Experimental Ewe Flock

The fertility, conception, lambing, and twinning rates of the experimental ewe flock were 93%, 100%, 114%, and 14.2%, respectively. The average litter size was 1.14, while the barrenness rate was recorded at 6.7%.

3.2 Serum Amino Acids in Awassi Ewes During Different Reproductive Stages

The concentration of essential amino acids exhibited a highly significant reduction ($P \leq 0.0001$) across the subsequent reproductive stages in comparison to the pre-mating phase. The data demonstrated a progressive decline in the levels of most essential amino acids as gestation advanced, with a relative increase observed by day 135 of pregnancy. Notably, isoleucine and valine concentrations exhibited a significant decline by day 75 of gestation, remaining substantially lower than the baseline levels observed on day 0 of the study. However, their concentrations began to increase again by day 135 of pregnancy (Table 1). Similar to the pattern

Table 1 Profile of serum essential amino acids in Awassi ewes across different reproductive stages (mean ±SE)

Serum essential amino acids (ppm)	Day 0 (n = 30)	Day 14 PM (n = 30)	Day 45 PM (n = 30)	Day 75 of pregnancy (n = 30)	Day 135 of pregnancy (n = 30)	P-value
Lysine	9.51 ±0.03A	8.57 ±0.06 B	8.34 ±0.02C	6.47 ±0.03D	8.29 ±0.01C	0.0001
Threonine	8.59 ±0.04A	6.50 ±0.007B	6.51 ±0.04B	4.45 ±0.04C	6.49 ±0.04 B	0.0001
Leucine	8.84 ±0.01A	6.54 ±0.04 B	6.40 ±0.03C	5.61 ±0.03D	6.42 ±0.04 C	0.0001
Isoleucine	12.45 ±0.02A	10.7 ±0.002 B	10.38 ±0.02C	8.43 ±0.04E	10.29 ±0.02D	0.0001
Valine	18.66 ±0.03A	16.13 ±0.01D	16.55 ±0.04B	13.69 ±0.01E	16.36 ±0.02C	0.0001
Methionine	9.36 ±0.01A	7.65 ±0.02 B	7.42 ±0.04C	5.46 ±0.03D	7.50 ±0.05 CB	0.0001
Histidine	7.62 ±0.02A	5.59 ±0.05 B	5.32 ±0.02C	4.36 ±0.02D	5.33 ±0.02 C	0.0001
Phenylalanine	10.3 ±0.02 A	8.50 ±0.05 B	8.45 ±0.02CB	6.50 ±0.04D	8.38 ±0.02 C	0.0001
Total	85.4 ±0.07 A	70.21 ±0.12B	69.39 ±0.13 C	54.99 ±0.1D	69.10 ±0.13C	0.0001

Means with different superscripts within each row indicate significant differences ($P \leq 0.0001$), PM – post-mating

Table 2 Profile of serum non-essential amino acids in Awassi ewes across different reproductive stages (mean ±SE)

Serum non-essential amino acids (ppm)	Day 0 (n = 30)	Day 14 PM (n = 30)	Day 45 PM (n = 30)	Day 75 of pregnancy (n = 30)	Day 135 of pregnancy (n = 30)	P-value
Aspartic acid	9.11 ±0.005A	8.29 ±0.027B	8.20 ±0.01C	6.33 ±0.02D	8.21 ±0.017C	0.0001
Asparagine	8.50 ±0.009A	7.32 ±0.02B	7.40 ±0.04B	5.66 ±0.02C	7.37 ±0.03B	0.0001
Serine	10.62 ±0.04A	8.50 ±0.05B	8.49 ±0.04B	6.18 ±0.005C	8.42 ±0.04B	0.0001
Alanine	10.33 ±0.01A	9.38 ±0.01B	9.47 ±0.05B	7.25 ±0.02C	9.48 ±0.04B	0.0001
Tyrosine	12.55 ±0.04A	10.24 ±0.01B	10.21 ±0.008B	8.51 ±0.001D	10.16 ±0.006C	0.0001
Arginine	10.20 ±0.007A	8.32 ±0.02B	8.29 ±0.02B	6.11 ±0.005C	8.31 ±0.01B	0.0001
Cysteine	9.71 ±0.01A	7.33 ±0.02C	7.70 ±0.008B	5.50 ±0.04D	7.64 ±0.01B	0.0001
Proline	6.29 ±0.02A	5.35 ±0.01B	5.21 ±0.001C	4.26 ±0.02D	5.21 ±0.01C	0.0001
Glycine	9.54 ±0.04A	6.57 ±0.04B	6.34 ±0.02C	4.42 ±0.02D	6.32 ±0.02C	0.0001
Total	86.85 ±0.07A	71.31 ±0.07B	71.33 ±0.1B	46.29 ±0.3C	71.15 ±0.07B	0.0001

Means with different superscripts within each row indicate significant differences($P \leq 0.0001$), PM – post-mating

observed for essential amino acids, the concentrations of all examined non-essential amino acids showed a highly significant decrease ($P \leq 0.0001$) throughout the different stages of pregnancy in ewes (Table 2). The data revealed that several non-essential amino acids, including asparagine, serine, L-tyrosine, and arginine, underwent a consistent and pronounced decline from day 0 (pre-gestation) through day 75 of gestation. The nadir of their concentrations was recorded on day 75, with values as follows: asparagine (8.20 ±0.01 ppm), serine (6.18 ±0.005 ppm), L-tyrosine (7.25 ±0.02 ppm), and arginine (6.11 ±0.005 ppm). Following the observed decline, a relative increase in the concentrations of these amino acids was noted by day 135 (the final month of gestation), although their levels remained below those recorded prior to pregnancy. On day 135, the concentrations were as follows: asparagine (7.37 ±0.03 ppm), serine (8.42 ±0.04 ppm), L-tyrosine (9.48 ±0.04 µmol/L), and arginine (8.31 ±0.01 ppm). In contrast, glycine and proline exhibited a marked reduction, with glycine declining to less than half of its baseline concentration by day 75, reaching 4.42 ±0.02 ppm compared to its initial level on day 0 (Table 2).

3.3 Serum Amino Acid Profile of Awassi Ewes Based on Litter Size and Offspring Sex

No significant differences were observed in the concentrations of essential amino acids between single and twin births, nor between male and female offspring (Table 3). With the exception of asparagine, which was significantly higher ($P \leq 0.003$) in ewes bearing male offspring (8.53 ±0.01 ppm) compared to those with female offspring (8.47 ±0.01 ppm), the concentrations of all other serum non-essential amino acids did not differ significantly based on litter size or offspring sex (Table 4).

3.4 Serum Fatty Acids Profile in Awassi Ewes During Different Reproductive Stages

The results demonstrated a highly significant increase ($P \leq 0.0001$) in the concentrations of saturated fatty acids across the stages of pregnancy (Table 5).

Table 3 Serum essential amino acid profile in Awassi ewes according to litter size and offspring sex (mean ±SE)

Serum essential amino acids (ppm)	Litter size			Offspring sex		
	single (n = 26)	twin (n = 4)	P-value	male (n = 11)	female (n = 17)	P-value
Lysine	9.50 ±0.03	9.51 ±0.09	0.6740	9.53 ±0.05	9.48 ±0.04	0.4668
Threonine	8.58 ±0.05	8.67 ±0.17	0.5594	8.54 ±0.07	8.64 ±0.06	0.2708
Leucine	8.84 ±0.01	8.81 ±0.05	0.5206	8.85 ±0.02	8.82 ±0.02	0.4904
Isoleucine	12.46 ±0.02	12.41 ±0.08	0.5256	12.46 ±0.04	12.44 ±0.03	0.6982
Valine	18.66 ±0.03	18.65 ±0.07	0.9023	18.66 ±0.05	18.66 ±0.04	0.9949
Methionine	9.366 ±0.01	9.35 ±0.02	0.6425	9.34 ±0.01	9.37 ±0.01	0.1973
Histidine	7.621 ±0.02	7.67 ±0.06	0.3823	7.58 ±0.02	7.66 ±0.02	0.0608
phenylalanine	10.37 ±0.02	10.39 ±0.05	0.7662	10.34 ±0.02	10.41 ±0.02	0.1174
Total	85.42 ±0.08	85.52 ±0.12	0.6893	85.34 ±0.1	85.53 ±0.09	0.2247

Table 4 Serum non-essential amino acid profile in Awassi ewes according to litter size and offspring sex (mean ±SE)

Serum non-essential amino acids (ppm)	Litter size			Offspring sex		
	single (n = 26)	twin (n = 4)	P-value	male (n = 11)	female (n = 17)	P-value
Aspartic acid	9.10 ±0.006	9.13 ±0.006	0.1278	9.10 ±0.009	9.11 ±0.006	0.4028
Asparagine	8.50 ±0.01	8.49 ±0.02	0.7461	8.53 ±0.01A	8.47 ±0.01B	0.003
Serine	10.63 ±0.05	10.53 ±0.07	0.5027	10.61 ±0.08	10.62 ±0.06	0.9025
Alanine	10.33 ±0.02	10.29 ±0.06	0.4673	10.31 ±0.02	10.33 ±0.02	0.6526
Tyrosine	12.57 ±0.04	12.34 ±0.05	0.0670	12.53 ±0.07	12.56 ±0.05	0.7401
Arginine	10.20 ±0.007	10.18 ±0.03	0.5141	10.19 ±0.01	10.20 ±0.01	0.7647
Cysteine	9.71 ±0.01	9.69 ±0.02	0.3900	9.72 ±0.02	9.70 ±0.01	0.3385
Proline	6.27 ±0.02	6.35 ±0.08	0.2660	6.29 ±0.03	6.27 ±0.03	0.6667
Glycine	9.52 ±0.04	9.63 ±0.13	0.3756	9.55 ±0.05	9.52 ±0.05	0.7402
Total	86.88 ±0.08	86.65 ±0.09	0.3426	86.87 ±0.13	86.82 ±0.10	0.7710

Means with different superscripts within each row indicate significant differences($P \leq 0.003$)

Table 5 Serum saturated and unsaturated fatty acids profile in Awassi ewes across different reproductive stages (mean ±SE)

Serum fatty acids (%)	Day 0 (n = 30)	Day 14 PM (n = 30)	Day 45 PM (n = 30)	Day 75 of pregnancy (n = 30)	Day 135 of pregnancy (n = 30)	P-value
Palmitic	3.42 ±0.02D	4.69 ±0.007C	4.85 ±0.007B	6.02 ±0.004A	4.86 ±0.006B	0.0001
Stearic	1.46 ±0.02D	2.51 ±0.01C	2.90 ±0.008B	4.16 ±0.006A	2.89 ±0.007B	0.0001
Saturated total	4.88 ±0.03D	7.20 ±0.02C	7.76 ±0.01B	10.19 ±0.00A	7.76 ±0.009B	0.0001
Oleic	6.64 ±0.02D	7.47 ±0.004C	7.69 ±0.02B	8.73 ±0.02A	7.69 ±0.02B	0.0001
Linoleic	11.47 ±0.02D	13.53 ±0.01C	13.93 ±0.01B	15.33 ±0.01A	13.88 ±0.01B	0.0001
α-Linoleic	7.69 ±0.03D	9.12 ±0.003C	9.53 ±0.008B	11.35 ±0.01A	9.54 ±0.008B	0.0001
Eicosapentaenoic	4.35 ±0.02D	5.91 ±0.008C	6.03 ±0.003B	7.65 ±0.02A	6.02 ±0.002B	0.0001
Arachidonic	0.29 ±0.005E	0.81 ±0.01D	0.92 ±0.009B	1.11 ±0.001A	0.89 ±0.009C	0.0001
Unsaturated Total	30.47 ±0.05D	36.85 ±0.01C	38.10 ±0.02B	44.17 ±0.04A	38.03 ±0.02B	0.0001

Means with different superscripts within each row indicate significant differences ($P \leq 0.0001$), PM – post-mating

Specifically, palmitic acid concentrations increased from 3.42 ±0.02% before mating to 6.02 ±0.004% on day 75 of gestation, followed by a decrease to 4.86 ±0.006% by day 135. Similarly, stearic acid levels rose from 1.46 ±0.02% at the pre-mating stage to 4.16 ±0.006% on day 75, then declined to 2.89 ±0.007% at day 135 (Table 5). Overall, total saturated fatty acids significantly increased from 4.88 ±0.03% before mating to a peak of 10.19 ±0.00% at day 75, followed by a decrease to 7.76 ±0.009% on day 135. These findings suggest that saturated fatty acid concentrations reach their highest levels during mid-gestation and begin to decline in the later stages of pregnancy. The analysis of unsaturated fatty acids also revealed a highly significant effect ($P \leq 0.0001$) of reproductive stage (Table 6). All unsaturated fatty acids showed a steady increase from the pre-mating period to day 75 of gestation, after which their concentrations declined toward the final stages of pregnancy (Table 6).

3.5 Serum Saturated and Unsaturated Acid Profile of Awassi Ewes Based on Litter Size and Offspring Sex

The current study found no statistically significant effects of litter size or offspring sex on the serum concentrations of saturated and unsaturated fatty acids (Table 6).

3.6 Serum Volatile Acids Profile in Awassi Ewes During Different Reproductive Stages

With respect to acetic acid, its concentration prior to mating was 5.51 ±0.02%. This level increased significantly ($P \leq 0.0001$) and peaked at 7.60 ±0.03% by mid-pregnancy (day 75), followed by a slight decline thereafter (Table 7). Similarly, butyric acid reached 4.62 ±0.01% at mid-pregnancy before decreasing in the later stage. Propionic acid levels also exhibited a steady increase, reaching 5.85 ±0.007% on day 75, followed by a subsequent decline. Total volatile acid concentrations increased progressively, reaching a maximum of 18.08 ±0.033% on day 75. By day 135, the level decreased to 14.56 ±0.019%, though it remained higher than the pre-mating baseline (Table 7).

Table 6 Serum saturated and unsaturated acid profile in Awassi ewes according to litter size and offspring sex (mean ±SE)

Serum fatty acids (%)	Litter size			Offspring sex		
	single (n = 26)	twin (n = 4)	P-value	male (n = 11)	female (n = 17)	P-value
Palmitic	3.40 ±0.02	3.54 ±0.01	0.0173	3.41 ±0.03	3.42 ±0.02	0.9484
Stearic	1.46 ±0.02	1.45 ±0.09	0.8576	1.43 ±0.04	1.48 ±0.02	0.3110
Saturated total	4.86 ±0.03	4.99 ±0.07	0.1853	4.85 ±0.05	4.90 ±0.03	0.4182
Oleic	6.66 ±0.03	6.56 ±0.06	0.2908	6.62 ±0.03	6.65 ±0.04	0.5880
Linoleic	11.46 ±0.03	11.56 ±0.01	0.2469	7.73 ±0.04	7.67 ±0.04	0.0499
α-Linoleic	7.72 ±0.03	7.55 ±0.08	0.0561	11.41 ±0.03	11.52 ±0.03	0.3097
Eicosapentaenoic	4.36 ±0.02	4.29 ±0.09	0.4008	4.36 ±0.04	4.34 ±0.03	0.6644
Arachidonic	0.29 ±0.005	0.28 ±0.01	0.4754	0.29 ±0.007	0.29 ±0.008	0.9037
Unsaturated total	30.49 ±0.06	30.25 ±0.04	0.1340	30.43 ±0.05	30.49 ±0.08	0.5984

Table 7 Serum volatile acids profile in Awassi ewes during different reproductive stages (mean ±SE)

Serum volatile acids (%)	Day 0 (n = 30)	Day 14 PM (n = 30)	Day 45 PM (n = 30)	Day 75 of pregnancy (n = 30)	Day 135 of pregnancy (n = 30)	P-value
Acetic	5.51 ±0.02E	6.16 ±0.007D	6.81 ±0.004B	7.60 ±0.03A	6.42 ±0.006C	0.0001
Butyric	2.15 ±0.006E	3.17 ±0.01D	3.4 ±0.01B	4.62 ±0.01A	3.48 ±0.01C	0.0001
Propionic	3.40 ±0.01E	4.19 ±0.009D	4.82 ±0.01B	5.85 ±0.007A	4.65 ±0.01C	0.0001
Total	11.07 ±0.02E	13.53 ±0.01D	15.48 ±0.02B	18.08 ±0.03A	14.56 ±0.01C	0.0001

Means with different superscripts within each row indicate significant differences($P \leq 0.0001$), PM – post-mating

3.7 Serum Volatile Acid Profile of Awassi Ewes Based on Litter Size and Offspring Sex

No significant differences were observed in the concentrations of acetic, butyric, propionic, or total volatile acids between singleton and twin-bearing ewes. Similarly, no significant differences were found between male and female offspring groups (Table 8).

This study is the first in Iraq and globally to investigate how different stages of pregnancy (days 0–135) affect the levels of amino acids, fatty acids, and volatile acids in Iraqi Awassi ewes. Additionally, it explores the relationship between these acids, various litter sizes, and the sex of the offspring. While previous studies (Mohammed et al., 2021; Khazaal et al., 2023) have addressed similar topics, they primarily focused on only a few stages of pregnancy, typically the last two months or specific periods (such as days 120 and 150). This study also examines how certain reproductive performance traits, including litter size and the sex of the fetus, influence these parameters. It is important to highlight that the current flock of ewes demonstrates excellent reproductive performance. The fertility rate stands at 93.3%, with a conception rate of 100%, a lambing rate of 114.2%, and a twinning rate of 14.2%. Additionally, the average litter size is 1.14, and the barrenness rate is 6.7%. These figures reflect the effectiveness of mating practices, as well as proper nutrition and healthcare management. Notably, these outcomes exceed those reported by Alkass et al. (2004) and Abdulkareem et al. (2023) for Awassi ewes in different regions of Iraq.

The results revealed that the concentrations of both essential and non-essential amino acids declined during the later stages of pregnancy compared to the pre-mating period. The lowest amino acid levels were recorded on day 75 of pregnancy. This suggests that amino acids play a crucial role in supporting the physiological processes involved in fetal tissue growth and development, particularly during the early stages of pregnancy (Ulwahhab and Khalil, 2020). Therefore, it is essential to ensure an adequate supply of amino acids throughout all stages of pregnancy to support healthy fetal development and maintain a normal gestational course. During early pregnancy, overall amino acid concentrations in the maternal bloodstream typically increase, a change that is at least partly due to the heightened demand for protein synthesis in the developing fetal muscles (Tain and Hsu, 2024). The primary period of fetal muscle development in sheep occurs during the first two trimesters of gestation, with most muscle fiber formation taking place within the first three months (Du et al., 2017). As the fetus matures and enters the later stages of pregnancy, its demand for amino acids decreases, leading to a normalization of amino acid concentrations in the ewe's bloodstream (Edwards et al., 2020). This is consistent with our observations of the current serum levels of most essential and non-essential amino acids on day 135 of pregnancy. Moreover, the uptake, metabolism, and transport of amino acids by the ovine placenta are essential processes that support fetal survival, growth, and development (Wang et al., 2012). Amino acids play a pronounced role in fetal energy metabolism and are essential building blocks for synthesizing important biomolecules, such as proteins, neurotransmitters, and polyamines, in both the fetus and the placenta (McCoard et al., 2016). Placental transport maintains fetal amino acid homeostasis (Bell and Ehrhardt, 2002). Changes in fetal circulating amino acid concentrations closely reflect those of the dam (Kwon et al., 2003), underscoring the significance of amino acid transport across the placental barrier. In ovine species, most amino acids are delivered to the fetus in quantities exceeding those necessary for net tissue accretion (Marconi et al., 1989). This process is energy-dependent (Regnault et al., 2005), resulting in higher fetal amino acid concentrations than maternal levels (Ashworth et al., 2011).

The decrease in serum concentrations of arginine and histidine between days 0 and 75 of pregnancy observed in the current study is consistent with findings by Gao et al. (2009) and Wang et al. (2015), who reported an 8- to 25-fold increase in the total amounts of these amino acids in uterine flushings between days 10 and 16 of pregnancy. This shift suggests a transfer from serum to the uterine environment, highlighting the essential role of arginine and histidine in supporting fetal growth and development. Arginine is a cationic amino acid that plays a critical role in implantation and pregnancy as a precursor for nitric

Table 8 Serum volatile acid profile in Awassi ewes according to litter size and offspring sex (mean ±SE)

Serum volatile acids (%)	Litter size			Offspring sex		
	single (n = 26)	twin (n = 4)	P-value	male (n = 11)	female (n = 17)	P-value
Acetic	5.51 ±0.02	5.52 ±0.04	0.8305	5.54 ±0.03	5.48 ±0.02	0.2010
Butyric	2.15 ±0.007	2.14 ±0.02	0.4642	2.16 ±0.008	2.14 ±0.01	0.2978
Propionic	3.40 ±0.01	3.38 ±0.04	0.6511	3.38 ±0.02	3.42 ±0.02	0.2941
Total	11.07 ±0.03	11.05 ±0.09	0.7785	11.08 ±0.04	11.05 ±0.04	0.6001

oxide, which promotes angiogenesis and vasodilation, as well as for polyamines involved in numerous cellular functions essential for conceptus development (Flynn et al., 2002). Adequate arginine levels during gestation are vital, as deficiencies can lead to intrauterine growth restriction and altered fetal gene expression (Wu et al., 2004). In this regard, Thureen et al. (2002) reported that the 4.2-fold greater increase in uteroplacental compared to net fetal arginine uptake may reflect preferential arginine metabolism by the uteroplacental tissues, a relatively limited placental-to-fetal arginine transport capacity compared to uterine uptake capacity, or both. Additionally, high levels of arginine during the late stages of pregnancy, specifically in the third trimester, are associated with increased birth weight, improved fetal blood flow, and enhanced nutrient transfer due to nitric oxide production (Thureen et al., 2002 and De Boo et al., 2005). Morovere, Erichsen et al. (2024) observed that maternal supplementation with arginine affected the expression of genes related to amino acid transport, placental efficiency, and angiogenesis. These changes may influence the placental transport capacity. Overall, the reduced oxidation of essential amino acids during late pregnancy suggests that there is effective retention and preferential deposition of nitrogen and amino acids in tissue proteins (Duggleby and Jackson, 2002).

Freetly and Ferrell (1998) found that the concentration of valine increases during the later stages of pregnancy, serving as an alternative energy source for the fetal brain (Goldansaz et al., 2022). This finding aligns with current data, which shows that valine and isoleucine serum concentrations were reduced during the first trimester (day 75 of pregnancy) but increased significantly in the third trimester (day 135). Valine and isoleucine, which are branched-chain amino acids (BCAAs), play a vital role in the growth and development of ovine fetuses. They serve as essential building blocks for protein synthesis and contribute to energy production. The availability of these amino acids affects placental transport, which in turn impacts fetal growth and development (Kwon et al., 2003).

A recent metabolomics study has identified certain serum biomarkers that can predict litter size in pregnant sheep (Zhai et al., 2023). However, the current findings indicate that neither litter size nor offspring sex affects amino acid levels. This conclusion contradicts the findings of Goldansaz et al. (2022), who reported that ewes-carrying twins had higher arginine concentrations (a non-essential amino acid) in their blood serum. Additionally, amino acids such as glutamine, glutamate, arginine, histidine, and leucine have been recognized as limiting factors in developing twin fetuses. Ewes that give birth to twins show an increased rate of fetal protein synthesis, resulting in a higher consumption of valine, leading to lower serum valine concentrations than ewes that give birth to a single lamb (Freetly and Ferrell, 1998).

Concentrations of saturated, unsaturated, and volatile acids have been observed to peak around day 75 of gestation in ewes, followed by a slightly decline toward day 135. The findings are consistent with those of Aparicio et al. (2021), who demonstrated a significant increase in both total saturated and unsaturated fatty acids from the first to the third trimester of pregnancy. In contrast, Duttaroy (2009) reported that placental lipase activity increases during the final trimester of pregnancy, probably enhancing placental fatty acid delivery during the maximal fetal fatty acid requirement period. Concomitantly, volatile acids, like propionic acid, influences fetal development and metabolic programming in pregnant women (Ziętek et al., 2021). During pregnancy, maternal plasma fatty acids play a crucial role in cell growth and development, cell signaling, and the formation of essential structural and functional components of the fetoplacental unit. In the first trimester, these fatty acids influence the early placental development by regulating angiogenesis. Additionally, the special transport of maternal plasma long-chain polyunsaturated fatty acids (LCPUFA) during the late pregnancy is vital for optimal fetal brain development. The critical need for LCPUFAs in the fetoplacental unit necessitates efficient transport from the mother to the fetus through the placenta. The placenta is vital in mobilizing fat stores from maternal adipose tissue and actively concentrating and directing essential LCPUFAs to the fetus. This process occurs through several mechanisms, including selective uptake by the trophoblast, intracellular metabolic transfer, and a targeted supply to the fetal circulation (Duttaroy and Basak, 2022).

The significant fluctuations in fatty acid profiles are not random but appear closely associated with the physiological demands and metabolic adjustments across different pregnancy stages. Further, the energy level and nutrient source available to ewes during gestation have been demonstrated to significantly influence lamb performance outcomes (Peñagaricano et al., 2014). In particular, omega-3 (n-3) polyunsaturated fatty acids, notably eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), are highly bioactive and capable of modulating key metabolic pathways. These fatty acids support energy metabolism by improving the gene expression related to fat breakdown and suppressing those related to fat storage, thereby enhancing the body's capability to use fats for energy., and shifting in metabolism leads to a higher energy output than other nutrients (Clarke, 2001). The slight

decline in fatty acids and volatile acids may be attributed to the increased metabolic demands of the fetus as pregnancy progresses. These changes could potentially have implications for fetal health and development. Moreover, a previous study found that calves born to cows supplemented with 190 grams per day (0.032% of body weight) of a mixture of unsaturated fatty acids, including linolenic acid, DHA, and EPA, showed a tendency to have increased average daily gains and live body weights during the final period of gestation (Marques et al., 2017).

Litter size and offspring sex had not effects on fatty acid levels in the current results. These findings were disagreed with those reported by Gulliver et al. (2013), who revealed that ewes fed on high dietary saturated fatty acids (linoleic and α-linoleic) for six week before and 3 weeks following conception produced a higher proportion of female lambs. Ewes carrying male fetuses may experience altered serum fatty acid levels during pregnancy compared to female fetuses. Specifically, Alon et al. (2023) suggest that ewes carrying a high male ratio (HMR) have lower plasma β-hydroxybutyrate and non-esterified fatty acid (NEFA) concentrations compared to those with a low male ratio (LMR). Additionally, maternal obesity in sheep can affect fetal fatty acid synthesis, potentially influencing the offspring's metabolism later in life.

4 Conclusion

The blood profiles of amino acids, fatty acids, and volatile acids may serve as valuable biomarkers for pregnancy detection and for evaluating the nutritional requirements of the developing embryo across different reproductive stages. These findings hold promise for enhancing reproductive management strategies and advancing sheep breeding programs. However, neither litter size nor offspring sex appeared to influence the profiles of these metabolites.

Acknowledgments

The authors would like to express their gratitude to the farm manager at Al-Itihad for their generous cooperation and assistance.

Conflict of Interest

The authors declare no conflict of interest in this manuscript.

Authors Contribution

Serum amino acids, fatty acids, and volatile acids assays, as well as original draft preparation and editing, were performed by Rwaida A. Ali. Experimental design, selection and analysis of specific microRNAs, statistical analysis, manuscript writing, and critical revisions were conducted by the corresponding author, Dr. Talal A. Abdulkareem.

AI and AI-Assisted Technologies Use Declaration

No generative AI tools/AI-assisted technologies were used during the preparation of the manuscript.

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