Praca oryginalna *Original paper*

Use of metabolomics tools in the evaluation of the fattening performance of lambs*

MÜCAHİT KAHRAMAN[1], AYDIN DAŞ[1], GÜLŞAH GÜNGÖREN[1], BESİME DOĞAN DAŞ[2], HAMZA YALÇIN[3], İSMAİL KOYUNCU[4], ŞÜKRÜ AKMEŞE[5]

[1]Department of Animal Husbandry, Faculty of Veterinary Medicine, Harran University, Şanlıurfa, Turkey
[2]Department of Animal Nutrition and Nutritional Diseases, Faculty of Veterinary Medicine, Harran University, Şanlıurfa, Turkey
[3]Department of Biostatistics, Harran University, Faculty of Agriculture, Harran University, Şanlıurfa, Turkey
[4]Department of Biochemistry, Faculty of Medicine, Harran University, Şanlıurfa, Turkey
[5]Program of Pharmacy Services, Vocational School of Health Services, Harran University, Şanlıurfa, Turkey

Received 23.03.2022 *Accepted 11.06.2022*

Kahraman M., Daş A., Güngören G., Doğan Daş B., Yalçin H., Koyuncu İ., Akmeşe Ş.
Use of metabolomics tools in the evaluation of the fattening performance of lambs

Summary

This study aimed to determine the metabolomic characteristics associated with the fattening performance of the Awassi lamb breed under intensive fattening for 90 days. Twenty-four lambs were used in the research. The lambs were divided into two groups according to their fattening performance (good fattening performance, n = 12, and poor fattening performance, n = 12), and their metabolomic properties were evaluated. The differences between the two research groups in the amino acids of alloisoleucine, aspartic acid, histidine, hydroxylysine, isoleucine, lysine, methionine, phenylalanine, tryptophan, valine, and 1-methylhistidine were found to be statistically significant ($P \leq 0.001$). Moderately significant negative correlations were found between daily concentrate feed intake and 3-methylhistidine ($r = -0.469$; $P = 0.021$), hydroxylysine ($r = -0.408$; $P = 0.048$), and serotonin ($r = -0.467$; $P = 0.021$); as well as between the average daily weight gain (ADWG) and alloisoleucine ($r = -0.528$; $P = 0.008$), 3-methylhistidine ($r = -0.440$; $P = 0.032$), and hydroxylysine ($r = -0.577$; $P = 0.003$). A moderate positive correlation was found between hydroxyisovalerylcarnitine and ADWG ($r = 0.476$; $P = 0.019$), and a negative correlation was found between hydroxyisovalerylcarnitine and the feed conversion ratio ($r = -0.430$; $P = 0.036$). Pathway analysis revealed that the most important biological pathway was the phenylalanine, tyrosine, and tryptophan biosynthesis pathway. The results of the research reveal the potential for plasma free amino acid and carnitine profiles to be used as candidate biomarkers in the evaluation of fattening performance in lambs.

Keywords: amino acid, carnitine, fattening performance, metabolomics, lamb

Feed intake and feed conversion ratio (FCR) are widely used to identify animals that are efficient at converting feed into products (20). Improved feed conversion efficiency can bring significant benefits for the industry by reducing feed costs while maintaining or increasing the level of production (24). Animals with a low FCR are considered efficient and eat less, whereas animals with a high FCR are inefficient and consumes more feed. The feed utilization rate is easy to calculate, but there are some practical limitations. Sheep breeding in flocks, feeding animals as a group, and labor costs make it difficult to calculate individual feed consumption (20). Therefore, alternative methods are required for determining feed consumption in livestock raising.

Sheep of the Awassi breed are of significant value in the Middle East and the Mediterranean region because of their meat and milk yields (5). Awassi breed sheep stand out for their resistance to adverse environmental conditions (2). Among the fat-tailed sheep, there is a large variation in yield characteristics in this genotype (15). Rapid progress is achieved by selection in flocks with wide variations in yield characteristics (1). Determining high-performance animals under breeding conditions is a very laborious process (27). It is important to investigate and apply new selection parameters for quantitative characteristics, such as growth and meat yield (31). Therefore, analyzing the correlation between yield characteristics and metabolites obtained from the classical performance test constitutes a significant shortcoming (8). Numerous researchers have reported that metabolomic parameters obtained

* This study was financially supported by HUBAP (Harran University Scientific Research Projects Unit) under project number 20002 (y. 2021).

by liquid and gas chromatographic methods can be used as markers in the selection of high-yielding farm animals (11, 12, 28).

In this study, we aimed to establish new selection criteria for fattening performance (FP) and determine the metabolomic characteristics (plasma free amino acid and carnitine profiles) associated with average daily weight gain (ADWG), daily concentrate feed consumption (DCFI), and FCR in Awassi breed lambs.

Material and methods

Animal care and feeding. Within the scope of the research, permissions necessary for all kinds of procedures to be applied to animals were obtained from the Harran University Animal Experiments Local Ethics Committee (21.01.2020 Date 2020/001 session, Decision No. 01-04).

To determine the FP characteristics of lambs, 24 3-month-old Awassi breed lambs born on close dates were used. The animals used in the study were selected by following their live weights from birth to the weaning period. Lambs weaned at an average age of 90 days were sprayed against internal and external parasites before starting feeding. After a 2-week eating habituation period, the lambs, which reached an average live weight of 22 kg, were placed in individual compartments of 150 × 150 cm for the fattening period, and the fattening work was commenced. During the fattening period, the lambs were fed fattening feed (17% raw protein, 2660 kcal/kg ME) and wheat straw *ad libitum*. The feed was weighed every morning and evening, and the feed left in the mangers was weighed again. Lamb feed from a commercial company was used as a solid feed, while coarse feed was obtained from the enterprise. The live weight of the lambs was determined every 2 weeks by individual weighing. The weighing was performed before feeding in the morning. At the end of the 90-day fattening period, the fattening weight of the lambs was determined. At the beginning of the fattening period, the lambs were at an average age of 14 weeks. The lambs were slaughtered at the end of the 3-month fattening period at the age of 26 weeks. Subsequently, the animals were divided into two groups according to their FP, and the metabolomic properties of good fattening performance (GFP, n = 12) and poor fattening performance (PFP, n = 12) were evaluated.

Collection of blood samples. Blood samples required for metabolomic analyses were collected at the end of the fattening period. The blood required for amino acid analysis was taken from the jugular vein into blood tubes containing 10 ml of ethylenediaminetetraacetic acid. The blood samples were collected within 15 minutes. The plasma was separated by centrifugation at 4500 rpm. Blood samples taken for carnitine analysis from the same vein were dried by dripping on solid paper. The plasma and blood samples were stored at –80°C until analysis.

Metabolomic analyses. Plasma samples collected at the end of fattening were used to determine the metabolomic properties. For the analysis, 100 µL of the sample was obtained and it was prepared in 0.1 M HCl and mixed with an internal standard mixture consisting of 20 amino acids containing C13 and N15 labeled atoms. To balance the pH of the mixture, basic organic buffer components prepared in propanol were added. Then, a chloroform/isooctane mixture containing 5% alkyl chloroformate as an active ingredient was added to the mixture and kept at room temperature for 3 minutes. Meanwhile, gas evolution was observed due to the carbon dioxide formed during the esterification of alkyl chloroformate and amino acids. The derived amino acids were centrifuged and transferred to the top phase. Later, 1 µL of this phase was injected into the liquid chromatography-mass spectrometry/mass spectrometry (LC-MS/MS) system. Chromatographic separation was performed in a Trimaris Amino Acid LC-MS/MS column (250 mm × 2 mm, 3 µM) containing C18 reverse phase filler. Mobile phase A content was determined as water : methanol (MeOH) : 1 Mammonium formate (85 : 14 : 1), and mobile phase B content was determined as MeOH.

With regard to the dried blood samples, a method based on derivatization using butanolic-HCl was used in an analysis performed with acylcarnitine. In this method, a spot sample with a diameter of 3.2 mm taken from the dry blood sample was kept in an organic extraction solvent containing a certain concentration of amino acid and acylcarnitine deuterium marked standards. After 30 minutes of extraction, the organic solvents were removed by solvent evaporation under nitrogen until dryness. After this stage, butanolic-HCl was incubated for 20 minutes at 60°C to differentiate amino acids and acylcarnitine. After re-evaporation, the samples were dissolved in the mobile phase, and 5 µL was injected into the LC-MS/MS system. Amino acid and acylcarnitine molecules were analyzed in the multiple reaction monitoring mode by the electrospray ionization method, and each acylcarnitine molecule was scanned according to its internal standard using device-specific software. In this analysis, the column was not used, and 90% MeOH was used as the mobile phase.

Statistical analyses. The independent samples t-test was used to determine whether there was a significant difference in FP between the research groups. Pearson correlation analysis was performed to determine the direction and strength of the relationship between milk quality characteristics and metabolomic parameters. SPSS v.24 and R computer package programs were used for all analyses. For multivariate statistical analysis, amino acid data obtained from the LC-MS/MS analysis were uploaded to the MetaboAnalyst 5.0 (https://www.metaboanalyst.ca/) server, but none of MetaboAnalyst's normalization protocols were applied. Principal Component Analysis (PCA) was first performed to detect the segregation and aggregation of the groups. Then, partial least squares-discriminant analysis (PLS-DA) was applied to maximize segregation and aggregation. The variable importance in projection (VIP) scores of the amino acids contributing to the separation of the groups were calculated. In addition, pathway analysis was performed to identify degradation in biological pathways.

Results and discussion

The FP values determined from the research groups are shown in Table 1. Male lambs performed better in terms of feed consumption, live weight, and feed utilization rate ($P < 0.01$; $P < 0.001$). The profiles of amino acids and carnitine determined in the fattening

Tab. 1. Descriptive values of fattening performance in the research groups

Group	Items	x̄ ± SEM		P
		Female	Male	
Sex	Average daily weight gain (kg)	0.20 ± 0.01	0.30 ± 0.01	0.00
	Daily concentrate feed intake (kg)	1.58 ± 0.04	1.79 ± 0.04	0.00
	Feed conversion ratio*	8.07 ± 0.42	6.02 ± 0.16	0.00
Group	**Items**	**x̄ ± SEM**		**P**
		Poor	**Good**	
Feed conversion	Average daily weight gain (kg)	0.20 ± 0.0	0.30 ± 0.01	0.00
	Daily concentrate feed intake (kg)	1.60 ± 0.0	1.77 ± 0.05	0.02
	Feed conversion ratio*	8.11 ± 0.4	5.98 ± 0.13	0.00

Explanation: * – concentrate feed consumed (kg) for 1 kg body weight gain

groups are shown in Table 2 and Table 4. Glycine was the highest amino acid determined in the research groups. The difference between the groups in terms of alloisoleucine, aspartic acid, histidine, hydroxylysine, isoleucine, lysine, methionine, phenylalanine, tryptophan, valine, and 1-methylhistidine amino acids was statistically significant ($P \leq 0.001$). Almost all amino acid profiles examined in the study were lower in the GFP group than in the PFP group. When the correlations between FP and blood free amino acid levels were examined, moderately significant negative correlations were found between DCFI and 3-methylhistidine, hydroxylysine, and serotonin, and between ADWG and alloisoleucine, 3-methylhistidine, and hydroxylysine. FCR and hydroxylysine amino acids showed moderate positive correlations (Tab. 3). The difference in free carnitine (C0) and acetylcarnitine (C2) values was found to be statistically significant in the fattening groups examined within the scope of the study ($P < 0.05$). The highest carnitine value in the research groups was the C0 value in the PFP group (Tab. 4). Successive PCA and PLS-DA were performed to visualize possible outlines and sample distribution in the amino acid and carnitine profiles (Fig. 1 and 2). Two-dimensional score charts of PCA and PLS-DA for the amino acids are shown in Figure 1A and 1B, respectively. The analysis showed that there was no clear separation and cluster-

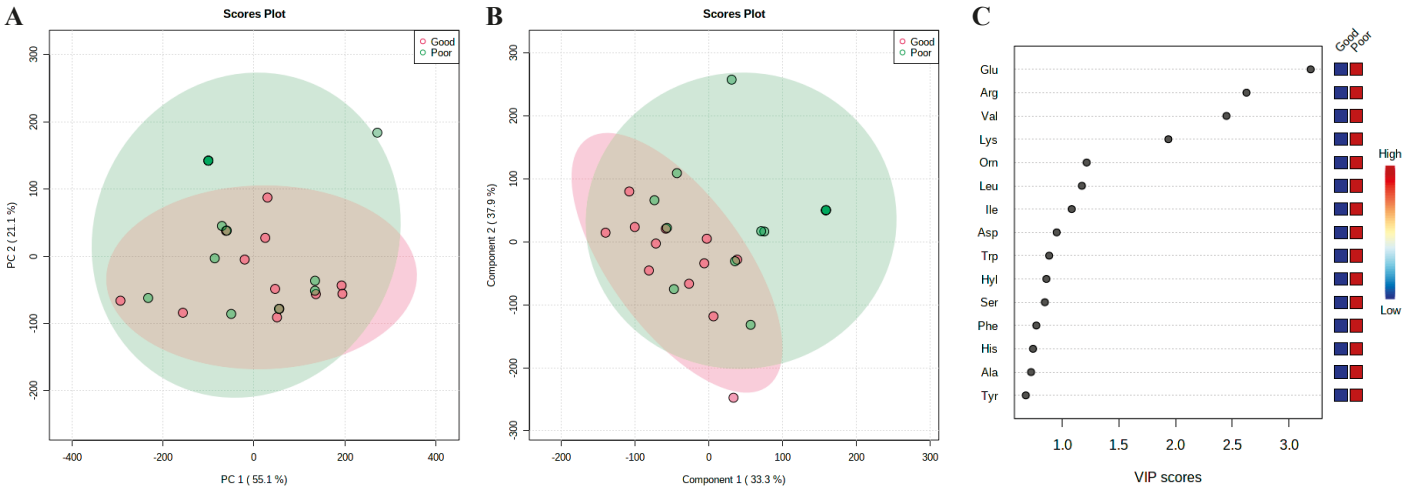

Fig. 1. Amino acids PCA and PLS-DA analyses for the good and poor fattening performance groups. PCA 2D (A) score graphs. PLS-DA 2D (B) score graphs. VIP table: essential amino acids (C)

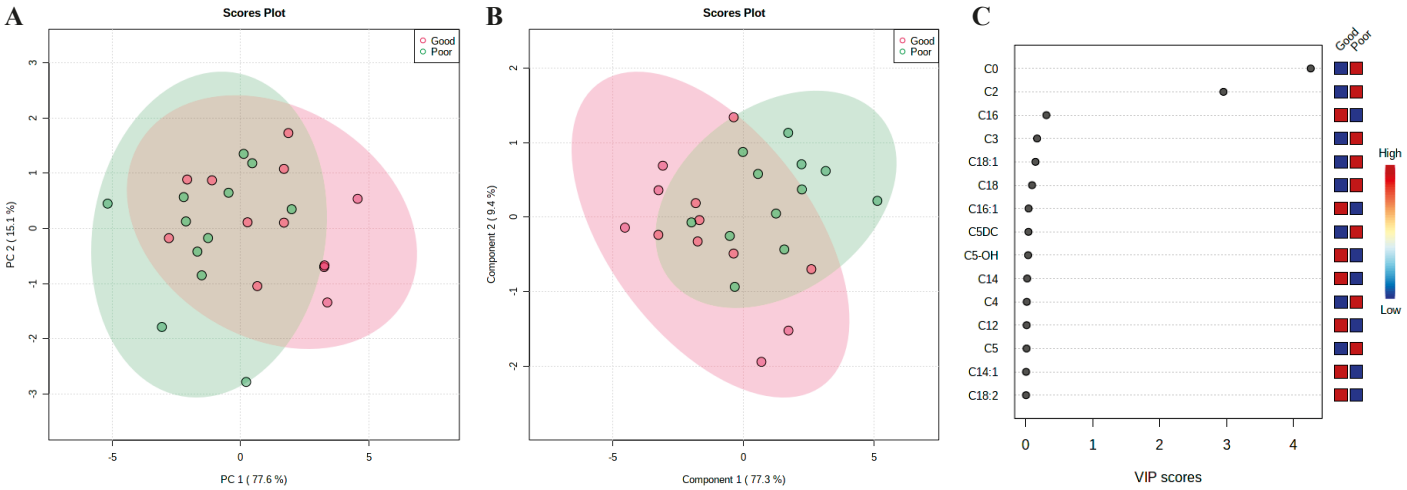

Fig. 2. Carnitines PCA and PLS-DA analyses for the good and poor fattening performance groups. PCA 2D (A) score graphs. PLS-DA 2D (B) score graphs. VIP table: essential carnitines (C)

Tab. 2. Plasma free amino acid profile in lambs

Amino Acid	Abbreviation	Mean ± SEM		P
		Good (n = 12)	Poor (n = 12)	
Alanine	Ala	147.57 ± 28.14	157.94 ± 25.90	0.36
Alloisoleucine	Allo-Ile	0.33 ± 0.08	0.47 ± 0.11	0.00
Alpha Aminobutyric Acid	A-Aba	1.67 ± 1.07	2.11 ± 1.56	0.43
Alpha Aminopimelic Acid	A-Apa	0.71 ± 0.09	0.77 ± 0.08	0.13
Anserine	Ans	5.39 ± 2.34	5.15 ± 2.35	0.80
Arginine	Arg	80.85 ± 31.27	118.34 ± 78.51	0.15
Asparagine	Asn	27.44 ± 12.41	28.08 ± 9.82	0.89
Aspartic Acid	Asp	15.38 ± 6.69	28.97 ± 20.80	0.04
Beta Alanine	B-Ala	10.93 ± 3.39	11.89 ± 3.87	0.53
Beta Aminoisobutyric Acid	B-Aiba	8.17 ± 4.49	12.70 ± 8.89	0.13
Citrulline	Cit	130.38 ± 51.97	132.95 ± 37.60	0.89
Cystathionine	Cys	0.10 ± 0.07	0.06 ± 0.06	0.17
Gamma Aminobutyric Acid	G-Aba	3.29 ± 1.42	3.48 ± 1.99	0.79
Glutamine	Gln	49.21 ± 22.83	55.27 ± 20.80	0.50
Glutamic Acid	Glu	155.44 ± 58.16	201.00 ± 74.58	0.11
Glycine	Gly	309.82 ± 122.92	311.52 ± 125.30	0.97
Histidine	His	25.22 ± 6.87	35.84 ± 13.13	0.02
Homocystine	Hcy	0.16 ± 0.13	0.26 ± 0.19	0.15
Hydroxylysine	Hyl	22.43 ± 5.43	34.73 ± 9.11	0.00
Hydroxyproline	Hyp	0.42 ± 0.25	0.46 ± 0.32	0.74
Isoleucine	Ile	37.62 ± 9.13	53.10 ± 12.68	0.00
Leucine	Leu	79.76 ± 30.69	96.53 ± 31.91	0.20
Lysine	Lys	63.34 ± 11.95	90.99 ± 42.10	0.04
Methionine	Met	17.40 ± 3.47	24.87 ± 10.25	0.03
Ornithine	Orn	43.01 ± 19.03	60.38 ± 22.47	0.05
Phenylanalanine	Phe	28.43 ± 4.62	39.45 ± 7.28	0.00
Proline	Pro	52.77 ± 19.21	59.59 ± 12.03	0.31
Sarcosine	Sar	1.88 ± 1.14	1.72 ± 1.04	0.71
Serine	Ser	43.61 ± 18.17	55.72 ± 20.51	0.14
Serotonin	5-Ht	0.01 ± 0.00	0.01 ± 0.01	0.14
Thiaproline	Tpro	4.96 ± 2.30	5.05 ± 1.57	0.91
Threonine	Thr	75.26 ± 56.98	74.66 ± 41.70	0.98
Tryptophan	Trp	20.90 ± 5.13	33.55 ± 11.76	0.00
Tyrosine	Tyr	45.78 ± 16.97	55.48 ± 10.38	0.11
Valine	Val	98.36 ± 23.46	133.32 ± 7.87	0.00
1-Methylhistidine	1-Mhs	0.68 ± 0.13	0.99 ± 0.31	0.01
3-Methylhistidine	3-Mhs	0.04 ± 0.02	0.07 ± 0.05	0.12
5-Hydroxytryptophan	5-Htp	6.78 ± 4.72	8.34 ± 2.69	0.33

Tab. 3. Correlations between fattening performance and blood free amino acid levels

	Average daily weight gain	Daily concentrate feed intake	Feed conversion ratio
Alanine	0.05	0.12	–0.06
Alloisoleucine	–0.53**	–0.38	0.39
Alphaaminoadipic Acid	0.37	0.28	–0.28
Alphaaminobutyric Acid	0.05	0.10	–0.05
Alphaaminopimelic Acid	–0.08	0.35	0.26
Anserine	0.10	0.20	–0.00
Arginine	–0.23	–0.05	0.20
Argininosuccinic Acid	–0.13	–0.05	0.12
Asparagine	0.06	0.09	–0.07
Aspartic Acid	–0.38	–0.34	0.23
Beta Alanine	–0.02	–0.12	–0.15
Beta Aminoisobutyric Acid	–0.14	–0.12	0.05
Citrulline	0.20	0.21	–0.15
Cystathionine	0.20	0.23	–0.15
Etanolamine	–0.04	0.04	0.06
Gamma Aminobutyric Acid	0.17	0.08	–0.22
Glutamic Acid	–0.07	0.01	0.05
Glutamine	–0.12	–0.29	–0.08
Glycine	0.03	0.34	0.14
Histidine	–0.26	–0.24	0.10
Homocystine	0.14	0.31	–0.07
Hydroxylysine	–0.58**	–0.41*	0.43*
Hydroxyproline	–0.13	–0.08	0.11
Isoleucine	–0.34	–0.07	0.37
Leucine	–0.39	–0.17	0.36
Lysine	–0.29	–0.07	0.25
Methionine	–0.16	–0.03	0.14
Ornithine	–0.39	–0.16	0.33
Phenylalanine	–0.16	–0.19	0.05
Phosphoetanolamine	0.09	–0.02	–0.19
Proline	–0.18	–0.05	0.12
Sarcosine	0.36	0.26	–0.35
Serine	–0.05	0.11	0.08
Serotonin	–0.22	–0.47*	0.01
Thiaproline	0.31	0.37	–0.12
Threonine	0.14	0.25	–0.04
Tryptophan	–0.20	–0.17	0.09
Tyrosine	–0.27	–0.40	0.04
Valine	–0.08	–0.08	0.04
1-Methylhistidine	0.20	0.22	–0.12
3-Methylhistidine	–0.44*	–0.47*	0.27
5-Hydroxy tryptophan	0.26	0.26	–0.16

Explanation: * – $p \leq 0.05$ (2-tailed); ** – $p \leq 0.01$ (2-tailed)

ing between the groups. VIP analysis generated from PLS-DA models was performed to show the amino acids separating the GFP and PFP groups. Glutamic acid, arginine, and valine were the amino acids with the highest scores (Fig. 1C). As a result of metabolic network simulation, it was determined that the most important pathway with impact value = 1 was the phenylalanine, tyrosine, and tryptophan biosynthesis pathway (Tab. 6).

Tab. 4. Plasma free carnitine profile in lambs

Common name	Abbreviation	Mean ± SEM		P
		Good (n: 12)	Poor (n: 12)	
Free carnitine	C0	10.24 ± 0.63	14.05 ± 1.80	0.02
Acetylcarnitine	C2	3.78 ± 0.31	5.63 ± 0.58	0.01
Propionylcarnitine	C3	0.24 ± 0.02	0.33 ± 0.03	0.05
Butyrylcarnitine	C4	0.07 ± 0.01	0.08 ± 0.00	0.25
Methylmalonylcarnitine	C4 DC	0.04 ± 0.01	0.05 ± 0.01	0.78
Isovalerylcarnitine	C5	0.05 ± 0.01	0.06 ± 0.00	0.14
Tiglylcarnitine	C5:1	0.02 ± 0.00	0.02 ± 0.00	0.98
Hydroxyisovalerylcarnitine	C5 OH	0.23 ± 0.06	0.13 ± 0.01	0.40
Glutarylcarnitine	C5 DC	0.07 ± 0.01	0.08 ± 0.01	0.07
Hexanoylcarnitine	C6	0.01 ± 0.00	0.01 ± 0.00	0.64
Adipoylcarnitine	C6 DC	0.01 ± 0.00	0.01 ± 0.00	0.10
Octanoylcarnitine	C8	0.01 ± 0.00	0.02 ± 0.00	0.82
Octenoylcarnitine	C8:1	0.04 ± 0.02	0.02 ± 0.00	0.77
Suberoylcarnitine	C8 DC	0.02 ± 0.00	0.01 ± 0.00	0.33
Decanoylcarnitine	C10	0.02 ± 0.01	0.01 ± 0.00	0.65
Decenoylcarnitine	C10:1	0.05 ± 0.02	0.03 ± 0.00	0.70
Sebacoylcarnitine	C10 DC	0.02 ± 0.00	0.02 ± 0.00	0.51
Dodecanoylcarnitine	C12	0.04 ± 0.01	0.04 ± 0.01	0.11
Myristoylcarnitine	C14	0.12 ± 0.02	0.12 ± 0.02	0.49
Myristoleylcarnitine	C14:1	0.03 ± 0.00	0.03 ± 0.01	0.28
Tetradecadienoylcarnitine	C14:2	0.01 ± 0.00	0.01 ± 0.00	0.51
Palmitoylcarnitine	C16	1.01 ± 0.17	1.02 ± 0.18	0.44
Palmitoleylcarnitine	C16:1	0.15 ± 0.02	0.13 ± 0.02	0.42
Stearoylcarnitine	C18	1.42 ± 0.19	1.68 ± 0.25	0.84
Oleylcarnitine	C18:1	0.87 ± 0.12	0.95 ± 0.13	0.68
Linoleylcarnitine	C18:2	0.14 ± 0.02	0.14 ± 0.02	0.90
Hydroxyoleylcarnitine	C18:1 OH	0.01 ± 0.00	0.01 ± 0.00	0.89

Tab. 5. Correlations between fattening performance and blood free carnitine levels

	Average daily weight gain	Daily concentrate feed intake	Feed conversion ratio
C0	–0.23	–0.14	0.15
C2	–0.36	–0.14	0.33
C3	–0.14	–0.10	0.04
C4	–0.16	–0.17	0.09
C4 DC	0.09	0.00	–0.14
C5	–0.12	–0.15	0.05
C5:1	–0.14	–0.11	0.07
C5 OH	0.48*	0.15	–0.43*
C5 DC	–0.25	–0.06	0.32
C6	0.17	–0.01	–0.21
C6 DC	0.20	0.08	–0.24
C8	0.24	0.15	–0.18
C8:1	0.11	0.19	–0.07
C8 DC	0.17	0.23	–0.11
C10	0.10	0.17	–0.03
C10:1	0.09	0.18	–0.05
C10 DC	0.06	0.07	–0.02
C12	–0.03	–0.03	0.01
C14	0.03	0.04	0.00
C14:1	0.05	–0.03	–0.03
C14:2	0.09	0.15	–0.06
C16	0.03	0.04	0.00
C16:1	0.10	0.09	–0.03
C18	–0.12	–0.06	0.12
C18:1	–0.08	–0.07	0.10
C18:2	–0.06	–0.01	0.12
C18:1 OH	–0.02	0.08	0.07

Explanation: * – $p \leq 0.05$ (2-tailed)

This study examined the relationships between blood free amino acid levels and carnitine profiles in Awassi lambs fed intensively for 90 days and it revealed new selection criteria important for sheep breeding.

In the fattening groups examined as part of the study, males performed better than females in terms of daily live weight increase. Superiority due to anabolic-acting hormones is expected in animals used as male breeding material (22). The values were higher than the those obtained in a study by Omar (29) or the one reported by Haddad and Husein (18). The differences in ADWG between the present study and literature reports are due to genotype, sex, fattening time, and feeding method. The FCR values determined during the 90-day fattening period were higher than those reported in a study (34) conducted to determine the FP, as well as slaughter and carcass characteristics of Awassi male lambs or in another study (25) comparing the FP of Awassi and Ost-Friz × Awassi (F1) crossbred lambs. This difference in FCR is due to the fact that the animals were fed *ad libitum* with concentrate feed during the fattening period, and the live weight gain was high.

Tab. 6. Impaired metabolic pathways

Pathway	P	Impact
Tryptophan metabolism	0.00	0.27
Phenylalanine, tyrosine and tryptophan biosynthesis	0.02	1.00
Phenylalanine metabolism	0.02	0.36
Cysteine and methionine metabolism	0.03	0.33
Beta-Alanine metabolism	0.03	0.40

Amino acids and products formed after amino acid metabolism play an important role in many vital processes, such as nutrient intake, food digestion and metabolism, cell signaling, gene expression, homeostasis, immune system and antioxidative responses, hormonal regulation, and reproduction (35). Leucine, isoleucine, and valine, which are branched chain amino acids, account for approximately 16% of the skeletal muscle. In addition to acting as a substrate for

protein synthesis, leucine from these amino acids acts as a signaling molecule that activates the mechanism of muscle protein synthesis (16). For amino acids to be used by the body, they must be absorbed from the gut into the bloodstream. This absorption, however, does not occur with the same efficiency for all amino acids. In addition, amino acids in the bloodstream are used with different efficiencies depending on the function for which they are used. Growth occurs faster in male sex farm animals with high FP. A high growth rate increases the need for protein in muscle, bone, and other tissues (13). This leads to differences between research groups in terms of amino acid levels. In the present study, the plasma amounts of almost all amino acids were lower in the GFP group. Specifically, the plasma levels of valine and isoleucine from the branched chain amino acid group were lower than they were in the PFP pigs. Studies in humans and laboratory animals have shown that branched chain amino acids stimulate muscle protein synthesis. Aspartic acid is an amino acid that acts as a common precursor for the synthesis of lysine, methionine, threonine, and isoleucine (17). Aspartic acid is an important source of energy in small intestinal epithelial cells and can be absorbed from the intestinal mucosa and converted into nutrients by dihydroxylation or amination (26). It has been reported that dietary aspartic acid supplementation in pigs improves growth performance and feed efficiency (21). Hydroxylysine, a hydroxylated derivative of the amino acid lysine, is a component of collagen and contributes to the molecular structure of crosslinks in fibers. Lysine, together with hydroxylysine, is thought to play a major role in the formation of bone tissue, which constitutes an important part of carcass and live weights (3). The gastrointestinal tract is the center where feed consumed by the animals is digested and the absorption of nutrients necessary for metabolism occurs (6). Good development of the digestive system, especially in ruminants, can aid in digestion and absorption of nutrients and increase feed use efficiency (9). It has been shown that phenylalanine can regulate pancreatic enzyme synthesis and promote starch digestion in adult ruminants (9).

When the correlations between FP and blood free amino acid levels were examined, moderately significant negative correlations were found between DCFI and 3-methylhistidine (r = –0.469; P = 0.021), hydroxylysine (r = –0.408; P = 0.048), and serotonin (r = –0.467; P = 0.021) and between ADWG and alloisoleucine (r = –0.528; P = 0.008), 3-methylhistidine (r = –0.440; P = 0.032), and hydroxylysine (r = –0.577; P = 0.003). The contractile proteins of skeletal muscle, actin and myosin, are methylated following peptide bond synthesis with the production of 3-methylhistidine (3-MeHis) and release 3-MeHis during intracellular degradation of these proteins (36). A moderate positive correlation was determined between FCR and the amino acid hydroxylysine (r = 0.430; P = 0.036).

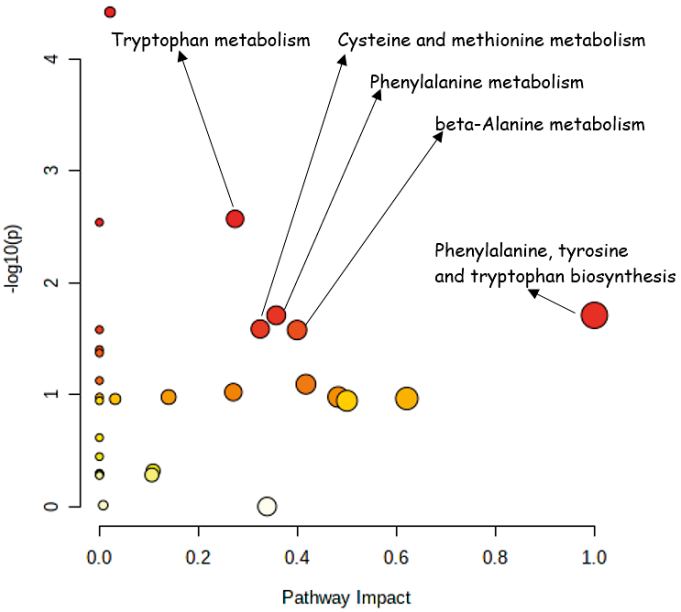

Fig. 3. Summary of pathway analysis

Maintaining the mass of skeletal muscle is of great importance in maintaining functional capacity and metabolic health. Muscle mass persistence is regulated by fluctuations in muscle protein synthesis and breakdown (7). Proteins obtained as a result of feed consumption stimulate muscle protein synthesis by providing substrates (i.e. amino acids) for the production of muscle proteins. In addition, insulin, which increases in the circulation after food intake, shows an inhibitory effect on muscle protein breakdown (16, 30). The amino acid differences and correlations detected in the GFP and PFP groups in our study overlap with mechanisms affecting the FP parameters, thus supporting our hypothesis that metabolomic data can be used to identify high-performance animals. As a result of the pathway analysis performed in this research, five pathways were identified. Phenylalanine, tyrosine, and tryptophan biosynthesis pathways had the highest impact value among these pathways. Amino acids and their metabolites are building blocks for proteins, but they also act as neurotransmitters. Therefore, they play an important role in neurological health and function (4). Concentrations of phenylalanine, tryptophan, and tyrosine are very important for maintaining the production of neurotransmitters. These amino acids have a profound effect on the food intake of animals by affecting nerve conduction (19).

The analysis of correlations between FP and carnitine levels revealed that hydroxyisovalerylcarnitine (C5 OH) was moderately positively correlated with ADWG (r = 0.476; P = 0.019) and negatively correlated with FCR (r = –0.430; P = 0.036). Isovalerylcarnitine is an intermediate product of branched chain amino acid metabolism (37). Branched chain amino acid catabolism involves the removal of the amino group and the subsequent disintegration of the resulting carbon skeleton. Unlike other amino acids, these amino acids are metabolized mainly by peripheral tissues

(especially muscle tissue) rather than by the liver (10). Transamination of leucine, isoleucine, and valine results in the production of α-keto acids. The second step in the catabolism of these amino acids is oxidative decarboxylation, which results in the removal of the carboxyl group of α-keto acids, which is catalyzed by the branched chain α-keto acid dehydrogenase complex. Oxidative decarboxylation of α-keto acids results in the production of isovaleryl coenzyme A, α-methyl butyryl CoA, and isobutyl CoA (10, 16).

Marker-assisted selection has been suggested for many years as a method to predict the phenotype of farm animals (33). Genetic markers and candidate genes explain only a small part of phenotypic variation in economically important quantitative traits (32). There is an increasing need to identify biomarkers for the selection of animals with good yield characteristics (23, 32). Metabolomic parameters can detect phenotypic variations more accurately because they express the end products that result from the effect of genetic mechanisms (23).

In this study, plasma free amino acid and carnitine profiles were found to be promising new molecular features as candidate biomarkers. To better understand the physiological roles of these metabolites, it is necessary to verify them with another dataset.

References

1. *Ahbara A., Bahbahani H., Almathen F., Al Abri M., Agoub M. O., Abeba A., Kebede A., Musa H. H., Mastrangelo S., Pilla F., Ciani E., Hanotte O., Mwacharo J. M.*: Genome-wide variation, candidate regions and genes associated with fat deposition and tail morphology in Ethiopian indigenous sheep. Front. Genet. 2019, 9, 699.
2. *Almeida A. M. de, Zachut M., Hernández-Castellano L. E., Šperanda M., Gabai G., Mobasheri A.*: Biomarkers of fitness and welfare in dairy animals: healthy living. J. Dairy Res. 2019, 86, 379-387.
3. *Bailey A. J., Wotton S. F., Sims T. J., Thompson P.*: Post-translational modifications in the collagen of human osteoporotic femoral head. Biochem. Biophys. Res. Commun. 1992, 185, 801-805.
4. *Bao X, Feng Z, Yao J., Li T., Yin Y.*: Roles of dietary amino acids and their metabolites in pathogenesis of inflammatory bowel disease. Mediat. Inflamm. 2017.
5. *Boyazoglu J, Morand-Fehr P.*: Mediterranean dairy sheep and goat products and their quality: A critical review. Small Rumin. Res. 2001, 40, 1-11.
6. *Bunting L. D., Tarifa T. A., Crochet B. T., Fernandez, J. M., Depew C. L., Lovejoy J. C.*: Effects of dietary inclusion of chromium propionate and calcium propionate on glucose disposal and gastrointestinal development in dairy calves. J. Dairy Sci. 2000, 83, 2491-2498.
7. *Burd N. A., Tang J. E., Moore D. R., Phillips S. M.*: Exercise training and protein metabolism: influences of contraction, protein intake, and sex-based differences. J. Appl. Physiol. 2009, 106, 1692-1701.
8. *Caboni P., Murgia A., Porcu A., Manis C., Ibba I., Contu M., Scano P.*: A metabolomics comparison between sheep's and goat's milk. Food Res. Int. 2019, 119, 869-875.
9. *Cao Y., Liu S., Yang X., Guo L., Cai C., Yao J.*: Effects of dietary leucine and phenylalanine on gastrointestinal development and small intestinal enzyme activities in milk-fed Holstein dairy calves. Biosci. Rep. 2019, 39, BSR20181733.
10. *Champe P. C., Harvey R. A., Ferrier D. R.*: Amino acid degradation and synthesis, [in:] Lippincott's Illustrated Reviews: Biochemistry, 4th edition 2008, 261-276.
11. *Consolo N. R. B., Buarque V. L. M., Silva J., Poleti M. D., Barbosa L. C. G. S., Higuera-Padilla A., Gomez J. F. M., Colnago L. A., Gerrard D. E., Saran Netto A., Silva S. L.*: Muscle and liver metabolomic signatures associated with residual feed intake in Nellore cattle. Anim. Feed Sci. Technol. 2021, 271, 114757.
12. *Elolimy A., Zeineldin M. M., Abdelmegeid M., Abdelatty A. M., Alharthi A. S., Bakr M. H., Elghandour M. M. M. Y., Salem A. Z. M., Loor J. J.*: Metabolomics and Proteomics Signatures in Feed-Efficient Beef and Dairy Cattle. Sustainable Agriculture Reviews 2021, 54, 153-165.
13. *Evans E. H., Patterson R. J.*: Use of dynamic modeling seen as good way to formulate crude protein, amino acid requirements for cattle diets. Feedstuffs 1985, 57, 24.
14. *Garlick P. J.*: The role of leucine in the regulation of protein metabolism. J. Nutr. 2005, 135, 1553S-1556S.
15. *Getachew T., Haile A., Meszaroz G., Rischkowsky B., Huson H. J., Gizaw S., Wurzinger M., Mwai A. O., Sölkner J.*: Genetic diversity, population structure and runs of homozygosity in Ethiopian short fat-tailed and Awassi sheep breeds using genome-wide 50k SNP markers. Livest. Sci. 2020, 232, 103899.
16. *Gorissen S. H., Phillips S. M.*: Branched-chain amino acids (leucine, isoleucine, and valine) and skeletal muscle, [in:] Nutrition and Skeletal Muscle. Academic Press 2019, 283-298.
17. *Gutierrez E., Miller T. C., Gonzalez-Redondo J. R., Holcombe J. A.*: Characterization of immobilized poly-L-aspartate as a metal chelator. Environ. Sci. Technol. 1999, 33, 1664-1670.
18. *Haddad S. G., Husein M. Q.*: Effect of dietary energy density on growth performance and slaughtering characteristics of fattening Awassi lambs. Livest. Prod. Sci. 2004, 87, 171-177.
19. *He W., Wu G.*: Metabolism of amino acids in the brain and their roles in regulating food intake. Adv. Exp. Med. Biol. 2020, 265, 167-185.
20. *Herd R. M., Bishop S. C.*: Genetic variation in residual feed intake and its association with other production traits in British Hereford cattle. Livest. Prod. Sci. 2000, 63, 111-119.
21. *Jiao Y., Li X., Kim I. H.*: Changes in growth performance, nutrient digestibility, immune blood profiles, fecal microbial and fecal gas emission of growing pigs in response to zinc aspartic acid chelate. Asian-australas. J. Anim. Sci. 2020, 33, 597.
22. *Karimi A., Abarghuei M. J., Boostani A.*: Growth performance and carcass traits of purebred and crossbred fattening lambs from Ghezel ram with Grey Shirazi ewe. Trop. Anim. Health Prod. 2022, 54, 1-10.
23. *Karisa B. K., Thomson J., Wang Z., Li C., Montanholi Y. R., Miller S. P., Moore S. S., Plastow G. S.*: Plasma metabolites associated with residual feed intake and other productivity performance traits in beef cattle. Livest. Sci. 2014, 165, 200-211.
24. *Knott S. A., Dunshea F. R., Leury B. J., Brien F. D., Suster D., Cummins L. J.*: Body composition influences net feed intake in terminal sire rams. Anim. Prod. 2004, 1, 274-274.
25. *Kul S., Akcan A.*: İvesive Ost-Friz x İvesiMelez (F1) kuzulardabesiperformansın, kesimvekarkasözellikleri. Uludag Univ. J. Fac. Vet. Med. 2002, 21, 1-7.
26. *Liu Y., Wang X., Hou Y., Yin Y., Qiu Y., Wu G., Hu C. A. A.*: Roles of amino acids in preventing and treating intestinal diseases: recent studies with pig models. Amino Acids 2017, 49, 1277-1291.
27. *Moore S. S., Mujibi F. D., Sherman E. L.*: Molecular basis for residual feed intake in beef cattle. J. Anim. Sci. 2009, 87, E41-E47.
28. *O'Callaghan T. F., O'Donovan M., Murphy J. P., Sugrue K., Tobin J. T., McNamara A. E., Yin X., Sundaramoorthy G., Brennan L.*: The bovine colostrum and milk metabolome at the onset of lactation as determined by 1H-NMR. Int. Dairy J. 2021, 113, 104881.
29. *Omar J. A.*: Utilization of corrugated cardboard in fattening rations of Awassi lambs. Small Rumin. Res. 2001, 42, 167-170.
30. *Phillips S. M., Tipton K. D., Aarsland A. S. L. E., Wolf S. E., Wolfe R. R.*: Mixed muscle protein synthesis and breakdown after resistance exercise in humans. Am. J. Physiol. Endocrinol. Metab. 1997, 273, E99-E107.
31. *Randhawa I. A., McGowan M. R., Porto-Neto L. R., Hayes B. J., Lyons R. E.*: Comparison of genetic merit for weight and meat traits between the polled and horned cattle in multiple beef breeds. Animals 2021, 11 (3), 870.
32. *Reverter A., Fortes M. R. S.*: Breeding and Genetics Symposium: building single nucleotide polymorphism-derived gene regulatory networks: Towards functional genome wide association studies. J. Anim. Sci. 2013, 91, 530-536.
33. *Sejian V., Bhatta R., Gaughan J. B., Dunshea F. R., Lacetera N.*: Adaptation of animals to heat stress. Animal 2018, 12, s431-s444.
34. *Tekel N., Şireli H. D., Vural M. E.*: Besi süresininivesi erkek kuzuların besi performansı ve karkas özelliklerine etkisi. Tarım Bilimleri Dergisi 2007, 13, 372-378.
35. *Wu R., Chen J., Zhang L., Wang X., Yang Y., Ren X.*: LC/MS-based metabolomics to evaluate the milk composition of human, horse, goat and cow from China. Eur. Food Res. Technol. 2021, 247, 663-675.
36. *Young V. R., Munro H. N.*: Ntau-methylhistidine (3-methylhistidine) and muscle protein turnover: an overview. Federation Proceedings 1978, 37, 2291-2300.
37. *Zanchi N. E., Gerlinger-Romero F., Guimaraes-Ferreira L., De Siqueira Filho M. A., Felitti V., Lira F. S., Seelaender M., Lancha A. H.*: HMB supplementation: clinical and athletic performance-related effects and mechanisms of action. Amino acids 2011, 40, 1015-1025.

Corresponding author: Mücahit Kahraman, Assistant Professor Dr., Department of Animal Husbandry, Faculty of Veterinary Medicine, Harran University, TR-63200, Şanlıurfa, Turkey; e-mail: mucahitkahraman@harran.edu.tr