Acta Scientiarum
http://periodicos.uem.br/ojs/acta
ISSN on-line: 1807-8672
Doi: 10.4025/actascianimsci.v44i1.56273

Novel missense variant L46Q of fatty acid synthase gene and fatty acids content in Awassi sheep

Tahreer Mohammed Al-Thuwaini* **and Zahraa Aqeel Kareem**

Department of Animal Production, College of Agriculture, Al-Qasim Green University, Babil, Iraq. *Author for correspondence. E -mail: tahrearmohammed@agre.uoqasim.edu.iq

ABSTRACT. This study was conducted to investigate the association between the polymorphism of the *FASN* gene with fatty acid content in Awassi sheep. A total of 100 male Awassi sheep between the ages of one and two and a half years old were used in this study. Phenotypic measurement was recorded at slaughter, and from each animal, the *longissimus dorsi* (LD) muscle samples were taken to analyze the fatty acid profile. Genotyping, sequencing reactions, and *in silico* tools were performed to confirm the variants in amplified fragments. The result of genotyping revealed two genotypes (AA and AB) of the ovine *FASN* gene (exon 3). Novel SNP (L46Q) was discovered only within the *FASN* gene (AB genotype). All utilized *in silico* tools revealed remarkably deleterious effects for the L46Q on the mutant protein structure, function, and stability. Association analysis revealed that the AB genotype has significantly ($p < 0.05$) higher levels of animal length and monounsaturated fatty acids (MUFA) with lower amounts of saturated fatty acids (SFA) content than the AA genotype. In conclusion, novel SNP (L46Q) was discovered within the *FASN* gene (AB genotype), made the animals that has the AB genotype associated with good meat quality traits and this polymorphism may serve as markers for meat quality.

Keywords: fatty acids composition; *FASN* gene; meat quality; rams.

Received on October 20, 2020.
Accepted on February 16, 2021.

Introduction

Small ruminants, especially native breed types, play an important role in the livelihoods of a considerable part of the human population in the tropics from socio-economic aspects (Ebrahimi, Mohammadabadi, & Esmailizadeh, 2017). Therefore, integrated attempt in terms of management and genetic improvement to enhance production is of crucial importance (Moghadaszadeh, Mohammadabadi, & Esmailizadeh, 2015). Economical and biological efficiency of sheep production enterprises generally improves by increasing productivity and reproductive performance of ewes (Mohammadabadi, 2016; Al-Thuwaini, 2022). Awassi breed is the most predominant sheep in the Middle East areas that are characterized by a high ability to cope with hardy conditions (Al-Thuwaini, 2021a), with high meat and milk production than the other surrounding breeds in the Middle East (Jawasreh, Al-Amareen, & Aad, 2019; Ajafar, Kadhim, & AL-Thuwaini, 2022a). Several genes of lipid metabolism regulate the fatty acid content of livestock meat (Quiñones, Bravo, Calvo Lacosta, & Sepúlveda, 2017; Ajafar, AL-Thuwaini, & Dakhel, 2022b). Among them, the fatty acid synthase (*FASN*) gene is used to improve fatty acid components (Shi et al., 2019). The *FASN* gene mapped on chromosome 11 in sheep and on chromosome 19 in cattle (Oztabak et al., 2014), which encodes enzymes responsible for fatty acid synthesis, elongation, and desaturation (Quiñones et al., 2017). Fatty acid synthase (FAS or FASN) is a multifunctional enzyme complex that organizes *de novo* biosynthesis of long-chain fatty acids (Raza et al., 2018) and synthesizes saturated and unsaturated fatty acids (Pećina & Ivanković, 2021). There are associates of genetic polymorphism with meat quality traits (Zalewska, Puppel, & Sakowski, 2021). The polymorphism of the TE-FASN gene in sheep revealed the impact of the genotypes on the fatty acid content (Esteves et al., 2019). Several variants in the bovine *FASN* gene correlated to the fat content and the composition of fatty acids in both milk and meat (Ciecierska et al., 2013). The g. 17924GG genotype in *FASN* led to lower saturated fatty acid (SFA) including Myristic and palmitic acids and higher levels of oleic acid as main monounsaturated fatty acids (MUFA) amounts in Korean cattle (Cho et al., 2010). In Korean and Angus cattle, the GG genotype of the *FASN* gene is associated with higher levels of MUFA and lower levels of SFA

(Ciecierska et al., 2013). On the other hand, determination of gene polymorphism is important in farm animals breeding (Shamsalddini, Mohammadabadi, & Esmailizadeh, 2016; Gholamhoseini, Mohammadabadi, & Asadi Fozi, 2018; Gooki, Mohammadabadi, Fozi, & Soflaei, 2019) to define genotypes of animals and their associations with productive, reproductive and economic traits (Pasandideh, Mohammadabadi, Esmailizadeh, & Tarang, 2015). Based on the above consideration, no research yet on the association of the *FASN* gene with the fatty acid content has been reported in Awassi sheep. Thus, the current study was conducted to evaluate the association of the *FASN* gene polymorphism and fatty acids content in Awassi sheep.

Material and methods

Animals

This study was performed according to regulations of the international recommendations for the care and use of animals under Al-Qasim Green University's approval (Agri, No. 015,3,12), at the College of Agriculture /Department of Animal Production for the period from October /2017 to June / 2018. A total of 100 male Awassi sheep between the ages of one and two and a half years old and weight between 25 to 40 kg were included in this study. The referred animals were randomly selected from three flocks in the middle Euphrates regions of Iraq. In each studied flock, 10–12 rams were randomly allocated to mate with about 20–25 ewes per ram, with male identification recorded. Animals were kept on seasonal grass during spring and autumn, while in winter, animals were kept indoors and fed concentrated food. Animals were slaughtered at abattoirs of Babylon, and from each animal, the *longissimus dorsi* (LD) muscle samples (~100 g) were taken between the 12 and 13th ribs at 45 min. post mortem, collected, and fractionated to analyze the fatty acid content. HPLC technique was used and the different fatty acids contents were calculated using the method of Salimon, Omar, and Salih (2017). The fatty acid composition was determined using the GS solution 2.42 software. Phenotypic measurements including body weight (BW), back fat thickness (BFT), body length (BL), abdominal fat (AF), and fat tail weight (FTW) were recorded at slaughter according to Al-Thuwaini et al. (2020).

DNA isolation and PCR amplification

The high salt method of Al-Shuhaib (2017) was conducted to isolate the genomic DNA from the whole blood. The primers used to amplify the ovine gene were designed using the Primer-BLAST online server (Ye et al., 2012) according to the sequence of the *FASN* gene (exon 3) for ovine (GenBank accession numbers NC_019468.2). The sequence of the used primer in this study was: F: 5'-AGGTCAGAGAATTAA AGCT-3', R: 5' GGAAGTGACAGTGGTTTT-3'. PCR experiments were conducted as follow: initial denaturation for 5 min, followed by 30 cycles for 30 sec of denaturation (95°C), annealing (56.7°C), and extension (72°C), with a final extension (72°C) for 5 min. The specificity of PCR amplicons was verified by electrophoresis on agarose gel then submitted to SSCP protocols (Al-Thuwaini, 2021b).

Genotyping and sequencing analysis

The SSCP experiment was performed according to Imran, Al-Thuwaini and Al-Shuhaib (2020) protocol. For single-stranded conformation polymorphism (SSCP) analysis, 10 µL of each amplification product was mixed with 10 µL of SSCP denaturing buffer heated for 7 min. at 95°C and then chilled on ice for 7 min. SSCP analysis was conducted in 10% polyacrylamide gels (37.5:1) at 200 V for 4h in TBE (0.5×) buffer at a constant temperature of 20°C. The silver staining of SSCP patterns on the gels was visualized by methods described by Byun, Fang, Zhou and Hickford (2009). For each genotype, the PCR products were sent for purification and sequencing of multiple sequence alignment programs, according to DNA Star, EditSeq. / ClustalW, with the sequences published in the GenBank database taken as a reference to identify the polymorphisms. The observed mutations were visualized and checked the novelty by SnapGene Viewer, ver. 4.0.4. (GSL. Biotech. LLC) and the ensemble genome browser 96 (https://asia.ensembl.org/index.html).

In silico prediction

Many computational tools were utilized to assess the consequences of the observed missense variants on the resulting mutant protein structures, functions, and stability, namely SIFT, PolyPhen-2, Provean, SNAP2, and I-Mutant2.0 (Imran et al., 2020).

Statistical analysis

The genetic diversity of the *FASN* gene was analyzed using PopGen32 software, v. 1.31 (Yeh & Yang, 1999). Association analysis was analyzed using SPSS v23.0 (IBM Corp, 2015). The significant effect of genotype on the various phenotypic parameters was performed using General linear mixed-effects models (GLMMs) with the following model*;*

$$Yijk = \mu + Gi + \alpha j + eijk$$

Where: Y*ijk* is the phenotypic trait, μ is the overall mean, Gi is the fixed effect associated with the ith SNP genotype(*i* = AA, AB),αj is the random effect of the jth sire and e*ij*= random error with assumed NID (0, σ2e). Normality was tested using the Kolmogorov–Smirnov test. Preliminary statistical analyses indicated that age, season, and nutrition were not found to affect phenotypic characteristics and thus they were not included in the model.

Results and discussion

Genotyping analysis of *FASN* gene

The SSCP analysis revealed two variations within the DNA samples that amplified by the ovine *FASN* (exon 3) specific primer pair (Figure 1).

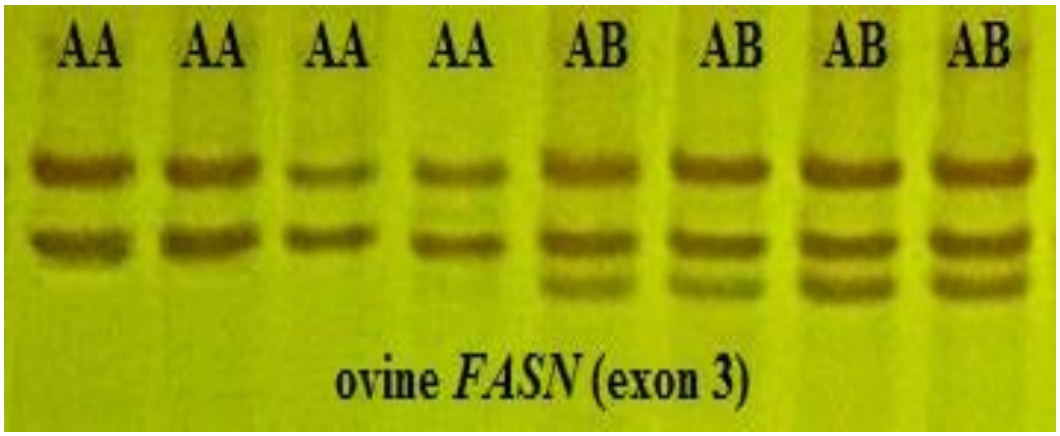

Figure 1. SSCP non-denaturing polyacrylamide gel electrophoresis of ovine *FASN* gene (exon 3) PCR fragments, which showed two SSCP banding patterns in Awassi sheep.

Results of genetic diversity and Hardy-Weinberg test for *FASN* (exon 3) gene were presented in Table 1. The genetic analysis showed that the predominant genotype was AA with a genotype frequency of 60%. The χ^2 test indicated that the polymorphism of *FASN* (exon 3) in Awassi sheep was not at Hardy-Weinberg equilibrium (Table 1).

Table 1. Genetic diversity parameters for the *FASN* gene in Awassi breed.

	Genotype frequencies (n)		Allele frequencies		*Ho*	*He*	*Ne*	*χ2*
	AA	AB	A	B	0.4000	0.321	1.470	6.07
Awassi	0.60 (60)	0.40 (40)	0.80	0.20				

Abbreviations: (n) – samples number, χ2 – chi-square, *Ho* – observed heterozygosity, *He* – Expected heterozygosity, *Ne*- effective allele number. Chi-square tests have one degree of freedom within the significance level $p < 0.05$.

Sequence and *in silico* analysis of *FASN* gene

The SSCP analysis reported two different genotypes in the studied animals. Sequence analysis of the ovine *FASN* locus identified four SNPs, between the two resolved genotypes and the *FASN* (exon 3) NCBI reference sequences (Figure 2) which confirmed the results of the SSCP analysis. The pattern of each SNP that discovered by sequencing was listed in (Table 2). Several SNPs were discovered in *FASN* (exon 3) reference in comparison with two genotypes AA and AB (Table 2).

Table 2. The differences of nucleic acids and the amino acid patterns between genotype AA and genotype AB in exon 3 of *Ovis aries* of the *FASN* gene.

No.	Wild type	Mutant	Genotype	Position in the PCR fragment	Position in the genome	Type of SNP	Amino acid change
1	T	A	AB	84	49890901	Exonic (L47)	L47Q
2	T	C	AA & AB	131	49890948	Exonic (S63)	S63P
3	G	A	AB	247	49891064	Intronic variant	-
4	T	G	AB	281	49891098	Intronic variant	-

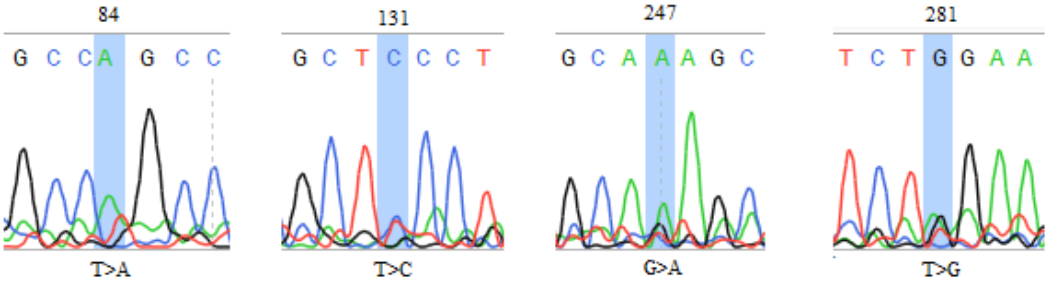

Figure 2. Sequences and SNP positions of two genotypes AA and AB in the Awassi sheep *FASN* (exon 3) gene.

Postulated three-dimensional structure of *Ovis aries* FASN protein was performed using protein homology/analogy recognition engine (Phere2), ver 2.0 and PyMOL-v1, 7.0.1 software (www.shrodinger.com) (Figure 3).

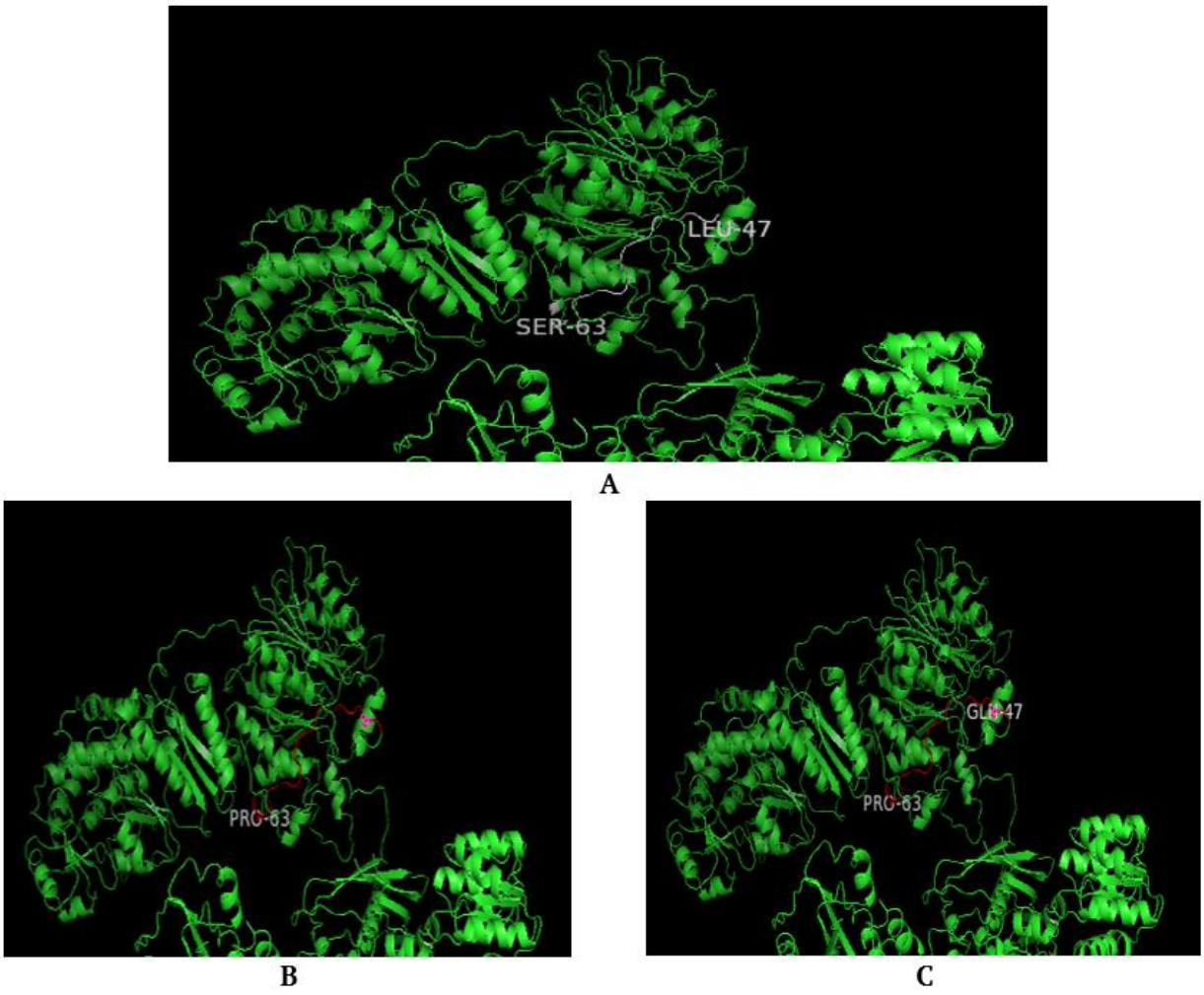

Figure 3. Virtual 3-D structure of ovine FASN. A) Reference type protein (Before mutation), B) mutant protein (in AA genotype), C) mutated protein (in AB genotype).

Consequences of the observed missense variants on the resulting altered protein structures, functions, and stability were evaluated and their results were shown in Table 3. Many bioinformatics tools were used to evaluate the consequences of the observed missense variants on the resulting altered protein structures, functions, and stability including SIFT, PolyPhen-2, Provean, SNAP2, and I-Mutant2.0 (Table 3).

Table 3. The *in silico* analysis of the observed nonsynonymous SNPs in FASN.

SNP	SIFT		PolyPhen-2		PROVEAN		SNAP2		I-Mutant2	
	score	prediction	score	prediction	score	prediction	score	prediction	score	prediction
L47Q	0.01	Affect protein function	0.924	Probably damaging	-5.700	Deleterious	67	Effect	-1.00 (DDG-kcal mol^{-1})	Decrease Stability
S63P	0.27	Tolerated	0.891	Probably damaging	-3.331	Deleterious	63	Effect	-3.14 (DDG-kcal mol^{-1})	Decrease Stability

Two missense mutation was found in two genotype AA and AB genotype. Only L47Q was found in AB genotype with an entire deleterious consequence on protein function and stability according to *in silico* tools (Table 3) and was predicted by the missense 3D server (Figure 3). Thus, it was found that there was an alteration between AA and AB genotypes regarding altered disordered positions, which may be responsible for this alteration in fatty acids content made AB genotype has good meat quality traits. This result is in concord with the study of Hayakawa et al. (2015) that studied the association between g.841G>C SNP in the *FASN* gene and associated with the fatty acid content of Japanese Black cattle. Bartoň, Bureš, Kott, & Řehák, (2016) identified one SNP in the *FASN* gene of Holstein cattle and revealed a significant relationship with fatty acid content in the *longissimus dorsi* muscle.

Association analysis

FASN (exon 3) gene polymorphism and animal traits

Table 4 refers to the effect of *FASN* gene polymorphism (exon3) on animal traits. Significant differences ($p < 0.05$) in the length of the animal for genotypes AA and AB of *FASN* (exon 3) gene showed that animals with AB genotype had a greater body length compared with AA genotypes ($p < 0.05$). While there was no significant association ($p > 0.05$) in the other animal traits among the genotypes (Table 4).

Table 4. Relationship between *FASN* gene polymorphism (exon 3) and animal traits in Awassi sheep.

Animal traits	Genotypes (LSM ± SE)		p-value
	AA (60)	AB (40)	
live body weight (kg)	29.32 ± 0.24	33.52 ± 0.31	0.13
body length (cm)	70.30 ± 1.45 [b]	82.53 ± 1.15 [a]	0.04 *
Fat tail (kg)	2.11 ± 0.05	2.43 ± 0.02	0.57
Abdominal fat (kg)	1.63 ± 0.03	1.45 ± 0.01	0.82
Back fat (kg)	1.42 ± 0.10	1.61 ± 0.01	0.29
Carcass weight (kg)	23.53 ± 0.37	25.11 ± 0.22	0.13

LSM, Least square mean ± SE standard error; * ($p < 0.05$); Different superscript in the same row refers to significant differences ($p < 0.05$).

Matsuhashi et al. (2011) that reveal polymorphisms in the *FASN* gene showed no effect on any animal traits that support our result. In contrast, another study reveals the three SNPs in the *FASN* gene that correlated to the subcutaneous fat thickness and growth traits in cattle (Souza et al., 2012). The TT genotype at g. 13232 C > T is correlated with higher intramuscular fat in Qinchuan cattle (Raza et al., 2018).

FASN gene polymorphism (exon 3) and lipid profile

The results of the current study showed significant differences ($p < 0.05$) in the lipid profile level between *FASN* (exon 3) genotypes. AA genotype had significantly higher ($p < 0.05$) levels of LDL concentration than AB genotype (72.68 ± 1.68 and 66.72 ± 0.93 (mg dL^{-1}) respectively, while no significant differences ($p > 0.05$) in other lipid profile concentrations among genotypes (Table 5).

Table 5. Association analysis between *FASN* polymorphism (exon 3) and lipid profile in Awassi sheep.

Indices	Genotypes (LSM ± SE)		p-value
	AA(60)	AB(40)	
Cholesterol (mg dL^{-1})	115.32 ± 1.21	111.17 ± 2.31	0.54
Triglyceride (mg dL^{-1})	77.53 ± 1.63	75.81 ± 1.01	0.23
High density lipoprotein (mg dL^{-1})	26.37 ± 0.79	26.54 ± 0.25	0.46
Very low density lipoprotein (mg dL^{-1})	15.79 ± 0.83	15.01 ± 0.77	0.22
Low density lipoprotein (mg dL^{-1})	72.68 ± 1.64 [a]	66.72 ± 0.93 [b]	0.01*

LSM, Least square mean ± SE standard error; * ($p < 0.05$); Different superscript in the same row refers to significant differences ($p < 0.05$).

Alter cellular cholesterol concentrations belong to the function of the FASN enzyme by increased mitochondrial oxidation of fatty acids (Carroll et al., 2018). Mahmoud, Mohammad, and Ezat (2016) refer to a significant association between FASN level with triglyceride and LDL concentrations.

FASN gene polymorphism (exon 3) and fatty acid composition

Statistical analysis for the fatty acid content of intramuscular in the *longissimus dorsi* muscle and *FASN* (exon 3) genotypes, shown in Table 6. The AB genotype had the lowest content of Capric acid (2.14 ± 0.04), Myristic C14:0 (0.56 ± 0.04) and Stearic C18:0 (0.001 ± 0.0001), while highest content of Oleic C18:1n9 (2.11 ± 0.05) than AA genotype. No significant association of the *FASN* genotypes was observed in other fatty acid composition.

Table 6. The relationship between *FASN* polymorphism (exon 3) and fatty acids composition in Awassi sheep.

Fatty acids composition	Genotypes (LSM ± SE)		p-value
SFA	AA(60)	AB(40)	
Caprylic	2.53 ± 0.01	1.35 ± 0.02	0.15
Capric	6.33 ± 0.02[b]	2.14 ± 0.04[a]	0.01*
Lauric	0.53 ± 0.05	0.47 ± 0.03	0.42
Myristic	1.53 ± 0.01[b]	0.56 ± 0.04[a]	0.02*
Palmitic	0.83 ± 0.02	0.94 ± 0.05	0.71
Stearic	0.02±0.003[b]	0.001±0.0001[a]	0.01*
Phytanic	0.84 ± 0.03	0.75 ± 0.04	0.59
MUFA			
Ricinoleic	0.45 ± 0.01	0.39 ± 0.02	0.31
Ricinelaidic	0.56 ± 0.03	0.70 ± 0.03	0.63
β-linolenic	1.99 ± 0.06	2.53 ± 0.04	0.25
α-linoleic	0.73 ± 0.07	0.78 ± 0.01	0.40
Oleic	1.32 ± 0.02[b]	2.11 ± 0.05[a]	0.03*
Petroselinic	0.73 ± 0.05	0.90 ± 0.01	0.61
Elaidic	0.11 ± 0.001	0.18 ± 0.004	0.42
Vaccenic	0.04 ± 0.0001	0.07 ± 0.002	0.78
PUFA			
α-linolenic	0.51 ± 0.03	0.52 ± 0.01	0.22

LSM, Least square mean ± SE standard error; SFA, saturated fatty acid; MUFA, monounsaturated fatty acid; PUFA, polyunsaturated fatty acid; * ($p < 0.05$); Different superscript in the same row refers to significant

Significant impacts reported of the *FASN* polymorphism on fatty acids content of the intramuscular in Japanese black cattle might indicate that this polymorphism might influence the β-ketoacyl reductase domain function in the *FASN* gene (Matsuhashi et al., 2011). A significant association is reported between fatty acid content and g.13232C > T SNP in the *FASN* gene in Qinchuan cattle (Raza et al., 2018). This suggested that the SNP g. 17924 G>A may change the activity in the TE domain of the *FASN* gene resulting in differences in fatty acids content between genotypes (Oztabak et al., 2014).

Conclusion

Novel SNP (L46Q) was discovered within the *FASN* gene (AB genotype), made the animals that have the AB genotype associated with good meat quality traits with the lowest content of SFA and the highest content of MUFA of Awassi sheep.

References

Ajafar, M. H., Kadhim, A. H., & AL-Thuwaini, T. M. (2022a). The Reproductive Traits of Sheep and Their Influencing Factors. *Reviews in Agricultural Science*, *10*, 82-89. DOI: https://doi.org/10.7831/ras.10.0_82

Ajafar, M. H., Al-Thuwaini, T. M., & Dakhel, H. H. (2022b). Association of OLR1 gene polymorphism with live body weight and body morphometric traits in Awassi ewes. *Molecular Biology Reports*, 1-5. DOI: https://doi.org/10.1007/s11033-022-07481-3

Al-Shuhaib, M. B. S. A. (2017). A universal, rapid, and inexpensive method for genomic DNA isolation from the whole blood of mammals and birds. *Journal of Genetics*, *96*(1), 171-176. DOI: https://doi.org/10.1007/s12041-017-0750-6

Al-Thuwaini, T. M. (2021a). The relationship of hematological parameters with adaptation and reproduction in sheep; A review study. *Iraqi Journal of Veterinary Sciences*, *35*(3), 575-580. DOI: https://doi.org/10.33899/ijvs.2020. 127253.1490

Al-Thuwaini, T. M. (2021b). Novel single nucleotide polymorphism in the prolactin gene of Awassi ewes and its role in the reproductive traits. *Iraqi Journal of Veterinary Sciences*, *35*(3), 429-435. DOI: https://doi.org/10.33899/ijvs.2020.126973.1423

AL-Thuwaini, T. M. (2022). Adiponectin and Its Physiological Function in Ruminant Livestock. *Reviews in Agricultural Science*, *10*, 115-122. DOI: https://doi.org/10.7831/ras.10.0_115

Bartoň, L., Bureš, D., Kott, T., & Řehák, D. (2016). Associations of polymorphisms in bovine DGAT1, FABP4, FASN, and PPARGC1A genes with intramuscular fat content and the fatty acid composition of muscle and subcutaneous fat in Fleckvieh bulls. *Meat Science*, *114*(1), 18-23.

Byun, S. O., Fang, Q., Zhou, H., & Hickford, J. G. H. (2009). An effective method for silver-staining DNA in large numbers of polyacrylamide gels. *Analytical Biochemistry*, *385*(1), 174-175. DOI: https://doi.org/10.1016/j.ab.2008.10.024

Carroll, R. G., Zasłona, Z., Galván-Peña, S., Koppe, E. L., Sévin, D. C., Angiari, S., ... & O'Neill, L. A. (2018). An unexpected link between fatty acid synthase and cholesterol synthesis in proinflammatory macrophage activation. *Journal of Biological Chemistry*, *293*(15), 5509-5521. DOI: https://doi.org/10.1074/ jbc.RA118.001921

Cho, Y. M., Lee, S. H., Park, E. W., Kim, N. K., Lim, D., Kim, K. H., ... & Yoon, D. (2010). Association of-867G> C,-877Gdel, and Exon 5G> T polymorphisms in the Stearoyl-CoA Desaturase (*SCD*) gene with fatty acid composition in the *M. longissimus* dorsi muscle of Hanwoo (Korean Cattle). *Korean Journal for Food Science of Animal Resources*, *30*(4), 655-660.

Ciecierska, D., Frost, A., Grzesiak, W., Proskura, W. S., Dybus, A., & Olszewski, A. (2013). The influence of fatty acid synthase polymorphism on milk production traits in Polish Holstein-Friesian cattle. *Journal of Animal and Plant Sciences, 23*(2), 376-379.

Ebrahimi, M. T. V., Mohammadabadi, M., & Esmailizadeh, A. (2017). Using microsatellite markers to analyze genetic diversity in 14 sheep types in Iran. *Archiv fuer Tierzucht*, *60*(3), 183-189. DOI: https://doi.org/10.5194/aab-60-183-2017

Esteves, C., Livramento, K. G., Paiva, L. V., Peconick, A. P., Garcia, I. F. F., Garbossa, C. A. P., & Faria, P. B. (2019). The polymorphisms of genes associated with the profile of fatty acids of sheep. *Arquivo Brasileiro de Medicina Veterinária e Zootecnia*, *71*(1), 303-313. DOI: https://doi.org/10.1590/1678-4162-9376

Gholamhoseini, F., Mohammadabadi, M. R., & Asadi Fozi, M. (2018). Polymorphism of the growth hormone gene and its effect on production and reproduction traits in goat. *Iranian Journal of Applied Animal Science*, *8*(4), 653-659.

Gooki, F. G., Mohammadabadi, M., Fozi, M. A., & Soflaei, M. (2019). Association of biometric traits with growth hormone gene diversity in raini cashmere goats. *Walailak Journal of Science and Technology, 16*(7), 499-508. DOI: https://doi.org/10.48048/wjst.2019.3791

Hayakawa, K., Sakamoto, T., Ishii, A., Yamaji, K., Uemoto, Y., Sasago, N., ... & Sasazaki, S. (2015). The g. 841G> C SNP of FASN gene is associated with fatty acid composition in beef cattle. *Animal Science Journal*, *86*(8), 737-746. DOI: https://doi.org/10.1111/asj.12357

IBM Corp. (2015). *IBM SPSS statistics for windows, version 23.0*. Armonk, NY: IBM Corp.

Imran, F. S., Al-Thuwaini, T. M., Al-Shuhaib, M. B. S., & Lepretre, F. (2020). A novel missense single nucleotide polymorphism in the *GREM1* gene is highly associated with higher reproductive traits in awassi sheep. *Biochemical Genetics, 59*(1), 422-436. DOI: https://doi.org/10.1007/s10528-020-10006-x

Jawasreh, K. I., Al-Amareen, A. H., & Aad, P. Y. (2019). Relationships between Hha1 calpastatin gene polymorphism, growth performance, and meat characteristics of Awassi sheep. *Animals*, *9*(9), 667. DOI: https://doi.org/10.3390/ani9090667

Mahmoud, A. A., Mohammad, A. N., & Ezat, M. A. W. (2016). Evaluation of circulating fatty acid synthase as a biomarker in non-alcoholic fatty liver disease. *Open Journal of Gastroenterology*, *6*(9), 229-237. DOI: https://doi.org/10.4236/ojgas.2016.69028

Matsuhashi, T., Maruyama, S., Uemoto, Y., Kobayashi, N., Mannen, H., Abe, T., ... & Kobayashi, E. (2011). Effects of bovine fatty acid synthase, stearoyl-coenzyme A desaturase, sterol regulatory element-binding protein 1, and growth hormone gene polymorphisms on fatty acid composition and carcass traits in Japanese Black cattle. *Journal of Animal Science*, *89*(1), 12-22. DOI: https://doi.org/10.2527/jas.2010-3121

Moghadaszadeh, M., Mohammadabadi, M. R., & Esmailizadeh, A. K. (2015). Association of exon 2 of *BMP15* gene with the litter size in the Raini Cashmere goat. *Genetics in the 3rd Millennium, 13*(3), 4062-4067.

Mohammadabadi, M. R. (2016). Inter-simple sequence repeat loci associations with predicted breeding values of body weight in Kermani sheep. *Genetics in the 3rd Millennium*, *14*(4), 4383-4390.

Oztabak, K., Gursel, F. E., Akis, I., Ates, A., Yardibi, H., & Turkay, G. (2014). FASN gene polymorphism in indigenous cattle breeds of Turkey. *Folia Biologica*, *62*(1), 28-34. DOI: https://doi.org/10.3409/fb62_1.29

Pasandideh, M., Mohammadabadi, M. R., Esmailizadeh, A. K., & Tarang, A. (2015). Association of bovine PPARGC1A and OPN genes with milk production and composition in Holstein cattle. *Czech Journal of Animal Science*, *60*(1), 97-104.

Pećina, M., & Ivanković, A. (2021). Candidate genes and fatty acids in beef meat, a review. *Italian Journal of Animal Science*, *20*(1), 1716-1729. DOI: https://doi.org/10.1080/1828051X.2021.1991240

Quiñones, J., Bravo, S., Calvo Lacosta, J. H., & Sepúlveda, N. (2017). Genetic polymorphism in meat fatty acids in Auraucano Creole sheeps.*The Journal of Animal and Plant Sciences, 27*(3), 743-746.

Raza, S. H. A., Gui, L., Khan, R., Schreurs, N. M., Xiaoyu, W., Wu, S., ... & Guo, H. (2018). Association between FASN gene polymorphisms ultrasound carcass traits and intramuscular fat in Qinchuan cattle. *Gene*, *645*(1), 55-59. DOI: https://doi.org/10.1016/j.gene.2017.12.034

Salimon, J., Omar, T. A., & Salih, N. (2017). An accurate and reliable method for identification and quantification of fatty acids and trans fatty acids in food fats samples using gas chromatography. *Arabian Journal of Chemistry*, *10*(1), S1875-S1882. DOI: https://doi.org/10.1016/j.arabjc.2013.07.016

Shamsalddini, S., Mohammadabadi, M. R., & Esmailizadeh, A. K. (2016). Polymorphism of the prolactin gene and its effect on fiber traits in goat. *Russian Journal of Genetics (Genetika)*, *52*(4), 461-465. DOI: https://doi.org/10.1134/S1022795416040098

Shi, B., Jiang, Y., Chen, Y., Zhao, Z., Zhou, H., Luo, Y., ... & Hickford, J. G. (2019). Variation in the fatty acid synthase gene (FASN) and its association with milk traits in Gannan yaks. *Animals*, *9*(9), 613. DOI: https://doi.org/10.3390/ani9090613

Souza, F. R. P., Chiquitelli, M. G., Fonseca, L. F. S., Cardoso, D. F., Silva Fonseca, P. D., Camargo, G. M. F., ... Albuquerque, L. G. (2012). Associations of FASN gene polymorphisms with economical traits in Nellore cattle (Bos primigenius indicus). *Molecular Biology Reports*, *39*(12), 10097-10104. DOI: https://doi.org/10.1007/s11033-012-1883-6

Ye, J., Coulouris, G., Zaretskaya, I., Cutcutache, I., Rozen, S., & Madden, T. L. (2012). Primer-BLAST: a tool to design target-specific primers for polymerase chain reaction. *BMC Bioinformatics*, *13*(1), 134. DOI: https://doi.org/10.1186/1471-2105-13-134

Yeh, F. C., & Yang, R. C. (1999). *POPGENE version 1.31, Microsoft window-based freeware for population genetic analysis*. Edmonton, AB: University of Alberta and Tim Boyle, Centre for International Forestry Research.

Zalewska, M., Puppel, K., & Sakowski, T. (2021). Associations between gene polymorphisms and selected meat traits in cattle - A review. *Animal Bioscience*, *34*(9), 1425-1438. DOI: https://doi.org/10.5713/ab.20.0672