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MSTN gene polymorphisms are associated with the feed efficiency of fattened lambs in Latvian sheep breeds

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Abstract. The economic benefit for sheep farmers depends on the level of feed efficiency of the lambs raised. Promoting breed selection for feed efficiency could provide sheep farmers with higher quality and more economically profitable lambs. In livestock production, marker-assisted selection employs genetic biomarkers linked to specific traits. Myostatin (MSTN), a vital transforming growth factor-beta superfamily member, is pivotal in regulating myogenesis and negatively impacts mammal muscle growth and development. The study aims to find molecular markers related to feed efficiency parameters in the MSTN gene for Latvian sheep breeds. DNA extraction was made from blood collected from 76 controlled fatten lambs, with 63.16% belonging to the Latvian Dark-head (LT) breed. A complete gene sequencing analysis was conducted to identify variations in loci across different sheep breeds, aiming to discover statistically significant associations between identified polymorphisms and feed efficiency indicators. Polymorphic variants were identified in 23 loci of the MSTN gene among Latvian lambs, with the discovery of a novel SNP. Notably, SNP rs404916326 T>A exhibited statistically significant associations with indicators such as Residual feed intake, Residual intake, and body weight gain, specifically in the LT breed. Furthermore, SNP rs408469734 G>A showed associations with relative growth rate and Kleiber's ratio within the lamb group. These findings suggest that SNPs rs404916326 and rs408469734 within the MSTN gene are promising molecular markers for marker-assisted selection strategies in sheep breeding to improve feed efficiency indicators.

Key words: marker-assisted selection (MAS), intensive fattening, feed efficiency, sheep genome, Latvian sheep, genetic markers.

INTRODUCTION

MSTN is a member of the TGFβ superfamily, and it exerts a detrimental influence on the growth of skeletal muscle (Sjakste et al., 2011; Osman et al., 2021; Guo et al., 2023). Myostatin controls muscle development at specific stages of pre-natal muscle growth, including muscle precursor proliferation, myoblast proliferation, and differentiation

(Aiello et al., 2018). Suppression of MSTN expression results in increased muscle cell number, hyperplasia, and increased size, or hypertrophy (Du et al., 2022). Mutations within the MSTN gene lead to a 'double muscle' trait, which is crucial for boosting the meat yield of livestock and providing humans with protein of exceptional quality (Chen et al., 2023; Chacko Kaitholil et al., 2024). The editing of the MSTN gene has led to a considerable increase in the diameter of myofibers and an improvement in the average daily gain (ADG) and body weight (BW) of sheep (Zhou et al., 2022).

Several natural mutation sites of *MSTN*, most in the non-coding areas, have been documented in different sheep breeds (Hadjipavlou et al., 2008; Chen et al., 2023). SNP c.*1232G>A (rs408469734) in the 3'UTR of the MSTN gene in Texel sheep leads to an unauthorised microRNA binding site and causes a significant decrease of around 2/3 in the level of MSTN protein in the bloodstream, resulting in increased muscularity (Masri et al., 2011). In Norwegian white sheep, identifying a frameshift mutation in the MSTN gene, specifically c.960delG, along with c.*1232G>A, correlates with reduced fat deposition and increased muscle mass (Boman et al., 2010).

According to Kumar et al. (2023), sheep farming plays a significant role in meeting the dietary requirements of rural communities, particularly in developing countries. Eurostat data (Eurostat, 2023a) reveals a decline in the sheep population in Latvia from 112.21 thousand in 2017 to 87.32 thousand in 2022 despite ten approved breeding programs. While Latvia produced 0.47 thousand tonnes of sheep and goat meat in 2022, a slight decrease from the 0.43 thousand tonnes recorded in 2017 was observed (Eurostat, 2023b). It is crucial to enhance the growth, production, and reproductive characteristics of Latvian sheep breeds to meet the rising demand for meat production amidst declining sheep numbers.

Feed expenses constitute a significant portion of daily costs in sheep breeding, particularly for meat or milk production. One strategy to reduce these expenses is to breed animals with improved feed efficiency (Berry & Crowley, 2012; Lima et al., 2017; Hervás et al., 2021). Marker-assisted selection (MAS) is a powerful technology that helps to identify the most feed-efficient lambs for breeding purposes (Wakchaure et al., 2015; Sahu et al., 2019; Osman et al., 2021).

Recent research indicates that genes involved in metabolic processes may influence various feed efficiency indicators (Chacko Kaitholil et al., 2024). These indicators include Feed efficiency (FE), Feed conversion ratio (FCR), Relative growth rate (RGR), Kleiber ratio (KR), Residual feed intake (RFI), Residual weight gain (RWG), and Residual intake and body weight gain (RIG) (Berry & Crowley, 2012). Despite challenges in measuring individual feed efficiency in group-reared sheep, a few genetic variations linked to these metrics have been identified (Zhang et al., 2023). Our study presents an opportunity to explore genetic variants associated with feed efficiency indicators in Latvian sheep breeds raised under controlled, intensive, fattening conditions.

Recent research indicates that the impact of genetic variations, such as MSTN gene polymorphisms, on metabolic processes, varies across breeds (Guo et al., 2023; Chacko Kaitholil et al., 2024), highlighting the necessity for breed-specific investigations. Prior studies (Sjakste et al., 2011) have examined the MSTN gene in the local Latvian sheep breed, the Latvian Dark-head (LT; *Latvijas tumšgalve*), primarily focusing on genetic variation identification. However, there has been a lack of comprehensive analysis on the complete MSTN gene sequence and its potential role in Latvian sheep breeds, including the national breed Latvian Dark-head. This study aims to elucidate the

distribution of SNPs across the entire MSTN gene in different Latvian sheep breeds and assess these genetic variants' potential roles under standardised feeding conditions. A further aim is to analyse the complete sequence of the MSTN gene in lambs representing six Latvian sheep breeds to determine potential associations between polymorphisms and feed efficiency indicators among intensively fattened lambs. Scientifically based knowledge of the relationship between feed efficiency indicators and genetic markers within a specific region can be used in MAS as a cost-effective and rapid method for improving breeds and increasing feed efficiency. Consequently, breeders can benefit from both time and cost savings in the breeding process.

MATERIALS AND METHODS

Animals of intensive fattening

Seventy-six lambs representing six distinct breeds (Latvian Dark-head (LT), Merinolandschaf (Ml), Île de France (IF), Charollais (Ch), Dorper (Do) and Texel (Tx)) underwent controlled fattening from March to October 2022. The study aimed to assess the offspring indicators of rams from each breed. The study group contained the most commonly grown breeds in Latvia with an approximately proportional distribution. The Latvian Dark-head breed accounted for the most significant number of lambs (48), followed by Merino Landscape (8), Île de France (6), Charolais (3), Dorper (5), and Texel (6). This investigation was conducted with the Latvian Sheep Breeders' Association at the ram breeding control station. The lambs underwent a fattening period lasting an average of 66.38 ± 11.05 days, with durations ranging from 44 to 83 days.

Following the fattening control technique outlined by LAAA (2022), lambs from the same sire ram were raised together in a pen with an approximate size of 4 m^2. The pen was equipped with loose silos containing mixed concentrate and slatted silos for hay, with straw provided as bedding. Upon completion of each group of lambs, the pen underwent thorough sanitisation. Natural ventilation was provided by the building's ceiling openings and insect-netted windows. All animal housing procedures during the research adhered to animal welfare regulations.

Details regarding the intensive fattening process of lambs and the computation of feed efficiency indicators using established formulas (Berry & Crowley, 2012; Lima et al., 2017) have been documented in earlier publications by our team (Trapina et al., 2023a, b).

DNA sequencing and SNP identification

Blood samples were obtained from each lamb's jugular vein after the fattening or a 24-hour fasting period before slaughter. These samples were utilised to extract genomic DNA using a genomic DNA extraction kit sourced from Fermentas, Lithuania. DNA quality and quantity were assessed through agarose gel electrophoresis and spectrophotometry techniques.

The complete sequencing of the MSTN gene was performed using the Illumina MiSeq DNA sequencing system (Illumina, USA). Clean reads were then aligned to the sheep reference genome (GCF_016772045.1_ARS-UI_Ramb_v2.0, NCBI), and variable loci were identified using Geneious Prime® 2023.2.1 (https://www.geneious.com).

The most recent and comprehensive genomic sequence of the sheep species (*Ovis aries*), ARS_UI_Ramb_v2.0 (breed: Rambouillet, France), can be accessed in the NCBI database (https://www.ncbi.nlm.nih.gov/) from 2022, as provided by Davenport et al. (2022). This sequence information was utilised to analyse and compare samples from Latvian sheep breeds collected during the investigation. The localisation of genetic variations was determined using the ARS_UI_Ramb_v2.0 sequence.

Statistical analyses

Genotypes and frequencies of the studied loci in individual genes were determined through direct counting. The mean and standard error (SEM) of feed efficiency indicators were computed for each single-locus genotype group using measurement data. Statistical tests such as *T-tests, ANOVA, Kruskal-Wallis* test, or *Median test* were chosen based on the normality and/or homogeneity of variances of the genotype group data to accurately assess the extent of the differences across all 76 DNA samples and the 48 LT DNA sample group. The coefficient *Eta* (η), ranging from 0 (no relationship) to 1 (perfect relationship), was employed to gauge the degree of association. A significance threshold of $P < 0.05$ was applied to determine significant results.

Haplotype analysis was conducted using DnaSP6.12.03 software (www.ub.edu/dnasp/) (Rozas et al., 2017). Additionally, linkage disequilibrium (LD) analysis was performed twice: once with DnaSP 6.12.03 and again with Haploview 4.1 (Barrett et al., 2005).

The General Linear Model (GLM) analysis assessed potential genetic effects statistically associated with feed efficiency. The *GLM* equation included variables such as breed membership, birth weight, and genotype SNP of the MSTN gene. Interactions between variables were evaluated to determine their potential significance for inclusion in the model. *GLM* analyses were conducted both with and without SNP information.

Statistical analysis was conducted using SPSS v.25 (IBM Corp., 2017).

RESULTS AND DISCUSSION

Polymorphisms and genetic diversity of MSTN gene

The MSTN gene is located on the long arm of chromosome 2 (2q32.2; NC_056055.1). According to the Ensembl database, the ENSOART00020014589.2 transcript comprises three exons spanning 376 amino acids. UniProt protein O18830 (www.uniprot.org) is encoded by three exons, encompassing amino acids 267–374.

The Ensembl database contains data on 204 polymorphic loci within the 6,757 bp-long MSTN gene region. In Latvian sheep DNA samples, 22 known SNPs were variable. In addition, a previously unidentified genetic variant was detected in exon 1 (Fig. 1; Table 1).

Analysing the positioning of the polymorphisms (refer to Fig. 1, Table 1), we observed a cluster of eight SNPs at the beginning of intron 1 (ranging from positions 18 to 323 within intron 1). Similar findings have been reported in previous studies (Gan et al., 2008; Sjakste et al., 2011; Farhadian & Hashemi, 2016; Osman et al., 2021), indicating that the first intron of the ovine MSTN gene is notably polymorphic across various sheep breeds. In our study of Latvian samples, six variations were identified, while no variability was detected for rs598751087 (c.373+101C>T) and rs407388367 (c.373+323C>T) in Latvian samples.

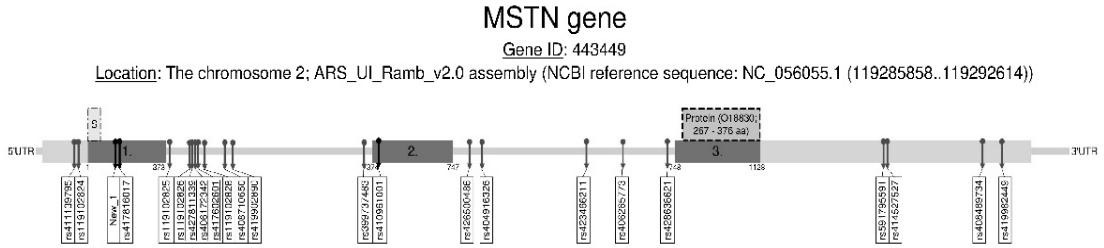

Figure 1. The MSTN gene structure exhibits exon regions with polymorphisms (indicated by arrows) identified in Latvian sheep breed samples. The numbers below the exons represent the nucleotide numbering in the cDNA, starting from the initial ATG; S – Signal peptide (1–23 aa), according to the NCBI database (https://www.ncbi.nlm.nih.gov/).

Exon 3 was identified as monomorphic in Latvian sheep, displaying no genetic variations. Comparable findings were reported in New Zealand Romney sheep and various Chinese sheep breeds (Gan et al., 2008; Sahu et al., 2019), although this was not the case in Nilagiri sheep (Sahu et al., 2019).

Table 1. The frequency (%) distribution of genotypes for polymorphisms in the IGF1 gene in Latvian sheep breeds

Gene	Polymorphisms ID number Alleles*	Genotype	Group of sheep (n) All (76)	LT (48)	Other (28)	Statistical significance (*P*)
MSTN	rs411139795	CC	36.84	25.00	57.14	1.88×10^{-2}
	c.-40C>A	CA	39.47	47.92	25.00	
		AA	23.68	27.08	17.86	
	rs119102824	TT	76.32	72.92	82.14	0.64
	c.-37T>C	TC	19.74	22.92	14.29	
		CC	3.95	4.17	3.57	
	New_1	GG	97.37	100.00	92.86	0.06
	c.84G>A	GA	2.63	0.00	7.14	
	p.Glu28=	AA	0.00	0.00	0.00	
	rs417816017	AA	97.37	100.00	92.86	0.06
	c.101A>G	AG	2.63	0.00	7.14	
	p.Glu34Gly	GG	0.00	0.00	0.00	
	rs119102825	GG	40.79	29.17	60.71	2.60×10^{-2}
	c.373+18G>T	GT	36.84	43.75	25.00	
		TT	22.37	27.08	14.29	
	LD1^	TT/GG/--	72.37	68.75	78.57	0.54
		Hetero	26.32	29.17	21.43	
		CC/AA/TT	1.32	2.08	0.00	
	rs427811339	GG	52.63	47.92	60.71	0.43
	c.373+243G>A	GA	34.21	39.58	25.00	
		AA	13.16	12.50	14.29	
	rs406172342	TT	75.00	72.92	78.57	0.84
	c.373+246T>C	TC	19.74	22.92	14.29	
		CC	5.26	4.17	7.14	

Table 1 (continued)

LD3^	TT/CC/AA/GG	98.68	100.00	96.43	0.19
	Hetero	1.32	0.00	3.57	
	CC/TT/CC/AA	0.00	0.00	0.00	
rs119102828	GG	59.21	43.75	85.71	1.24×10^{-3}
c.373+259G>T	GT	19.74	29.17	3.57	
	TT	21.05	27.08	10.71	
LD2^	GG/CC	75.00	72.92	78.57	0.86
	GA/CT	21.05	22.92	17.86	
	AA/TT	3.95	4.17	3.57	
rs410961001	AA	98.68	100.00	96.43	0.19
c.384A>G	AG	1.32	0.00	3.57	
p.Leu128=	GG	0.00	0.00	0.00	
rs426500486	AA	42.11	29.17	64.29	7.41×10^{-3}
c.747+164A>G	AG	38.16	43.75	28.57	
	GG	19.74	27.08	7.14	
rs404916326	TT	81.58	70.83	100.00	2.39×10^{-3}
c.747+309T>A	TA	17.11	27.08	0.00	
	AA	1.32	2.08	0.00	
rs423466211	CC	42.11	29.17	64.29	7.41×10^{-3}
c748-810C>T	CT	38.16	43.75	28.57	
	TT	19.74	27.08	7.14	
rs406265773	AA	52.63	47.92	60.71	0.38
c.748-475A>C	AC	35.53	39.58	28.57	
	CC	11.84	12.50	10.71	
rs408469734	GG	89.47	100.00	71.43	2.08×10^{-4}
c.*1232G>A	GA	3.95	0.00	10.71	
	AA	6.58	0.00	17.86	

n – Sample number in group; * – Nucleotide in position in gene cDNA: common allele (1) to rear allele (2); *P* – the statistical significance between LT (Latvian dark-head) and other breed groups; ^LD1 – SNP rs119102826 (c.373+241T>C)/ rs408710650 (c.373+563G>A)/ rs591795591 (c.*707DelT); LD2: rs419902890 (c.373+607G>A)/ rs399737483 (c.374-54C>T); LD3: rs417602601 (c.373+241T>C)/ rs428638621 (c.748-54C>T)/ rs414527527 (c.*709A>C)/ rs419982449 (c.*1316G>A).

Within the MSTN gene, three groups exhibit complete linkage disequilibrium (LD; D' = 1.00; r^2 = 1.00) among all polymorphisms (refer to Fig. 2), comprising nine SNPs. Consequently, only one SNP representing each LD group was included in the association analysis. The first LD (LD1) group consist of three SNPs: rs119102826 (c.373+241T>C), rs408710650 (c.373+563G>A), and rs591795591 (c.1128+707DelT). The second group (LD2) includes two: rs419902890 (c.373+607G>A) and rs399737483 (c.374-54C>T), while the third group (LD3) encompasses four SNPs: rs417602601 (c.373+241T>C), rs428638621 (c.748-54C>T), rs414527527 (c.*709A>C), and rs419982449 (c.*1316G>A), all exhibiting perfect linkage disequilibrium with each other. In all three groups, the homozygous form of the most common alleles was found to predominate (Table 1), resulting in the dominance of the most common haplotype (Fig. 2). LD1 and LD2 exhibited common alleles pairing to form haplotypes, observed in 85.5% of cases. In contrast, LD3 featured rare alleles detected in only 0.7% of cases, exclusively in the heterozygous form.

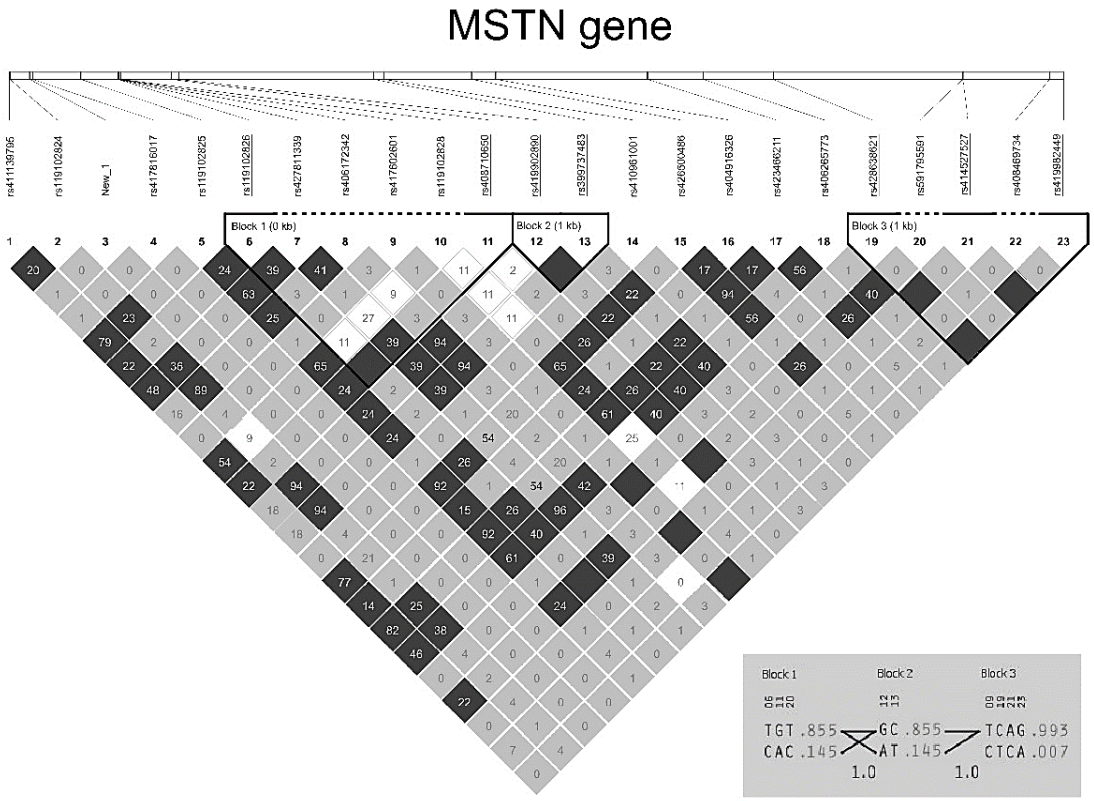

Figure 2. The linkage disequilibrium analysis of MSTN gene polymorphisms is represented graphically. The colour of each square indicates the value of *D*' (ranging from black for 1.00 to white for 0.00), while the numerical value within the square represents the value of r^2 *100.

The Ensembl database contains linkage disequilibrium data for numerous SNPs, including those from the International Sheep Genomics Consortium (ISGC) study in Composite and Romney sheep groups (https://www.sheephapmap.org/). No LD information was found after examining each SNP within the LD2 group in the database. However, for the LD1 group, complete LD was exclusively observed for the rs408710650 SNP. Regarding specific SNPs, no evidence suggests that the ISGC group exhibits perfect linkage disequilibrium with any SNPs found in the Latvian sheep. Conversely, the Romney sheep group displayed perfect LD with ten additional SNPs, three of which were within the MSTN gene. Moreover, all these SNPs (rs426500486, rs423466211, and rs406265773, refer to Fig. 1, Table 1) exhibited variability in our experimental group of sheep.

In the LD3 group, four SNPs exhibited perfect linkage disequilibrium among each other. According to the Ensembl database, three specific genetic markers, rs417602601, rs428638621, and rs419982449, were identified to be in complete LD in both Composite and Romney sheep DNA samples. The comprehensive LD analysis also encompassed 27 SNPs, including two additional SNPs from the MSTN gene region. Several studies have determined multilocus genotype combinations, particularly in intron 1 (Clop et al., 2006; Hickford et al., 2010; Sjakste et al., 2011; Grochowska et al., 2019; Kolenda et al., 2019). However, no LD analysis data were available. In this study, we did not analyse multilocus combinations due to the focus on the significance of individual SNPs.

Based on the genotyping data presented in Table 1, seven rare SNPs were identified in the MSTN gene among the experimental cohort of Latvian sheep, each accounting for less than 5% of the population. These include New_1, rs417816017, rs410961001, and the LD3 group: rs417602601, rs428638621, rs414527527, and rs419982449. Notably, SNPs rs417816017 and rs410961001 were also classified as rare in the Ensemble database, unlike the SNPs from the LD1 group. Interestingly, none of these six SNPs were detected in the LT samples in this study; however, they were observed in other breeds, with New_1 and rs417816017 exclusively found in Merinolandschaf, rs410961001 in Dorper, and LD1 group SNPs detected in Charolais.

Our study revealed a statistically significant difference between the LT breed group and other breeds for seven specific SNPs. In addition, a significantly higher proportion of heterozygous genotypes for six polymorphisms was found in the LT group than in other breeds.

In the group of LT sheep, SNP rs408469734 (c.*1232G>A) was represented only by the homozygous genotype for the common allele. According to early studies, this polymorphism in the 3'UTR was found to correlate with carcass and/or growth characteristics in different sheep breeds (Masri et al., 2011; Kolenda et al., 2019). Thus, in the Kamieniec breed, the heterozygous genotype was more favourable for average daily gain and correlated with an average gain of 20–40 grams (Kolenda et al., 2019). Research suggests that a sequence in this specific region is targeted by certain microRNAs depending on the SNP allele, resulting in increased levels of MSTN protein production and improved growth characteristics in the mutant allele (Tellam et al., 2012).

The frequency of the rs408469734 A allele in Latvian sheep breeds was significantly lower (8.55%) than in other more frequently studied breeds. However, in our collection of Texel breed sheep, the frequency of the A allele was 91.67%. These findings align with the results reported by Clop et al. (2006). Moreover, the rare A allele in Charolais lambs from our experimental cohort was observed at a frequency of 33.33%. Similarly, Hadjipavlou et al. (2008) reported comparable frequencies. However, the allele A frequency varies significantly across sheep breeds. For instance, Han et al. (2013) found a much lower frequency of 2.79% in New Zealand Romney sheep, while Kijas et al. (2007) reported a frequency of 12.5% in Lincoln, Poll Dorset, and White Suffolk breeds. Interestingly, in some breeds, such as Colored Polish Merino sheep (Grochowska et al., 2019), Iranian sheep breeds (Miar et al., 2014), and Suffolk sheep (Hadjipavlou et al., 2008), only the GG genotype of this locus was detected. These findings highlight the significant variation in the allele frequency of SNP rs408469734 between different sheep breeds.

In our study, we identified promoter SNPs rs411139795 (c.-40C>A) and rs119102824 (c.-37T>C), which have been frequently examined in other research. We made several notable observations by comparing our findings with those of a previous study on the LT breed conducted by Sjakste et al. (2011). First, our study identified a rare allelic homozygote for SNPs rs411139795 and rs119102824, which had not previously been observed in the LT breed. In addition, in the present study, the frequency of the CC genotype related to the rs411139795 polymorphism was approximately 2.5 times lower compared to the study mentioned above. Furthermore, when referring to the Ensemble database within the NextGen project, we discovered that Iranian and Moroccan sheep also exhibit an increased proportion of heterozygous samples for rs411139795.

Association with feed efficiency indicators

The general experimental cohort found a statistically significant association between the 3'UTR SNP rs408469734 (c.*1232G>A) and RGR and KR indicators. However, when focusing specifically on the LT breed, a different SNP, rs404916326 (c.747+309T>A), located in intron 2, exhibited a significant association with RFI and RIG indicators (Fig. 3).

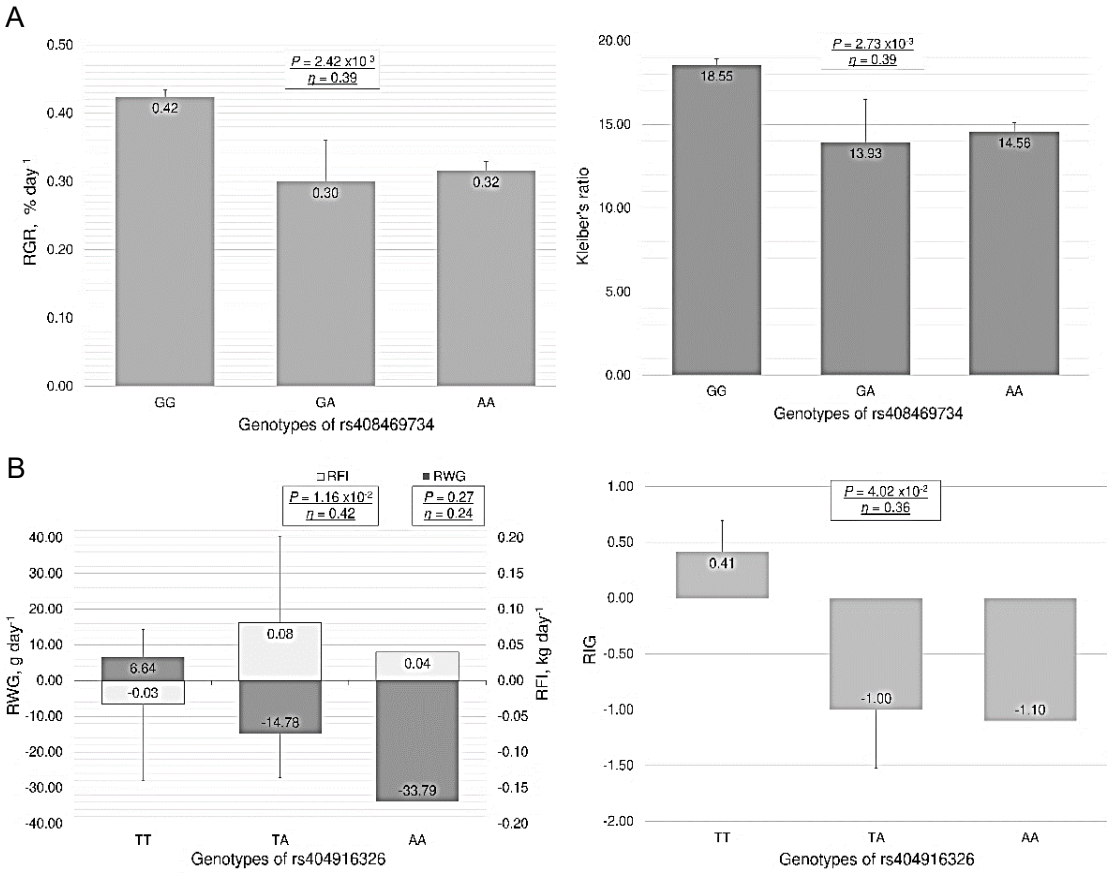

Figure 3. Gene MSTN SNP rs408469734 association with (A) Relative growth rate (RGR) and Kleiber ratio in the overall Latvian sheep, and SNP rs404916326 association with (B) Residual feed intake (RFI) and Residual weight gain (RWG), and Residual intake and body weight gain (RIG) in the overall Latvian dark-head. *P* – the statistical significance of the mean with the *ANOVA* test; η – a measure of association.

Statistically significant differences were observed between the average relative growth rate and Kleiber ratio values in a cohort of lambs carrying homozygous genotypes for the common allele (see Fig. 3, A). In particular, lambs with the GG genotype of SNP rs408469734 demonstrated 0.10% higher relative growth rate compared to lambs with the homozygous genotype (AA) for the rare allele ($P = 2.42 \times 10^{-3}$). Interestingly, the growth rate in lambs with the heterozygous GA genotype was also 0.12% higher than in carriers of the AA genotype.

Lambs carrying the rs408469734 GG genotype exhibited higher Kleiber coefficient values than other genotypes, prevailing by at least 4 points ($P = 2.73\times10^{-3}$). This suggests that these lambs experience more significant weight gain while maintaining a consistent metabolic rate, potentially reducing feeding costs. Consequently, significant weight gain is achieved without a proportional increase in energy consumption for maintenance (Talebi, 2012).

The above SNP rs408469734 or c.*1232G>A has been extensively studied in relation to growth and carcass traits across various sheep breeds, yielding significant associations. For instance, Hadjipavlou et al. (2008) discovered a link between this SNP and muscle and fat depth in the third lumbar vertebra of Charolais lambs. Kolenda et al. (2019) found an association between SNP and body weight on day 56 in the Kamieniec breed. Masri et al. (2011) reported a connection between this SNP and various tissue area measurements and computed tomography scan parameters in Texel and Poll Dorset lambs. Additionally, an Austrian study by Kijas et al. (2007) investigated the genetic distribution of rs408469734 and its association with traits like fat depth and meat tenderness across 12 sheep breeds.

The study revealed a significant association between SNP rs404916326 or c.747+309T>A and feed efficiency indicators RIF and RIG in Latvian Dark-headed sheep (refer to Fig. 3, B). Notably, the minor allele was exclusively detected in this cohort. Among the various genotypes, lambs with the homozygous TT genotype for the common allele of rs404916326 exhibited the most optimal, namely negative, average value of the RIF parameter. This genotype was present in 70.83% of animals in the LT cohort, with an average RFI of -0.03 kg per day. Consequently, lambs with the TT genotype consumed approximately 300 g less dry material intake (DMI) per day compared to the other two genotypes, with differences ranging from 0.07 to 0.11 kg of dry food per day ($P = 1.16\times10^{-2}$).

Lambs with the homozygous TT genotype for the common allele SNP rs404916326 exhibited an optimal RIG indicator value of 0.41. Conversely, lambs carrying genotypes TA and AA of SNP rs404916326 showed negative average RIG values, suggesting a positive residual feed intake, meaning they consume more feed than expected. This negative residual weight gain indicates that these lambs are not gaining the expected weight (Berry & Crowley, 2012). Consequently, these animals take longer to reach their target weight, resulting in lower average daily gain (ADG) while consuming more feed than high-performing animals. In the case of LT breed lambs, those with the TT genotype of the rs404916326 polymorphism displayed a negative RFI indicator value, indicating a lower required dry matter intake (DMI) for growth and a positive RWG (reflecting greater ADG). Conversely, lambs with the TA and AA genotypes of rs404916326 exhibited positive RFI and negative RWG (refer to Fig. 3, B).

Significant information regarding SNP rs404916326 in sheep was lacking. While genotypic variation data existed in projects like ISGC and NextGen, no studies have previously analysed this SNP's association with specific animal traits. Therefore, our research marks the first confirmation of the association of the *MSTN* SNP rs404916326 gene with feed efficiency indicators in a sheep breed.

Prediction of feed efficiency indicator value

The General Linear Model *(GLM)* analysis was conducted in our study to evaluate the impact of statistically significant SNPs on associated indicators included in our study.

Initially, it was observed that the choice of the ram significantly influences the prediction, with a determination coefficient (R^2) value of approximately 70–75%. However, data regarding ram parameters at around 5–6 months, when lambs complete intensive fattening, are not consistently available. This lack of information challenges incorporating ram indicators into algorithms for predicting lamb outcomes. Consequently, we excluded rams as an independent variable in the prediction models, allowing us to focus on assessing the significance of SNP effects.

Our *GLM* study integrated lamb birth characteristics, including breed, birth weight, litter size, and SNP genotype, which exhibited statistical significance. Information from various breeds was leveraged to evaluate the cumulative impact of all samples within the experimental lamb cohort. The analysis aimed to identify the optimal *GLM* model with the highest R^2 value while assessing each variable's main effect and interaction. In none of the models, including the MSTN gene SNP, breed affiliation was not the primary effect indicator. Specifically, removing breed identity individually from the indicators or incorporating it in an interaction form resulted in a higher coefficient of determination.

The *GLM* analysis revealed (Table 2) that by incorporating the genotypes of SNP rs408469734 (c.*1232G>A) and the mentioned indicators, it is possible to predict or account for 15.9% of the variation in relative growth rate values and 16.7% of the variation in Kleiber ratio values across the experimental animals. While none of the included measures were statistically significant in the models, their collective contribution formed a statistically significant model.

Table 2. The General Linear Model analysis of statistically significant polymorphisms of the MSTN gene

Feed efficiency indicators	Model*	Adjusted R^2		
		with SNP	without SNP	difference
rs408469734 (All samples)				
RGR	*MSTN* SNP + BirthNr + Breed*BWbirth	0.159	0.162	-0.003
KR	*MSTN* SNP + BWbirth + Breed*BirthNr + Breed*BWbirth	0.167	n.s.	-
rs404916326 (Latvian dark-head samples)				
RFI	***MSTN* SNP** + BirthNr	0.145	n.s.	-
RIG	***MSTN* SNP**	0.095	n.s.	-

* – in statistically significant ($P < 0.05$) model in bold statistically significant ($P < 0.05$) variables; BirthNr – number of lambs in litter; BWbirth – body weight at birth. n.s. – no significant results.

Upon removal of SNP rs408469734 from the model, there was a marginal increase of 0.3% in the model's predictive capacity for RGR. This slight enhancement suggests a potentially functional role for the SNP in the 3′UTR region of the MSTN gene. It is plausible that constructing multilocus combinations directly from the 3'UTR SNPs might yield a more accurate prediction.

Excluding SNP rs408469734 from the prediction model resulted in a loss of statistical significance in the KR prediction model. This suggests that the effects of this SNP play a crucial role in explaining the observed pattern. Similar findings regarding the significance of SNPs in prediction models were reported for the Charolais sheep breed. In a mixed model analysis, the genotype of SNP rs408469734 explained 14% of genetic variation for muscle depth and 2.1% for fat depth (Hadjipavlou et al., 2008).

Statistically significant prediction models incorporating rs404916326 were established for the LT breed. These models can accurately predict or explain 14.5% of the variation in RFI and 9.5% of the variation in RIG. The model for the RFI indicator gave the best coefficient of determination when considering this SNP and the number of lambs in the litter. Conversely, only rs404916326 was included in the model for the RIG indicator. Both models demonstrate statistically significant SNP relevance. When the effect of SNP rs404916326 was removed from the model, statistical significance was no longer observed in the model for the RFI indicator.

While gene polymorphisms as molecular markers in sheep breeding are not extensively employed, some studies calculate the estimated breeding value (EBV) by integrating genomic DNA changes into statistical models like Best Linear Unbiased Prediction (BLUP). This involves SNP arrays with thousands of SNPs (Carracelas et al., 2022; Kaseja et al., 2023). However, these models cannot currently assess the individual impact of each SNP.

This study showed the importance of MSTN gene SNPs in determining feed efficiency indicators. However, like other quantitative traits, feed efficiency is influenced by multiple genetic loci (Rosa, 2015), and its molecular mechanisms are complex, often influenced by various factors (Zhang et al., 2023). Hence, further analysis of putative molecular markers is needed to improve the accuracy of predictive models.

CONCLUSIONS

The MSTN gene was fully sequenced in Latvian lambs, uncovering 22 variable loci, including the novel c.84G>A (p.Glu28=) in exon 1.

SNP rs408469734 (c.*1232G>A) showed non-variability in the Latvian Dark-Head breed.

Seven SNPs showed significant genotype distribution differences between LT and other breeds, with a higher proportion of heterozygous genotypes in the LT sheep.

The 3'UTR SNP rs408469734 was significantly associated with feed efficiency indicators (RGR and KR) in Latvian sheep, while rs404916326 in intron 1 was associated with RFI and RIG specifically in LT sheep.

General Linear Model analysis revealed that rs408469734, alongside birth weight, breed and number of lambs in the litter, accounted for 15.9% (R^2 = 0.159) of RGR and 16.7% (0.167) of KR in intensive fattening lambs of different Latvian breeds. In comparison, rs404916326 explained approximately 10% of RFI and 9.5% of RIG in LT breed lambs.

The findings of our study suggest that the SNPs rs408469734 and rs404916326 in the MSTN gene have the potential to serve as molecular markers for marker-assisted selection in Latvian sheep breeding, specifically for improving feed efficiency indicators.

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