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IGF1 and IGF2 gene polymorphisms are associated with the feed efficiency of fattened lambs in Latvian sheep breads

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Abstract. Feed efficiency is an economically important indicator in sheep farming. The most effective technology for selecting the best feed-efficient lambs for breeding is marker association selection of genetic variations in the sheep genome as potential biomarkers. In tissue growth and differentiation, insulin-like growth factors (IGFs) play a major role: IGF1 mediates the effects of growth hormone, and IGF2 is a growth regulator, regulating skeletal muscle growth. The study aims to find possible molecular markers for feed efficiency indicators in IGF1 and IGF2 genes for Latvian sheep breeds. The exonic regions of the IGF1 and IGF2 genes were sequenced for the first time in the genomic DNA of 76 controlled, intensively fattened lambs, to search for possible genetic biomarkers. Seven polymorphic loci in the IGF1 gene and sixteen in the IGF2 gene were detected. Statistically significant associations of the IGF1 SNP rs600896367 were found with residual indicators: Residual feed intake, Residual weight gain (RWG), and Residual intake and body weight gain (RIG), and with feed efficiency and feed conversion ratio in the overall group of samples. Additionally, IGF2 SNPs New_7 and rs429576107 exhibited associations with RWG and RIG specifically in the Latvian dark-head sheep group. On average, effect of the IGF1 SNP on associated feed efficiency residuals is 3.9%, with the most pronounced impact observed in RFI. In contrast, the influence of IGF2 SNPs is comparatively lower. Our results indicate that rs600896367 and New7/rs429576107 are potential molecular markers for marker-assisted selection in sheep breeding for residual feed efficiency indicators.

Key words: breeding, fattening, feed efficiency, insulin-like growth factor, Latvian sheep, polymorphisms.

INTRODUCTION

Sheep breeding plays an important role in meeting the needs of rural populations through the supply of meat (Kumar et al., 2023). According to Eurostat, the sheep population in Latvia was 112.21 thousand in 2017, but decreased to 87.32 thousand in 2022 (Eurostat, 2023a). Sheep and goat meat production in Latvia was 0.47 thousand tons in 2022, and 0.43 thousand tons in 2017 (Eurostat, 2023b). The number of sheep

has been decreasing in recent years, therefore, improving the growth, production, and reproduction performance of Latvian sheep breeds a necessary need. There are currently ten sheep breeding programs in Latvia (LAAA, 2022) which include regularly controlled trials of fattening ram offspring to evaluate them.

In sheep breeding, more precisely, in fattening sheep for meat production, a large part of the daily costs are the feed costs; one of the possibilities for reducing them is to breed animals with higher feed efficiency (Berry & Crowley, 2013; Lima et al., 2017). For farmers to have access to higher feed efficiency animals, it is necessary to carry out breeding work with a specific goal - to increase the feed efficiency of the breeds (Wakchaure et al., 2015). Feed efficiency 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), reduced the ratio of average weight gain and amount of feed required what in final reduce production costs (Berry & Crowley, 2013).

The inheritance value of feed efficiency indicators varies among different breeds and ranges from 0.10 to 0.45. However, traits inherited from parents represent genetic variations in the genome, the combinations of which determine the quality of feeding efficiency parameters (Chacko Kaitholil et al., 2024). It is considered that feeding efficiency is a combinative trait controlled by a large number of genes, as well as environmental influences (Rosa, 2015). One of the very effective technologies for selecting the best feed-efficient lambs for breeding is marker association selection (MAS) of genetic variations in the sheep genome, as potential biomarkers for defining this economically important performance trait (Wakchaure et al., 2015).

Studies suggest that molecular markers associated with feed efficiency may be genetic variations in genes involved in various metabolic processes (Chacko Kaitholil et al., 2024). In tissue growth and differentiation, insulin-like growth factors (IGFs) play a major role. Insulin-like growth factor 1 (IGF1) mediates the effects of growth hormone. Polymorphism in the IGF1 is reported to affect growth and production traits in several livestock species (Li et al., 2021). Several studies investigate the effect of polymorphisms in 5'UTR or intron regions of the IGF1 gene on growth characteristics in different sheep breeds (Li et al., 2021). In the 5'UTR region were found SNPs associated with growth traits in Makui (Hajihosseinlo et al., 2013) and Makooei sheep (Negahdary et al., 2013), but not in Hulun Buir sheep (Ding et al., 2022), Polish Pomeranian coarse-wool sheep (Proskura & Szewczuk, 2014) or Palu sheep (Malewa & Awaluddin, 2022).

Insulin-like growth factor 2 (IGF2), growth regulator A, or somatomedin A, regulates feta development and skeletal muscle growth (Wei et al., 2018; Zhao et al., 2024), and acts as a growth factor, as an autocrine signal to promote muscle cell growth and differentiation by increasing the expression of MyoD and myogenin (Wei et al., 2018). There are important studies on the association of polymorphisms of IGF*2* with feed efficiency in cows and pigs, but the limited number of studies on this topic relates to the sheep genome. There is a study on the association of five SNPs in the 5'UTR region with body weight indices in 6-12-month-old Chinese Tibetan sheep (Zhao et al., 2024).

Elucidation of the molecular mechanisms of the feed efficiency trait, in the context of the genes involved and their polymorphisms, is important for farm profitability, environmental cleanliness, and breeding assistance (Zhang et al., 2023). Therefore, the discovery of genes and genetic variations underlying feed efficiency is an important breeding strategy in sheep farming. At the same time, it is important to consider the

differences between the mechanisms or effects of genes and their variations in their influence on the studied productive tract in different breeds (Chacko Kaitholil et al., 2024). In sheep, only a few genomic variations associated with feed efficiency have been identified because it was difficult to accurately record individual feed intake in group-reared herds (Zhang et al., 2023). Our study provides an opportunity to search for genomic variations of sheep breeds bred in Latvia related to feed efficiency indicators, as the lambs have been reared under controlled conditions with intensive fattening. Feed efficiency parameters analysed in lambs can be used as an economical and rapid breeding tool.

Currently, there is a lack of studies that have examined the sequence of the IGFs genes and its potential impact on sheep breeds specifically bred in Latvia, including the national breed known as the Latvian Darkh-Head (LT; Latvijas tumšgalve). This study attempted to determine the distribution of the SNPs of the IGF1 and IGF2 genes in six most popular Latvian sheep breeds. Additionally, it sought to analyse the potential functions of these genetic variants under standardised feeding settings. The study aims to analyse for the first time the sequences of the exon region of the IGF1 and IGF2 genes in lambs of breeds bred in Latvia, as well as to find out the possible relationship of polymorphisms in both gene regions with feed efficiency indicators in intensively fattened lambs of Latvian sheep breeds. Science-based knowledge of statistically significant associations between gene polymorphisms and feed efficiency indicators can be used as an economical and rapid breeding tool to promote sheep breeding with higher feed efficiency indicators. In this way, breeders can systematically improve the sheep breed with each generation by using MAS in future.

MATERIALS AND METHODS

Animals of intensive fattening

76 lambs from six breeds: Latvian dark-head (48 lambs), Merinolandschaf (MSL; 8 lambs), Île de France (IF; 6 lambs), Charollais (CH; 3 lambs), Dorper (DOR; 5 lambs) and Texel (TE; 6 lambs), were included in controlled fattening from March to October 2022. This study was carried out in cooperation with the Latvian Sheep Breeders' Association at the ram breeding control station including in a specific group, all Latvia's most frequently grown varieties with an approximate proportional distribution. Lambs were fattened for 66.38 ± 11.05 days with an interval of 44 to 83 days.

According to the fattening control technique (LAAA, 2022), all offspring from the same ram were fattened together in an enclosure with an approximate area of 4 square metres. The pen was equipped with a detachable container for blended concentrate and a grated container for hay. Straw is used as bedding. Once each batch of lambs is finished, the cage is meticulously sanitised. The structure incorporates natural ventilation through ceiling apertures and windows equipped with insect-proof screens. The housing of animals during the research adhered to animal welfare requirements. The health of the animals during fattening is monitored by a certified veterinarian of the Latvian Association of Sheep Breeders. No health problems were reported during this fattening.

Information on the intensive fattening of lambs and the calculation of feed efficiency indicators is described in previous publications (Trapina et al., 2023a, 2023b). Feed efficiency, Feed conversion rate, Relative growth rate, Kleiber coefficient, and residuals: Residual feed intake, Residual weight gain, and Residual intake and live

weight gain were calculated using previously published formulas (Berry & Crowley, 2013; Lima et al., 2017; Trapina et al., 2023b).

DNA sequencing and SNP identification

At the end of the fattening or 24 h fasting before slaughter, blood samples from the jugular vein were taken from each lamb for genomic DNA extraction with a kit for genomic DNA extraction (Fermentas, Lithuania). DNA quality and quantity were determined using agarose gel electrophoresis and spectrophotometry.

IGF1 and IGF2 gene exons, including at least 100 bp of introns of each end, were sequenced using the Illumina MiSeq DNA (Illumina, USA) sequencing system. By using Geneious Prime® 2023.2.1 (https://www.geneious.com), clean reads were mapped to the sheep reference genome (GCF_016772045.1_ARS-UI_Ramb_v2.0, NCBI), and variable loci were detected.

Statistiscal analyses

Single-locus genotypes and allele frequencies were estimated by direct counting. Mean and standard error (*SEM*) of feed efficiency indicators of the group of the single-locus genotype were calculated from the measurement data. Appropriate statistical tests (*T-tests, ANOVA, Kruskal-Wallis, or Median test*) were used to determine the magnitude of the difference between genotype group data depending on data normality and/or homogeneity of variances in all 76 samples and 48 LT sample group. A significant result was defined as $P < 0.05$.

All haplotypes of the collection samples were created with the software DnaSP6.12.03 (www.ub.edu/dnasp/) (Rozas et al., 2017), but linkage disequilibrium (LD) analysis was performed twice: with DnaSP 6.12.03 and with Haploview 4.1. (Barrett et al., 2005)

Possible statistically associated genotype effects on feed efficiency were determined using *General Lineal Model* analysis. The *GLM* equation as individual covariates were included breed affiliation, birth weight, and IGF1 or IGF2 gene-associated SNP genotypes. In *GLM*, the possible significance of the interaction between variables was tested to include or not include them in the model. The *GLM* analysis was performed with/without SNP information including.

Analytical statistics were performed with SPSS v.25 (IBM Corp., 2017).

RESULTS AND DISCUSSION

Polymorphism detection and genetic diversity of IGF1 and IGF2 genes

The latest and most complete sequence ARS_UI_Ramb_v2.0 (breed: Rambouillet, France) of the sheep (*Ovis aries*) genome can be found in the NCBI database (https://www.ncbi.nlm.nih.gov/), which was posted there in 2022 (Davenport et al., 2022). This sequence information was used when analysing and comparing the sequences of samples of Latvian sheep breeds obtained in the study. However, since 2017, the NCBI database no longer stores information on animal (non-human) genome variations. Therefore, variation information was obtained from the Ensembl database (www.ensembl.org), which has a large amount of information but uses an older and non-updated sheep genome sequence. Accordingly, the localisation of variation in genes is determined by the ARS_UI_Ramb_v2.0 sequence.

The IGF1 gene is located on the 3rd chromosome (NC_056056) on the negative strand. The Ensembl database lists five transcripts, but the UniProt protein P10763 (www.uniprot.org) is annotated to ENSOART00020015334.2 with four exons of 154 amino acids. The database contains information on 1,084 allelic variations or polymorphisms for a given transcript. Of these polymorphisms, seven (7) SNPs were variable in Latvian sheep samples, including two SNPs in the second exon in the active protein region (Fig. 1, A).

The IGF2 gene is located on the 21st chromosome (NC_056074.1). The UniProt protein P10764 is annotated to the one (ENSOART00020011909.2) of three transcripts of the Ensembl database, a transcript containing five (5) exons. In the case of a particular transcript, the database contains information on 116 allelic variations or polymorphisms. Of these polymorphisms, nine (9) SNPs were found to be variable in Latvian sheep samples, including three SNPs localised in exons (Fig. 1, B). In addition to the IGF2 gene, seven previously unknown variable loci were found.

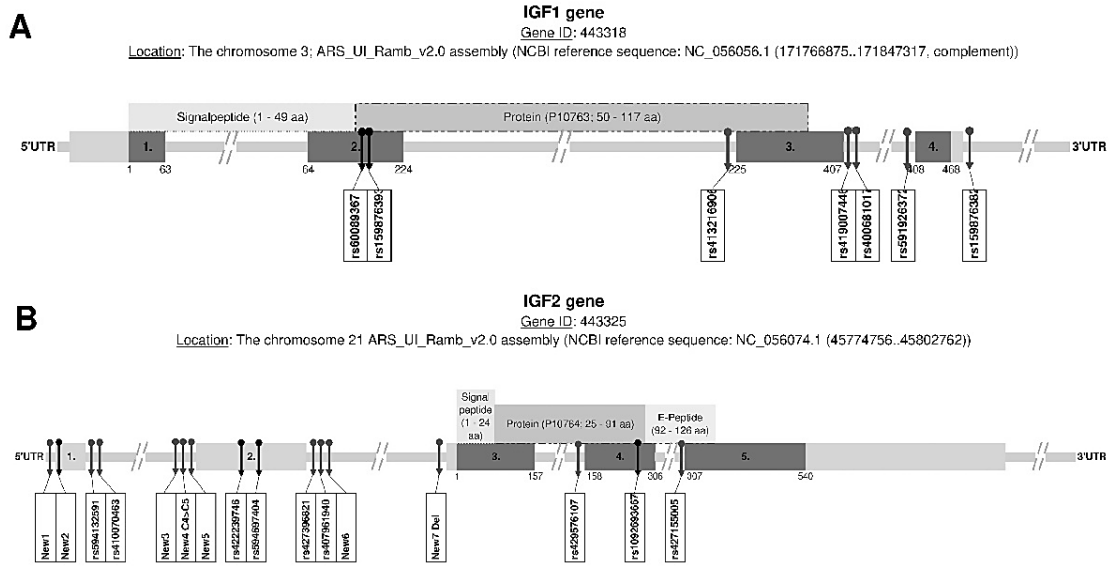

Figure 1. Gene IGF1 (A) and IGF2 (B) structure of exon regions with polymorphisms (arrows) found in Latvian sheep bread samples. The numbers below the exons reflect the numbering of nucleotides in the cDNA, starting with the first ATG number (according to NCBI database, https://www.ncbi.nlm.nih.gov/).

In the case of the IGF1 gene, a group of three polymorphisms with complete linkage disequilibrium (LD; D' = 1.00; r = 1.00) was found among the seven SNPs, while in the IGF2 gene, a total of two LD groups with two SNPs each were found (Fig. 2). Accordingly, only one SNP from each group was included in the association analysis. Complete LD group in the IGF1 gene were formed by rs413216906, rs419007446, and rs400681017, but in the case of IGF*2*, two new polymorphisms generated blocks with two known SNP: New2 with rs410070463 and New7 with rs429576107. In the IGF1 LD block, the haplotype from the common alleles of all three SNPs occurs in 86.80% of lambs' DNA samples examined, and the haplotype from the rare alleles is 13.20%, respectively.

Also, in the LD blocks of the IGF2 gene, haplotypes are formed from frequent and rare alleles. In the first block, formed from New2 and rs410070463, the haplotype of the most frequent alleles is present in 96.70% of investigated Latvian lambs, and the second block - 85.50%, respectively. SNPs inside LD blocks are examined together.

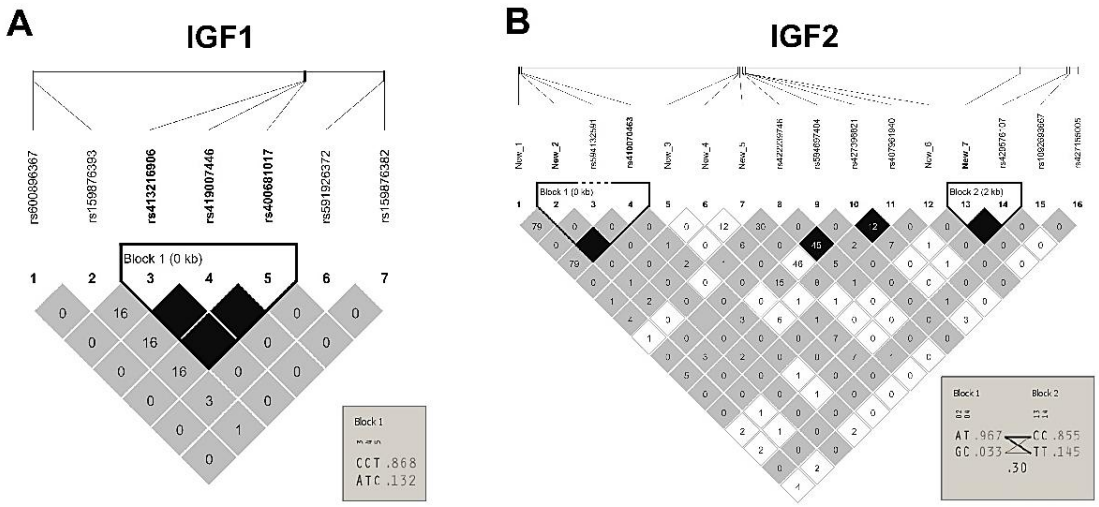

Figure 2. Linkage disequilibrium analysis of polymorphisms of (A) IGF1 and (B) IGF2 genes. The colour of the square indicates the value of D' (black - 1.00; white 0.00), and the value in the square is the value of r2 *100.

Information on LD for IGF1 gene SNPs is limited. For Hulun Buir sheep (Ding et al., 2022) were analysed three IGF1 SNPs, with no significant LD. Additionally, for Santa Inês lambs (Machado et al., 2020) two LD blocks of 11 SNPs were identified in the Intron 1 region. In Chinese Tibetan sheep, an LD including several SNPs was also identified in the 3'UTR region of the IGF2 gene (Zhao et al., 2024).

According to results of genotyping (Table 1), for all SNPs of the IGF1 gene, except rs159876393, the most common genotype in Latvian sheep samples is the homozygous genotype of the common allele.

For three SNPs: rs600896367, rs591926372, and rs159876382, no homozygous form of the rare allele was detected. Also, in cases of rs600896367 and rs159876382, all samples of Latvian dark-head (LT) were monozygotic, but in the case of rs591926372 - no LT breed samples were monozygotic.

Genotyping analysis by the International Sheep Genome Consortium International Sheep Genomics Consortium (ISGC, https://www.sheephapmap.org/) also failed to detect the homozygous form of the rare allele for rs600896367. The homozygous form of the rare allele was detected in Coopworth sheep for rs591926372 and one for the Wiltshire sheep sample at rs159876382.

A large difference was found between the IGF1 rs159876393 genotype distribution of the LT breed and other variety breeds (Table 1). In the case of the LT breed, 54.17% of the lambs were found to have the heterozygous form, which was found in only 16.67% of animals of the other Latvian breeds. In ISGC genotyping, the heterozygous genotype was detected most frequently (39.06%).

Table 1. Frequency (%) of genotypes of polymorphisms of IGF1 gene of sheep of Latvian breeds

Gene	Polymorphisms		Group of sheep (n)			Statistical
	ID number Alleles*	Genotype	All (76)	LT (48)	Other (28)	analyses (*P*)
IGF1	rs600896367	GG	97.37	100.00	92.86	0.06
	c.147G>A	GA	2.63	0.00	4.17	
	p.Ala=	AA	0.00	0.00	0.00	
	rs159876393	TT	38.16	22.92	64.29	1.43×10^{-3}
	c.153T>C	TC	44.74	54.17	16.67	
	p.Pro51=	CC	17.11	22.92	4.17	
	LD1^	CC/CC/TT	77.63	68.75	92.86	4.65×10^{-2}
		CA/CT/TC	18.42	25.00	4.17	
		AA/TT/CC	3.95	6.25	0.00	
	rs591926372	TT	89.47	83.33	100.00	2.23×10^{-2}
	c.408-54T>C	TC	10.53	16.67	0.00	
		CC	0.00	0.00	0.00	
	rs159876382	CC	96.05	100.00	89.29	2.07×10^{-2}
	c.468+72G>G	CG	3.95	0.00	6.25	
		GG	0.00	0.00	0.00	

n – Sample number in group; * – Nucleotides in position in gene cDNA: common allele (1) to Rear allele (2) as second; *P* – the statistical significance between LT (Latvina dark-head) and other group; LD1^ – SNP rs413216906 (c.225-47C>A), rs419007446 (c.407+7C>T) and rs400681017 (c.407+88T>C).

An LD block of three SNPs was identified as having combinations of genotypes included rear allele only in the LT breed, but not in lambs from other Latvian breeds. Accordingly, about six times more samples of LT heterozygotes were identified.

From these analysed SNPs of the IGF1 gene, we have found that rs600896367 has been analysed in Hulun Buir sheep (Ding et al., 2022). The results are similar to ours because, in Hulun Buir, sheep were found in only 0.02% of heterozygote samples and without rear homozygote samples. SNP rs159876393 and one of LD block - rs413216906 have been analysed in Hulun Buir sheep (Ding et al., 2022) and New Zealand Romney sheep (Li et al., 2021).

Genotype distribution related to rs413216906 heterozygotes in Hulun Buir sheep concerning LT sheep was found to be similar, however, with the higher prevalence of common allele homozygotes. The rs159876393 genotype distribution is more similar to the common cohort of all Latvian breeds (Ding et al., 2022).

The locus rs159876393 genotype distribution in the Zealand Romney sheep (Li et al., 2021) and the distribution in the LT sheep breed are similar, with a definite presence of 50% heterozygous samples in both cases. On the other hand, with almost 85% showing the common allele for rs413216906, the allele results are more similar to the total eksperimantal group of Latvian lambs.

In the case of 16 SNP polymorphisms of the IGF2 gene, the 15 most common genotypes were determined to be homozygous for common alleles (Table 2).

A deviation occurs with the SNP rs407961940, where the most frequent genotype corresponds to an allele that is not present in the reference sequence or a homozygous genotype of a mutant, usually rare, allele. Furthermore, this scenario is observed in samples from both the LT variety and for samples of other Latvian varieties. There is no information on the genotypic distribution of this SNP in the database, nor is there any scientific publication.

Table 2. Frequency (%) of genotypes of polymorphisms of IGF2 gene of sheep of Latvian breeds

Gene	Polymorphisms		Group of sheep (n)			Statistical
	ID number Alleles*	Genotype	All (76)	LT (48)	Other (28)	analyses (*P*)
IGF2	New_1	AA	94.74	91.67	100.00	0.12
	c.1-21654A>G	AG	5.26	8.33	0.00	
		GG	0.00	0.00	0.00	
	LD1^	AA/TT	93.42	91.67	96.43	0.42
		AG/TC	6.58	8.33	3.57	
		GG/CC	0.00	0.00	0.00	
	rs594132591	GG	98.68	100.00	96.43	0.19
	c.1-21538G>A	GA	1.32	0.00	3.57	
		AA	0.00	0.00	0.00	
	New_3	GG	94.74	93.75	96.43	0.51
	c.1-12231G>A	GA	2.63	4.17	0.00	
		AA	2.63	2.08	3.57	
	New_4	C4C4	63.16	66.67	57.14	0.69
	c.1-12148C4 > C5	C4C5	13.16	12.50	14.29	
		C5C5	23.68	20.83	28.57	
	New_5	CC	56.58	62.50	46.43	0.29
	c.1-12148C>T	CT	14.47	10.42	21.43	
		TT	28.95	27.08	32.14	
	rs422239746	GG	73.68	77.08	67.86	0.26
	c.1-12032G>A	GA	13.16	14.58	10.71	
		AA	13.16	8.33	21.43	
	rs594697404	CC	98.68	100.00	96.43	0.19
	c.1-11998C>T	CT	1.32	0.00	3.57	
		TT	0.00	0.00	0.00	
	rs427396821	CC	43.42	45.83	39.29	0.26
	c.1-11887C>T	CT	43.42	45.83	39.29	
		TT	13.16	8.33	21.43	
	rs407961940	AA	5.26	6.25	3.57	0.14
	c.1-11886A>G	AG	26.32	18.75	39.29	
		GG	68.42	75.00	57.14	
	New_6	CC	92.11	87.50	100.00	0.051
	c.1-11882C>T	CT	7.89	12.50	0.00	
		TT	0.00	0.00	0.00	
	LD2#	CC/CC	73.68	75.00	71.43	0.90
		C-/CT	23.68	22.92	25.00	
		--/TT	2.63	2.08	3.57	
	rs1092693667	CC	98.68	100.00	96.43	0.19
	c.258C>A	CA	1.32	0.00	3.57	
	p.Ala86=	AA	0.00	0.00	0.00	
	rs427155005	CC	77.63	72.92	85.71	0.20
	c.307-15C>T	CT	22.37	27.08	14.29	
		TT	0.00	0.00	0.00	

n – Sample number in group; * – Nucleotides in position in gene cDNA: common allele (1) to Rear allele (2) as second; *P* – the statistical significance between LT (Latvina dark-head) and other group; LD1^ – SNP New_2 (c.1-21608A>G) and rs410070463 (c.1-21466T>C); LD2#: New_7 (c.1-75DelC) and rs429576107 (c.158-29C>T).

Four of all analysed IGF2 gene SNPs (rs594132591, New_3, rs594697404 and rs1092693667) can be considered rare, as the frequency of rare genotypes for all samples together is less than 5%.

Among 16 specific genetic variations analyzed in DNA samples of Latvian sheep breeds, seven single nucleotide polymorphisms (SNPs) have not been documented or described in any previous studies. Among these SNPs, one (New_3 G>A) is a rare SNP (frequency of rare genotypes <5%), but with all three possible genotypes; other three new SNPs have high genetic variability found specifically in the LT variety samples.

When comparing the genetic diversity of Latvian sheep breed samples with the genotype distributions from the ISGC project, notable differences arise, particularly in the occurrence of homozygous genotypes for rare alleles. Three SNPs exhibited significant distinctions, with the homozygous genotype of the rare allele being absent in Latvian samples but present in the ISGC samples, with a rarity of around 1%, primarily in specific breeds. This variance may be attributed to our study's smaller sample size. In cases where the homozygous genotype of the rare allele was detected in ISGC samples, it was rare - around 1%, and only in specific breeds. However, for one SNP, the homozygous genotype of the rare allele was not detected in ISGC samples but was found in 13.16% of Latvian sheep samples, specifically in the LT, TE, MSL, and DOR breeds, excluding IF and CH breeds.

IGF2 gene polymorphisms have been infrequently examined in previous studies. Presently, information is limited to a single study on SNP analysis within the 3'UTR region of the IGF2 gene in Chinese Tibetan sheep (Zhao et al., 2024). Contrastingly, other investigations of IGF2 have predominantly focused on the protein's functional aspects (Chen et al., 2008; Wei et al., 2018).

In contrast to the SNP distribution observed in the IGF1 gene, the variation in the IGF2 gene is significantly smaller in Latvian sheep breeds, particularly between LT and other breeds.

Associations with Feed Efficiency indicators

IGF1 gene SNP rs600896367 was statistically significantly associated (Fig. 3) with Feed efficiency, Feed conversion ratio and residual indicators: Residual feed intake, Residual weight gain and Residual intake and body weight gain in the overall Latvian sheep group. Sheep with SNP rs600896367 heterozygote GA genotype showed on average a need for less than 1.60 kg dry material intake to gain 1 kg of body weight ($P = 2.97\times10^{-2}$), and the gain from 1 kg of dry material intake was greater on average for 90 grams ($P = 1.57\times10^{-3}$) than sheep with the homozygous GG genotype of the common allele (Fig. 3, A). In the case of a particular SNP, no homozygote of the rare allele was detected either among Latvian sheep breeds or in the ISGC study.

Sheep with SNP rs600896367 heterozygote GA genotype had negative RFI ($P = 4.47\times10^{-5}$) and positive RWG ($P = 2.22\times10^{-3}$) compared to sheep with the most common CC genotype (Fig. 3, A). The obtained differences also add up to a statistically significant RIG difference: -0.13 for sheep with GG genotype versus 4.80 for sheep with GA genotype. The findings suggest that sheep with the GA genotype are not only economically superior when assessed individually based on RWG and RFI but also according to the RIG index. (Fig. 3, C). The variation in RIG index among lambs with different genotypes exceeds 4.5-fold, indicating that lambs with the heterozygous GA genotype require a shorter feeding time (i.e. faster ADG) and a lower daily feed

intake compared to what is expected, considering potential differences in maintenance (i.e., BW) requirements (Berry & Crowley, 2012).

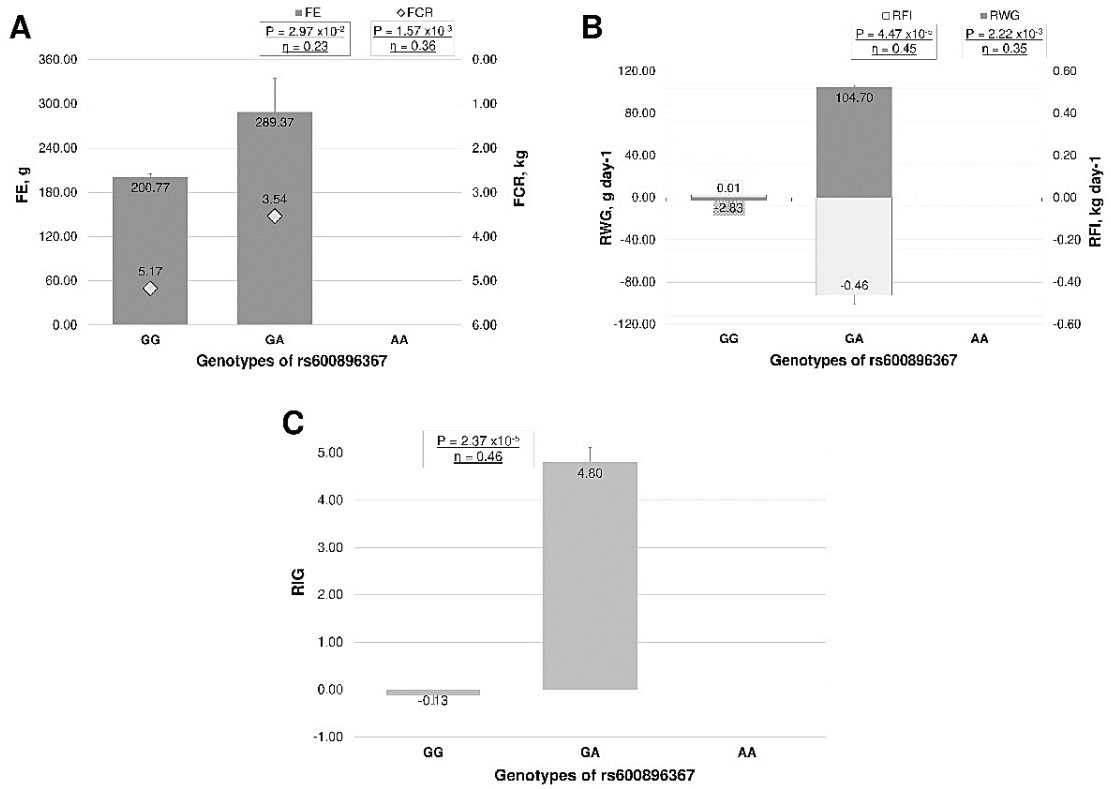

Figure 3. Gene IGF1 SNP rs600896367 association with (A) Feed efficiency (FE) and Feed conversion ratio (FCR), (B) Residual feed intake (RFI) and Residual weight gain (RWG) and (C) Residual intake and body weight gain (RIG) in the overall Latvian sheep. *P* – the statistical significance of mean with *ANOVA* or *T-test* test; η – a measure of association.

In our study, the relationship between IGF1 gene polymorphisms and feed efficiency indicators is analysed for the first time. In the case of the IGF1 gene, we have identified a potential marker that is probably unrelated to a specific sheep breed. Previous studies have presented divergent findings concerning the correlation between variations in the IGF1 gene and growth traits in sheep. Specifically, there are findings on the association of the IGF1 gene with weight gain during various periods of growth in Makooei sheep (Hajihosseinlo 2013; Negahdary et al., 2013), Munjalai sheep (Kumar et al., 2023), Barki and Farafra sheep (Darwish et al., 2023), Turkey meat sheep breeds (Kader Esen & Esen, 2023), Hulun Buir sheep (Ding et al., 2022), New Zealand Romney lambs (Li et al., 2021). Simultaneously, certain studies (Proskura & Szewczuk, 2014; Malewa & Awaluddin, 2022; Kumar et al., 2023) have reported a lack of association between various IGF1 polymorphisms and parameters that characterize sheep growth.

In current study, IGF2 gene LD2 block with SNPs New_7 and rs429576107 were found in statistically significant associations with RWG ($P = 4.46\times10^{-2}$) and RIG ($P = 4.06\times10^{-2}$) in the Latvian dark-head sheep, but the association with RFI was borderline statistically significant (Fig. 4). Association analysis indicates that 75% of all

LT lambs carry the desired more common genotype CC/CC of LD2 block, with mean RWG and RFI scores three times better than those of heterozygous or rare allele homozygous genotypes (Fig. 4, A). Lambs harboring the LD2 block genotype CC/CC exhibit a threefold increase in RIG compared to lambs with other genotype variations (Fig. 4, B). This justifies a statistical correlation not only with RWG but also with RFI.

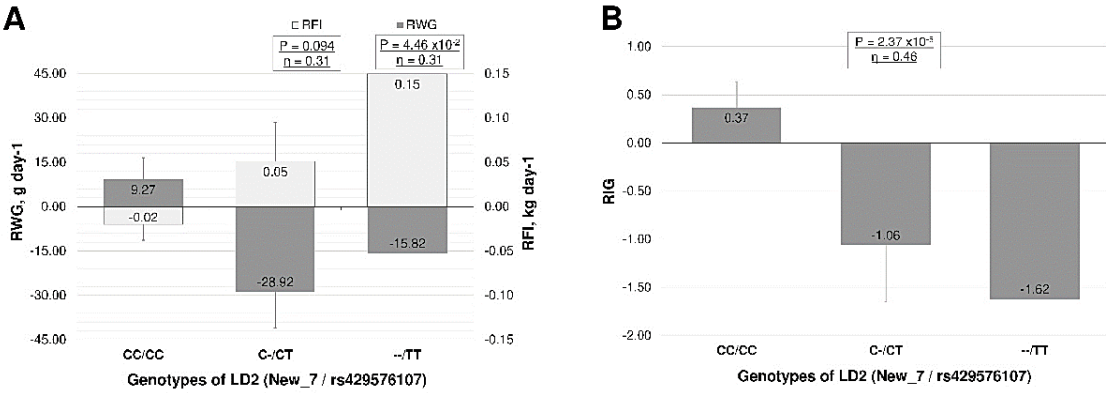

Figure4. Gene IGF2 LD block of two SNP: New_7 / rs429576107, association with (A) Residual feed intake (RFI) and Residual weight gain (RWG) and (B) Residual intake and body weight gain (RIG) in the overall Latvian sheep. *P* – the statistical significance of mean with *ANOVA* or *T-test* test; η – a measure of association.

Research on the IGF2 gene and its polymorphisms in sheep breeds is limited. Zhao et al. (2024) found three 5'UTR region SNPs associated with body weight indices in 6-12-month-old Chinese Tibetan sheep.

The current study suggests an association between IGF1 and IGF2 gene polymorphisms and feed efficiency indicators implying their potential use as genetic markers in marker-assisted selection for the breed improvement of Latvian sheep. Especially, for consumer demand for lamb meat (up to 8 months; pre-slaughter weight between 40 and 55 kg), RIG is an indicator recommended for use as a sheep classifier with the aim of higher feed efficiency with higher weight gain (Gurgeira et al., 2022). The statistically significant association of SNPs with RIG indicates that using the polymorphism as a molecular marker in the MAS process, it is possible to improve the economic performance of lambs, considering that the improvement in the efficiency for RIG indicator leads to a reduced DMI and increased ADG (do Nascimento et al., 2020).

Prediction of value of feeding efficiency indicators

The GLM analysis was used to determine SNPs' statistically significant effect size on feed efficiency indicators. The evidence strongly indicates that choosing a ram recognized by sheep breeding specialists and utilized in breeding significantly impacts (R^2 approximately 70–75%) the prediction of feed efficiency indicators. However, the productivity parameters of the ram at the age around 5–6 months (the age of lambs at the finish of fattening in our study) are not always known, which prevents the use of ram indicators in lambs' prediction algorithms. Accordingly, we did not use rams as an influencing variable in the prediction models. In this way, we were able to determine the significance level of the SNP effect.

The GLM analysis included variables detectable at lamb birth: breed affiliation, birth weight, number of lambs in the litter, and genotype of the statistically significant SNP. When analysing the effect of IGF1 gene SNPs, all samples and all six sheep breeds were used, but in the case of IGF2 gene SNPs, the analysis was performed only on LT samples. In addition, the analysis found the best GLM model with the largest R^2 value, considering the main effect and interaction of each variable.

When creating prediction algorithms using the *GLM* method, it was observed that the IGF1 gene SNP reached statistical significance in the RFI and RIG prediction models. However, it was not possible to create a statistically significant model for the FCR parameter (Table 3). For the FE and RWG parameters, a statistically significant model with the highest R^2 or coefficient of determination was developed, however, none of the included variables demonstrated statistical significance.

The most substantial distinction in the value of R^2 for prediction models, whether including or not including the IGF1 gene SNP rs600896367 was identified in RFI prediction. This presence of this difference allowed for the creation of a model with the highest R^2.

Table 3. The General Linear Model analysis of statistically significant polymorphisms of IGF1 and IGF2 genes

Feed efficiency indicators	Model*	Adjusted R^2		
		with SNP	without SNP	difference
	IGF1 gene SNPrs600896367			
FE	IGF1 SNP + BirthNr + Breed + BWbirth	0.119	0.106	0.013
FCR	No significant modele	-	-	-
RWG	IGF1 SNP + BirthNr*Breed*BWbirth	0.185	0.172	0.013
RFI	**IGF1 SNP** + BirthBW + **Breed*BirthNr**	0.502	0.425	0.077
RIG	**IGF1 SNP** + BirthNr + **Breed*BWbirth**	0.350	0.297	0.053
	IGF2 gene SNPs New_7 / rs42957610			
RWG	**IGF2 SNPs** + BirthNr	0.135	n.s.	-
RIG	**IGF2 SNPs**	0.094	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.

The *GLM* including SNP rs600896367 explained 50.2% of the determination ($R^2 = 0.50$) for the RFI parameter, while the model without it explained 42.5% ($R^2 = 0.425$), indicating a 7.7% increase in the explained proportion for FE and RWG. Inclusion of SNP rs600896367 in *GLM* changed the explained proportion by 1.3% for RFI and 5.3% for RIG. These data establish the significance of the rs600896367 SNP of the IGF1 gene in determining feed efficiency parameters.

SNPs New 7 and rs42957610 of the IGF2 gene have a significantly lower effect on RWG and RIG prediction, and the significance of lamb birth weight was not identified in GLM analysis.

Constructing a GLM, a statistically significant model was achieved for RWG, incorporating IGF2 SNPs New_7 and rs42957610 along with the number of lambs born in a litter. However, a statistically significant model for RIG was established only by including genotypes of the SNPs New_7 and rs42957610 together. For both indicators

of feed efficiency, the explanatory part with the specific GLMs did not reach greater than 15% ($R^2 < 0.15$), indicating a relatively small impact of SNPs on these parameters.

Although the utilization of gene polymorphisms as molecular markers in sheep breeding is not widespread, there are studies that incorporate genomic DNA variations into the estimation of breeding values (EBV). For instance, researchers like Carracelas et al. (2022) and Kaseja et al. (2023) have explored Best Linear Unbiased Prediction (BLUP) models, applying DNA variations obtained from SNP arrays containing thousands of SNPs. However, such models do not analyse the individual effect of each SNP.

The importance of SNPs of the IGF1 and IGF2 in determining feed efficiency has been established. Consideration must be given to the fact that indicators of feed efficiency are often characterized by multilocus traits, with complex molecular mechanisms influenced by various factors (Zhang et al., 2023). Consequently, while each SNP may have minimal significance, their common influences effect remains significant in understanding and improving feed efficiency traits. Further research is needed to verify the obtained results in an additional group of samples and to clarify the possibility of IGF1 and IGF2 gene SNPs as molecular markers and it use in MAS.

CONCLUSIONS

The first sequencing of the exon region of the IGF1 and IGF2 genes in DNA samples of lambs from Latvian sheep breeds revealed known seven polymorphisms in the IGF1 gene and 16, including seven not reported, in the IGF2 gene.

Genetic variability of sheep samples of Latvian national breed Latvian dark-head differs from other varieties for the IGF1 gene, but the distribution is similar for the *IGF2*.

IGF1 gene SNP rs600896367 (c.147G>A) is statistically significantly associated with Feed efficiency, Feed conversion ratio and residual indicators: Residual feed intake, Residual weight gain and Residual intake and body weight gain, in the overall Latvian sheep group, but IGF2 gene LD2 block with SNPs New_7 (c.1-75DelC) and rs429576107 (c.158-29C>T) is statistically significant associations with RWG and RIG in the Latvian dark-head sheep.

The General Linear Model analysis showed that the SNP rs600896367, alongside with birth body weight, lamb breed, and birth number, explains 50.2% ($R^2 = 0.50$) of the Residual Feed Intake of intensive fattening. The potential individual impact of this SNP is estimated to be approximately 7.7%. In contrast, the LD2 block of the IGF2 gene, with SNPs New_7 and rs429576107 included, contributes to around 10% of the Residual Weight Gain.

Our results indicate that rs600896367 of the IGF1 gene and New7/rs429576107 of the IGF2 gene are potential molecular markers for marker-assisted selection in sheep breeding for residual feed efficiency indicators.

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