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The genotypes of Myostatin gene are associated with weight gain indicators of intensively fattened lambs of Latvian sheep

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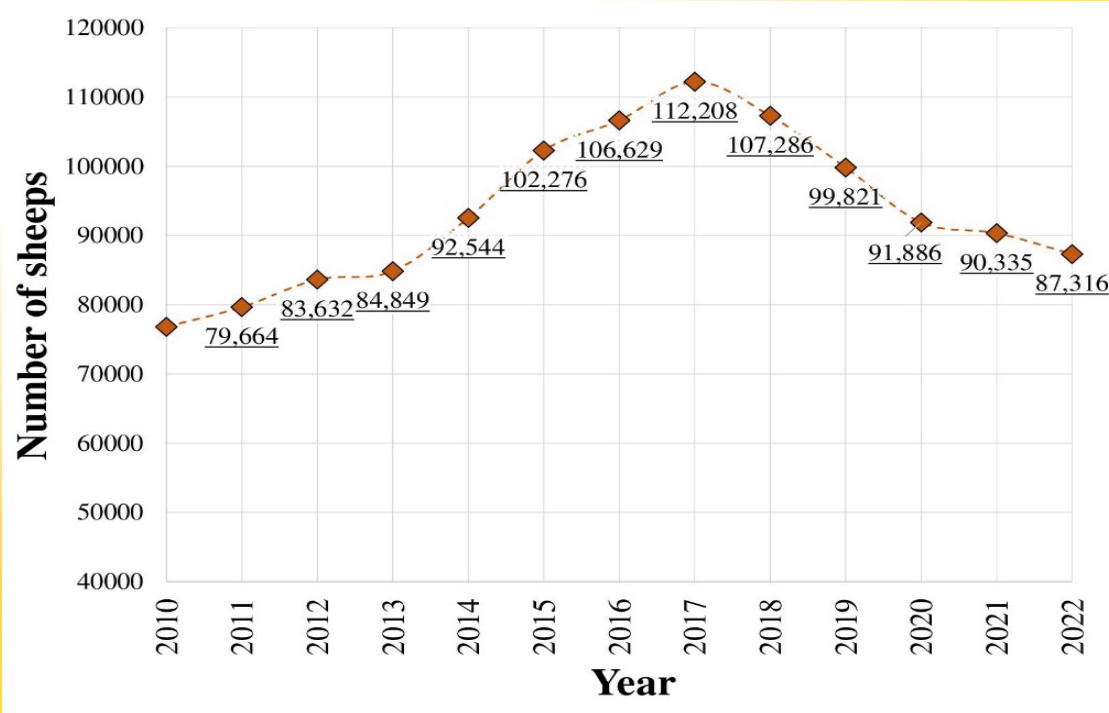
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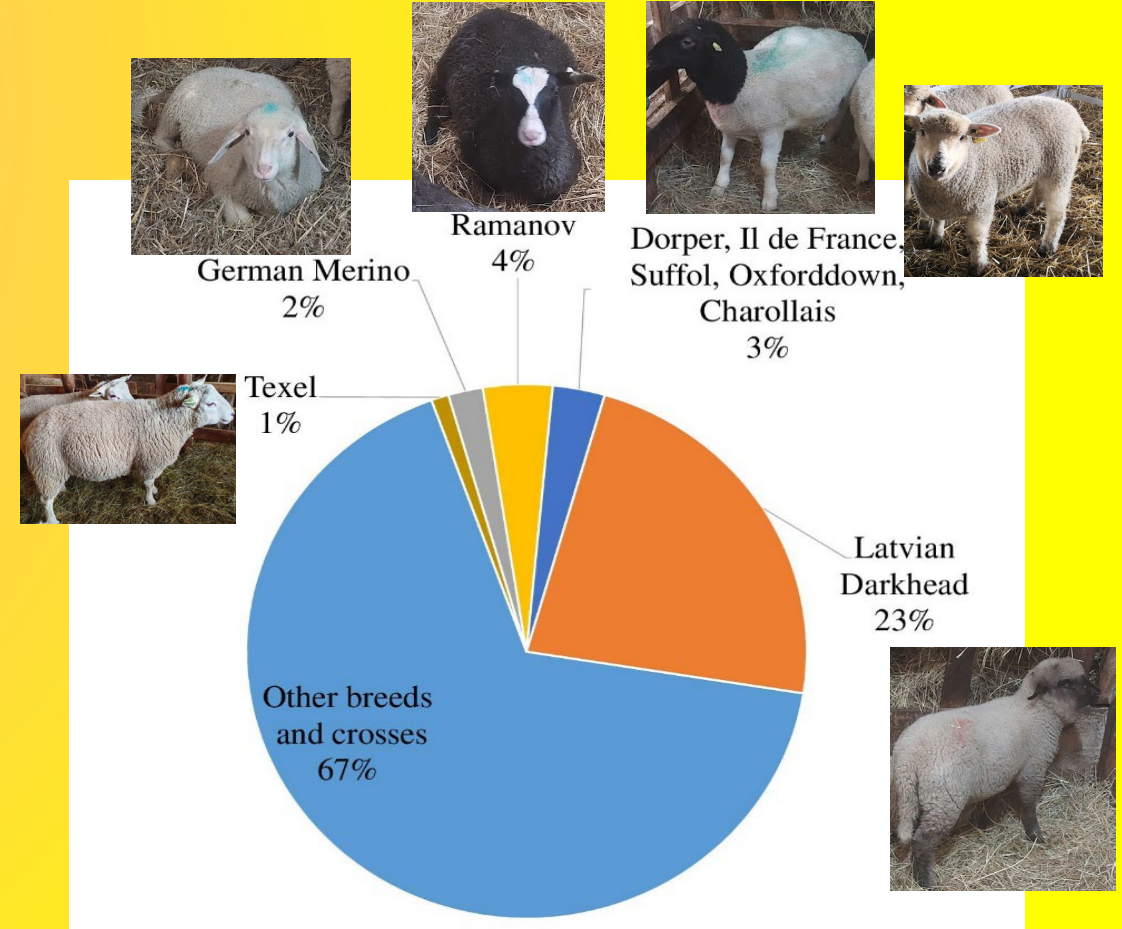
Sheep in Latvia

In December of 2022, 87.32 thousands of sheep of different breeds.



Type of Sheep's **farmers**:

1. Selection by breeder;
2. Production – meat, wool and other.



From G.Jansone presentation at «XIX Baltic Animal Breeding Conference», 2022

Weight and feed efficiency

Feed efficiency is a crucial economic trait in animal husbandry because a **feed-efficient** animal will eat less while maintaining production and health and emitting less methane. Improving feed efficiency parameters is highly important because **sire rams** with better feed efficiency **produce offspring** with higher values of this indicator.



From lamb

Feed accounts for **65–70% of the cost** in the sheep industry

Till adult sheep/ram

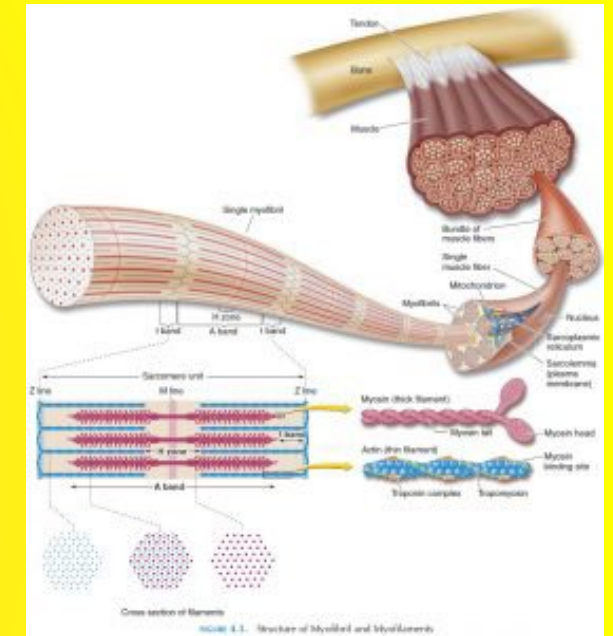
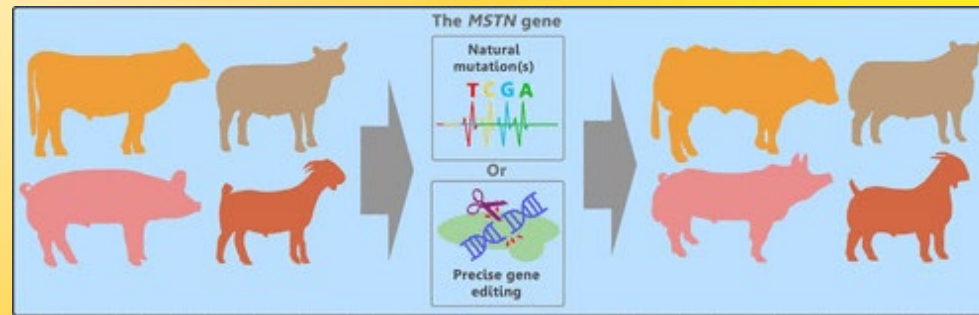
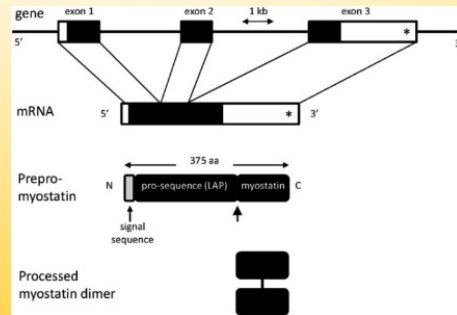


Feed efficiency is a multifactorial and complex animal trait, and differences between animals result from the interaction of many biological processes, which in turn are influenced by the physiological state.

Improving **Feed efficiency** by 5% can bring economic benefits up to four times higher than a 5% increase in average daily gain for animals.

Myostatin (MSTN)

Myostatin (MSTN) is a well-preserved member of the transforming growth factor-beta superfamily. This protein plays a crucial role in controlling the process of muscle formation and functions as an inhibitor of muscle growth and development in animals. The editing of the MSTN gene led to a considerable increase in the diameter of myofibers, as well as an improvement in the average daily gain (ADG) and body weight (BW) of sheep



Several natural mutation sites of *MSTN* have been documented in different sheep breeds. Most of these changes were observed in the non-coding areas and did not impact *MSTN* activity. The increased muscular development results from genetic variation in the MSTN gene, specifically identified as a quantitative trait nucleotide in Texel rams.



Aim of the study

To search for an association between multi-locus genotypes of the MSTN gene and weight gain indicators in Latvian sheep.

The **purpose of full project** is to determine **genetic and molecular markers** to identify individual animals in **sheep** herds with the maximum **predisposition** to **feed** digestibility or **efficiency** (FE) and weight gain, with the aim of their introduction into breeding.

Materials and methods

76 lambs of six Latvian sheep breed:

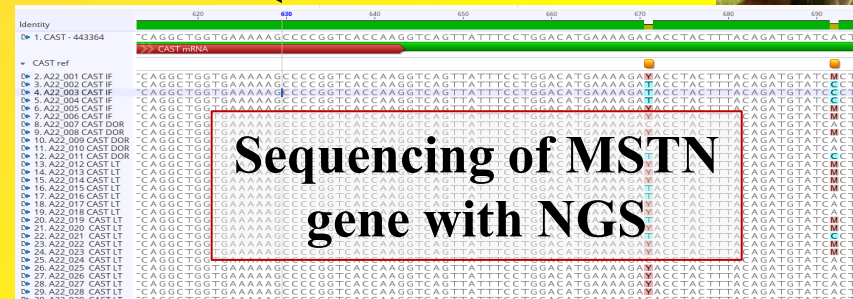
The lambs were subjected to a period of fattening lasting 66.38 ± 1.27 days, with a range of 44 to 83 days.

Intensive fattened with straw, mineral fodder and combined fodder without limitations in ram control breeding station “Klimpas” in the summer months of 2022

At average weight, 45 – 50 kg of lambs of one ram
Phenotypic parameters AND Blood sample for DNA

- Calculation of growing parametrs
- Calculation of Feed efficiency indicators from phenotypic indicators

Breed	Lambs	
	N	%
Latvian dark-head	48	63.2
Merinolandschaf	8	10.5
Île-de-France	6	7.9
Charollais	6	7.9
Dorper	5	6.6
Texel	6	7.9
Total	76	100.0

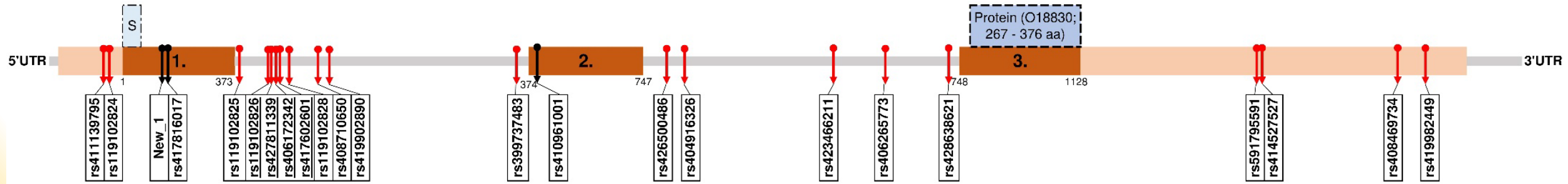


Statistical analyses for
the association between multi-loci genotypes and indicators

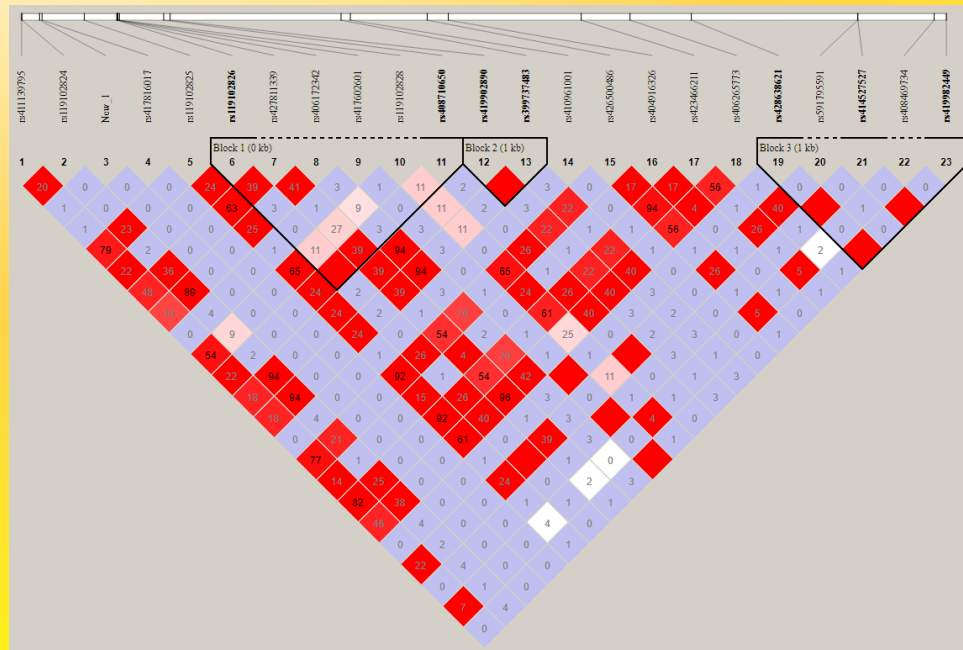
Trait	Formula ¹
Feed conversion ratio (FCR; kg DM/kg)	$\frac{DMI}{ADG}$
Relative growth rate (RGR; kg BW/day)	$100 \times \left[\frac{(\log BW_f - \log BW_i)}{(\text{Days in test})} \right]$
Kleiber's ratio (KR; g gain/kg BW ^{0.75})	$100 \times \left[\frac{ADG}{BW^{0.75}} \right]$
Residual feed intake (RFI; kg DM/day)	$DMI - DM_{fe}$
Residual weight gain (RWG; kg gain/day)	$ADG - ADG_{fe}$
Residual intake and BW gain (RIG)	$RWG + [(-1) \times RFI]$

Results

MSTN gene structure with polymorphisms in LV breed



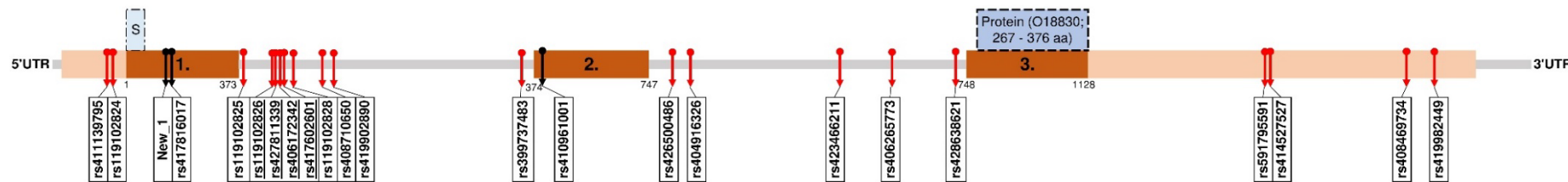
- The Ensembl database contains data on 204 polymorphic loci within the 6,757 bp-long MSTN gene region.
- In Latvian sheep DNA samples, 22 known SNPs were variable, and one previously unidentified genetic variant was detected in exon 1.



In the MSTN gene, between all polymorphisms are three complete linkage disequilibrium (LD; $D' = 1.00$; $r^2 = 1.00$) groups, including a total of nine SNPs.

Results

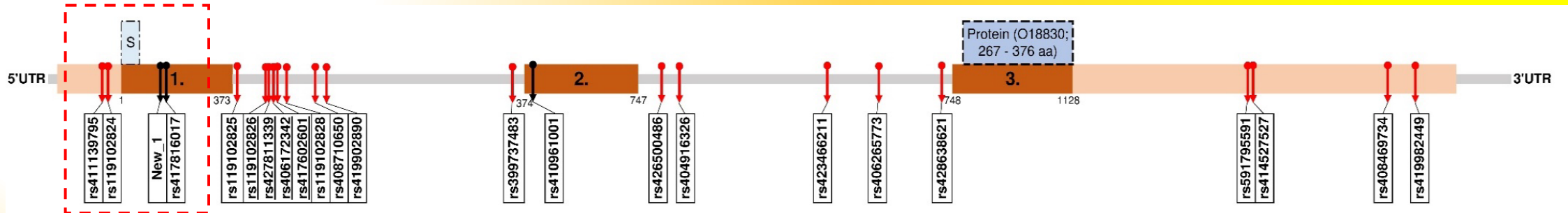
MSTN gene polymorphisms in Latvian breeds



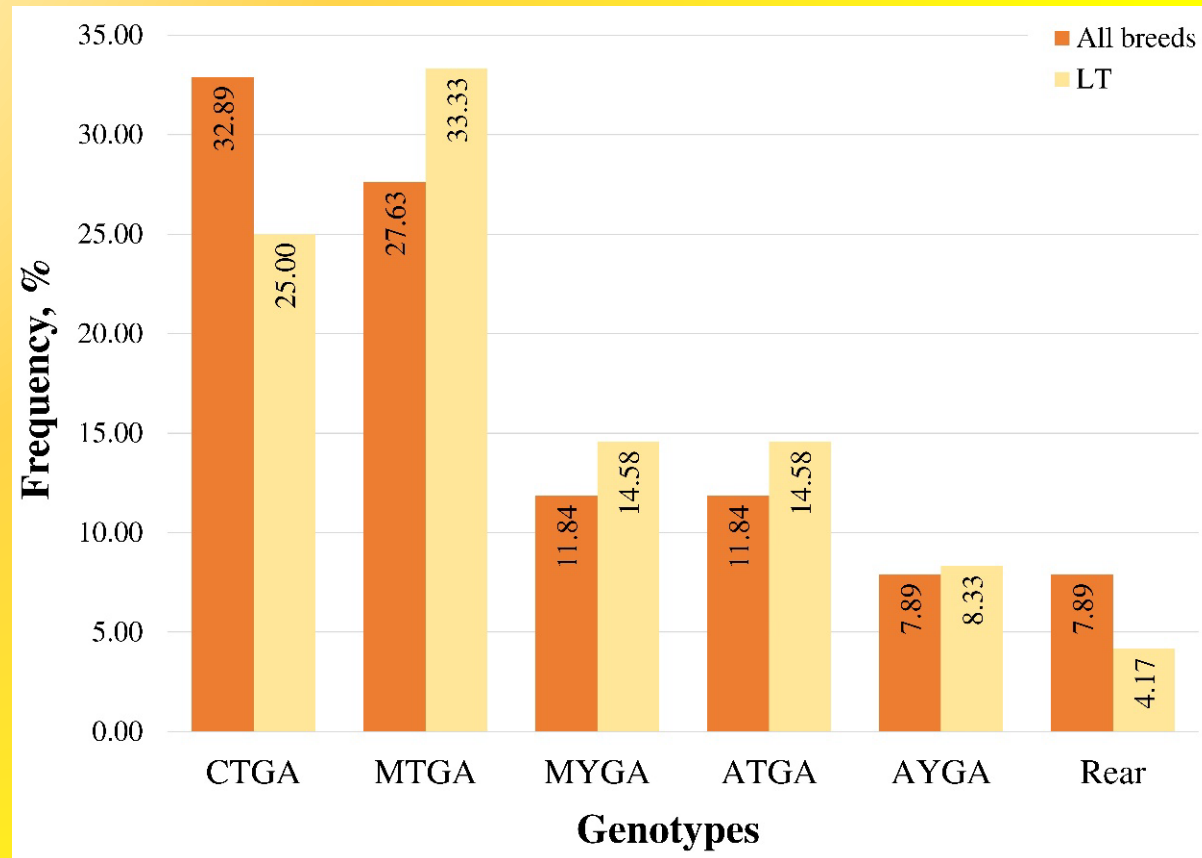
	Promoter + 1ex				1.introns									2ex	2.introns					3'UTR						
	Ms1	Ms2	Ms3	Ms4	Ms5	Ms6	Ms7	Ms8	Ms9	Ms10	Ms11	Ms12	Ms13	Ms14	Ms15	Ms16	Ms17	Ms18	Ms19	Ms20	Ms21	Ms22	Ms23			
	rs411139795	rs119102824	New_1	rs417816017	rs119102825	rs119102826	rs427811339	rs406172342	rs417602601	rs119102828	rs408710650	rs419902890	rs399737483	rs410961001	rs426500486	rs404916326	rs423466211	rs406265773	rs428638621	rs591795591	rs414527527	rs408469734	rs419982449			
	C>A	C>T	G>A	A>G	G>T	T>C	G>A	T>C	T>C	G>T	G>A	G>A	C>T	A>G	A>G	T>A	C>T	A>C	C>T	DelT (T>-)	A>C	G>A	G>A			
Latvian dark-head	X	X			X	X	X	X		X	X	X	X		X	X	X	X		X						
Île-de-France	X	X			X	X	X	X		X	X	X	X		X		X	X		X						
Texel	X				X	X	X				X				X		X	X		X		X				
Charollais	X	X			X		X	X	X	X		X	X		X		X	X	X		X	X	X			
Dorper	X	X			X		X	X		X		X	X	X	X		X	X								
Merinolandschaf	X		X	X	X	X	X				X				X		X	X		X						
Haplotypes																										
H1	C	T	G	A	G	T	G	T	t	G	g	G	c	a	A	T	C	A	c	t	a	G	g			
H2	A	T	G	A	T	C	A	T	t	T	a	G	c	a	G	T	T	C	c	c	a	G	g			
H3	A	T	G	A	T	T	G	T	t	T	g	G	c	a	G	A	T	A	c	t	a	G	g			
H4/5	A	C	G	A	T	T	A	C	t/c	T	g	A	t	a	G	T	T	C	c/t	t	c/a	G	g/a			
H6	C	T	G	A	G	T	G	T	t	G	g	G	c	a	A	T	C	A	c	t	a	A	g			
H7	A	T	G	A	T	C	A	T	t	G	a	G	c	a	G	T	T	C	c	c	a	G	g			
H8	A	C	G	A	T	T	A	C	t	G	g	A	t	a	G	T	T	C	c	t	a	G	g			
H9	A	T	G	A	G	T	G	T	t	G	g	G	c	a	A	T	C	A	c	t	a	G	g			
H10	C	T	G	G	G	T	G	T	t	G	g	G	c	a	A	T	C	A	c	t	a	G	g			
H11	C	T	A	A	G	T	G	T	t	G	g	G	c	a	A	T	C	A	c	t	a	G	g			
H12	A	C	G	A	T	T	A	C	t	G	g	A	t	g	A	T	T	C	c	t	a	G	g			
H13	A	T	G	A	T	T	G	T	t	T	g	G	c	a	G	T	T	A	c	t	a	G	g			
H14	C	T	G	A	T	T	A	C	t	G	g	G	c	a	A	T	C	C	c	t	a	A	g			
H15	C	T	G	A	T	T	A	C	t	T	g	A	t	a	G	T	C	C	c	t	a	G	g			
H16	A	T	G	A	T	T	A	T	t	T	g	G	c	a	A	T	C	A	c	t	a	G	g			
																							All		LT	
																							68	44.74	49	51.04
																							15	9.87	14	14.58
																							15	9.87	15	15.63
																							14	9.21	8	8.33
																							12	7.89	0	0.00
																							7	4.61	2	2.08
																							6	3.95	5	5.21
																							6	3.95	2	2.08
																							2	1.32	0	0.00
																							2	1.32	0	0.00
																							1	0.66	0	0.00
																							1	0.66	1	1.04
																							1	0.66	0	0.00
																							1	0.66	0	0.00
																							1	0.66	0	0.00
																							152		96	

Results

Multi loci genotype of promoter and 1st exon of MSTN gene



			Alelles (heterozygot)
Promoter + 1ex	Ms1	rs411139795	C>A (M)
	Ms2	rs119102824	C>T (Y)
	Ms3	New_1	G>A (R)
	Ms4	rs417816017	A>G (R)

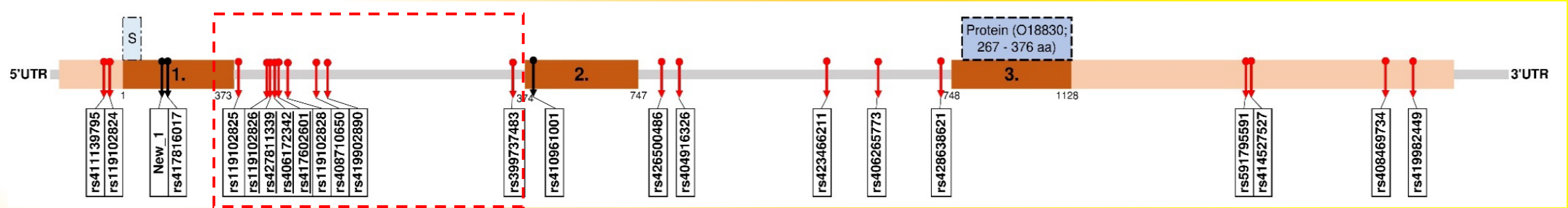


Rear genotypes:
ACGA
CTGR
CTRR
CTRA

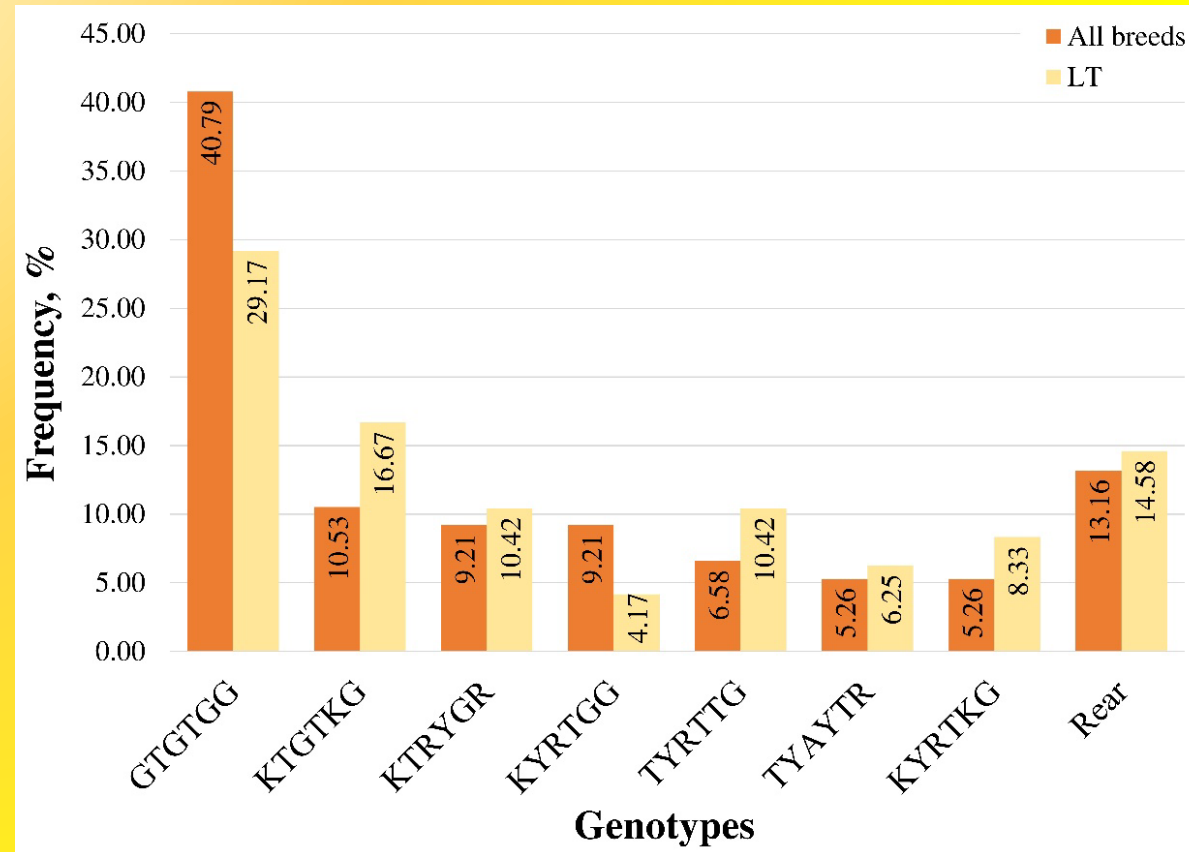
Genotypes in IUB/IUPAC nucleic acid codes format – one letter represents two alleles.

Results

Multi loci genotype of 1st intron of MSTN gene



			Alelles (heterozygot)
1st intron	Ms5	rs119102825	G>T (K)
	Ms6	rs119102826	T>C (Y)
	Ms7	rs427811339	G>A (R)
	Ms8	rs406172342	T>C (Y)
	Ms9	rs417602601	T>C (Y)
	Ms10	rs119102828	G>T (K)
	Ms11	rs408710650	G>A (R)
	Ms12	rs419902890	G>A (R)
	Ms13	rs399737483	C>T (Y)
	rare or LD group SNPs are crossed out		



Rear genotypes:

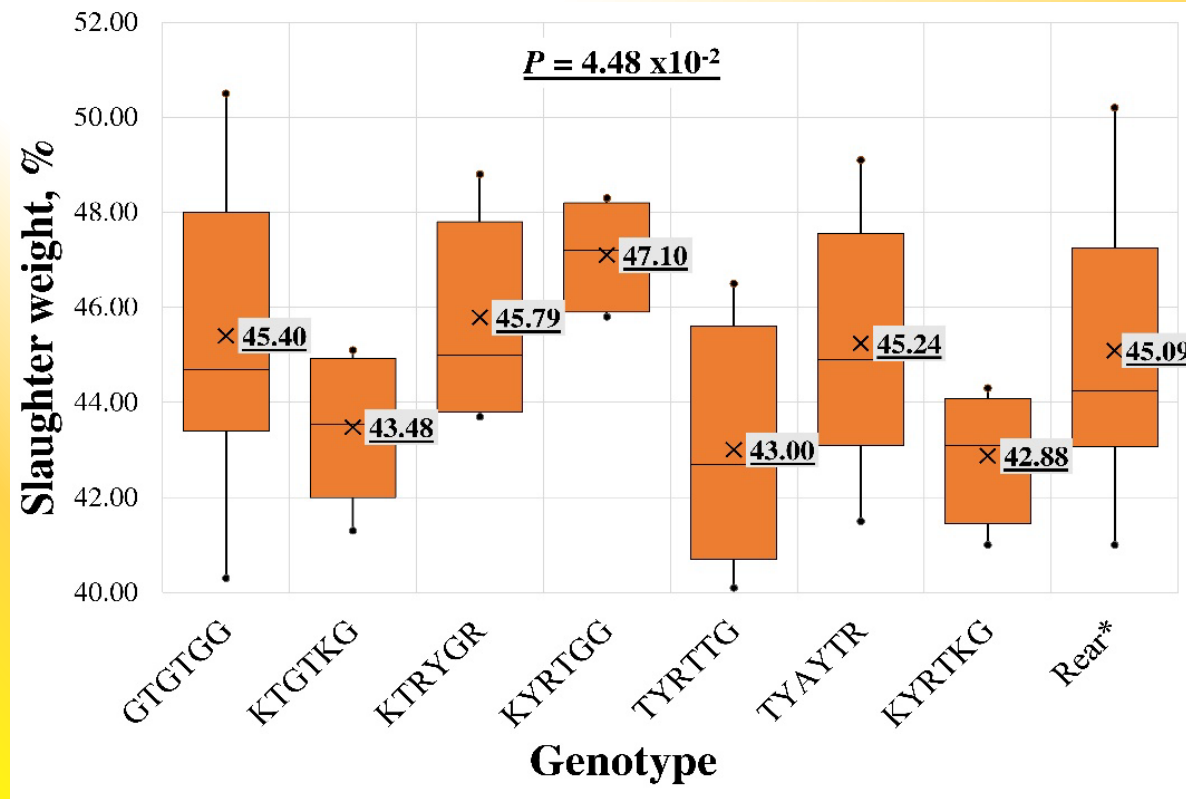
TTACTA
 TTGTTG
 KTRYKR
 TTACKR
 TTAYTR
 TTRYTR
 TCATTG

Genotypes in IUB/IUPAC nucleic acid codes format – one letter represents two alleles.

Results

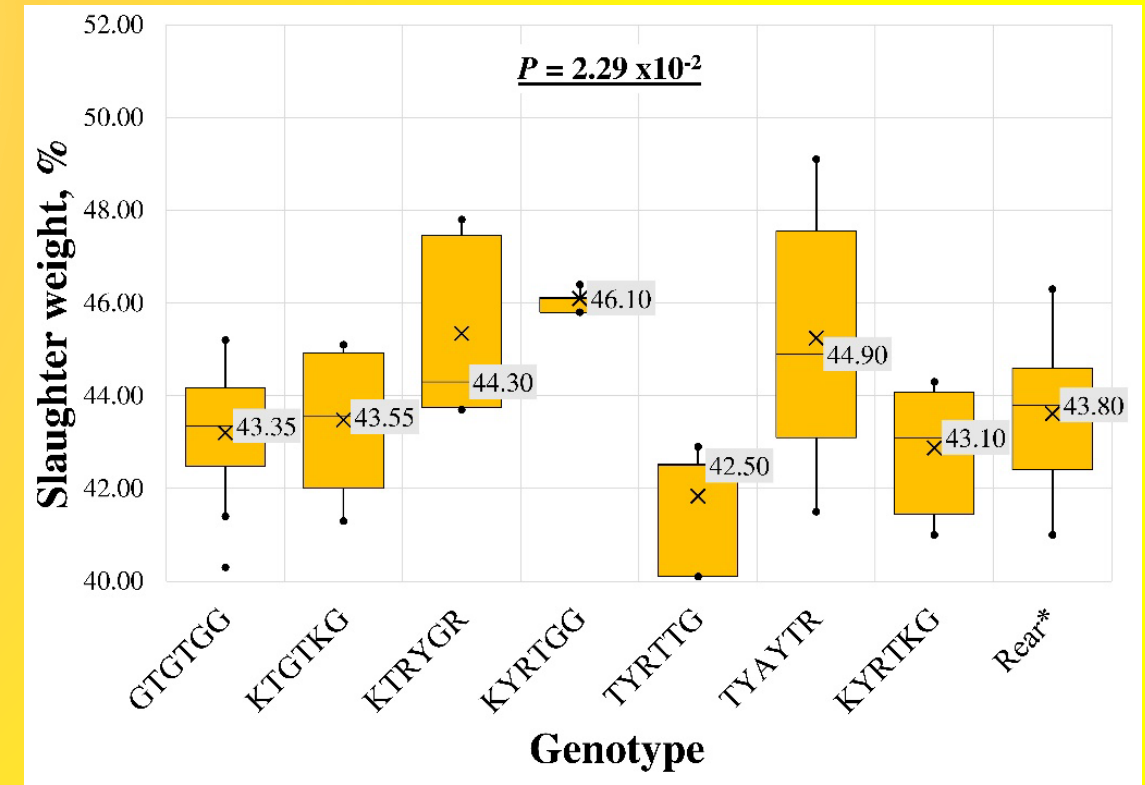
Associations of MSTN gene polymorphisms of 1st intron

All breeds



In the analysis of all samples, the highest slaughter weight, %, from live weight was $47.10 \pm 1.07\%$, was determined for samples of the KYRTGG genotype and the lowest, $42.88 \pm 1.39\%$ for the samples of the KYRTKG genotype. There is a difference between genotypes, specifically for the rs119102828 SNP.

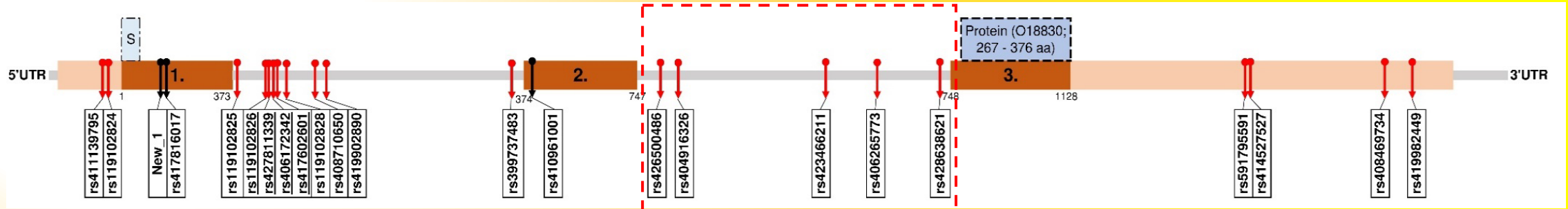
Latvian dark-head



In the LT group, the highest percentage of slaughter weight, 46.10 (IQR 0.10) %, was found in the case of the genotype KYRTGG, as well as for all samples together, while the lowest value, or 42.50 (1.51) % was found in the genotype TYAYTR.

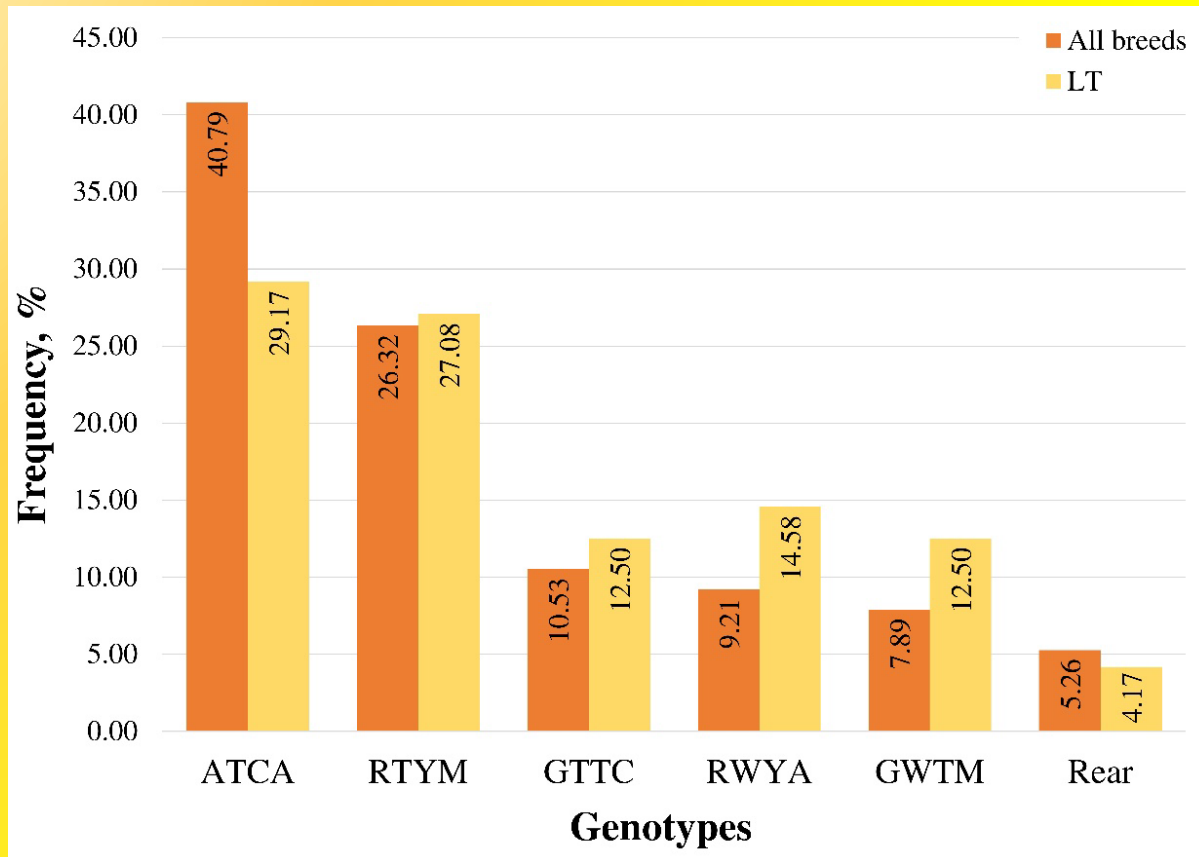
Results

Multi loci genotype of 2nd intron of MSTN gene



			Alelles (heterozygot)
2nd intron	Ms15	rs426500486	A>G (R)
	Ms16	rs404916326	T>A (W)
	Ms17	rs423466211	C>T (Y)
	Ms18	rs406265773	A>C (M)
	Ms19	rs428638621	C>T (Y)

rare or LD group SNPs are crossed out



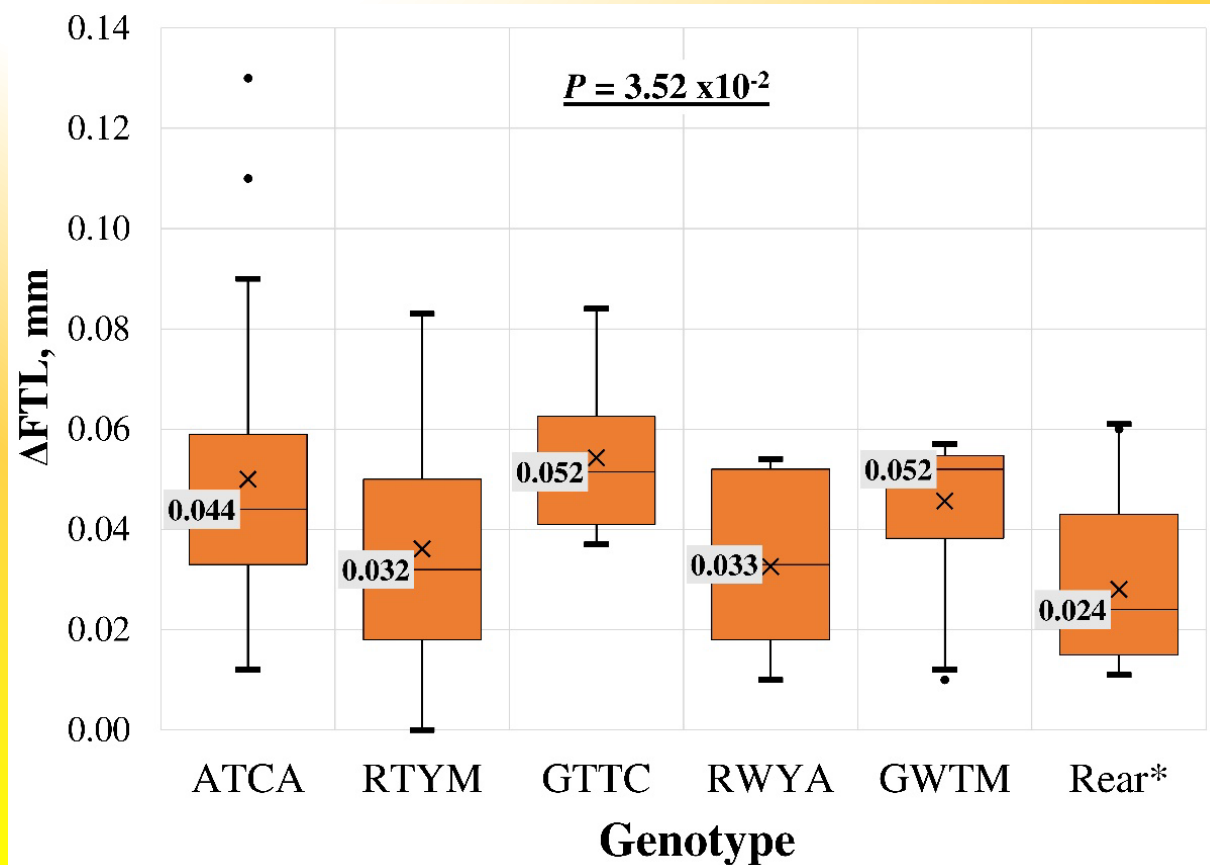
Rear genotypes:
ATYM
GATA
RTYA
RTCC

Genotypes in IUB/IUPAC nucleic acid codes format – one letter represents two alleles.

Results

Associations of MSTN gene polymorphisms of 2nd intron in Latvian breeds

Increase of adipose tissue depth against 13 ribs

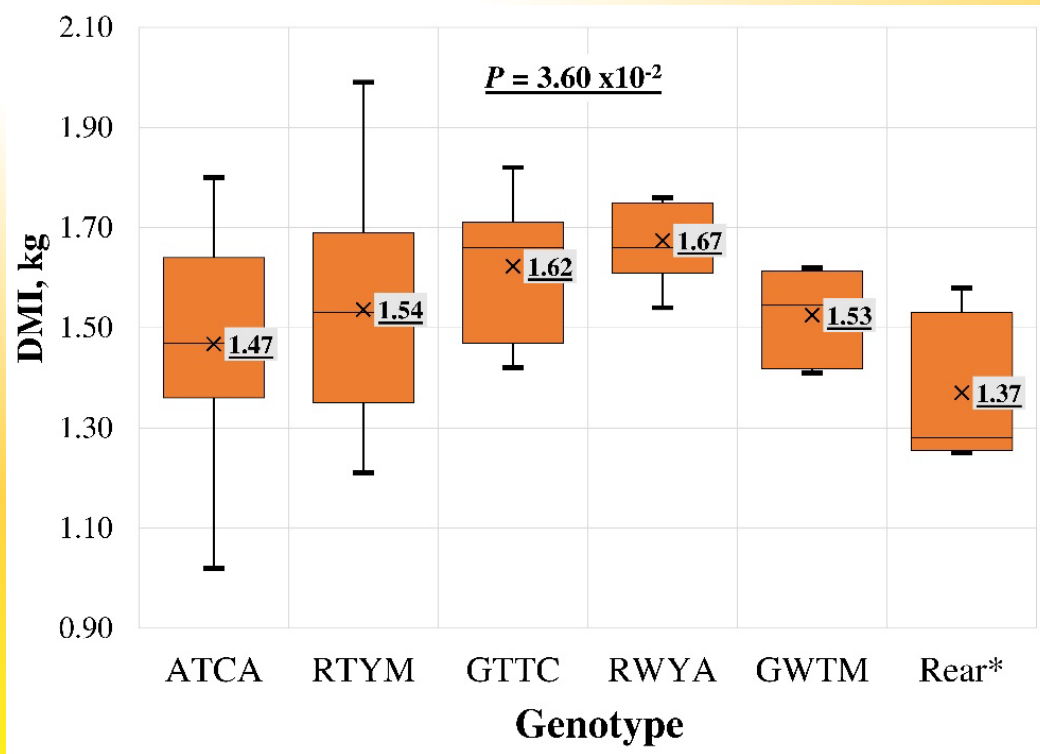


The rare genotypes show the smallest increase in adipose tissue depth, with an average increase of 0.022 mm (median). The RTYM genotype shows the second-smallest increase, 0.032 mm. The greatest increase, 0.052 mm, is found in GTTC, where the rare allele is homozygous at three loci, and GWTM.

Results

Associations of MSTN gene polymorphisms of 2nd intron in Latvian breeds

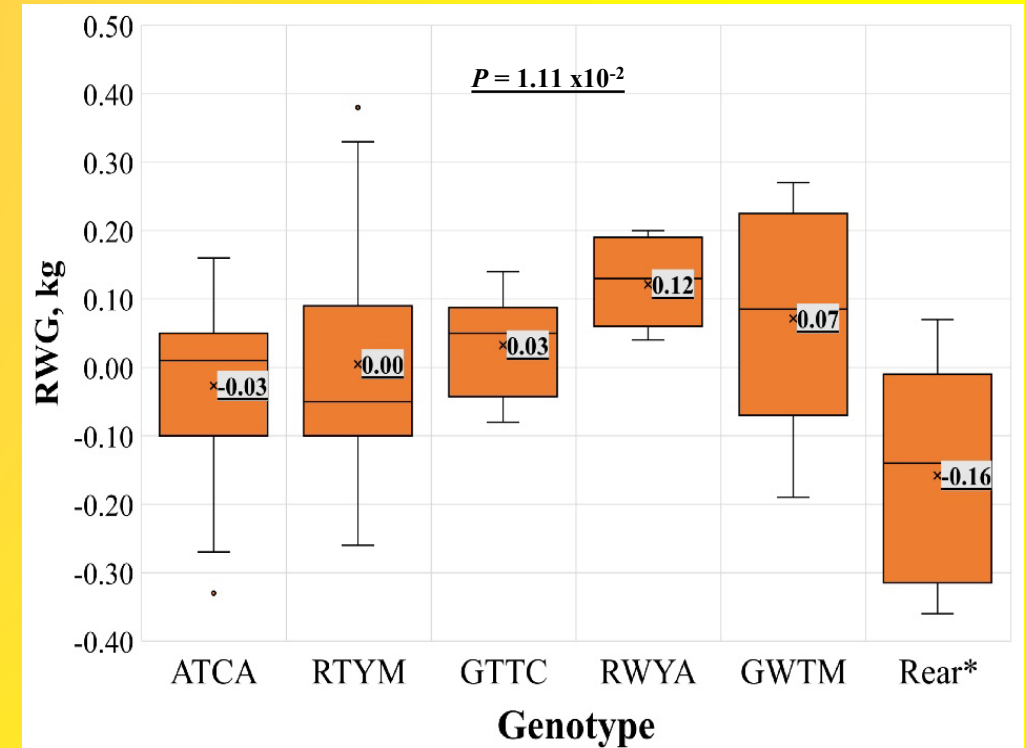
Dry matter intake



The lowest DMI, 1.40 ± 0.16 kg, was for lambs with Rare genotypes, and the second lowest, 1.47 ± 0.21 kg, was for ATCA. The highest or 1.67 ± 0.08 kg DMI is group with RWYA genotype.

On average, the difference is 0.20 kg per day, which is 1 kg in 5 days.

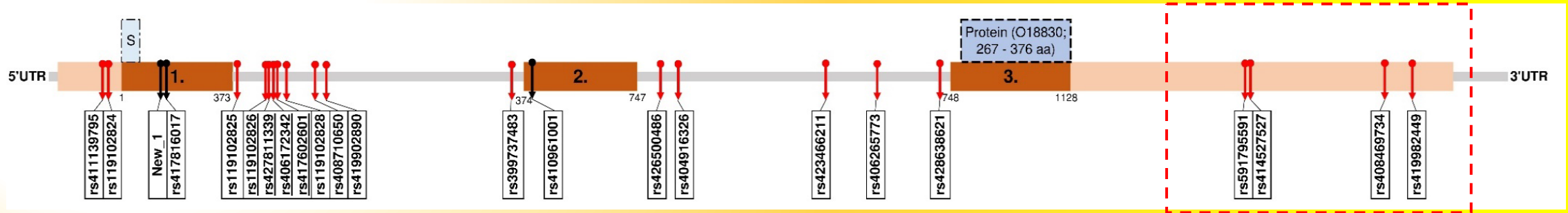
Residual weight gain



The higher RWG result is more desirable – the highest result, or 0.121 ± 0.06 kg, was found in lambs with the RWYA genotype. The lowest result of -0.108 ± 0.14 kg is for rare genotype lambs, and the second lowest is ATCA genotype lambs with RWG - 0.027 ± 0.13 kg.

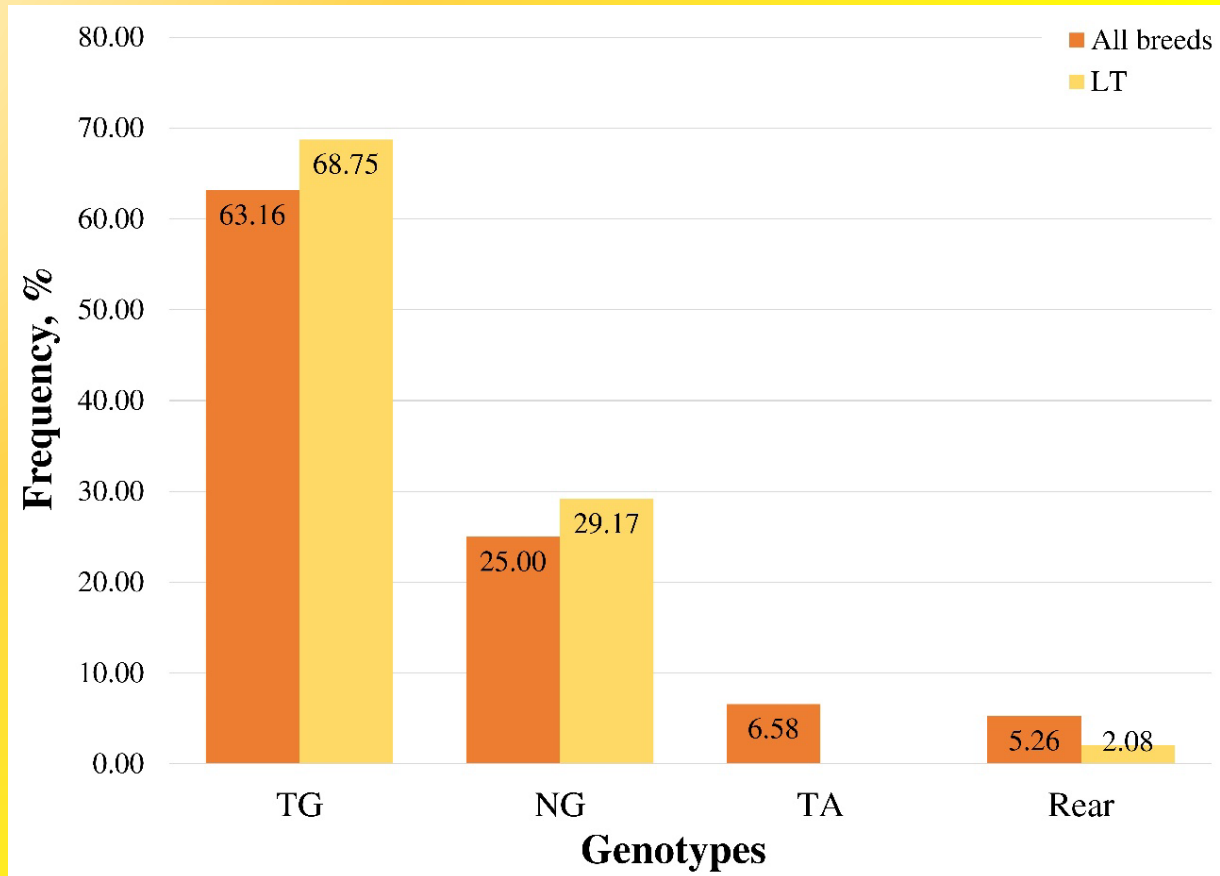
Results

Multi loci genotype of 3'UTR of MSTN gene



		Alleles (heterozygot)	
3'UTR	Ms20	rs591795591	DelT or T>- (N)
	Ms21	rs414527527	A>C (M)
	Ms22	rs408469734	G>A (R)
	Ms23	rs419982449	G>A (R)

rare or LD group SNPs are crossed out



Rear genotypes:
TR
-G
NR

Genotypes in IUB/IUPAC nucleic acid codes format – one letter represents two alleles.

Results

Associations of MSTN gene polymorphisms of 3'UTR region in LT breeds

Parameter		Genotype of 3'UTR				P
		TG	NG	TA	Rear*	
Birth weight, kg	Mean ± SD	<u>4.34 ± 0.81</u>	<u>4.32 ± 0.56</u>	5.76 ± 0.57	4.85 ± 0.13	4.76 x10 ⁻⁴
	Median (IQR)	4.30 (1.15)	4.20 (1.00)	5.60 (1.00)	4.85 (0.25)	-
DMI, kg	Mean ± SD	1.54 ± 0.19	<u>1.55 ± 0.16</u>	1.27 ± 0.18	1.37 ± 0.28	2.35 x10 ⁻³
	Median (IQR)	1.53 (0.27)	1.53 (0.26)	1.24 (0.34)	1.42 (0.53)	-
Slaughter weight, %	Mean ± SD	<u>44.64 ± 2.30</u>	<u>44.92 ± 2.66</u>	48.76 ± 2.08	46.55 ± 2.64	2.29 x10 ⁻³
	Median (IQR)	44.15 (3.38)	44.90 (4.40)	49.4 (3.40)	46.05 (4.80)	-
Muscle development	Mean ± SD	2.84 ± 0.42	2.89 ± 0.27	1.90 ± 0.55	2.38 ± 0.75	-
	Median (IQR)	<u>3.00 (0.00)</u>	<u>3.00 (0.00)</u>	2.00 (0.75)	2.50 (1.38)	1.00 x10 ⁻⁴
Degree of the adipose deposition	Mean ± SD	2.95 ± 0.38	2.97 ± 0.61	2.40 ± 0.22	2.50 ± 0.58	-
	Median (IQR)	3.00 (0.00)	3.00 (0.50)	2.50 (0.25)	2.50 (1.00)	3.70 x10 ⁻³
ADG, kg	Mean ± SD	0.33 ± 0.07	0.35 ± 0.07	0.26 ± 0.03	0.28 ± 0.10	-
	Median (IQR)	0.32 (0.11)	0.35 (0.08)	<u>0.27 (0.07)</u>	0.32 (0.17)	3.72 x10 ⁻²
Relative growth rate, kg % day ⁻¹	Mean ± SD	0.35 ± 0.10	0.37 ± 0.08	<u>0.24 ± 0.02</u>	0.26 ± 0.09	2.02 x10 ⁻²
	Median (IQR)	0.33 (0.14)	0.38 (0.13)	0.25 (0.04)	0.28 (0.16)	-
Kleiber's ratio	Mean ± SD	18.50 ± 2.88	18.83 ± 2.58	15.02 ± 1.26	15.79 ± 4.78	-
	Median (IQR)	18.67 (3.75)	19.13 (3.94)	<u>15.72 (2.39)</u>	17.05 (8.54)	2.03 x10 ⁻²
Residual weight gain, kg	Mean ± SD	0.02 ± 0.14	-0.01 ± 0.12	<u>-0.14 ± 0.20</u>	-0.06 ± 0.17	9.94 x10 ⁻³
	Median (IQR)	0.04 (0.18)	-0.03 (0.17)	-0.26 (0.37)	-0.03 (0.31)	-

**TA > Rear >
NG ~ TG**

**NG ~ TG >
rear > TA**



Conclusion

- ✓ **Higher slaughter weight**, %, from live weight is for lambs with KYRTGG genotype of 1st intron of MSTN gene.
- ✓ The results obtained for **DMI and RWG** indicate that the RWYA genotype of 2nd intron is preferable regarding feed efficiency because (1) there is more weight gain when eating less and (2) the fattening time, therefore, is reduced.
- ✓ For **birth weight, slaughter weight, muscle development and the degree of the adipose tissue layer**, the relationship of genotypes of 3'UTR can be expressed as $TA > Rear > NG \sim TG$, but for **DMI, ADG, RGR and RWG**: $NG \sim TG > rear > TA$.

Our results indicate that *MSTN* SNPs in the regions are essential for intensive lamb fattening, and their use in breeding can improve Latvian sheep breeds and economic yield.

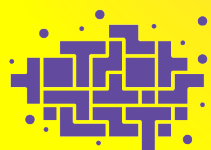
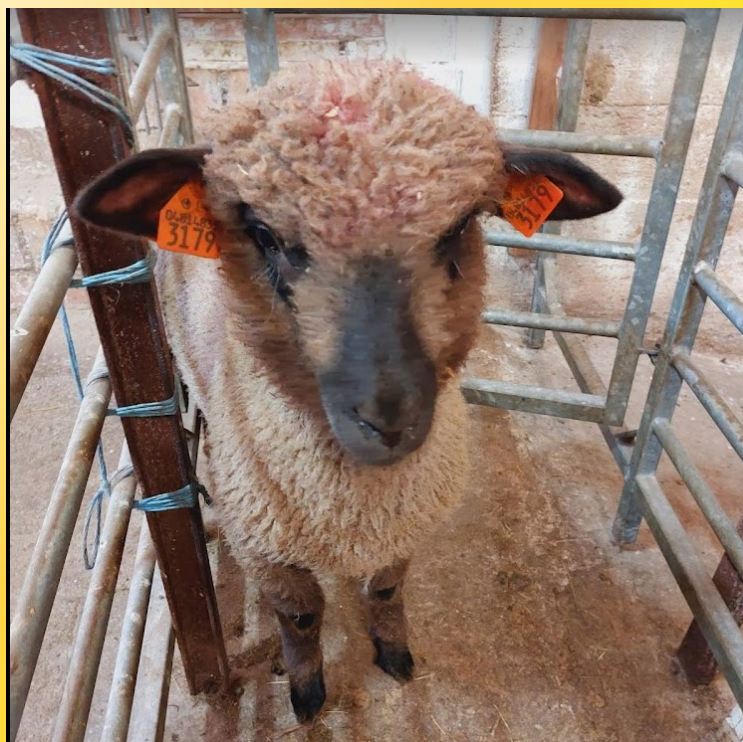


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Thank you for your attention!



FLPP
FUNDAMENTAL AND
APPLIED RESEARCH
PROJECTS

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„Development of an innovative approach to identify biological determinants involved in the between-animal variation in feed efficiency in sheep farming”