



Expression of CAST, MTOR and UPC3 genes and their association with SNPs of gene and feed efficiency in sheep intensive fattening in Latvia

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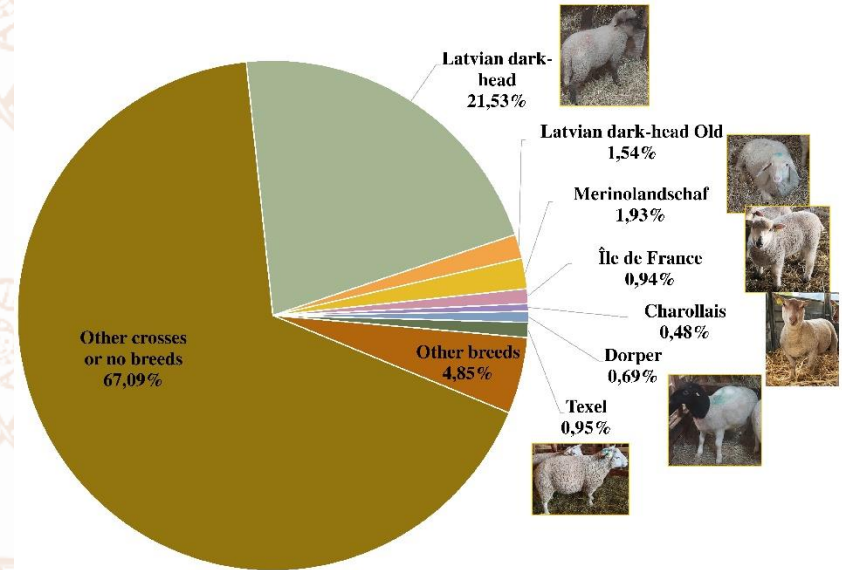
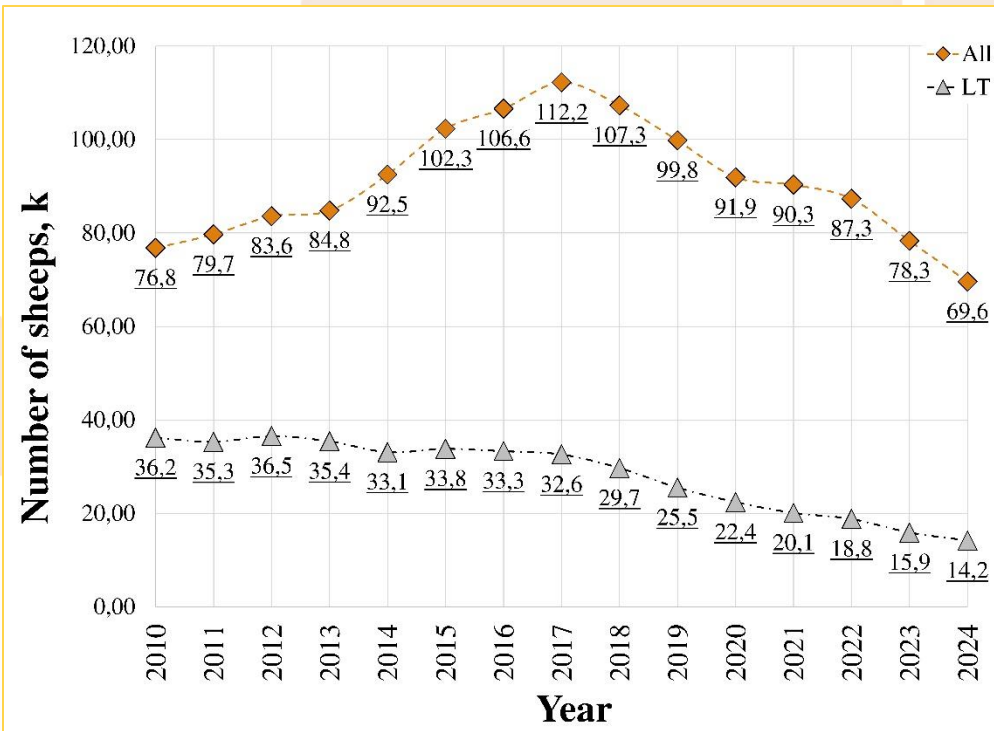
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Sheep in Latvia

In December of **2024**, **69,6 thousand** sheep of different breeds and crossbreeds



In **2001**, the **Sheep Breeders Association** officially began operating in Latvia, which is authorized to carry out selection work in sheep breeding. Currently, **10 sheep breed breeding programs** have been approved. Only breed selected in Latvia – Latvian dark-head (Latvijas tumšgalve; LT).

Feed efficiency

Feed efficiency is a vital economic characteristic in animal husbandry, as a **feed-efficient animal** consumes less while sustaining productivity and health, and **produces lower methane** emissions. **Enhancing feed efficiency metrics is crucial**, as sire rams exhibiting superior feed efficiency yield progeny with elevated levels of this indicator.



From lamb

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



Till adult sheep/ram

or



By creating a farm or breed of **highly feed-efficient sheep**, you can get up to approximately **13% more** income from meat sales compared to **low feed-efficient sheep**. Also, the average daily gain (ADG) is **130 grams higher** for the same amount of feed.

Feed efficiency is a complicated, multifactorial feature in animals, with variations arising from the interplay of numerous biological processes, which are further affected by the physiological condition.

Feed efficiency indicators

Table 1 - Definition of the indicator traits of feed efficiency

Trait	Formula ¹	Definition
Feed conversion ratio (FCR; kg DM/kg)	$\frac{DMI}{ADG}$	Amount of feed consumed divided by the weight gain. Lower values are favorable.
Relative growth rate (RGR; kg BW/day)	$100 \times \left[\frac{(\log BW_f - \log BW_i)}{(Days\ in\ test)} \right]$	Growth potential in relation to degree of maturity. Higher values are favorable.
Kleiber's ratio (KR; g gain/kg BW ^{0.75})	$100 \times \left[\frac{ADG}{BW^{0.75}} \right]$	ADG, in grams, proportional to each kilogram of metabolic weight. Higher values are favorable.
Residual feed intake (RFI; kg DM/day)	$DMI - DMi_e$	Difference between observed and estimated DMI based on ADG and BW ^{0.75} . Lower values are favorable.
Residual weight gain (RWG; kg gain/day)	$ADG - ADG_e$	Difference between observed and estimated ADG based on DMI and BW ^{0.75} . Higher values are favorable.
Residual intake and BW gain (RIG)	$RWG + [(-1) \times RFI]$	Simple index including RFI and RWG whose variance is adjusted at 1. Higher values are favorable.

Adapted from Grion et al. (2014).

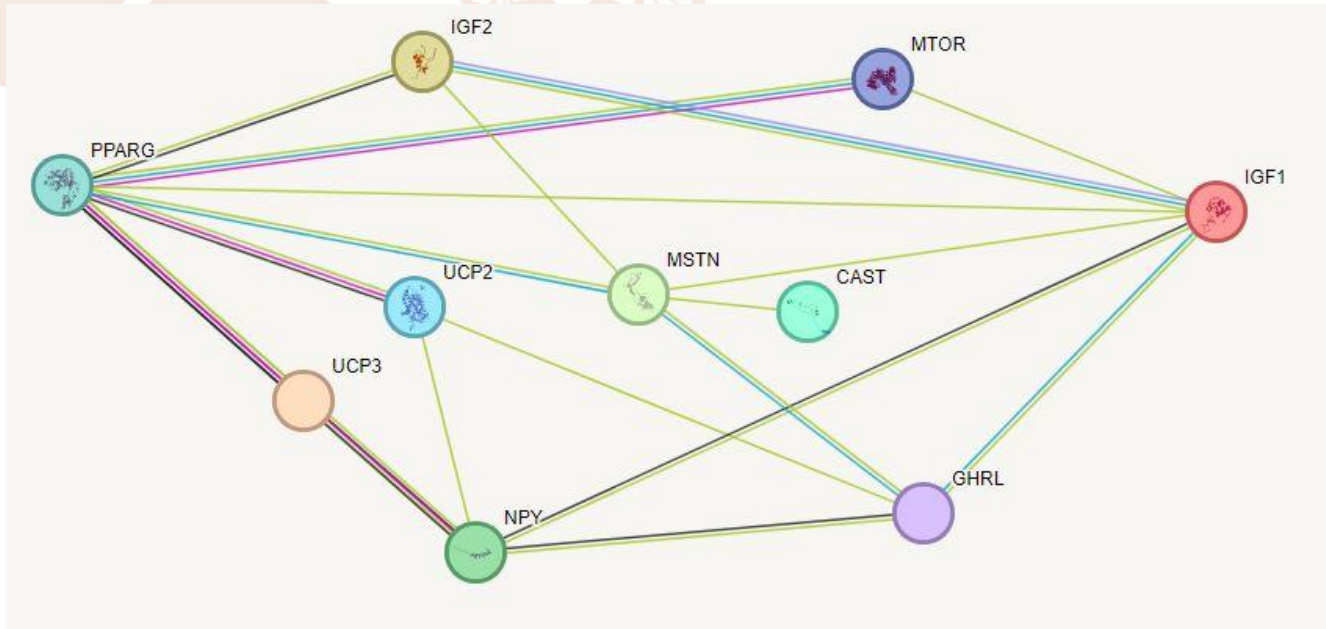
DM - dry matter; ADG - average daily weight gain; DMI - dry matter intake; BW - body weight; BW_f - final BW; BW_i - initial BW; BW^{0.75} - metabolic BW; DM_i_e - estimated DMI; ADG_e - estimated ADG.

Lima et al., 2017

Economic analysis, performance, and feed efficiency in feedlot lambs. *Revista Brasileira de Zootecnia*. 46(10). 821–829.

<https://doi.org/10.1590/S1806-92902017001000005>

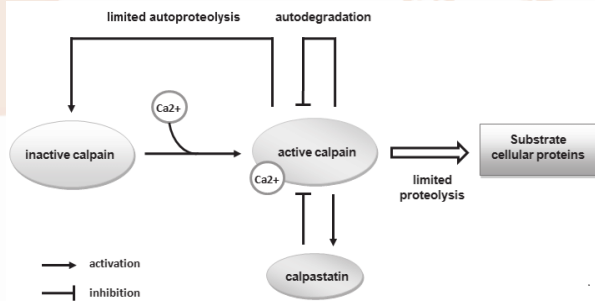
Genes analyzed in the project



Gene	In the formation/development of muscle tissue	Adipose tissue formation/development	Food intake or metabolism/energy metabolism
CAST	+		
GHRL	+	+	+
MSTN	+		
IGF1	+	+	+
IGF2	+	+	
MTOR	+		+
NPY	+	+	+
UPC2		+	+
UPC3	+	+	+
PPARG			+

Calpastatin (CAST)

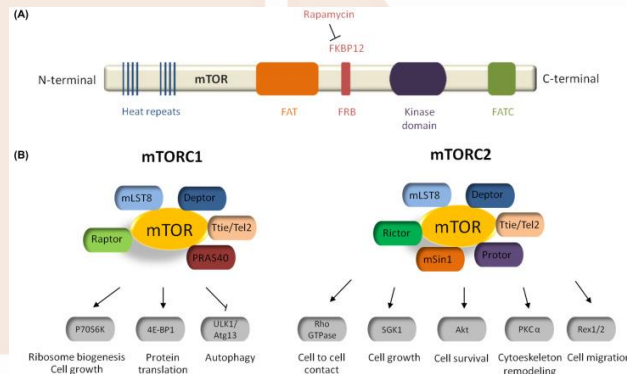
Calpastatin is a **regulatory protein**, a highly specific endogenous μ - and m- **calpains inhibitor** (calcium-dependent neutral proteinase), which doesn't affect other proteases, but is a negative regulator of calpain activity



Increasing Calpastatin activity **reduces muscle proteolysis**, which promotes animal growth and develops muscles.

However, the activity of the CAST protein, or its level, **negatively affects** post-mortem meat **tenderness**.

Mechanistic target of rapamycin kinase (MTOR)



mTOR is a **protein kinase** that **regulates cell growth**, survival, metabolism, and immunity, playing a crucial role in modulating cell growth and metabolism in **response to growth factors and nutritional status**, thereby **influencing** the biological and physiological **responses** of cells and organs.

mTOR forms two distinct complexes, called mTOR complex 1 (**mTORC1**) and 2 (**mTORC2**), by binding to several proteins.

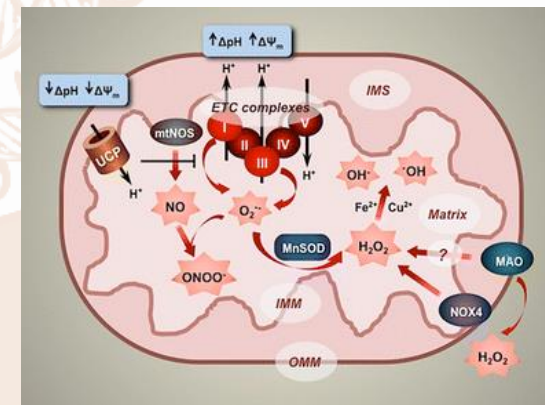
Uncoupling protein 3 (UPC3)

Uncoupling proteins (UCPs) are **mitochondrial transporters** that uncouple oxidative phosphorylation by net discharge of the proton gradient.

UCP3 equivalents are inversely related to fat oxidative capacity.

Its levels are reduced by endurance training.

UCP3 can also be upregulated by a high-fat diet and increased levels of circulating free fatty acids.



Aim of the study

Analyse the expression levels of CAST, MTOR, and UPC3 genes and their relationship with genes' SNPs and intensive fattened lambs' Feed Efficiency values in sheep raised in Latvia

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 and 84 lambs of six sheep breeds raised in Latvia:

Breed	2022 year (A22) Age of lambs ~150 day		2023 year (A23) Age of lambs ~81 day	
	N	%	N	%
Latvian dark-head	48	63,16	52	61.90
Merinolandschaf	8	10,53	4	4.76
Île de France	6	7,89	12	14.29
Charollais	3	3,94	4	4.76
Dorper	5	6,58	6	7.14
Texel	6	7,89	6	7.14

Intensive fattened with straw, mineral fodder and combined fodder without limitations in ram control breeding station “Klimpas” in the summer months of 2022/2023

At average weight, 45 – 50 kg (around 150 days of age) of lambs of one ram **growing** parameters were measured for the calculation of **Feed efficiency indicators**

Term	Formula ^a
Feed conversion ratio (FCR, kg DM/kg)	$\frac{DMI}{ADG}$
Relative growth rate (RGR, kg BW/day)	$100 \times \left[\frac{(\log BW_f - \log BW_i)}{(\text{Days to end})} \right]$
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)	$DMI - DMI_e$
Residual weight gain (RWG, kg gain/day)	$ADG - ADG_e$
Residual intake and BW gain (RIG)	$RWG + [(-1) \times RFI]$

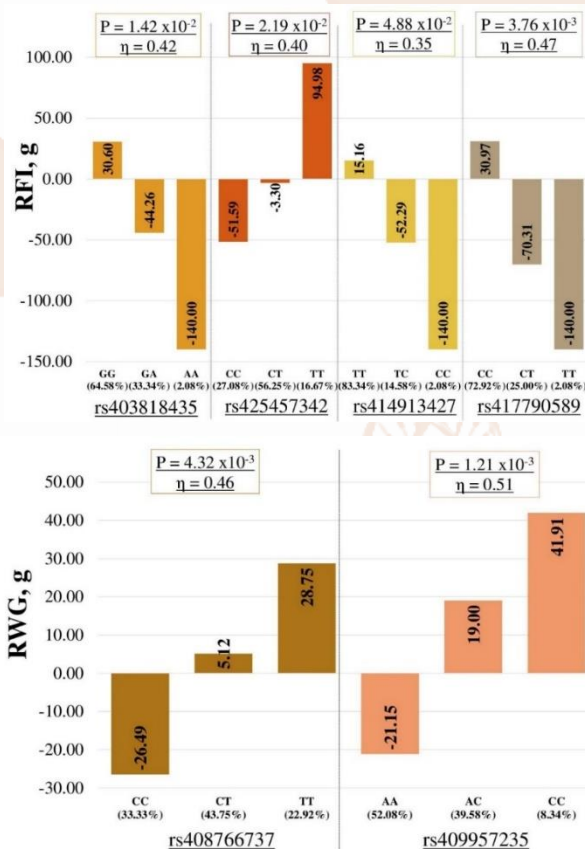
- Sequencing of the genes with **NGS** in the A22 group.
- Genotyping SNP of the three genes with **Restriction fragment length polymorphism**.
- Genes expression (blood taken before and after intensive fattening) measuring with **qPCR** (GAPDH gene as reference)

Statistical analyses with SPSS 25.0 for the **association between polymorphisms/expression of genes and Feed efficiency indicators**

Results: polymorphisms of

CAST gene

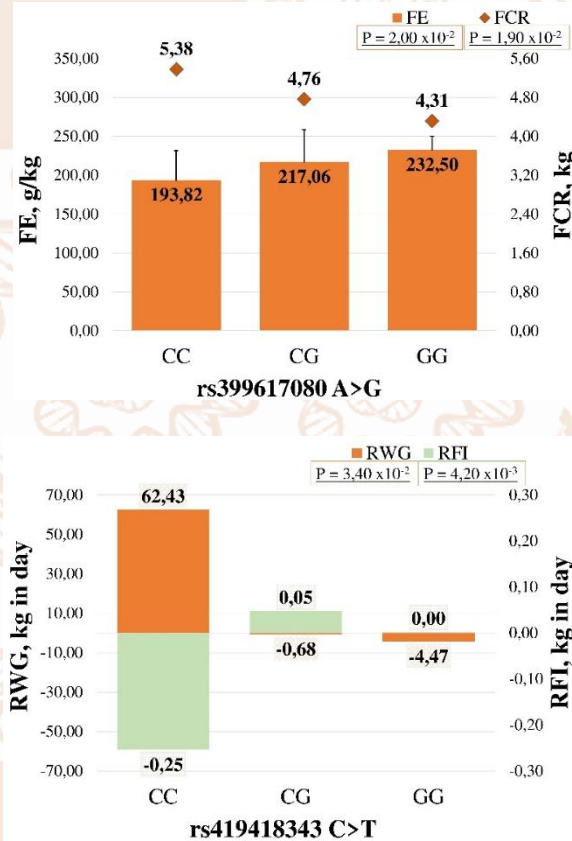
In the CAST gene, were found **83 SNPs**, from which **12** were associated with one of the **FE** indicators.



7 SNPs were chosen

MTOR gene

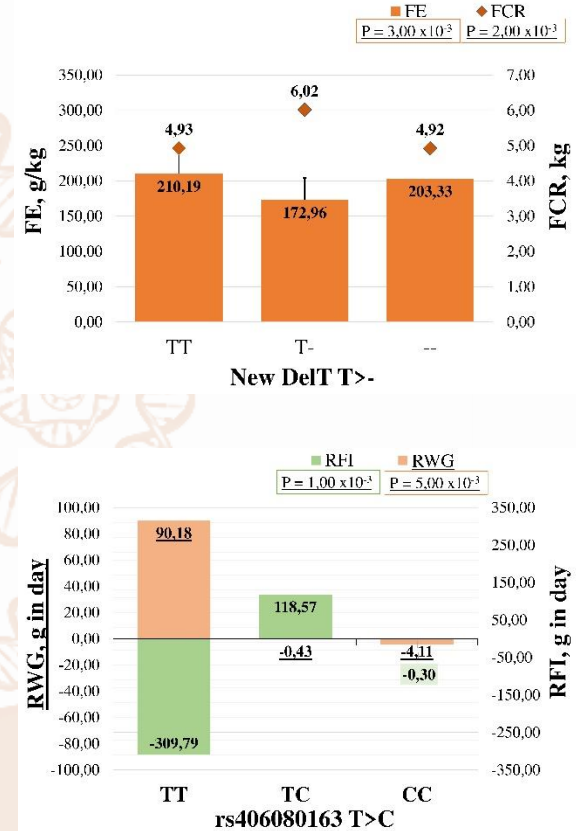
In the MTOR gene, 77 SNPs were identified, of which **20** were associated with one of the **FE** indicators.



14 SNPs were chosen

UPC3 gene

In the UPC3 gene, 137 SNPs were identified, of which **14** were associated with one of the **FE** indicators.

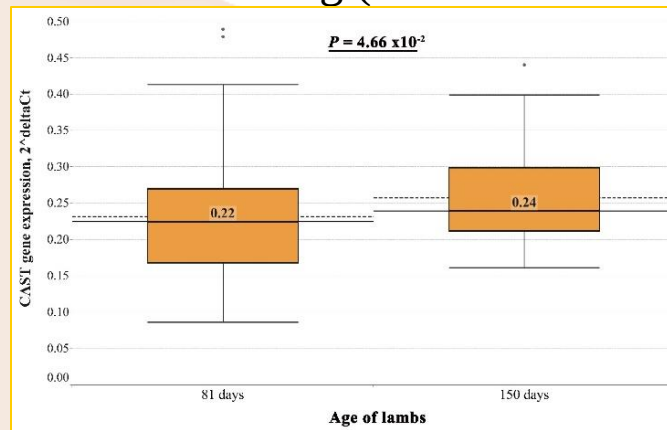


13 SNPs were chosen

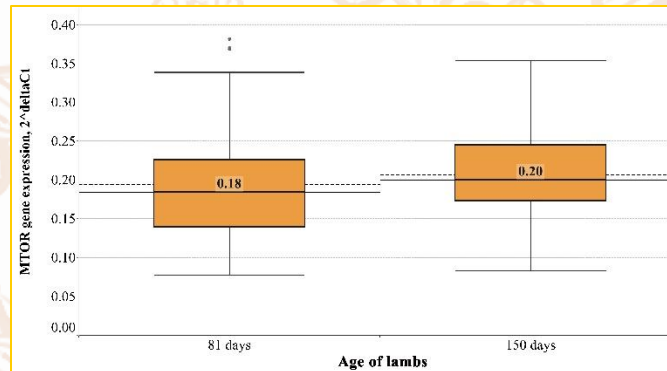
Results: expression

When comparing expression between two groups of lambs with different ages (average 81 and 150 days) or the time of intensive feeding (before and after), it was found that:

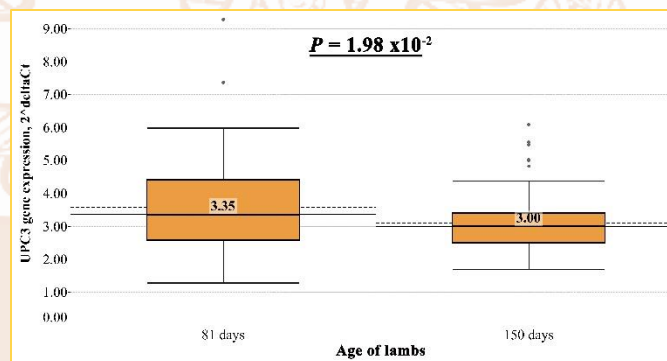
CAST gene expression was **higher after feeding**



MTOR gene expression was **higher after feeding**

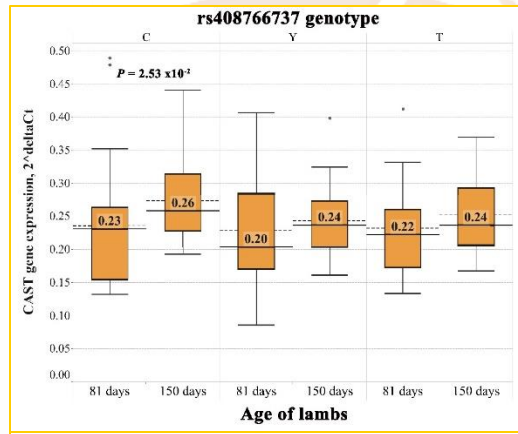


UPC3 gene expression was **higher before feeding**

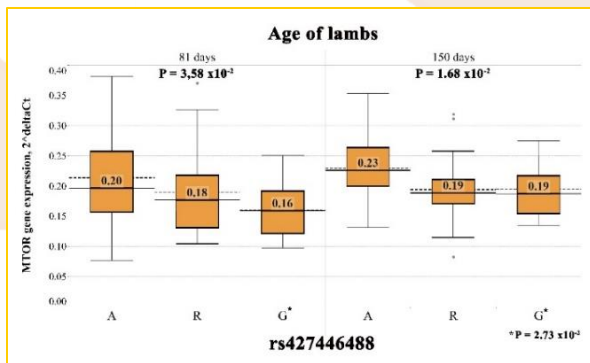
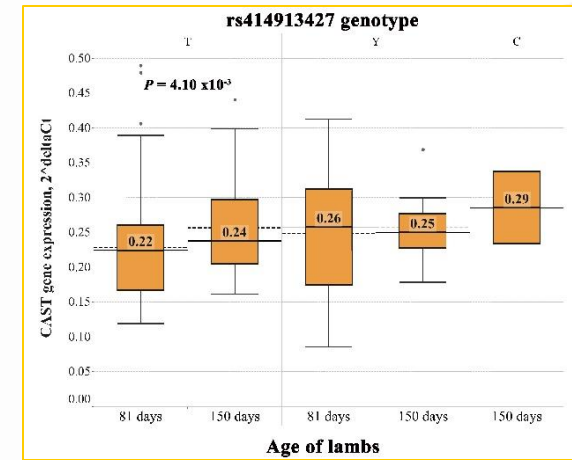


Confirming the need for CAST and MTOR protein, and its gene expression increases as body or muscle mass increases, but reduces the need for UPC3 in this process due to a reduction in fat proportion.

Results: expression's association with FE-related variation

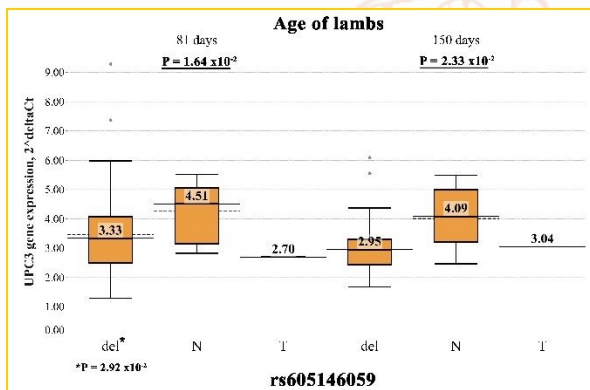
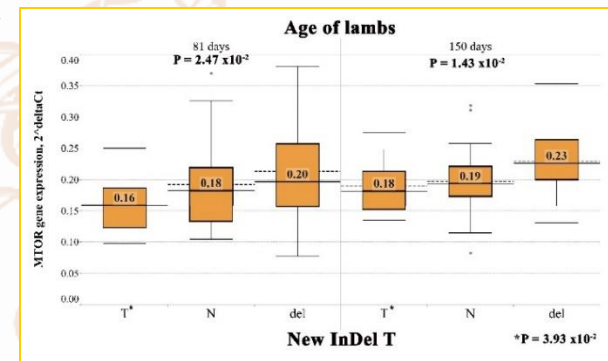


In the case of the **CAST** gene, 6 SNP genotypes (common homozygotes) were found to potentially affect the **increase** in the CAST gene during the intensive fattening period.



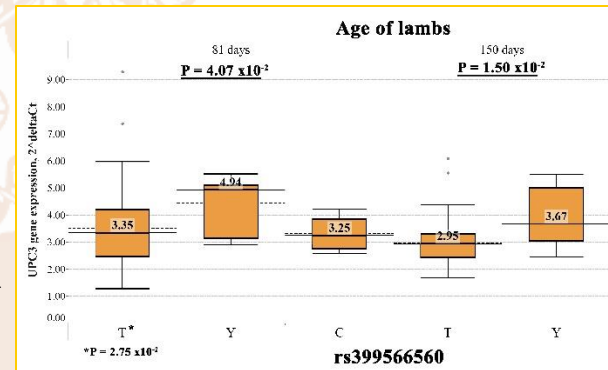
MTOR gene expression is statistically different between lambs with varying genotypes for 7 SNPs.

Genotypes of eight SNPs were identified as potentially affecting the **increase** in gene expression during the intensive fattening period.



UPC3 gene expression is statistically different between lambs with varying genotypes for 5 SNPs in both age or at 81 day of age.

Genotypes of 13 SNPs were identified as potentially affecting the **decrease** in gene expression during the intensive fattening period.

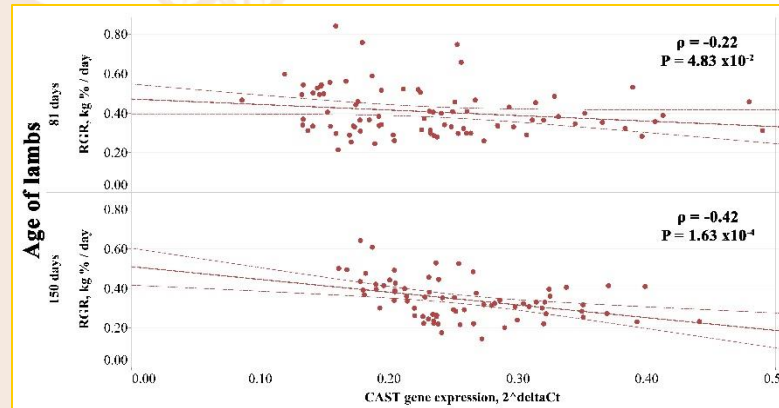


Genotypes in IUB/IUPAC nucleic acid codes format – one letter represents two alleles.

Results

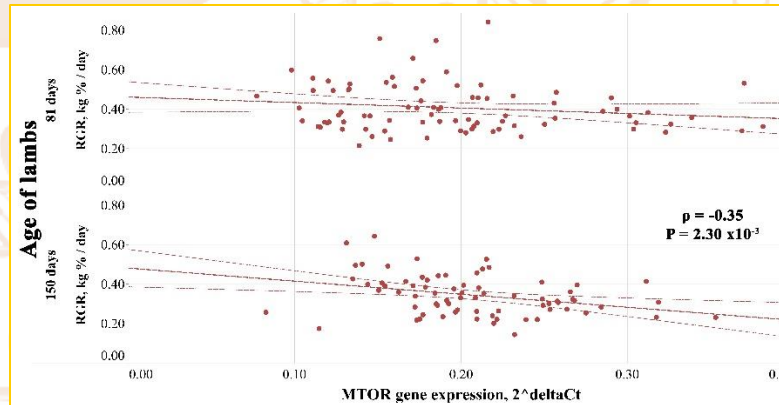
Gene expression correlation with Relative growth rate

There is a weak to moderate correlation between **CAST** gene expression at 81 days and 150 days of lamb age with the Relative growth rate.



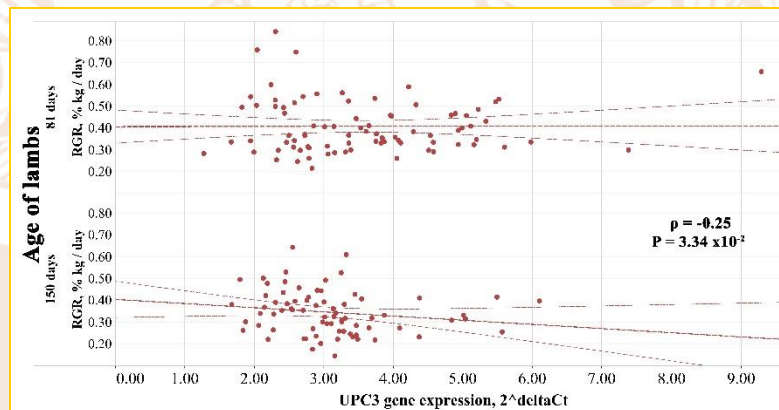
In case of all genes, there is significant ($P < 0.05$) regression or the coefficient of determination for the age of 150 days are around 11 – 15,5%.

There is a weak correlation between **MTOR** gene expression at 150 days lamb age with the Relative growth rate.



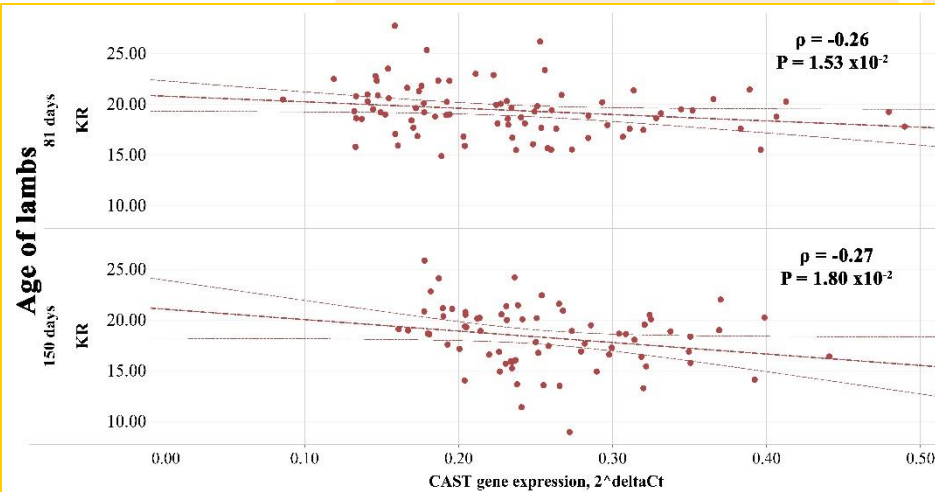
This indicates that as protein expression increases and muscle mass increases, RGR decreases more quickly, as the growth process remains slower, but muscle mass has grown and specific proteins are needed.

There is a weak correlation between **UPC3** gene expression at 150 days of lamb age with the Relative growth rate.

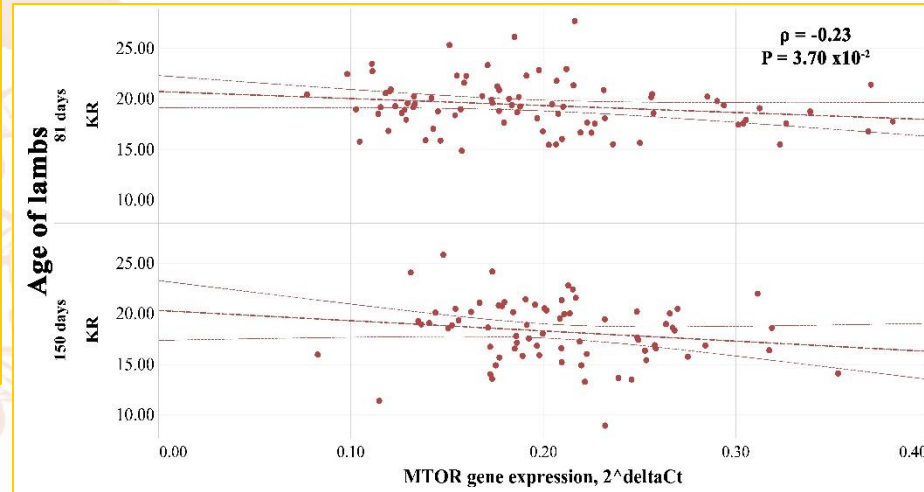


Results

Gene expression correlation with Kleiber's ratio



There is a weak correlation between **CAST** gene expression at 81 days and 150 days of lamb age with the Kleiber's ratio.



There is a weak correlation between **MTOR** gene expression at 81 days of lamb age and the Kleiber's ratio.

This indicates that as growth efficiency (value of Kleiber's ratio) increases, the body's need for specific gene expression results decreases.

Conclusion

- ❁ **CAST and MTOR gene expressions** is **higher** in lambs around 150 days of age or after intensive fattening, but **UPC3 gene expression** is **higher** at the age of 81 days or before fattening.
- ❁ For **eight SNPs** in the **MTOR** gene and **five** in the **UPC3** gene, statistically significant changes in **gene expression** were observed among **all lambs in the 81 and/or 150 day groups** when **all genotypes** were divided and analysed for variation.
- ❁ In the case of **six CAST, seven MTOR, and three UPC3** gene **SNPs**, statistically significant alterations in gene expression are observed **for one genotype** of variation **between lambs aged 81 days and 150 days**.
- ❁ **RGR, KR and RWG** after intensive fattening in lambs are likely influenced by **CAST gene expression** at approximately **81 days of age**; as well, as **KR** is likely influenced by **MTOR gene expression** at the same age.
- ❁ **RGR** indicators during intensive fattening in lambs are **correlated** with **CAST, MTOR, and UPC3 gene expression** at the **end of fattening**; additionally, the **KR** indicator is **correlated with MTOR gene expression**.

Conclusion



The results indicate changes in CAST, MTOR, and UPC3 gene expression during the growth period, depending on SNP genotypes, which could be a useful genetic marker for breeding programs.



Thank you for your attention!



This research is funded by the Latvian Council of Science projects:

- No lzp-2021/1-0489: „Development of an innovative approach to identify biological determinants involved in the between-animal variation in feed efficiency in sheep farming.”
- No lzp-2024/1-0092: „Genetic tools for feed efficient and sustainable meat production in Latvian sheep breeds.”