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Article

Relationship between some myostatin variants and meat production related calving, weaning and muscularity traits in Charolais cattle

Tamás Csürhés[1], Ferenc Szabó[2*], Gabriella Holló[3*], Edit Mikó[4], Márton Török[1], and Szabolcs Bene[3]

[1]National Association of Hungarian Charolais Cattle Breeders, Miskolc, Hungary; csurhest@yahoo.com (T.Cs.); info@charolais.hu (M.T.)
[2]Széchenyi István University, Albert Kázmér Faculty of Agriculture and Food Sciences, Mosonmagyaróvár, Hungary; szabo.ferenc@sze.hu (F.Sz.)
[3]Hungarian University of Agriculture and Life Sciences, Kaposvár, Keszthely, Hungary;
hollo.gabriella@uni-mate.hu (G.H.); bene.szabolcs.albin@uni-mate.hu (Sz.B.)
[4]University of Szeged, Faculty of Agriculture, Hódmezővásárhely, Hungary; miko.edit@mgk.u-szeged.hu (E.M.)
*Correspondence: szabo.ferenc@sze.hu, hollo.gabriella@uni-mate.hu

Simple Summary: The objective of this study was to evaluate the effect of different myostatin alleles on muscularity of four body parts and overall muscularity, moreover on calving ease, birth weight, and 205-day weaning weight of weaned calves in Hungarian Charolais population. Five myostatin alleles of 2046 calves were involved in the study. Among the myostatin alleles, the effect of Q204X was statistically proved ($p<0.01$ and $p<0.05$) on the 205-day weaning weight, muscle score of back, muscle score of thigh, loin thickness score and overall muscle development percentage. The other alleles had no significant effect on the evaluated weaning and muscularity traits.

Abstract: The slaughter value of live animals can be assessed during visual conformation scoring, as well as by examining different molecular genetic information, e.g. myostatin gene, which can be responsible for muscle development. In this study F94L, Q204X, nt267, nt324 and nt414 alleles of the myostatin gene were examined in relation to birth weight (BIW), calving ease (CAE), 205-day weaning weight (CWW), muscle score of shoulder (MSS), muscle score of back (MSB), muscle score of thigh (MST), roundness score of thigh (RST), loin thickness score (LTS), and overall muscle development percentage (OMP) of Charolais weaned calves in Hungary. Multi-trait analysis of variance (GLM) and weighted linear regression analysis were used to process the data. Calves carrying the Q204X allele in heterozygous form achieved approximately 0.14 points higher MSB, MST and LTS and 1.2% higher OMP and gained 8.56 kg more CWW than their counterparts not carrying the allele ($p<0.05$). As for the F94L allele, there was a difference of 4.08 kg in CWW of the heterozygous animals, but this difference could not be proved statistically. The other alleles had no significant effect on the evaluated traits.

Keywords: myostatin alleles; Q204X; F94L; muscularity scores; calving and weaning traits

1. Introduction

The value of the slaughter animals, that is the carcass composition, and meat quality of meat-producing farm animals, such as slaughter cattle, can be reliably evaluated with post-slaughter muscle and fat measurements and laboratory tests. In reality, however, slaughterhouse evaluation and laboratory meat quality testing are often impossible in the trade, as the animals are marketed on live basis. Despite of failing the mentioned objective evaluation possibilities both the sellers and the buyers must be able to visually or other ways appraise the meat production, the value of these animals.

Meat production, value of slaughter animals can be evaluated with a high degree of accuracy based on a number of external or internal characteristics seen, measured and estimated on them. A large number of literary sources, research results support the fact that the age, weight, sex, conformation, condition, muscle mass and shape of live animals provide reliable information about their meat production, however some environmental factors can also play an important role [1]. The mentioned traits can be easily assessed by visual scoring. At the same time some major genes or quantitative trait loci (QTL) were identified related to meet quantity and quality [2,3]. The latter situation gives us the opportunity to perform tests on live animals, as DNA can be isolated from blood or other tissue and the gene or gene variants affecting meat production can be detected. Such kind of tests can be carried out early, before slaughter at a young age of animals.

An indicator of slaughter value could be the myostatin which is an extracellular cytokine mostly expressed in skeletal muscles and known to play a crucial role in the negative regulation of muscle mass [4,5].

Sellick et al. [6] studying the different variants of myostatin genes found that F94L was the only polymorphism consistently related to increased muscling. Wiener et al. [7] found that the myostatin allele with the 11-bp deletion (MH) segregating in the South Devon breed affects several traits related to beef production. The MH allele was associated with heavier calves at birth but slower growth, leading to lighter adult animals. Allais et al. [8] found superiority of carcass traits of calves carrying one copy of the mutated allele (Q204X or nt821) over noncarrier animals was approximately +1 SD in the Charolais and Limousin breeds but was not significant in the Blonde d'Aquitaine. In the Charolais breed, for which the frequency was the greatest (7%), young bulls carrying the Q204X mutation presented a carcass with less fat, less intramuscular fat and collagen contents, and a clearer and more tender meat than those of homozygous-normal cattle. Hales et al. [9] reported that average daily gain measured in Limousin heifers across the whole study (121 day) was greater with 2 copies of the F94L (homozygous) variant. According to Ceccobelli et al. [10] the heterozygous myostatin gene in Marchigiana bulls showed slight superiority in the carcass weight (heterozygote 426 kg and normal 405 kg) and meat quality parameters, although not always with statistical significance.

Looking at the relevant literature, despite the fact that there is a lot of information, research results available on the effect of myostatin on meat production in cattle especially in double-muscled cattle [11,12], relatively less is known about the effect of certain alleles in Charolais. Based on previous data [13-15], it seems that there are significant differences between the phenotypic performance of individuals carrying and not carrying the myostatin alleles [16].

The objective of the present study was to evaluate some myostatin alleles such as F94L and Q204X and others on birth weight, calving ease, 205-day weaning weight and muscle score of some body part (shoulder, back, thigh, loin) and overall muscularity showing muscle development and trend of these traits in Charolais beef cattle population in Hungary.

2. Materials and Methods

2.1. The database

Data processed during the work were collected from the pedigree database of the National Association of Hungarian Charolais Cattle Breeders. The available and evaluated initial database contained pedigree, weaning, conformation traits and molecular genetic information. In the study there were of 2046 EU registered weaned Charolais calves (688 male 1358 female) born between 2015-2021.

2.2. The studied traits

During the study birth weight of calves (BIW), calving ease of dams (CAE), 205-day weaning weight of calves (CWW) moreover muscle score of shoulder (MSS), muscle score of back (MSB), muscle score of thigh (MST), roundness score of thigh (RST), loin thickness score (LTS) and overall muscle development percentage (OMP) as a phenotypic traits of weaned calves were evaluated in relation with myostatin mutations.

(1)

The conformation traits were scored at the weaning. The scoring of the mentioned body parts was carried out according to Conformation Scoring Guideline of National Association of Hungarian Charolais Cattle Breeders [17]. Each animal for each trait was scored from 1 to 10 points depending on the mass and shape of the muscles. However, the values of the OMP were calculated by the sum of the scores of each body part and the ratio of the maximum possible total score in per cent as follows:

$$\text{OMP} = (\text{MSS} + \text{MSB} + \text{MST} + \text{RST} + 2 \times \text{LTS}) / 60$$

The calving ease of cows was scored as follows: normal light calving 1, calving with assistance 2 and difficult calving 3.

2.3. The molecular genetic informations

The molecular genetic information of the 2046 weaned calves was determined with the Weatherbys Scientific Bovine VersaSNP 50K chip. The description of the method and the possibilities of interpreting the results were described in detail by [18].

The genetic database contained information on 117 different alleles. In the course of this study five relevant alleles of the gene encoding the myostatin protein (growth differentiation factor 8; GDF8), F94L, Q204X, nt267, nt324 and nt414 were examined [19,20]. Based on the available information [21-23], it seems that these alleles can have a significant impact on muscle growth, including the development of muscularity. In each case, it was indicated in the database whether the individuals carry the F94L, Q204X, nt267, nt324 and nt414 alleles in homozygous or heterozygous form, or not. The distribution of these alleles by sex of calves is shown in Table 1.

Table 1. Occurrence of myostatin alleles in the examined population.

Myostatin allele	**Genotype**	**Male calves**	Female calves	Total
		Number of animals		
F94L	**Non carrier**	**651**	1282	1933
	Heterozygous	37	76	113
	Homozygous	0	0	0
Q204X	Non carrier	606	1185	1791
	Heterozygous	82	173	255
	Homozygous	0	0	0
nt267	Non carrier	633	1318	1981
	Heterozygous	25	40	65
	Homozygous	0	0	0
nt324	Non carrier	547	1060	1607
	Heterozygous	132	277	409
	Homozygous	9	21	30
nt414	Non carrier	357	705	1062
	Heterozygous	277	548	825
	Homozygous	54	105	159
Total		688	1358	2046

2.4. The effect of different factors

Before evaluating the database the basic statistical parameters of the examined traits (mean, standard deviation, CV%, etc.) were calculated. To check the normality of the data Kolgomorov-Smirnov test, to check the homogeneity of the variances Levene test were used (Table 2).

Table 2. Basic statistics of the examined traits (number of animals for each trait 2046).

Trait	Mean	SD	CV%	Min	Max	Norm*	Hom#
BIW (kg)	43.63	5.99	13.74	21	70	0.07	0.11
CAE (score)	1.16	0.45	38.55	1	3	0.51	0.00
CWW (kg)	258.15	44.30	17.16	125	404	0.03	0.00
MSS (score)	5.54	1.10	19.91	2	9	0.18	0.06
MSB (score)	5.13	1.05	20.39	2	8	0.19	0.02
MST (score)	5.36	1.16	21.71	2	10	0.17	0.27
RST (score)	5.35	1.12	21.01	2	9	0.18	0.33
LTS (score)	5.26	1.07	20.45	2	9	0.18	0.13

OMP (%)	53.15	9.62	18.10	20	87	0.05	0.04

BIW = birth weight; CAE = calving ease; CWW = 205-day weaning weight; MSS = muscle score of shoulder; MSB = muscle score of back; MST = muscle score of thigh; RST = roundness score of thigh; LTS = loin thickness score; OMP = overall muscle development percentage; * Normality test: if p>0.05, the normal distribution is confirmed; #Homogenity test: if p>0.05, the homogenity is confirmed.

To evaluate the database multifactor analysis of variance (General Linear Model) was applied [24]. During this work, the birth year and sex of the calves, as well as the genotype determined on the basis of the myostatin alleles (mentioned above) were incorporated into the model as fixed effects [16]. The nine examined traits were treated separately from each other, and in all 9 cases separated models were performed. The general formula of the models used was as follows:

$$\hat{y}_{hijklmn} = \mu + Y_h + S_i + F_j + Q_k + N_l + M_m + T_n + e_{hijklmn} \quad (2)$$

where $\hat{y}_{hijklmn}$ = trait of a weaned calf of "h" year, "i" sex, "j" F94L, "k" Q204X, “l” nt267, “m” nt324 and “n” nt414 genotypes; μ = average of all observations; Y_h = effect of birth year of calves; S_i = effect of sex of calves; F_j = effect of F94L allele; Q_k = effect of Q204X allele; N_l = effect of nt324 allele; M_m = effect of nt324 allele; T_n = effect of nt414 allele; $e_{hijklmn}$ = random error [10].

2.5. Estimation of phenotypic trends and phenotypic correlations

For all nine traits of the evaluated calves born in the same year were analyzed and averaged. Weighted one-way linear regression analysis was used to estimate of the phenotypic trends. The dependent variable was the evaluated trait, the birth year of calves was considered as an independent variable, and the weight was the number of individuals per year.

Among the nine evaluated traits, Pearson's phenotypic correlation values (r) were also determined.

2.6. The used softwares

The data were prepared using Microsoft Excel 2003 and Word 2003. The evaluation of the database was performed with the statistical software package SPSS 27.0 [25].

3. Results

For all traits, the influence of the sex of the calf was statistically verifiable ($p<0.01$) and played a decisive role (62.27-96.74%) in the development of the phenotype (Table 3). The effect of the year of birth of the calves on the tested traits was also significant ($p<0.01$). Among the myostatin alleles, the effect of Q204X was statistically proved ($p<0.01$ and $p<0.05$) on the traits CWW, MSB, MST, LTS and OMP. The other alleles had no effect on the evaluated weaning and muscularity traits.

Table 3. Effect of the examined factors on the calving, weaning and the muscularity traits.

Factors	**BIW**	**CAE**	**CWW**	**MSS**	**MSB**	**MST**	**RST**	LTS	OMP
	p								
Birth year of calves	**<0.01**	**<0.01**	**<0.01**	**<0.01**	**<0.01**	**<0.01**	**<0.01**	<0.01	<0.01
Sex of calves	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
F94L	NS	NS	NS	NS	NS	NS	NS	NS	NS
Q204X	NS	NS	<0.01	NS	<0.05	<0.05	NS	<0.05	<0.05
nt267	NS	NS	NS	NS	NS	NS	NS	NS	NS
nt324	NS	NS	NS	NS	NS	NS	NS	NS	NS
nt414	NS	NS	NS	NS	NS	NS	NS	NS	NS
Factors	the ratio of the examined factors in phenotype (%)								
Birth year of calves	8.53	19.19	3.84	1.95	1.26	1.52	6.63	2.44	1.97
Sex of calves	90.37	62.27	87.90	96.18	96.74	94.43	92.32	95.53	96.49
F94L	0.24	1.39	0.68	0.12	0.39	0.01	0.21	0.50	0.24
Q204X	0.00	2.05	5.29	0.63	1.04	1.79	0.04	1.07	0.81
nt267	0.01	6.12	0.03	0.10	0.03	1.30	0.07	0.10	0.16
nt324	0.07	1.02	1.17	0.35	0.06	0.24	0.07	0.08	0.03
nt414	0.16	4.54	0.38	0.40	0.21	0.19	0.28	0.03	0.07
Error	0.62	3.42	0.71	0.27	0.27	0.52	0.38	0.25	0.23
Total	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0

BIW = birth weight; CAE = calving ease; CWW = 205-day weaning weight; MSS = muscle score of shoulder; MSB = muscle score of back; MST = muscle score of thigh; RST = roundness score of thigh; LTS = loin thickness score; OMP = overall muscle development percentage

The adjusted overall mean values (±SE) of the examined traits was as follows (Table 4 and Table 5): BIW 43.65±0.63 kg, CAE 1.12±0.05 points, CWW 269.07±4.73 kg, MSS 5.90±0,11 points, MSB 5.39±0.11 points, MST 5.65±0.12 points, RST 5.54±0.12 points, LTS 5.52±0.11 points and OMP 55.86±0.96%.

Regarding CWW, the calves carrying the Q204X allele in heterozygous form in the studied population gained 8.56 kg more weight than their counterparts not carrying the allele. From the point of view of the F94L allele, there was a difference of 4.08 kg in favor of the heterozygous individuals, but this difference could not be verified statistically. The weight of the individuals carrying the nt324 and nt414 alleles in homozygous form was higher (10.43 kg and 2.92 kg, respectively) than the non-carriers, but these differences were not significant either.

Table 3. The effect of different factors on the calving and weaning traits.

Factors	N	Calving and weaning traits		
		BIW (kg)	CAE (score)	CWW (kg)
Adjusted overall mean (±SE)	2046	43.65±0.63	1.12±0.05	269.07±4.73
		deviation from the overall mean		
Birth year of calves				
- 2015	195	-0.98	+0.16	-6.02
- 2016	51	-0.37	-0.10	-9.20
- 2017	139	-2.36	-0.02	-4.12
- 2018	296	+0.46	+0.00	-2.01
- 2019	540	-0.06	+0.04	+4.67
- 2020	597	+0.76	-0.02	+6.93
- 2021	228	+2.54	-0.05	+9.74
Sex of calves				
- male	688	+1.67	+0.05	+11.54
- female	1358	-1.67	-0.05	-11.54
F94L				
- non carrier	1933	+0.17	+0.01	-2.04
- heterozygous	113	-0.17	-0.01	+2.04
Q204X				
- non carrier	1791	-0.01	-0.01	-4.28
- heterozygous	255	+0.01	+0.01	+4.28
nt267				
- non carrier	1981	-0.05	+0.04	-0.54
- heterozygous	65	+0.05	-0.04	+0.54
nt324				
- non carrier	1607	+0.08	+0.00	-4.58
- heterozygous	409	-0.07	-0.02	-1.27
- homozygous	30	+0.00	+0.02	+5.85
nt414				
- non carrier	1062	+0.13	+0.02	-0.67
- heterozygous	825	+0.11	+0.02	-1.57
- homozygous	159	-0.24	-0.04	+2.25

BIW = birth weight; CAE = calving ease; CWW = 205-day weaning weight

In terms of muscularity scores, it could be established that calves carrying the Q204X allele in heterozygous form achieved approximately 0.14 points higher MSB, MST and LTS and 1.2% higher OMP than those their non-carrying partners. Despite the fact that the F94L allele had no statistically verifiable effect on muscularity parameters, it was striking that non-carrier calves showed higher values in almost all muscularity scores than heterozygous carriers. In the case of the nt267, nt324 and nt414 alleles, the muscularity score of the heterozygous, but even more so the homozygous carrier calves was - although not significantly - higher than that of the non-carrier individuals.

Table 5. The effect of different factors on the muscularity traits.

Factors	N	Muscularity traits					
		MSS (score)	MSB (score)	MST (score)	RST (score)	LTS (score)	OMP (%)
Adjusted overall mean (±SE)	2046	5.90± 0.11	5.39± 0.11	5.65± 0.12	5.54± 0.12	5.52± 0.11	55.86± 0.96
		deviation from the overall mean					
Birth year of calves							
- 2015	195	+0.13	+0.15	+0.04	-0.19	+0.12	+0.61
- 2016	51	+0.06	+0.14	-0.25	+0.00	+0.20	+0.57
- 2017	139	+0.19	+0.06	+0.06	+0.41	+0.07	+1.44
- 2018	296	+0.09	+0.01	+0.22	+0.36	+0.08	+1.40
- 2019	540	-0.02	-0.03	-0.04	+0.03	+0.03	+0.00
- 2020	597	-0.21	-0.18	-0.05	-0.24	-0.28	-2.06
- 2021	228	-0.24	-0.16	+0.02	-0.36	-0.22	-1.97
Sex of calves							
- male	688	+0.47	+0.44	+0.35	+0.41	+0.47	+4.34
- female	1358	-0.47	-0.44	-0.35	-0.41	-0.47	-4.34
F94L							
- non carrier	1933	+0.03	+0.06	-0.01	+0.04	+0.07	+0.43
- heterozygous	113	-0.03	-0.06	+0.01	-0.04	-0.07	-0.43
Q204X							
- non carrier	1791	-0.06	-0.07	-0.07	-0.01	-0.07	-0.60
- heterozygous	255	+0.06	+0.07	+0.07	+0.01	+0.07	+0.60
nt267							
- non carrier	1981	-0.04	-0.02	-0.11	-0.03	-0.04	-0.46
- heterozygous	65	+0.04	+0.02	+0.11	+0.03	+0.04	+0.46
nt324							
- non carrier	1607	-0.11	-0.04	+0.00	+0.02	+0.00	-0.23
- heterozygous	409	-0.07	-0.01	-0.06	+0.05	-0.04	-0.29
- homozygous	30	+0.17	+0.05	+0.06	-0.06	+0.04	+0.52
nt414							
- non carrier	1062	-0.06	-0.03	-0.03	-0.05	+0.00	-0.27
- heterozygous	825	+0.03	+0.03	-0.03	-0.01	-0.02	-0.01
- homozygous	159	+0.03	+0.00	+0.05	+0.06	+0.01	+0.28

MSS = muscle score of shoulder; MSB = muscle score of back; MST = muscle score of thigh; RST = roundness score of thigh; LTS = loin thickness score; OMP = overall muscle development percentage.

In the case of all traits, we observed considerable differences between the individual born in different years. This was also supported by the results of the phenotypic trend calculation (Table 6), according to which 6 of the 9 examined traits were statistically reliable ($p<0.05$ and $p<0.01$) and fairly well matched ($R^2 = 0.57$- 0.93) regression functions were obtained. In the case of BIW and CWW, the slope of the straight lines (b) was in a positive direction, while in the case of the other traits it was in a negative direction. Here must be note that in the case of muscularity parameters, the annual decrease is very small, typically -0.05, or -0.07 points/year.

Table 6. The phenotypic trend of the estimated traits.

Traits	Slope (bX)			Intercept (a)			Fitting	
	b	SE	p	a	SE	p	R^2	p
BIW (kg)	+0.54	0.20	<0.05	-1042.52	4407.67	<0.05	0.59	<0.05
CAE (score)	-0.01	0.02	NS	29.82	31.44	NS	0.14	NS
CWW (kg)	+3.18	0.44	<0.01	-6146.81	885.23	<0.01	0.91	<0.01
MSS (score)	-0.06	0.02	<0.05	134.90	38.18	<0.05	0.70	<0.05
MSB (score)	-0.06	0.01	<0.01	122.69	14.19	<0.01	0.93	<0.01
MST (score)	+0.01	0.03	NS	-16.01	59.19	NS	0.03	NS
RST (score)	-0.05	0.06	NS	103.80	115.26	NS	0.13	NS
LTS (score)	-0.07	0.02	<0.05	150.65	36.77	<0.01	0.76	<0.05
OMP (%)	-0.51	0.20	<0.05	1077.82	401.49	<0.05	0.57	<0.05

BIW = birth weight; CAE = calving ease; CWW = 205-day weaning weight; MSS = muscle score of shoulder; MSB = muscle score of back; MST = muscle score of thigh; RST = roundness score of thigh; LTS = loin thickness score; OMP = overall muscle development percentage.

Based on the obtained phenotypic correlation values (Table 7), it could be established that the calving and weaning traits did not show a close relationship with each other or with the muscularity traits (r = 0.00-0.24). On the other hand, there was a close (r = 0.61-0.92) and statistically reliable (p<0.01) correlation between the muscularity scores.

Table 7. Phenotypic correlation values between the estimated traits.

r	CAE	CWW	MSS	MSB	MST	RST	LTS	OMP
BIW	*0.13	*0.24	*0.13	*0.15	*0.08	*0.13	*0.13	*0.14
CAE		0.00	*0.09	*0.09	0.04	*0.08	*0.09	*0.09
CWW			*0.21	*0.20	*0.17	*0.24	*0.21	*0.24
MSS				*0.86	*0.61	*0.68	*0.80	*0.90
MSB					*0.63	*0.66	*0.82	*0.91
MST						*0.67	*0.62	*0.79
RST							*0.65	*0.82
LTS								*0.92

*p<0.01; BIW = birth weight; CAE = calving ease; CWW = 205-day weaning weight; MSS = muscle score of shoulder; MSB = muscle score of back; MST = muscle score of thigh; RST = roundness score of thigh; LTS = loin thickness score; OMP = overall muscle development percentage.

4. Discussion

Similar to the results of our work, several previous sources [8,21,26] contain information on the statistically verifiable effect of the Q204X allele on the meat production related traits. Contrary to our results, several previous studies [6,16] found the effect of the F94L allele to be significant on some muscularity-related parameters. Among the alleles belonging to the "small" myostatin group, we only found information on the effect of the double-muscled related allele nt821 in existing sources [27-29], however, this allele did not occur in the is the tested Charolais stock. The genetic structure of the nt267, nt324 and nt414 alleles was previously described by Dunner et al. [21], but no literature data were found on their effect on the phenotypic results.

The results of our work are similar to the findings of Casas et al. [12], according to which myostatin alleles in heterozygous form can have a favorable effect on weaning traits. Contrary to the results of Allais et al. [8], we could not detect the effect of the Q204X allele on birth weight in the examined Charolais herd. Similar to the results of Esmailizadeh et al. [22], the effect of the F94L allele on birth and weaning traits was not found to be significant.

Our results for the weaning weight of Charolais calves were similar to the data found in most of the relevant literary sources [30-32]. On the basis of the calving ease score observed during our work, it seems that there were fewer difficult calvings in the studied herd than what was found in the literature [33,34] in the case of the Charolais breed.

We found very little information available in the literature about the conformation of Charolais calves related to their muscularity. Arango et al. [35] and Vallée et al. [36] published data on purebred and crossbred Charolais herds, but due to the different methodology, we did not have the opportunity to compare them with our results.

The differences by birth year and sex of calves in weaning weight obtained during our work are very well known in the literature [37,38]. However, we did not find any data for this kind of evaluation of the muscularity parameters of Charolais calves.

Similar to our results, Gutiérrez et al. [39] and Chud et al. [40] did not find a close correlation between BIW, CAE and CWW traits in the case of the Asturiana de los Valles breed of cattle, and in the case of the Nellore breed.

5. Conclusions

Of the five myostatin alleles examined during this research, Q204X clearly proved to have the greatest effect on calving, weaning and muscularity-related traits. The allele was only present in heterozygous form in the evaluated Charolais population, so according to the available literature information, it did not affect CAE and BIW, but it clearly had a favorable effect on CWW. The effect of the allele on muscularity-related traits was also positive, although to a much lesser extent. Therefore, we think it would be advisable to pay attention to this allele in the breeding strategy, to increase the proportion of carriers from generation to generation. It would be advisable to repeat this test periodically, because based on literature data, it seems that the allele in its homozygous form could cause calving difficulty.

Based on our results, the favorable effect of the F94L allele shown in previous research was not detectable in our study. One of the reasons for this may be that the proportion of animals carrying the allele (about 5.5%) was very small in the studied population. On the other hand, based on previous studies, the better phenotypic performance of individuals carrying the allele was more evident in the fattening and slaughter traits.

The proportion of calves carrying the nt324 and nt414 alleles was quite high (21.5% and 48.1%, respectively) in the examined Charolais population. In the literature, there was very little information about their effect on phenotypic performance. Based on our results, it seems that homozygous carrier individuals may have better growth performance-related traits than non-carrier individuals.

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Data Availability Statement: The data presented in this study are available on request from the National Association of Hungarian Charolais Cattle Breeders.

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References

1. Cunningham, M.; Latour, M.A.; Acker, D. *Animal science and industry*. 7th Edition; Pearson Prentice Hall, New Yersey, USA, 2005.
2. Georges, M.; Grobet, L.; Poncelet, D.; Royo, L.J.; Pirottin, D.; Brouvers, B. Positional candidate cloning of the bovine mh locus identifies an allelic series of mutations disrupting the myostatin function and causing double-muscling in cattle. 6th World Congress on Genetics Applied to Livestock Production, Armidale, Australia, 11-16 January 1998, *26*, 195–204.
3. Stinckens, A.; Luyten, T.; Bijttebier, J.; van den Maagdenberg, K.; Dieltiens, D.; Janssens, S.; Buys, N. Characterization of the complete porcine MSTN gene and expression levels in pig breeds differing in muscularity. *Anim. Genet.* **2008,** *39*, 586–596. https://doi.org/10.1111/j.1365-2052.2008.01774.x

4. Mirhoseini, S.Z.; Zare, J. The role of myostatin on growth and carcass traits and its application in animal breeding. *Life Sci.* **2012**, *9*, 2353–2357. https://doi.org/10.7537/marslsj090312.340
5. Elkina, Y.; von Haehling, S.; Anker, S.D.; Springer, J. The role of myostatin in muscle wasting: an overview. *J. Cachexia Sarcopenia Muscle* **2011**, *2*, 143–151. https://doi.org/10.1007/s13539-011-0035-5
6. Sellick, G.S.; Pitchford, W.S.; Morris, C.A.; Cullen, N.G.; Crawford, A.M.; Raadsma, H.W.; Bottema, C.D.K. Effect of myostatin F94L on carcass yield in cattle. *Anim. Genet.* **2007**, *38*, 5.440–446. https://doi.org/10.1111/j.1365-2052.2007.01623.x
7. Wiener, P.; Woolliams, J.A.; Frank-Lawale, A.; Ryan, M.; Richardson, R.I.; Nute, G.R.; Wood, J.D.; Homer, D.; Williams, J.L. The effects of a mutation in the myostatin gene on meat and carcass quality. *Meat Sci.* **2009**, *83*, 127–134. https://doi.org/10.1016/j.meatsci.2009.04.010
8. Allais, S.; Levéziel, H.; Payet-Duprat, N.; Hocquette, J.F.; Lepetit, J.; Rousset, S.; Denoyelle, C.; Bernard-Capel, C.; Journaux, L.; Bonnot, A.; Renand, G. The two mutations, Q204X and nt821, of the myostatin gene affect carcass and meat quality in young heterozygous bulls of French beef breeds. *J. Anim. Sci.* **2013**, *88*, 446–454. https://doi.org/10.2527/jas.2009-2385
9. Hales, K.E.; Tait, R.G.; Lindholm-Perry, A.K.; Freetly, H.C.; Brown-Brandl, T.M.; Bennett, G.L. Effects of the F94L Limousin associated myostatin gene marker on metabolic index in growing beef heifers. *Appl. Anim. Sci.* **2020**, *36*, 851–856. https://doi.org/10.15232/aas.2020-02046
10. Ceccobelli, S.; Perini, F.; Trombetta, M.F.; Tavoletti,S.; Lasagna, E.; Pasquini, M. Effect of myostatin gene mutation on slaughtering performance and meat quality in Marchigiana bulls. *Animals* **2022**, *12*, 518. https://doi.org/10.3390/ani12040518
11. Grobet, L.; Poncelet, D.; Royo, L.J.; Brouwers, B.; Pirottin, D.; Michaux, C.; Ménissier, F.; Zanotti, M.; Dunner, S.; Georges, M. Molecular definition of an allelic series of mutations disrupting the myostatin function and causing double-muscling in cattle. *Mamm. Genome* **1998**, *9*, 210–213. https://doi.org/10.1007/s003359900727
12. Casas, E.; Keele, J.W.; Fahrenkrug, S.C.; Smith, T.P.L.; Cundiff, L.V.; Stone, R.T. Quantitative analysis of birth, weaning, and yearling weights and calving difficulty in Piedmontese crossbreds segregating an inactive myostatin allele. *J. Anim. Sci.* **1999**, *77*, 1686–1692. https://doi.org/10.2527/1999.7771686x
13. Short R.E.; MacNeil M.D.; Grosz M.D.; Gerrard D.E.; Grings E.E. Pleiotropic effects in Hereford, Limousin and Piedmontese F2 crossbred calves of genes controlling muscularity including the Piedmontese myostatin allele. *J. Anim. Sci.* **2002**, *80*, 1–11. https://doi.org/10.2527/2002.8011
14. Mosher D.S.; Quignon P.; Bustamante C.D.; Sutter N.B.; Mellersh C.S.; Parker H.G.; Ostrande E.A. A mutation in the myostatin gene increases muscle mass and enhances racing performance in heterozygote dogs. *PLoS Genetics* **2007**, *3*, e79. https://doi.org/10.1371/journal.pgen.0030079
15. Tozaki, T.; Miyake, T.; Kakoi, H.; Gawahara, H.; Sugita, S.; Hasegawa, T.; Ishida, N.; Hirota, K.; Nakano, Y. A genome-wide association study for racing performances in Thoroughbreds clarifies a candidate region near the MSTN gene. *Anim. Genet. Suppl.* **2010**, *41*, 28–35. https://doi.org/10.1111/j.1365-2052.2010.02095.x
16. Lines, D.S.; Pitchford, W.S.; Kruk, Z.A.; Bottema, C.D.K. Limousin myostatin F94L variant affects semitendinosus tenderness. *Meat Sci.* **2009**, *81*, 126–131. https://doi.org/10.1016/j.meatsci.2008.07.007
17. Domokos, Z.; Tőzsér, J. *Conformation Scoring Guideline of National Association of Hungarian Charolais Cattle* Breeders. Miskolc, Hungary, 2004, 25–33. [in Hungarian]
18. Kusza, Sz.; Hegedűs, B.; Domokos, Z. *A guide to interpreting gene variants on the Weatherbys Scientific Bovine VersaSNP 50K chip*. National Association of Hungarian Charolais Cattle Breeders, Miskolc, Hungary, 2020, 32–39. [in Hungarian]
19. Arnold, H.; Della-Fera, M.A.; Baile, C.A. (2001): Review of myostatin history, physiology and applications. *Int. Arch. Biosci.* **2001**, *1*, 1014–1022.
20. Hadjipavlou, G.; Matika, O.; Clop, A.; Bishop, S.C. Two single nucleotide polymorphisms in the myostatin (GDF8) gene have significant association with muscle depth of commercial Charollais sheep. *Anim. Genet.* **2008**, *39*, 346–353. https://doi.org/10.1111/j.1365-2052.2008.01734.x

21. Dunner, S.; Miranda, M.E.; Amigues, Y.; Canón, J.; Georges, M.; Hanset, R.; Williams, J.; Ménissier, F. Haplotype diversity of the myostatin gene among beef cattle breeds. *Genet. Sel. Evol.* **2003**, *35*, 103–118. https://doi.org/10.1186/1297-9686-35-1-103
22. Esmailizadeh, A.K.; Bottema, C.D.K.; Sellick, G.S.; Verbyla, A.P.; Morris, C.A.; Cullen, N.G.; Pitchford, W.S. Effects of the myostatin F94L substitution on beef traits. *J. Anim. Sci.* **2008**, *86*, 1038–1046. https://doi.org/10.2527/jas.2007-0589
23. Aiello, D.; Patel, K.; Lasagna, E. The Myostatin gene: an overview of mechanisms of action and its relevance to livestock animals. *Anim. Genet.* **2018**, *49*, 505–519. https://doi.org/10.1111/age.12696
24. Bene, Sz.; Polgár, J.P.; Szűcs, M.; Márton, J.; Szabó, E.; Szabó, F. Population genetic features of calving interval of the Limousin beef cattle breed in Hungary. *Acta Vet. Hung.* **2022**, *70*, 113–120. https://doi.org/10.1556/004.2022.00008
25. IBM SPSS Statistics for Windows. Version 27.0, IBM Corporation, Armonk, New York, USA, 2020.
26. Charlier, C.; Coppieters, W.; Farnir, F.; Grobet, L.; Leroy, P.L.; Michaux, C.; Mni, M.; Schwers, A.; Vanmanshoven, P.; Hanset, R.; Georges, M. The mh gene causing double-muscling in cattle maps to bovine chromosome 2. *Mamm. Genome* **1995**, *6*, 788–792. https://doi.org/10.1007/BF00539005
27. Miranda, M.E.; Amigues, Y.; Boscher, M.Y.; Ménissier, F.; Cortés, O.; Dunner, S. Simultaneous genotyping to detect myostatin gene polymorphism in beef cattle breeds. *J. Anim. Breed. Genet.* **2002**, *119*, 361–366. https://doi.org/10.1046/j.1439-0388.2002.00352.x
28. Bellinge, R.H.S.; Liberles, D.A.; Iaschi, S.P.A.; O'Brien, P.A.; Tay, G.K. Myostatin and its implications on animal breeding: a review. *Anim. Genet.* **2005**, *36*, 1–6. https://doi.org/10.1111/j.1365-2052.2004.01229.x
29. González-Berríos, C.L.; Rivera-Serrano, A.; Casas-Guérnica, A.; Sonstegard, T.; Pagán-Morales, M. Molecular breeding values distribution in slick male and female Senepol cattle differing in musculature. *J. Anim. Sci. Suppl.* **2016**, *94*, 152. https://doi.org/10.2527/jam2016-0317
30. Dodenhoff, J.; van Vleck, L.D.; Gregory, K.E. Estimation of direct, maternal and grand maternal genetic effects for weaning weight in several breeds of beef cattle. *J. Anim. Sci.* **1999**, *77*, 840–845. https://doi.org/10.2527/1999.774840x
31. Donoghue, K.A.; Bertrand, J.K. Investigation of genotype by country interactions for growth traits for Charolais populations in Australia, Canada, New Zealand and USA. *Liv. Prod. Sci.* **2004**, *85*, 129–137. https://doi.org/10.1016/S0301-6226(03)00133-7
32. Fördős, A.; Fürst-Waltl, B.; Baumung, R.; Bene, Sz.; Szabó, F. Estimation of genetic parameters for weaning traits in Austrian Charolais cattle fitting sire x year interaction as an additional random effect. *Züchtungskunde* **2010**, *82*, 181-194.
33. Phocas, F.; Bloch, C.; Chapelle, P.; Bécherel, F.; Renand, G.; Ménissier, F. Developing a breeding objective for a French purebred beef cattle selection programme. *Liv. Prod. Sci.* **1998**, *57*, 49-65. https://doi.org/10.1016/S0301-6226(98)00157-2
34. Eriksson, S.; Näsholm, A.; Johansson, K.; Philipsson, J. Genetic parameters for calving difficulty, stillbirth, and birth weight for Hereford and Charolais at first and later parities. *J. Anim. Sci.* **2004**, *82*, 375–383. https://doi.org/10.1093/ansci/82.2.375
35. Arango, J.A.; Cundiff, L.V.; van Vleck, L.D. Breed comparisons of Angus, Charolais, Hereford, Jersey, Limousin, Simmental and South Devon for weight, weight adjusted for body condition score, height, and body condition score in beef cows. *J. Anim. Sci.* **2002**, *80*, 3123–3132. https://doi.org/10.2527/2002.80123123x
36. Vallée, A.; van Arendonk, J.A.M.; Bovenhuis H. Genetic parameters for calving and conformation traits in Charolais x Montbéliard and Charolais x Holstein crossbred calves. *J. Anim. Sci.* **2013**, *91*, 5582–5588. https://doi.org/10.2527/jas.2013-6490
37. Lee, C.; van Tassel, C.P.; Pollak, E.J. Estimation of genetic variance and covariance components for weaning weight in Simmental cattle. *J. Anim. Sci.* **1997**, *75*, 325–330. https://doi.org/10.2527/1997.752325x
38. Teixeira, B.B.M.; MacNeil, M.D.; da Costa, R.F.; Dionello, N.J.L.; Yokoo, M.J.; Cardoso, F.F. Genetic parameters and trends for traits of the Hereford and Braford breeds in Brazil. *Liv. Sci.* **2018**, *208*, 60–66. https://doi.org/10.1016/j.livsci.2017.12.008
39. Gutiérrez, J.P.; Goyache, F.; Fernández, I.; Alvarez; I., Royo, L.J. Genetic relationships among calving ease, calving interval, birth weight, and weaning weight in the Asturiana de los Valles beef cattle breed. *J. Anim. Sci.* **2007**, *85*, 69–75. https://doi.org/10.2527/jas.2006-168

40. Chud, T.C.S.; Caetano, S.L.; Buzanskas, M.E.; Grossi, D.A.; Guidolin, D.G.F.; Nascimento, G.B.; Munari, D.P. Genetic analysis for gestation length, birth weight, weaning weight, and accumulated productivity in Nellore beef cattle. *Liv. Sci.* **2014**, *170*, 16–21. https://doi.org/10.1016/j.livsci.2014.09.024