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THE EFFECTS OF POLYMORPHISMS IN CALPAIN CALPASTATIN AND GROWTH HORMONE GENES ON GROWTH TRAITS IN ANGUS COWS

***S. Y. Ruban, **A. M. Fedota, **N. G. Lysenko, ***A. I. Kolisnyk, ****I. V.Goraichuk**

** Institute of Animal Breeding and Genetics nd. a. M.V.Zubets of NAAN, Chubynske, Ukraine*
*** V. N. Karazin Kharkiv National University, Kharkiv, Ukraine*
**** PE "Agrofirma Svitanok", Novoselivka, Ukraine*
***** National Scientific Center 'Institute of Experimental and Clinical Veterinary Medicine', Kharkiv, Ukraine*
e-mail: rubansy@gmail.com

Abstract: *The study was aimed to determine the allele and genotype frequencies of SNPs for gene calpain (CAPN316), calpastatin (CAST282) and growth hormone (GH L127V) in Aberdeen-Angus (n=52) and to evaluate their impact on body weight dynamics until age of 5 years. The allele and genotype frequencies was CAPN316: C = 0.45, G = 0.55; CC = 19.2%, CG = 51.9%, GG = 28.9%, CAST282: C = 0.75, G = 0.25; CC = 53.8%, CG = 42.3%, GG = 3.9%, GH L127V: C = 0.34, G = 0.66; CC = 9.6%, CG = 48.1%, GG = 42.3%. There is correlation between the number of C-alleles for GH L127V and increase in body weight from birth till two years. The CAPN316 and CAST282 alleles associated with meat tenderness effect on live body weight increase after two year age. A significant effect of genotype CC CAPN316 is observed at the age of three and four years. It was concluded, that selection aimed to improve meat quality does not lead to a significant reduction in live body weight.*

Keywords: *angus cattle, growth traits, calpain, calpastatin, growth hormone.*

INTRODUCTION

Calpain and its inhibitor calpastatin [1] are calcium-dependent intracellular proteases that breake one or two peptide bonds in the target protein molecule to modulate its function. Calpains are involved in regulation of cell differentiation, apoptosis, synaptic transmission, muscle protein metabolism, morphogenesis and other processes [3]. Growth hormone secreted by the anterior pituitary plays a key role in the regulation of growth and animal metabolism, lactation, mammary gland development and cows' fertility [4; 6]. The combined effect of calpain, calpastatin and growth hormone affects the quantity and quality of meat produced. When corresponding genes are expressed the SNPs can affect the structure and function of the final products.

The SNP *CAPN316* of calpain gene localized in 29th BTA encodes guanine (G) to cytosine (C) change at position 5709 in 9th exon, resulting in glycine to alanine substitution in the amino acid sequence of a μ- calpain large catalytic subunit associated with increased activity of this enzyme. Calpastatin gene (*CAST*) is located in the 7th chromosome BTA. SNP *CAST282* is characterized by replacement of guanine (G) with cytosine (C) at position 282 of the 5th intron, resulting to the nonfunctional calpastatin molecule synthesis. These SNPs are proved as indicators of the meat tenderness by Warner-Bratzler shear force [2; 8]. Calpain is activated by Ca^{2+} concentration increasing after cell death when Ca^{2+} ions leave intracellular depot, and destroys myofibrils that result to more tender meat. Calpastatin is activated by Ca^{2+} concentration required to achieve the half of μ-calpain maximal activity. Desirable alleles for meat tenderness are *C*-alleles of *CAPN316* and *CAST282*, as formed during the expression of these alleles

hyperactive calpain and non-functional calpastatin lead to the more intensive muscle fiber degradation.

Growth hormone (*GH*) gene is located in 19 BTA and consists of five exons, separated by four introns. SNP *L127V* codes cytosine (*C*) to guanine (*G*) substitution in the position 2141 of the 5th exon, resulting in a leucine to valine substitution at 127th position of the polypeptide. In this case, the desirable allele is allele *C*, since the *G* allele results to synthesis of molecule with a modified structure difficultly recognized by growth hormone receptor.

The purpose of the study was to analyze the effect of individual genotypes and allelic variants of SNPs *CAPN316*, *CAST282* and *GH L127V* in Aberdeen-Angus breed on the growth dynamics from birth to the age of five years.

MATHERIAL AND METHODS

The study object was Aberdeen-Angus cattle (n = 52) bred in Kharkiv region, Ukraine. Body weight was recorded at birth, 8, 12, 15 and 18 months, 2, 3, 4 and 5 years. DNA was extracted from blood samples using DNA extraction kits "Diatom DNA Prep 100" ("Isogene", Russia). For the SNP genotyping, PCR-RFLP methods were set up, using primer pairs [5; 7; 8] and restriction endonucleases RsaI, BtgI and AluI ("Fermentas", Lithuania). Electrophoretic analysis was performed on 2% agarose gel. The deviation of allele frequencies from Hardy-Weinberg equilibrium was tested using Pearson's chi-squared test. For data distribution it was used normality test assessment. Statistical analysis was performed with t-test, correlation analysis and analysis of variance.

RESULTS AND DISCUSSION

Allele and genotype frequencies of SNPs *CAPN316*, *CAST282* and *GH L127V* for Aberdeen-Angus herd studied are given in Table 1. When tested deviation from the Hardy-Weinberg equilibrium using Pearson's chi-squared test there were no statistically significant differences between actual and expected genotype frequencies for both genes. The genotype frequencies in group studied was found to be at equilibrium, thus frequency of desirable allele will not change without object-oriented selection.

Table 1. Allele and genotype frequencies of SNPs *CAPN316*, *CAST282* and *GH L127V* in the Aberdeen-Angus herd of Kharkiv region and parameters for Hardy-Weinberg equilibrium (χ^2).

Parameter	Single nucleotide polymorphism								
	CAPN316			*CAST282*			*GH L127V*		
Allele	*C*		*G*	*C*		*G*	*C*		*G*
frequency	0.45		0.55	0.75		0.25	0.34		0.66
Genotype	*CC*	*CG*	*GG*	*CC*	*CG*	*GG*	*CC*	*CG*	*GG*
$n_{act.}$	10	27	15	28	22	2	5	25	22
%	53.8	51.9	28.9	53.8	42.3	3.8	9.6	48.1	42.3
$n_{exp.}$	10	26	16	29	20	3	6	23	23
%	19.3	50.0	30.7	55.7	38.5	5.8	11.6	44.2	44.2
$\chi^2_{act.}$*	0.051			0.312			0.155		

Notes: n – number of animals, df=2, $\Sigma\chi^2_{st.}$= 5,99 for p = 0,05.

Our observations have shown that animals' body weight with genotype *CC* of SNP *GH L127V* exceeds that one for genotypes *CG* and *GG*. The differences between the groups in live weight at birth is 4.8-5.2 kg or 14.6-16.1% (*CC*: 35.2±1.3 kg, *CG*: 30.4±1.0 kg, *GG*: 29.9±0.9 kg; p = 0.044). While ageing the differences in live body weight decreases and reaches 3-25 kg or 1-5% between *CC* and *CG* groups, and 10-25 kg, or 2-

7% between *CC* and *GG* groups. This observation is explained by the more intense secretion of growth hormone in animals with *CC* genotype [4].

Analysis of the SNPs *GH L127V* and *CAPN316* in combination revealed that animals with genotypes *CC/CC* and *CC/CG* had greater live body weight till two-years age, but after two years animals with genotype *CG/CG* (Table 2). Population is in Hardy-Weinberg equilibrium state ($\chi^2_{act.} = 5.9$; $\chi^2_{st.} = 15.5$; $df = 8$; $p > 0.05$). Until two years of age differences between the groups is approximately 20-30 kg (up to 10% of body weight). After two years of age differences between the groups increase and reach 40-120 kg or 10-20%. Statistically significant effect of genotype on the body weight is observed in the age of three ($F = 5.9$; $p < 0.001$), four ($F = 14.4$; $p < 0.001$) and five years ($F = 12.7$; $p < 0.001$). The groups of animals also vary significantly on weight gain (Max: CC/CC = 966 g, Min: CG/CC = 683 g; $F = 21.6$; $p < 0.001$). Differences between animals with different genotypes increases because of later calpastatin effect realization, when expression of growth hormone gene is reduced.

Table 2. Body weight dynamics in Aberdeen-Angus by SNPs *GH L127V* and *CAPN316*, $\bar{x} \pm s_x$.

Weight, kg	Genotype *GH L127V / CAPN316*								
	CC/ CC	*CC/ CG*	*CC/ GG*	*CG/ CC*	*CG/ CG*	*CG/ GG*	*GG/ CC*	*GG/ CG*	*GG/ GG*
n	1	2	2	3	13	9	6	12	4
Birth	35.0	35.5 ±3.5	35.0 ±2.0	34.0 ±6.1	29.9 ±1.2	29.9 ±1.3	29.8 ±1.0	29.3 ±1.5	32.0 ±0.7
8 month	269.0	213.5 ±26.5	204.0 ±12.0	247.5 ±44.5	206.1 ±5.5	216.1 ±2.7	202.8 ±9.4	209.1 ±5.7	215.3 ±6.4
12 month	-	299.5 ±45.5	278.0 ±17.0	261.0	277.1 ±6.1	288.3 ±6.5	276.7 ±19.2	268.8 ±7.9	283.3 ±4.9
15 month	347.0	349.0 ±56.0	332.5 ±25.5	337.3 ±25.9	319.5 ±6.2	326.0 ±6.8	323.8 ±8.4	314.0 ±6.4	323.8 ±9.7
18 month	389.0	384.0 ±76.0	373.5 ±18.5	386.3 ±17.8	361.7 ±5.5	374.1 ±14.2	364.2 ±9.0	357.2 ±5.7	375.3 ±4.8
2 year	435.0	435.0 ±50.0	401.0 ±21.0	432.7 ±41.7	411.3 ±6.9	417.8 ±9.7	415.7 ±8.9	412.8 ±16.4	411.3 ±5.2
3 year *	467.0	459.5 ±55.5	455.0	560.0	436.3 ±9.9	448.6 ±9.6	467.5 ±21.3	442.8 ±16.4	436.7 ±5.9
4 year *	512.0	506.5 ±43.5	495.0	570.0	479.8 ±13.7	490.6 ±13.1	521.7 ±40.4	471.9 ±11.8	467.7 ±13.0
5 year *	535.0	560	628.5 ±78.5	597.5 ±98.5	563.3 ±24.9	521.3 ±12.8	649.0 ±36.7	559.2 ±33.5	551.7 ±16.9

Notes: n – number of animals per group; $\bar{x} \pm s_x$ – mean ± standard error; * differences are significant at $p < 0.05$.

When genotypes of *GH L127V* and *CAST282* in combination were analyzed it was not found animals with *CC/GG* combination. This can be explained by rare *G* allele in the population analyzed. The maximum body weight was shown in animals with genotype CC/CC (Table 3). Population is in Hardy-Weinberg equilibrium state ($\chi^2_{act.} = 2.1$; $\chi^2_{st.} = 15.5$; $df = 8$; $p > 0.05$). Differences between groups in live body weight reach 30-65 kg, or less than 10% of body weight. These observations are result of calpastatin secondary function as a modulator of calpain activity, being activated by higher Ca^{2+} concentration, thus indirectly affects the growth rate processes. A statistically significant effect on the body weight is observed in the age of four ($F = 12.3$; $p < 0.001$) and five years ($F = 6.9$; $p < 0.001$). Animals from selected groups also differ by the average daily gain (Max: GG/CG = 799 g, Min: CG/GG = 676 g; $F = 11.3$; $p < 0.001$).

Table 3. Body weight dynamics in Aberdeen-Angus by SNPs *GH L127V* and *CAST282*, $\bar{x} \pm s_x$.

Weight, kg	Genotype *GH L127V / CAST282*							
	CC/ CC	*CC/ CG*	*CG/ CC*	*CG/ CG*	*CG/ GG*	*GG/ CC*	*GG/ CG*	*GG/ GG*
n	3	2	15	9	1	10	11	1
Birth	35.3 ±2.0	35.0 ±2.0	29.9 ±1.0	28.6 ±2.2	24.0	29.8 ±1.1	29.8 ±1.4	33.0
8 month	232.0 ±24.0	204.0 ±12.0	209.6 ±4.3	190.4 ±10.0	185.0	209.1 ±3.2	208.3 ±10.9	207.0
12 month	299.5 ±45.5	278.0 ±17.0	279.0 ±4.9	241.7 ±8.9	250.0	273.4 ±4.7	274.4 ±13.7	255.0
15 month	348.3 ±32.3	332.5 ±25.5	319.1 ±5.1	287.6 ±9.4	295.0	320.3 ±5.1	321.7 ±6.9	280.0
18 month	385.7 ±43.9	373.5 ±18.5	364.6 ±6.3	325.0 ±13.1	340.0	365.0 ±4.7	363.0 ±7.0	335.0
2 year	435.0 ±28.9	401.0 ±21.0	408.7 ±6.1	370.6 ±14.1	390.0	404.0 ±5.1	424.1 ±13.6	395.0
3 year	462.0 ±32.1	455.0	442.1 ±8.5	394.3 ±17.3	410.0	439.6 ±4.8	460.0 ±22.1	407.0
4 year *	508.3 ±25.2	495.0	486.1 ±11.2	429.6 ±19.9	455.0	469.9 ±6.3	497.9 ±24.6	445.0
5 year *	547.5 ±12.5	628.5 ±78.5	573.6 ±31.1	436.5 ±21.3	-	576.8 ±32.2	581.6 ±31.6	-

Notes: n – number of animals per group; $\bar{x} \pm s_x$ – mean ± standard error; * differences are significant at $p < 0.05$.

There were positive correlation between the number of *C* alleles of SNP *GH L127V* and animal body weight (Table 4). The table shows that the gene effect is clearly manifested in the age of two years, in the period of intensive animal growth. When analyzed the effect of the number of preferred *C* alleles on live body weight at different ages for the growth hormone and calpain genes, growth hormone and calpastatin genes it was observed a significant correlation at the age from one and two years (Table 4).

Table 4. Correlation of C-alleles number for *GH L127V, CAPN316* и CAST282 and body weight in Aberdeen-angus.

Parameter	Genotype					
	GH L127V		*GH L127V + CAPN316*		*GH L127V + CAST282*	
Statistics	*r*	*T*	*r*	*t*	*r*	*t*
Birth weight, kg	0.90	2.08	0.96**	3.43	0.30	0.32
8 month weight, kg	0.99***	7.16	0.88	1.82	0.75	1.14
12 month weight, kg	0.99***	39.75	0.57	0.69	0.96*	3.45
15 month weight, kg	0.95**	3.08	0.96**	3.68	0.91*	2.21
18 month weight, kg	0.98***	6.08	0.93*	2.65	0.95*	3.17
2 year weight, kg	0.98***	6.67	0.83	1.51	0.80	1.34
3 year weight, kg	0.86	1.75	0.54	0.65	0.89	1.85
4 year weight, kg	0.98***	5.77	0.71	1.00	0.91*	2.24
5 year weight, kg	0.26	0.27	- 0.18	0.18	0.72	1.05

Notes: *r* – Pearson's correlation coefficient; t – Student's t-test; differences are significant at * $p<0.05$, ** $p<0.01$ and *** $p<0.001$.

CONCLUSION

It was found the positive effect of *C* allele of SNPs *CAPN316*, *CAST282* and *GH L127V* in Aberdeen-Angus breed on animal's body weight at different ages. It was concluded, that selection aimed to improve meat quality, *i. e.* for *CC* genotypes of *CAPN316* and *CAST282*, would not lead to a significant reduction in live body weight. There is correlation between the number of *C*-alleles for *GH L127V* and increase in body weight from birth till two years.

REFERENCES

1. Barendse W., Harrison B. E., Hawken R. J., Ferguson D. M., Thompson J. M., Thomas M. B., Bunch R. J. (2007) Epistasis between calpain 1 and its inhibitor calpastatin within breeds of cattle. *Genetics*, vol. 176, no. 4, pp. 2601-2610.
2. Gill J. L., Bishop S. C., McCorquodale C., Williams J. L., Wiener P. (2009) Association of selected SNP with carcass and taste panel assessed meat quality traits in a commercial population of Aberdeen Angus-sired beef cattle. *Genetics Selection Evolution*, vol. 41. Available at: http://www.gsejournal.org/content/41/1/36
3. Goll D. E., Thompson V. F., Li H., Wei W., Cong J. (2003) The calpain system. *Physiological Review*, vol. 83, pp. 731-801.
4. Hadi Z., Atashi H., Dadpasand M., Derakhshandeh A., Ghahramani Seno M. M. (2015) The relationship between growth hormone polymorphism and growth hormone receptor genes with milk yield and reproductive performance in Holstein dairy cows. *Iranian Journal of Veterinary Research*, vol. 16, no. 3, pp. 244-248.
5. Komisarek J., Michalak A., Walendowska A. (2011) The effects of polymorphisms in DGAT1, GH and GHR genes on reproduction and production traits in Jersey cows. *Animal Science Papers and Reports*, vol. 29, no. 1, pp. 29-36.
6. Leea J.-H., Lee Y.-M., Lee J.-Y., Oh D.-Y., Jeong D.-J., Kim J.-J. (2013) Identification of Single Nucleotide Polymorphisms (SNPs) of the Bovine Growth Hormone (bGH) Gene Associated with Growth and Carcass Traits in Hanwoo. *Asian-Australasian Journal of Animal Sciences*, vol. 26, pp. 1359-1364.
7. Miquel M. C., Villareal E., Mezzadra C., Melucci L., Soria L., Corva P., Schor A. (2009) The association of CAPN1 316 marker genotypes with growth and meat quality traits of steers finished on pasture. *Genetics and Molecular Biology*, vol. 32, no. 3, pp. 491–496.
8. Schenkel F. S., Miller S. P., Jiang Z., Mandell I. B., Ye X., Li H., Wilton J. W. (2006) Association of a single nucleotide polymorphism in the calpastatin gene with carcass and meat quality traits of beef cattle. *Journal of Animal Science*, vol. 84, pp. 291-299.