A NEW POLYMORPHISM IN *MYOSTATIN* INFLUENCES BEEF TRAITS IN A BLONDE D'AQUITAINE-HOLSTEIN CROSSBRED POPULATION

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Abstract – The effects on beef traits of the recently discovered mutation in the *myostatin* gene of Blonde d'Aquitaine cattle were estimated in a population segregating this mutation. Three F1 Blonde d'Aquitaine-Holstein crossbred sires were mated either to F1 or Holstein cows to obtain F2 or back-cross calves. Calves were fattened for veal production and slaughtered at 22 weeks of age. Live, slaughter traits and muscle characteristics were recorded. Genotypes at the new polymorphism were determined by RFLP: 19 T/T; 30 T/G and 8 G/G among the 56 calves with genotypes and phenotypes (43 F2 and 13 back-cross). The mutation was shown to have pleiotropic on most of the recorded traits except growth. Muscling and carcass yield were significantly affected positively by the mutation, while fatness and skeletal development were negatively affected. The mutation effects were partially recessive. The mutation also influenced muscle characteristics with higher proportion of fast white glycolytic muscle fibers. All these effects are coherent with published results of former known mutations in *myostatin* gene and can explain the superiority of Blonde d'Aquitaine muscling.

Key Words – beef traits, cattle, myostatin

I. INTRODUCTION

The genetic determinism of muscle hypertrophy in cattle has been extensively studied and the observed results were explained by pleiotropic genetic effects of mutations in the *myostatin* gene, a member of transforming growth factor located on bovine chromosome 2 [1-3]. Among the high number of polymorphisms discovered in this gene, six were located in the coding region and found to be disruptive, resulting in a lack of *myostatin* function [4]. Animals carrying two copies of these disruptive mutations exhibit extreme double-muscling phenotypes. These mutations are mostly breed specific: Belgian Blue cattle / nt821 mutation, Charolais / Q204X, Piedmontese / C313Y, Maine-Anjou / E226X [5]. Another mutation (F94L), specific of the Limousin cattle, was found to determine an intermediate, non-double muscling phenotype [6]. A new mutation *T3811>G3811* was recently found in intron 2 [7]. It produces an abnormal transcript harboring a premature termination codon. Translation of this aberrant transcript predicts a nonfunctional protein lacking the entire bioactive region. This mutation was shown to be almost fixed in Blonde d'Aquitaine cattle and absent in other breeds.

The superior muscularity of Blonde d'Aquitaine cattle is well known. In several sire breed comparison studies, the Blonde d'Aquitaine progeny were characterized with high carcass yields and high lean to fat ratios [8]. These results were intermediate between continental (Charolais or Limousin) and double-muscled (Belgian Blue or Piedmontese) crossbred progeny.

The recently detected mutation in the *myostatin* gene is obviously suspected to contribute to the superior muscularity of Blonde d'Aquitaine animals. An experiment has been conducted for estimating its polymorphism effects on beef traits. A Blonde d'Aquitaine x Holstein crossbred population was produced expecting segregation of the mutated allele among homozygous and heterozygous genotypes.

II. MATERIALS AND METHODS

In order to get the three genotypes (homozygous non mutated T/T; heterozygous T/G and homozygous mutated G/G) crossbreeding was

performed between Blonde d'Aquitaine sires, with G/G genotype, and Holstein cows, with T/T genotype. Three Blonde d'Aquitaine sires were mated to Holstein cows. Three F1 males, one per sire, were used to inseminate either F1 females to obtain F2 calves or Holstein cows to obtain back-cross calves. F1 females were poly-ovulated and F2 embryos implanted into beef heifers.

Soon after birth, calves were brought in a fattening barn where they received a complete milk diet and were intensively fattened. Eventually they were slaughtered at a fixed age (156 ± 5 days) in a commercial slaughterhouse.

The following traits were recorded on calves: birth weight (BW), average daily gain (ADG) and slaughter weight (SW). Three different traits were scored (15 point scale) the last week of fattening: muscularity (LM), fatness (LF) and shank bone thinness (BT). At slaughter carcass weight (CW) and yield (CY) were recorded and carcass length (CL) measured. The leg length (LL) and leg width (LW) were also measured. Carcass muscularity (CM), carcass colour (CC), carcass subcutaneous (CF) and internal fat (IF) were scored (15 point scale). Colour scale spreads from extremely pale to extremely red. Samples of *Longissimus thoracis* (LT) and *Triceps brachii* (TB) muscles were excised for subsequent measure of muscle characteristics: Isocitrate dehydrogenase (ICDH: oxidative) and Lactate dehydrogenase (LDH: glycolytic) activity, proportions of myosin heavy chain isoforms (MyHC) I, IIa, IIx.

The genotypes at the *T3811>G3811* mutation were determined by RFLP-PCR. All the traits were analyzed with the SAS GLM procedure in a linear model including the following fixed effects: sex; 5 contemporary groups; breed composition (F2 or back-cross); 3 sires and 3 genotypes (T/T T/G G/G). The additive substitution effect was estimated as half the difference between homozygous calves a = (G/G – T/T)/2 and the dominance effect was estimated as the difference between the heterozygous and the average of homozygous calves d = T/G – (T/T + G/G)/2.

III. RESULTS AND DISCUSSION

Sixty one calves entered the fattening barn: 45 F2 and 16 backcross calves. The genotypes of the 3 F1 sires and 9 F1 dams were T/G as expected. Among the 61 calves, genotypes could not be assessed for 3 calves due to twinning chimerism. The repartition of genotypes was 10 T/T, 25 T/G and 10 G/G in the F2 population and 8 T/T and 5 T/G in the back-cross population as expected: (¼, ½, ¼) and (½, ½).

Two calves had to be discarded of the analysis due to health problems that affected seriously their growth (-2.5 residual standard deviation, rsd). Both calves had a G/G genotype. The genotype frequencies in the phenotyped calves were: 18 T/T; 30 T/G and 8 G/G.

The breed composition had a significant effect on carcass yield and morphology. With ¾ of Holstein genetic background, back-cross calves had longer carcass, with poorer muscularity and lower dressing percentage. Sex had an effect on numerous traits. The most significant sex effect was on fatness scores: male calves were markedly leaner than females. There was no significant difference among the 3 sires.

Table 1 Least square mean values of T/T genotype, and additive (a) and dominance (d) effects of the G allele on live and carcass traits

Trait		T/T	rsd	a	Prob	d	Prob
BW	kg	43.2	3.7	0.77	0.001	0.07	0.80
ADG	g/d	1,048	88	-0.19	0.46	0.50	0.12
SW	kg	211	14	-0.04	0.87	0.28	0.40
LM	/15	5.9	1.3	1.36	<.001	-0.51	0.02
LF	/15	8.1	1.7	-0.88	<.001	-0.15	0.58
BT	/15	7.3	1.1	0.73	0.003	-0.75	0.01
CW	kg	126	11	0.69	0.005	-0.10	0.72
CY	%	59.9	2.7	1.46	<.001	-0.66	<.001
CL	cm	1,041	28	-0.62	0.01	0.52	0.10
LL	cm	694	13	-0.35	0.18	0.20	0.54
LW	cm	198	11	1.20	<.001	-0.44	0.05
CM	/15	5.8	1.6	1.32	<.001	-0.55	0.01
CC	/15	7.4	1.5	-0.78	0.001	-0.27	0.36
SF	/15	8.2	1.7	-0.79	0.001	0.32	0.27
IF	/15	8.1	1.6	-0.58	0.02	0.32	0.29

RSD: residual standard deviation

In table 1 are reported the estimates of effects associated with the *T3811>G3811* polymorphism. The additive (a) and dominance (d) effects of the G mutation are standardized in unit of residual standard deviations (rsd) that were obtained in a previous GLM including only contemporary group, sex and crossbreeding type effects.

The most important additive effects, above 1.2 rsd, are observed on carcass yield and muscling traits. For these traits, dominance effects are significant and negative, around -0.5 rsd, indicating the mutation impact is partially recessive. For these traits the heterozygous calves are 1.0 rsd above T/T calves while G/G calves exceed T/T by more than 2.4 rsd. The mutation has an opposite effect on fatness with a significant negative additive effect (around -0.8 rsd) and a positive, but not significant, dominant effect. Similar studies were reported by Short et al. [9] in an experimental population segregating the Piedmontese C313Y mutation and Esmailizadeh et al. [10] in an experimental population segregating the Limousin F94L mutation. They found that both mutations are partially recessive, and both increase muscling traits and reduce fat depots. Although not directly comparable, the additive effects of the present *T3811>G3811* mutation are not as high as the C313Y effects and are higher than F94L effects. In a commercial Charolais population where the Q204X was segregating, Allais et al. [11] estimated that differences of muscling and fatness between heterozygous and homozygous non mutated calves were of the same magnitude of the differences found in the present study for the *T3811>G3811* mutation.

Vinet et al. [12] analyzed the genetic determinism of beef traits in the INRA95 strain herd made up by crossbreeding highly muscled Charolais, Belgian Blue, Limousin and Blonde d'Aquitaine animals. They ranked the effects of the mutations that were known at that time (Q204X, nt811 and F94L). A direct comparison of the *T3811>G3811* mutation with the former known mutations is therefore possible in this population if calves could be genotyped for the new polymorphism.

Although birth weight is positively affected by the mutation, no effect was observed on the growth performances of the young calves. The positive effect on carcass weight is entirely a consequence of an improved dressing percentage of carcasses. This mutation has also a significant effect on skeletal characteristics although less important: carcass length is reduced and bones are thinner. Similar effects on skeletal characteristic were described in double-muscled cattle [13].

It is worth to notice the effect of the mutation on the carcass colour that is significantly paler. This effect on the whole carcass muscle colour, can be explained by its effects on muscle characteristics (Table 2).

Table 2 Least square mean values of T/T genotype, and additive (a) and dominance (d) effects of the G allele on muscle characteristics of *LT* and *TB* muscles

Trait		T/T	rsd	a	Prob	d	Prob
Longissimus thoracis (LT) muscle							
ICDH		9.2	3.9	-0.34	0.19	0.02	0.94
LDH		4,834	578	0.14	0.58	0.29	0.37
M I	%	14.2	4.8	0.00	0.99	0.31	0.34
M IIa	%	40.1	10.3	-0.40	0.12	0.41	0.19
M IIx	%	45.7	11.2	0.36	0.15	-0.51	0.11
Triceps brachii (TB) muscle							
ICDH		12.9	2.6	-0.97	<.001	-0.06	0.83
LDH		4,824	438	0.08	0.73	0.79	0.01
M I	%	15.7	3.5	-0.60	0.01	0.80	0.007
M IIa	%	25.4	3.7	-0.80	0.002	0.06	0.83
M IIx	%	58.9	5.7	0.89	<.001	-0.54	0.06

RSD: residual standard deviation

At the level of muscle characteristics the impact of the mutation is more important, and significant, on *TB* muscle as compared to *LT* muscle. Muscles of calves carrying the G allele have a lower oxidative activity and a higher proportion of myosin heavy chain II-x, i.e. a higher proportion of fast white, glycolytic muscle fibers. These muscle characteristics are coherent with the observed paler colour of carcass and with previous data on muscle fibre characteristics in Blonde d'Aquitaine young bulls [14]. These characteristics are coherent with the known effects of double muscling on muscle characteristics [15]. In double-muscled cattle the effect of *myostatin* mutation is different according to the muscles. As observed in the present study, the effects are less pronounced in the *LT* muscle which is not hypertrophied in double-muscled cattle [16].

IV. CONCLUSION

The abnormal transcript produced by the *T3811>G3811* mutation was shown to impair *myostatin* function and consequently suspected to enhance muscling. The present experiment gives the evidence of pleiotropic effects of this mutation on a range of traits related to muscle and skeletal

growth. Muscling and carcass yield are the most impacted traits. Similarly to other myostatin mutations, this new mutation is partially recessive. This recessive property is detectable primarily on traits that are the most impacted by the mutation.
The amplitude of this mutation effect is apparently intermediate between the effect of disruptive mutations (C313Y, Q204X) and effect of the F94L mutation. A fair comparison could be obtained using the multibreed INRA95 strain.
This mutation is apparently almost fixed in the Blonde d'Aquitaine breed. A genetic survey is presently engaged with the breeding organisms for estimating its frequency in the Blonde d'Aquitaine breed. The impact of this polymorphism on beef traits in commercial feed lots and in commercial cow herds will be quantified in order to optimize the mutation management.

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