Animal Science Papers and Reports vol. 21 (2003) no. 3, 147-157
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New polymorphism in the bovine *STAT5A* gene and its association with meat production traits in beef cattle*

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(Received August 14, 2003; accepted September 10, 2003)

STAT5 is a group of transcription factors that mediate signals from prolactin and growth hormone. Therefore, STAT5A gene is a candidate marker for quantitative traits in farm animals. In this study the new nucleotide sequence polymorphism was found in the coding region of the bovine STAT5A gene - substitution C→T at position 6853 within the exon 7. As this mutation creates new *Ava*I/*Dde*I restriction site it could be easily detected with PCR-RFLP analysis.
The RFLP-*Ava*I polymorphism was studied in cattle (n = 146) belonging to eight breeds, including two considered as Polish native. The overall frequencies of alleles C and T were 0.82 and 0.18, respectively. The genotype TT was found exclusively in both native breeds (Polish Red and White-Back).
Moreover, for the first time an association was reported between *STAT5A* gene polymorphism and beef production traits in cattle (n = 71). In the animals of the CC genotype the live body weight at the age of 9 and 15 months, live weight gain (0-15 months), dressing percentage and four carcass traits were found more favourable than in CT animals. Individuals CC used less feed for maintenance and meat production.

KEY WORDS: carcass traits / cattle / growth rate / PCR/RFLP / polymorphism / *STAT5A* gene

Genetic improvement of livestock has been accomplished by selecting animals that express superior phenotypes. Techniques in molecular genetics now enable the actual genotype of an animal to be determined for specific *loci*. This may accelerate future

* Supported by the State Committee for Scientific Research, grant PBZ-KBN-036/P06/12 and Polish Academy of Sciences Institute of Genetics and Animal Breeding, project No. S.I.-2.2.

genetic improvement of livestock through marker-assisted selection (MAS). STAT5 is a key intracellular mediator of prolactin signalling and can activate transcription of milk protein genes in response to prolactin [Wakao *et al*. 1994]. STAT5 is also known as a main mediator of growth hormone (GH) action on target genes [Argetsinger and Carter-Su 1996]. STAT5 exists in isoforms A and B that differ by few amino acids in the carboxylic end of the protein molecule and are coded by separate genes. In the cattle STAT5A gene has been assigned to chromosome 19q17 within 40 Kpz *STAT locus* which also contains STAT3 and STAT5B genes [Seyfert *et al*. 2000, Moleenar *et al*. 2000]. The STAT5 factors interact and functionally synergize with receptors for glucocorticoid and insulin [Lechner *et al*. 1997, Chen *et al*. 1996]. Nucleotide sequence polymorphism has been detected in the bovine STAT5A gene. MCCracken *et al*. [1997] found TG repeats of different length within *STAT5A* intron 12, and Antoniou *et al*. [1999] described two SSCP variants of the gene fragment that encodes SH_2 domain in bovine STAT5A protein. Detection of additional polymorphisms is necessary to help the investigating the role of STAT5A variation in the cattle production traits. In the present study a new nucleotide sequence polymorphism in exon 7 of the bovine STAT5A gene is reported. Moreover, for the first time a significant difference is described between various *STAT5A* genotypes and meat production traits in cattle.

Material and methods

Animals

For studying STAT5A gene polymorphism used were cattle belonging to the following breeds: Polish Black-and-White (BW, n = 30), Polish Red (PR, n = 30), Polish White-Back* (WhB, n = 15), Charolaise (Ch, n = 18), Limousine (L, n = 16), Red Angus (RA, n = 10), Hereford (H, n = 16) and Simmental (S, n = 11). BW, PR and WhB were dairy cows, while the remaining animals were young bulls included in a feed intake and conversion test. BW, Ch, L, RA, H and S were maintained at the Institute Experimental Farm, Jastrzębiec, while PR cattle at the Research Station for Ecological Agriculture and Preserve Animal Breeding, Popielno, Poland. WhB cows belonged to small farmers from Eastern Poland (Lubelski and Białostocki regions).

Approximately 10 ml blood was withdrawn from each animal by authorized veterinarian to the tube containing K_2EDTA. All experimental procedures involving animals were approved by the Local Ethics Commission (permission No. 67/2001).

Associations between *STAT5A/Ava*I gene polymorphism and meat production traits were studied in Ch, L, RA, H and S bulls (n = 71). Bulls of each breed were the randomly chosen progeny of 5-7 sires. The animals were born in beef herds, artificially reared on milk replacer, concentrate and hay and transferred to the Institute farm, Jastrzębiec, at the age of 6-7 months. After reaching the age of 9 months the animals were fed *ad*

**Białogrzbiety*.

libitum a total mixed ration (TMR), consisting of 75% corn silage, 20% concentrates and 5% hay. From the beginning of 13th to the end of 14th month of life the bulls were subjected to the 60-day test period for feed intake and conversion. Over that time the individual intake was recorded daily and chemical composition of the TMR was analysed weekly. One kg TMR contained 451 g DM, 0.36 feed units for maintenance and meat production (UFV, INRA) and 40.95 g PDI (INRA). The bulls were weighed monthly and slaughtered at the age of 15 months at the local abattoir. Right (cold) carcass-sides were obtained and measured, and their valuable cuts measured and dissected into lean, fat and bone. A total of 17 carcass indicators were analysed (Tab. 2).

Characteristics of the bulls have been presented in details by Oprządek *et al*. [2001].

Search for nucleotide sequence polymorphism

PCR reactions and SSCP analysis. DNA was isolated from blood leukocytes according to Kanai *et al*. [1994]. The single-strand conformation polymorphism (SSCP) analysis was carried out as described by Flisikowski and Zwierzchowski [2002]. The 215 bp DNA fragment was PCR amplified using the following primers:

STAT5-7.1: Forward: 5'-CTGCAGGGCTGTTCTGAGAG-3';

STAT5-7.2: Reverse: 5'-TGGTACCAGGACTGTAGCACAT-3'.

Ten μl of PCR product was denatured with formamide and subjected to the electrophoresis in the 8% polyacrylamide gels at the constant temperature 12°C. The gels were stained using the Silver Staining System (Kucharczyk Co.). The patterns of SSCP bands were observed and documented by the Molecular Imager System FX (BioRad).

DNA sequencing. PCR products of different *STAT5A* genotypes were purified with QIAquick® PCR Purification Kit (QIAGEN) and automatically sequenced in ABJ377 sequencer (APPLIED BIOSYSTEMS, USA). Sequence was analysed using Sequence Analyser 2.01 programme (BioRad).

RFLP analysis. Ten μl of PCR product were digested with 10 U of *Ava*I restriction nuclease (New England BioLabs, USA) for three hours at 37°C. The restriction fragments were subjected to electrophoresis in 2% agarose/ethidium bromide gel (GIBCO, BRL, England) in 1 × TBE buffer (0.09 M Tris-boric acid, 0.002 M EDTA). Gels were visualised under UV light and documented in FX Molecular Imager apparatus (BIO-RAD).

Statistical

Associations between meat production traits and *STAT5A* genotypes were estimated in a group of 71 bulls belonging to beef (Ch, L, RA, H) or dual-purpose (S) breeds. The data were analysed with the SAS procedure according to the following model:

$$Y_{ijk} = \mu + G_i + R_j + \beta(x_{ij} - x) + e_{ijk}$$

where: Y_{ijk} – studied trait;

μ – overall mean;
G_i – effect of *STAT5A* *i*-th genotype (*i* = 1, 2);
R_j – effect of *j*-th breed (*j* = 1,...5);
$\beta(x_{ij} - x)$ – regression on the live body weight at the age of 9 months;
e_{ijk} – random error.

The reference *STAT5A* genotypes were CC and CT. Data for all 71 bulls were analysed jointly and the effect of a breed has been included into statistical model.

Results and discussion

RFLP-*Ava*I polymorphism in bovine *STAT5A* gene

PCR-SSCP method was used to identify the polymorphism in intron 6 and exon 7 of the bovine STAT5A gene. First we obtained a specific PCR product of the desirable size of 215 bp. Then the PCR product was denatured and subjected to polyacrylamide gel electrophoresis to find SSCP polymorphism. Results are shown in Figure 1. The number of bands and their position in the gel very clearly show the occurrence of DNA sequence variation. The DNA samples representing different SSCP variants were sequenced. The nucleotide substitution C→T at position 6853 of the *STAT5A* gene was identified (Fig. 2). The polymorphic site was located in the exon 7. The SSCP variant

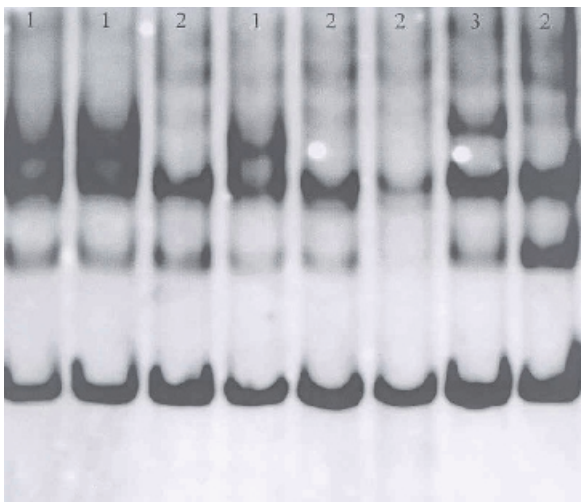

Fig. 1. SSCP polymorphism within intron 6/exon 7 of the bovine STAT5A-encoding gene. Shown are the polyacrylamide gel electrophoretic patterns of the 215-bp gene fragment amplified from DNA of eight animals. The PCR products were denatured, resolved in non-denaturing 8% polyacrylamide gels, and stained with silver. Three different SSCP patterns (genotypes) - 1, 2 and 3 - were observed.

1 and 3 appeared in the CC and TT homozygotes, respectively. The SSCP variant 2 was identified as CT heterozygote. Comparison of the restriction maps of both homozygous variants revealed that C→T substitution creates a new restriction site for *Ava*I and *Dde*I nucleases. Thus, digestion with restriction nuclease *Ava*I (*Eco*88I) enabled PCR-RFLP analysis of the polymorphism (Fig. 3). The nuclease cuts the 215 bp amplification product into 181 and 34 bp fragment for allele T, while allele C remains uncut. Analysis of a reading frame has not shown a change in the amino acid sequence in the protein coded by the polymorphic gene; CCC and CCT triplets both code for the amino acid proline.

Using the PCR-RFLP method the polymorphism was studied in 146 individuals from different breeds. Results are shown in Table 1. The estimated allele frequencies for eight breeds were 0.82 for C and 0.18 for T. The TT genotype was found only in Polish native breeds – PR and WhB. However, due to the small numbers of animals tested in each breed the genotype and allele frequencies cannot be considered as representative for a breed.

Effect of *STAT5A* gene polymorphism on beef production traits

Means for all traits analysed are given in Table 2. The significant effects of *STAT5A* C/T genotypes on growth performance, feed conversion and carcass traits are given in Table 3. At the age of 9 and 15 months the mean live weights of CC bulls were by 33.8 and 27.5 kg higher, respectively, than those of CT heterozygotes. Moreover, CC genotype was associated with significantly faster growth. During the feed intake and conversion test (month 13 and 14) the CC bulls gained daily 1.47 kg as compared to

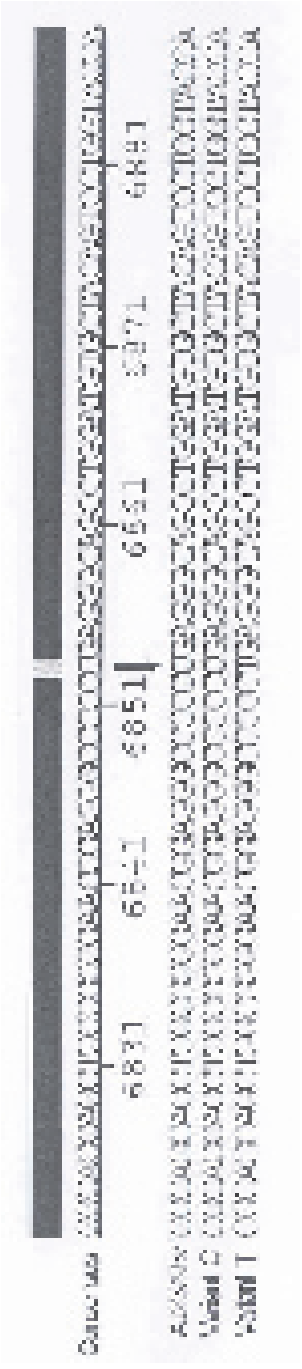

Fig. 2. Sequence alignment of the bovine STAT5A-encoding gene, variants C and T, with the GenBank sequence No. AJ237937.

Fig. 3. Agarose gel (2%) showing genotypes in bovine STAT5A-encoding gene after digestion of the PCR products with *Ava*I nuclease. Genotypes (CC, CT, TT) are shown at the bottom of each line. M – 26-501 bp DNA marker (*Msp*I digest of pUC19). PCR – undigested PCR product.

Table 1. Genotype and allele frequencies at the bovine *locus STAT5A/AvaI*

Genotype and allele	Black-and-White	Polish Red	Polish White-Back	Charolaise	Limousine	Red Angus	Hereford	Simmental
				Number of animals				
CC	20	14	10	13	12	7	14	7
CT	10	12	4	5	4	3	2	4
TT	–	2	1	–	–	–	–	–
				Allele frequency				
C	0.83	0.73	0.8	0.840	0.875	0.85	0.935	0.81
T	0.17	0.27	0.2	0.140	0.125	0.15	0.065	0.19

Table 1. Overall means and their standard deviations of analyzed traits in bulls (n = 71)

(No.)	Trait	Mean	SD
	Live weight (kg)		
(1)	age: 9 months	381.6	51.1
(2)	age: 11 months	417.1	59.4
(3)	age: 15 months	511.1	57.0
	Daily live weight gain (kg)		
(4)	0-9 months	0.84	0.18
(5)	11-15 months	1.41	0.17
(6)	0-15 months	1.04	0.11
	Daily feed intake, records 1-3 and 1-4		
(7)	consumed (kg)	10.69	1.15
(8)	dry matter (kg)	9.41	1.10
(9)	crude protein (kg)	1.01	0.11
(10)	feed units for maintenance and meat production (UFV)	8.46	1.15
(11)	PDI[1] (g)	670.7	86.7
	Feed conversion, records 1-3 and 1-4		
(12)	consumed (kg/kg LWG[2])	15.7	5.7
(13)	dry matter (kg/kg LWG)	7.1	1.4
(14)	crude protein (kg/kg LWG)	0.77	0.17
(15)	feed units for maintenance and meat production (kg/kg LWG)	6.10	1.10
(16)	PDI[1] (g/LWG)	506.0	17.0
	Carcass traits, age: 15 months		
(17)	dressing percentage (cold)	59.9	1.01
(18)	cold carcass (kg)	301.6	41.7
(19)	valuable cuts of carcass-side (kg)	86.1	12.8
(20)	valuable cuts share in carcass-side (%)	60.8	1.4
(21)	loin of valuable cuts (kg)	6.10	1.11
(22)	loin share in valuable cuts (%)	7.17	0.9
(23)	ribs of valuable cuts (kg)	10.1	2.1
(24)	ribs share in valuable cuts (%)	13.1	2.8
(25)	bone of valuable cuts (kg)	11.9	1.8
(26)	bone share in valuable cuts (%)	16.1	1.6
(27)	carcass length (cm)	161.0	5.7
(28)	carcass length from pubic symphysis to 1st lumbar vertebra (cm)	59.1	2.5
(29)	carcass width over the III thoracic vertebra (cm)	67.8	2.1
(30)	carcass length of the thoracic-lumbar section (cm)	104.1	4.4
(31)	width of round (cm)	41.4	4.0
(32)	depth of round (cm)	15.8	1.9
(33)	circumference of round (cm)	112.7	5.7
(34)	length of round (cm)	76.6	2.1

[1] PDI – protein digested in the small intestine. Source: Jamroz (1989)
[2] Live weight gain

1.26 kg found in CT animals. The test showed that CC bulls are superior over the CT heterozygotes. They consumed less total feed as well as dry matter and protein, simultaneously showing better conversion indicators – 6.06 feed units for maintenance and meat production *vs*. 7.4 in CT bulls.

As shown in Table 3 the C/T genotype at exon 7 of the STAT5A-encoding gene significantly affected five out of 17 carcass traits considered in this study. Most important is the weight of cold carcass (by some 20 kg higher in CC than in CT bulls) and lean share in valuable cuts (by 3.9 per cent points higher in CC). Thus, the results presented here show the superiority of the CC genotype, and possibly of C allele, for important meat production traits in beef cattle. Growth rate, feed conversion, as well as four out of 17 carcass traits occurred in favour of CC bulls. We suppose that this phenomenon might concern cattle in general, since the bulls tested in this study were either beef or dual-purpose (Simmental) animals. Unfortunately, no homozygous TT genotype was found in all 71 bulls considered. It is supposed that comparing animals of two homozygous genotypes – CC and TT – could have strengthen the conclusion of the superiority of the CC genotype.

Hormones, growth factors, and other regulatory proteins associated with „soma-

Table 3. Least squares means (LSM) and their standard errors (SE) for traits affected significantly by *STAT5A/MwoI* genotypes

(No.)	Trait	Genotype CC LSM	CC SE	CT LSM	CT SE
	Live weight (kg)				
(1)	age 9 months	298.2[A]	6.2	264.4[B]	10.4
(3)	age 15 months	522.3[A]	5.9	494.8[B]	9.7
	Daily live weight gain (kg)				
(4)	0-9 months	0.88[A]	0.02	0.79[B]	0.03
(5)	13-15 months (test period)	1.47	0.05	1.26	0.08
(6)	0-15 months	1.07[A]	0.01	1.00[B]	0.02
	Intake/kg LWG[2] (test period)				
(12)	total feed (kg)	15.2[A]	0.8	18.6[B]	1.3
(13)	dry matter (kg)	6.8[A]	0.3	8.3[B]	0.6
(14)	crude protein (kg)	0.74[A]	0.04	0.9[B]	0.06
(15)	feed units for maintenance and meat production (UFV)	5.06[A]	0.31	7.4[B]	0.5
(16)	PDI[1] (g)	485.3[A]	25	588.6[B]	4.1
(17)	Dressing percentage (cold)	60.2[A]	0.2	59.3[B]	0.4
(18)	Cold carcass (kg)	298.9[A]	4.02	279.7[B]	6.64
(22)	Lean share in valuable cuts (%)	63.1[A]	0.9	59.2[B]	1.5
(25)	Bone of valuable cuts (kg)	14.0[A]	0.18	13.4[B]	0.3
(33)	Circumference of round (cm)	113.3[A]	0.6	111.1[B]	0.9

[A,B] Within rows means bearing the same superscript differ significantly at P≤0.05.
[1]PDI – protein digested in the small intestine. Source: Jarrige [1989].
[2]Live weight gain.

totropic axis" are candidate markers for quantitative traits in farm animals. Genes encoding for growth hormone (GH), GH receptor (GHR), transcription factor Pit1, insulin-like growth factor 1 (IGF1), and perhaps presently unknown genes coding for GH signal transduction pathways, may contribute to the progress in genetic selection of farm animals. In spite of this, only few studies have been carried out of the effect of these genes on growth performance and carcass traits in cattle. Schlee *et al*. [1994] showed a significant effect of GH L/V genotype on breeding value for meat production in Simmental bulls. In their study the heterozygous LV genotype was superior to LL and VV genotypes in both carcass gain and meat quality. However, when classification score was considered, the LL genotype appeared significantly more desirable than LV or VV. In the same breed, Chrenek *et al.* [1998] reported that bulls of VV genotype reached a lower live weight than those of LL or LV genotypes. In our previous study on Black-and-White (Friesian) bulls, body weight and feed intake strongly depended on the GH genotype; the LV heterozygotes were heaviest and consumed most feed [Zwierzchowski *et al*. 1998]. We also showed that LL and LV genotypes were related to higher meat deposition in Friesian bulls [Oprządek *et al*. 1999], but in beef bulls the VV animals were heavier and showed higher daily live weight gain as compared to LL and LV genotypes [Zwierzchowski *et al*. 2001]. Hale *et al*. [2000] have reported a correlation between the microsatellite marker in the P1 promoter of the bovine GH receptor gene and growth rate in Angus steers. In the study by Parmentier *et al*. [1999] the superior effect of Pit1 transcription factor allele A was shown on body depth, angularity, and rear leg set in Holstein Friesian cattle.

Transcription factors STAT5 may be considered as members of the „somatotropic axis". They mediate the growth promoting action of the pituitary growth hormone on the target cells. Therefore, genes encoding for STAT5A and STAT5B are candidate markers for quantitative traits in cattle. Only in few cases nucleotide sequence polymorphism has been detected in the bovine STAT5A-encoding gene, but no studies were performed to date of the effect of the *STAT5A* polymorphism on production traits of cattle or of any other farm animal. In our previous report [Flisikowski and Zwierzchowski 2002] observed were six different SSCP patterns in a fragment of the STAT5A gene encompassing parts of intron 6 and exon 7. In the present study we precisely identified and localized the STAT5A polymorphic site. The C/T nucleotide substitution appeared at position 6853 within the exon 7 of the bovine STAT5A gene. Exon 7 codes for amino acids 250-480 of the STAT5A molecule in the DNA-binding domain responsible for binding of this transcription factor to promoters of the target genes [Pellegrini *et al*. 1997]. As reported here the RFLP method with the use of *Ava*I restriction nuclease was applied to detect the new polymorphism. Moreover, we found an association existing between the novel polymorphism in exon 7 of the gene and meat production traits in cattle. Our results show the value of the *STAT5A locus* as a marker for growth performance, feed conversion, as well as important carcass traits in beef cattle.

Acknowledgement. Authors are grateful to Mrs Beata Żelazowska for technical assistance.

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Nowy polimorfizm w genie STAT5A bydła i jego związek z cechami produkcyjności mięsnej

S t r e s z c z e n i e

Czynniki transkrypcyjne STAT5 pośredniczą w działaniu prolaktyny na ekspresję genów białek mleka i w aktywacji docelowych genów przez hormon wzrostu (GH). Gen STAT5A jest przeto kandydatem na marker cech ilościowych.

W eksonie 7 genu STAT5A bydła opisano nowy polimorfizm. Najpierw polimorfizm analizowano metodą PCR-SSCP, a następnie próbki DNA różniące się wzorem SSCP poddawano sekwencjonowaniu. Analiza sekwencyjna wykazała, że polimorfizm powodowany jest tranzycją C®T w pozycji 6853. Substytucja znajduje się w kodonie proliny CCC, nie wywołuje jednak zamiany aminokwasu w białku. Mutację można rozpoznać metodą RFLP, stosując nukleazy *Ava*I i *Dde*I. W grupie 146 osobników należących do ośmiu ras bydła stwierdzono zróżnicowaną frekwencję genotypów. Genotyp TT *STAT5A* wystąpił jedynie u bydła polskiego czerwonego (ze Stacji Badawczej PAN w Popielnie) i białogrzbietego (należącego do drobnych hodowców z dawnych województw białostockiego i lubelskiego).

Związek między polimorfizmem genu STAT5A a cechami produkcyjnymi badano na materiale złożonym łącznie z 71 buhajów opasowych ras charolaise, limousine, red angus, hereford i simental. Zwierzęta o genotypie CC charakteryzowały się korzystniejszymi cechami użytkowości mięsnej niż należące do genotypu CT. Buhaje o genotypie CC *STAT5A/Ava*I były w wieku 9 i 15 miesięcy istotnie cięższe, szybciej przyrastały, charakteryzowały się większą wydajnością rzeźną, lepszymi parametrami tuszy i lepiej wykorzystywały paszę w przeliczeniu na potrzeby bytowe i produkcję mięsa niż buhaje CT.