

# Ligand-binding activity of growth hormone receptor (GH-R) in bulls of different breeds with identified GH-R genotypes

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## ABSTRACT

The relationship between the ligand-binding activity of growth hormone receptor (GH-R) and polymorphism in *AluI*, *AccI* and *StuI* sites in the 5' flanking region of bovine GH-R gene was examined. The study was performed on twenty six 15-month old bulls of dairy (Friesians) and beef breeds (Charolais, Limousine, Piemontese, Aberdeen Angus, Hereford). Receptor binding capacity ( $B_{max}$ ) and dissociation constant ( $K_d$ ) for GH-R were determined by the Scatchard method. The polymorphism in the GH-R gene was analyzed by PCR-RFLP technique. Our results showed significant differences in the liver GH-R  $B_{max}$  and  $K_d$  between the dairy and beef breeds.  $B_{max}$  was greater ( $P \leq 0.05$ ) in Polish Friesians as compared to the beef breeds, while  $K_d$  revealed the lowest figure in the dairy breed ( $P \leq 0.01$ ). No significant differences were found in the  $B_{max}$  and  $K_d$  values between genotypes within the 5' flanking region of bovine GH-R gene. In conclusion, ligand-binding activity of the liver GH receptors was shown to differ between the dairy and beef bulls. Furthermore, the polymorphism in the 5' regions of GH-R gene seems to have no influence on ligand-binding properties of the liver GH receptor in cattle.

KEY WORDS: growth hormone receptor, gene polymorphism, cattle

## INTRODUCTION

The biological effects of growth hormone (GH) can be broadly classified as either somatogenic or metabolic. The somatogenic effects, those in which GH

stimulates cell proliferation, are essentially mediated by insulin like growth factor-I (IGF-I). Many of the metabolic effects are direct actions of GH that involve a variety of tissues and the metabolism of all nutrient classes: carbohydrate, lipid, protein and minerals. These coordinated changes in tissue metabolism alter nutrient partitioning and thus play a key role in increasing growth performance or milk yield (Etherton and Bauman, 1998). Therefore, there is interest in using growth hormone to improve production traits in cattle. Moreover, the gene encoding for GH was considered to be a promising candidate as a marker for selection purposes (Grochowska et al., 2001).

Growth hormone actions on various target tissues involve the GH receptor (GH-R) (Burton et al., 1994) and are either directed or indirected *via* IGF-I which acts as an autocrine/paracrine regulator (Butler and Le Roith, 2001). Binding of GH to the GH-R causes its dimerization, activation of the GH-R-associated JAK2 tyrosine kinase, and tyrosyl phosphorylation of both JAK2 and GH-R. These events recruit and/or activate a variety of signaling molecules, including MAP kinases, insulin receptor substrates, phosphatidylinositol 3'-phosphate kinase, diacylglycerol, protein kinase C, intracellular calcium and Stat transcription factors. In a target cell, these signaling molecules contribute to the GH-induced changes in enzymatic activity, transport function and gene expression that ultimately culminate in changes in growth and metabolism (Carter et al., 1996).

The GH receptor is a single transmembrane domain cytokine/haematopoietin superfamily receptor comprising a 246-amino acid (aa) extracellular hormone binding domain, a 24-aa transmembrane domain and a long cytoplasmic domain (Kopchick and Andry, 2000). The bovine GH-R gene consists of 9 exons and is associated to chromosome 20 (Moody et al., 1995). Its transcription is controlled by multiple promoters: liver-specific promoter P1 and promoters P2, P3 driving ubiquitous expression of the GH-R gene (Jiang et al., 1999).

Heterogeneity in the 5' untranslated region (UTR) of the growth hormone receptor gene has been shown in different species of mammals. Nine variants of GH-R mRNAs were identified in humans and in cattle (V1-V9 and 1A-11, respectively) (Goodyer et al., 2001; Jiang and Lucy, 2001a). In the cattle, variant 1A is exclusively expressed in the liver and transcriptionally controlled by the liver-specific transcription factor hepatocyte - nuclear factor-4 (HNF-4) (Jiang and Lucy, 2001b). Moreover, it has been suggested that expression of different transcripts is under developmental- and tissue-dependent regulation (Goodyer et al., 2001; Me-non et al., 2001). Despite a growing amount of information concerning the isoforms of GH-R mRNA, so far no data are available on 5'-UTR heterogeneity in relation to the functional activity of GH-R.

Several polymorphic sequences have been identified in the bovine GH-R gene. Falaki et al. (1996) reported nine genotypes (*TaqI* RFLP polymorphism) within the GH-R gene sequences coding for the intracellular C-terminal part of the receptor.

The effect of this polymorphism on breeding value for milk protein was highly significant in Italian Holstein Friesian bulls. Moisisio et al. (1998) detected three bovine GH-R gene variants differing in the length of a 3' flanking region (311, 320 and 325-bp fragments) and one differing in a base C→G substitution at position +2313. Three polymorphic sites (single nucleotide substitutions) in 5' flanking region of bovine GH-R gene, recognized by RFLP with restriction nucleases: *AluI* (A→T, at -1182), *AccI* (C→T, at -892), and *StuI* (C→T, at -232), were identified by Aggrey et al. (1999). The polymorphism at *AluI* site was shown to be associated with milk production traits. Holstein bulls with *AluI* (+/+) genotype had a higher breeding value for milk fat than Holstein bulls with (-/-) genotype. Hale et al. (2000) reported in Angus cattle the presence of the polymorphic TG-repeat microsatellite, which is located 90 bp upstream of a major transcription start site in the GH-R gene. According to their results the (TG)<sub>11</sub> growth hormone receptor allele decreased growth rates in Angus steers. Ge et al. (1999), using DGGE (denaturing gradient gel electrophoresis) polymorphism analysis, identified three polymorphic variants of bovine GH-R gene promoter region, located within -90 to -308 bp upstream of the starting site of exon 1. The precise nature of this polymorphism and its possible effect on cattle production traits has not yet been studied.

In the present study selected bulls representing 6 cattle breeds differing in sequences within the 5' flanking region of bovine GH-R gene were used. The ligand-binding activity of the liver growth hormone receptor (GH-R) was characterized in the different breeds and GH-R genotypes.

## MATERIAL AND METHODS

### *Animals*

The study was performed on twenty six 15-month old bulls of dairy (Friesians) and beef breeds (Charolais, Limousine, Piemontese, Aberdeen Angus, Hereford). The animals were born at different farms and housed from the age of 4 months until slaughter between March 1997 and June 1999 at experimental farms of the Institute of Genetics and Animal Breeding (Table 1). Animals were fed according to Polish standards (Feeding Standards, 1993). Water was available *ad libitum*. The ration consisted of commercial concentrates (POLFARM, Grodzisk Mazowiecki, Poland), maize silage and hay, and was supplemented with vitamins and minerals. The average metabolizable energy contents and crude protein per kg dry matter were 5.3 MJ and 164.5 g for concentrates, 6.4 MJ and 11.9 g for silage and 3.3 MJ and 88.9 g for hay, respectively.

Blood samples for DNA genotyping were collected from jugular vein by an authorized veterinarian. All bulls were slaughtered at the local abattoir after 24 h-

TABLE 1

Characteristics of dairy and beef bulls used for determination of ligand-binding activity of growth hormone receptor

Item	Breed					
	Polishn Friesian n	Charolais	Limousine	Piemontese	Aberdeen Angus	Hereford
N <sup>1</sup>	6	6	4	3	3	4
Age at slaughter, days						
mean <sup>2</sup>	486	456	456	412	456	457
SD <sup>3</sup>	41	4	3	34	6	4
Body weight at slaughter, kg						
mean	463	527	466	496	474	464
SD	45	57	20	14	47	17

<sup>1</sup> number of bulls

<sup>2</sup> overall mean

<sup>3</sup> standard deviation

fasting. The samples of liver tissue were collected from the same middle part of the small lobe within 15 min after slaughter, frozen in liquid nitrogen and stored at -70°C until assayed. The animal treatments and procedures followed both local and international regulations (Council Directive 86/609/EEC) regarding the protection of animals used for experimental and other scientific purposes.

#### *Tissue preparation*

Frozen liver samples (1g) were homogenized in 10 ml of buffer A (25 mM Tris, 150 mM NaCl and 5 mM MgCl<sub>2</sub>, pH 7.5) 3 times for 5 sec, with 30 sec intervals using Ultra-Turrax T 125 homogenizer (Janke and Kunkel IKA). The homogenates were first centrifuged for 15 min at 150 g to remove nuclear fraction and cell debris and then for 10 min at 50,000 g. The pellets were resuspended in 5 volumes of buffer A and centrifuged for 20 min at 3,000 g. The obtained fraction membrane preparations were suspended in 5 volumes of buffer and used for binding analysis.

#### *Liver GH-R assay*

Recombinant bovine GH (Monsanto, St. Louis, USA) was used as an unlabelled standard as well as the <sup>125</sup>I-labeled ligand, prepared by iodination with the

chloramine T method. The incubation was performed in triplicates by adding to plastic tubes: 0.1 ml of  $^{125}\text{I}$  GH (specific activity 70 TBq/mM), 0.1 ml of buffer A or GH solution, and 0.1 ml of membrane preparation. The range of GH concentrations in incubates of each membrane preparation was from 5 to 800 pmol/l. Incubation was done during 16 h at room temperature in a total volume 500  $\mu\text{l}$  in buffer A containing 0.1% BSA (Sigma, St Louis, USA). Nonspecific binding was measured in the presence of 1000-fold excess of unlabelled bGH and was always less than 5% of total activity added. Separation of bound from free ligand was by filtration (under vacuum) through Whatman GFA glass fibre filters (Whatman, Clifton, CJ). At the end of incubation 1 ml of 25% polyethylene glycol (PEG 6000), dissolved in PBS (pH 7.5) was added, vortex mixed and the mixture was then applied to GFA filters presoaked with 2% BSA in buffer A. The retained activity was measured in a  $\gamma$ -counter (Cobra II, Packard) with a counting efficiency of 85% for  $^{125}\text{I}$ . Receptor binding capacity -  $B_{\text{max}}$  (fmol/g tissue) and dissociation constant ( $K_d \times 10^{-11}$  mol/l) for GH-R were determined by the Scatchard method (Snochowski, 1985).

The statistics used to describe the precision of the estimates of the apparent equilibrium dissociation constant ( $K_d$ ) and maximum number of binding sites ( $B_{\text{max}}$ ) were standard error (S.E.) and 95% confidence interval, respectively. The slope of the plot was tested for difference from zero, and the data obtained were not classified as significant unless the slope was different from zero with 95% confidence.

#### *Determination of GH-R polymorphism*

The polymorphism in the 5' flanking region of the growth hormone receptor gene was analyzed by PCR-RFLP techniques. DNA was isolated from cattle blood according to the method of Kanai et al. (1994). For the GH genotyping, the method previously described by Aggrey et al. (1999) was adapted and modified so that one long 1934-bp DNA fragment was amplified instead of two shorter fragments. The amplified DNA extends from position -1871 to +63 in the GH-R gene and harbored 5 *AluI* sites, 3 *AccI* sites and 1 *StuI* site (analyzed by HIBIO DNASIS, Hitachi, version 2.10). The sequences of primers were as follows:

forward - 5' - TGCGTGACAGCAGCTCAACC-3';

reverse - 5' - GGCAAACAGTGC GGGGTTGGA-3'.

PCR was performed in a volume of 12  $\mu\text{l}$  using 1.4  $\mu\text{l}$  (approx. 100 ng) template DNA, 0.25  $\mu\text{M}$  primers, PCR buffer (50 mM KCl; 10 mM Tris-HCl, pH 8.0; 1.5 mM  $\text{MgCl}_2$ ), 2.5 mM dNTPs, and 1 unit of Taq polymerase (InGen, Sieradz, Poland). Amplification was carried out for 35 cycles: 92°C for 60 sec, 66°C for 80 sec, and 72°C for 120 sec. The 10- $\mu\text{l}$  aliquots of the PCR product were separately digested at 37°C with 5 units of the restriction enzymes: *AluI*, *AccI* and *StuI* (BioLabs, New England, USA) for 3 h. The digested DNA fragments were then

separated by electrophoresis in 2% agarose (Gibco, BRL, England) in  $1 \times$  TBE buffer (0.09 M Tris-boric acid, 0.002 M EDTA) with 0.5 mg/ml ethidium bromide (Et-Br) added to the gels, and visualized under UV light.

### *Statistical analysis*

The data were processed by analysis of variance using the GLM procedure of SAS program (1989). The models contained the fixed effects of GH-R genotype and breed. Body weight at slaughter was treated as a covariate. Since bulls were slaughtered at the same age the effect of age was not considered in the analysis. Similarly, the effect of station had no influence on traits studied. All data were expressed as least square means with standard errors. The GH-R allele frequencies were calculated by allele counting according to the Hardy-Weinberg equilibrium (Falconer and MacKay, 1996).

## RESULTS

There were significant differences in the liver GH-R  $B_{\max}$  and  $K_d$  between dairy and beef breeds (Table 2).  $B_{\max}$  was significantly higher ( $P \leq 0.05$ ) in Friesians (dairy breed) compared to beef breeds, except for the Hereford breed. Furthermore,  $K_d$  revealed the lowest value in the dairy breed ( $P \leq 0.01$ ). No significant differences in ligand-binding of GH-R in the liver were found within beef breeds with the exception for a tendency to greater  $B_{\max}$  in the Hereford compared to the Charolais breed ( $P \leq 0.05$ ).

The results of PCR-RFLP of the 5' flanking region of GH-R gene are shown in Figure 1. *AluI* digestion resulted in 6 main restriction fragments for heterozygous (+/-) genotype (1100, 630, 500, 430, 190, 120 bp). The (+/+) homozygotes differed by the lack of the 1100-bp fragment and in the digests of (-/-) DNA 630-bp and 500-bp fragments were absent. *AccI* (+/-) heterozygotes exhibited 5 restriction fragments (1340, 1040, 430, 310, and 290 bp). In (+/+) and (-/-) homozygotes 1340-bp and 1040/430 bp fragments, respectively, were cut by the nuclease. Digestion with *StuI* showed two genotypes only, characterized by the presence of 1440-bp fragment (+/+) or 1600-bp and 1440-bp fragments (+/-). No (-/-) homozygotes were found.

No clear tendency in the distribution of genotype and allele frequencies of polymorphisms *AluI*, *AccI* and *StuI* in GH-R gene was observed within breeds or between beef and dairy breeds (Table 3). Friesians seemed to have higher frequency of (+) alleles at *AluI* (except for Herefords) and at *AccI* sites polymorphism (except for Aberdeen Angus) than other beef breeds, but the difference was not statistically significant.

TABLE 2

Binding capacity ( $B_{max}$ ; fmol/g tissue) and binding affinity ( $K_d$ ;  $\times 10^{-11}$  mol/l) of growth hormone receptor in the liver of bulls from dairy and beef breeds tested<sup>1</sup>

Breed	$B_{max}$	$K_d$
Polish Friesian	43.39 $\pm$ 4.88 <sup>a</sup>	10.66 $\pm$ 3.04 <sup>A,a</sup>
Charolais	24.26 $\pm$ 7.78 <sup>b</sup>	30.31 $\pm$ 4.68 <sup>B</sup>
Limousine	29.63 $\pm$ 8.59	21.97 $\pm$ 5.20 <sup>b</sup>
Piemontese	36.86 $\pm$ 7.03	18.49 $\pm$ 4.23
Aberdeen Angus	36.81 $\pm$ 9.83	29.67 $\pm$ 5.95 <sup>B</sup>
Hereford	48.63 $\pm$ 9.92 <sup>a</sup>	28.23 $\pm$ 6.01 <sup>B</sup>

<sup>1</sup> values are least square means  $\pm$  standard errors; means within a column followed by different letters were significantly different from one another; <sup>A,B</sup>  $P \leq 0.01$ , <sup>a,b</sup>  $P \leq 0.05$

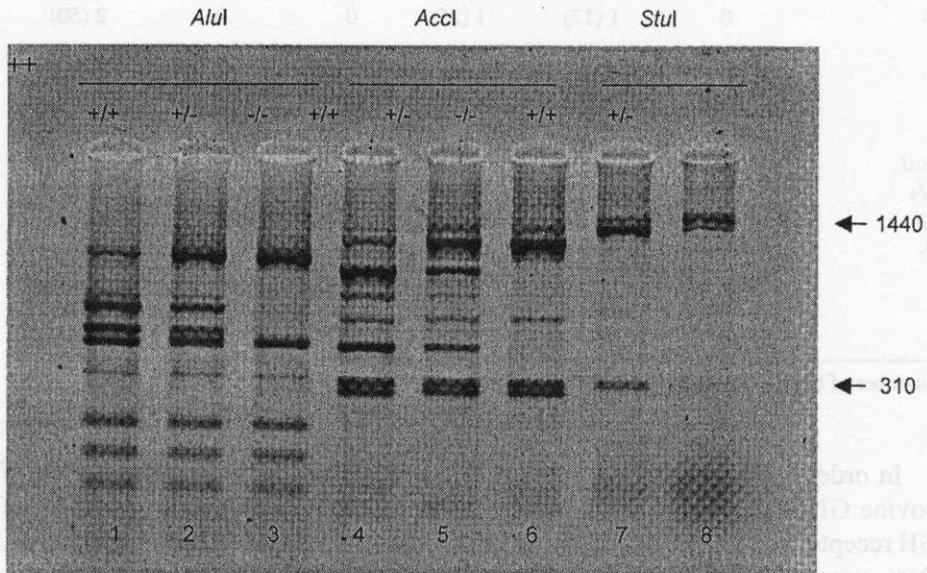


Figure 1. PCR-RFLP analysis of sequence polymorphism within 5' flanking region of the growth hormone receptor (GH-R) gene. Amplified 1934-bp DNA fragment (position from -1871 to +63 in GH-R gene) was digested separately with *AluI*, *AccI* or *StuI* restriction nucleases, resolved electrophoretically in 2% agarose gels, and stained with ethidium bromide. 1-3 - *AluI* restriction fragments; lanes 1, 2, 3 represent (+/+), (+/-), and (-/-) genotypes, respectively; 4-6 - *AccI* restriction fragments; lanes 4, 5, 6 represent (+/+), (+/-), and (-/-) genotypes, respectively; 7, 8 - *StuI* restriction fragments; lanes 7 and 8 represent (+/+) and (+/-) genotypes, respectively. In all cases alleles (+) are cut, and allele (-) are not cut by the respective enzymes

TABLE 3

Distribution of genotype and allele frequencies of polymorphisms *AluI*, *AccI* and *StuI* in the 5'-flanking region of the bovine growth hormone receptor (GH-R) gene for the sample of dairy and beef bulls<sup>1</sup>

Genotypes and alleles of GH-R gene	Breed					
	Polish Friesian	Charolais	Limousine	Piemontese	Aberdeen Angus	Hereford
<i>AluI</i>						
+/+	3 (50)	1 (17)	1 (25)	0	0	2 (50)
+/-	2 (33)	4 (66)	2 (50)	2 (67)	2 (67)	2 (50)
-/-	1 (17)	1 (17)	1 (25)	1 (33)	1 (33)	0
+	0.67	0.50	0.50	0.33	0.33	0.75
-	0.33	0.50	0.50	0.67	0.67	0.25
<i>AccI</i>						
+/+	3 (50)	3 (50)	1 (25)	1 (33)	3 (100)	0
+/-	3 (50)	2 (33)	2 (50)	2 (67)	0	2 (50)
-/-	0	1 (17)	1 (25)	0	0	2 (50)
+	0.75	0.67	0.50	0.67	1.00	0.25
-	0.25	0.33	0.50	0.33	0.00	0.75
<i>StuI</i>						
+/+	5 (83)	6 (100)	4 (100)	3 (100)	3 (100)	4 (100)
+/-	1 (17)	0	0	0	0	0
-/-	0	0	0	0	0	0
+	0.92	1.00	1.00	1.00	1.00	1.00
-	0.08	0.00	0.00	0.00	0.00	0.00

<sup>1</sup> number of bulls with percentage in parentheses

In order to find out whether the polymorphism in the 5' flanking region of bovine GH-R gene may influence GH receptor characteristics,  $B_{\max}$  and  $K_d$  of GH receptor were estimated in the liver of bulls with identified GH-R genotypes. Data were analyzed jointly for all breeds tested. Despite the apparent differences (Table 4), no statistically significant effect of GH-R genotypes on either  $K_d$  or  $B_{\max}$  was observed.

In further analyses all beef breeds were tested together (Charolais, Limousine, Piemontese, Aberdeen Angus and Hereford) and compared to the results obtained for Polish Friesians. As shown in Table 5,  $B_{\max}$  did not differ significantly between GH-R genotypes in both groups – beef and dairy breeds. Significant differences were found in the  $K_d$  value between (+/+) and (+/-) genotypes within

TABLE 4

Genotypic values of homozygotes and heterozygotes within the growth hormone receptor (GH-R) gene for binding capacity ( $B_{max}$ ; fmol/g tissue) and binding affinity ( $K_d$ ;  $\times 10^{-11}$  mol/l) of GH-R in the liver of all bulls tested <sup>1</sup>

GH-R genotypes		$B_{max}$	$K_d$
<i>AluI</i>	+/+	35.27 ± 7.06	18.92 ± 4.20
	+/-	37.00 ± 5.28	26.04 ± 3.05
	-/-	37.40 ± 7.28	21.71 ± 4.34
<i>AccI</i>	+/+	36.11 ± 5.40	23.04 ± 3.31
	+/-	40.39 ± 5.80	25.42 ± 3.45
	-/-	30.05 ± 10.20	18.96 ± 6.17
<i>StuI</i>	+/+	36.47 ± 3.36	22.54 ± 1.94
	+/-	39.76 ± 13.92	36.99 ± 7.87
	-/-	n.d.	n.d.

<sup>1</sup> values are least square means ± standard errors

n.d. – not determined

TABLE 5

Genotypic values of homozygotes and heterozygotes within the growth hormone receptor (GH-R) gene for binding capacity ( $B_{max}$ ; fmol/g tissue) and binding affinity ( $K_d$ ;  $\times 10^{-11}$  mol/l) of GH-R in the liver of bulls from dairy and beef breeds tested <sup>1</sup>

GH-R genotypes	$B_{max}$		$K_d$		
	dairy cattle	beef cattle	dairy cattle	beef cattle	
<i>AluI</i>	+/+	41.83 ± 7.17	32.04 ± 9.10	8.14 ± 4.46 <sup>a</sup>	23.67 ± 5.46
	+/-	48.82 ± 11.33	34.58 ± 5.53	19.53 ± 6.74 <sup>b</sup>	26.24 ± 3.27
	-/-	33.24 ± 13.16	36.05 ± 8.11	3.38 ± 7.91 <sup>a</sup>	24.84 ± 4.87
<i>AccI</i>	+/+	41.21 ± 6.94	30.31 ± 5.86	8.38 ± 4.42 <sup>a</sup>	27.74 ± 3.56
	+/-	43.75 ± 7.99	39.56 ± 6.20	13.98 ± 4.85 <sup>b</sup>	22.96 ± 3.75
	-/-	n.d.	32.09 ± 8.86	n.d.	24.34 ± 5.42
<i>StuI</i>	+/+	40.21 ± 6.17	34.43 ± 3.75	7.44 ± 3.81 <sup>a</sup>	25.37 ± 2.22
	+/-	48.70 ± 10.69	n.d.	19.04 ± 6.36 <sup>b</sup>	n.d.
	-/-	n.d.	n.d.	n.d.	n.d.

<sup>1</sup> values are least square means ± standard errors; means within a column followed by different letters were significantly different from one another (<sup>ab</sup> for  $P \leq 0.05$ )

n.d. – not determined

the 5' flanking region of bovine GH-R gene in Polish Friesians ( $P \leq 0.05$ ). In all cases (polymorphism in *AluI*, *AccI* and *StuI* sites) heterozygotes had greater  $K_d$  than (+/+) homozygotes. A significant difference was also observed between heterozygotes and (-/-) homozygotes in *AluI* polymorphism ( $P \leq 0.05$ ). Due to the small number of observations such differences could not be analyzed in other GH-R genotypes.

## DISCUSSION

Our previous study (Grochowska et al., 1999) showed significantly higher GH secretion parameters in young Friesian heifers and bulls than in Piemonteses. Kazmer et al. (1986) also reported that in Holstein cows selection for high milk yield resulted in a greater GH serum concentration in contrast to the level observed in cows selected for low milk yield or to control animals. This suggests that a higher serum GH concentration appears to be characteristic for dairy cattle when compared with beef cattle, and reflects a genetic potential for high milk production. In the present study we analyzed a possible relation between cattle breed, GH-R gene polymorphism and ligand-binding parameters of the growth hormone receptor.

First, the identification of animals differing in the sequences within the 5' flanking region of bovine GH-R gene was performed on dairy and beef breeds. The data obtained showed that all alleles of GH-R were present in all breeds, except for (-/-) *StuI* allele. High variability of the occurrence of different GH-R genotypes between five beef cattle breeds tested may suggest the possible significant differences might be valid for larger populations of animals. Nevertheless, our results showed that GH-R genotype does not represent the biological parameter, being breed-specific.

In general, the receptor binding data showed no relation between the GH binding to the functional liver GH receptor and the polymorphism in the 5' flanking region of bovine GH-R gene. However, when beef and dairy bulls were analyzed separately, a significant difference was found in the  $K_d$  value between Polish Friesians carrying (+/+) and (+/-) genotypes.

Differences in the  $B_{max}$  and  $K_d$  values observed between dairy and beef bulls may reflect, at least partially, different hormonal status of these animals. The high receptor binding found in the Herefords in comparison with the other beef breeds tested may indicate other genetic features that might be responsible for receptor activity.

The results of functional analysis of GH receptor provide two important pieces of information describing the capacity and the affinity of the receptor. Both parameters characterize the cellular ability to respond to the hormonal signal.

From the physiological point of view the higher capacity informs about the cellular potential to respond to the hormonal stimulus while the higher affinity reflects the higher sensitivity of the liver cell to the GH signal. Greater density of GH receptors and greater affinity of the GH ligand-binding in the liver cells of Friesian bulls than in beef bulls may be related to the remodelling of cellular physiology to match the cell's capacity to respond to the high GH concentration of the highly productive dairy animals. Recently a direct role for GH receptor in mediating the effect of GH on milk production and secretion has been reported in bovine mammary tissue post partum (Sinowatz et al., 2000) with a relatively constant GH-R mRNA content during mammogenesis and lactation.

It was expected to find significant differences in ligand-binding activity of the liver GH-R between different breeds of beef bulls. With the exception of the Charolais breed no such differences were found in this study. The tested breeds of beef bulls represented two different types of farm animals differing in their growth performance. Charolais, Piemontese and Limousine cattle are usually fed intensively and reach much larger body weight than extensively fed Angus and Hereford cattle. It should be noted, however, that no differences of body weight at slaughter were evident between cattle breeds or types, except for the higher weight of Charolais bulls. According to Ohlson et al. (1981) representatives of a larger, faster growing breed of cattle (Simmental) exhibited greater secretory activity of GH than smaller, slower growing breeds, e.g. Herefords. Based on our results it is not possible to conclude that larger breeds represent greater binding capacity and affinity values of the liver GH-R compared to smaller ones.

The trigger for cellular response to GH is its binding to its specific receptor in the cell membrane (Thomas, 1998; Wójcik and Postel-Vinay, 1999) while endocytosis of the GH-R depends on a functional ubiquitin conjugation at the cell surface that is regulated together with the endocytotic machinery (van Kerkhof et al., 2001). It has been shown in humans that short but membrane-anchored forms of the GH receptor might exist in addition to growth hormone binding protein (Mercado et al., 1994; Ross et al., 1997). These short isoforms may modulate the function of GH receptor, as they have been shown to act as dominant negative inhibitors of the full-length receptor. If so, 'functional receptor' activity is a balance among a variety of interactions occurring at the level of target cell plasma membrane and intracellular signalling pathways. The method applied in the present study allows an evaluation of the functional activity of GH receptor, which reflects the responsiveness of the liver to GH regulatory action (Baumeister and Meyerhof, 2000).

It can be concluded that ligand-binding activity of the liver GH receptors may differ between breeds but those differences are not related to polymorphism within the 5' flanking region of bovine GH-R gene. A search for the other genetic indicators for GH-R activity is therefore warranted.

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## STRESZCZENIE

**Reaktywność receptorów hormonu wzrostu (GH-R) w wątrobie buhajów różnych ras bydła o zidentyfikowanych genotypach GH-R**

Celem przeprowadzonych badań było określenie związku między reaktywnością receptorów GH-R a polimorfizmem obszaru flankującego 5' genu GH-R. W badaniach wykorzystano 26 buhajów, w tym 6 rasy mlecznej (czarno-biała) oraz 20 wybranych ras mięsnych (charolais N=6, limousine N=4, piemontese N=3, aberdeen angus N=3, hereford N=4) w wieku 15 miesięcy. W analizie uwzględniono wartość największej liczby miejsc wiążących ligand ( $B_{max}$ ; fmol/g tkanki) oraz stałą dysocjacji ( $K_d$ ;  $\times 10^{-11}$  mol/l), określone metodą Scatcharda. Do identyfikacji genotypów GH-R zastosowano technikę PCR-RFLP. Wykazano istotne różnice w reaktywności GH-R w wątrobie bydła rasy czarno-białej (cb) i ras mięsnych. Stwierdzono, że buhaje rasy cb charakteryzowały się większym  $B_{max}$  ( $P \leq 0,05$ ) oraz mniejszym  $K_d$  ( $P \leq 0,01$ ) w porównaniu z buhajami ras mięsnych. Nie stwierdzono istotnych różnic w liczbie miejsc wiążących ligand oraz stałej dysocjacji między osobnikami o różnych genotypach GH-R. Na podstawie przeprowadzonych badań można sądzić, że polimorfizm obszaru flankującego 5' genu GH-R nie wpływa istotnie na właściwości wiążące receptorów hormonu wzrostu w wątrobie bydła.