SZENT ISTVÁN EGYETEM

AluI polymorphism of bovine growth hormone and growth hormone receptor genes in a Hungarian Holstein-Friesian bull dam population

Ph.D. Theses

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1. Premises and aims of the dissertation

The highlight of physiological background of lactation is highly important due to the primary selection for milk production. Development of mammary gland and its milk synthesis are under endocrine control, in which steroid and protein hormones (i.e. growth hormone) have central roles. Patterns of synthesis of these proteins are genetically determined. Milk production is a typical polygenic trait. Therefore candidate genes with close linkage of the encoding loci for milk production are searched to estimate future lactational performance.

Debates relating to growth hormone generate numbers of studies in this topic in last decades. As a result of a succeeded campaign, it is forbidden to inject exogenous somatotropine in animals in the EU due to human and animal health reasons. Contrary to this, the use of this hormone is absolutely legal and authorised in the United States.

On the basis of these, the aim of my dissertation were to examine growth hormone and growth hormone receptor gene whether they are appropiate markers for milk synthesis and production. I searched for associations between milk production and some reproductive traits and two loci**.**

The aims in detail were as follows:

- Adaptation of methods for the identification of the known polymorphisms of bovine growth hormone and growth hormone receptor genes to Hungarian circumstances on the basis of methods published in the literature.
- Genotyping of the Hungarian Holstein-Friesian bull dams for growth hormone and growth hormone receptor genes. Determination of genotype frequencies in the studied

population. Search for association between the studied genotypes and milk production and composition.

- Investigation for relationships between polymorphisms of growth hormone and growth hormone receptor genes and reproductive traits in the representative bull dam population.

2. Material and methods

2.1. Animals, samples, identification of GH and GHR genotypes

A representative sample of the Hungarian Holstein-Friesian bull dam population was used in this study (365 dams). Animals originated from 6 herds throughout Hungary and represent the whole Holstein-Friesian dam herd. Blood samples were collected from all animals with veterinarian assistance and then DNA isolation was performed from white blood cells.

Lactation and some reproductive data are recorded to analyse the potential differences in performance. This data package included 6 herds, 4 lactation periods and 4 seasons of calving.

PCR-RFLP method was used to amplify the two desired DNA fragments. The GH primers were designed to amplify a 427 bp fragment using the published DNA sequence of the bGH gene. The 427 bp target DNA contained the site of interest in exon 5, 55 bp of exon 4 and the entire intron 4. GHR primers generated a 286 bp fragment which included the 10. exon with the mutated site.

The fragments were spliced by *Alu*I endonuclease in both cases, resolved in agarose gels stained with ethidium bromide and visualised under UV light.

Genotype frequencies of growth hormone and growth hormone receptor genes were determined in the studied population.

2.2. Databases and statistical analysis

Lactation and some reproduction data were collected to analyse the potential differences in performance.

Lactation data contained days in milking, persistency, mean and maximum milk kg of test milkings, 305 days milk yield (kg), 305 days milk fat kg and percent and 305 days milk protein kg and percents. Lactation performance data were recorded in 10 consequent lactational periods, but only the first four lactations of cows were involved in statistical analysis. Reproduction data included age at first calving (days) and calving interval (days) as well. Database included totally 721 lactation and reproduction data of 365 genotyped bull dams.

Genotyping of samples was followed by population genetic calculi on the base of Hardy-Weinberg equilibrium and χ^2 test.

Method of multivariate analysis of variance and predicted marginal means were used to prove statistical relationship between the studied loci (GH-*Alu*I and GHR-*Alu*I) and milk production and reproduction traits. Calculations were carried out by SPSS 11.0 for Windows software.

General Linear Model analysis used the following model in case of growth hormone *Alu*I locus:

$$y_{ijklm} = \mu + GH_i + number\ of\ lactations_j + GH_i * number\ of\ lactations_j + birth\ year_k + calving\ season_l + farm_m + e_{ijklm}$$

This model was modified in case of growth hormone receptor AluI locus as follows:

$$y_{ijklm} = \mu + GHR_i number\ of\ lactations_j + GHR_i * number\ of\ lactations_j + birth\ year_k + calving\ season_l + farm_m + e_{ijklm}$$

Dominance and additive effect of alleles of each loci were also calculated. Finally, the calculation of significance levels of these values was made by Fisher's least square analysis.

3. Results

3-3 patterns were identified as the result of *Alu*I digestion in case of both loci. (GH gene: LL, LV, VV; GHR gene: AA, AG, GG). The following allele frequencies were detected in the sample population: LL: 87,05%; LV: 12,40% és VV: 0,55%; AA: 77,8%; AG: 20,6% és GG: 1,6% .

Population genetic studies (X^2-test) proved Hardy-Weinberg equilibrium in case of both loci.

Results of association analyses between loci and quantitative traits were as follows.

3.1. GH gene, AluI polymorphism

According to the results of multivariate analysis of variance, it can be stated that GH *Alu*I locus influenced reproductive traits significantly ($p<0,05$) (age at first calving, calving interval).

When I analysed production traits (days in milking, dry period, average and maximum of test milkings, persistency, 305 days milk yield, 305 days milkfat kg and percent, 305 days milkprotein kg and percent) together, it was revealed that variants of GH gene again significantly ($p<0,05$) affected milk production performance.

If the different GH-*Alu*I genotypes were compared to each other, significant ($p<0,05$) differences were detected between genotypes in milking days, dry period and test milk kg. VV genotype had significantly more days in milking than the others. However the low incidence of VV genotype must be considered! LV cows, compared to LL ones, showed higher values in case of dry period and test milk kg. We shal then

assume a relationship between the presence of V allele and higher test milk quantity.

305 days milk yield, milk protein- and milk fat percent were showed to have significant ($p<0,05$) differences between the performance of GH genotypes. LV individuals produced higher quantity of milk compared to LL genotype if 305 days milk yield was studied. Except that mean value of VV genotype was not reliable from statistical point of view, this result may lead to the conclusion that the presence of V allele positively affected milk yield. 305 days milk fat and milk protein percents of LL animals were significantly higher than in case of LV cows. These findings together with literature results seem to confirm the association between the presence of L allele and higher milk composition data.

Analysis of dominance showed that correction effect of LV genotype was 514 kg milk in case of 305 days milk yield. LL genotype increased 305 days milk fat percent by 0.08% and 305 days milk protein percent by 0.13%. These effects were proved to be significant ($p<0.05$).

3.2. GHR gene, AluI polymorphism

According to the results of multivariate analysis of variance, it can be stated that GHR *Alu*I locus did not really influenced reproductive traits (age at first calving, calving interval) only a tendency could be observed.

After comparing least square means of reproductive traits, significant difference ($p<0.05$) was found in calving interval between GHR genotypes. GG cows had longer calving interval than AA and AG genotypes.

When production traits were analysed (milking days, dry period, average and maximum of test milkings, persistency, 305 days milk yield, 305 days milkfat kg and percent, 305 days milkprotein kg and percent) together, it was revealed that variants of GHR gene individually had no or only tendential significant effect on milk production performance. However the interaction of GHR genotype and number of calvings significantly ($p<0.05$) influenced milk production traits.

305 days milk yield, milk protein- and milk fat percent were showed to have significant ($p<0,05$) diferences between the performance of GHR genotypes. GG dams produced more milk compared to AA and AG genotypes if 305 days milk yield was studied. This result may lead to the conclusion that the presence of G allele positively affected milk yield. This effect mainly prevail in case of homozygotes, where G allele is presented in two copies. 305 days milk fat and milk protein percents of GG animals were significantly lower than in case of AA and AG cows. These findings seem to indicate an association between the presence of A allele and higher milk composition data.

Results of additive effect confirmed that correction effect of GG genotype was 514 kg milk in case of 305 days milk yield. AA genotype increased 305 days milk fat percent by 0.13% and 305 days milk protein percent by 0.09%. Analysis of dominance also showed the enhancing effect of GG genotype in milk yield. These effects were proved to be significant ($p<0.05$).

A paralel tendency could be noted in lactation milk production in case of both loci (GH-*Alu*I and GHR-*Alu*I). In both cases, the presence of alleles with low incidence (GH: Vallele; GHR: G allele) seems to increase 305 days milk yield.

4. New scientific results

1. Identification methods of AluI polymorphisms in growth hormone and growth hormone receptor genes were successfully modified on the basis of published PCR-RFLP conditions.
2. Allele frequencies and genotype distribution in Holstein-Friesian bull dam population were calculated in case of AluI loci of growth hormone and growth hormone receptor genes first in Hungary.
3. Significant relationship was identified between growth hormone AluI variants and milk production traits. LV cows had higher results comparing to LL in dry period, maximum and mean vslue of test milkings. It seems to be a close relationship between the presence of V allele and higher test milkings.
4. Again significant association was found between GH-AluI genotypes in 305 days production. LV animals showed significantly better production results in 305 days milk yield comnparing to LL genotype. If statistically unreliable means of VV cows are omitted, this result will lead to the conclusion thet the presence of V allele positively influences milk quantity.
5. There was no significant relationship between AluI genotypes of growth hormone gene and the studied reproductive traits.
6. Significant differences were found between AluI genotypes of growth hormone receptor gene in case of lactation production. GG dams had significantly higher 305 days milk yield results than AA and AG dams. It is concluded that presence of G allele may influence the

quantity of produced milk. This effect is prevailed mainly in case of homozygotes when G allele is presented in two copies. In case of 305 days milk fat and milk protein percent GG cows produced higher values comparing to AA and AG genotypes.

7. If least square means of reproductive traits were compared between GHR genotypes, it revealed that GG dams had higher calving interval comparing to AA and AG animals.

5. Conclusions and proposals

Frequencies of V and G alleles are fairly low in case of both loci. In the studied Holstein-Friesian bull dam population (V allele: 7%; G allele: 12%). This confirms literature data and indicates that breeding aim and improvement of quantitative traits favoured the spread of L and A alleles.

In both cases of growth hormone and growth hormone receptor genes, allele frequency calculi proved the studied null-hypothesis that sample population is in Hardy-Weinberg equilibrium. Consequently, selection pressure for the two loci could not be detected. This equilibrium, however, could further indicate that the studied population is representative and describe well the Hungarian Holstein-Friesian bull dams herd and studied animals represents the majority of the above mentioned herd.

Significant relationship were detected again in case of both loci between gene variants in 305 days milk yield, milk protein and milk fat percents. A paralell tendency could be observed between the two studied loci in case of lactation milk production.

The presence of V allele (in case of growth hormone gene) and G allele (in case of growth hormone receptor gene) seemed to influenced positively milk quantity.

A növekedési hormon gén esetében a V, a növekedési hormon receptor gén esetében pedig a G allél (mindkét esetben a kis százalékban előforduló allél) jelenléte valószínűleg pozitívan befolyásolja a termelt tej mennyiségét. In case of GHR gene, this effect is prevailed mainly in case of homozygotes when G allele is presented in two copies.

Literature results in this topic together with my findings might confirm that presence of L allele (GH gene) seem to be attached to higher milk fat and milk protein percents. This relationship also exist in case of GHR gene, where the presence of A allele could be associated with more concentrated milk.

However, further examinations, taking into greater ratio of Hungarian Holstein-Friesian milk producing population, are needed to draw the final conclusion. It would also be worth to increase sample number because of rare genotypes.

On the basis of results of this dissertation, it could be suggested to build growth hormone and growth hormone receptor genotypes, as markers of milk production, in breeding indices (e.g. Holstein Global Index).

6. List of publications

Papers published in periodicals:

1. **Kovács K.**, Fésüs L., Zsolnai A., Györkös I. (2001): A szarvasmarha növekedési hormont (szomatotropin) kódoló gén. Szemlecikk. Álattenyésztés és Takarmányozás, 50. 105-113.
2. **Kovács K**., Zsolnai A., Bölcskey K., Györkös I., Fésüs L. (2002): A szarvasmarha szomatotropin gén AluI polimorfizmusa és a termelési tulajdonságok közötti összefüggés magyarországi holstein-fríz bikanevelő tehenekben. Állattenyésztés és Takarmányozás, 51. 1-7.
3. Balogh O., **Kovács K**., Kulcsár M., Kátai L., Zsolnai A., Gáspárdy A., Reiczigel J., Fésűs L., Huszenicza Gy. (2004): Interrelations of the STH genotype (*Alu*I polymorphism) and first ovulation in postpartum dairy cows. Abstract. Biotechnology, Agronomy, Society and Environment, 8. 36.
4. Balogh O., **Kovács K**., Kulcsár M., Kátai L., Zsolnai A., Gáspárdy A., Reiczigel J., Fésűs L., Huszenicza Gy. (2004): Interrelation of the STH genotype (*Alu*-I polymorphism) and first ovulation in postpartum dairy cows. Abstract. Reprod. Dom. Anim., 39. 260-261.
5. Balogh O., **Kovács K**., Kulcsár M., Zsolnai A., Gáspárdy A., Reiczigel J., Kátai L., Fésűs L., Huszenicza Gy. (2005) A növekedési hormon genotípus (*Alu*-I polimorfizmus) hatása az ellés utáni első ovuláció idejére holstein-fríz tehenekben. Állattenyésztés és Takarmányozás, 54. 237-245.
6. **Kovács K.**, Völgyi-Csík J., Zsolnai A., Györkös I., Fésüs L. (2006): Associations between the AluI polymorphism of growth hormone gene and production and reproduction traits in a Hungarian Holstein-Friesian bull dam population. Archiv für Tierzucht, accepted, under publication

Conference papers:

1. **Kovács K**., Zsolnai A., Györkös I., Fésüs L. (1999): A szarvasmarha növekedési hormon gén alléljeinek gyakorisága magyar holstein-fríz populációkban. Kitörési pontok a magyar állattenyésztésben, Tudományos Konferencia, MTA, Budapest, november 24.
2. **Kovács K**., Fésüs L., Györkös I. (2000): A szarvasmarha növekedési hormon génnel kapcsolatos kutatási eredmények. 73. OMÉK Fiatal Agrárkutatók Fóruma, Budapest, szeptember 2-7.
3. **Kovács K**., Zsolnai A., Bölcskey K., Györkös I., Fésüs L. (2001): Association of the bovine growth hormone gene polymorphism with production traits in Hungarian Holstein Friesian cows. Állattenyésztők Társaságának 52. Konferenciája, Budapest, augusztus 26-29.
4. **Kovács K**., Fésüs L., Völgyi-Csík J., Györkös I., Zsolnai A. (2003): A szarvasmarha novekedési hormon gén AluI polimorfizmusának vizsgálata bikanevelő HF teheneknél. Akadémiai Beszámolók, SZIE Állatorvostudományi Kar, Budapest, január 21.
5. **Kovács K**., Zsolnai A., Völgyi-Csík J., Györkös I., Anton I., Fésüs L. (2003): A szarvasmarha szomatotropin gén AluI polimorfizmusa magyarországi bikanevelő holstein-fríz teheneknél. Magyar Genetikai Konferencia, Siófok, április 14-15.
6. **Kovács K**., Zsolnai A., Völgyi-Csík J., Petró T., Fésüs L. (2004): A szarvasmarha növekedési hormon gén AluI polimorfizmusa magyar holstein-fríz bikanevelő populációkban. X. Ifjúsági Tudományos Fórum, Veszprémi Egyetem Georgikon Mezőgazdaságtudományi Kar, Keszthely, április 29.

7. Balogh O., **Kovács K**., Kulcsár M., Kátai L., Zsolnai A., Gáspárdy A., Reiczigel J., Fésüs L., Huszenicza Gy. (2004): A növekedési hormon genotípus (*Alu*-I polimorfizmus) hatása az ellés utáni első ovuláció időpontjára holstein fríz tehenekben. MTA Állatorvos-tudományi Bizottsága beszámoló ülés, SZIE-ÁOTK, Budapest, január 26.
8. Balogh O., **Kovács K**., Kulcsár M., Kátai L., Zsolnai A., Gáspárdy A., Reiczigel J.., Fésüs L., Huszenicza Gy. (2004): Interrelation of the STH genotype (*Alu*-I polymorphism) and first ovulation in postpartum dairy cows. Oral presentation. 8th Annual Conference of the European Society for Domestic Animal Reproduction, Agricultural University, Varsó, Lengyelország, szeptember 23-25.
9. Balogh O., **Kovács K**., Kulcsár M., Gáspárdy A., Zsolnai A., Reiczigel J., Kátai L., Fésüs L., Huszenicza Gy. (2004): Possible role of the STH genotype (*Alu*I polymorphism) in the length of postpartum acyclic period in dairy cows. Proc. of the 7th EAAP/ASAS/COST Workshop: Biology of Lactation, Bled, Szlovénia, szeptember 9-10.; pp. 22-23.
10. **Kovács K**., Völgyi-Csík J., Zsolnai A., Fésüs L. (2005): Associations between AluI polymorphism of bovine growth hormone (GH) gene and production and reproduction traits in a Hungarian Holstein-Friesian dam population. Ph.D. Hallgatók 5. Nemzetközi Konferenciája, Miskolc, augusztus 14-20.

Other papers:

1. **Kovács K.** (2005): A szarvasmarha szomatotropin tejelő tehenekre gyakorolt hatása. Holstein Magazin, 12. 6. 27-29.