IDENTIFICATION OF BETA-LACTOglobulin AND KAPPA-CASEIN GENOTYPES USING PCR-RFLP IN HOLSTEIN CATTLE

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MILK

All mammals produce milk to feed their young. The milk is used to help the young mammal grow and develop, and so contains all necessary nutrients to enable maturation of the young until they are able to feed themselves.
Milk proteins:

- Caseins (%80)
  - $\beta$ - Caseins (%27)
  - $\alpha_s$ - Caseins (%43)
  - $\kappa$ - Caseins (%10)

- Whey Proteins (%20)
  - Lactoalbumins (%2)
  - Lactoglobulins (%15)
  - Proteose-Peptones (%3)
\( \beta \)-lactoglobulin;

- The major protein of bovine milk whey, is found in a number of genetic variants of which A & B predominant.

- A & B variants of \( \beta \)-LG may affect milk composition and properties.

- \( \beta \)-LG AA genotypes; \( \uparrow \) \( \beta \)-LG, \( \downarrow \) caseins, \( \downarrow \) fat

- \( \beta \)-LG BB genotypes; \( \uparrow \) caseins

- \( \beta \)-LG B allele and BB genotype mastitis resistance \( \uparrow \)
The gene encoding β-lactoglobulin has been mapped on chromosome 11 (BTA 11) in cattle.
- **β-LG A** variant differs from **B** variant by two amino acid only Aspartic acid-64 and Valine-118.

<table>
<thead>
<tr>
<th>64. Codon</th>
<th>118. Codon</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>β-LG A</strong></td>
<td><strong>β-LG B</strong></td>
</tr>
<tr>
<td>GAT</td>
<td>GGT</td>
</tr>
<tr>
<td>(Aspartic acid)</td>
<td>(Glycine)</td>
</tr>
</tbody>
</table>

- **Hph I** restriction site
- **Hae III** restriction site
κ-casein;

- CSN3 plays an important role in preserving the other caseins from precipitation.

- Treatment of milk with rennin cleaves CSN3, resulting in curd formation.

- CSN3 variants affect the cheese making properties of milk.

- CSN3 B allele was reported to have favourable and significant effect on both milk and milk protein yield.
k-casein gene has been mapped on chromosome 6 (BTA 6).
CSN3 A variant differs from B variant by two amino acid only Threonine-136 and Aspartic acid-148.

<table>
<thead>
<tr>
<th></th>
<th>136. Codon</th>
<th>148. Codon</th>
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</thead>
<tbody>
<tr>
<td>CSN3 A</td>
<td>ACC</td>
<td>GAT</td>
</tr>
<tr>
<td></td>
<td>(Threonine)</td>
<td>(Aspartic acid)</td>
</tr>
<tr>
<td>CSN3 B</td>
<td>ATC</td>
<td>GCT</td>
</tr>
<tr>
<td></td>
<td>(Isoleucine)</td>
<td>(Alanine)</td>
</tr>
</tbody>
</table>

*Hinf I* restriction site

*Hind III* restriction site
AIM

The aim of this study was to determine genotypic and allelic frequencies of $\beta$-LG and CSN3 in Holstein cattle populations in Turkey.
MATERIALS & METHODS
<table>
<thead>
<tr>
<th>Province</th>
<th>Enterprise</th>
<th>Sample Size</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ankara</td>
<td>Bala Agricultural Enterprises</td>
<td>78</td>
</tr>
<tr>
<td>Şanlıurfa</td>
<td>Ceylanpınar Agricultural Enterprises</td>
<td>89</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td></td>
<td><strong>167</strong></td>
</tr>
</tbody>
</table>
Blood Samples

- Blood samples were collected by puncture of jugular vein into sterile tubes containing EDTA.

Genomic DNA Isolations

- Salting-out
  - 1% agarose gel electrophoresis
  - spectrophotometer at $A_{260} / A_{280}$ nm
Amplification of $\beta$-LG gene by PCR

**Forward primer:**
5' ACC TGG AGA TCC TGC TGC AGA AAT G 3'

**Reverse primer:**
5' CAT CGA TCT TGA ACA CCG CAG GGA T 3'

<table>
<thead>
<tr>
<th>Temperature</th>
<th>Time</th>
<th>Stage</th>
</tr>
</thead>
<tbody>
<tr>
<td>94 °C</td>
<td>3 min.</td>
<td>Initial denaturation</td>
</tr>
<tr>
<td>94 °C</td>
<td>1 min.</td>
<td>Denaturation</td>
</tr>
<tr>
<td>61 °C</td>
<td>30 sec.</td>
<td>Annealing</td>
</tr>
<tr>
<td>72 °C</td>
<td>2.5 min.</td>
<td>Extension</td>
</tr>
<tr>
<td>72 °C</td>
<td>5 min.</td>
<td>Final extension</td>
</tr>
</tbody>
</table>

30 cycles
**Amplification of CSN3 gene by PCR**

**Forward primer:**
5' GTG CTG AG(T/C) AGG TAT CCT AG 3'

**Reverse primer:**
5' GTA GAG TGC AAC AAC ACT GG 3'

<table>
<thead>
<tr>
<th>Temperature</th>
<th>Time</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>95 °C</td>
<td>5 min.</td>
<td>Initial denaturation</td>
</tr>
<tr>
<td>94 °C</td>
<td>1 min.</td>
<td>Denaturation</td>
</tr>
<tr>
<td>57 °C</td>
<td>1 min.</td>
<td>30 cycles</td>
</tr>
<tr>
<td>74 °C</td>
<td>3 min.</td>
<td>Annealing</td>
</tr>
<tr>
<td>72 °C</td>
<td>5 min.</td>
<td>Extension</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Final extension</td>
</tr>
</tbody>
</table>
**Incubation of β-LG PCR products with Hph I restriction enzyme**

15 µl PCR + 10 µl Hph I restriction mix

37 °C for 1 h.

5' ....GGTGA(N)$_8$↓.....3'

3' ....CCACT(N)$_7$↑.....5'

\[ \xrightarrow{Hph I} \]

5' .. GGTGA(N)$_8$ + ....3'

3' .. CCACT(N)$_7$ + N....5'
Incubation of CSN3 PCR products with Hind III restriction enzyme

10 µl PCR + 10 µl *Hind* III restriction mix
37 ºC for 1 h.

\[ \begin{align*}
5' \cdots A & \downarrow AGCTT \cdots 3' \\
3' \cdots TTCTGA & \uparrow A \cdots 5'
\end{align*} \]

\[ \begin{align*}
5' \cdots A & 5' \cdots A \\
3' \cdots TTCTGA & + AGCTT \cdots 3' \\
A \cdots 5' & A \cdots 5'
\end{align*} \]
Detection of alleles at the β-LG locus

F. Primer

Exon 2

Hph I Restriction Site

961 bp

Hph I

Exon 3

R. Primer

Allele A

741 bp

220 bp

Allele B

741 bp

166 bp

54 bp
Detection of alleles at the κ-CN locus

Hind III Restriction Site

gtgctgag(t/c)aggtctctag

Exon 4

Amplified DNA (874 bp)

ggtcacaacaacgtagatg

Allele A

874 bp

Allele B

521 bp

353 bp
Statistical Analysis

Counting the number of gene was used to estimate gene and genotype frequencies of β-LG and CSN3. The $\chi^2$ test was used to check whether the populations were in Hardy-Weinberg equilibrium or not.
RESULTS
Identification of β-LG Genotypes

Identification of β-lactoglobulin genotypes on %2 agarose gels by PCR-RFLP (M 100 bp DNA marker).
Identification of κ-casein genotypes on % 2 agarose gels by PCR-RFLP (M 100 bp DNA marker).
<table>
<thead>
<tr>
<th>Herd</th>
<th>No. of animals</th>
<th>Genotype β-LG</th>
<th>Genotype CSN3</th>
<th>Allele frequency β-LG</th>
<th>Allele frequency CSN3</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>AA BB AB AA BB AB A B A B</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bala</td>
<td>78</td>
<td>17 15 46 50 3 25</td>
<td>0.51 0.49 0.80 0.20</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ceylanpınar</td>
<td>89</td>
<td>10 39 40 65 5 19</td>
<td>0.34 0.66 0.84 0.16</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*p<0.05*
DISCUSSION

- Bala and Ceylanpınar populations were found to be polymorphic in two loci.
- In the β-LG locus, both populations were in Hardy-Weinberg equilibrium, while in the CSN3 locus only one of the populations was in the Hardy-Weinberg equilibrium.
This seemingly unexpected result might be, either due to the sampling error, or, due to that the bull who gives his sperm to the cows was a selected certain one, not randomly taken. Whereas in the case of bull being selected, not only one, but both loci are expected to be biased from the Hardy-Weinberg equilibrium.
So we could say that the bias from Hardy-Weinberg equilibrium was a sampling error. If you have decided the rejection criteria as the probability $p<0.01$ instead of $p<0.05$, you have accepted the hypothesis that the population was in Hardy-Weinberg equilibrium.
Further studies looking at the relation of the various yield and quality features of milk with the genetic variation in the milk proteins such as β-Lactoglobulin and K-casein can give rise to getting some molecular genetic markers as selection criteria for milk production.
THANK YOU...