COMPOSITION OF PROTEIN FRACTIONS OF BLOOD PLASMA IN HEIFER CALVES NATURALLY INFECTED WITH BOVINE LEUKAEMIA VIRUS

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Abstract

This study was aimed at determining the dynamics of changes in the level of protein fractions and total protein content in blood plasma, during growth and development of heifers naturally infected and non-infected with bovine leukaemia virus (BLV). Investigations were carried out on 64 heifers 3-7 months old, originating from one herd. The BLV infection was diagnosed using the ELISA and PCR test. The study demonstrated the effect of age of young animals on the dynamics of changes in the composition of protein fractions and total protein content in their blood plasma. It refers in particular, to the fraction of $\beta_1$-globulins and total protein. However, changes induced by natural BLV infection in young heifers were not great and concerned only the fraction of $\beta_2$-globulins.

Key words: heifers, bovine leukosis, age, protein content, protein fractions.

Infection with bovine leukaemia virus (BLV) results in dysregulation of the host immune system at both humoral and cellular levels (11, 17), in which the major function is ascribed to cytokines. These substances affect the extent, character, and time span of an immunological response by influencing the activation, proliferation, and differentiation of cells (1, 8). As a consequence, cytokines affect the concentration of acute-phase proteins (APP) that participate in the inflammatory response induced by infection, neoplastic disease, and other factors (4, 16). The proteins occur in blood plasma and their concentration changes under particular physiological and pathological conditions. This is reflected in the quantitative and qualitative composition of protein fractions in blood plasma (2, 20).

The present study was undertaken to determine the dynamics of changes in the content of protein fractions and total protein in blood plasma during growth and development of heifers naturally infected with bovine leukaemia virus.

Material and Methods

Material and tests. The experiments were carried out on 64 heifers 3-7 months old, originating from one herd. The animals were kept indoors with maintained welfare conditions. Blood to be analysed was collected from the jugular vein in the third, fourth, fifth, and seventh month of life of the heifers. Heparin was used as an anticoagulant.

Infection with BLV was diagnosed with the ELISA and PCR technique. The heifers with positive ELISA or PCR results were denoted as BLV-positive (BLV+), whereas those showing negative results during 4 months were denoted as BLV-negative (BLV-). The ELISA was performed in a specialist laboratory.

DNA isolation. Isolation of DNA was conducted with the use of a commercial reagent kit (Wizard Genomic DNA Purification Kit–Promega, USA) following the manufacturer’s recommendations. The isolated DNA was analysed quantitatively (GeneQuant apparatus – Pharmacia) and qualitatively (electrophoresis).

PCR protocol. In the PCR reactions, a fragment of a gag gene of the virus with a length of 364 bp and a fragment of $\kappa$ gene of milk casein with the length of 273 bp were amplified from genomic DNA. The applied primers, composition of a reaction mixture, and thermal profile of the reaction were consistent with earlier descriptions (6).

Blood plasma protein fractions. The major protein fractions of blood plasma were determined by means of a Cormay Gel Protein 100 kit and according to the procedure provided by the manufacturer (CORMAY). The total protein content in blood plasma (g/L) and the densitometric measurement of electrophoretic fractions (%) were used in the calculations of the individual concentrations (g/L) of protein fractions.

Statistical analysis. The statistical analysis included arithmetic mean, standard deviation, and data distribution fit tests with the normal curve. The use was
also made of two- and one-factorial analysis of variance (ANOVA/MANOVA for factorial systems and ANOVA), in which the consideration was given to the effect of BLV infection and/or age of heifers and interactions between these factors on the levels of protein fractions and total protein in blood plasma. Statistical analyses were done with STATISTICA 7.1 software.

Results

In the herd examined, 41 heifers (64.06%) were diagnosed as BLV+ and 23 heifers (35.94%) as BLV-.

The composition of protein fractions in blood plasma was within reference values and accounted for albumins – 35.70 g/L, α-globulins – 13.84 g/L, β-globulins – 22.92 g/L, and γ-globulins – 10.62 g/L (reference values: 32–49 g/L, 7–23 g/L, 11–33 g/L, and 10–32 g/L, respectively) (18).

In comparing the concentration of total protein and composition of protein fractions in blood plasma between animals infected and non-infected with BLV, negligible differences were observed between levels of albumins, α₁- and α₂-globulins, β₁-globulins, and γ-globulins (Table 1). The exception was the fraction of β₂-globulins, whose concentration in the BLV+ heifers was lower than that in the BLV- ones.

Table 1

<table>
<thead>
<tr>
<th>Fractions of protein</th>
<th>Total protein (g/L)</th>
<th>Results of diagnostic test</th>
<th>Month of life</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>x ± SD</td>
<td>BLV+</td>
<td>BLV-</td>
</tr>
<tr>
<td>Total protein</td>
<td>83.17 ± 11.40</td>
<td>82.83 ± 11.91</td>
<td>83.75 ± 10.53</td>
</tr>
<tr>
<td>Albumins</td>
<td>35.70 ± 5.58</td>
<td>36.04 ± 5.16</td>
<td>35.13 ± 6.21</td>
</tr>
<tr>
<td>%</td>
<td>43.55 ± 6.43</td>
<td>44.06 ± 6.60</td>
<td>42.69 ± 6.21</td>
</tr>
<tr>
<td>α₁-globulins</td>
<td>5.87 ± 1.47</td>
<td>5.94 ± 1.50</td>
<td>5.75 ± 1.42</td>
</tr>
<tr>
<td>%</td>
<td>7.07 ± 1.46</td>
<td>7.15 ± 1.54</td>
<td>6.94 ± 1.30</td>
</tr>
<tr>
<td>α₂-globulins</td>
<td>7.97 ± 1.65</td>
<td>7.83 ± 1.67</td>
<td>8.19 ± 1.60</td>
</tr>
<tr>
<td>%</td>
<td>9.55 ± 1.32</td>
<td>9.43 ± 1.28</td>
<td>9.74 ± 1.36</td>
</tr>
<tr>
<td>β₁-globulins</td>
<td>8.29 ± 1.49</td>
<td>8.24 ± 1.53</td>
<td>8.38 ± 1.42</td>
</tr>
<tr>
<td>%</td>
<td>9.99 ± 1.40</td>
<td>9.98 ± 1.50</td>
<td>10.02 ± 1.21</td>
</tr>
<tr>
<td>%</td>
<td>17.39 ± 3.67</td>
<td>16.93b ± 3.70</td>
<td>18.16b ± 3.52</td>
</tr>
</tbody>
</table>

Mean values denoted with the same capital or small letters are significant at P≤0.01 or P≤0.05, respectively. The interaction of result of diagnostic test x month of life were not significant. Significant differences (P≤0.01) were observed between the youngest and the oldest animals.
The analysis of the impact of age on the plasma concentrations of total protein and protein fractions demonstrated statistically significant differences in the contents of total protein, albumins, $\beta_1$-globulins, and $\gamma$-globulins (Table 1). The concentrations of total protein, albumins, and $\gamma$-globulins increased, whereas those of $\beta_1$-globulin decreased along with the age of heifers. The dynamics of changes in the values of those parameters was, however, slightly different in the infected and non-infected animals (Figs 1, 2, and 3). In the BLV- heifers, concentrations of albumins and total protein increased, whereas in the BLV+ heifers they remained at a similar level in the subsequent months of life (Figs 1, 2, and 3). Contrary dynamics of changes was reported in respect of the $\gamma$-globulin fraction whose level was observed to increase along with the age of BLV+ heifers (Figs 3a and 3b). In the BLV- animals, its level was relatively equal, and the differences between the youngest and the oldest heifers were not high and statistically insignificant (Figs 2a and 2b). In the analysed months of life of the heifers, considerable changes were also observed in the concentration of $\beta_1$-globulins. They occurred irrespective of the diagnosed BLV infection (Figs 2a, 2b, 3a, and 3b).

**Fig. 1.** Concentration of total protein in heifers (mean ± SD). The values denoted with the same capital or small letters are significant at $P \leq 0.01$ or $P \leq 0.05$, respectively.

**Fig. 2.** Composition of protein fractions in blood plasma in BLV- heifers (mean ± SD). The values denoted with the same capital or small letters are significant at $P \leq 0.01$ or $P \leq 0.05$, respectively.
Discussion

The study demonstrated a relatively low effect of natural infection with BLV on the content of total protein and composition of protein fractions in blood plasma. The effect referred only to the fraction of $\beta_2-$globulins whose concentration was lower in the BLV+ than in the BLV- heifers (Table 1). A similar tendency was reported for leukaemic cows in previous investigations (9). The C3 component of a complement and part of IgA, IgM, and IgG immunoglobulins migrate with the fraction of $\beta_2-$globulins. The C3 component is an important element of the complement system and participates in its activation. Its concentration is the highest one amongst all proteins of the complement system and affects the electrophoregram’s picture. It seems that the lower concentration of $\beta_2-$globulin fraction recorded in the BLV+ heifers may be linked with the concentration of that component as well as with its higher utilisation as a result of an intensified defence reaction of the body induced by the bovine leukaemia virus infection in young animals.

In the heifers examined, no effect was found of BLV infection on the concentration of the $\gamma-$globulin fraction. However, the differences between BLV-infected and non-infected animals have been observed earlier in cows (9). Leukaemic cows were characterised by a lower level of the $\gamma-$globulin fraction compared to the clinically healthy animals ($P \leq 0.05$), which might have resulted from the dysfunction of B lymphocytes, typical of chronic lymphocytosis phase, and from a lower level of IgM synthesis registered by other authors (7, 13, 17). In addition, the concentration of IgM immunoglobulins is negatively correlated with the number of B-lymphocytes (15, 17).

It may be supposed, that small differences in the concentrations of protein fractions in blood plasma between BLV-infected, and non-infected heifers are elicited by a short-term infection with BLV, resulting from the young age of the animals and, consequently, the early phase of enzootic bovine leukosis (EBL) development.

Greater differences in the concentrations of total protein and protein fractions were also reported in the period of the growth and development of the heifers.
(Figs 1, 2, 3). The changes referred to the levels of total protein, albumins, β1-globulins, and γ-globulins and the direction of these changes (except for β1-globulins) were; however, slightly different in the BLV+ and BLV-heifers. An increase in the concentration of the γ-globulin fraction in the BLV+ heifers is probably, evoked by an increased concentration of immunoglobulins (mainly IgM, IgG, and IgA) whose level in blood plasma increases to a significant extent, as a result of organism response to the infection. In addition, with the fractions of γ-globulins, C-reactive protein (CRP) migrates amongst the acute phase proteins. This protein acts on target cells (e.g. neutrophils, monocytes, NK cells, blood platelets) that serve anti-inflammatory and post-inflammatory functions in modulating the immunological response (10). The increasing level of the γ-globulins fraction in the subsequent months of life of the BLV+ heifers was likely to indicate an intensified reaction of the immune system to the development of BLV infection. In turn, the negligible differentiation of γ-globulins concentration in the non-infected animals, observed in the subsequent months of their life, seemed to result in that period from sustaining some level of immaturity of the immune system, the development of which is affected, apart from age, by other immunostimulatory and immunosuppressive factors of the surrounding environment.

An opposite dynamics of changes was demonstrated by β1-globulins. The highest level of this fraction was recorded in the youngest heifers, whereas the lowest level was in the oldest animals, irrespective of BLV infection. Interpretation of the results obtained is intricate and requires more detailed analyses, since transferrin and β-lipoprotein (linked with LDL) migrate with the fraction of β1-globulins and changes in their levels affect the contribution of this fraction onto an electrophoreogram. Transferrin plays a key role in the iron metabolism in the body. Its level is determined by the age, physiological condition, and sex of an animal (14). This protein displays bacteriostatic properties and is included among non-specific humoral factors (12). In addition, transferrin is one of the negative acute phase proteins, whose role consists in the repair of damaged tissues and organs in order to restore homeostasis in the body. In the inflammatory processes induced by infection, a decrease was observed in plasma transferrin level along with time since the infection onset (12, 14). In the present study, however, no differences were reported between the BLV+ and BLV- heifers.

The observed decrease in β1-globulin levels may also be linked with changes in the hormonal profile proceeding in the body of animals during their growth and development. A thyroid-stimulating hormone (TSH) migrates with that fraction (3), stimulates the proliferation of thyroid cells, and is responsible for the transport of iodine and synthesis of triiodothyronine (T3) and thyroxine (T4). The thyroid hormones are regulators of a variety of biological functions of an organism, among others, embryonic and postnatal growth, and its reproductive functions (5, 19).

In summarising the results obtained, it may be concluded that the age of young animals exerts a decisive effect on the dynamics of changes in the level of some protein fractions and total protein in the blood plasma. It refers, in particular, to a fraction of β1-globulins and total protein. However, changes induced by natural infection with BLV in young heifers were not great and concerned only the fraction of β1-globulins.

References


