The Usefulness of the γ-glutamyltransferase Activity and Total Proteinemia in Serum for Detection of the Failure of Immune Passive Transfer in Neonatal Calves

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SUMMARY
The aim of this study was to explore the usefulness of serum γ-glutamyltransferase activity (GGT) and total serum protein (TSP) as markers of hypogammaglobulinaemia in early neonatal calves. These investigations were performed on 40 Holstein-fresian 1 day old calves. Blood samples were collected by jugular venipuncture at 24 h and immunoglobulin G (IgG) concentrations, activities of GGT and TSP values in sera were determined. Calves were divided into three groups according to the serum IgG concentrations. Two calves had complete failure of passive transfer (IgG : < 8 g/P), 18 calves had partial failure (IgG : > 16 g/P) and 20 calves had normal passive transfer (IgG : > 16 g/P). There was a positive significant (p < 0.05) correlation between serum IgG scores and serum GGT activities (r = 0.566). In addition there was a positive significant (p < 0.05) correlation between serum IgG scores and serum proteinemia (r = 0.462). The sensitivity and specificity of GGT activities measured in serum were 65 % and 95 % when the threshold of decision was 1047 U/l (i.e. the inferior limit of the confident interval obtained in normal 24 h old calves). For serum proteinemia, the chosen threshold of decision was 60 g/l and sensitivity and specificity of this biological test were 90 % and 95 % respective- ly. Consequently, the probability that a calf effectively had a defective passive transfer was great when GGT activity was below 1047 U/l or when proteinemia was below 60 g/l. As a conclusion, it is showed that the detection of serum GGT activity and TSP, could give additional information on neonatal calf immune status.

KEY-WORDS : neonatal calves - passive transfer - γ-glutamyltransferase - proteinemia - IgG.

1. Introduction
The calves diseases and losses have always been an important cause of economic losses in neonatal period thorough veterinary services use of drugs and dead [8, 12, 32]. The diarrhea and septicaemia were associated with inadequate serum immunoglobulin concentrations (hypogammaglobulinaemia) in neonatal period [2, 3]. Prevention of diarrhea in neonates depends to a large extend on lactogenic immunity obtained by ingestion of colostral immunoglobulins [11, 21, 29]. Neonatal calves, like most domestic animals, are born lacking circulating immunoglobulins, because syndesmocho- rial type placenta does not permit the immunoglobulin trans- fer [1, 5, 10, 14, 18]. Therefore they must absorb maternal immunoglobulins via intestine from colostrum for passive immunity during the neonatal period [7, 13, 25].

The ability of the newborn calf to absorb colostral immu- noglobulin decreases quite rapidly following birth and essen- tially stops by about 24 hours of age [6, 24]. Early ingestion
of colostrum is essential for the newborn calf [20, 22, 27].

The ingestion of adequate amounts of colostrum by calves during the first 24 hours of life is crucial for their resistance to diseases, rate of growth and survival [9, 31]. If adequate colostrum cannot be ingested, failure of passive transfer (FPT) occurs [4, 19, 23, 26]. Early detection prior to the installation of immunoglobulin deficiency in calves is great importance, because it can enable supplementation with colostrum and correction of the immunoglobulin deficiency. Early recognition of neonatal calves with FPT is the most critical control point [16, 17].

It has been shown that following colostral ingestion, serum immunoglobulin concentrations increased. Similarly, the enzyme GGT, whose concentration in the colostrum is very high, is also absorbed by intestine and gets to calf serum. This enzyme is readily absorbed across the intestinal barrier in the first few days of life [17]. Serum GGT activities in calves that ingested colostrum are 60-160 times greater than healthy adult cattle [30].

The detection of calf’s serum immunoglobulin concentration at postpartum 24 h is important for evaluate immune status of calves before and after weaning. Several tests for the evaluation of colostral ingestion are available (zinc sulfate turbidity test, sodium sulfate precipitation test). Determination of serum GGT activity and TSP would be additional and referent methods in the assessment of passive transfer in calves. The aim of this study was to explore the usefulness of serum GGT activity and TSP as an indicator of FPT in 24 h old calves.

2. Material and method

Blood samples were collected from 40 Holstein-fresian calves by jugular venipuncture at 24 h, old age. Serum was obtained by centrifugation of blood samples. Sera were stored at -18 °C until used. Serum concentrations of immunoglobulin G (IgG) were determined by use of a commercial radial immunodiffusion kit (SRID kit, VMRD Inc. Pullman, Washington). Activity of GGT in serum was measured by a kinetic colorimetric test method (GGT reagent, Ciba Corning Diagnostics, Ohio). Total serum protein values were assessed with a refractometer. SPSS for Windows program (statistical packet program, version 10.0) was used for statistical determination. Results were considered as significant when p values were less than 0.05.

Calves were divided into three groups according to the serum IgG levels; failure of passive transfer (< 8 g/l), partial failure (8-16 g/l), and normal passive transfer (> 16 g/l). Total protein concentration over 60 g/l would mean that immunoglobulin absorption was adequate, less than 50 g/l would mean a minimal absorption and proteinemia between 50 and 60 g/l are marginal; GGT concentration over 2000 U/I signified a normal passive transfer, 1500-2000 U/I, a partial failure and concentration less than 200 U/I, a failure of passive transfer.

3. Results

The findings are demonstrated in table I. Two calves have failure of passive transfer (Group 1), 18 calves have partial failure (Group 2) and 20 calves have normal passive transfer (Group 3). In groups of calves with partial or complete failure of immune passive transfer, serum GGT activities significantly decreased according to the degree of insufficiency of immune passive transfer (p < 0.01, figure 1). Furthermore, there was a positive significant correlation between serum IgG concentrations and serum GGT activities (R = 0.566).

In the same way, total proteinemia was significantly lower when the passive transfer failed (p < 0.01, figure 2). Proteinemia was maximal in Group 3 (normal passive transfer) and minimal in Group 1 (complete failure of passive transfer). In addition, there was a positive significant correlation between serum IgG concentrations and TSP (R = 0.462).

In calf with normal passive transfer (Group 3), the distributions of serum GGT activities and value of proteinemia were considered as normal distributions (p < 0.01, with Kolmogorov-Smirnov test). Consequently, normal values for serum GGT activities and for total serum proteinemia in 24 h old calves were established. The confident intervals with a risk of 5 % were: [1047 U/I; 4476 U/I] for serum GGT activities and [55,51 g/l; 89,19 g/l] for total serum proteinemia.

For GGT activities in serum of calves, the inferior limit of the confident interval with a risk of % 5 was considered as the threshold of decision. If value was below this limit, the result was considered as positive. In calves with partial or complete failure of immune passive transfer, 13 animals had a serum GGT activity below 1047 U/I and were considered as true positive results. When the passive transfer was normal, only one calf presented a value below the threshold and constituted a false positive (Table II). With a threshold of decision equal to 1047 U/I, the sensitivity (Se) of this biological test (i.e. the probability to have a positive test in a calf certainly ill, Se = True positive /20 X 100) was 65 %. The specificity (Sp) (i.e. the probability to have a negative test in a calf certainly healthy, Sp = True negative /20 X 100) was 95 %. As far as total serum proteinemia (TSP) was concerned, the threshold of decision chosen was 60 g/l. In this case, 10 calves with a defective passive transfer give true positive results (i.e. below 60 g/l), whereas only one calf with a normal passive transfer showed a proteinemia inferior to the threshold (false positive) (Table III). Sensibility and specificity of TSP as biological marker were determined with a threshold equal to 60 g/l and were 50 % and 95 % respectively.

Consequently, when serum GGT activity measured was less than 1047 U/I or TSP was less than 60 g/l, the probability that the animal was certainly ill and presented a failure of passive transfer is great. But when results were above 1047 U/I for GGT activity or 60 g/l for TSP, it is difficult to conclude that the passive transfer is quite normal.

4. Discussion

There was a predisposition for diseases in neonatal period in calves that have insufficiently ingested colostrum and/or have ingested bad quality colostrum in early days, which reflected a defective passive transfer. The detection of hypogammaglobulinaemia is very important at proper lifetime in order to decrease neonatal calf losses. LOMBARDI et al.
demonstrated that, in dairy cattle, 25-34% of the calves fail to suckle by 6-8 hours of age and consequently suffer from low serum immunoglobulins at their early critical stage of life.

There are several indirect and direct methods for detection of FPT. There are several procedures for determining serum immunoglobulin concentrations in the neonatal calf. The most accurate measurement of immunoglobulins is using single radial immunodiffusion. However, indirect assessment of serum immunoglobulins by means of the refractometer, zinc sulfate turbidity test, sodium sulfite precipitation test or glutaraldehyde coagulation tests are cost, technical expertise, and the delay necessary for performing test (24 to 48h) are limitations.

The GGT enzyme, whose concentration in the colostrum is very high, is absorbed by intestine and reaches blood flow in

<table>
<thead>
<tr>
<th>Gr. 1: failure of passive transfer</th>
<th>Gr. 2: partial failure</th>
<th>Gr. 3: normal passive transfer</th>
</tr>
</thead>
<tbody>
<tr>
<td>calves number</td>
<td>2</td>
<td>18</td>
</tr>
<tr>
<td>IgG g/L</td>
<td>8 ± 0</td>
<td>13.75 ± 2.05</td>
</tr>
<tr>
<td>GGT U/L</td>
<td>167.5 ± 62.9</td>
<td>1494.2 ± 1234.9</td>
</tr>
<tr>
<td>TSP g/L</td>
<td>49 ± 5</td>
<td>63 ± 8</td>
</tr>
</tbody>
</table>

TABLE I. — The relationship among serum IgG scores, serum GGT activities and TSP (total serum proteinemia) in neonatal calves.

<table>
<thead>
<tr>
<th>GGT (UI)</th>
<th>TSP (g/l)</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt; 1047 U/L</td>
<td>1</td>
</tr>
<tr>
<td>&gt; 1047 U/L</td>
<td>19</td>
</tr>
</tbody>
</table>

TABLE II. — Contingency table of serum GGT activities.
The chosen threshold of decision was 1047 U/L. Calves with defective passive transfer, which presented serum GGT activities below this limit were considered as true positive animals, whereas serum GGT activities of calves with normal passive transfer below 1047 U/L were false positive values. When serum GGT activities were above the threshold, results were considered as negative: true negative in the case of calves with normal passive transfer, and false positive in the case of calves with defective passive transfer. Sensibility (Se): true positive results (in percent) /20; Specificity (Sp): true negative results (in percent) /20.

<table>
<thead>
<tr>
<th>TSP (g/l)</th>
<th>&lt; 60 g/L</th>
<th>&gt; 60 g/L</th>
</tr>
</thead>
<tbody>
<tr>
<td>Calves with normal passive transfer</td>
<td>1</td>
<td>10</td>
</tr>
<tr>
<td>Calves with defective passive transfer</td>
<td>19</td>
<td>10</td>
</tr>
</tbody>
</table>

TABLE III. — Contingency table of total serum proteinemia (TSP).
The chosen threshold of decision was 60 g/L. Calves with defective passive transfer, which presented TSP below this limit were considered as true positive animals, whereas TSP of calves with normal passive transfer below 60 g/L were false positive values. When TSP were above the threshold, results were considered as negative: true negative in the case of calves with normal passive transfer, and false negative in the case of calves of with defective passive transfer. Sensibility (Se): true positive results (in percent) /20; Specificity (Sp): true negative results (in percent) /20.
the same time that colostral antibodies [6, 15]. This enzyme originates from the active epithelial cells in the mammary gland [21].

BOGIN et al. [6], studying the activity of the enzyme in calf serum before and after colostral ingestion, concluded that GGT can serve as a marker for colostral intake. In the present study a positive correlation was detected between IgG levels and GGT activity in serum (R = 0.566). Furthermore, sensibility and specificity of serum GGT activities are 65% and 95%, indicating that a value below the threshold of decision (i.e. 1047 U/P) has a great probability to be obtained in calves with complete or partial failure of passive transfer. This result indicated that determination of the serum activity of GGT gave information of calf immune status. In addition, determination of serum GGT activity is cheaper and reliable than other direct methods (ELISA, SRID).

A positive correlation (R = 0.462) was determined between serum IgG and TSP. This demonstrated that TSP gave indirect information about the calf immune status. In the same way because of an elevated specificity (95%) of the biological test, total serum proteinemia below 60 g/l would help to diagnosis of a defect in immune passive transfer with a great probability. However, refractometric determination of TSP can be misleading, because other plasma substrates, such as glucose, urea and creatinine could contribute to refractive index. In addition, dehydrated calves have also high total serum proteinemia.

As a result, the determination of serum GGT activity and TSP give information on the neonatal calf immune status and these methods are cheaper and more rapid than other indirect and direct methods.

5. References


9. CROWLEY M.L., FISHER L.J. and OWEN B.D. : Blood-derived immunoglobulins in milk replacer, or by injection for improved per-