Histochemical and biochemical study of rabbit intestine in healthy and affected by epizootic enteropathy animals

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ABSTRACT. Rabbits are suffering from Epizootic Rabbit Enteropathy (ERE), a new gastro-intestinal syndrome of unknown aetiology. ERE has not yet been investigated thoroughly from the patho-physiological point of view of the intestine malfunction during the intestine dysbiosis. For this reason, the aim of the present research was to study the haematological profile, the intestine histopathology and certain biochemical parameters (α-amylase activity, blood glucose concentration) of animals affected by ERE in comparison with those of healthy rabbits of the same age. In six healthy and six, affected by ERE, crossbreed New Zealand X
California 35-day old rabbits, the following analyses were performed: Total blood count, histopathological morphology of the intestine, alkaline phosphatase (ALP) distribution in intestine brush border and α-amylase activity in blood plasma and small intestine epithelium, respectively. Total blood count showed significantly (at 95% confidence level) decreased values for all blood parameters except for the white blood cell count, which proved to be significantly higher compared in ERE rabbits to that of normal rabbits. The α-amylase activity and concentration in blood plasma and intestinal epithelium were significantly (a=0.05) decreased, in contrast to blood glucose concentration, that was found to be significantly increased in ERE rabbits. Stomach was full of watery content, intestine presented non-specific enteropathy and mainly the small intestine was full of gas and watery content. Caecal and colon presented impaction and mucus was present in the colon. The histopathological evaluation of the ileum, sacculus rotundus, caecum and colon presented, mainly, lamina propria mononuclear cell infiltration and swelling, flattening and denuding of the enterocytes as well as oedematous lymphoid tissues. Duodenum had necrotic villi and deep infiltration with mononuclear and polymorphonuclear cells, within the lamina propria. Also swelling, vacuolation, flattening, enterocytes denuding and oedematous lymphoid tissues were observed. Jejunum had no lesions. The caecum and the colon presented an ALP positive reaction along the brush border of the epithelial cells. Small intestine presented a positive reaction along the brush border of the intestinal glands-upper part-cells only and the bases of a few of the villi.

**Keywords**: haematological parameters, intestine, α-amylase activity, ALP- activity, Epizootic Rabbit Enteropathy

**INTRODUCTION**

Since 1997, a new gastro-intestinal syndrome called Epizootic Rabbit Enteropathy (ERE) has appeared in Europe with a high incidence of morbidity and mortality in affected rabbit farms. ERE is characterized by phenomena of gut dysbiosis, appearing mainly in kits during/after weaning period (Coudert et al., 1997). The main aetiology is still unknown (Maertens et al., 2005, Alvarez et al., 2006). Ten years after its description in rabbitries in Europe, it can be concluded that it could be considered as a syndrome due to multifactorial parameters, such as housing system, pathological agents [virus, bacteria including their toxin, parasites] (Licois, 2004), high stock density, genetic sensibility of the animals, nutritional factors (Boisot et al., 2003, Alvarez et al., 2006, Colin and Prigent, 2006), age of weaning (Garrido et al., 2006), exogenic toxin as suggested by Marlier et al. (2003) and unidentified causes etc (Coudert et al., 1997). That is why it could be considered a syndrome due to genetic and environmental factors, as well as to the genetic sensibility of the animals and the type of the housing system. The aetiology of ERE is still unknown, despite the numerous studies that have been conducted during the last years. The main point is the fluctuation of the incidence of this disease, which is related with the genetic sensibility of the animals, the type of the housing system and the environmental factors. The importance of this disease is due to the high incidence of morbidity and mortality in affected rabbit farms, and the economic impact that it has on the rabbit industry. Therefore, the aim of this study was to investigate the aetiology of ERE and to determine the most important risk factors for its occurrence.

Since 2003 ERE has also affected Greek rabbit farms with severe economic losses (Xylouri and Fragkiadakis, 2006). The outbreak of the disease in Greece was due to the continuous imports of rabbits' parent stock from other European countries with reported cases of ERE or similar digestive problems (Lebas and Coudert, 1997, Maertens et al., 2005, Cerioli & Lavazza, 2006).

The aim of this research was the study of the haematological profile, the intestinal histopathology, some anatomical characteristics, certain biochemical parameters as α-amylase activity, blood glucose concentration, as well as the ALP intestine distribution of animals affected by ERE in Greece. Some of these parameters were compared to those of the same age healthy rabbits.

MATERIALS AND METHODS

Six healthy and six affected by ERE crossbreed New Zealand X California 35-day old rabbits, were used. The rabbits were housed in closed rabbit house in flat-deck wire cages. The animals were provided a typical industrial diet and water ad libitum.

Blood sample was taken, for haematological and biochemical analysis, from the ear veins using heparin as anticoagulant and was analysed within 30 minutes after sampling, by using a SYSMEX AD-270 automating apparatus. Plasma was tested for the determination of biochemical parameters according to standard methods (Nemi, 1986, Van Kampen and Zijlstra, 1961, 1965). The laboratory scales with 0,01g accuracy were used to determine the body and the pancreas weight. Also the small intestine length was measured.

All animals were slaughtered with the use of chloroform in a glass container. The abdominal cavity was immediately opened and the alimentary tract from the cardia of the stomach to the rectum was removed. The intestine was then unravelled and released from mesentery and (a) Segments of duodenum, jejunum, ileum, caecum and colon were fixed in 10% buffered formalin for 24 hours. They were then rinsed in tap water, dehydrated with increasing concentration of ethanol and embedded in paraplast. The blocks were cut at 4μm of thickness with a Reichert-Jung microtome-mod 2055/Autocut. A modified coupling Azo dye method was used for revealing the site of alkaline phosphatase activity. This technique (Pearse, 1961), previously used on cold formalin fresh frozen sections or freeze dried paraffin sections, was used on paraplast sections from the small and large intestine. 10-20 mg sodium a-naphthyl phosphate was dissolved in 20 ml 0.1 M stock «Tris» buffer (pH 10). 20mg of the stable diazotate of 5-chloro-o-toluidine (Fast Red) were added and stirred well. The resulting solution was filtered onto the slides. The well-flooded sections were then incubated at room temperature for one hour, after which they were rinsed in tap water for two minutes, stained by haematoxylin for five minutes and differentiated in acid-alcohol. Finally, after being rinsed in distilled water and washed in running water for 30 minutes, they were mounted in glycerin jelly. The sites of alkaline phosphatase activity were stained brown. (b) Small pieces of the small intestine (jejunum) of each animal were removed and their mucosa were homogenized using Ringer solution at pH 7,0 for the determination of the α-amylase activity in the epithelium according to the kinetic method (Marshall et al., 1977, 1978) and all the necessary dilutions took place in the same solution. This method was performed by using the non - natural substrate p-nitro-phenylmaltoheptaoside and the combined action of glucoamylase and α-glucosidase. The final product of the hydrolytic reaction, performed at +37°C, showed the α-amylase activity measured by using a spectrophotometer at 405 nm. The measurements took place once per minute for three times, in order to measure the linear phase of the reaction, according to the instructions of the kit.

The plasma blood glucose concentration was measured using the method of glucose oxidase (Trinder, 1969).

RESULTS

Average complete blood count, morphological, biochemical and histochemical results are summarized in the following Tables 1, 2, 3 and 4.

Total blood count showed significantly (at 95% confidence level) decreased values for all blood parameters, except for the white blood cell count, which proved to be significantly higher, compared to normal rabbits (Table 1).

Intestine length was increased in affected animals, whereas measurements for pancreas weight and its proportion compared to the total body weight proved
Table 1. Weaning Weight, S. Intestine Length, Pancreas Weight, Amylase Activity of Small Intestine, Blood Serum Amylase, Haematological profile of 35 days old rabbits

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Animals affected by ERE</th>
<th>Significance</th>
<th>Healthy animals</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean Value</td>
<td>Stand. Error</td>
<td>Mean Value</td>
</tr>
<tr>
<td>Weaning Weight (g)</td>
<td>799.17</td>
<td>29.54</td>
<td>1284.17</td>
</tr>
<tr>
<td>S. Intestine Length (cm)</td>
<td>237.50</td>
<td>10.70</td>
<td>179.17</td>
</tr>
<tr>
<td>Pancreas Weight (g)</td>
<td>0.62</td>
<td>0.06</td>
<td>0.70</td>
</tr>
<tr>
<td>Amylase Activity of Small Intestine</td>
<td>385.0</td>
<td>20.12</td>
<td>146.09</td>
</tr>
<tr>
<td>Epith. Tiss. Amylase Activ. (LU/mg)</td>
<td>-</td>
<td>-</td>
<td>1219.50</td>
</tr>
<tr>
<td>Blood Serum Amylase (x10³LU/l)</td>
<td>7.37</td>
<td>0.48</td>
<td>NS</td>
</tr>
<tr>
<td>Glucose (mM/l)</td>
<td>14.22</td>
<td>0.66</td>
<td>8.96</td>
</tr>
<tr>
<td>Hematocrit</td>
<td>31.98</td>
<td>2.81</td>
<td>44.38</td>
</tr>
<tr>
<td>Hemoglobin (g/l)</td>
<td>4.80</td>
<td>0.46</td>
<td>5.79</td>
</tr>
<tr>
<td>Mean Corpuscular Hemoglobin Concentration (%)</td>
<td>23.57</td>
<td>0.56</td>
<td>29.50</td>
</tr>
<tr>
<td>Mean Corpuscular Volume (fl)</td>
<td>66.93</td>
<td>2.06</td>
<td>76.64</td>
</tr>
<tr>
<td>Mean C. H (µg)</td>
<td>15.78</td>
<td>0.62</td>
<td>22.57</td>
</tr>
</tbody>
</table>

NS: not significant, *: P<0.05, **: P<0.01, ***: P<0.001.

The α-amylase’s activity and concentration in blood plasma and intestinal epithelium were significantly (a=0.05) decreased, in contrast to blood glucose concentration that was proved to be significantly increased in rabbits with Enteropathy (Table 3).

Small intestine presented an ALP positive reaction along the brush border of the epithelial cells laying the upper part of the intestinal glands and the bases of some villi as well. Cæcum and colon presented an ALP positive reaction along the brush border of the epithelial cells (Table 4).

no significant difference (Table 2).
Table 2. Live Body weight, small intestine length and pancreas weight of 35 days old rabbits

Πίνακας 2. Ζων βάρος, μήκος λεπτού εντέρου και βάρος παγκρέατος, ηλικίας 35 ημερών κουνελιών

<table>
<thead>
<tr>
<th>Rabbits</th>
<th>Body weight (g)</th>
<th>Small intestine Length (cm)</th>
<th>Pancreas weight (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Healthy</td>
<td>ERE</td>
<td>Healthy</td>
<td>ERE</td>
</tr>
<tr>
<td>1</td>
<td>1375</td>
<td>885</td>
<td>206</td>
</tr>
<tr>
<td>2</td>
<td>1100</td>
<td>735</td>
<td>170</td>
</tr>
<tr>
<td>3</td>
<td>1380</td>
<td>785</td>
<td>185</td>
</tr>
<tr>
<td>4</td>
<td>1385</td>
<td>860</td>
<td>172</td>
</tr>
<tr>
<td>5</td>
<td>1235</td>
<td>700</td>
<td>150</td>
</tr>
<tr>
<td>6</td>
<td>1230</td>
<td>830</td>
<td>192</td>
</tr>
<tr>
<td>Mean</td>
<td>1284,17</td>
<td>799,17</td>
<td>179,17</td>
</tr>
</tbody>
</table>

Table 3. α-amylose activity, glucose concentration

Πίνακας 3. Δραστικότητα α-αμυλάσης, συγκέντρωση γλυκόζης

<table>
<thead>
<tr>
<th>RABBITS</th>
<th>PANCREAS WEIGHT OF BODY WEIGHT (%)</th>
<th>α-AMYLASE ACTIVITY OF HOMOGENIZED SMALL INTESTINE EPITHELIUM (1000 IU)</th>
<th>α-AMYLASE ACTIVITY IN SMALL INTESTINE EPITHELIUM (IU/mg)</th>
<th>α-AMYLASE ACTIVITY IN BLOOD PLASMA ACTIVITY (1000 IU)</th>
<th>α-AMYLASE ACTIVITY IN PLASMA and HOMOGENIZED INTESTINE</th>
<th>GLUCOSE IN BLOOD (mmol/l)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.07</td>
<td>29.78</td>
<td>320</td>
<td>7.8</td>
<td>0.26</td>
<td>16.76</td>
</tr>
<tr>
<td>2</td>
<td>0.09</td>
<td>48.14</td>
<td>380</td>
<td>8.15</td>
<td>0.17</td>
<td>13.3</td>
</tr>
<tr>
<td>3</td>
<td>0.11</td>
<td>41.69</td>
<td>390</td>
<td>9.05</td>
<td>0.22</td>
<td>14.61</td>
</tr>
<tr>
<td>4</td>
<td>0.07</td>
<td>38.78</td>
<td>360</td>
<td>6.05</td>
<td>0.16</td>
<td>15.16</td>
</tr>
<tr>
<td>5</td>
<td>0.07</td>
<td>44.19</td>
<td>470</td>
<td>6.14</td>
<td>0.14</td>
<td>13.07</td>
</tr>
<tr>
<td>6</td>
<td>0.055</td>
<td>39.27</td>
<td>390</td>
<td>7.03</td>
<td>0.18</td>
<td>12.42</td>
</tr>
<tr>
<td>Mean diseased animals</td>
<td>0.078</td>
<td>40.31</td>
<td>385</td>
<td>7.37</td>
<td>0.19</td>
<td>14.22</td>
</tr>
<tr>
<td>Mean healthy animals</td>
<td>0.060</td>
<td>135.09</td>
<td>1163</td>
<td>6.27</td>
<td>0.05</td>
<td>8.96</td>
</tr>
</tbody>
</table>

Clinical signs-Gross lesions-Histological examination:

Clinical signs: The first sign of ERE affected animals was the reduction of feed intake which was followed by anorexia. The abdominal cavity was enlarged and diarrhea appeared. When animals were kept by one hand and shook, "paflasmos" was heard viz. a characteristic noise stemming from the motion of the watery content of the stomach (bowel rumbling noises). This noise was characterized as "burborygmos" by other researchers. The animals were euthanized before their death.

Gross lesions: In the post-mortem examination a non-specific enteropathy was observed, without
Table 4. Alkaline Phosphatase reaction in small and large intestine.

<table>
<thead>
<tr>
<th>Reaction</th>
<th>35 days Normal rabbits</th>
<th>35 days affected rabbits</th>
</tr>
</thead>
<tbody>
<tr>
<td>Duodenum</td>
<td>4+</td>
<td>- *</td>
</tr>
<tr>
<td>Jejunum</td>
<td>4+</td>
<td>- *</td>
</tr>
<tr>
<td>Ileum</td>
<td>4+</td>
<td>- *</td>
</tr>
<tr>
<td>Caecum</td>
<td>2+</td>
<td>2+</td>
</tr>
<tr>
<td>Colon Proximal</td>
<td>3+</td>
<td>2+</td>
</tr>
<tr>
<td>Colon Distal</td>
<td>アポメチオニンメノコヨロ</td>
<td>-</td>
</tr>
</tbody>
</table>

*2+ Glands (upper part only) and bases of few villi
*2+ Αδένες (άνω τμήμα μόνο) και οι βάσεις σε λίγες λάχνες

intestinal inflammatory lesions. Cecal impaction, non-specific reaction in the gut associated lymphoid tissue and often massive mucus in the colon were showed. Stomach was full of watery content and, primarily the small intestine, was full of gas and watery content as well. Impaction and mucus were present into the colon.

Histological examination: The apical epithelium of duodenal villi was necrotic and lamina propria infiltration of mononuclear and polymorphonuclear cells were noticed as well as swelling, vacuolation, flattening, denuding of the enterocytes and also oedematous lymphoid tissue were visible. Similar histopathological aspects were noticed in ileum, sacculus rotundus, caecum and colon. Jejunum had no lesions.

STATISTICAL ANALYSIS OF DATA:
The comparison of means using t-test showed a significant statistical difference (P-value < 0.05) in the following haematological data:

RBC (Red Blood Cells), Hb (Haemoglobin), WBC (White blood cells), MCHC (Mean Corpuscular Haemoglobin Concentration), MCV (Mean Corpuscular Volume), MCH (Mean Corpuscular Haemoglobin).

Similar conclusions were extracted using Mann-Whitney (Wilcoxon, W) test for the comparison of medians, with P-value <0.05 for RBC, Hb, WBC, MCHC, MCV, MCH.

In contrast, there was no significant difference in Red Blood Cells count (p-value=0.13 using t-test, p-value=0.11 with W-test).

Regarding the biochemical profile of the affected rabbits, there was a significant statistical difference using t-test in:

- Body weight, intestine length, α-amylase activity in homogenized small intestine epithelium (in LR’s solution), α-amylase activity in small intestinal epithelium, α-amylase plasma activity/ α-amylase activity in homogenized small intestine epithelium and glucose blood concentration. Similar results were extracted using W-test.

- There was no significant statistical difference in α-amylase activity in blood plasma (p-value = 0.3), in pancreas weight (p-value = 0.173) and in pancreas' weight proportion compared to body weight (p-value = 0.174). The same conclusions were confirmed after comparing the medians using W-test.

DISCUSSION
The comparative study of the above mentioned haematological values, biochemical parameters, body and pancreas weight, intestine length, α-amylase activity, concentration and ALP distribution in healthy and ERE/sick rabbits, revealed that:

The total blood count showed significantly (at 95% confidence level) decreased values for all blood parameters except for the white blood cell count, which proved to be significantly higher to ERE compared to normal rabbits. These results are in agreement with those of (a) Jobert et al., (2001), who in an experimental reproduction of ERE in 35 day old rabbits observed a decrease in the number of erythrocytes and an increase of the mean corpuscular volume, in the number of leucocytes as well as in the number of neutrophils and (b) with those of Marlier et al. (2003), who observed an increase in the number of neutro-
phils. Our results of healthy/control rabbits were within the normal range and in accordance with the results of the recent publication (Suvegova et al., 2004, Sabatakou et al., 2007) for the haematological parameters in normal fattening rabbits.

The intestine length was increased in affected animals whereas measurements for pancreas weight and its proportion compared to the total body weight proved no significant difference.

According to our findings, the length of the intestine of the diseased animals is longer than that of the healthy ones and this may be due to the influence of endogenous or exogenous toxin as suggested by Marlier et al., (2003).

The α-amylase activity in (A) 35-day old healthy rabbits showed that

α-amylase activity of small intestine epithelium was: 146,09±32,28 (10^3 X IU/Lit), (P>0,05), α-amylase activity of epithelial tissue was: 1219,5±204,42 (IU/mgr) and α-amylase activity of blood serum was: 6,27±0,88 (10^3 X IU/Lit), (P<0,01). These results are in agreement with those of other researchers (Kosa et al., 2004, Sabatakou et al., 2006, Debray et al., 2001), who described a significantly increase of α-amylase activity according to the progress of age up to 35-day old rabbits, at the level of intestine. (B) On the contrary, the α-amylase activity in 35-day old ERE rabbits showed that the α-amylase activity and concentration in both blood plasma and intestinal epithelium were significantly (a=0.05) decreased (Philips and Fuller, 1983, Rodeheaves and Wyatt, 1986).

The main pathological agent for ERE consists of clostridia growth in the alimentary tract. Weaning rabbits excrete a low amount of amylase. Feed containing high starch concentration results in an increased amount of indigested starch in intestine content (Tester et al., 2004). Indigested starch in the caecum is a good medium for clostridia growth (Agnoletti, 2006). Furthermore, high levels of proteins in diet induce an increase in trypsin secretion, which enhances C. spiroforme pathogenicity (Agnoletti, 2006).

Concerning the blood glucose concentration in 35-day old healthy rabbits, it was 8,96±0,22 (mM/Lit) vs. 14,22±0,66 (mM/Lit) in 35-day old ERE rabbits. It was found to be substantially increased due to the activation of homeostatic body mechanisms. Hyperglycemia may occur due to the increase of glucocorticoids as ERE animals were stressed (stress hyperadrenocorticism) (Meyer and Harvey, 1992).

Limited numbers of reports regarding the distribution of the Alkaline Phosphatase enzyme in the gut of rabbits (Jervis 1963, Lafont and Moretti, 1970, Sabatakou et al., 1999) are published. Our observations for Alkaline Phosphatase in the gut of the healthy/control rabbits were in accordance with the findings that (Jervis 1963, Lafont and Moretti, 1970, Sabatakou et al., 1999, 2007) are published. A strong Alkaline Phosphatase reaction was observed along the brush border of the villous epithelial cells of small intestine, caecum and proximal colon.

Regarding the present observations for Alkaline Phosphatase in the gut of ERE/sick rabbits, the small intestine presented a positive reaction along the brush border of epithelial cells laying the bases of some villi and the upper part of glands, while the deep gland cells showed no such reaction. The caecum and the colon presented an Alkaline phosphatase positive reaction along the brush border of the epithelial cells. To the best of our knowledge, there is no report in the literature on the distribution of the Alkaline Phosphatase enzyme in the gut of ERE/sick rabbits. Nevertheless the biochemical study of Toofanian (1985) on the experimental mucoid enteropathy in rabbits (6-8 weeks of age) can be mentioned. This report proved that Alkaline Phosphatase was significantly lower in rabbits’ small intestine with mucoid enteropathy. Similar observations on intestinal Alkaline Phosphatase distribution have been made in the present study, since Alkaline Phosphatase was significantly lower in ERE/sick rabbits’ small intestine in comparison to the control ones.

As far as the physiological role of Alkaline Phosphatase is concerned, it was thought earlier to be involved in sugar absorption and later it was suggested that it plays a role in cell adhesion. Crane (1968) suggested the possibility of its being a digestive enzyme on the grounds that the other enzymes found in the alimentary tract. Weaning rabbits excrete a low amount of amylase. Feed containing high starch concentration results in an increased amount of indigested starch in intestine content (Tester et al., 2004). Indigested starch in the caecum is a good medium for clostridia growth (Agnoletti, 2006). Furthermore, high levels of proteins in diet induce an increase in trypsin secretion, which enhances C. spiroforme pathogenicity (Agnoletti, 2006).

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Regarding the present observations for Alkaline Phosphatase in the gut of ERE/sick rabbits, the small intestine presented a positive reaction along the brush border of epithelial cells laying the bases of some villi and the upper part of glands, while the deep gland cells showed no such reaction. The caecum and the colon presented an Alkaline phosphatase positive reaction along the brush border of the epithelial cells. To the best of our knowledge, there is no report in the literature on the distribution of the Alkaline Phosphatase enzyme in the gut of ERE/sick rabbits. Nevertheless the biochemical study of Toofanian (1985) on the experimental mucoid enteropathy in rabbits (6-8 weeks of age) can be mentioned. This report proved that Alkaline Phosphatase was significantly lower in rabbits’ small intestine with mucoid enteropathy. Similar observations on intestinal Alkaline Phosphatase distribution have been made in the present study, since Alkaline Phosphatase was significantly lower in ERE/sick rabbits’ small intestine in comparison to the control ones.
ALP in the fetus and the suggestion of Deren (1968) that it has a part in differentiation as well as that it plays a chemical role during development cannot be ignored. It is perhaps possible for Alkaline Phosphatase to have a dual role viz. Not only does it assist in the differentiation, but it also contributes to digestive activity. It has been established that Alkaline Phosphatase is expressed by active and mature mucosal enterocytes and is therefore indicative of enterocyte functional activity. Also, Wieser (1973) and Traber et al., (1991), cited by Uni et al., (1998) regarded that the presence of Alkaline Phosphatase as expressing the maturity of the absorptive cells. The clinical signs of our present ERE affected animals were typical and in accordance with the findings of other researchers (Coudert and Licois 2004, Licois et al., 2005). Bowel rumbling noises were heard, that are considered to be a pathognomonic symptom. This symptom that is referred as "Borborygmos" by other researchers (Coudert and Licois 2004, Licois et al., 2005). Bowel rumbling noises were heard, that are considered to be a pathognomonic symptom. This symptom that is referred as "Borborygmos" by other researchers (Coudert and Licois 2004), is a greek word meaning the automatic, active movement of a watery content while the animal is alive. On the other hand, according to the etymological point of view, it would be better expressed by the word "paflasmos", a greek word also, meaning the noise coming out of the movement of a watery content in dead animal.

At necropsy, a non-specific enteropathy could be observed, without inflammatory lesions in the small intestine. Caecal impaction, non-specific reaction in the gut associated lymphoid tissue and often mucus into the colon could be observed (Wyers, 1998). In the present study, stomach was full of watery content and intestine, mainly the small one, was full of gas and watery content as well. Impaction and mucus were present into the colon. These gross lesions as well as the following observations of the histopathological examinations are in agreement with the observations of other authors (Wyers, 1998, Jobert et al., 2001, Licois et al., 2001, Licois et al., 2005).

The histopathological evaluation of the ileum, sacculus rotundus, caecum and colon presented, mainly, lamina propria mononuclear cell infiltration and swelling, vacuolation, flattening and denuding of the enterocytes, as well as edematous lymphoid tissues. Duodenum had necrotic villi and deep infiltration with mononuclear and polymorphonuclear cells, within the lamina propria. Also, cell swelling, vacuolation, flattening, denuding of the enterocytes and oedema of lymphoid tissue were observed. Jejunum had no lesions. Atrophy of enteric villi was noticed and it was in accordance with other researchers (Wyers 1998, Jobert et al., 2001, Marlier et al., 2003 and Licois et al., 2005).

CONCLUSION

In view of the current study, it is concluded that:

All blood and biochemical parameters were significantly decreased in ERE affected animals, with the exception of the WBC and blood glucose levels.

Intestine length was increased in affected animals.

Measures for pancreas weight and its proportion compared to the total body weight, proved no significant difference.

The affected rabbits suffered from severe hypochromic anaemia, leucocytosis, weight loss, emaciation and hyperglycemia. Histopathological lesions were sub acute or chronic.

The decreased activity of α-amylase in small intestine epithelium of the infected rabbits and the decreased body weight point to a general digestive malfunction.

The hypochromic anaemia was the clinical result of the reduced protein synthesis.

The blood glucose concentration that was found to be significantly elevated (hyperglycemia) is, probably, due to compensatory gluconeogenetic process, considering that - after the destruction of the intestinal epithelium and the resulting food malabsorption - the organism must be well set up with the indispensable reserves of energy. In other words, it concerns a physiological / pathological mechanism of stress confrontation.

The Alkaline Phosphatase activity was significantly lower or absent.

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