

Journal of the Hellenic Veterinary Medical Society

Vol 59, No 4 (2008)



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

E. M. XYLOURI (Ε. Μ. ΞΥΛΟΥΡΗ), O. A. SABATAKOU (Ο. ΣΑΜΠΑΤΑΚΟΥ), E. KALDRYMIDOU (Ε. ΚΑΛΔΡΥΜΙΔΟΥ), K. A. SOTIRAKOGLU (Α. Κ. ΣΩΤΗΡΑΚΟΓΛΟΥ), G. M. FRAGKIADAKIS (Μ. Γ. ΦΡΑΓΚΙΑΔΑΚΗΣ), P. N. NOIKOKYRIS (Π. Ν. ΝΟΙΚΟΚΥΡΗΣ)

doi: [10.12681/jhvms.14970](https://doi.org/10.12681/jhvms.14970)

To cite this article:

XYLOURI (Ε. Μ. ΞΥΛΟΥΡΗ) Ε. Μ., SABATAKOU (Ο. ΣΑΜΠΑΤΑΚΟΥ) Ο. Α., KALDRYMIDOU (Ε. ΚΑΛΔΡΥΜΙΔΟΥ) Ε., SOTIRAKOGLU (Α. Κ. ΣΩΤΗΡΑΚΟΓΛΟΥ) Κ. Α., FRAGKIADAKIS (Μ. Γ. ΦΡΑΓΚΙΑΔΑΚΗΣ) Γ. Μ., & NOIKOKYRIS (Π. Ν. ΝΟΙΚΟΚΥΡΗΣ) Π. Ν. (2017). Histochemical and biochemical study of rabbit intestine in healthy and affected by epizootic enteropathy animals. *Journal of the Hellenic Veterinary Medical Society*, 59(4), 357–366.
<https://doi.org/10.12681/jhvms.14970>

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

Xylouri M. E.¹ DVM, MSc., PhD, Sabatakou A. O.² DVM, MPhil., PhD,
Kaldrymidou E.³ DVM, MSc., PhD, Sotirakoglou A. K.⁴ Maths, PhD, Fragkiadakis G. M.¹ BSc Agron,
Noikokyris N. P.⁵ Biochemistry PhD

¹ Dep. of Anatomy & Physiology of Farm Animals, Faculty of Animal Science & Aquaculture, Agricultural University of Athens

² Institute of Veterinary Research of Athens, 25, Neapoleos str., Agia Paraskevi, Athens, Greece

³ Lab. of Pathology, Veterinary Medicine, Aristotle's University of Thessaloniki, Greece

⁴ Department of Mathematics and Statistics, Faculty of Science, Agricultural University of Athens

⁵ Dep. of Nutrition of Farm Animals, Faculty of Animal Science, Agricultural University of Athens, 75, Iera Odos Str., 118 55, Athens, Greece

Ιστοχημική και βιοχημική μελέτη του εντέρου υγιών κουνελιών και προσβεβλημένων από την επιζωοτική εντεροπάθεια των κουνελιών

E. M. Ξυλούρη¹ DVM, MSc., PhD, αναπλ. καθηγήτρια ΓΠΑ, O. Σαμπατάκου² DVM, MPhil., PhD,
E. Καλδρυμίδου³ DVM, MSc., PhD, καθηγήτρια Α.Ε.Ι.,
A. K. Σωτηράκογλου⁴ Maths, PhD, επίκ. καθηγήτρια Α.Ε.Ι., M. Γ. Φραγκιαδάκης¹ BSc Agron,
Π. Ν. Νοικοκύρης⁵ Biochemistry PhD

¹ Εργ. Ανατομίας & Φυσιολογίας Αγροτικών Ζώων, Τμήμα Επιστήμης Ζωικής Παραγωγής και Υδατοκαλλιεργειών, Γεωπονικό Πανεπιστήμιο Αθηνών

² ΕΘΙΑΓΕ, Αττική

³ Εργ. Παθολογικής Ανατομικής, Κτηνιατρική Σχολή, Αριστοτέλειο Πανεπιστήμιο Θεσσαλονίκης

⁴ Εργ. Μαθηματικών, Γενικό Τμήμα, Γεωπονικό Πανεπιστήμιο Αθηνών

⁵ Εργ. Διατροφής Αγρ. Ζώων, Τμήμα Επιστήμης Ζωικής Παραγωγής και Υδατοκαλλιεργειών, Γεωπονικό Πανεπιστήμιο Αθηνών

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

Correspondence: E. Xylouri

Dep. of Anatomy & Physiology of Farm animals, Faculty of Animal Science & Aquaculture, Agricultural University of Athens, 75, Iera Odos Str., 118 55, Athens, Greece. Tel.: +30 210 5294386, Fax: +30 210 5294388, E-mail: cfxil@aua.gr

Αλληλογραφία: Ε. Μ. Ξυλούρη

Εργ. Ανατομίας & Φυσιολογίας Αγροτικών Ζώων, Τμήμα Επιστήμης Ζωικής Παραγωγής και Υδατοκαλλιεργειών, Γεωπονικό Πανεπιστήμιο Αθηνών, Ιερά Οδός 75, 118 55 Αθήνα. Τηλ.: 210 5294386, Fax: +30 210 5294388, E-mail: cfxil@aua.gr

Submission date: 21.01.2009

Approval date: 08.05.2009

Ημερομηνία υποβολής: 21.01.2009

Ημερομηνία εγκρίσεως: 08.05.2009

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 ($\alpha=0.05$) 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, vacuolation, 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 tissue were observed. Jejunum had no lesions. The caecum and the colon presented an ALP positive reaction along the brush border of the epithelial cells. The small intestine presented a positive reaction along the brush border of 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

ΠΕΡΙΛΗΨΗ. Τα κουνέλια πάσχουν από την Επιζωοτική Εντεροπάθεια των Κουνελιών (EEK) (Epizootic Rabbit Enteropathy, ERE), η οποία αποτελεί σύνδρομο του γαστρεντερικού σωλήνα αγνώστου αιτιολογίας. Η EEK δεν έχει ακόμη πλήρως μελετηθεί όσο αναφορά τη παθοφυσιολογία της δυσλειτουργίας του εντέρου, κατά τη διάρκεια της διαταραχής της ισορροπίας της εντερικής βακτηριακής χλωρίδας. Για το λόγο αυτό, ο σκοπός της παρούσας έρευνας ήταν η μελέτη των αιματολογικών παραμέτρων, της ιστοπαθολογίας του εντέρου και ορισμένων βιοχημικών παραμέτρων (όπως η α -αμυλάση και η συγκέντρωση γλυκόζης στο αίμα) προσβεβλημένων από επιζωοτική εντεροπάθεια κουνελιών σε σύγκριση με τις αντίστοιχες υγείων κουνελιών ίδιας ηλικίας. Σε έξι υγεία και έξι προσβεβλημένα από EEK κουνέλια, υβρίδια Νέας Ζηλανδίας Χ Καλιφόρνιας ηλικίας 35 ημερών, μελετήθηκαν: οι αιματολογικές παράμετροι, η ιστοπαθολογία του εντέρου, η κατανομή της αλκαλικής φωσφατάσης (ALP) στην ψηκτροειδή παρυφή του εντέρου και η δραστηριότητα της α -αμυλάσης στο πλάσμα του αίματος και στο εντερικό επιθήλιο αντίστοιχα. Η πλήρης αιματολογική εξέταση έδειξε (σε επίπεδο εμπιστοσύνης 95%) σημαντικά μειωμένες τις τιμές όλων των αιματολογικών παραμέτρων εκτός από τις τιμές των λευκών αιμοσφαιρίων, οι οποίες ήταν στατιστικώς σημαντικά υψηλότερες στα προσβεβλημένα από EEK σε σύγκριση με τα φυσιολογικά κουνέλια. Η δραστηριότητα της α -αμυλάσης και η συγκέντρωση της στο πλάσμα του αίματος και στο εντερικό επιθήλιο είχε στατιστικώς σημαντικά ($\alpha=0.05$) μειωθεί, σε σχέση με τη συγκέντρωση της γλυκόζης στο αίμα, η οποία βρέθηκε σημαντικά αυξημένη στα προσβεβλημένα από EEK κουνέλια. Ο στόμαχος ήταν πλήρης υδαρούς περιεχομένου, το έντερο παρουσίαζε μη ειδική εντεροπάθεια και κυρίως το λεπτό ήταν πλήρες αερίων και υδαρούς περιεχομένου. Το τυφλό και το κόλον περιείχαν συμπαγές περιεχόμενο και βλέννα υπήρχε στο κόλο. Η ιστοπαθολογική αξιολόγηση του ειλεού, (λεμφικός ιστός πλησίον της ειλεοτυφλικής βαλβίδας) του *sacculus rotundus*, του τυφλού και του κόλου αποκάλυψε κυρίως μονοκύτταρη διήθηση του χορίου και οίδημα, κενοτοπίωση εκφύλιση, πλάτυνση και αποφολίδωση των εντεροκυττάρων, όπως επίσης και οίδημα των λεμφικών ιστών. Το δωδεκαδάκτυλο παρουσίαζε νέκρωση των λάχνων και έντονη διήθηση του χορίου με μονοκύτταρα και πολυμορφοπύρρηνα κύτταρα. Επίσης, παρατηρήθηκαν οίδημα, κενοτοπίωση, πλάτυνση και αποφολίδωση των εντερικών κυττάρων και οίδημα του λεμφικού ιστού. Η νήστιδα δεν είχε αλλοιώσεις. Το τυφλό και το κόλον παρουσίαζαν θετική ALP αντίδραση κατά μήκος της ψηκτροειδούς παρυφής των επιθηλιακών κυττάρων. Το λεπτό έντερο παρουσίαζε θετική αντίδραση κατά μήκος της ψηκτροειδούς παρυφής των εντερικών αδένων -στα κύτταρα της άνω μοίρας μόνο- και των βάσεων μερικών λαχνών.

Λέξεις ευρετηρίασης: αιματολογικές παράμετροι, έντερο, δραστηριότητα α -αμυλάσης, ALP- δραστηριότητα, Επιζωοτική Εντεροπάθεια κουνελιού

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 generally dysbiosis of intestine flora (frequent use of antibiotics) or unidentified causes etc (Coudert et al.,

1997, Coudert et al., 2000).

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 4 μ m 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 α -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 oxydase (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

Πίνακας 1. Βάρος απογαλακτισμού, Μήκος λεπτού εντέρου, Βάρος παγκρέατος, Δραστικότητα αμυλάσης λεπτού εντέρου, Δραστικότητα αμυλάσης επιθηλιακού ιστού, Αμυλάση ορού αίματος, αιματολογικό προφίλ ηλικίας 35 ημερών κουνελιών

Parameters Παράμετροι	Animals affected by ERE Κουνέλια που εμφάνιζαν ΕΕΚ		Significance	Healthy animals Υγιή ζώα	
	Mean Value	Stand. Error		Mean Value	Stand. Error
Weaning Weight (g) Βάρος απογαλακτισμού	799.17	29.54	***	1284.17	47.21
S. Intestine Length (cm) Μήκος λεπτού εντέρου	237.50	10.70	**	179.17	7.97
Pancreas Weight (g) Βάρος παγκρέατος	0.62	0.06	NS	0.70	0.16
Amylase Activity of Small Intestine (10x ³ I.U/l) Δραστικότητα αμυλάσης λεπτού εντέρου	385.0	20.12	***	146.09	32.28
Epith. Tiss. Amylase Activ. (I.U/mg) Δραστικότητα αμυλάσης επιθηλιακού ιστού	-	-		1219.50	204.42
Blood Serum Amylase (x10 ³ I.U/l) Αμυλάση ορού αίματος	7.37	0.48	NS	6.27	0.88
Glucose (mM/l) Γλυκόζη	14.22	0.66	***	8.96	0.22
Hematocrit Αιματοκρίτης	31.98	2.81	**	44.38	1.13
Red Blood Cells (x10 ⁶) Ερυθρά αιμοσφαίρια	4.80	0.46	*	5.79	0.04
Hemoglobin (g/l) Αιμοσφαιρίνη	7.47	0.52	***	13.07	0.11
Mean Corpuscular Hemoglobin Concentration (%) Ποσοστιαία αναλογία αιμοσφαιρίνης	23.57	0.56	**	29.50	0.68
Mean Corpuscular Volume (fl) Μέσος όγκος ερυθρού	66.93	2.06	*	76.64	1.80
Mean C. H (pg) Μέση συγκέντρωση αιμοσφαιρίνης	15.78	0.62	***	22.57	0.27

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

no significant difference (Table 2).

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. Caecum and colon presented an ALP positive reaction along the brush border of the epithelial cells (Table 4).

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

Results / Αποτελέσματα						
	Body weight (g) Βάρος σώματος		Small intestine Length (cm) Μήκος λεπτού εντέρου		Pancreas weight (g) Βάρος Παγκρέατος	
Rabbits Κουνέλια	Healthy Υγιή	ERE Νοσούντα	Healthy Υγιή	ERE Νοσούντα	Healthy Υγιή	ERE Νοσούντα
1	1375	885	206	282	1,301	0,632
2	1100	735	170	224	0,840	0,66
3	1380	785	185	221	0,431	0,876
4	1385	860	172	257	0,378	0,602
5	1235	700	150	225	0,971	0,512
6	1230	830	192	216	0,295	0,457
Mean value Μέσος όρος	1284,17	799.17	179,17	237.5	0,70	0.623

Table 3. α-amylase activity, glucose concentration

Πίνακας 3. Δραστικότητα α-αμυλάσης, συγκέντρωση γλυκόζης

BIOCHEMICAL RESULTS

ΑΠΟΤΕΛΕΣΜΑΤΑ: ΒΑΡΟΣ ΠΑΓΚΡΕΑΤΟΣ ΚΑΙ ΒΙΟΧΗΜΙΚΩΝ ΑΝΑΛΥΣΕΩΝ

RABBITS	PANCREAS WEIGHT OF BODY WEIGHT (%)	α-AMYLASE ACTIVITY OF HOMOGENIZED SMALL INTESTINE EPITHELIUM (1000 IU)	α-AMYLASE ACTIVITY IN SMALL INTESTINE EPITHELIUM (IU/mg)	α-AMYLASE BLOOD PLASMA ACTIVITY (1000 IU)	α-AMYLASE ACTIVITY IN PLASMA and HOMOGENIZED INTESTINE	GLUCOSE IN BLOOD (mmol/l)
1	0.07	29.78	320	7,8	0,26	16,76
2	0.09	48.14	380	8,15	0,17	13,3
3	0.11	41.69	390	9,05	0,22	14,61
4	0.07	38.78	360	6,05	0,16	15,16
5	0.07	44.19	470	6,14	0,14	13,07
6	0.055	39.27	390	7,03	0,18	12,42
Mean diseased animals	0.078	40.31	385	7.37	0.19	14.22
Mean healthy animals	0.060	135.09	1163	6.27	0.05	8.96

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 "borborygmus" 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.
Reaction: +mild, 2+positive, 3+strong, 4+very strong, -no reaction

Πίνακας 4. Αντίδραση Αλκαλικής φωσφατάσης στο λεπτό και παχύ έντερο.
Αντίδραση: + ήπια, 2+θετική, 3+ ισχυρή, 4+ πολύ ισχυρή, - καμία αντίδραση

	35 days Normal rabbits 35 ημερών φυσιολογικά κουνέλια	35 days affected rabbits 35 ημερών πάσχοντα κουνέλια
Duodenum Δωδεκαδάκτυλο	4+	- *
Jejunum Νήστιδα	4+	- *
Ileum Ειλεός	4+	-*
Caecum Τυφλό	2+	2+
Colon Proximal Εγγύς κόλον	3+	2+
Colon Distal Απομακρυσμένο κόλον	-	2+

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

intestinal inflammatory lesions. Caecal 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: $146,09 \pm 32,28$ ($10^3 \times \text{IU/Lit}$), ($P \geq 0,05$), α -amylase activity of epithelial tissue was: $1219,5 \pm 204,42$ (IU/mgr) and α -amylase activity of blood serum was: $6,27 \pm 0,88$ ($10^3 \times \text{IU/Lit}$), ($P \leq 0,01$). 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 ($\alpha=0.05$) decreased (Philips and Fuller, 1983, Rodeheaves and Wyatt, 1986).

The main pathological agent for ERE consists of the facilitated factors for clostridia growth in the alimentary track. 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 $8,96 \pm 0,22$ (mM/Lit) vs. $14,22 \pm 0,66$ (mM/Lit) in 35-day old ERE rabbits. It was found to be substantially increased due to the activation of homeostatic body mechanisms. Hyper-

glycemia 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 substantial amounts in the brush border, all have a digestive function catalyzing the hydrolysis of a wide variety of phosphorylated compounds. On this basis it seems that the function of Alkaline Phosphatase is that of a digestive enzyme cleaving a non - penetrating molecule into transportable components. Nevertheless, it is difficult to attribute a digestive role to the

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), 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 gluconeogenic 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.

ACKNOWLEDGEMENT: the COST ACTION 848 for supporting the present study. ■

REFERENCES - ΒΙΒΛΙΟΓΡΑΦΙΑ

- Agnoletti F, (10. 2. 2006) Update on rabbit enteric pathology of bacterial origin. Aggiornamenti sulle patologie enteriche nella moderna conigliocultura: Spagna, Francia e Italia a confronto CEVA SANTE ANIMALE, Sirmione del Garda, Brescia, Italy.
- Alvarez JL, I. Margüenda, P. Garc_a-Rebollar, R. Caraba_o, JC De Blas Y A.I. Garc_a-Ruiz (2006) Effect of level and type of fibre on growth performance and the ileal digestibility in growing rabbits. XXXI Symposium de Cunicultura, May Lorca, Spain, pp 79-87.
- Boisot P, Licois D, Gidenne T (2003) Une restriction alimentaire réduit l'impact sanitaire d'une reproduction expérimentale de l'entéropathie épizootique (EEL) chez le lapin en croissance. 10èmes Journées de la Recherche Cunicole, 19-20 nov. 2003, (ITAVI Ed.) Paris, 267-270.
- Ceroli M and A. Lavazza (2006) Ruolo dei virus nelle patologie enteriche della moderna conigliocultura. Aggiornamenti sulle patologie enteriche nella moderna conigliocultura: Spagna, Francia e Italia a confronto. CEVA SANTE ANIMALE, Sirmione del Garda, Brescia, Italy.
- Colin M, AY Prigent (2006) Effect of dietary supplementation of essential oils or plant extract mix on mortality and growth performance in growing rabbits, XXXI Symposium de Cunicultura, Lorca, Spain, pp 111-116.
- Coudert P, Lebas F, Licois D (1997) Une nouvelle pathologie ravage les eveages. La profession se mobilise. Cuniculture, 24 :225-229.
- Coudert P, Licois D (2004) Study of early phenomena during experimental epizootic rabbit Enteropathy: preliminary results. 8th World Rabbit Congress, Puebla, Mexico, pp. 520 -525.
- Coudert P, Peeters J, Rossel J, Arguello JL, Badiola JJ, Blas E, Cuervo L, Drouet-Viard F, Licois D, Provo F (2000) Enfermedades del aparato digestivo: causas parasitarias y otras. 219-263, IN: Enfermedades del conejo, tomo II Enfermedades. Ediciones Mundi-Prensa.
- Crane RK, (1968) A concept of the digestive-absorptive surface of the small intestine. Handbook of Physiology - Alimentary canal V - chapter 118: 2535-2539, American Physiological Society, Washington, D. C
- Debray L, Gidenne, T, Fortun-Lamothe L, (2001) Evolution of intestinal enzymatic digestive capacity of rabbit around weaning. COST Action 848, 2nd meeting, Workshop. Nutrition and Pathology, 37-38, Godollo, Hungary.
- Deren, JJ (1968) Development of intestinal structure and function. Handbook of Physiology, Section 6, Alimentation canal III: 1099-1123, American Physiological Society, Washington D.C.
- Garrido S, Nicodemus N, Chamorro S, De Blas JC (2006) Effect of weaning age (25 vs 35), the farm and the period on mortality and performance of growing rabbits. XXXI Symposium de Cunicultura, Lorca, Spain, pp 95-101.
- Jervis HR, (1963) Enzymes in the mucosa of the small intestine of the rat, the guinea pig and the rabbit. J. Histochem Cytochem 11: 692-699.
- Jobert JL, F Eone, G Legall-Recule, M (2001) Guttet Reproduction expérimentale du syndrome d' Entérocite Epizootique du Lapin: étude anatomo-clinique et hématologique. 9emes Journ. Cunicole Paris.
- Kaneko, JJ (1989) In: Clinical Biochemistry of Domestic Animals. Fourth Edition. Academic Press,
- Kósa E, Kovács M, Rigó Z (2004) Effect of a vegetable extract (Trade Name: Immunovet-Hbm®) upon activity of digestive enzymes in rabbits. 8th World Rabbit Congress, Puebla, Mexico, pp 1087-1090.
- Lafont, J, and Moretti, J, (1970) Etude de la distribution de la phosphatase alcaline le long de l'intestine grêle. C. R. Seanc. Soc. Biol., 164 : 1589-1581.
- Lebas F, Coudert P, (1997) Enterocolite: les donnees recentes. Cuniculture, 24, 269-272.
- Licois D, (2004) Domestic rabbit enteropathies. In: Proc. 8th World Rabbit Congress Puebla, Mexico, 2004, pp 385-403.
- Licois D, Coudert P (2001) Entéropathie épizootique du lapin: reproduction expérimentale, symptômes et lésions observées. 9èmes Journées de la Recherche Cunicole en France, 28-29 Novembre, (ITAVI Ed.) Paris, 139-142.
- Licois D, Wyers M, Coudert P (2005) Epizootic Rabbit Enteropathy: Experimental transmission and clinical characterization. Vet. Res. 36, 601-613 (INRA, EDP Sciences).
- Maertens L, Cornez B, Vereecken M, Van Oye S (2005) Efficacy study of soluble bacitracin (Bacivet S®) in a chronically infected epizootic rabbit Enteropathy environment. World Rabbit Science 13:165-178.
- Marlier D, Dewree R, Licois D, Coudert P, Lassence C, Poulipoulis A, Vindevogel H (2003) L' Entéropathie Epizootique du Lapin: un bilan provisoire des résultats après 20 mois de recherches. 10èmes Journées de la Recherche Cunicole, 19-20 November (ITAVI Ed.) Paris, 247-250.
- Marshall JJ (1978) Quantitative enzymatic synthesis of 2-acetamido-2-deoxy-a-D-glycopyranosyl-1,6-diphosphate. J. Analytical Chemistry, 85, 541-549.
- Marshall JJ, Iodice AP, Whelan WJ (1977) A serum a-amylase assay of high sensitivity. Clinica Chim. Acta, 76, 277 -283.
- Meyer JD, and JW Harvey (1992) Veterinary Laboratory Medicine 2nd Edition, W. B. Saunders Company, 190.
- Nemi C Jain, (1986) Schalm's Veterinary Haematology. Fourth edition. Editor: Lea and Febiger. Philadelphia Press.
- Pearse A.G.E. (1961) Histochemistry theoretical and applied. London eds: J & A Churchill EDS, 104 Gloucester Place W1, London
- Philips SM and Fuller R, (1983) The activities of amylase and a trypsin like protease in the gut contents of germ-free and conventional chickens. British Poultry Science, 24: 115-121
- Rodeheaver DP, Wyatt RD, (1986) Distribution of alpha amylase activity in selected broiler tissues. Poultry Science, 65 (2): 325-9.
- Sabatikou AO, Xylouri ME, Sotirakoglou AK, Fragkiadakis GM, Noikokyris NP Histochemical and biochemical characteristics of weaning rabbit intestine. World Rabbit Sci. 2007, 15: 173 – 178.
- Sabatikou O, Xylouri E, Paraskevaki E and K Papantonakis (1999) The distribution of alkaline Phosphatase in the cells of the small and the large intestine of the rabbit. J. Submicrosc. Cytol. Pathol. 31(3), 335-344.
- Suvegova K, Jurcik R, Chrenek P, Gazovicova Z, Rafay J, Hanusova E, (2004) "Comparison of inner organs weigh and some haematological and haematological blood parameters of transgenic and non transgenic rabbits" 8th World Rabbit Congress, Puebla, Mexico, pp 632-638.
- Tester RF, J Karkalas and X Qi, (2004) Starch structure and digestibility. Enzyme - Substrate relationship. Journal World's Poultry Science, 60: 186 – 195.
- Toofanian F, (1985) Intestinal Disaccharidase and Alkaline Phosphatase Activities in Experimental Rabbit Mucoid Enteropathy. Laboratory Animal Science 35(6):624-626.

- Traber PG, Gumucio DL and Wang W, (1991) Isolation of intestinal epithelial cells for the study of different gene expression along the crypt-villus axis. *Am. J. Physiol.* 260:895-903, (quoted by Uni Z. et al., 1998).
- Trinder P, (1969) Determination of glucose in blood using glucose oxydase with an alternative oxygen acceptor. *Ann. Clin. Biochem.* 6:24-26.
- Uni Z, Ganot S and Sklan D, (1998) Post hatch development of mucosal function in the broiler small intestine. *Poultry Science* 77 (1): 75-82.
- Van Kampen EJ, and Zijlstra WG, (1961) The Hemoglobincyanide method: *Clinical Chem. Acta*, 6:538.
- Van Kampen EJ, and Zijlstra WG, (1965) Determination of Hemoglobin and its derivatives, *Adv. Clin. Chem.*, 8, 141.
- Wieser MM, (1973) Intestinal epithelial cell surface membrane glycoprotein synthesis. *J. Biol. Chem.* 248:2536-2541, (quoted by Uni Z. et al., 1998).
- Wyers M, (1998) Trois questions à propos de l'histopathologie de l'entérocite du lapin. Interview de v. Dedet. *La Semaine Vétérinaire*, 9 mai 1998. Cited by Licois D, (2001).
- Xylouri E, Fragkiadakis M (2006) Epizootic rabbit enteropathy - Current data and control. *Journal Hellenic Veterinary Medical Society*, 57 (4) 302-314.