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# Nutritionally induced enteropathy in the growing rabbit: impact on caecal microbial activity and blood metabolic profile

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## SUMMARY

Caecal microbial activity and blood metabolic profiles were assessed according to the health status (diarrhoeic vs. healthy) in growing rabbits after induction of a nutritional enteropathy. Rabbits were fed ad libitum from weaning (28 days) to 70 days of age with a standard fibre content diet (n = 150) or with a deficient fibre diet (n = 209) known to induce non specific enteropathy. The reduction of fibre intake caused a significantly delayed weight growth and markedly increased the health risk index (mortality + morbidity). In diarrhoeic rabbits, dry matter and total volatile fatty acid (tVFAs) concentrations in the caecum dramatically decreased by 27 and 40%, respectively whereas the caecal ammonia concentration, minor VFA proportions and pH markedly increased by 2 and 8.5 times and by 0.7 unit, respectively. In parallel, the C3:C4 ratio increased fourfold and the pectinase activity was significantly depressed. Blood plasma glucose, potassium and albumin concentrations significantly decreased in diseased animals compared to the healthy ones while urea and total cholesterol concentrations showed a two-fold increase suggesting preferential metabolic utilisation of lipids and proteins. These results show that the caecal function and blood metabolic profiles were greatly affected during nutritionally induced enteropathy and that the observed changes were globally similar to those induced by coccidiosis or colibacillosis.

**Keywords:** Growing rabbit, fibre deficiency, enteropathy, caecal fermentation, caecal microbial activity, blood parameters.

## RESUME

L'activité bactérienne caecale ainsi que les profils métaboliques sanguins ont été déterminés en fonction de l'état général des lapins en croissance après induction d'une entéropathie nutritionnelle (animaux avec diarrhée ou sains). Les lapins ont été nourris ad libitum du sevrage (à 28 jours) jusqu'à 70 jours avec un aliment ayant une teneur standard en fibre (n = 150) ou avec un aliment déficient en fibres (n = 209) connu pour induire une entéropathie non fonctionnelle. La réduction de la consommation de fibres a causé un retard significatif de la croissance pondérale et a nettement accru le risque de morbidité et de mortalité. Chez les animaux diarrhéiques, le contenu caecal en matière sèche et la concentration des acides gras volatils ont chuté de façon importante, respectivement de 27 et de 40%, alors que la concentration caecale en ammoniac, les proportions en acides gras volatils mineurs et le pH ont nettement augmenté, respectivement, 2 et de 8.5 fois et de 0.7 unités. Parallèlement, le rapport C3:C4 a été multiplié par 4 et l'activité pectinase a été grandement diminuée. Les concentrations plasmatiques de glucose, de potassium et d'albumine ont significativement diminué chez les animaux malades alors que l'urémie et la cholestérolémie ont été multipliées par 2 environ, suggérant une utilisation métabolique préférentielle des lipides et des protéines. Ces résultats montrent que la fonction caecale et les profils sanguins métaboliques ont grandement été affectés lors d'une entéropathie nutritionnelle et que ces modifications sont globalement analogues à celles observées lors de coccidiose ou de colibacillose.

**Mots-clés :** Lapin en croissance, déficit en fibres, entéropathie, fermentation caecale, activité microbienne caecale, paramètres sanguins.

## Introduction

Diarrhoea is the main clinical sign in rabbits affected by digestive disorders [21, 24] and is mostly observed during the first three weeks after weaning [20]. It is possible to distinguish enteropathies originating from specific pathogenic agents such as coccidia or enteropathogenic *Escherichia coli* (like O<sub>15</sub> or O<sub>103</sub> serogroups) from those where no clear pathogenic origin is detected. For these non specific enteropathies change in bacterial community balance and activity is assumed in the caecal ecosystem. In addition, since the frequency of digestive troubles is higher in the young animals, several authors try to describe the relationship between caecal ecosystem parameters around weaning [29] and the diet composition [9]. Among the nutritional factors, the dietary fibre level is the most important in order to modulate the digestive health in the young rabbit [13, 15] and

caecal microbial community [2]. Besides, some studies report metabolic changes, in particular in hydromineral metabolism in rabbits suffering from diarrhoea [11], coccidiosis [22, 23, 31] and colibacillosis [30].

However, few studies analysed the physiological status of young rabbits affected by digestive troubles (diarrhoea), and particularly the changes in the caecal ecosystem associated with metabolic criteria. Thus the aim of the present study is to describe the caecal activity (fermentation, end-products levels, and bacterial fibrolytic activity) and metabolic blood profile according to a nutritionally induced enteropathy model in the growing rabbit. It is also intended to compare the physiological impact of specific (coccidiosis, etc.) to that of non-specific enteropathy.

## Material and Methods

### ANIMALS, DIETS AND EXPERIMENTAL DESIGN

Hybrid conventional rabbits (White New-Zealand x Californian) from INRA (strain INRA A1067) were used in this experiment. Two hundred and seventy nine rabbits were placed in collective cages (C) (5 to 6 animals/ cage) and 80 other rabbits were housed in individual cages (I) (26 × 33 × 65 cm, l × h × b) under a temperature of 18 ± 2°C, and a 12:12 light: dark cycle (lights on 07:00 h), and a minimum of 0.8 m<sup>3</sup> air changes per hour from weaning (day 28) till 70 days of age.

Before weaning, kits and does were fed with a commercial diet. At weaning (day 28), each group (C and I) was allotted into two-sub groups, according to the kit origin and weaning weight, and they were fed, either with a diet having a standard fibre concentration (diet SF, 120 and 30 rabbits, respectively, for C and I groups), or with a fibre deficient diet (diet DF, 159 and 50 rabbits, respectively, for C and I groups). The two experimental diets were prepared in pelleted form and formulated to avoid any changes in the fibre quality, but only in the fibre quantity. For instance, the lignocellulosic content of the diet SF (189 g.kg<sup>-1</sup>) corresponded to the current recommendations [13] while that of the DF diet was only

88 g.kg<sup>-1</sup>. The origin and proportions of fibre fractions were similar between diets, as well as the ratio of digestible protein/digestible energy (Table I). No additive was included in the diet except a coccidiostatic (Robenidine<sup>®</sup>). Feeds and water were available *ad libitum*.

### CONTROL OF PERFORMANCES AND HEALTH STATUS

Live-weight and feed intake were controlled each week, and mortality and morbidity were controlled individually every day from 28 to 70 days of age. Morbidity control consisted of a global external examination (dynamic or prostrated animal), followed by a precise observation of all clinical signs of digestive troubles or sickness. In addition, animals without visible digestive troubles, but showing severe disturbances in feed intake or growth (loss of weight during a week or with abnormally low growth) were classed as morbid. The morbidity rate was expressed as the number of sick rabbits divided by the initial number of animals, and an animal was considered morbid only once (within a period), even if diarrhoea lasted several days. Dead animals were only counted for the mortality rate (within a period), even when they exhibited clinical signs of diarrhoea before death. Therefore, the Health Risk index (HRi) corresponding to the sum of morbid and dead animals, taking into account that each animal was counted only once was calculated.

	Experimental diets	
	Standard in fibres (SF)	Deficient in fibres (DF)
<b>Ingredients (%)</b>		
Dehydrated alfalfa meal	30.0	9.0
Dehydrated beet pulp	20.0	7.5
Wheat bran	20.0	5.0
Wheat	12.4	54.3
Soybean meal	10.0	19.0
Wheat straw	6.0	2.0
DL Methionine	0.2	0.2
Minerals	0.9	2.5
Oligoelements and vitamins <sup>1</sup>	0.5	0.5
<b>Chemical analysis (g.kg<sup>-1</sup>air dry basis)</b>		
Dry matter	902	893
Organic matter	824	837
Crude protein (N x 6.25)	159	177
Starch	96	320
NNCC <sup>2</sup>	286	467
Crude fibre	162	72
Neutral-detergent fibre (NDF)	379	193
Acid-detergent fibre (ADF)	189	88
Acid-detergent lignin (ADL)	34	15
<b>Nutritive value (air dry basis)</b>		
Digestible crude proteins (DP) (g.kg <sup>-1</sup> )	118	152
Digestible energy (DE) (MJ.kg <sup>-1</sup> )	10.26	13.06
DP/DE (g.MJ <sup>-1</sup> )	11.50	11.63

<sup>1</sup>containing a coccidiostatic (66 mg of robenidine<sup>®</sup>/kg) but no antibiotics added; <sup>2</sup>Non Nitrogenous Cellular Content = Organic matter - NDF - Crude Proteins

TABLE I: Ingredients and chemical composition of experimental diets given to rabbits after weaning.

## SAMPLING AND MEASUREMENTS OF CAECAL MICROBIAL ACTIVITY

A total of 130 healthy rabbits of each cage and each diet group were killed without fasting after a lethal injection of anaesthetic solution (sodium thiopental) in the marginal ear vein, at the end of the caecotrophy period (about 11:00 h). The pH of the caecal digesta was measured immediately after laparotomy, with a glass electrode pH meter (pH 95, WTW, Weilherm, RFA). Caecal digesta samples (1-3 g fresh matter) were collected in three different tubes containing respectively: 10 mL of 0.025 M of an anaerobic buffer MES-DTT (2-N-Morpholinoethanesulfonic acid-DL-Dithiothreitol), 2 mL of  $H_3PO_4$  (2% v/v) and 3 mL of  $H_2SO_4$  (2% v/v), used as storage solution, for further analysis of bacterial fibrolytic activity (BFA), volatile fatty acids (VFAs) and ammoniacal nitrogen ( $NH_3$ -N), respectively. The portions of the caecal content were stored at  $-80^\circ C$  for BFA and at  $-20^\circ C$  for VFAs and  $NH_3$ -N analyses. The same sampling procedure was performed on agonising rabbits ( $n = 19$ ) between 36 and 65 days and on rabbits which had died less than 10 hours before sampling ( $n = 37$ ) between 32 and 67 days. The dead rabbits were classified in 2 groups: Group 1 ( $n = 17$ ) corresponding to animals still lukewarm and sampled 1 to 3 hours after death, and group 2 ( $n = 20$ ) corresponding to animals that were on *rigor mortis* state (between 4 and 10 hours after death).

Dry matter (DM) was analysed on feed and caecal content by heating at  $103^\circ C$  for 24 hours. Organic matter (OM) was determined by ashing samples at  $550^\circ C$  for 5 hours. On feeds, nitrogen was analysed by the DUMAS combustion method using a Leco apparatus (model FP-428, Leco Corp., St Joseph, MI, USA), converted to crude protein (CP) using the factor 6.25, and energy was measured by an adiabatic calorimeter (PARR Instrument; Moline, IL). Starch was enzymatically measured by quantitative hydrolysis with glucoamylase (after gelatinisation and autoclaving), and the released glucose was then measured using the hexokinase (EC.2.7.1.1)-glucose-6-phosphate dehydrogenase (NAD, EC1.1.1.49) system (Boehringer Mannheim). Different fractions of the fibre cell-wall (neutral detergent fibre: NDF, acid detergent fibre: ADF, acid detergent lignin: ADL) were analysed by the VAN SOEST method [32] and according to the EGRAN procedure [10].

Fibrolytic bacterial activity was assayed from bacteria, after extraction of cellular content from caecal samples [17]. Cellulolytic, xylanolytic and pectinolytic activities were measured, respectively, on carboxymethylcellulose, wood xylose and citrus pectin substrates. After incubation at  $39^\circ C$  for 60 minutes, released sugars (glucose, xylose, galacturonic acid) for each enzymatic activity were measured spectrophotometrically at 410 nm after 5 minutes of incubation at  $100^\circ C$  to stop the reaction. The quantity of released sugars was expressed as  $\mu M$  of reducing sugars per g of dry matter of caecal content per hour.

Volatile fatty acids were extracted from caecal liquid digesta and measured by gas phase chromatography (P9000, Chrompack, Middelburg, The Netherlands) on semi-capillary columns [1].  $NH_3$ -N concentration was analysed spectrophotometrically at 660 nm [33].

## SAMPLING AND ANALYSIS OF BLOOD PARAMETERS

Blood was sampled from the central ear artery, from 121 healthy rabbits at the end of the caecotrophy period (about 11:00 h) at 28, 42, 56 and 70 days of age (8 to 20 rabbits/diet and age). Rabbits did not fast before sampling. Blood samples were also taken on diarrhoeic rabbits, between 38 and 65 days of age (93 animals). Blood serum was obtained after 1 hour refrigeration of samples at  $4^\circ C$  and centrifugation at 1500 g for 10 minutes at  $4^\circ C$ . Serums were sampled in sterile tubes then frozen at  $-20^\circ C$  for later analysis.

The haematocrite value was determined by the capillary micro-haematocrite method, after centrifugation at 15 000 g for 10 minutes with a micro-haematocrite centrifuge and the packed cell volume was read using a micro-haematocrite reader. The haematocrite was expressed as the percentage of packed cell volume on the total blood volume in the tube.

Serum albumin (green of bromocresol, Bayer), urea (urease, Bayer), glucose (Glu-cinet, glucose oxydase, Bayer), cholesterol (cholesterol oxydase, cholesterol esterase, Bayer), triglycerides (GPO, Bayer) were analysed on serum (automatic analyzer Technicon R.A 1000, Ireland). Sodium and potassium concentrations were measured after sample dilution, with an atomic absorption spectrophotometer (PU 9200, Pye-Unicam, Cambridge, England).

## STATISTICAL ANALYSIS

When data followed a normal distribution, a variance analysis was carried out, using the GLM procedure of SAS, and means were compared using the Scheffe test. Without a normal distribution, data was analysed by non parametric procedure (Mann & Whitney test). Health control data (mortality, morbidity, HRi) was performed using the Catmod procedure and  $Chi^2$ .

Likewise, in order to compare unhealthy with healthy rabbits and according to the age of diarrhoeic animals, rabbits were separated in 3 classes of age: 28 to 42 days old, 43 to 56 days old and 57 to 70 days old.

Microbial activity (BFA, VFAs), caecal traits and blood parameters were analysed according to the type of housing (individual, collective), to the age (42, 56 and 70d) for healthy rabbits, to the diets (SF and DF), to the time of sampling (dying, 1-3 h after death, > 4 h after death) and to the health status (healthy, diarrhoeic). Differences were considered as significant when p value was less than 0.05.

## Results

### EFFECT OF DIETARY FIBRE DEFICIENCY ON GROWTH PERFORMANCE AND HEALTH STATUS

As expected, the feed intake of rabbits fed with the DF diet was 33% lower than for those fed with the control diet (Table II,  $p < 0.001$ ), and although the growth was impaired (-10% from 28 to 70 days,  $p = 0.021$ ), the feed conversion remained 25% better ( $p < 0.0001$ ). Accordingly, the body weight was 7% lower ( $p = 0.053$ ) at 70 days for animals fed with the fibre deficient diet. Compared to the control, rabbits fed with the DF diet showed a higher incidence of digestive perturbations after the weaning, leading to significant increases in morbidity ( $p = 0.01$ ) and in morbidity ( $p < 0.01$ ) and resulting in a double higher health risk index ( $p < 0.001$ , Table II).

### IMPACT OF SAMPLING DELAY AFTER DIGESTIVE TROUBLES ON THE CAECAL MICROBIAL ACTIVITY

Except the  $\text{NH}_3\text{-N}$  which increased progressively by two ( $p < 0.001$ , figure 1) between dying rabbits and rabbits of group 2 (> 4 hours *post-mortem*), other parameters of the caecal microbial activity (CMA) did not evolve according to the time of the death. Significant modifications of CMA were observed between agonising rabbits having acute digestive trouble and rabbits of the group 1 (corresponding to dead animals still lukewarm and sampled 1 to 3 hours after death (figure 1). When the caecal content was sampled between 1 and 3 hours *post-mortem*, tVFAs (total volatile fatty acids), acetate ( $\text{C}_2$ ), propionate ( $\text{C}_3$ ), butyrate ( $\text{C}_4$ ), and  $\text{NH}_3\text{-N}$  increased by 79, 65, 76, 146 and 68%, respectively, comparatively to caecal measurements from dying rabbits ( $p < 0.001$ ). Inversely, the caecal pH did not vary significantly (means 6.72, result not shown).

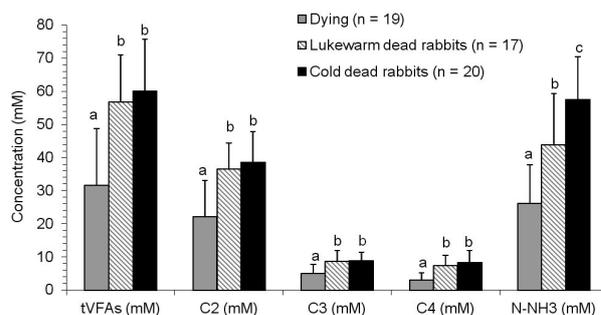


FIGURE 1: Caecal fermentative activity for dying and dead rabbits after diarrhoea, between 32 and 67 days of age. Results are expressed as means  $\pm$  standard deviations.

Different superscripts a,b,c indicate significant differences ( $p < 0.05$  or more) between groups.

In parallel, BFA gradually increased with the time delay for caecal sampling (figure 2): a two fold increase was found for cellulolytic and xylanolytic activities ( $p < 0.05$ ) in group 2 compared to dying rabbits. However, no significant change in pectinolytic activity was observed among the 3 groups (mean: 106  $\mu\text{M}$  of reduced sugar/g DM/h). Additionally, BFA values were relatively variable, with a residual coefficient of correlation of 72% for cellulase, 74% for xylanase and 53% for pectinase.

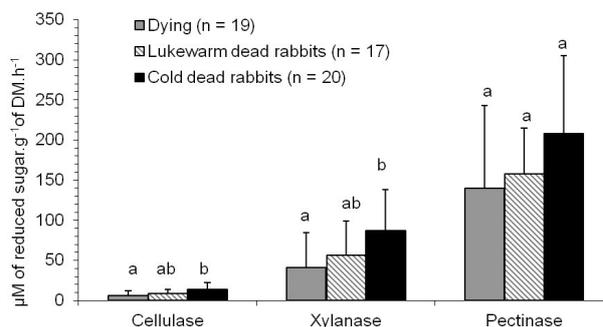


FIGURE 2: Bacterial fibrolytic activity (BFA) in dying and dead rabbits after diarrhoea, between 32 and 67 days of age. Results are expressed as means  $\pm$  standard deviations.

Different superscripts a,b,c indicate significant differences ( $p < 0.05$  or more) between groups.

	Individually housed rabbits fed with		P
	SF (n = 30)	DF (n = 50)	
Weight at weaning (g)	625 $\pm$ 10	636 $\pm$ 6	NS
Weight at slaughter (g)	2372 $\pm$ 64	2210 $\pm$ 35	NS
Weight gain (g/day)	41.7 $\pm$ 1.5	37.5 $\pm$ 0.8	< 0.05
Feed intake (g/day)	122.7 $\pm$ 3.1	82.5 $\pm$ 1.8	< 0.001
Feed conversion (g/g)	2.97 $\pm$ 0.06	2.21 $\pm$ 0.03	< 0.001
Mortality (%)	7.5	22	0.01
Morbidity (%)	35.5	76	< 0.01
HRI (%)	42.9	98	< 0.001

SF: Diet with standard fibre level; DF: Diet deficient in fibre level; HRI: Health Risk index; NS: not significant.

TABLE II: Growth, feed intake and health status of growing rabbits (period from the 28<sup>th</sup> day to the 70<sup>th</sup> days of age) according to the dietary fibre level. Results are expressed as means  $\pm$  standard deviation from animals individually housed.

## EFFECTS OF HEALTH STATUS ON CAECAL TRAITS AND MICROBIAL ACTIVITY

For caecal measurements, no significant interactions were detected between age-diet, age-housing and housing-diet. Therefore pooled data from diarrhoeic or healthy rabbits were presented in Tables III and IV, and in figure 3. The effects of health status on caecal development and CMA were evaluated only on dying rabbits with diarrhoea between 36 and 65 days of age (n = 19) and compared to healthy ones between 42 and 56 days of age (n = 56) from the 2 groups of diets. The majority of these dying rabbits were those of the DF group (n = 17). In diarrhoeic rabbits, the body weight loss and caecal dry matter content reached 16% (p = 0.04) and 27% (p < 0.001) respectively, compared to the healthy rabbits (Table III). However, caecum weight and content weight were not affected, averaging 23.6 and 96.2 g respectively. The caecal fermentative profile was deeply disturbed in sick rabbits (Table IV). For these animals, tVFAs decreased by 40% (p < 0.001) and originated in a fall of C<sub>2</sub> and C<sub>4</sub> concentrations (-49% and -61%, p < 0.001, respectively). Proportion of C<sub>3</sub> increased by 2.5 times (p < 0.001, Table IV), leading to a reverse C<sub>3</sub>:C<sub>4</sub> ratio in diarrhoeic rabbits compared to healthy ones (2.02 vs. 0.54, p < 0.001). Moreover, a sharp increase (8.5 times) in minor VFAs (isobutyric (IC<sub>4</sub>), valeric (C<sub>5</sub>) and isovaleric (IC<sub>5</sub>) acids) was observed in dying rabbits (p < 0.001). In addition, the concentration of NH<sub>3</sub>-N doubled

in sick rabbits compared to healthy ones (p < 0.001) and in parallel, an alkalinisation of caecal content by 0.7 unit (p < 0.001) was observed. Among the three bacterial fibrolytic activity assessed here, only the pectinolytic activity decreased (-32%, p < 0.05, figure 3) in diarrhoeic rabbits. Furthermore, an increased variability of BFA was observed (rCV% between 51 and 96%).

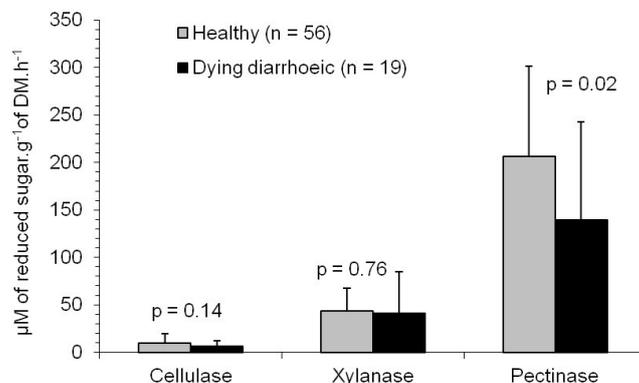


FIGURE 3: Bacterial fibrolytic activity (BFA) in healthy rabbits (n = 56) between 42 and 56 days and dying diarrhoeic rabbits (n = 19) between 36 and 65 days of age fed with standard or deficient fibre diets (pooled data). Results are expressed as means ± standard deviations.

	Healthy rabbits (n = 56)	Dying diarrhoeic rabbits (n = 19)	p
Age	42-56 days	36-65 days	-
Body weight (BW) (g)	1452 ± 423	1223 ± 202	< 0.05
Organ weight (OW) (g)	24.9 ± 6.0	22.3 ± 6.0	NS
Ratio BW/OW (%)	1.99 ± 2.10	1.91 ± 0.40	NS
Content weight (g)	92.3 ± 25.0	100.2 ± 53.0	NS
Dry matter content (%)	23.07 ± 2.00	16.83 ± 5.00	< 0.001

NS: not significant.

TABLE III: Caecal characteristics according to the health status of the growing rabbits fed with standard or deficient fibre diets (pooled data). Results are expressed as means ± standard deviations.

	Healthy rabbits (n = 56)	Dying diarrhoeic rabbits (n = 19)	p
Age	42-56 days	36-65 days	-
pH	6.0 ± 0.4	6.7 ± 0.5	< 0.001
NH <sub>3</sub> -N (mM)	10.6 ± 2.1	26.2 ± 11.7	< 0.001
Total VFAs (mM)	53.1 ± 21.0	31.7 ± 15.7	< 0.001
C <sub>2</sub> (%)	79 ± 5	70 ± 7	< 0.001
C <sub>3</sub> (%)	6 ± 3	15 ± 3	< 0.001
C <sub>4</sub> (%)	14 ± 4	9 ± 4	< 0.001
C <sub>3</sub> :C <sub>4</sub>	0.54 ± 0.54	2.02 ± 1.52	< 0.001
minor VFAs (%)	0.6 ± 0.9	5.2 ± 0.3	< 0.001

VFAs: Volatile fatty acids; NS: not significant.

TABLE IV: Caecal fermentative activity according to the health status of the growing rabbits fed with standard or deficient fibre diets (pooled data). Results are expressed as means ± standard deviations.

## EFFECTS OF HEALTH STATUS ON SOME BLOOD PARAMETERS

Because an interaction between age (42, 56 and 70 days) and the amount of dietary fibre on the blood profile in healthy rabbits (haematocrite, glycaemia and uraemia) was previously established [4], the effect of the health status on blood parameters was here analysed only in diarrhoeic rabbits, between 7 and 8 weeks old ( $n = 59$ ), and compared to healthy ones at the corresponding ages (i.e. 56 days of age,  $n = 34$ ). As no statistical effect was detected between rabbits in acute or in finishing diarrhoea, on blood parameters, they were considered together (Table V). Nearly half of rabbits housed in collective cages and individual cages have exhibited diarrhoea (166/359, which 78% of them were from DF group, results not shown). When possible, a blood sampling was performed on diseased rabbits at different stages of diarrhoea (beginning, clear and finishing). In this case, blood parameters were taken on 93 sick rabbits in which 74 originating from the DF group. Moreover, clear diarrhoea was recorded in 75% of the diseased animals. In addition, appearance of diarrhoea according to the age of rabbits showed that 62% of them were sick between 49 and 56 days of age ( $n = 59$ ). The majority of blood parameters were affected in diarrhoeic rabbits (Table V), except plasma triglyceride and sodium concentrations. The plasma glucose, potassium and albumin concentrations decreased by 6, 21 and 4% respectively compared to the healthy ones ( $p < 0.05$  and more). Conversely, urea and total cholesterol concentrations ( $p < 0.0001$ ) as well as haematocrite ( $p < 0.01$ ) markedly increased.

## Discussion

Some authors have reported an increased incidence in digestive disturbances according to a low dietary fibre level, and without any change of the proportions of the cell wall [3, 6, 16, 19]. The present results were in agreement with these authors. Indeed, a sharp decrease in the fibre level (19 to 9% ADF) doubled the risk of digestive troubles after weaning and impaired the growth performances [12].

Diarrhoeic rabbits stopped their solid consumption but they continued to consume water. The result was a drop in body weight and an important dilution of caecal content compared to the healthy rabbits. Indeed, LICOIS *et al.* [22] noted the same observation with an experimental coccidiosis.

Regardless of the health status, the pectinolytic activity was prevalent and was around 4 to 22 times higher than xylanolytic and cellulolytic ones, respectively, as previously reported [17, 26]. This hierarchy in fibrolytic activities also corresponds to that of the digestive efficacy of cell wall constituents in rabbits (pectins > hemicelluloses > cellulose), and also to results of microbiological enumeration reporting higher counts of pectinolytic and hemicellulolytic bacteria compared to cellulolytic bacteria [7]. In diarrhoeic rabbits, only pectinolytic activity was reduced. This reduction was also observed with deficient fibre content in diet [5]. As already reported [16], it seems that the decline of the substrate in the caecum firstly reduced the pectinase activity and consequently also decreased the production of VFAs. However, this lower pectinolytic activity could be related to a break in feed intake for diseased rabbits. For instance, GIDENNE and BELLIER [14] mentioned a similar modification of CMA in some healthy adulthood rabbits which fasted during 5 to 8 hours, without appearance of any digestive troubles.

Compared to the healthy animals, the caecal fermentative profile of diarrhoeic rabbits was highly disturbed, with sharp reductions of  $C_2$  and  $C_4$  proportions, suggesting a severe dysbiosis. These results were probably related to a decrease in available substrates for caecal microflora and a modification of the microbial community balance. BENNEGADI *et al.* [2] noticed for these diseased rabbits, a decrease in total caecal bacteria, in fibrolytic bacteria such as *Ruminococcus* genus and Archaea population (methanogenic population). Thus, the activity of some classes of specialised bacteria such as *Butyrivibrio* [34] which produced butyrate could be reduced. The consequence of this reduction was an inversion of the ratio of  $C_3:C_4$  compared to the healthy rabbits, which

	Healthy rabbits (n = 34)	Diarrhoeic rabbits (n = 59)	p
Age (days)	56	49-56	-
Haematocrite (%)	35.4 ± 3.7	37.9 ± 3.4	< 0.01
Glucose (mM)	7.94 ± 2.0	7.44 ± 5.72	< 0.01
Urea (mM)	4.50 ± 1.33	9.82 ± 8.82	< 0.0001
Cholesterol (mM)	2.22 ± 0.9	3.80 ± 2.02	< 0.0001
Albumin (g.L <sup>-1</sup> )	43.5 ± 3.5	41.6 ± 5.1	< 0.05
Triglycerides (g.L <sup>-1</sup> )	0.45 ± 0.18	0.70 ± 0.67	NS
Potassium (mM)	6.32 ± 1.01	4.98 ± 1.34	< 0.0001
Sodium (mM)	137.4 ± 12.6	136.4 ± 9.9	NS

NS: not significant

TABLE V: Blood profile in healthy and diarrhoeic rabbits aged around 7 weeks fed with standard or deficient fibre diets (pooled data). Results are expressed as means ± standard deviations and no distinction was performed between diarrhoeic rabbits with clear acute diarrhoea or at the end of the diarrhoea.

corresponded to the finding of several authors [12, 27]. This ratio seems to be a reliable indicator of an unbalance of the digestive ecosystem in the growing rabbit. In addition, BELLIER [1] noticed similar results in morbid rabbits. On the other hand, an increased  $\text{NH}_3\text{-N}$  concentration and an alkalinisation of the caecal content were observed in diseased rabbits compared to the healthy ones. Similar conclusions were reached by GIDENNE [12] and GIDENNE and LICOIS [18] in non-specific or specific rabbit enteropathies, respectively. In addition, it seems that the caecal fermentative profile evolved to a more "proteolytic" profile in diarrhoeic rabbits. Among minor VFAs, the proportions of isoacids which originated essentially from fermentation of branched amino acids (valine, leucine, isoleucine) [28] have increased and high ammonia caecal concentration also supported this hypothesis. Moreover, modifications of the composition of the caecal microflora and of the origin and quantity of substrates entering the caecum (fall of polysaccharides and oligosaccharides, rise of endogenous proteins) can explain the changes in the caecal fermentative pattern.

Compared to the dying rabbits with digestive disturbances, caecal microbial activity of dead rabbits was deeply modified and more specifically between 1-6h after death, when fermentation end-products (VFAs,  $\text{NH}_3\text{-N}$ ) and fibrolytic activities (cellulase and xylanase) increased with time delay after death. Indeed, the bacterial fermentations persisted in the caecum during the first hours after the death of rabbits leading to an hydrolysis of carbohydrates and endogenous proteins (mucins, epithelial cells, free amino acids,...). Since the increase of tVFAs was balanced with that of ammonia, the pH of the caecal medium remained unaffected.

In this study, a hypoglycaemia was noticed for rabbits affected by nutritional enteropathy, probably related to a very low (or a break in) feed intake. This was in agreement with the study of TAMBUR *et al.* [31] on rabbits with a coccidiosis intestinal infestation. By contrast, COUDERT *et al.* [8] have not shown any disturbances. They deduced that a constant glycaemia was related to a rapid metabolic vicariousness between carbohydrate and lipid metabolisms. Increases in plasma urea and total cholesterol concentrations in diarrhoeic rabbits compared to the healthy ones were found in agreement with previous reports [8, 30]. In diarrhoeic rabbits, a high uraemia associated with an intense proteolytic catabolism (decrease of the body weight) or with a renal dysfunction had been mentioned by COUDERT *et al.* [8] and by RENAULT *et al.* [30], respectively. In addition, in agreement with COUDERT *et al.* [8], the elevated blood lipemia may be related to an important lipolysis because rabbits stopped their feed consumption. In rabbit coccidiosis, albumin and haematocrite values were reduced because of haemodilution [8, 22, 30]. However, in the present study, diarrhoeic rabbits, compared to the healthy ones, exhibited a slight haemoconcentration coupled to a normal natremia, generally related to extracellular dehydration caused by diarrhoea. Nevertheless, a low plasma albumin concentration can be also noticed in dying rabbits suggesting a severe

hepatic insufficiency. On the other hand, kaliemia dropped by 21% compared to the healthy ones. This reduction was systematically shown in diarrhoeic rabbits and was generally more intense (a reduction of 30 to 50%) in the case of coccidiosis [23]. According to LICOIS and MONGIN [25], hypokaliemia in rabbits inoculated with *Eimeria spp.*, is explained by a mechanism of compensation which results in an exchange between sodium and potassium ions through the caecal wall. Consequently sodium is retained to the detriment of potassium which is lost during diarrhoea.

As a conclusion, the caecal microbial activity was highly impaired by nutritional enteropathy, caused by a dietary fibre deficiency. The metabolism modifications were similar to those observed in animals suffering from specific enteropathies (e.g. colibacillosis). In the future, it would be advisable to investigate factors of resistance to the enteropathies, and more particularly to the nutritional factors which can control the activity of the caecal microbial community.

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