

## Impact of fibre deficiency and sanitary status on non-specific enteropathy of the growing rabbit

Nadia BENNEGADI<sup>a</sup>, Thierry GIDENNE<sup>a\*</sup>, Dominique LICOIS<sup>b</sup>

<sup>a</sup> Station de Recherches Cunicoles, INRA Toulouse, BP 27, 31326 Castanet-Tolosan, France

<sup>b</sup> Laboratoire de Pathologie du Lapin, Station de Pathologie Aviaire et de Parasitologie, INRA Tours, 37380 Nouzilly, France

(Received 22 January 2001; accepted 3 August 2001)

**Abstract** — The effects of a sharp reduction in dietary fibre level (19 to 9% ADF resp. for “S” and “D” diet) were studied on the health status and performances of two groups of rabbits with different sanitary statuses: conventional “C” ( $n = 224$ ) and specified pathogen free ‘SPF’ ( $n = 72$ ) from 28 to 70 days of age. Digestive pathology was monitored daily and individually along the whole fattening period (28–70 d old) to precisely quantify the impact of a fibre deficiency on rabbit health. Diarrhoea was the most common symptom of enteritis due to fibre deficiency (90% of cases). The major digestive lesions were dilatation of the caeco-colic segment (30%) and a watery intestinal and caecal digesta (60%). The digestive troubles had a similar expression with respect to sanitary status or diet effect. The mortality and the Health Risk index (HRi = mortality + morbidity rate) respectively reached 25 and 60% with the fibre deficient diet “D” (28–70 d period) compared to 9 and 41% for the standard diet “S” ( $P < 0.05$ ). The digestive disturbances appeared mainly during the period 28–42 d for SPF rabbits, and later for the C rabbits (43–56 d). Morbidity, HRi and the duration of diarrhoea were respectively 7, 2 and 3 times higher for C rabbits as compared to the SPF rabbits. A synergistic effect of fibre deficiency and conventional sanitary status was observed on the incidence and intensity of digestive troubles. For individually caged rabbits, morbidity seemed to be mainly linked to the sanitary status of the animal (SPF or conventional), while the mortality level was enhanced by a fibre deficiency. The fibre supply thus seemed to prevent morbidity from evolving into mortality. In conclusion, non-specific enteropathy depends jointly on sanitary and nutritional status of the animals.

**rabbit / fibre deficiency / sanitary status / digestive pathology / nutrition**

**Résumé** — **Incidence d’une déficience en fibres alimentaires et du statut sanitaire sur les entéropathies non-spécifiques du lapin en croissance.** L’incidence d’une forte baisse du niveau de fibres alimentaires (19 à 9 % d’ADF respectivement pour l’aliment témoin « S » et déficient en fibres « D ») sur le statut sanitaire et les performances a été étudiée sur deux groupes de lapins en croissance, différant par leur statut sanitaire : des lapins conventionnels « C » ( $n = 224$ ) et des lapins Exempts d’Organismes Pathogènes Spécifiés « EOPS » ( $n = 72$ ). Les pathologies digestives ont été contrôlées quotidiennement et individuellement, durant la totalité de la période de croissance (28 à

\* Correspondence and reprints

Tel.: 33 (0)5 61 28 51 77; fax: 33 (0)5 61 28 53 19; e-mail: gidenne@toulouse.inra.fr

70 j d'âge), afin de quantifier avec précision l'impact de la déficience en fibre sur l'état sanitaire du lapin. La diarrhée est le symptôme le plus fréquemment observé dans les entérites provoquées par la réduction du taux de fibres (90 % des cas). Les lésions digestives sont essentiellement, une dilatation du segment caeco-colique (30 % de cas) et une liquéfaction du contenu digestif intestinal et caecal (60 % de cas). La mortalité et le Risque Sanitaire (HRi = mortalité + morbidité) atteignent respectivement 25 et 60 % chez les lapins nourris avec le régime déficient en fibres « D » (période 28–70 j d'âge), contre 9 et 41 % chez ceux nourris avec le régime témoin « S » ( $P < 0,05$ ). L'intensité des entérites atteint son maximum entre 28 et 42 j d'âge pour les EOPS, alors que chez les lapins C, elle est plus tardive (43 à 56 j). Chez les lapins C, la morbidité, le risque sanitaire, et la durée de la diarrhée sont plus importants que chez les EOPS d'environ 7, 2 et 3 fois. Un effet de synergie négatif, de la déficience en fibres combinée à un statut sanitaire conventionnel, est observé sur l'intensité et la fréquence des diarrhées. Pour les animaux logés individuellement, la morbidité semble essentiellement liée à leur statut sanitaire (EOPS ou conventionnels), alors que le taux de mortalité est accru par la déficience en fibres alimentaires. L'apport de fibre semble agir en tant que facteur de prévention de l'évolution de la morbidité en mortalité. En conclusion, les diarrhées non-spécifiques dépendent conjointement du statut sanitaire et nutritionnel des animaux.

**lapin / déficience en fibres / statut sanitaire / pathologie digestive / nutrition**

## 1. INTRODUCTION

Digestive pathology is the most important problem encountered in rabbit breeding [20], and occurs mainly in young rabbits after weaning (4–8 weeks of age). Enteropathology is mainly responsible for an increase in mortality rate after weaning that reaches 14% in France [19] but may frequently exceed 20% in cases of specific epidemic. Digestive disorders are also responsible for a significant morbidity (depression of growth and poor feed conversion). Although the losses in performance and efficiency due to transient diarrhoea are costly, they are difficult to evaluate in field conditions. Diarrhoea is the main clinical symptom of enteropathy and could have several origins. It is possible to distinguish enteritis originating from a specific pathogenic agent (for example coccidia, *Escherichia coli* O103, *Clostridium spiroforme*...) from those where no clear pathogenic origin is detected [21, 33, 38].

The reduction of the dietary fibre level is a major factor that increases the incidence of non-specific enteritis in the growing rabbit [18, 40]. However, few studies have precisely addressed the relation between the

nutrition and the digestive pathology [23, 32, 35]. Some physiological factors implicated in digestive troubles have been reviewed [14], but several points remain unclear. For instance, the presence of potential pathogenic flora could presumably increase the impact of enteritis.

Therefore, our study was aimed at describing the digestive troubles more precisely, using the daily control of the health status of conventional rabbits. We used a fibre deficient diet as a model to increase the incidence of the digestive troubles [18] that we compared to a control diet having a standard fibre level and without change in the origin or nature of the cell wall. We also replicated the study with specified pathogen free 'SPF' rabbits exempt of the most known pathogens, in order to evaluate whether sanitary status could interact with the dietary factor.

## 2. MATERIALS AND METHODS

### 2.1. Experimental design and diets

A set of two trials was designed to test the impact of two diets with different fibre

**Table I.** Design of the experiments.

Cage	Experiment 1				Experiment 2	
	Individual				Collective	
Sanitary status	Conventional		SPF		Conventional	
Location	A		B		A	
Diet	Standard	Deficient	Standard	Deficient	Standard	Deficient
Number of rabbits	30	50	26	46	60	84

levels on rabbit performances and health (Tab. I). In the first one, the diet effect was studied on rabbits with different sanitary statuses and bred in individual cages: conventional (group C from location A) and specified pathogen free rabbits (group SPF from location B). In the second experiment, the diet effect was studied only on conventional rabbits bred in collective and individual cages in location A.

The two experimental diets differed mainly in their fibre levels: a standard diet S (ADF = 19%) corresponding to current recommendations [9, 15, 28, 29] and a fibre deficient diet D (ADF = 9%). In both diets, wheat and dehydrated alfalfa meal were the main sources of starch and fibre. The origin and proportions of fibre fractions were similar among diets, as well as the ratio of digestible protein/digestible energy (Tab. II). The diets were given in pelleted form (3 mm of diameter), ad libitum throughout the experiments.

## 2.2. Animals and housing

A total of 72 Specified Pathogen Free rabbits (SPF) and 224 conventional rabbits of both sexes were bred from weaning (28 days of age) to slaughter (70 d) (Tab. I): conventional rabbits were New Zealand White × Californian hybrid (Toulouse, France, strain INRA A1067), and SPF were New Zealand White produced at INRA,

Tours, France. The SPF rabbits were obtained free of coccidia, oxyuris, *Pasteurella*, *Clostridium spiroforme* and enteropathogenic *Escherichia coli* (O103, O132, O128, O26, O15) [8]. The SPF status was obtained under highly protected conditions of breeding (hygiene and prophylaxy). The rabbits were individually identified and housed in wired individual or collective cages at a room temperature of  $18 \pm 2$  °C and were subjected to 12 h of light. The density of rabbits per cage was 16 rabbits·m<sup>-2</sup>. At 28 days of age, the rabbits of each experiment were allotted to diets according to litter origin and weaning weight.

## 2.3. Control of the health status and zootechnical performances

Live-weight and feed intake were controlled weekly. Mortality and morbidity were controlled individually every day from 28 to 70 days of age. Morbidity control consisted in a global external examination (dynamic or prostrate animal), followed by a precise observation of all clinical signs of digestive troubles or sickness: light, acute or finishing diarrhoea; constipation (caecal impaction); and presence of mucus in excreta. 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

**Table II.** Ingredients, chemical composition and nutritional value of the experimental diets.

	Diets	
	Standard (S)	Fibre deficient (D)
<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
Soya bean meal	10.0	19.0
Wheat straw	6.0	2.0
DL Methionine	0.2	0.2
Minerals	0.9	2.5
Oligo element 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 × 6.25)	159	177
Starch	96	320
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)<sup>2</sup></b>		
DP (g·kg <sup>-1</sup> ) <sup>3</sup>	118	152
DE (MJ·kg <sup>-1</sup> ) <sup>4</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 per kg), <sup>2</sup> value measured on 16 rabbits per diet (conventional and SPF), <sup>3</sup> DP = digestible crude protein, <sup>4</sup> DE = digestible energy.

morbidity rate was expressed as the number of ill rabbits over the initial number of animals, and an animal was considered morbid only one time (within a period), even if diarrhoea lasted several days. Dead animals were only accounted for in the mortality rate, even when they exhibited clinical signs of diarrhoea before death. Therefore, we calculated a Health Risk index (HRi) corresponding to the sum of morbid and dead animals, knowing that each animal was deducted only once and categorised either as dead or morbid. Mortality and morbidity

were calculated within periods of two weeks (28–42 d, 43–56 d, 57–70 d) and for the whole fattening period (28–70 d). In addition, since health status was controlled daily, we calculated the mean number of days with diarrhoea (NDD) to evaluate the global impact of the digestive troubles: (sum of days with diarrhoea on morbid and dead rabbits)/(initial number of rabbits). We also attempted to qualify the digestive troubles themselves, by calculating the mean duration of diarrhoea (MDD) for two classes of rabbits (ill or dead): (sum of days with

diarrhoea)/(number of rabbits, either ill or dead).

The rabbits observed as recently dead (within few hours) or to be dying were subjected to an autopsy, so that symptoms and lesions of each part of the digestive tract associated with digestive disturbances could be described. The dry matter level and the pH of the caecal content were measured, and compared to caecal content of healthy rabbits slaughtered at the same age (28–42–56–70 d of age). The frequency of lesions and symptoms was calculated and presented as a clinical table (Tab. III). In cases of haemorrhagic caecal content, the research of pathogenic flora such as *E. coli* and *Clostridium* sp. was performed. In addition, the counting of *E. coli* was carried out after dilution ( $10^{-2}$ – $10^{-6}$  of 1 g of fresh caecum content) and cultured on solid DRIGALSKI medium (Diagnostics Pasteur, France) at 37 °C during 24 h. *Clostridium* sp. was detected by the GRAM coloration method.

#### 2.4. Analytical methods

The chemical analysis of organic matter (OM) was determined by ashing samples at 550 °C for 5 h. Measurements of fibre fractions (neutral detergent fibre: NDF, acid detergent fibre: ADF, acid detergent lignin: ADL) were made according to the sequential

Van Soest procedure [42] using an amylolytic pre-treatment with a thermostable amylase [1]. Nitrogen was determined by the DUMAS combustion method using a Leco apparatus (model FP-428, Leco Corp., St Joseph, MI, USA), and converted to crude protein (CP) using the factor 6.25. Gross energy was measured by an adiabatic calorimeter (PARR Instrument; Moline, IL). Starch was enzymically 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).

The nutritive values of the two diets were obtained by measuring total tract apparent digestibility on 8 conventional and SPF rabbits per diet [11].

#### 2.5. Statistical analysis

Data on growth, feed intake, mean number of days with diarrhoea, and mean duration of diarrhoea were examined by analysis of variance using the GLM procedure of SAS (Statistical Analysis System) [39]. No data from morbid animals were excluded from growth performance calculation, to evaluate the overall impact of the fibre deficiency. Descriptive statistics (mortality, morbidity, health risk, digestive lesions...) were

**Table III.** Clinical table of digestive lesions in sick rabbits\*.

Digestive segment Lesions (%)	Stomach	Duodenum	Jejunum	Ileum	Caecum		Colon	
					anterior	posterior	proximal	distal
Watery content	55	70	65	70	84	69	61	41
Dilatation	7	39	36	41	42	35	42	20
Impaction	0	2	6	7	6	20	2	0
Congestion	0	0	1	1	14	15	2	0
Mucus	0	2	3	6	3	1	7	7

\* Measurements from 98 necropsy, done on recently dead or dying animals (experiment 1 and 2).

performed using the Catmod procedure and  $\text{Chi}^2$  [39].

The effect of the breeding cage (individual vs. collective) for conventional rabbits of location A, and sanitary status (conventional vs. SPF) of experiment 1 were statistically analysed in relation to the diet and their interaction.

### 3. RESULTS

According to dietary formulation constraints, the fibre level decreased by 50% from the standard to the deficient diet, and the proportions of the different fractions of fibre remained unchanged as determined with the Van-Soest criteria (Tab. II). We expressed, however, the fibre level using only the ADF level for simplicity and because this unit is commonly used to define fibre recommendations.

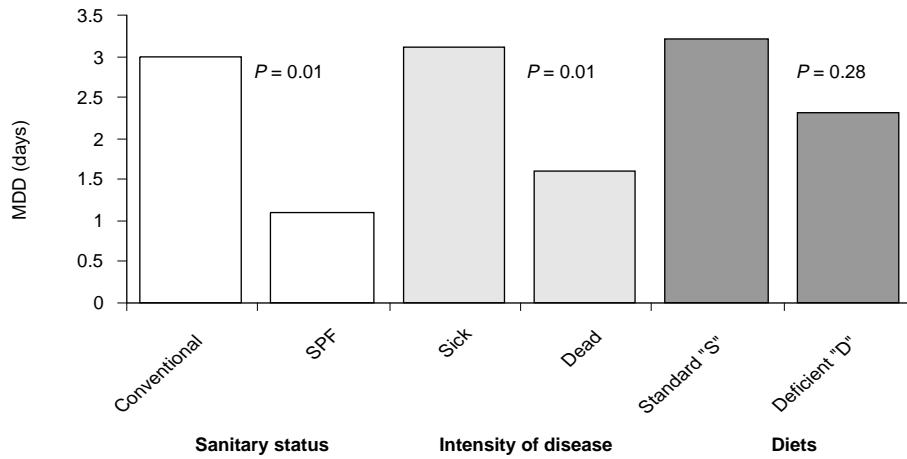
#### 3.1. Characterisation and clinical description of digestive troubles

The individual and daily control of rabbits from 28 to 70 days of age permitted to detect and precisely follow the digestive disturbances. The clinical expression of the enteritis syndrome was essentially diarrhoea (over 90%) independently of the diets or of the sanitary status. Dying and dead rabbits with enteritis were immediately autopsied. After laparotomy, the entire tract was photographed and the gastrointestinal segments were more particularly examined (Tab. III). We did not find any typical case of Rabbit Epizootic Enteropathy (REE) and any typical lesions of coccidia. Some microbial cultures were done on samples from animals with a congestive caecum wall. The number of *E. coli* found in the DRIGALSKI culture remained at a normal level (less than  $10^6$  bacteria per g of fresh caecal content). The research of *C. spiroforme* by the method of GRAM coloration was negative.

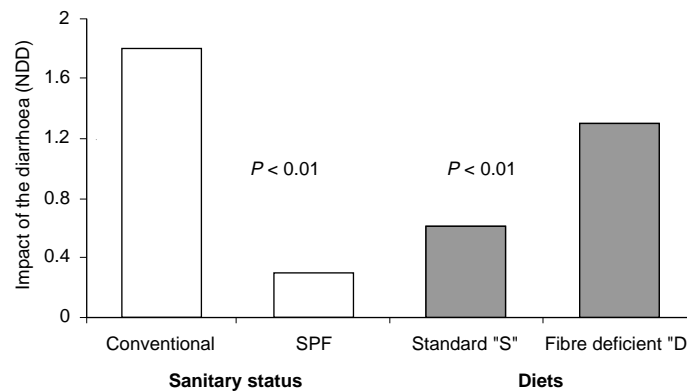
On 98 autopsied rabbits, about 98% had diarrhoea and 48% had a distended abdomen. A dilated ileo-caeco-colic tract (means 40%) and a watery content (means 70%) were the most frequent observations (Tab. III). Besides, we detected some occurrence of caecal impaction (20%), congestion of the caecum (15%) and some mucus in the colon (7%). Congestion of the caecum and its impaction were never found in the same animal. The dilatation of the anterior part of caecum and its proximal segments (ileum and proximal colon) was caused essentially by gas, mucus or a watery digestive content. No lesions were detected in the other organs. The dry matter level in caecal content for sick rabbits was lower than for healthy ones (14% vs. 23%;  $P < 0.001$ ). The pH of the caecal content for sick rabbits tended to be neutral, and was higher than for the healthy ones (6.76 vs. 6.01,  $P < 0.001$ ).

A sharp decrease in the feed intake and live weight was observed two or three days before the first symptoms of diarrhoea (90% of cases) and during diarrhoea. Morbid animals were generally prostrate. In 90% of cases, the morbid animal had diarrhoea for 2 or 3 days. The 10% of the remaining cases were loss of caecotrophes, excretion of mucus or a decrease in feed intake and live weight. Diarrhoea generally has 3 phases: light or beginning diarrhoea followed by an acute phase and then by a finishing phase. The diarrhoea was liquid, and stained the rectal and caudal region. Animals surviving the acute diarrhoea recovered their initial feed intake and weight gain within 2 to 3 days after the finishing phase of diarrhoea.

The effects of diet and sanitary status were not significant on clinical expression of the enteritis. The mean duration of diarrhoea (MDD) was similar regardless the type of cage and the diet, and no interactions were detected between sanitary status and the intensity of the disease (Fig. 1). For conventional animals of experiment 1, the diarrhoea appeared longer ( $3.0 \pm 1.7$  d) compared to SPF rabbits ( $1.1 \pm 0.3$  d;  $P = 0.018$ ).



**Figure 1.** Mean duration of diarrhoea (MDD, days) in dead or morbid animals, according to sanitary status, health status and diets (experiment 1).



**Figure 2.** Impact of diarrhoea (NDD: Mean number of days with diarrhoea), according to sanitary status and diets (experiment 1).

The death of rabbits during the acute phase of diarrhoea obviously led to a shorter duration of diarrhoea ( $1.6 \pm 1.1$  d) compared to the morbid ones ( $3.1 \pm 1.8$  d,  $P = 0.015$ ).

### 3.2. Quantification of the impact of enteritis

Four indicators of health status were used: number of days with diarrhoea (NDD), mortality rate, morbidity rate and the health risk index (HRi). For NDD, no interactions were detected between the sanitary status

and the diet (Fig. 2). The NDD of conventional rabbits was 1.8 d whereas for SPF rabbits it was only 0.3 d ( $P < 0.01$ ). The reduction of dietary fibre level for the NDD was twice that of the control diet ( $P < 0.01$ ).

No interactions were observed between the sanitary status and the diet, neither for mortality and morbidity nor for HRi (Tab. IV). The mortalities remained similar according to sanitary status, and were respectively 16 and 22% for conventional and SPF rabbits. The morbidity and the HRi were 7 times and 2.3 times less in SPF rabbits



**Table IV.** Health status of growing rabbits (period 28–70 d of age) according to the dietary fibre level and sanitary status.

<i>n</i>	Sanitary status		Diets		Statistical level		
	Conventional 80	SPF 72	S 56	D 96	Sanitary stat.	Diet	San. × Diet
Mortality (%)	16.6	22.2	9.4	25.0	0.43	0.024	0.89
<i>n</i>	13	16	5	24			
Morbidity (%)	55.9	8.3	31.7	34.4	< 0.01	0.84	0.27
<i>n</i>	45	6	18	33			
HRi* (%)	72.5	30.6	41.1	59.4	< 0.01	0.017	0.37
<i>n</i>	58	22	23	57			

*n*: number of rabbits (data from experiment 1).

\* HRi: Health Risk index.

compared to the conventional ones ( $P < 0.01$ ). For conventional rabbits, HRi was caused essentially by morbidity which represented 2/3 of HRi (1/3 by the mortality). However for SPF, HRi corresponded mainly to mortality.

Mortality and HRi were respectively 2.7 times ( $P = 0.024$ ) and 1.4 times ( $P = 0.017$ ) higher with the deficient diet compared to the standard one, whereas no effect of diet on morbidity was observed (mean 33%). If we consider that excretion of caecotrophes for 3 successive days is a symptom of digestive troubles, then morbidity level increased by 30% and reached 98% (28–70 d period) for conventional rabbits fed diet D. With the standard diet, morbidity was similar with or without considering the caecotrophes in the calculation of morbidity (41% vs. 37%). Digestive troubles were distributed differently along with growth for conventional and SPF rabbits. For SPF rabbits, digestive troubles appeared mainly during the 28–42 d period, whereas for conventional rabbits, enteritis was expressed essentially between 43–56 d of age. These two periods represented about 50% of the digestive disturbances, with the fibre deficient diet.

Although, the interaction between the diet and caging effect tended to be significant

for mortality and morbidity ( $P < 0.10$ , Tab. V), the mortality rate was higher in collective cages compared to individual ones (34.6 vs. 17.0%,  $P < 0.01$ ) for the whole fattening period. Conversely, morbidity appeared significantly higher for individually caged rabbits (+ 20 units), resulting in a similar global incidence of digestive troubles (means of HRi = 70%).

### 3.3. Feed intake and growth performances

A relatively high variability affected the zootechnical performances, since data from morbid animals were not discarded. We observed an interaction between the sanitary status and the diet for intake, growth and final live weight (Tab. VI). For conventional rabbits fed the standard diet, the intake, the final live weight and the total weight gain were 5% higher compared to SPF rabbits. With the fibre deficient diet, the SPF rabbits had a higher intake and higher final live weight and growth (respectively + 15%, + 6% and + 10%;  $P < 0.001$ ).

Globally, the reduction of the dietary ADF level (19 vs. 9%) led to a 3-fold reduction in ADF intake (28–70 day period), while the starch intake only increased two



fold (means 11 to 28 g·d<sup>-1</sup>). The feed conversion (FC) and the energetic conversion index (ECi, MJ DE·kg<sup>-1</sup> LW gain) were respectively 0.74 and 1.37 units lower for the D diet compared to the S diet (2.24 vs. 2.98, and 29.26 vs. 30.63 MJ DE·kg<sup>-1</sup> LW gain,  $P < 0.01$ ).

During the period 43–56 d, parallel to the peak of enteritis, decreases of 44% and 24% ( $P < 0.01$ ) were observed respectively for the feed intake and growth with the D diet compared to the S diet. The peak of enteritis with the D diet was two weeks earlier (28–42 d) in SPF rabbits, but the intake

**Table V.** Health status of growing rabbits (period 28–70 d of age) according to the breeding cages and dietary fibre level.

Period 28–70 d <i>n</i>	Breeding cages		Diets		Statistical level		
	Collective <i>144</i>	Individual <i>80</i>	S <i>90</i>	D <i>134</i>	Cage	Diet	C × D
Mortality (%) <i>n</i>	34.6 <i>50</i>	17.0 <i>13</i>	29.1 <i>26</i>	27.5 <i>37</i>	< 0.01	0.32	0.053
Morbidity (%) <i>n</i>	32.7 <i>47</i>	55.9 <i>45</i>	26.3 <i>24</i>	50.9 <i>68</i>	< 0.01	< 0.01	0.074
SRi (%) <i>n</i>	67.3 <i>67</i>	72.5 <i>58</i>	55.3 <i>50</i>	78.4 <i>105</i>	0.49	< 0.01	0.67

*n*: number of rabbits (data from location A, experiment 1 + 2).

**Table VI.** Growth and feed intake of growing rabbits (period 28–70 d of age), according to dietary fibre level and sanitary status (experiment 1).

Sanitary status Diets <i>n</i>	Conventional		SPF		RMSE <sup>a</sup>	Statistical level		
	S <i>22</i>	D <i>37</i>	S <i>23</i>	D <i>33</i>		Sanit. stat	Diet	San. × D
Weight at weaning (g)	632	636	591	596	47.3	< 0.001	0.59	0.99
Weight at slaughter (g)	2 385	2 210	2 258	2 354	198.7	0.82	0.30	< 0.001
Weight gain (g·d <sup>-1</sup> )	41.7	37.5	39.7	41.8	4.5	0.17	0.22	< 0.001
Feed intake (g·d <sup>-1</sup> )	122.5	82.5	119.2	95.1	11.50	0.035	< 0.01	< 0.001
(MJ DE·d <sup>-1</sup> )	1.26	1.08	1.22	1.24	0.13	0.012	< 0.001	< 0.001
Feed conversion (kg·kg <sup>-1</sup> LW gain)	2.96	2.21	3.00	2.27	0.21	0.27	< 0.001	0.72
(MJ DE·kg <sup>-1</sup> LW gain)	30.43	28.89	30.83	29.69	2.56	0.22	< 0.01	0.67

<sup>a</sup> Root mean square error; *n*: number of rabbits.

was only reduced by 28% and growth by 8% ( $P < 0.01$ ). In addition, the variability (Root Mean Square Error) of the final weight seemed lower in SPF rabbits than in conventional ones (111 vs. 255 g).

## 4. DISCUSSION

### 4.1. Methodology and diagnostic of digestive disorders

The methods employed in this study were aimed at precisely describing the digestive troubles caused by a fibre deficiency in conventional and SPF rabbits. Although not complex, this approach was time consuming since daily control of health was essential. Evaluating the morbidity and mean duration of diarrhoea is not original itself, and has been extensively used in some other species (e.g. pigs). However, few studies have used this methodology to describe non-specific enteritis in the growing rabbit [13, 36, 40]. Furthermore, the calculation of a health risk index ( $\text{HRi} = \text{mortality} + \text{morbidity}$ ) for a group of animals improves the evaluation of the health status and the impact of enteritis, that are greatly underevaluated if only mortality is recorded. Here, morbidity represented more than 60% of the health problems. Till now, no studies have controlled the daily rabbit health status during the whole fattening period, except that of Rémois et al. [37].

In agreement with Licois [22], diarrhoea was the predominant and constant clinical sign observed in rabbits with enteritis. The clinical table was consistent with a previous study of Vörös [43]. In diarrhoeic rabbits, we also found a higher caecal pH, which is habitual in sick animals [26, 31], that could be related to a lower VFA level as described by Gidenne [14].

While descriptions of diarrhoea have been widely carried out on infectious diseases in the rabbit [5, 24, 25, 34, 41], little is known about diarrhoea linked to nutritional factors [26, 31, 40]. In our experiment, more

than 90% of morbid rabbits had diarrhoea. Only 10% agonising rabbits showed symptoms of infection (such congestive caecum), but microbial culture did not reveal a significant level of *E. coli* or *C. spiroforme*, the most frequent bacteria encountered in rabbits and known to induce infectious enteritis. Besides, the clinical expression of the digestive pathology here was similar with respect to the sanitary status or the effect of the diet.

### 4.2. Effect of sanitary status

Pathogenic agents, such as *E. coli* or *Clostridia*, are frequently detected at low levels in healthy rabbits. To test if the impact of a non specific enteropathy, caused by fibre deficiency, could be dependant or not on a potentially associated pathogenic flora, we compared conventional and SPF rabbits. Whatever the diet, the post-weaning mortality was not significantly affected by the sanitary status, contrary to morbidity which was seven times lower for SPF. Furthermore, the intensity of the digestive troubles was weaker for SPF than for conventional rabbits (duration of diarrhoea and the number of days with diarrhoea). The sanitary status interacted with the dietary fibre level only for growth parameters. The increased incidence of non-specific enteropathy for conventional rabbits impaired their growth and their final weight, as previously observed by Gidenne et al. [18]. Conversely, SPF rabbits were less affected by the fibre deficiency since they showed a similar growth for both diets. Our results were in agreement with the observations of Licois et al. [27] who reported a lower mortality and morbidity for SPF rabbits (10–15%) than for conventional ones (60%), when they were contaminated by Rabbit Epizootic Enteropathy. This effect of the sanitary status could be linked to the balance of the ecosystem (flora free of pathogenic agents), as well to the strict sanitary conditions of breeding of SPF rabbits before weaning [8]. In return, conventional rabbits potentially having

pathogenic microbes, showed a lower resistance to non-specific enteropathy.

### 4.3. Impact of dietary fibre level

Few authors have studied the digestive disturbances in relation to the dietary fibre level, without change of the proportions of the cell wall [3, 17, 18]. Here, a sharp decrease of the fibre level (19 to 9%) doubled the risk of digestive troubles after weaning. The fibre deficient diet also increased the incidence of digestive disorders by losses of caecotrophes, and then morbidity reached 98% for conventional rabbits. Maître et al. [30] found that mortality increased from 7.5 to 14.4% when the level of dietary fibre decreased (21 to 15% of ADF). Gidenne and Jehl [16] indicated that HRi and morbidity tended to increase between 28–70 d of age, even when mortality remained not significantly affected by the reduction of the dietary fibre level (20 to 16% of ADF).

Moreover, an increased incidence of enteropathy with a fibre deficient diet led to losses of performances in conventional rabbits, as previously described [10, 15, 18]. Some hypotheses were suggested to explain the effect of dietary fibre on rabbit enteropathy. A direct effect of a fibre level reduction is a slowing down of the digestive transit, corresponding mainly to a longer retention time in the caecum [2, 12, 18]. Long retention time would probably be induced by a higher proportion of fine particles in a low-fibre diet, thus inducing change in the caecal motility pattern. On the contrary, fibre deficiency greatly affects the caecal fermentation pattern and impairs the caecal microbial activity [2, 15, 18] and the level of cellulolytic flora [4]. All these changes in microbial activity could thus lead to an unbalanced ecosystem and to a weak barrier effect of the caecal flora, finally favouring the incidence of enteropathy.

Conversely, the level of the fibre in the diet was generally inversely correlated to the level of the starch. Cheeke and Patton

[6] suggested that a high starch level in the diet would promote a carbohydrate overload in the hindgut, and may favour the proliferation of a pathogenic agent such as *Clostridium* sp. However, with low fibre feeds, Gidenne et al. [18] showed a very high ileal digestibility of starch (> 93%) in the adult, and they estimate that starch ileal flow in 6 week old rabbits remained under  $2 \text{ g}\cdot\text{d}^{-1}$ , even with a 30% dietary starch level. Nevertheless, before 6 weeks of age, the secretion of pancreatic amylase is not well established [7] and the starch ileal flow could be higher and may partly explain the appearance of enteropathy two weeks post-weaning. On the contrary, Pinheiro and Gidenne [36] have found an interaction between the age and nature of starch. They detected a higher ileal level of starch for 42 d than for 70 d old rabbits (resp. 1.4 vs. 0.8%), but using feed containing a resistant starch (crude potato) or starch from wheat. The ileal starch digestion remained very high, even with a 14% dietary addition of resistant starch. Therefore, the relationship between resistant starch addition and morbidity was moderate, and was not significant for mortality. Furthermore, with respect to rabbit age, the peak of appearance of enteropathy could vary largely with fibre deficiency among the studies: after 42 d of age for Gidenne et al. [18] and around 43–56 d of age for the present study (for conventional rabbits). This did not support the role of starch overload on enteropathy, since after 6 weeks of age, starch digestion is almost complete in the rabbit intestine.

In conclusion, a fibre deficiency clearly impairs the health status of the conventional growing rabbit (caged collectively), and more particularly morbidity after weaning. Morbidity, such as transient diarrhoea or growth disturbances, represented more than 50% of the health problems. Compared to SPF rabbits, the presence of potentially pathogenic flora in conventional animals (caged individually) clearly promoted morbidity and not mortality. A correct fibre supply acts as a factor of prevention so that the

background morbidity level (mainly linked to the sanitary status) does not evolve into mortality. Thus, non-specific diarrhoea depends jointly on sanitary and nutritional status of the animals. The digestive microflora and the role of fibre on microbial activity should be further studied to clarify the relationship between fibre intake and digestive pathology of the rabbit.

### ACKNOWLEDGEMENTS

The authors thank P. Aymard and A. Lapanouse (INRA, Station de Recherches Cunicoles) and M. Dupuy and J.-P. Molteni (INRA, Pathologie Aviaire et Parasitologie) for their technical collaboration.

### REFERENCES

- [1] AFNOR, Norme Française homologuée, Aliments des animaux. Détermination séquentielle des constituants pariétaux. Méthode par traitement aux détergents neutre et acide et à l'acide sulfurique, AFNOR publ., Paris, NF V 18-122, 1997, p. 11.
- [2] Bellier R., Gidenne T., Consequences of reduced fibre intake on digestion, rate of passage and caecal microbial activity in the young rabbit, *Brit. J. Nutr.* 75 (1996) 353-363.
- [3] Blas E., Cervera C., Fernandez Carmona J., Effect of two diets with varied starch and fibre levels on the performances of 4-7 weeks old rabbits, *World Rabbit Sci.* 2 (1994) 117-121.
- [4] Boulahrouf A., Fonty G., Gouet P., Establishment, counts and identification of the fibrolytic bacteria in the digestive tract of rabbit. Influence of feed cellulose content, *Current Microb.* 22 (1991) 1-25.
- [5] Carman J.R., Borriello S.P., *Clostridium spiroforme* isolated from rabbits with diarrhoea, *Vet. Rec.* 11 (1982) 461-462.
- [6] Cheeke P.R., Patton N.M., Carbohydrate overload of the hindgut. A probable cause of enteritis, *J. Appl. Rabbit Res.* 3 (1980) 20-23.
- [7] Corring T., Lebas F., Courtot D., Contrôle de l'évolution de l'équipement enzymatique du pancréas exocrine du lapin de la naissance à 6 semaines, *Ann. Biol. Anim. Biochim. Biophys.* 12 (1972) 221-231.
- [8] Coudert P., Licois D., Besnard J., Establishment of a specified pathogen free breeding colony (SPF) without hysterectomy and hand-rearing procedures, in: Holdas S. (Ed.), 4th World Rabbit Congress, Budapest, Hungary, 10-14 October, 1988, pp. 137-148.
- [9] De Blas C., Garcia J., Carabaño R., Role of fibre in rabbit diets. A review, *Ann. Zootech.* 48 (1999) 3-13.
- [10] De Blas J.C., Santoma G., Carabaño R., Fraga M.J., Fiber and starch level in fattening rabbit diets, *J. Anim. Sci.* 63 (1986) 1897-1904.
- [11] EGRAN, European reference method for in vivo determination of diet digestibility in rabbits, *World Rabbit Sci.* 3 (1995) 41-43.
- [12] Gidenne T., Effets d'une réduction de la teneur en fibres alimentaires sur le transit digestif du lapin. Comparaison et validation de modèles d'ajustement des cinétiques d'excrétion fécale des marqueurs, *Reprod. Nutr. Dev.* 34 (1994) 295-306.
- [13] Gidenne T., Effect of fibre level reduction and gluco-oligosaccharide addition on the growth performance and caecal fermentation in the growing rabbit, *Anim. Feed Sci. Technol.* 56 (1995) 253-263.
- [14] Gidenne T., Caeco-colic digestion in the growing rabbit: impact of nutritional factors and related disturbances, *Livest. Prod. Sci.* 51 (1997) 73-88.
- [15] Gidenne T., Recent advances and perspectives in rabbit nutrition: Emphasis on fibre requirements, *World Rabbit Sci.* 8 (2000) 23-42.
- [16] Gidenne T., Jehl N., Réponse zootechnique du lapin en croissance face à une réduction de l'apport de fibres, dans des régimes riches en fibres « digestibles », in: Perez J.M. (Ed.), 8<sup>es</sup> Journées de Recherches Cunicoles, Paris, France, 9-10 juin, ITAVI publ., 1999, pp. 109-113.
- [17] Gidenne T., Jehl N., Caecal microbial activity of the young rabbit. Incidence of a fibre deficiency and of feed intake, in: Blasco A. (Ed.), 7th World Rabbit Congress, Valencia, Spain, 4-7 July, Universidad politécnica de Valencia publ., Vol. C, 2000, pp. 233-239.
- [18] Gidenne T., Pinheiro V., Falcão e Cunha L., A comprehensive approach of the rabbit digestion: consequences of a reduction in dietary fibre supply, *Livest. Prod. Sci.* 64 (2000) 225-237.
- [19] Guerder F., Renalap : la marge économique dégagée par le lapin s'améliore, *Cuniculture* 153 (2000) 105-113.
- [20] Lebas F., Gidenne T., Perez J.M., Licois D., Nutrition and pathology, in: De Blas C., Wiseman J. (Eds.), *The nutrition of the rabbit*, Chapter 11, CABI Publishing, Wallingford, 1998, pp. 197-214.
- [21] Lelkes L., A review of rabbit enteric-diseases: A new perspective, *J. Appl. Rabbit Res.* 10 (1987) 55-61.
- [22] Licois D., Affections digestives d'origine infectieuse et/ou parasitaire chez le lapin, *Bull. GTV* 91 (1991) 73-88.

- [23] Licois D., Gidenne T., L'emploi d'un régime déficient en fibres par le lapereau augmente sa sensibilité vis-à-vis d'une infection expérimentale par une souche *Escherichia coli* entéropathogène, in: Perez J.M. (Ed.), 8<sup>es</sup> Journées de Recherches Cunicoles, Paris, France, 9–10 juin, ITAVI publ., 1999, pp. 101–104.
- [24] Licois D., Coudert P., Mongin P., Changes in hydromineral metabolism in diarrhoeic rabbits. 1. A study of the changes in water metabolism, *Ann. Rech. Vet.* 9 (1978) 1–10.
- [25] Licois D., Coudert P., Mongin P., Changes in hydromineral metabolism in diarrhoeic rabbits. 2. Study of the modifications of electrolyte metabolism, *Ann. Rech. Vet.* 9 (1978) 453–464.
- [26] Licois D., Coudert P., Colin M., Essai d'induction de la diarrhée chez le lapereau à l'aide d'aliments comportant différentes teneurs en cellulose, *Ann. Rech. Vet.* 10 (1980) 279–284.
- [27] Licois D., Coudert P., Céré N., Vautherot J., Epizootic enterocolitis of the rabbit: Review of current research, in: Blasco A. (Ed.), 7th World Rabbit Congress, Valencia, Spain, 4–7 July, Universidad politécnica de Valencia publ., Vol. B, 2000, pp. 299–306.
- [28] Maertens L., Rabbit nutrition and feeding: a review of some recent developments, in: 5th Congress of the WRSA, 25–30 July, Corvallis, Oregon, *J. Appl. Rabbit Res.* 15 (1992) 889–913.
- [29] Maertens L., Lebas F., Mesure de la valeur énergétique des aliments et des matières premières chez le lapin. Une approche critique, *Cuni-Sci.* 5 (1989) 35–46.
- [30] Maître I., Lebas F., Arveux P., Bourdillon A., Duperray J., Saint Cast Y., Taux de lignocellulose (ADF de Van-Soest) et performances de croissance du lapin de chair, in: 5<sup>es</sup> Journées de Recherches Cunicoles, Paris, France, 12–13 déc., ITAVI publ., 1990, pp. 56.1–56.11.
- [31] Morisse J.P., Boilletot E., Maurice R., Alimentation et modifications du milieu intestinal chez le lapin (AGV, NH<sub>3</sub>, pH, flore), *Rec. Med. Vet.* 161 (1985) 443–449.
- [32] Padilha M.T., Étude des relations entre la microflore et l'activité fermentaire caecale chez le lapereau, pendant la période péri-sevrage, thèse de Doctorat, Univ. F. Rabelais, Tours, 1995, 160 p.
- [33] Peeters J.E., Etiology and pathology of diarrhoea in weaning rabbits, in: Auxilia T. (Ed.), Rabbit production systems including welfare, CEC publ., report EUR 10983, 1987, pp. 127–137.
- [34] Peeters J.E., Recent advances in intestinal pathology of rabbits and further perspectives, in: Holdas S. (Ed.), 4th World Rabbit Congress, Budapest, Hungary, 10–14 Oct., WRSA publ., 3, 1988, pp. 293–315.
- [35] Peeters J.E., Orsenigo R., Maertens L., Gallanzzì D., Colin M., Influence of two iso-energetic diets (starch vs. fat) on experimental colibacillosis (EPEC) and iota-enterotoxemia in early weaned rabbits, *World Rabbit Sci.* 1 (1993) 53–66.
- [36] Pinheiro V., Gidenne T., Substitution of wheat by potato starch for growing rabbits: effect on performances, digestion, and health, in: Blasco A. (Ed.), 7th World Rabbit Congress, Valencia, Spain, 4–7 July, Universidad Politécnica Valencia publ., Vol. C, 2000, pp. 391–398.
- [37] Rémois G., Abiven N., Ledan L., Lafargue-Hauret P., Bourdillon A., Effect of dietary fibre and energy content on mortality and growth performances of rabbits in case of epizootic rabbit enterocolitis, in: Blasco A. (Ed.), 7th World Rabbit Congress, Valencia, Spain, Universidad politécnica de Valencia publ., Vol. C, 2000, pp. 399–405.
- [38] Renault L., Maire C., Vaissaire J., Labadie J.P., Albouy R., Contribution à l'étude des troubles digestifs des lapereaux en début d'engraissement, in: 1<sup>er</sup> Congrès International Cunicole, Dijon, France, ASFC, publ., 31 mars–2 avril, 1976, comm. 46.
- [39] SAS, Inc., SAS/STAT. Guide for personal computers, version 6.03 Edition SAS (Statistical Analysis System) Institute Inc., Cary, NC, 1988, 1028 p.
- [40] Sinkovics G., Szeremi Z.S., Medgyes I., Factors predisposing for rabbit dysentery. Part I, in: Proceedings II World's Rabbit Congress, Barcelona, Spain, 16–18 April, ASESCU publ., Vol. 2, 1980, pp. 358–366.
- [41] Thouless M.E., Digiacomò R.F., Deeb B.J., The effect of combined rotavirus and *Escherichia coli* infections in rabbits, *Lab. Anim. Sci.* 46 (1996) 381–385.
- [42] Van Soest P.J., Robertson J.B., Lewis B.A., Methods for dietary fiber, neutral detergent fiber, and non starch polysaccharides in relation to animal Nutrition, *J. Dairy Sci.* 74 (1991) 3583–3597.
- [43] Vörös G., Investigation relating to diseases of the digestive system at weaning rabbits, in: 1<sup>er</sup> Congrès International Cunicole, Dijon, France, ASFC publ., 31 mars–2 avril, 1976, comm. 42.