NUTRIONNALY INDUCED ENTEROPATHY IN THE GROWING RABBIT: IMPACT ON CAECAL MICROBIAL ACTIVITY AND METABOLIC PROFILE

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ABSTRACT

Caecal microbial activity, metabolic and mineral blood profiles were studied according to the health status of growing rabbits (diarrhoeic *vs.* healthy). Conventional rabbits (n=359) were housed in collective cages and 80 rabbits in individual cages. A standard fibre-diet SF (ADF=19g.kg^{-1} DM) or a deficient-fibre diet DF (ADF= 9g.kg^{-1} DM) as a model to reproduce non-specific enteropathy were distributed *ad libitum* from weaning (28 days) to 70 days of age. In agonising rabbits, dry matter and total volatile fatty acids concentration in the caecum decreased, as well as bacterial pectinasic activity, while caecal ammonia concentration, minor VFAs proportions, C_3:C_4 ratio and pH increased.
Regarding the blood profile, glucose, potassium and albumin plasma decreased in diarrhoeic rabbits compared to healthy ones, while urea and total cholesterol showed a two-fold increase. Thus the metabolic profile of the rabbit affected by a non specific enteropathy is similar to that previously described for rabbit specific enteropathy such coccidiosis or colibacillosis.

Key words: Blood parameters, caecal fermentation, diarrhoea, fibre deficiency, growing rabbits.

INTRODUCTION

Diarrhoea is the main clinical sign in rabbits affected by digestive disorders (Licois, 2006). It is possible to distinguish enteropathies originating from specific pathogenic agents such as coccidia or enteropathogenic *Escherichia coli,* 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. Among the nutritional factors, the dietary fibre level is the most important to modulate the digestive health in the young rabbit (Gidenne *et al.,* 2010) and caecal microbial community (Bennegadi *et al.,* 2003). Besides, some studies report metabolic changes, in particular for hydromineral metabolism in rabbits suffering from specific enteropathy, such as coccidiosis (Licois *et al.*, 1978ab) and colibacillosis (Renault *et al.,* 1976). However, few studies analysed the physiological status of young rabbits affected by non specific enteropathy, and particularly the changes in the caecal microbial ecosystem associated to metabolic criteria. Thus we aimed to describe caecal microbial activity (fermentation end-products levels, bacterial fibrolytic activity) and metabolic blood profile according to a nutritionally induced enteropathy model in the growing rabbit.

MATERIALS AND METHODS

Animals, diets, and experimental design

In this experiment, 359 rabbits (strain INRA A1067) were housed in collective cages (C), (5 to 6 animals/cage) and 80 rabbits housed in individual cage (I), (26×33×65 cm, l×h×b) under controlled environmental conditions from weaning (day 28) till 70 days of age. Rabbits from C and I groups were

allotted into two-sub groups, and were fed *ad libitum* either a diet having a standard fibre concentration (diet SF) or a fibre deficient diet (diet DF). The two pelleted diets were composed of alfalfa, beet pulp, wheat bran, wheat, wheat straw and soya bean meal. Diets were formulated to avoid no changes in fibre quality, but only in fibre concentration. The lignocellulosic content of the diet SF was 18.9%, while that of the DF diet was only 8.8%. The origin and proportions of fibre fractions were similar among diets, as well as the ratio of digestible protein/ digestible energy (Table 1).

Table 1. Chemical composition of the experimental diets ($g.kg^{-1}$ air dry basis).

	Diet SF	Diet DF		Diet SF	Diet DF
Dry matter	902	893	Neutral-detergent fibre (NDF)	379	193
Organic matter	824	837	Acid-detergent fibre (ADF)	189	88
Crude protein (Nx6.25)	159	177	Acid-detergent-lignin (ADL)	34	15
Starch	96	320	Digestible Energy (DE), g/MJ	10.68	12.81

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, at the end of the caecotrophy period. The pH of the caecal digesta was measured immediately after laparotomy. Fresh caecal digesta samples (1-3g) were collected in 3 different tubes containing respectively: 10ml of 0.025M of an anaerobic buffer MES-DTT (2-N-Morpholinoethanesulfonic acid–DL-Dithiothreitol), 2ml of H_3PO_4 (2%, *v/v)* and 3ml 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 same sampling procedure was performed on agonising rabbits and on rabbits which died less than 10 hours before sampling. These rabbits were classified in 2 groups: Group 1 corresponding to animals still lukewarm and sampled 1 to 3 hours after death, and group 2 corresponding to animals that were on *rigor mortis* state (between 4 and 10 hours after death).

Sampling and analysis of blood parameters.

Blood was sampled from central ear artery, from 121 healthy rabbits at the end of the caecotrophy period at 28, 42, 56 and 70 days of age (8 to 20 rabbits per diet and age). Rabbits were not fasted before sampling. Blood samples were also taken on diarrhoeic rabbits, between 38 and 65 days of age (93 animals). The haematocrit value was determined by the capillary micro-haematocrit method. Albumin, urea, glucose, cholesterol, triglycerides were analysed on serum with an automatic analyzer. Sodium and potassium serums were analysed, after sample dilution, with an atomic absorption spectrophotometer.

Biochemical analysis.

Dry matter, ash, crude protein, crude fibre, neutral-detergent fibre, acid-detergent fibre and acid detergent lignin of feeds were analysed according to E.G.R.A.N. (2001). Fibrolytic bacterial activity was assayed from bacteria, after extraction of cellular content from caecal samples (Gidenne *et al.*, 2002). Volatile fatty acids were extracted from cæcal liquid digesta and measured by gas phase chromatography on semi-capillary columns (Bellier, 1994). NH_3-N concentration was analysed spectrophotometrically at 660 nm.

Statistical analyses.

When data followed a normal distribution, records were analysed using a model of variance analysis with fixed effects and interaction (GLM procedure according to SAS, 1991). Otherwise, date were analysed by non parametric procedure (Mann & Whitney test). 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: class d42, class d56 and class d70. Microbial activity, caecal traits and blood parameters were analysed according to the type of housing, to the age for healthy rabbits, to the diets, to the time of sampling and to health status.

RESULTS AND DISCUSSION

Effect of health status on caecal traits and microbial activity

Compared to dying rabbits with digestive disturbances, caecal microbial activity (CMA) of dead rabbits was deeply modified and more specifically between 1-6h after death, when fermentations end-products (VFAs, NH_3-N) and fibrolytic activities (cellulase and xylanase) increased with time delay after death (results not shown). For this reason, caecal characteristics results were compared only between dying rabbits and healthy ones from the 2 groups of diets. For caecal measurements, no significant interactions were detected between age-diet, age-housing and housing-diet. Therefore pooled data from diarrhoeic or healthy rabbits are presented in table 2.

Diarrhoeic rabbits stopped their solid consumption but continued to consume water. The result was a drop of body weight by 16% (P=0.04) and an important dilution of caecal content compared to healthy rabbits (P<0.001; Table 2). Indeed, Licois *et al.* (1978a) described the same observation with an experimental coccidiosis. For these sick animals, tVFAs decreased by 40% (P<0.001, Table 2) and originated in a fall of C_2 and C_4 concentrations (-49% and -61%, P<0.001, respectively). The consequence is a reverse C_3:C_4 ratio for diarrhoeic rabbits compared to healthy ones. These results were probably related to a decrease of available substrate for caecal microflora and a modification of microbial community balance (Bennegadi *et al.*, 2003; Licois *et al.*; 2006). On the other hand, the concentration of NH_3-N doubled in sick rabbits compared to healthy ones (P<0.001). Similar conclusions were reached by Gidenne and Licois (2005). Moreover, a sharp increase (8.5 times, P<0.001) of minor VFAs proportions was observed for dying rabbits. It seems that in diarrhoeic rabbits, caecal fermentative profile evolved to a more "proteolytic" profile (Nordgaard *et al.*1995). Among the three bacterial fibrolytic activity assessed here (Table 2), only the pectinolytic activity decreased (-32%, P=0.022) in diarrhoeic rabbits. This reduction was also observed with deficient fibre in diet (Bennegadi-Laurent *et al.*, 2004). It seems that the decline of the substrate in the caecum first reduced the pectinase activity and was already been seen by Gidenne et Jehl (2000).

Table 2. Caecal parameters according to the health status in growing rabbit [1]

	Healthy (42 to 56d) n=56	Dying diarrhoeic (36 to 65d) n=19	*P value*
Caecal characteristics			
Body weight, BW (g)	1451.8±423	1223.1±202	*0.04*
Organ weight, OW (g)	24.9±6	22.3±6	*0.14*
Ratio OW/BW (%)	1.99±2.1	1.91±0.4	*0.88*
Content weight (g)	92.3±25	100.2±53	*0.41*
Dry matter (%)	23.07±2	16.83±5	*<0.001*
Caecal fermentative activity			
pH	6.0±0.4	6.7±0.5	*<0.001*
NH_3-N ($mM.l^{-1}$)	10.6±2.1	26.2±11.7	*<0.001*
Total VFAs ($mM.l^{-1}$)	53.1±21.0	31.7± 15.7	*<0.001*
C_2 (%)	79±5	70±7	*<0.001*
C_3 (%)	6±3	15±3	*<0.001*
C_4 (%)	14±4	9±4	*<0.001*
C_3:C_4 ratio	0.54±0.54	2.02±1.52	*<0.001*
Minors VFAs (%)	0.6±0.9	5.2±0.3	*<0.001*
Bacterial fibrolytic activity *(µM of reduced sugar.$g^{-1}$of DM.h^{-})*			
Cellulase	9.9±9.4	6.1±5.6	*0.14*
Xylanase	43.3±24.4	40.7±44.0	*0.76*
Pectinase	206.1±95.6	139.6±103.4	*0.02*

Results were performed by mean ± standard deviation (SD);
[1] : Pooled data from SF and DF groups, and from C and I groups; n: number of animals

Effect of health status on blood parameters

The major part of blood parameters was affected in diarrhoeic rabbits (Table 3), except triglycerides and sodium plasma. A hypoglycaemia was noticed for rabbits affected by nutritional enteropathy, probably related to a very low (or a break) feed intake. This was in agreement with the study of Tambur *et al.* (1999) on rabbits with a coccidiosis intestinal infestation. In addition, albumin and haematocrit values were reduced because of a hemodilution (Licois *et al.*, 1978a). In contrast, in our study, diarrhoeic rabbits had a slight haemocentration. A drop of kaliemia in dying rabbits was systematically showed in diarrhoeic rabbits and was generally more intense (a reduction of 30 to 50%) in the case of coccidiosis (Licois *et al.*, 1978b). In our study, this reduction was around 21%. An increase of urea and total cholesterol in diarrheic rabbits compared to healthy ones was noticed. These results confirm those already obtained by Licois *et al.* (2006). In addition, according to Coudert *et al.* (1978), the strong blood lipemia was related to an important lipolysis because rabbits stopped their feed consumption.

Table 3. Blood profile in healthy and diarrhoeic rabbits aged around 7 weeks [1]

	Healthy (56d) n=34	Diarrhoeic (49-56d) [2] n=59	*P value**
Haematocrit (%)	35.4±3.7	37.9±3.4	*0.002*
Glucose ($g.l^{-1}$)	1.43±0.36	1.34±1.03	*0.002*
Albumin ($g.l^{-1}$)	43.5±3.5	41.6±5.1	*0.016*
Urea ($g.l^{-1}$)	0.27±0.08	0.59±0.53	*<0.0001*
Triglycerides ($g.l^{-1}$)	0.45±0.18	0.70±0.67	*0.56*
Total cholesterol ($g.l^{-1}$)	0.86±0.33	1.47±0.78	*<0.0001*
Potassium ($mM.l^{-1}$)	6.32±1.01	4.98±1.34	*<0.0001*
Sodium ($mM.l^{-1}$)	137.4±12.6	136.4±9.9	*0.90*

Results were performed by mean ± standard deviation (SD); [1]: Pooled data from SF and FD groups, and from C and I groups; [2]: animals in phase of clear acute diarrhoea and at the end of the diarrhoea; n*:* number of animals; *: Mann-Whitney test (no parametric test).

CONCLUSIONS

Caecal microbial activity was deeply affected by nutritional enteropathy, caused by a dietary fibre deficiency. These 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 resistances to the enteropathies, and particularly to the nutritional factors which can control the activity of the caecal microbial community.

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