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Original article

Assessment of the activity of a fructo-oligosaccharide on different caecal parameters in rabbits experimentally infected with *E coli* 0.103

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Summary — Thirty 51-d-old rabbits, fed since the 18th d on a mixed diet containing a fructo-oligosaccharide (FOS) added at 0.25% (FOS group) and 30 51-d-old rabbits without FOS (C group) were infected *via* oesophagal route, with 2 x 10^7 *E coli* O.103 strain B_{10}. Seven d after challenge the first digestive symptoms were observed, and 20% of the animals died in each group; in the survivors, the number of rabbits without any clinical signs was significantly higher in the FOS group (35.7% *versus* 14.8% in the C group $P < 0.05$). In the FOS group the body weights of survivors were 95 g more than the controls weights (2 454 g *versus* 2 359 g). At 80 d, 14 animals were euthanised in each group and different caecal parameters were compared. In the FOS group the following were observed:
– a lower pH: 6.04 *versus* 6.26 ($P < 0.05$);
– a strong rise in total VFA: 73.4 mmol/kg *versus* 56.2 mmol/kg ($P < 0.05$);
– a marked decrease of NH_3: 11.1 mmol/kg *versus* 17.0 mmol/kg ($P < 0.001$);
– an increase in saprophytic *E coli* counts: $10^{4.2}$/g *versus* $10^{2.5}$/g ($P < 0.001$). The results confirm the possibility that FOS increases the rate of VFA production in the caecum. With this increase is associated a higher *E coli* population ($P < 0.001$), characteristic of a favourable biochemical environment (pH, VFA, NH_3) and considered by the authors as able to reduce the morbidity (but not the mortality) induced by an enteropathogenic *E coli* infection.

fructo-oligo-saccharide / caecal parameter / rabbit / colibacillosis

Résumé — Évaluation de l'activité d'un fructo-oligo-saccharide sur différents paramètres cæcaux chez le lapin soumis à une colibacillose expérimentale. *Trente lapereaux de 51 j ayant reçu depuis le 18e j un aliment contenant 0,25% d'un fructo-oligo-saccharide (FOS) et 30 lapereaux recevant un aliment témoin (C) ont reçu par voie œsophagienne 2 x 10^7* E coli *O103 souche B10. Sept jours après l'épreuve expérimentale, les premiers troubles digestifs apparurent et les pertes furent équivalentes dans les deux lots (20%); cependant, chez les survivants, le nombre de sujets n'ayant extériorisé aucun trouble digestif a été significativement plus élevé dans le lot FOS (35,7%* versus *14,8% pour le lot témoin* $P < 0,05$*). À J80, 14 sujets dans chaque lot ont été sacrifiés et des contrôles ont été réalisés sur différents paramètres cæcaux. Chez les sujets du lot FOS ont été observés : un pH cæcal plus faible : 6,04* versus *6,26 (*$P < 0,05$*); une élévation du taux de AGV : 73,4 mmol/kg* versus *56,2 mmol/kg (*$P < 0,05$*); une diminution de l'ammoniac : 11,1 mmol/kg* versus *17,0 (*$P < 0,001$*); une augmentation de la densité des colibacilles non pathogènes $10^{4,2}$/g* versus *$10^{2,5}$/g*

(P < 0,001). Ces résultats confirment l'aptitude du fructo-oligo-saccharide étudié à augmenter la production des AGV au niveau du cæcum. À cette augmentation du taux des AGV est associé, dans les conditions de l'étude, un développement significatif de la flore colibacillaire (P < 0,001). Les auteurs émettent l'hypothèse que le niveau atteint par la flore colibacillaire dans un environnement biochimique favorable (pH, AGV, NH3), permet de limiter la morbidité (mais non la mortalité) liée à une infection expérimentale par un colibacille entéropathogène.

fructo-oligo-saccharide / paramètre cæcaux / colibacillose

INTRODUCTION

The prevention of enteritis is of utmost economic importance for rabbit producers as intestinal troubles are responsible for average losses of ≈ 12% of animals between the ages of weaning and slaughtering (Koel, 1991).

The intensive use of antibiotics to prevent or control enteritis has many disadvantages such as poor efficiency, risks of antibioresistance and residues left in the meat.

An anarchistic development of enteropathogenic *E coli* (O.103, O.15) and/or *Clostridia* (mainly *C spiroforme*) (Carman and Borriello; Renault *et al,* 1983; Milon *et al,* 1990; Peeters and Geeroms, 1991) due to various causes such as stress, inadequate diet or intolerance to different antibiotics (Morisse *et al,* 1979, 1985) is commonly observed in enteritis.

As dysbacteriosis is usually related to a severe reduction of volatile fatty acids (VFA) in the caecum, to a strong rise in caecal ammonia and to a rise in the pH (Prohaszka, 1980), the prevention of enteritis has been attempted either by direct acidification of the intestinal content (Morisse *et al,* 1979) or by providing the animals with a suitable glucide balance (cellulose and starch) in order to induce high levels of VFA in the intestine (Morisse *et al,* 1985).

In a previous study (Morisse *et al,* 1990) we have shown that several parameters in the caecum, *ie* pH, VFA, ammonia) could be modulated by the utilization of a mixture of fructo-oligo-saccharides (FOS) (Profeed, ® Beghin-Meiji Industries).

FOS are non hydrolysable compounds of glucose (G) and fructoses (F), *eg* GF_2, GF_3, GF_4, etc (Takahisa *et al,* 1989). They exist naturally in many kinds of plants such as onions, asparagus roots, bananas, etc and are industrially obtained from sucrose by enzymatic action of β-fructofuranosidase from *Aspergillus niger.*

In human intestinal flora, the selective utilization of FOS by intestinal bacteria leads to a remarkable increase in bifidobacteria, a reduction of *Clostridium perfringens* and of putrefaction products; and the rate of VFA also rises markedly (Hidaka, 1986).

The aim of the present study was to try to reproduce the caecal modifications already observed in rabbits and to test the resistance of these FOS-treated animals to an experimental infection with an enteropathogenic *E coli* O.103.

MATERIAL AND METHODS

Determination of the infective dose and of the age of the challenged animals

The setting up of the experimental model required several experimentations with the aim of determining the adequate infective concentration of *E coli* O.103 $s.B_{10}$ and the age of opti-

mum susceptibility required to induce a medium-intensity rate of mortality–morbility.

A preliminary trial (see *Annex*) involving 4 groups of 10 37-d-old rabbits: 1 non inoculated control and 3 groups respectively inoculated with 2 x 10^4 2 x 10^5 and 2 x 10^6 *E coli*, resulted in a mortality rate not exceeding 1/10 in any group within an observation period of 17 d.

A second experiment performed in 4 groups of 37-d-old animals, inoculated under identical conditions gave similar results.

In addition, higher infective doses (10^8 and 10^9 *E coli*) were tested in 2 groups of 6 rabbits. The mortality rates were respectively 2 and 3 animals and the morbidity (number of d of enteritis) ranged linearly in function of the dose from 10^4–10^9 (see *Annex*).

In spite of the similarity of the experimental design, the susceptibility of our rabbits under our feeding conditions was demonstrated to be much lower than that of animals used by Licois *et al* (1990).

A third experiment performed with an infective dose of 2 x 10^7 *E coli* in 18 37-d-old and in 18 51-d-old rabbits demonstrated that under our conditions, mortality and morbility were lower at 37 d than at 51 d of age (see *Annex*).

From these preliminary adjustments (summarized in the *Annex*) the experimental conditions were determined as follows: age: 51 d, infective dose: 2 x 10^7 *E coli* O.103 s B_{10}.

Animals, housing and diet

Thirty 51-d-old rabbits fed since the 18th d on a mixed diet containing Profeed, a FOS compound (FOS group) and 30 51-d-old rabbits receiving the same diet without FOS (C group), were housed 6 per cage in an absolute air filtered chamber (0.22 µm) for 29 d.

FOS was added to the basic diet (table I) at the rate of 0.25% of a specific mixture of GF_2, GF_3 and GF_4 (0.7% Profeed).

Challenge

On the day after their transfer to the air-filtered chamber, each animal was infected *via* oesophagal route with 2 x 10^7 *E coli* O.103 strain B_{10} cultivated in trypticase-soya medium.

Recordings

The animals were observed daily for abnormalities and the growth of survivors was controlled by individual weighings at d 51 and 80.

Dead animals were autopsied and attempts made to recover *E coli* O.103 by serotyping.

Table I. Composition and analytical characteristics of the diet.

Ingredients	%	*Analysis*	%
Alfalfa 17	25.5	Dry matter	87.4
Wheat bran	25.5	Crude protein	15.9
Oats	14.5	Fat	4.7
Wheat	7	Fiber	14.2
Soya 48 meal	6	Neutral deterg fiber	32.1
Sunflower meal	5.5	Acid deterg fiber	17.8
Citrus meal	2.5	Lignin	4.8
Cocoa hull	2.5	Starch	15.5
Wheat straw	2	Soluble sugars	5.4
Soya oil	1.5	Digestible energy	2580
Sugar	1.5	Kcal/kg	
Corn gluten	1		
Mineral–vitamin mixture	3		

At d 80, 14 animals from each groups were selected on the basis of the visible absence of any signs of enteritis; the animals were killed and the following caecal investigations were carried out: measurement of pH (electrode of the pHmeter inserted into the caecum); amount of VFA and ammonia (gas chromatography); *E coli* count in Na desoxycholate plate medium.

Statistical analysis

Mortality, morbidity and biochemical results were compared using the χ^2 test.

RESULTS AND DISCUSSION

Sanitary results

As a consequence of the inoculation by the oesophagal route, 5 animals died or were eliminated within the first 2 d after challenge. Observations were performed respectively on 28 rabbits for the C group and 27 for the FOS group. Results are given in table II.

Mortality

Five animals in group C and 6 animals in the FOS group died with clinical signs of enteritis during the observation period.

At *post-mortem* examination there was evidence of acute typhlitis and *E coli* O.103 was recovered from caecal liquid contents.

Neither the mortality rate (18 and 22%) nor the course of the disease in the dead animals differed between the 2 groups.

On average, the first clinical signs appeared at the 7th d post-challenge and mortality occurred 5–7 d after an acute diarrhoea.

Morbidity

Among the surviving rabbits, the number of animals exhibiting clinical signs of diarrhoea was statistically lower in the FOS group: 4/27 (14.8%), compared with 13/28 (46.4%) in the group C ($P < 0.05$) (table II).

Weight gain of survivors

In rabbits free of clinical signs, the mean body weight at d 80 in the FOS group was 95 g more than that in the group C: 2 454 g compared with 2 359 g respectively; but the difference was not significant (table III).

Caecal parameters

The tests performed on caecal contents at 80 d of age showed several statistically

Table II. Sanitary results.

Group	*No of animals at d 51*	*Dead animals*		*Animals with enteritis*		*Animals without enteritis*	
		No	*%*	*No*	*%*	*No*	*%*
C	28	5	17.9	13	46.4	10	35.7
FOS	27	6	22.2	4**	14.8	17	14.8

** $P < 0.01$.

significant differences between the 2 groups (table IV). The FOS group had:

– a lower pH: 6.04 *versus* 6.26 ($P < 0.05$);

– a higher rate of VFA production: 73.4 mmol/kg of crude material *versus* 56.2 mmol/kg ($P < 0.05$);

– a marked decrease in ammonia production: 11.1 mmol/kg *versus* 17.0 mmol/kg ($P < 0.001$);

– an increase in saprophytic *E coli* counts: $10^{4.2}$/g *versus* $10^{2.5}$/g ($P < 0.001$) and a more regular distribution of *E coli* within the FOS group.

The data recorded in the FOS group were characteristic of healthy rabbits whereas in the group C, pH, ammonia and VFA rates were representative of critical values.

In digestive disorders, several changes are routinely observed in caecal content: a rise in pH (up to 7) a marked development of *E coli* (107–109/g) a rise in ammonia levels and a drop in VFA production rate (40–45 mmol/kg *versus* 70–80 mmol.kg).

Rabbits are generally considered as differing from all the other species as far as *E coli* counts are concerned: while *E coli* counts in the caecal contents are $\approx 10^5$/g in most species under normal conditions, practically no *E coli* occur in healthy rabbits (Smith and Crabb, 1961; Prohaszka, 1980). In fact, from our previous studies on feeding and intestinal environment (Morisse *et al*, 1985, 1990) we are of the opinion that the limited *E coli* population or its absence in many animals is not physiological but is the consequence of a relative inadequacy of intensive feed on the digestive physiology of rabbits. The abnormally small *E coli* population could explain

Table III. Growth of survivors without clinical signs.

Group	*No d 51*	*Body weight (g) d 51*	*No of healthy rabbits d 80*	*Body weight d 80 (g)*	*Average daily gain (g/d)*
C	28	1478 ± 108	10	2359 ± 156	32.6 ± 4.3
FOS	27	1477 ± 131	17	2454 ± 216	33.5 ± 17

Table IV. Summary of changes observed in the caecal contents.

Group	*No of animals*	*pH*	E coli *(log_{10}/g)*	*NH_3 (mmol/kg)*	*Total VFA (mmol/kg)*	*VFA mmol/kg*		
						C_2	*C_3*	*C_4*
C	14	6.26 ± 0.31	2.5 ± 0.3	17.0 ±4.0	56.2 ± 24.8	45.7 ± 16.9	1.3 ± 0.01	9.2 ± 8.1
FOS	14	6.04 ± 0.17*	4.2 ± 0.1***	11.1 ±3.4***	73.4 ± 16.7*	57.6 ± 13.0*	1.8 ± 0.01	19.8 ± 4.9*

* $P < 0.05$; *** $P < 0.001$.

the extreme fragility of the intestinal flora balance and the increased opportunity for enteropathogenic *E coli* to develop strongly.

In 'farmer rabbits' fed on vegetables and plants, and in wild rabbits, caecal pH are generally lower than in intensively fed rabbits: 5.6–5.8 *versus* 6.2–6.5 and *E coli* counts are substantially higher: 10^4–10^5/g *versus* $\leq 10^{2.5}$/g (Morisse *et al*, 1979, 1985).

In rabbits reared in this manner, enteritides of bacterial origin are not reported as having great significance.

The differences observed in *E coli* counts between the 2 groups have been studied according to the sanitary status of animals after the experimental infection.

Although none of the rabbits presented any visible signs of enteritis when killed, 11 in the C group had been recorded as sick animals *versus* only 3 in the FOS group.

Consequently, the increase observed in the *E coli* counts in the latter group seems to be related to the FOS treatment and cannot be considered as a result of the disease; for this reason and in the absence of serotyping the increased *E coli* counts can only be supposed to be non pathogenic.

We consider that all means (dietary balance or feed additive) whereby the biochemical characteristics of the caecal content are maintained in a steady state are a contribution towards the non therapeutic control of digestive pathologies in rabbits.

When biochemical parameters (pH, VFA, ammonia) reach their optimum level, the *E coli* flora is more abundant than classically reported (Smith and Crabb, 1961); and although its level remains modest (10^5/g), it suggests a kind a barrier effect which prevents the development of pathogenic *E coli*.

CONCLUSIONS

Under experimental conditions, the incorporation of a fructo-oligo-saccharide preparation (Profeed) in rabbit feed, partially reduced the pathogenic effect of *E coli* O.103. In survivors, FOS allowed the restoration of several caecal parameters (pH, NH_3, VFA) to their proper physiological levels. The present work suggests that the physiological rise in the saprophytic *E coli* population (barrier effect) could be of interest for the non-antibiotic control of bacterial enteritis. Nevertheless more research is necessary to determine the optimum dosage of FOS and to confirm the present results.

ANNEX

Preliminary determination of the experimental conditions

Experiment 1. Mortality and morbility observed in 4 groups of 10 37-d-old rabbits inoculated with 0,2 x 10^4, 2 x 10^5 and 2 x 10^6 *E coli* O.103 strain B_{10}.

Infective doses	*No of animals*	*No of dead animals*	*No of surviving animals*	
			With enteritis	*Without enteritis*
0 (Control)	10	1	0	9
2 x 10^4	10	1	2	7
2 x 10^5	10	1	3	6
2 x 10^6	10	1	5	4

Experiment 2. Mortality and morbility recorded in 37-d-old rabbits infected with various doses of *E coli* O.103 strain B_{10}.

Infective doses	*No of animals*	*No of dead animals*	*No of surviving animals*	
			With enteritis	*Without enteritis*
0 (Control)	12	0	0	12
2×10^4	12	0	1	11
2×10^5	12	1	2	9
2×10^6	11	2	6	3
2 x 108	6	2	3	1
2 x 109	6	3	3	0

Experiment 3. Mortality and morbility observed with a unique infective dose (2×10^7 *E coli* O.103 strain B_{10}) in 37- and 51-d-old rabbits.

Age (d)	*No of animals*	*No of dead animals*	*No of surviving animals*	
			With enteritis	*Without enteritis*
37	18	2	4	12
51	18	5	11	2

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