Proceedings 5th World Rabbit Congress, 25-30 July 1992, Corvallis – USA, 1137-1143.

EFFECT OF A FRUCTO-OLIGO-SACCHARIDES COMPOUND IN RABBITS EXPERIMENTALLY INFECTED WITH *E. COLI* 0.103

J.P. MORISSE, R. MAURICE, E. BOILLETOT and J.P. COTTE

CENTRE NATIONAL D'ETUDES VETERINAIRES ET ALIMENTAIRES
National Center of Veterinary and Food Researches
B.P. 53 – 22440 PLOUFRAGAN-FRANCE

ABSTRACT

Works carried out previously in rabbits, showed that the administration of fructo-oligo-saccharides (FOS) had the following effects in the intestine : an increased production of volatile fatty acids (VFA), a decrease in ammonia levels (NH_3) and a rise in the numbers of saprophytic *E. coli* in the caecal contents.

In the present experiments, the possibility of controlling *E. coli* infections and their consequences without administration of antibiotics was investigated using a similar procedure.

Thirty 51 days-old rabbits, fed since the 18th day on a mixed diet containing FOS added at 0,25 percent* (FOS group) and thirty 51 days-old rabbits without FOS (C group) were infected, by the oral route, with 2 x 10^7 *E. coli* 0.103 strain B_{10}. The experiments were performed in a filtered air (0.22 μ) chamber for a period of 29 days.

Seven days after challenge the first digestive symptoms were observed, and 20 percent 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 percent versus 14,8 percent in the C group $P<0,05$) and their final body weights were 95 g more than the controls weights (2454 g versus 2359 g).

In the sick animals, only the lightest rabbits of the FOS group exhibited diarrhoea.

At 80 days, 14 animals were euthanased in each group and different caecal parameters were compared.

In the FOS group, the following differences were observed compared with the C group :

- A lower pH (6.04 versus 6.26 $P < 0,05$)
- A strong rise in total VFA (73,4 mmol/kg versus 56.2 $P < 0,05$)
- A marked decrease of NH_3 (11.1 mmol/kg versus 17 $P < 0,001$)
- An increase in saprophytic *E. coli* counts ($10^{4,2}$/g versus $10^{2.5}$ $P < 0,001$).

These results confirm the possibility that FOS increases the rate of VFA production in the caecum and therefore allows the developpment of a higher sapprophytic *E. coli* population, inducing a better resistance against the event of an enteropathogenic *E. coli* invasion.

---

* PROFEED ® R. BEGHIN MEIJI Industries at 0,7 percent.

Proceedings 5th World Rabbit Congress, 25-30 July 1992, Corvallis – USA, 1137-1143.

EFFECT OF A FRUCTO-OLIGO-SACCHARIDES COMPOUND IN RABBITS EXPERIMENTALLY INFECTED WITH *E. COLI* 0.103.

J.P. MORISSE, R. MAURICE, E. BOILLETOT and J.P. COTTE

CENTRE NATIONAL D'ETUDES VETERINAIRES ET ALIMENTAIRES
National Center of Veterinary and Food Researches
B.P. 53 – 22440 PLOUFRAGAN-FRANCE

Introduction

The prevention of the enteritis is of the utmost economical importance for rabbit producers as intestinal troubles are responsible for average losses of about 12 per cent of animals between the ages of weaning and slaughtering (3).

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

An anarchic development of enteropathogenic *E. coli* (0.103, 0.15) and/or *Clostridia* (mainly *C. spiroforme*) (4–8–10–1) due to various causes such as : stress, inadequate diets or intolerance to different antibiotics (5–8) is commonly observed in enteritis.

Because dysbacteriosis is usely related to a severe reduction of volatile fatty acids (VFA) in the caecum, to a strong rise of caecal ammonia and to a rise in the pH (9), the prevention of enteritis has been attempted either by the direct acidification of the intestinal content (5) or by providing the animals with a suitable balance of glucides (cellulose and starch) in order to induce high levels of VFA in the intestine (6).

In a previous work (7) we have shown that several parameters in the caecum i.e : pH, VFA, ammonia) could be modulated by the utilization of a mixture of fructooligosaccharides (FOS.)*.

F.O.S. are non hydrolysable compounds of glucose (G) and fructose (F) in variable numbers : GF_2, GF_3, GF_4, etc... (6–11). They exist naturally in many kinds of plants such as onions, asparagus roots, bananas, etc... and they are industrially obtained from sucrose by enzymatic action of the β–fructofuranosidase from "*Aspergillus niger*".

In human intestinal flora the selective utilization of FOS by intestinal bacteria leads to a remarkable increase in bifidobacteria, to a reduction of *Clostridium perfringens* and of putrefaction products, and also the rate of VFA rises markedly (2–12).

The aim of the present work 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* 0.103.

---

* PROFEED® R. BEGHIN–MEIJI Industries

Material and methods

Animals, housing and diet

Thirty 51 days old rabbits fed since the 18th day on a mixed diet containing a FOS compound* (FOS group) and thirty 51 days old rabbits receiving the same diet without FOS (C group), were housed by 6 in collective cages in an absolute air filtered chamber (0.22 μ) for 29 days.

FOS was added to the basic diet (table 1) at the rate of 0.25 per cent of a specific mixture of GF_2, GF_3 and GF_4 (0.per cent of PROFEED ® R)*.

Challenge

On the day after their transfer into the air filtered chamber, each animal was infected by the oral route with 2×10^7 *E. coli* 0.103 strain B_{10}.

Recordings

The animals were observed for abnormalities and weighted daily.

Dead animals were autopsied and attempts made to recover *E.coli* 0.103 by serotyping.

At day 29, 14 animals in each group were sacrified and following caecal investigations were carried out :

- measurement of pH (electrode of pHmeter inserted into the caecum)
- amount of VFA and ammonia (Gas Chromatography)
- count of *E. coli* in Na desoxycholate plate medium.

Statistical analysis

Mortality, morbidity and biochemical results were compared using the $\aleph^2$ test.

Results and discussion

1. Sanitary results

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

Mortality

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

Post mortem examinations gave evidence of an acute *typhlitis* and *E. coli* 0.103 was recovered from caecal liquid contents.

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

On average, the first clinical signs appeared at the 7th day post challenge and the death occurred 5 to 7 days later of an acute diarrhoea.

Morbidity

Among the surviving animals, the numbers of animals exhibiting clinical signs of *enteritis* were statistically lower in the FOS group : 4/27 (14,8 per cent), compared with 13/28 (46,4 per cent) in the group C ($P < 0.05$) (table 2).

2. Weight gain of survivors

In rabbits without any clinical signs, the mean body weight at day 80 in the FOS group was 95 g more than that in the group C : 2454 g compared with 2359 g respectively ($P < 0.02$) (table 3).

3. Caecal parameters

The tests performed on caecal contents at 80 days of age showed several differences of statistical significance between the two groups. 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 materiel versus, 56.2 mmol/kg ($P < 0.05$).
- A marked decrease of ammonia production : 11.1 mmol/kg versus 17 ($P < 0.001$)
- An increase in saprophytic *E. coli* counts : $10^{4.2}$/g versus $10^{2,5}$ ($P < 0.001$) and a more regular repartition of *E. coli* within the FOS group.

The data recorded in the FOS group were quite 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 the caecal content : a rise of pH (up to 7) a marked development of *E. coli* (10^7–10^9/g) a rise of ammonia and a drop in the 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 of the caecal contents are about 10^5/g in most species under normal conditions, practically no *E. coli* occur in healthy rabbits (11–9). In fact from our previous works on feeding and intestinal environment (6–7) we believe that the smallness of the *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 abnormal smallness of the *E. coli* population could explain the extreme fragility of the intestinal flora balance and the increased opportunity for entero pathogenic *E. coli* to develop invasively.

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 substancially higher $10^4 - 10^5$ versus $\leq 10^{2,5}$ (5–6).

In rabbits reared this way, enteritis of bacterial origin have little or no significance.

From the present results, the relative abundance of *E. coli* in healthy rabbits gives evidence that the small number or the absence of *E. coli* (classical in the literature from the initial work of Smith and Crabb) (11) is not a a typical feature of the rabbit species. According to recent advances concerning the possible vaccination against enteritis by administration of saprophytic *E. coli* (4), we believe that any feed additive having a favourable influence on the development of saprophytic *E. coli* populations up to the physiological limit of about 10^5 g (i.e enhancing the barrier effect of *E. coli*) is a contribution towards the non therapeutic control of digestive pathologies in rabbits.

Conclusions

Under experimental conditions, the incorporation of a fructo–oligo–saccharide preparation (PROFEED® R) in a rabbit feed, partially reduced the pathogenic effect of *E. coli* 0.103. In survivors, FOS had allowed the restoration of several caecal parameters (pH, NH_3, VFA) to their proper physiological levels. The present work confirms that the physiological rise of 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.

Acknowledgements

The authors are undebted to Professor A. MILON from the National Veterinary School of Toulouse for supplying the *E. coli* O.103 strain B_{10}.

References

1 CARMAN, R.J., BORRIELLO, S.P. (1982) : *Clostridum spiroforme* isolated from rabbits with diarrhoea. Vet. Rec., 114 : 461–462.

2 HIDAKA, H. (1986) : Effects of Fructo Oligo Saccharides on intestinal flora and human health. Bifidobacteria Microflora, Vol. 15 (1) : 37–50.

3 KOEL, P.F. (1991) : Gestion Technico Economique : Bilan et résultats 1990. Cuniculture 99, 18 (3) : 129–137

4 MILON, A., CAMGUILHEM, R., ESSLINGER, J. (1990) : Vaccination du lapereau contre la colibacillose 0.103 : rôle de l'immunité anti LPS et de l'effet barrière

exercée par des souches vivantes non pathogènes. Cuniculture 18, 5ème Journées de la Recherche Cunicole, 12–13 Déc. 1990 PARIS.

5 MORISSE, J.P., BODOLEC, J.L., ANDRIEUX, J., LE FLOCH, B., (1979) : Essais de prévention d'une entérite colibacillaire chez le lapin par l'acide acétique, le lactulose et le lactose. Rec. Méd. Vét., 155 (12) : 943–954.

6 MORISSE, J.P., BOILLETOT, E., MAURICE, R. (1985) : Alimentation et modification du milieu intestinal chez le lapin (AGV, NH_3, pH, Flore). Rec. Méd. Vét. 161 (5) : 443–449.

7 MORISSE, J.P., LE GALL, G., MAURICE, R., COTTE, J.P., BOILLETOT, E. (1990) : Action chez le lapereau d'un mélange de fructo oligo saccharides sur certains paramètres intestinaux et plasmatiques. 5ème Journées de la Recherche Cunicole, Communication 53, 12–13 Déc. 1990 PARIS.

8 PEETERS, J.E., GEFROMS, R., (1991) : Traitement et éradication de la colibacillose 0.15 sur le terrain. Cuniculture 101, 18 (5) : 227–231.

9 PROHASZKA, L. (1980) : Antibacterial effect of volatils fatty acids in enteric *E. coli*–infections of rabbits. Zbl. Vét. Méd., 27 : 631–639.

10 RENAULT, L., ROUX, J., DE BOURHIS, E., COUDERT, P., LICOIS, D., GUILLOT, J.F. (1983) : Description d'un sérogroupe (0.103) d'*E. coli* entéropathogène chez le lapin au sevrage. Bull. Acad. Vét. de France, 56 : 387–400.

11 SMITH, N., CRABB, N.E. (1961) : The caecal flora of animals and men. J. of Path. Bact., 82, 53 : 53–66.

12 TAKAHISA TOKUNAGA, TSUNEYUKI OKU and NORIMASA HOSOYA (1989) : Utilization and excretion of a new sweetener fructoligosaccharides (Neosugar) in Rats. J. Nutr., 199 : 553–559.

Table 1. Composition and analytical characteristics of the diet

Ingredients	p.cent	Analysis	p.cent
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
Soja 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
Soja oil	1.5	Digestible energy Kcal/kg	2580
Sugar	1.5		
Corn gluten	1		
Minerals vitamins mixture	3		

Table 2. Sanitary results

Groups	Number of animals at J51	An. dead		An. with enteritis		An. without enteritis	
		Number	p.cent	Number	p.cent	Number	p.cent
C	28	5	17.9	13	46.4	10	35.7
FOS	27	6	22.2	4*	14.8	17	14.8

* $P < 0.02$ test Khi2

Table 3. Growth of survivors without clinical signs

Group	Number day 51	Body weight (g) day 51	Number of healthy rabbits day 80	Body weight day 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 4. Summary of changes observed in the caecal contents

	Number of animals	pH	*E. coli* Log10/g	NH3 mmol/kg	Total VFA mmol/kg	VFA p.cent		
						C2	C3	C4
C	14	6.26 ±0.31	2.5 ±0.3	17.0 ±4.0	56.2 ± 24.8	81.2	2.4	16.2
FOS	14	6.04* ±0.17	4.2** ±0.1	11.1*** ±3.4	73.4* ±16.7	78.4*	1.8	19.8*

* $P < 0.05$
*** $P < 0.001$