## EFFECTS OF FRUCTO-OLIGOSACCHARIDES ON PERFORMANCES OF GROWING RABBITS

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# ABSTRACT

A common problem in rabbits is the occurrence of digestive disorders just after weaning. This problem is associated with instability of the cecal microflora, and characterized by diarrhea, loss of appetite and increased mortality. The supplementation of diets with fructo-oligosaccharides (FOS) can improve animal's health and performances by selective stimulation of beneficial endogenous bacteria in the cecum. A trial with the objective of evaluating the effects of FOS on growth performances and intestinal morphometry of growing rabbits was conducted. A total of 144 mixed-sex growing rabbits (817±35 g) were submitted from 35 to 70 days of age to 2 experimental treatments. The 2 treatments were applied to a common basal diet as follow: 1) Control (no FOS); 2) FOS (0.36 g.kg<sup>-1</sup> diet). Experimental diets were offered ad libitum. No statistical differences on mortality and diarrhea frequency were observed during the essay. The Control and FOS rabbits had similar live weights at 35 (821 vs. 812 g), 49 (1107 vs. 1146 g) and 70 days (2223 vs. 2234 g), weight gain and feed intake. However, the feed efficiency on the growing period tended to be higher with FOS diet (0.274 vs. 0.304; P=0,056). No significant effects are observed on morbidity and mortality. FOS did not affect the weights of liver, cecum, small intestine and stomach. Also the ileal villi measurements did not show differences between treatments. Results of this study suggest that FOS increase feed efficiency and have negligible effects on growth performance and microbial activity.

Key words: fructo-oligossacarides, rabbit, growth, microbial activity, villi measurement.

# INTRODUCTION

Growing rabbits are currently affected by digestive pathologies. The intestinal pathology is mostly encountered after weaning, leading to high mortality and morbidity. In rabbits, cecal microbial ecosystem has an important role in the nutrition and health, particularly around the weaning period and can easily be altered by feed factors. The mortality rate after weaning reaches 14%, but may exceed 20%. Loss of performance (low growth, low feed intake and poor feed efficiency) due to transient diarrhea is also costly.

Fructo-oligosaccharides (FOS) are  $\beta$ -linked fructose units to the fructose moiety of sucrose not hydrolysed by mammalian digestive enzymes. The supplementation of diets with FOS can improve animal's health and performances by selectively stimulating growth and activity of beneficial endogenous bacteria in the cecum, avoiding enteric

processes and, thus acting as a prebiotic (GIBSON and ROBERFROID, 1995). FOS has been shown to increase volatile fatty acids (VFA) production, reduce pH and modify VFA patterns in the lower gastro intestinal tract (reduce proportion of acetate and increase the proportion of propionate), creating a non favourable environment to pathogen microorganisms, and increasing bifidobacterias (HOUDIJK, unpublished cited by SUTTON *et al.*, 1999). Additionally, absorbed VFA can contribute to cover the energetic needs of the animals. Different works have shown improvements in health and performances in rabbits (MORISSE *et al.*, 1992; LEBAS, 1993). Unbalancing microbial species within the alimentary tract had adverse effects on the animal efficiency through the following mechanisms; modifying the gut wall structure with considerable changes in ultrastructure, limiting the turnover of the intestinal epithelial layer with sparing effects on the metabolic energy costs to maintain the gut (ARMSTRONG, 1986). In our previous study (ALVES *et al.*, 2003) rabbits slaughtered at 49 days fed with a diet supplemented with a FOS complex showed a significant (P=0.04) great ileum villi height (546.6  $\mu$ m) than the fed with a Control diet (382.3  $\mu$ m).

The objective of this trial was to evaluate the effects of FOS on growth performances, microbial activity and intestinal morphometry development of growing rabbits.

# MATERIAL AND METHODS

## Diet

Two treatments were applied to a common basal diet. The two treatments were as follows: 1) Control (no FOS); 2) FOS (commercial FOS; 0.36 g.kg<sup>-1</sup> diet). The basal diet met the nutritive requirements for fattening rabbits (Table 1). Animals were fed *ad libitum*.

#### Table 1. Main ingredients and estimated nutritive value of basal diet.

Composition (g.kg <sup>-1</sup> diet)						
Dehydrated alfafa 14/15	300	Grape-seed meal	40			
Wheat bran	114	Soybean	40			
Sunflower meal	105	Corn destiller's grain dehydrated	30			
Wheat	82	Sunflower hulls	27			
Citrine pulp	60	Soybean meal 44	21			
Molasse	60	Animal fat	7.5			
Barley	50	Soybean oil	5.4			
Estimated nutritive value* (g.kg <sup>-1</sup> diet)						
Dry matter	890.4	Starch	120.3			
Protein	152.5	ADF	214.9			
Fat	43.1	NDF	344.7			
Sugar	67.4	Digestible energy (kcal.kg <sup>-1</sup> diet)	2354			

\*based on tabled values for raw materials (INRA, 1989)

# Animals

A total of 144 weaned mixed-sex rabbits 35 days old were housed in groups of 4 in 18 cages per treatment until they reach 70 days. Animals had a daily light period of 14 hours.

# Methods

Visual health inspections of rabbits were performed daily. Mortality was controlled daily. Rabbit's weight and feed consumption were controlled on days 35, 49 and 70. When a rabbit death occurred, the weight of remaining rabbits and feed in the cage were controlled, which permit to calculate correctly the weight gain and feed intake. Average weight, weight gain, feed consumption per cage and feed efficiency (weight gain/feed consumption) were determined. At 49 and 70 days of age five rabbits per treatment were randomly chosen and slaughtered between 11 and 12 hours a.m. Liver, small intestine and stomach and cecum were weighted, cecal VFA was determined and histological exams (villi development) of ileum were done. For histological exams segments from ileum were cut longitudinally at the mesenteric attachment. The villi measurements were performed according to a modification of the method by Jaeger et al. (1990) and Iji et al. (2001). For VFA analysis, samples of cecal content were collected immediately after slaughters and stored at -20°. VFA analysis was conducted according to JOUANY (1982). Concentration of acetic, propionic and butyric acids were determined using pivalic acid as internal standard. Mortality and diarrhea were analysed with  $\chi^2$  test. The remaining data was analysed as a completely randomised design with 2 treatments. Analysis of variance was performed using the general linear models procedure of SYSTAT 5.0 (1992).

# **RESULTS AND DESCUSSION**

Mortality and incidence diarrhea during the growing period were not affected (P>0.05) by treatments (Table 2). Similar results were observed by MENDEZ et al. (1993) and ALVES et al., (2003). Almost all death and diarrhea cases occurred after weaning, between d35 and d49, as expected. With both treatments mortality reached higher values than the average results normally observed on commercial farms.

Table 2 Death rabbits and diarrhea (n=72 per treatment).								
	Diarrhea			Death				
	35 – 49 d 49 - 70 d 35 – 70 d 35 – 49 d 49 - 70 d 35 - 70 d							
Control diet	24	0	24	12	2	14		
FOS diet	29	0	29	12	0	12		
$\chi^2$ test <sup>1</sup>	ns		ns	ns	ns	ns		
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 $\frac{1}{\chi^2}$  test probability: ns – not significant (P>0.05).

There was no effect of FOS or Control (P>0.05) on weight gain, feed intake and live weights at d35, d49 and d70 of rabbits under experimental conditions (Table 3). However, the feed efficiency on the growing period showed a trend to be higher with FOS diet (P=0,056). Similar results (live weight at 70d 2020 vs 2097; P=0.08) were observed in our previous essay (ALVES *et al.*,2003) and by LEBAS (1993; 1996) and ROCA (1994). Nevertheless, MENDEZ *et al.* (1993) and AGUILAR (1996) also observed improvements on growth rate.

Table 3. Live weight of rabbits (g) weight gain (g) and feed intake (g) per cage and
feed efficiency (weight gain/feed intake) from d35 to d70 (n=18 per treatment).

	Period	Control diet	FOS diet	SEM <sup>1</sup>	F Test <sup>2</sup>
Rabbit live weight	35 d	821	812	6.1	ns
-	49 d	1107	1146	22.5	ns
	70 d	2223	2234	23.4	ns
Cage weight gain	35 – 49 d	586	810	103.1	ns
	35 - 70 d	4144	4545	238.4	ns
Cage feed intake	35 – 49 d	3477	3358	107.4	ns
-	35 - 70 d	13784	14253	618.1	ns
Feed efficiency	35 – 49 d	0.146	0.230	0.028	0.134
-	35 - 70 d	0.274	0.304	0.008	0.056

<sup>1</sup> Standard error of mean; <sup>2</sup> F test probability; ns – non significant (p>0.05)...

The weights of liver, small intestine and stomach and cecum  $(g.kg^{-1} \text{ live weight})$ , total content of cecum and small intestine and stomach at d49 and d70 were not affected (P>0.05) by treatments (Table 4). Only dry mater concentration (%) of cecal contents was significantly higher (P<0.05) with FOS diet at d70. Also, there were no significant effects of the treatments on villi measurement (Table 5) at d49 or d70 (P>0.05). These results do not confirm our previous work (ALVES *el al.*,2003), where a significant effect was observed. Consequently, it is assumed that FOS effect on development of gastro intestinal tract could be positive, but is not always significant.

Table 4. Weight (g/kg live weight) of the small intestine and stomach (SIS), liver	
and cecum at d49 and d70 (n=5 per treatment).	

	<b>Control diet</b>	FOS diet	SEM <sup>1</sup>	F Test <sup>2</sup>
SIS empty	6.49	6.81	0,34	ns
SIS Total content	8.04	6.33	0,63	ns
Liver	3.36	4.33	0,35	ns
Cecum empty	0.20	0.21	0,01	ns
Cecal content	90.1	90.1	7,10	ns
Dry matter of cecal content (%)	21.9	19.4	1,02	ns
SIS empty	2.77	2.50	0,15	ns
SIS Total content	2.34	2.70	0,20	ns
Liver	2.58	2.25	0,14	ns
Cecum empty	0.13	0.15	0,01	0.111
Cecal content	43.9	49.2	2,97	ns
Dry matter of cecal content (%)	20.6 <sup>b</sup>	23.9 <sup>ª</sup>	2,11	0.034
	Liver Cecum empty Cecal content Dry matter of cecal content (%) SIS empty SIS Total content Liver Cecum empty Cecal content	SIS empty6.49SIS Total content8.04Liver3.36Cecum empty0.20Cecal content90.1Dry matter of cecal content (%)21.9SIS empty2.77SIS Total content2.34Liver2.58Cecum empty0.13Cecal content43.9	SIS empty6.496.81SIS Total content8.046.33Liver3.364.33Cecum empty0.200.21Cecal content90.190.1Dry matter of cecal content (%)21.919.4SIS empty2.772.50SIS Total content2.342.70Liver2.582.25Cecum empty0.130.15Cecal content43.949.2	SIS Total content8.046.330,63Liver3.364.330,35Cecum empty0.200.210,01Cecal content90.190.17,10Dry matter of cecal content (%)21.919.41,02SIS empty2.772.500,15SIS Total content2.342.700,20Liver2.582.250,14Cecum empty0.130.150,01Cecal content43.949.22,97

<sup>1,2,</sup> ns: see table 3.

There was no significant effect of diet on total VFA concentration and proportion of cecal samples at 49 days and 70 days (P>0.05). This agrees with the similar total VFA ob-

served by ALVES *et al.*, (2003). Acetic acid was the predominant VFA, being followed by butyric and propionic acids (Table 6). Only at 49 days rabbits fed with FOS diet showed a tendency (P=0,117) for a higher proportion of acetic acid. However, it is assumed that FOS effect on cecal microbial activity was not significant.

	<b>Control diet</b>	FOS diet	SEM <sup>1</sup>	F Test <sup>2</sup>
Villus height	416.5	464.5	20.19	ns
Villus width at tip	117.2	120.6	5.06	ns
Villus width at crypt/villus junction	105.7	101.2	4.54	ns
Crypt depth	165.8	170.8	11.68	ns
Villus height/ crypt depth	2.59	2.8	0.17	ns
Villus height	635.2	647.3	24.95	ns
Villus width at tip	134.2	150	8.82	ns
Villus width at crypt/villus junction	98.9	113.6	6.15	ns
Crypt depth	171.5	172.6	8.52	ns
Villus height/ crypt depth	3.74	3.85	0.21	ns
	Villus width at tip Villus width at crypt/villus junction Crypt depth Villus height/ crypt depth Villus height Villus width at tip Villus width at crypt/villus junction Crypt depth	Villus height416.5Villus width at tip117.2Villus width at crypt/villus junction105.7Crypt depth165.8Villus height/ crypt depth2.59Villus height635.2Villus width at tip134.2Villus width at crypt/villus junction98.9Crypt depth171.5	Villus height416.5464.5Villus width at tip117.2120.6Villus width at crypt/villus junction105.7101.2Crypt depth165.8170.8Villus height/ crypt depth2.592.8Villus height635.2647.3Villus width at tip134.2150Villus width at crypt/villus junction98.9113.6Crypt depth171.5172.6	Villus height416.5464.520.19Villus width at tip117.2120.65.06Villus width at crypt/villus junction105.7101.24.54Crypt depth165.8170.811.68Villus height/ crypt depth2.592.80.17Villus height635.2647.324.95Villus width at tip134.21508.82Villus width at crypt/villus junction98.9113.66.15Crypt depth171.5172.68.52

### Table 5. Villi measurements (µm) at d49 and d70 (n=5 per treatment)

 $^{1,2}$ , ns: see table 3.

Table 6 . Total volatile fatty acids concentration and proportion of caecal samples at d49 and d70 (n=5 per treatment).

		Control diet	FOS diet	SEM <sup>1</sup>	F Test <sup>2</sup>
49 days	Total VFA (mmol.l <sup>-1</sup> )	64,2	81,2	6,97	ns
	Acetic acid (%)	83	86,5	1,08	0,117
	Propionic acid (%)	7,3	4,8	1,09	ns
	Butyric acid (%)	9,7	8,7	0,78	ns
	Pool (mmol) <sup>3</sup>	4,9	6,4	0,67	ns
	Propionate/butyrate	0,85	0,63	0,17	ns
70 days	Total VFA (mmol.l <sup>-1</sup> )	68,8	64,6	4,36	ns
	Acetic acid (%)	80,1	82,6	1,03	ns
	Propionic acid (%)	5,4	4,9	0,29	ns
	Butyric acid (%)	14,4	12,5	0,88	ns
	Pool (mmol) <sup>3</sup>	5,0	5,7	0,39	ns
12	Propionate/butyrate	0,38	0,42	0,03	ns

<sup>1,2,</sup> ns: see table 3. <sup>3</sup> pool = total VFA/total caecal liquid content

# CONCLUSIONS

In conclusion, FOS have not a significant effect on morbidity and mortality, confirming the observation of MENDEZ *et al.* (1993). Also FOS did not improve weight gains, live weight and feed intake. However, FOS increased the feed efficiency (weight gain/feed intake). Similar results were observed by LEBAS (1993; 1996) and ROCA (1994). The several parameters of the fermentative activity measured (VFA concentration, VFA proportions and pool, propionate/butyrate proportion) were not affected by treatments. Con-

sequently, it is assumed that FOS effect on microbial activity was not significant. However, in future studies, cecal ammonium nitrogen, acid lactic concentration, pH and quantify cecal microbial populations should be determined for a better knowledge of FOS effects on microbial activity. Data from this study showed the capability of FOS to increase feed efficiency, without effects on growth performance and microbial activity. No significant effects are detected on morbidity and mortality neither on digestive organ development.

## ACKNOWLEDGEMENTS

The authors are grateful to the Provimi Group for financial help and FOS furniture.

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