

## DEGRADATION OF DIETARY OLIGOFRUCTOSE AND INULIN IN THE GASTRO-INTESTINAL TRACT OF THE RABBIT AND THE EFFECTS ON CAECAL pH AND VOLATILE FATTY ACIDS

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**ABSTRACT:** Three experimental fattening diets were each fed *ad libitum* to 12 animals of 8-9 weeks of age (initial weight  $2,257 \pm 87$  g). The diets contained 0% (Control), 2% oligofructose (OF) (Raftifeed®OPS) or 2% inulin (Raftifeed®IPS). After an adaptation period of 10 days, the individually caged rabbits received the same diets with  $Cr_2O_3$  as a marker. For the 24 h before they were euthanised (between 08:30 and 09:00), rabbits wore a plastic collar to prevent caecotrophy. Dietary treatment affected gut acidity only in the caecum where a tendency to a reduced pH in the inulin-fed rabbits was observed. The total caecal concentration of volatile fatty acids was similar among dietary treatments but a significant ( $P < 0.05$ ) change occurred in inulin-fed rabbits with an increase of butyrate proportion (17.6, 20.2 and 22.6% for control, OF and inulin rabbits, respectively) at the expense of acetate (75.0, 72.3 and 70.1%, respectively). Fructans were not detected in the ileum, caecum or faeces of control rabbits, indicating that both types of oligosaccharides originating from the raw materials were degraded quickly. Significant amounts of the  $\beta(2-1)$ -fructans were still present in the ileum content of both OF and inulin fed rabbits (1.78 and 1.63% DM). Apparent ileal digestibility of fructans was 100% in control rabbits but significantly lower ( $P < 0.01$ ) in OF (35.3%) and inulin (49.2%) fed rabbits. The absence of fructans from caecal and faecal samples confirms their complete fermentation by the caecal microbial flora. However, with the methodology used (which increased variability) and the limited number of replicates (4 pooled samples/diet), no significant difference in the degradation due to the chain length of the inuline-type fructans was detected.

**Key words:** inulin, oligofructose, degradation, fermentation, rabbit.

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## INTRODUCTION

Growing opposition to the prophylactic use of antibiotics as additives in livestock feed has prompted the search for effective alternatives, such as probiotics, prebiotics, dietary acids and plant extracts. Prebiotic oligosaccharides are defined as non-

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digestible food ingredients that stimulate selectively the growth and (or) activity of potentially health-enhancing intestinal bacteria (FLICKINGER *et al.*, 2003). They are not digested hydrolytically in the upper intestinal tract of monogastric animals and are thus available for fermentation by the hindgut flora (FISHBEIN *et al.*, 1988). A wealth of information exists concerning the significance of prebiotic oligosaccharides, mainly fructans, in modulating various functions of the human body, and their potential value in livestock and companion animal nutrition and health (MILNER and ROBERFROID, 1999; VAN LOO *et al.*, 1999; FLICKINGER *et al.*, 2003).

One mode of action in the promotion of health that has been ascribed to fructans is their positive effect on the production of short-chain fatty acids (FLICKINGER *et al.*, 2003). However, the studies using rabbits yielded controversial results. Dietary additions of galacto-oligosaccharides (GOS) or fructo-oligosacchides (FOS) affected the caecal fermentation pattern (MORISSE *et al.*, 1990, 1993; MAERTENS and PEETERS, 1992), whereas no effect was found in other experiments (LEBAS, 1993; GIDENNE, 1995). All these oligosaccharides have quite a low degree of polymerisation and a limited chain length. Fructans with various chain lengths are extracted from chicory roots. The short-chain fraction (also called oligofructose) has an average degree of polymerisation of about 4, in contrast to inulin-type fructans with a value of around 10.

Both types of fructans (oligofructose and inulin) are commercially available. The aim of the present experiment was to investigate if there was a difference in their degradation in the rabbit intestine and if a dietary inclusion affected the caecal concentration of volatile fatty acids.

## MATERIALS AND METHODS

The experiment was performed in June 2002. The Animal Use and Care Advisory Committee of the Centre for Agricultural Research approved all animal care procedures.

### Assayed fructans and experimental diets

One 600 kg batch of fattening rabbit feed was prepared at the Institute. The ingredients and the analytical composition of the control diet are presented in Table 1. The meal was divided into 3 parts to produce: (1) the experimental diet without additive (control); (2) oligofructose (OF) (Raftifeed®OPS) added at 4 kg/196 kg control diet; or (3) inulin (Raftifeed®IPS) added at 4 kg/196 kg. Both fructans were provided “blind” (in coded boxes) by Orafti (Tienen, Belgium). Chromium-marked diets were obtained by adding 2% of Cr<sub>2</sub>O<sub>3</sub> to 20 kg of each experimental meal before pelleting. All diets were pelleted (3.2 mm Ø) and the first 10 kg of each diet was removed to avoid any contamination.

**Table 1:** Ingredient composition and analytical characteristics of the control diet.

Ingredients	%	Analysis	g kg <sup>-1</sup> DM
Alfalfa meal 15	35.0	DM	919
Wheat shorts	17.0	Crude protein	180
Cassava	16.0	Ash	102
Sunflower meal 27	12.0	Ether extract	41
Soybean meal 44	4.0	NDF	305
Full-fat soybeans	7.5	ADF	180
Flax chaff	2.0	ADL	51
Molasses	4.0	Fructans	9.8
Minerals and vitamins	2.5	DE (MJ/kg DM)*	10.95

\*Calculated according to MAERTENS *et al.* (2002).

Raftifeed®IPS, or native chicory inulin, is a polydisperse mixture of linear molecules, all with the same basic chemical structure, which is symbolised as G-F<sub>n</sub> (G, glucosyl moiety; F, fructosyl moiety; and *n*, number of fructose units linked together through β(2-1) bonds). The degree of polymerisation (*DP*) of native chicory inulin ranges between 3 and 65, with an average (*DP*<sub>av</sub>) of 10. Raftifeed®OPS is a short-chain fraction (also called oligofructose) that is obtained by partial enzymatic hydrolysis of native chicory inulin. It is composed of linear G-F<sub>n</sub> and F-F<sub>n</sub> chains, with *DP* ranging from 2 to 8 (*DP*<sub>av</sub> about 4) (FLICKINGER *et al.*, 2003).

### **Animals and housing**

Forty-eight hybrid rabbits (final cross of the Institutes' lines, MAERTENS, 1992) of 8-9 weeks of age (initial weight: 2,257 ± 87 g) were used. In each experimental group, the 12 rabbits out of 16 with the highest feed intake were finally used for the measurements and sampling. All rabbits were housed individually in fattening cages measuring 40 cm × 60 cm × 50 cm height, had a light/dark cycle of 11 h/13 h, with the light period starting at 07:00. The rabbits were fed the experimental diets for 10 days. For 48 h before sampling, rabbits received the same experimental diet *ad libitum* but marked with chromium. For 24 hours before sampling, each rabbit was fitted with a plastic collar to prevent caecotrophy, as described by GIDENNE and LEBAS (1987).

### **Measurements and sampling**

Rabbits were euthanised with T61®(Intervet, Belgium) in the ear vessel between 08:30 and 09:00. The digestive tract was dissected immediately and pH was measured by introducing a glass electrode into the stomach and caecum *via* a small slit at a fixed place. The last 80 cm of the small intestine was emptied, by gentle squeezing, and pH was measured in pools of 3 rabbits. For the determination of volatile fatty acids (VFA), individual samples of caecal content were diluted 1:1 (v/v) with distilled water. After adding 3 drops of toluene, samples were stored at -20°C. In order to have sufficient quantity, samples of ileum (last 80 cm of the small intestine) and caecum were pooled for 3 rabbits, homogenised, lyophilised and milled (Retsch laboratory mill). Faecal samples were collected under the cages and pooled for 3 rabbits. Faecal samples were oven-dried (24 h per 70°C), homogenised and milled

before analysis.

### Analytical procedures

Official EC-methods were applied for proximate analyses of the feed. Residual moisture was determined by oven drying at 103°C for 4 h. Crude protein (CP = N x 6.25) was determined following Kjeldahl. Crude fat was extracted for 6 h with petroleum ether. NDF, ADF and ADL were determined sequentially with the filterbag method (Ankom, New York) in the presence of heat stable  $\alpha$ -amylase but without sodium sulphite and were expressed on an ash-free basis (VAN SOEST *et al.*, 1991). Caecal VFA were determined after acidification with 25% (w/v) metaphosphoric acid and centrifugation twice at 12,000 rpm (GETACHEW *et al.*, 2001). Subsequent determination of the concentration of VFA in the resulting purified and diluted caecal content was carried out by gas chromatography (Varian Star 3400) using an EC-1000 capillary column and FID. Chromium was determined quantitatively after complete oxidation by a mixture of nitric and perchloric acid, and titration with Mohr's salt, according to the procedure described by FRANÇOIS *et al.* (1978). Inulin and oligofructose were quantified by ion exchange chromatography as described by HOEBREGS (1997).

### Calculations and statistical analysis

The digestibility coefficients (DC) of the fructans in the different parts of the gastrointestinal (GI) tract were calculated in relation to the Cr determined in the diet and with the following formula:

$$DC = 100 \times \frac{(\text{Cr in GI} / \text{Fructans in GI}) - (\text{Cr in diet} / \text{Fructans in diet})}{\text{Cr in GI} / \text{Fructans in GI}}$$

where the Cr and Fructans values are percentages on DM basis. The digestibility of the added fructans was calculated as proposed by VILLAMIDE *et al.* (2001).

Data were submitted to analysis of variance and mean comparisons were made using contrasts.

**Table 2:** Fructans (g/100 g DM) in the diets, ileum and caecum.

	Diets		
	Control	Oligofructose	Inulin
Diet	0.90	2.20	2.40
Ileum	0	1.78	1.63
Caecum	0	0	0

## RESULTS AND DISCUSSION

The dietary content of fructans is presented in Table 2. The control diet contained 0.90% fructans, mainly from wheat shorts. In the experimental diets, 2.20% and 2.40% fructans were determined and the real enrichment with OF and inulin reached only 1.30% and 1.50%, respectively, in comparison with 2% expected. The average live weight at the end of the trial and values for feed intake during the 24 h preceding the sampling are presented in Table 3. Rabbits reached slaughter weight (2.5-2.6 kg) at the end of the trial.

**Table 3:** Animal weight at slaughter and pH in the gut tracts.

	Diets			SEM	<i>P</i> -value		
	Control	OF	Inulin		C vs T	C vs I	OF vs I
Live weight <sup>1</sup> (g)	2694	2517	2502	56	NS	NS	NS
Feed intake <sup>1</sup> (g/d)	122	116	141	6.3	NS	0.06	NS
pH in							
- Stomach <sup>1</sup>	1.88	2.01	1.93	0.38	NS	NS	NS
- Ileum <sup>2</sup>	7.55	7.37	7.22	0.08	NS	NS	NS
- Caecum <sup>1</sup>	5.72	5.80	5.65	0.03	NS	0.09	0.03

<sup>1</sup>n = 12; <sup>2</sup>n = 4.

SEM: Standard error of the mean.

C vs T: Control diet vs other diets; C vs I: Control diet vs Inulin diet; OF vs I: Oligofructose diet vs Inulin diet.

NS: Non significant (*P*>0.10).

The feed intake showed a large individual variability (SEM = 6.1 g). Rabbits were trained once (7 days before the sampling) in wearing the plastic anti-caecotrophy collar. Although the collar was adapted to the weight of each rabbit, it still provoked stress, as demonstrated by the considerable reduction (25 to 40%) in voluntary feed intake during the 24 h preceding the collection as compared with average feed intake data of rabbits of the same age (MAERTENS and VILLAMIDE, 1998).

The acidity in the stomach, ileum and caecum is presented in Table 3. The pH values in the different digestive tracts are in line with those reported for healthy rabbits (LEBAS *et al.*, 1998; GARCIA *et al.*, 2002). Differences between diets were not significant in stomach ( $P=0.11$ ) and ileum. Caecal pH was lower in the inulin fed rabbits than in the control rabbits ( $P=0.09$ ), in line with results reported by MORISSE *et al.* (1993) and MAERTENS and PEETERS (1992) using a dietary addition of fructo- or galacto-oligosaccharides, respectively. A significant reduced pH was determined in inulin fed rabbits compared to OF rabbits ( $P=0.03$ ) that might indicate an increased fermentation.

The total caecal VFA concentration did not differ significantly among dietary treatments (Table 4). However, a significant change in the proportions of different VFA occurred in inulin-fed rabbits, with an increase of butyrate at the expense of acetate. OF-fed rabbits showed acetate and butyrate concentrations intermediate

**Table 4:** Volatile fatty acid concentration and molar proportion in the caecum.

	Diets			SEM	P-value		
	Control	OF	Inulin		C vs T	C vs I	OF vs I
Total VFA (mmol/l)	115.1	108.8	107.8	3.2	NS	NS	NS
- Acetic acid (% mol.)	75.0	72.3	70.1	0.52	0.01	0.02	NS
- Propionic acid (% mol.)	7.5	7.5	7.3	0.15	NS	NS	NS
- Butyric acid (% mol.)	17.6	20.2	22.6	0.62	0.02	0.03	NS

n = 12

SEM: Standard error of the mean.

C vs T: Control diet vs other diets; C vs I: Control diet vs Inulin diet; OF vs I: Oligofructose diet vs Inulin diet.

NS: Non significant ( $P>0.10$ ).

between those of control and inulin-fed rabbits. The caecal butyrate concentration increased significantly from 20.2 to 22.1 and 24.3 mmol/l, while acetate decreased from 86.3 to 78.6 and 75.6 mmol/l in control, OF and inulin rabbits, respectively.

The short-chain oligosaccharides in the basal diet originating from the raw materials, mainly from wheat, alfalfa meal and wheat shorts (HUSSEIN *et al.*, 1998), were not detected in the gut or caecum, indicating that they are degraded in the upper part of the intestinal tract (Table 2). As a consequence of the absence of fructans in the ileum of control rabbits, ileal digestibility amounted to 100% (Table 5). MAROUNEK *et al.* (1995) demonstrating that some significant fibrolytic activity exists even in the stomach and intestine. Indeed, at the ileal level, uronic acids are already highly digested (25-60%) (GIDENNE, 1992; CARABAÑO *et al.*, 2001). This fibrolytic (pectinolytic) activity in the upper part of the GI should be partly related to the intake of micro-organisms through the caecotrophes. Moreover, BACON (1978) suggested that fructans are acid-labile compounds that are partially hydrolysed in the gastric environment. In our study, caecotrophy was prevented during the 24 h before sampling, but there seems to remain a certain fermentation activity that could explain the early degradation of the fructans present in the raw materials.

On the other hand, for OF and inulin fed rabbits, the concentration of fructans in the ileum content was similar:  $1.78 \pm 0.36\%$  DM and  $1.63 \pm 0.77\%$  DM, respectively.

**Table 5:** Digestibility coefficients of the total fructans in the different tracts of gut.

	Diets			SEM	<i>P</i> -value		
	Control	OF	Inulin		C vs T	C vs I	OF vs I
Ileum	100	35.3	49.2	5.90	0.001	0.001	NS
Caecum	100	100	100	-	-	-	-
Faeces	100	100	100	-	-	-	-

n = 4

SEM: Standard error of the mean.

C vs T: Control diet vs other diets; C vs I: Control diet vs Inulin diet; OF vs I: Oligofructose diet vs Inulin diet.

NS: Non significant ( $P > 0.10$ ).



This is reflected also in the similar ileal digestibility of the total fructans in the OF and inulin diets, which was  $35.3 \pm 15.9\%$  and  $49.2 \pm 23.3\%$ , respectively (Table 5). An apparently similar proportion of both added fructans reached the caecum, where they were fermented totally (100% caecal digestibility).

In the present experiment, the total amount of VFA was apparently not modified by the added fructans. This may be due to the experimental conditions. Previously, in experiments with infected (colibacillosis) rabbits, a significant rise of caecal VFA concentration has been observed and attributed to the dietary addition of oligosaccharides (MAERTENS and PEETERS, 1992; MORISSE *et al.*, 1993). ALVES *et al.* (2003), however, did not observe an increase of the caecal VFA concentration after a simultaneous dietary addition of inulin and OF, but a significant increase of the height of ileal mucosa was observed. However, this effect can be induced by an increased presence of VFA and, particularly, butyrate (GALFI and NEOPRADY, 1995). Stronger indications of the presence of greater or smaller amounts of VFA are the structural changes in the gut architecture that they may have caused, such as the ones observed by ALVES *et al.* (2003).

A significant effect on the relative composition of the VFA pool was observed in the present experiment. The molar ratio of acetate to butyrate was modified significantly. This can be ascribed only to the effects of the chicory fructans on caecal fermentation. The effect is markedly more pronounced with the longer-chain inulin fraction than with the oligofructose, suggesting that in the rabbit the long chains have a more important impact than the short chains on caecal fermentation. This may be due to the particular fermentation induced by the two types of carbohydrates. Indeed, the long chains ( $DP > 10$ ) in inulin are fermented more slowly than oligofructose with a chain length of less than 8 fructose moieties (ROBERFROID *et al.*, 1998). As they are fermented more slowly, they also stimulate different bacteria in a different way, and they maintain caecal saccharolytic fermentation for a longer time. However, with the methodology used (which increased variability) and the limited number of replicates (4 pooled samples/diet), no significant difference in the degradation due to the chain length of the inuline-type fructans was detected in our trial. Apparently, inulin fermentation reduces caecal pH more markedly than OF

fermentation (Table 3), and inulin has a stronger impact on VFA pattern (Table 4).

Another aspect is that for rabbits, having a particularly long small intestine containing a rather dense bacterial population (up to 8–9 log CFU) (GOUET and FONTY, 1979), rapidly fermented short-chain oligosaccharides are degraded (e.g. depolymerised) to a larger extent than long-chain, more slowly fermented, non-digestible soluble carbohydrates. As the longer-chain inulins arrive more intact in the caecum, they can maintain a moderate caecal fermentation for longer and, as a consequence, they can keep the pH relatively low (around 5.5). This is the major aim of the use of prebiotics in rabbit feed, that is, to create conditions in the caecum that are not favourable to clostridia development.

## CONCLUSIONS

The addition of inulin to rabbit feed tended to reduce caecal pH and modified significantly the molar proportions of VFA, characterised by a higher proportion of butyrate. However, differences in the degradation due to the chain length of the inuline-type fructans were not detected with our methodology. Further research should focus on comparing the effect of short chain *vs* long chain fructans on the prevention of clostridia proliferation in a controlled challenge experiment.

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