

## Research Article

### ***In-vitro* Gas Production of Different Dietary Fibre Sources Incubated with Faecal Microflora to Test Applicability of Preventing DIOS in Patients Suffering from Pancreatic Exocrine Insufficiency – Studies in Minipigs Used as A Model for Humans**

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## Abstract

Patients suffering from cystic fibrosis (CF) often suffer from an exocrine pancreatic insufficiency (PEI) and have an increased risk for distal intestinal obstruction syndrome (DIOS). To ensure a high energy density in the diet to prevent malnutrition and meteorism in these patients the dietary fibre supply is low which might exacerbate constipation and DIOS. The aim of this study was to test the *in-vitro* gas production of different fibre concentrates incubated with porcine faecal microflora. To test whether PEI affects fermentation, faeces of pigs with experimentally induced PEI were used alongside healthy controls (C). Four fibre sources were tested in this study: Pure methylcellulose [MC], two lignocellulose products (FibreCell® [FC] OptiCell® [OC] and a pea fibre [PF]). The *in-vitro* gas production was continuously measured manometrically over 48 hours and recorded every minute using the ANKOM gas production system®. *In-vitro* gas production was highest when 1 g PF was used as substrate (127 ± 36.3 ml in C, 123 ± 47.9 ml in PEI pigs) while values were significantly lower (< 4 ml/g substrate) for all the other fibre sources tested. The PEI did not affect *in-vitro* gas production in this study. To conclude, the use of highly concentrated fibre sources (60 % crude fibre) might be an option to increase fibre supply in patients suffering from PEI in case of CF with no risk of bloating or meteorism and no need to markedly increase the amount of food intake. As *in-vitro* gas production differed remarkably between fibre sources the fibre used for dietary measures should be chosen carefully.

**Keywords:** Pancreatic Exocrine Insufficiency; Cystic Fibrosis; Distal Intestinal Obstruction Syndrome; Fibre Supply; Gas Production; Nutrition; Animal Model; *In-vitro*; Fermentation

## Introduction

Pancreatic exocrine insufficiency (PEI) is a disease seen on the one hand in adults as a result of chronic pancreatitis but on the other hand in patients with cystic fibrosis (CF) who suffer to a great extent (85-90%); [1,2] from PEI most often directly from birth. PEI causes maldigestion and malabsorption of nutrients resulting in symptoms like diarrhoea, steatorrhoea, azotorrhoea, small intestinal bacterial overgrowth (SIBO), meteorism, and eventually malnutrition and increased susceptibility to infectious disease [3,4]. Pancreatic enzyme replacement therapy (PERT) reduces the risk of SIBO and fermentation due to enhanced enzymatic digestion and reduction in malabsorption (reduced availability of nutrients for fermentation) but it often does not result in complete normalisation [5,6]. Another symptom seen in CF patients is *distal intestinal obstruction syndrome* (DIOS); which was precisely characterised in 2009 [7] as an acute complete or incomplete faecal obstruction in the ileocaecum while constipation was defined as faecal impaction of the total colon by the same authors. DIOS primarily occurs in adolescents and adults [8]. The pathophysiology of DIOS is complex (pancreatic enzyme dose, nutritional, motility and intestinal wall factors). In the case of DIOS the digesta material is strongly connected to the crypts and villi [9] and therefore resists physiological transport. Meconium ileus at birth and DIOS are significantly correlated [10]. In many cases the DIOS is a recurrent condition with intermittent symptoms [9]. To prevent this obstruction an increased fibre supply might be beneficial [11,12] as well as for preventing chronic faecal retention (megacolon syndrome) and reduced bowel frequency [13,14]. However, it has to be emphasised that the impact of fibre intake in CF on DIOS is controversially discussed in literature, [15] found low fibre intake associated with DIOS, [16] and [17] found no effect of fibre intake on constipation in CF patients. In a multicentre study 92 % of paediatric patients suffering from DIOS were pancreatic insufficient [7] and also other authors [18] found PEI in all patients with DIOS in their study. Nonetheless, [9] stated that evidence for relevance of PEI needs to be critically proven as DIOS cases were also observed in pancreatic sufficient patients. The recommendation to put patients on high fat and low fibre diets might be relevant for the increasing incidence of DIOS as well [15]. To prevent the painful disturbances in digesta passage an increased fibre supply is recommendable. Nevertheless, due to the higher fermentative capacity seen in these patients there is a higher risk of meteorism resulting in abdominal pain. The current recommendations for CF patients include a diet low in fibre to ensure a high energy density [11,19,20]. The use of fibre concentrates might be a suitable way of providing patients with fibre without need to relevantly increase the amount of food intake.

The pancreatic duct ligated (PL) pig or minipig is an established model to study effects of PEI [21-29]. As in human PEI-patients a SIBO is observed [29] that could be demonstrated also by increased hydrogen exhalation in PL-pigs [30]. As faecal samples

represent fairly well the large intestinal microflora it is an established method to use faeces as inoculum for *in-vitro* studies to check the effect of different substrates on fermentation [31].

The present study aimed to test the cumulative *in-vitro* gas production of different fibre sources, incubated with faeces of healthy control pigs and PL-pigs treated with pancreatic enzymes, to identify fibre sources that can be used for fibre supply in patients suffering from PEI without causing an increased gas production. Furthermore, the study set out to check whether experimentally induced PEI affects the extent or pattern of fermentation.

## Animals, Material and Methods

All efforts were made to minimise both the stress for the individual animal as well as the numbers of animals used. The procedures used in this study were conducted in accordance with the German Animal Welfare Act and with the European Council Directive of 24 November, 1986 (86/609/EEC) and were approved by the Ethics Committee on Animal Welfare of the Hannover District Government.

### Animals

The animals used in this study (n=6) were adult female minipigs (Ellegaard®) that were housed individually without litter. The housing without litter ensured a completely standardised feeding and prevented fibre intake from the litter by the animals.

In some pigs (n=3) a pancreatic duct ligation (PL) was performed according to the method described by [29]. This was carried out at least several months before starting the trial to induce a PEI, while 3 other minipigs with intact pancreatic function were used as controls (C). The activity of faecal chymotrypsin was measured in all pigs after surgery (test kit purchased from Immundiagnostik AG, Wiesenstrasse 4, 64625 Bensheim, Germany, catalogue No. K6990). Only minipigs with a chymotrypsin activity < 0.900 U/g faeces were defined as PL-pigs.

### Feeding

All pigs were fed a commercial complete diet (per kg dry matter: 150 g crude protein, 42.4 g crude fat, 352 g starch, 35.5 g crude fibre) and were given at least 10 days to get accustomed to it. The diet was offered twice a day (at 07:00 and 15:00) at an amount of 220 g dry matter per animal. PL-pigs received pancreatic enzyme replacement therapy (PERT) with a porcine multienzyme product at a dosage of ~ 5000 U lipase/g fat). Water was offered ad libitum via a nipple drinker.

### Fibre sources

Four fibre sources were tested in this study: Methylcellulose [MC] - Methocel® (98 % pure methylcellulose; Roth, Karlsruhe,

Germany) two different lignocellulose products (FibreCell® [FC; 65 % crude fibre, produced from fresh wood] and OptiCell® [OC; 59 % crude fibre, 30 % lignin; contains fermentable and non-fermentable fibres according to supplier's (agromed Austria GmbH, Kramsmünster, Austria) information) - as well as pea fibre [PF] supplied by LaVita GmbH (Kumhausen, Germany). The chemical analysis of the fibre sources used is given in table 1.

**Table 1.** Chemical analysis of fibre sources (g/kg dry matter [dm]) used in the study.

|     | Methocel® (MC)*  | FibreCell® (FC) | OptiCell® (OC) | Pea fibre (PF) |
|-----|------------------|-----------------|----------------|----------------|
| NDF | not detectable** | 838             | 900            | 661            |
| ADF | 172              | 702             | 741            | 573            |
| ADL | not detectable** | 270             | 243            | 2.00           |
| NfE | not detectable   | 273             | 276            | 432            |

\* 98 % methycellulose, \*\* due to the very fine structure of this product

NDF: neutral detergent fibre; ADF: acid detergent fibre; ADL: acid detergent lignin; NfE: nitrogen free extractives

### Inoculum material

Buffer used to prepare the faecal suspension according to [32] and [33]. The buffer consisted of two buffers which were mixed directly before preparing the incubation suspension. The pH of the buffer mix was 6.8.

Fresh faeces (100 g wet weight) from C-pigs (n=3) and PL-pigs (n=3), were used separately as inoculum and mixed with the buffer (450 ml) according to [32, 33]. The suspension was filtered through a sieve with pore sizes of 200 µm. The filtrate was diluted at a ratio of 1:5 with the buffer. Every preparation step was followed by rinsing with CO<sub>2</sub> to enable an anaerobic milieu.

### Incubation and measurement of gas production

100 ml of the prepared faeces suspension were filled in glass bottles which each contained 1 g of the substrate to be tested. All fibre sources were tested in parallel as triple measurements with identical faeces suspension. Faeces suspension without any added substrate was used to measure the origin *in-vitro* gas production and to calculate the net gas production caused by fermentation of the added substrate.

Pressure sensor modules of the ANKOM Gas Production System<sup>RF</sup> (Ankom Fairport, Technology, Macedon, New York, USA) were placed on all bottles. Flushing of bottles with CO<sub>2</sub> was performed again before closure to achieve anaerobic conditions. Bottles were placed on a magnetic stirrer plate in a

warming-cupboard (38 °C). In case of gauge pressure (defined as 100 mbar) an automatic pressure release was performed in order to avoid negative effects on fermentation process. Gas production was detected manometrically continuously over 48 hours and data were transferred over a wireless network to the computer and saved electronically at intervals of 1 minute using the software of ANKOM gas production system<sup>RF</sup> 9.7.1. Cumulative gas production after 48 hours was calculated as well.

### Statistical analysis

Statistical analysis was performed using SAS<sup>®</sup> version 9.3 (SAS Institute Inc., Cary, NC; USA). The Shapiro-Wilk test from the procedure UNIVARIATE was used to test for normality. For variables with normal distributions of the residuals of the linear model estimated by the GLM procedure a two sample t-test was used – for those being non-normally distributed a Wilcoxon's two sample test was used.

### Results

Cumulative *in-vitro* gas production within 48 hours did not differ between control- and PL-pigs and was highest in both groups when PF was used as substrate, while MC, OC and FC caused only very low values in both groups of animals (see table 2).

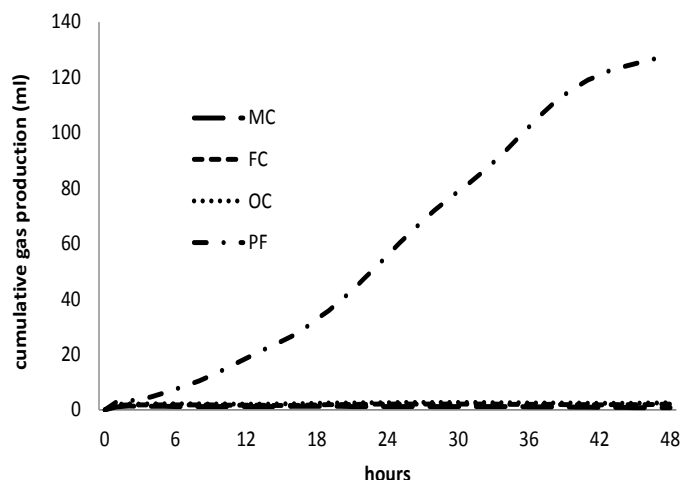
**Table 2.** Cumulative gas production (ml/48 h)\*\* when using different fibre sources (mean ±SD).

|         | Methocel® (MC) | FibreCell® (FC) | OptiCell® (OC) | Pea fibre (PF) |
|---------|----------------|-----------------|----------------|----------------|
| C-pigs  | 0.723 ± 0.447a | 2.02 ± 0.750a   | 2.53 ± 1.88a   | 127 ± 36.3b    |
| PL-pigs | 1.24 ± 6.98a   | 0.063 ± 3.56a   | 3.89 ± 8.02a   | 123 ± 47.9b    |

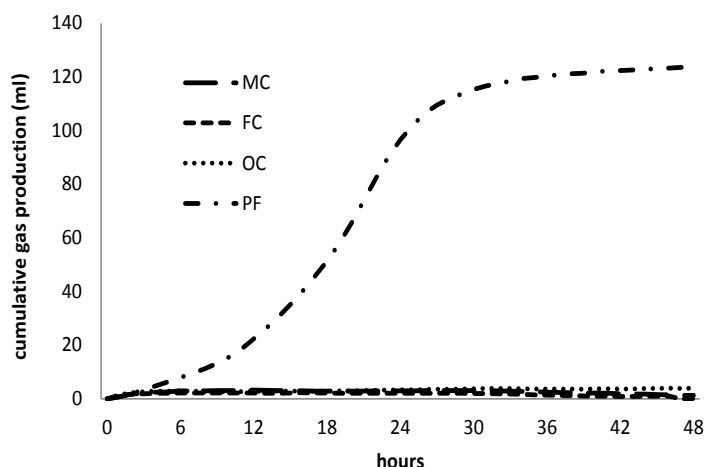
\*\*Net-values, i.e. corrected by blank value (faeces suspension without substrate)

Different letters mark significant differences between the fibre sources used (*p*-value < 0.05). There was no significant effect (*p* > 0.05) of animal group when same fibre source was added.

The kinetic of *in-vitro* gas production differed markedly depending on the fibre source used: While PF showed a distinct increase, the other fibre sources used (MC, FC and OC) caused only very low, irrelevant increases (see figures 1 and 2). While the cumulative *in-vitro* gas production did not differ between both groups of animals after 48 hours, the kinetic tended to differ. In control-pigs PF caused an *in-vitro* gas production of 55.8 ± 45.9 ml after 24 hours, while incubation with faeces of PL pigs resulted in 96.5 ± 33.1 ml after the same duration.



**Figure 1.** Kinetic of *in-vitro* gas production after incubation of faecal suspension of C-pigs each with 1 g of substrate (net gas production).



**Figure 2.** Kinetic of *in-vitro* gas production after incubation of faecal suspension of PL-pigs each with 1 g of substrate (net gas production).

### pH-value of incubation suspension

The pH value of the incubation suspension was measured at the end of the incubation period (48 h) to get more information about fermentation intensity (as fermentative degradation results in acid products like short chain fatty acids). While three fibre sources tested (MC, FC, OC) did not cause any changes in pH value compared to the values of the faecal suspension incubated without substrate (blank), the addition of PF resulted in a significant decrease of pH (see table 3). PEI had no effect on pH of incubation suspension as values of C- and PL-pigs did not differ when identical fibre was added.

**Table 3.** pH value of incubation suspension after 48 h of fermentation process when using different fibre sources as substrate (mean  $\pm$ SD).

|         | Blank (without substrate) | Methocel® (MC)    | Fibre Cell® (FC)  | Opti Cell® (OC)   | Pea fibre (PF)    |
|---------|---------------------------|-------------------|-------------------|-------------------|-------------------|
| C-pigs  | 6.95 $\pm$ 0.142a         | 6.99 $\pm$ 0.095a | 6.93 $\pm$ 0.105a | 6.93 $\pm$ 0.101a | 5.72 $\pm$ 0.116b |
| PL-pigs | 6.99 $\pm$ 0.073a         | 7.01 $\pm$ 0.113a | 6.95 $\pm$ 0.132a | 6.93 $\pm$ 0.121a | 5.96 $\pm$ 0.500b |

Different letters mark significant differences between the fibre sources used ( $p$ -value < 0.05). There was no significant effect ( $p$  > 0.05) of animal group when same fibre source was added.

### Feeding trial

In follow-up feeding trials 10 g of MC, FC or OC were added to the meal (220 g dm, fed twice a day) and no negative effects were observed. The faeces quality differed after feeding MC, FC and OC: the addition of FC and OC improved the faeces quality (higher amounts, softer consistency, well formed), whereas MC resulted in a very sticky consistency.

### Discussion

DIOS is a multifactorial complication seen in CF patients. The overall incidence of DIOS is 6.2 episodes per 1000-patient-years or 5-12 episodes per 1000 juvenile patients per year [7] with higher rates (35.5 / 1000 patients) in adults [12]. As incidence was higher than in a former study [34] it was discussed that the higher enzyme dosage used in more recent studies might result in a promotion of faecal impaction due to reduced amounts of undigested food in the gut lumen. However, the role of PERT dosage in DIOS is controversially discussed. An inadequate dose of PERT is seen as a possible causation for DIOS as well as for constipation [12] and acquired megacolon by altering the digesta passage via induction of ileal brake due to reduced fat absorption [35]. The recommendation to put patients on low fibre diets might be relevant for the increasing incidence of DIOS as well [15].

Nutrition of CF patients is challenging as the sufficient supply of energy is crucial [36] – resulting in the recommendations to offer low fibre diets [19] which might be a risk factor for DIOS. However, this is controversially discussed: [15] found a correlation between low fibre intake and abdominal pain and DIOS in CF children while [16] and [17] found no effect of fibre intake on constipation in CF patients. It has to be taken into account that in the study of [15] the mean daily fibre intake was much lower (7 g / day) than in the study of [16] with CF patients showing a mean daily fibre intake of 17 g / day. From these findings it can be speculated that the fibre uptake in the Belgian children was above a critical value. Interestingly, in this study patients with DIOS tended to have a higher daily fibre

intake [16] which might be a response to constipation in these patients although fibre supplements were not routinely prescribed.

The challenge in nutrition of CF patients with PEI is therefore to enrich the diet with moderate levels of fibre (10-30 g / day) to ensure passage rate and to minimise risk of obstipation or constipation [11, 13] without negative effects like enhancing the amount of food intake needed, satiety [37] and forced intestinal gas production. Another side effect that might be of relevance is the reduction in enzyme activity of substituted enzymes by dietary fibre. However, this effect is strongly related to the type of dietary fibre used with 1.5g% of pure cellulose having no effect on amylase and trypsin activity and only very little effect on lipase activity [38]. In patients with PEI a SIBO and an increased bacterial fermentation is often observed resulting in symptoms like diarrhoea, meteorism, abdominal pain, flatulence and impaired wellbeing [3]. With modern pancreatic enzyme replacement therapy (PERT) maldigestion and malabsorption as well as SIBO can be controlled, but in some patients problems still occur [1]. The further characterisation of fibre sources seems to be of greatest relevance as meteorism and flatulence not only affect patients' wellbeing, but also might impair food intake. The fibre enrichment of the diet should be done deliberated as energy expenditure / requirements are increased in patients with CF and the intake of food rich in fibre raises the risk for malnutrition as diets rich in fibre are in general less digestible and have a lower energy density. The use of highly concentrated fibre sources might be way out to achieve a sufficient fibre supply to prevent DIOS without causing negative side effects like uptake of bulky food or food with a low energy density which might raise the risk of reduced energy uptake or malnutrition. There are several fibre concentrates available – differing in chemical composition. The two lignocellulose products used in this study did not cause any relevant *in-vitro* gas production and had positive effects on faeces quality (soft but formed faeces). Taking into account the very high fibre content of these supplements (~ 60 %) it is possible to enrich the diet with a relevant amount of fibre (10 g) without markedly increasing the amount of food. This is especially relevant when considering the recommendation of opting for a diet with high energy density for patients with PEI [19].

Regarding low *in-vitro* gas production MC, FC, OC can be recommended for a fibre enrichment of the diet. Interestingly, PF – which is a common fibre source in human dietetics – caused the highest *in-vitro* gas production. Furthermore pH dropped significantly after addition of PF – also indicating a much higher fermentation rate. Taking all data into account FC and OC seem to be recommendable to increase fibre intake even in patients with PEI.

Determining the *in-vitro* gas production is a completely non-invasive method and allows differentiation of the extent of fermentation of different fibre sources. The screening of different foodstuffs under the aspect of minimising gas production is

easy to perform and might help to improve patients' wellbeing. It seems noteworthy that even the addition of only 1 g of PF as a "fibre source" caused a net gas production of 120 ml of gas in this study. It was previously shown [33] that fermentation of starch caused very high *in-vitro* gas production when incubated with ileal chyme of PL-pigs (up to 160 ml / g substrate). As a good nutritional status is crucial for CF patients [21,36] introducing fibre into the diet should be critically proven by a dietetic supervision and not recommended for all patients. Nonetheless, in patients with symptoms of DIOS or abdominal pain due to extensive loading of the colon with faeces the enrichment of diet with a lignocellulose fibre might be helpful to normalise gut function although [16,17] found no association between fibre intake and DIOS or constipation in CF. However, it should be taken into account that diets for CF patients are low in fibre in general according to recommendations and that this fact might mimic effects of fibre in the cohort (as all patients have a low fibre supply). [16] stated a lower fibre uptake in patients with DIOS, indicating that an increased fibre supply might be feasible. More precise characterisation of fibre seems to be crucial to optimise fibre supply in CF and PEI patients. Although fermentable fibre is of benefit for healthy people, in patients with maldigestion and SIBO the use of non-fermentable fibre seems to be more beneficial. Looking forward it seems necessary to do more experimental and clinical work to estimate the practical consequences of an enrichment of the diet with fibre. Aspects like effects on satiety, passage rate, water binding capacity as well as intestinal microflora should be taken into account. The use of *in-vitro* tests to quantify gas production might be a valuable measure to screen different fibre sources and to exclude those fibre sources that might have negative side effects like excessive gas production.

## Conclusions

The use of a highly concentrated fibre source (lignocellulose) might reduce the negative side effects of fibre sources usually used while promoting passage rate and reducing the risk of DIOS. These fibre sources are neutral in flavour and taste and can be easily added to drinks or food.

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