

## Original Article

# Effects of convenience rice congee supplemented diets on guinea pig whole animal and gut growth, caecal digesta SCFA and *in vitro* ileal contractility

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The aim of the study was to feed convenience baby food brown rice (BC) and white rice (WC) congee diets compared to egg custard (EC) and baked bean (BB) diets to newborn guinea pig pups. Diets were isocaloric and formulated to contain equal macronutrient content of carbohydrate, protein, fat and fibre. Diets were supplemented with essential nutrients, fruit and vegetables and decrementally with standard chow for palatability. We investigated the acceptability of the diets and specifically whether the different natural fibre content of these diets could influence whole animal and small intestinal growth, caecal digesta properties and specifically *in vitro* ileal contractility. After 8 weeks of feeding, the mean body weight of WC group was significantly lower than the BB group. WC group had lower small intestine weight than both BC group and BB group resulting in lower small intestine density compared to BB group. Caecal digesta pH and total short chain fatty acid (SCFA) concentration were similar. However, butyrate was higher in the BB group compared to the other diets. Contractility studies revealed a small but significantly higher voltage was required to initiate ileal contraction of BC group compared to both the EC and BB groups. All dietary groups responded similarly to acetylcholine, histamine, serotonin, PGE<sub>2</sub>, PGF<sub>2α</sub>, and 8-*iso*-PGE<sub>2</sub>. There were no differences on inhibition of electrically-driven contraction by morphine or epinephrine. The newborn guinea pig model was an effective system for testing, with limitations, supplemented convenience baby foods with variable natural fibre content that demonstrated significant effects on animal growth, caecal digesta SCFA and intestinal contractility.

**Key Words:** ileum, contractility, guinea pig, fibre, eicosanoid, short chain fatty acids, SCFA.

## Introduction

The major staple in Asia is rice and mothers use rice-based porridge or congee as domestic infant food that is introduced around six months of age.<sup>1</sup> Congee is made in the home by boiling and straining rice several times in a laborious and time-consuming process. The congee is then often supplemented with fish, meat and vegetables. However, some of these preparations may be contaminated, contain antinutrient phytates that limit the bioavailability of minerals and may be inadequate in vitamins such as riboflavin<sup>2,3</sup> and be implicated in child malnutrition.<sup>4</sup> Nonetheless, because of their potential nutritional qualities, rice based dishes have been recommended for the management of some diet based diseases.<sup>5</sup> The products of fibre and resistant starch fermentation have important implications in the pathogenesis of colorectal cancer and other diseases of the large bowel, which are uncommon in Asia and Africa but have a high prevalence in Western populations.<sup>6</sup> Therefore, there may be a strong demand for commercially produced bottled infant foods, provided these products are nutritionally comparable or superior to congee made domestically.

Processing conditions are known to increase the formation of resistant starch from rice<sup>7,8</sup> while animal dietary studies have shown that different sources of resistant

starch and dietary fibre from rice, for example, can alter properties of growth, gastrointestinal morphology, enzyme activity and bowel health.<sup>9-11</sup> Recent research is confirming that short chain fatty acid (SCFA) production from carbohydrate as resistant starch that enters the caecum and large bowel may be responsible for these health benefits<sup>12-14</sup> and may also play an important role in regulatory feedback loops of gut motility.<sup>15</sup> However, knowledge of how dietary fibre or resistant starch influences small intestinal absorption of nutrients and colonic fermentation is sparse.<sup>11,14</sup> Furthermore, whether fibre and resistant starch sources of SCFA influence adaptive change to animal and human gut motility that can persist is even less evident. Guinea pigs are relatively advanced when born, compared to the rat or the pig,<sup>16,17</sup> so diets can be introduced to new-born at a very early stage of development.<sup>18</sup> The guinea pig ileum is also an established model for measuring small intestinal

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contractility. The aim of this study was to feed the convenience infant foods brown rice and white rice congee diets compared to egg custard and baked beans diets containing varying amounts of natural fibre to newborn guinea pig pups and to investigate small intestine growth, caecal digesta SCFA and further to evaluate the *in vitro* small intestine contractile responses to electrical stimulation and gastrointestinal effectors.

## Methods

### Animals

A total of 16 pregnant guinea pigs were obtained from the Institute of Medical and Veterinary Science (Gilles Plains, Adelaide, South Australia, Australia) and housed at the CSIRO small animal colony in floor pens lined with straw containing shelters. The dams had *ad libitum* access to commercial guinea pig chow as pellets (Ridley Agri Products, Murray Bridge, South Australia, Australia) supplemented with daily rations of fruit and vegetable matter (15g). Water was freely available. The dams were subjected to 12 h light/12 h dark schedule at 23°C. The litters of 1 to 4 guinea pig pups were allocated to the four experimental diets at 3 to 5 litters per dietary group and matched with regards to sex ratio and age. The animal housing, feeding and experimentation regimes were conducted with the approval and guidance of the CSIRO Health Sciences and Nutrition animal ethics committee.

### Analysis of industrially supplied dietary components

Protein, fat, starch, sugar and fibre (as total neutral non-starch polysaccharide) contents were determined analytically for the white and brown rice congees with beef while these components of egg custard and baked beans were determined from the food table values of Food Australia, 1990 (Table 1). Briefly, protein was determined by Dumas oxidation using a Carlo Erba nitrogen analyser. Fat was measured by gravimetric determination following extraction with a chloroform/methanol mixture (AOAC method 983.23). An enzymatic digestion of starch was

measured colorimetrically (AACC method 76-12). Sugars were measured by HPLC following extraction in 80% ethanol. Fibre was determined by gas liquid chromatography (AOAC method 994.13).

### Diets

Half of the dry matter of each formulated diet was provided by industrial, powdered preparation of white rice congee (with beef), brown rice congee (with beef), egg custard or baked beans (Heinz Wattie's Australasia, Malvern, Victoria, Australia). From the nutrient analysis of these products (Table 1) extra protein, fat, starch, sucrose, fibre, vitamins and minerals were added as required to achieve isocaloric formulations with uniform macronutrient content with the nutritional requirements necessary for guinea pig development.<sup>18,19</sup> The formulation of the experimental diets contained 30% protein, 6-7% fat, 37-38% carbohydrate and 15% fibre (Table 2).

### Experimental design

After an acclimatisation time of 2 days *post partum*, the guinea pig pups were introduced to the experimental diets in their litter groups with their mothers to give total animal numbers of 9 to 13 pups for the 4 dietary groups. The dietary treatments included the infant weaning foods egg custard (EC) as negative control, white rice congee (WC), brown rice congee (BC) and the human food baked

**Table 1.** Composition of industrially prepared dried foods<sup>1</sup>

Component	Egg Custard	White rice Congee <sup>2</sup>	Brown rice Congee <sup>2</sup>	Baked Beans
Protein	15.9	17.7	17.7	20.8
Fat	13.6	6.3	6.3	2.1
Starch	11.4	53.8	51.9	29.2
Sugar	54.5	8.2	7.6	18.8
Fibre Total	0.0	3.2	3.2	20.8
Other	4.5	10.8	13.3	8.3

<sup>1</sup>Data is shown as percentage of total weight of dried product from the mean of two duplicate determinations or derived from food tables.

<sup>2</sup>The Rice congee preparations were formulated with beef.

**Table 2.** Composition of experimental diets<sup>1</sup>

Component	Egg Custard	White Rice Congee	Brown Rice Congee	Baked Beans
	<i>g/kg diet</i>			
Dried commercial diet	500	500	500	500
Casein	227	193	193	211
Corn starch	46	0	0	0
Glucose	0	0	0	68
Cane sugar	0	67.5	67.5	23.5
Safflower oil	0	31	31	60.5
α-Cellulose	150	131.5	131.5	60
L-Arginine	3	3	3	3
DL-Methionine	3	3	3	3
Choline chloride	1	1	1	1
Minerals (AIN-93M-MX)	60	60	60	60
Vitamins (AIN-93-VX)	10	10	10	10

<sup>1</sup>These formulated diets as mixed powder are isocaloric and have equal final quantities of protein, carbohydrate, fat and fibre. They were then constituted with 50% or 25% milled commercial guinea pig pellets (chow). The formulated diets and milled chow were bound with a small quantity of water, mixed, pelleted and dried overnight at 35°C.

beans (BB) as positive control (Table 2). The dams and their pups were fed 50% chow: 50% diet formulation mix for 7 days followed for a further week with 75% formulated test diet with 25% chow. For the remaining time of the feeding trial (approximately 6-7 weeks) the litters were fed 100% formulated test diet. This equated to 6-7% fat, 30% protein, and 37.5% carbohydrate with 15% fibre. From feeding experience in our and other laboratories, the guinea pig diets were supplemented with a daily ration of 15g of the same fruit and vegetables per animal per day and water *ad libitum* supplemented with vitamin C and with a measured ration of lucerne hay provided twice weekly for dietary variation and bedding.<sup>18,19</sup> The developing guinea pigs were weaned from their mothers at 18 days of age or 180 g body weight providing 34-40 days on the diets alone not supplemented with mother's milk. The amount of diet and fruit and vegetables consumed was measured for each litter group from day two until sacrifice.

#### **Measurement of gut length and weight and sampling for *in vitro* contractility and SCFA analysis**

At the completion of the feeding period the young guinea pigs were weighed and then euthanased by cervical dislocation and exsanguination. The whole small intestine was removed, flushed with saline, measured unstretched on saline soaked paper towel along side a tape measure and then weighed after gentle blotting. Gut density was defined as weight divided by the length. Sections of the ileum 10 cm from the ileo-cecal junction were dissected for electro-physiological recordings<sup>20</sup> and stored in Krebs-Henseleit-bicarbonate buffer at room temperature gassed with O<sub>2</sub>:CO<sub>2</sub> (95:5 v/v) as described below. Samples of 3-4g of the caecal digesta were collected for pH and SCFA analysis from the experimental groups and from five dams for comparison at the end of the experiment and stored at -80°C.

#### **Caecal digesta pH and short chain fatty acid analysis**

Caecal digesta was diluted in three volumes of 0.06% heptanoic acid (pH 7.0) and homogenised by Ultra-Turrax. This mixture was centrifuged at 4000 rpm in a Beckman GRP bench top centrifuge at 5°C for 10 min. The sample supernatant was then assessed for pH. A 0.1 mL aliquot of supernatant was placed into a 5 mL round bottom Quickfit flask and acidified with 20 µL 1 M phosphoric acid and shell frozen in ethanol at -70°C. The sample was then attached to a manifold and the contents evacuated whilst cooling the flask. The flask was then sealed from the pump and transferred to a 50°C water bath and the contents distilled into a cooled flask. A 1 µL sample was injected into a HP5710 GC for SCFA content using a 2 m x 2 mm column of Tenax TA coated with 1% phosphoric acid.

#### **Electrophysiological recording of ileal contractility**

The ileum was stored immediately at room temperature in Krebs-Henseleit-bicarbonate buffer and bubbled with 95%:5% O<sub>2</sub>:CO<sub>2</sub> for at least 30 minutes to fully oxygenate the tissue. Two lengths of ileum of approximately 5 cm were cannulated at either end with a flanged plastic tubing and mounted vertically in duplicate organ baths containing the above buffer at 37°C and continuously gassed

with 95%:5% O<sub>2</sub>:CO<sub>2</sub> and electrically stimulated to contract.<sup>20</sup> The tissue outflow tube was connected to a Harvard isotonic transducer and force of contraction was determined via a BIOPAC System connected to computer and recorded using the AcqKnowledge® 3.01 program. At the end of the recording the tissue was blotted and weighed and the force of contraction was determined and standardised as voltage per gram (V/g) of ileal tissue or as a percentage of maximal contraction per particular treatment.<sup>20</sup>

#### **Electrical stimulation threshold**

After a tissue equilibration period of 30 min the electrical stimulation voltage was turned to zero and then increased incrementally up to 60V to determine the threshold of electrical stimulation and maximal electrically induced contraction.<sup>20</sup>

#### **Effect of gastrointestinal agonists**

A series of gastrointestinally active agonists were added cumulatively to quiescent ileal tissue in the organ bath until maximal contraction had been achieved.<sup>20</sup> The organ bath was flushed out between each treatment and 5-10 min allowed for the tissue to equilibrate. The tissue was then electrically stimulated and only tissues that retained the initial maximal contraction were kept for further treatment. The tissue was then contracted by electrical stimulation and morphine or epinephrine added cumulatively to the bath until maximal inhibition of contraction had been achieved.<sup>20</sup> Morphine inhibition was reversed by naloxone (10-100 nmol/L) and epinephrine inhibition was reversed by phentolamine (100-1000 nmol/).

#### **Data analysis**

Statistical evaluation was performed by one-way analysis of variance (ANOVA) using GraphPad InStat3 for Windows 98 (GraphPad Software, San Diego CA USA). When significance was detected ( $P < 0.05$ ), differences between individual means were analysed by the Bonferroni multiple comparison test. The EC<sub>50</sub>, IC<sub>50</sub> and maximal contraction values for electrically-driven and effective agents on ileal contraction were determined using graph fits in GraphPad PRISM 3.01 using R<sup>2</sup> values >0.99. Data are shown as mean with SEM together with the number of animals as indicated.

## **Results**

#### **Animal age, food acceptability and live weight gain**

The experimental dietary groups also tolerated an incremental increase in the diet groups from 50% to 75% and finally 100% (with the concomitant decreasing supplementation of guinea pig chow).<sup>20</sup> Daily rations of measured quantities and types of fruit and vegetables and periodic supply of lucerne hay were also supplied as a requirement for guinea pig development,<sup>18-21</sup> and should not confound the use of the different formulations of egg custard, rice congee or baked bean diets. There was no significant difference between initial guinea pig birth weights or the ages of the experimental groups at the completion of the dietary trial (Table 3). The daily dietary intake per animal per experimental diet over the last three weeks were as follows (g/day, diet): 13.9 ± 1.4, EC; 15.2 ± 2.4, WC; 16.8 ± 1.6, BC; and 15.1 ± 1.9, BB which was

**Table 3.** Birth weight, final weight, age, and small intestinal physical properties of guinea pig dietary groups<sup>1</sup>

Dietary group	Birth weight g	Final weight g	Age Days	Small intestine		
				Length mm	Weight g	Density g/m
Egg Custard	99± 6	491± 29	52.2± 2.1	1421± 36	10.3± 0.5	7.2± 0.3
White Rice Congee	95± 5	443± 28 <sup>a</sup>	51.5± 1.7	1413± 26	9.4± 0.5 <sup>ab</sup>	6.6± 0.3 <sup>a</sup>
Brown Rice Congee	102± 7	499± 12	50.3± 1.5	1511± 24	11.3± 0.5 <sup>a</sup>	7.4± 0.2
Baked Beans	106± 5	546± 16 <sup>a</sup>	49.9± 0.6	1474± 14	11.6± 0.2 <sup>b</sup>	7.9± 0.1 <sup>a</sup>

<sup>1</sup>Values are means ± SEM for N=9-13 animals per dietary group. Significant differences exist between dietary groups within the same column with the same letter superscript. The terminal weight of White Rice Congee was significantly lower than the Baked Beans ( $P<0.05$ ). For small intestine weights, the White Rice Congee was significantly lower than the Brown Rice Congee ( $P<0.05$ ) and the Baked Beans ( $P<0.01$ ). For small intestine density, White Rice Congee was significantly lower than the Baked Beans ( $P<0.01$ ).

not significantly different. However, at the end of the feeding trial the WC group body weights were lower than BB group (Table 3) but were within the normal range.<sup>21</sup>

#### **Small intestinal weight, length and density**

There was no significant difference in final small intestine length. However, intestine weight of WC group and BC group were significantly lower than BB group (Table 3). This is reflected in a significantly lower small intestine density (weight/length) for WC group compared to BB group.

#### **Caecal digesta pH and short chain fatty acid (SCFA) levels**

The pH of caecal digesta was not significantly different across the dietary groups (Table 4). For acetate, which usually represents about 60-70% of caecal total SCFA, and for propionate there was no differences between the dietary groups (Table 4). However, feeding baked beans led to butyrate being significantly higher compared to the other three dietary groups. Although elevated in the BB group, the caecal total SCFA (with small quantities of isobutyrate, valerate, isovalerate and caproate, results not shown) content was not significantly different between the dietary groups. For comparison, the caecal digesta of

five of the dams who were fed standard chow for at least 4-5 weeks were also analysed. The final live weight of the dams was 1015 ± 61 g with pH of caecal digesta 6.72 ± 0.10 with SCFA (mmol/L) measurements of: acetate, 56.8 ± 5.0; propionate, 12.9 ± 0.6; and butyrate 10.6 ± 1.1 with a total SCFA content of 80.3 ± 3.2. There was no significant difference between caecal digesta pH or propionate concentration of the dams compared to the experiment diets, however the acetate and total SCFA concentration was higher ( $P<0.05$ ) than the congee diets, as were the butyrate levels compared to the rice congee and egg custard diets ( $P<0.001$ ) as determined by ANOVA.

#### **Ileal contraction induced by electrical stimulation**

Higher voltage was required to initiate ileal contraction of the BC (13.9±0.2) and WC (13.3±0.2) groups compared to the EC (12.1±0.4) and BB (12.5±0.4) groups (Fig. 1) with statistical significance noted for the BC group versus EC ( $P<0.01$ ) and BB groups ( $P<0.05$ ).

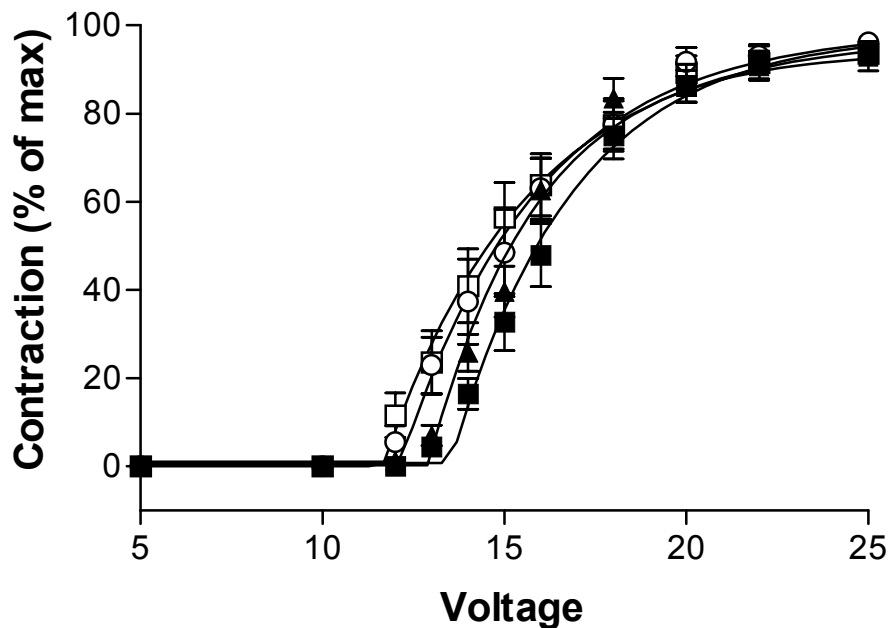
#### **Ileal contraction induced by gastrointestinal agonists**

There was no significant difference in the EC<sub>50</sub> values across the four dietary groups for acetylcholine, histamine, serotonin, the prostaglandins PGE<sub>2</sub> or PGF<sub>2α</sub>, or

**Table 4.** Caecal digesta pH, and individual and total short chain fatty acids of guinea pigs fed experimental diets<sup>1</sup>

Dietary group	pH	Acetate	Propionate	Butyrate	Total
Egg Custard	6.72 ± 0.07	38.5 ± 2.1	8.9 ± 0.4	5.6 ± 0.5 <sup>a</sup>	53.0 ± 2.9
White Rice Congee	6.93 ± 0.06	33.2 ± 2.8	8.7 ± 0.9	5.5 ± 0.4 <sup>b</sup>	47.4 ± 3.7
Brown Rice Congee	6.88 ± 0.08	34.4 ± 2.4	9.3 ± 0.7	5.1 ± 0.5 <sup>c</sup>	48.7 ± 3.1
Baked Beans	6.70 ± 0.07	41.3 ± 7.1	11.5 ± 1.5	8.2 ± 0.9 <sup>abc</sup>	61.0 ± 9.4

<sup>1</sup>The results are expressed as mean ± SEM for n = 9-13 per dietary group measured in duplicate. The total is the sum of the major SCFAs, acetate, propionate and butyrate. Significant differences exist between the dietary groups within the same column with the same letter superscript. The level of butyrate in Baked Beans was significantly higher than the White Rice Congee and Brown Rice Congee groups ( $P < 0.01$ ) and Egg Custard group ( $P < 0.05$ ).



**Figure 1.** Effect of increasing voltage on contraction of isolated intact guinea pig ileum driven at 0.1Hz for 5 ms. The dietary treatments were: Egg Custard ( $\square$ ); White Rice Congee ( $\blacktriangle$ ); Brown Rice Congee ( $\blacksquare$ ); and Baked Beans ( $\circ$ ). The variation between the groups was statistically significant ( $P=0.0018$ ) by ANOVA. Using Bonferroni multiple comparison as post-test, Brown Rice Congee was significantly different from both Egg Custard ( $P<0.01$ ) and Baked Beans ( $P<0.05$ ). Results shown are mean  $\pm$  SEM of the combined data points for each dietary group, whereas the voltage thresholds are calculated as a mean from each individual voltage curve in each dietary group.

8-*iso*-PGE<sub>2</sub> induced contraction of quiescent tissue (Table 5). Irrespective of dietary group, the relative order of potencies (EC<sub>50</sub>) was PGE<sub>2</sub> > acetylcholine > PGF<sub>2 $\alpha$</sub>  > histamine > 8-*iso*-PGE<sub>2</sub>  $\geq$  5-HT as determined previously.<sup>58</sup> For maximal contraction (V/g of ileum), the order of potency was histamine  $\geq$  acetylcholine  $\geq$  5-HT > PGE<sub>2</sub> > 8-*iso*-PGE<sub>2</sub>  $\geq$  PGF<sub>2 $\alpha$</sub>   $\gg$  8-*iso*-PGF<sub>2 $\alpha$</sub> . The guinea pig ileum preparation was found to have a relatively small response to 8-*iso*-PGF<sub>2 $\alpha$</sub>  with maximal contraction (V/g of ileum) values of  $0.81 \pm 0.30$  (EC),  $0.66 \pm 0.33$  (WC)  $0.83 \pm 0.21$  (BC) and  $0.42 \pm 0.20$  for (BB) which were not significantly different for  $n=6-8$  animals performed in duplicate.

#### **Inhibitors of electrically-driven contraction**

Electrically-driven ileal tissue was more sensitive to inhibition by the catecholamine, epinephrine than to the opioid, morphine. Although not significant by ANOVA analysis ( $P=0.095$ ), the EC<sub>50</sub> values for morphine inhibition of electrically-driven contractions of BC and WC groups were less than that required for the EC or BB groups (Table 5). The BC group was only marginally more sensitive to adrenaline inhibition than BB group, but this difference was not statistically significant. Morphine inhibition was reversed by naloxone to  $97.4 \pm 5.9\%$  (EC),  $93.7 \pm 5.5\%$  (BC),  $94.6 \pm 6.0\%$  (WC) and  $99.5 \pm 3.3\%$  (BB) while epinephrine inhibition was reversed by phenolamine to  $92.9 \pm 3.9\%$  (EC),  $93.8 \pm 7.9\%$  (BC),  $95.5 \pm 6.2\%$  (WC) and  $97.9 \pm 3.1\%$  (BB) of maximal electrically-driven contraction and were not significantly different.

#### **Discussion**

Although guinea pigs are a suitable model for animal development and measurement of ileal contractility, they are basically herbivorous and are fastidious eaters that

establish food preferences early in life.<sup>18,21</sup> The incorporation of dietary chow in the initial diet rendered it acceptable, which was not the case with the 100% rice congee manufactured diets. As a mandatory requirement for diet acceptability and guinea pig nutrition,<sup>18,21</sup> and to avoid pregnancy toxemia in the dams, daily weighed rations of fruit and vegetables were given to the dietary groups as a supplement along with a small quantity of lucerne hay. All groups consumed similar amounts of supplemental fruit, vegetables and lucerne. Accordingly, the only dietary differences assumed between the experimental groups were that due to the treatments.

Because guinea pigs require 12-16% fibre in the diet,<sup>18,19,21</sup> this study included 15% fibre in the experimental diets with 60% of the fibre from baked beans, 8% from white and brown rice congee and none from egg custard with the remainder made up with  $\alpha$ -cellulose as insoluble source of fibre.<sup>6</sup> Gut length and muscle thickness increases with newborn age<sup>22</sup> and proliferation of gastrointestinal mucosa is stimulated by a host of growth factors and hormones<sup>23</sup> directly or by stimulating release of these agents. In this study, the animals receiving the BB diet were heavier at sacrifice and had denser small intestines than the WC group. The denser small intestine in the BB group may be related to release of stimulating factors or the generation of substrate since there was no indication of a higher dietary intake. Conversely, the WC diet that led to a lesser growth rate may be due to a problem with micronutrient deficiency or due to the presence of some anti-nutritional factor(s) generated during congee preparation.

In this study, there was no significant difference in the caecal digesta pH irrespective of the source of dietary fibre. Depending on the diet and animal model employed,



**Table 5.** Effect of hormones and neuroeffectors on contraction of quiescent tissue and inhibition of electrically-driven contraction of intact, isolated guinea pig ileal tissue

Effector	Dietary Groups							
	Egg Custard	White Rice Congee	Brown Rice Congee	Baked Beans	Egg Custard	White Rice Congee	Brown Rice Congee	Baked Beans
	Stimulation of contraction of quiescent ileum <sup>1</sup>							
	Sensitivity - EC <sub>50</sub> <i>nmol/L</i>				Maximum contraction <i>V/g ileum</i>			
Acetylcholine	31.6 ± 6.3	20.8 ± 2.2	25.4 ± 4.0	26.0 ± 5.9	10.9 ± 1.3	10.6 ± 0.8	11.0 ± 1.2	11.3 ± 0.8
Histamine	228.9 ± 38.5	174.5 ± 20.9	194.8 ± 25.4	148.4 ± 21.8	11.3 ± 1.3	11.2 ± 1.1	11.6 ± 1.4	13.3 ± 1.3
Serotonin	612.9 ± 143.3	603.2 ± 124.6	596.1 ± 105.4	633.9 ± 138.4	7.8 ± 1.1	9.2 ± 1.1	8.5 ± 1.1	11.2 ± 1.2
PGE <sub>2</sub>	21.5 ± 6.4	12.0 ± 2.1	12.6 ± 3.2	18.5 ± 3.8	6.3 ± 1.3	8.1 ± 0.5	9.0 ± 1.3	7.2 ± 0.9
PGF <sub>2α</sub>	77.7 ± 16.0	123.0 ± 35.9	90.6 ± 35.7	81.3 ± 25.0	4.5 ± 1.3	4.0 ± 0.5	4.3 ± 0.8	4.5 ± 0.8
8- <i>iso</i> -PGE <sub>2</sub>	330.6 ± 117.0	570.3 ± 139.5	601.6 ± 88.9	453.4 ± 116.2	4.9 ± 1.0	5.6 ± 0.8	5.4 ± 1.3	6.8 ± 1.2
	Inhibition of electrically-driven ileum <sup>2</sup>							
	IC <sub>50</sub> <i>nmol/L</i>				Maximum inhibition %			
Morphine	77.1 ± 9.6	54.7 ± 12.5	47.6 ± 11.1	69.9 ± 9.6	87.3 ± 3.6	88.4 ± 3.0	90.6 ± 3.0	84.3 ± 1.8
Epinephrine	18.3 ± 3.0	23.7 ± 6.4	14.5 ± 3.6	25.7 ± 3.7	93.1 ± 2.4	93.4 ± 2.6	94.5 ± 1.5	93.9 ± 2.4

<sup>1</sup>Results are Mean ± SEM for *N*=9-13; <sup>2</sup>*N*=7-10 guinea pigs per dietary group measured in duplicate.





an increased caecal pH has been found associated with decreased gastrointestinal transit time<sup>24</sup> whereas a lowering of colonic pH has been correlated with reduced large bowel transit time<sup>25</sup> and reduced incidence of cancer.<sup>26,27</sup> The fact that the rice grain congees, baked beans and egg custard diets are formulated 1:1 with added components up to the standard recommended guinea pig formulation and the further addition of a fruit and vegetable ration may have diluted potential differences in SCFA levels.

Significantly however, the BB diet caused an increase in the concentration of butyrate in the caecal digesta. SCFA have been implicated in the control of gastrointestinal contractility and motility at level of the rumen<sup>28</sup> and stomach,<sup>29</sup> small intestine<sup>30</sup> and the colon.<sup>31</sup> Dietary baked beans have also been shown to increase digesta butyrate in normal pigs<sup>32</sup> and to lower cholesterol in normal and hypercholesterolemic models.<sup>33,34</sup> The physiological effects of dietary fibre and the complicated interplay between substrate generation and gastro-intestinal modulator release are yet to be fully enunciated.<sup>35</sup> It is of interest to note the discovery of SCFA receptors in a variety of tissues including small intestine, which has been shown to express GPR41 which is associated with acetate and propionate binding.<sup>36</sup>

It was found that the ileal tissue of the guinea pigs fed the congee rice diets required more voltage to initiate contraction compared to the EC and BB groups. This may indicate that there may be some intrinsic property differences between the gut tissues with regards to release of acetylcholine or other gastrointestinal mediators or the ionic handling properties of the smooth muscle membranes involved with depolarisation and contraction between the animals. This is difficult to explain, but may be a result of different natural dietary fibre or possibly the subtle differences in the fatty acid composition of dietary fat that has translated into the membrane.<sup>37,38</sup> In an effort to deduce any possible mechanism underlying the difference in voltage sensitivity and to validate these findings, an extensive range of gastrointestinal agonists involved in intestinal contractility and pathophysiology were tested in the *in vitro* ileal model.<sup>20</sup>

The major muscarinic parasympathetic effector in the myenteric plexus of small intestinal smooth muscle is acetylcholine.<sup>39,40</sup> However, there was no significant difference in the responses to acetylcholine across the four diets. Histamine and 5-HT which are also strong effectors of ileal contractility and have been implicated in intestinal secretion,<sup>41,42</sup> vomiting,<sup>43,44</sup> bowel inflammation and disease,<sup>45-47</sup> as well as normal motility,<sup>48,49</sup> also had no significant effects across the dietary groups. These negative effects may imply a presynaptic effect at the myenteric plexus to release neurotransmitter(s) that elicit smooth muscle contraction or a post receptor modification.

Prostaglandins are believed to also play an important role in the maintenance of human gastric mucosal homeostasis,<sup>50</sup> mucous secretion<sup>51</sup> and smooth muscle tone of isolated jejunum in animal models.<sup>52</sup> Eicosanoids have been shown to affect the migrating motor complex in human and animal studies.<sup>53</sup> However, the physiological role eicosanoids play in gastrointestinal motility remains

unclear.<sup>50,54</sup> PGE<sub>2</sub> and PGF<sub>2α</sub> were also found to have similar effects on contractility across the four dietary groups.

The isoprostane, 8-*iso*-PGE<sub>2</sub>, is formed by free radical mediated peroxidation of arachidonic acid. Isoprostanes have been monitored as an indication of oxidant stress<sup>55,56</sup> but also have potent biological activity of their own.<sup>57</sup> Although not reaching significance, the egg custard diet had the highest sensitivity to 8-*iso*-PGE<sub>2</sub>. Having no inherent natural fibre of its own, the egg custard diet was totally supplemented with the insoluble fibre source, α-cellulose. However, recent results from our laboratory have demonstrated that ileal sensitivity to 8-*iso*-PGE<sub>2</sub> and electrically-driven stimulation was altered when a relatively low amount of fish oil (1.5% of total dietary fat) was supplemented into the brown rice congee diet.<sup>58</sup> Therefore, it is important to note that the influences of dietary fat and even protein should not be ruled out in this study. The diets contained added protein in the form of casein between 193 g/Kg for the rice congee diets up to 227g/Kg for the egg custard diet which is only an 18% difference. In regards to fat, however, the congee diets were supplemented with 3.1% safflower oil and the baked bean diet fully supplemented with 6% safflower oil. Whilst the fatty acid composition of ileal tissue was not measured in this study, we have recently described for the rat that a relatively higher supplementation of dietary fish oil (~5% of total dietary intake) increased ileal contractility in response to muscarinic and eicosanoid neuromuscular effectors compared to a vegetable or an animal fat with a concomitant increase in n-3 polyunsaturated fatty acid incorporation into ileal tissue.<sup>59</sup>

Two agonists that inhibit electrically-driven contraction of guinea pig ileum; epinephrine<sup>60</sup> and morphine, had similar profiles of inhibition across the four dietary groups. The opioids and catecholamines have long been known to interfere with normal gut contractility and motility, but intrinsic activity has not been implicated in this study with the alterations in sensitivity to electrically-driven contraction.

In summary, this study has demonstrated that guinea pigs can be fed industrially prepared rice congee diets *post partum* if care is taken to initially formulate diets with an acceptable mix of normal chow. The four diets tested had inherent fibre content ranging from zero for egg custard to approximately 20% for baked beans that were ultimately formulated to a final concentration of 15% fibre using α-cellulose. The terminal weight and intestinal density of the WC group was lower than the BB groups but were within the normal range.<sup>18,21</sup> As found by others, the BB group had higher caecal butyrate.<sup>32</sup> The rice congee groups required more voltage to initiate contraction than both the control EC group and BB group. These changes in electrical sensitivity could not be explained by differences in sensitivities to the range of gastrointestinal effectors employed and may be a result of presynaptic or postsynaptic changes. The subtle physiological changes described herein have not been reported before and their functional significance and the role of dietary fibre in gut motility are as yet to be fully elucidated especially in relation to other dietary factors such as the type of fat.

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### References

1. Michaelsen KF, Friis H. Complementary feeding - a global perspective. *Nutrition* 1998; 14: 763-766.
2. Gibson RS, Ferguson EL, Lehrfeld J. Complementary foods for infant feeding in developing countries - their nutrient adequacy and improvement. *Eur J Clin Nutr* 1998; 52: 764-770.
3. Lartey A, Manu A, Brown KH, Peerson JM, Dewey KG. A randomized, community-based trial of the effects of improved, centrally processed complementary foods on growth and micronutrient status of Ghanaian infants from 6 to 12 mo of age. *Am J Clin Nutr* 1999; 70: 391-404.
4. Al-Kanhal MA, Al-Mohizea IS, Al-Othaimen AI, Khan MA. Nutritive value of various rice based dishes in Saudi Arabia. *Ecol Food Nutr* 2000; 38: 223-235.
5. Ahmed R, Segal I, Hassan H. Fermentation of dietary starch in humans. *Am J Gastroenterol* 2000; 95: 1017-1020.
6. Choi YS, Cho SH, Kim HJ, Lee HJ. Effects of dietary fibers on lipid metabolism and activities of intestinal disaccharidases in rats. *J Nutr Sci Vitaminol* 1998; 44: 591-600.
7. Mangala SL, Malleshi NG, Mahadevamma, Tharanathan RN. Resistant starch from differently processed rice and ragi (finger millet). *Zeitschr Lebensmittel-untersuchung Und-forschung A - Food Res Technol* 1999; 209: 32-37.
8. Bird AR, Hayakawa T, Marsono Y, Gooden JM, Record IR, Correll RL, Topping DL. Coarse brown rice increases fecal and large bowel short-chain fatty acids and starch but lowers calcium in the large bowel of pigs. *J Nutr* 2000; 130: 1780-1787.
9. Yalcin, Sehu A, Onol AG. Straw degradability as a predictor of intake and growth rate in sheep. *Anim Sci* 1998; 67: 485-490.
10. Yu B, Tsai CC, Hsu JC, Chiou PWS. Effect of different sources of dietary fibre on growth performance, intestinal morphology and caecal carbohydrases of domestic geese. *Br Poultry Sci* 1998; 39: 560-567.
11. Bird AR, Brown IL, Topping DL. Starches, resistant starches, the gut microflora and human health. *Curr Issues Intest Microbiol* 2000; 1: 25-37.
12. Archer SY, Meng S, Shei A, Hodin, RA. p21(WAF1) is required for butyrate-mediated growth inhibition of human colon cancer cells. *Proc Nat Acad Sci USA* 1998; 95: 6791-6796.
13. Böhmig GA, Krieger PM, Säemann MD, Wenharardt C, Pohanka E, Zlabinger GJ. n-Butyrate down regulates the stimulatory function of peripheral blood-derived antigen-presenting cells: a potential mechanism for modulating T-cell responses by short-chain fatty acids. *Immunology* 1997; 92: 234-243.
14. Topping DL, Clifton P. Short-chain fatty acids and human colonic functions: roles of resistant starch and nonstarch polysaccharides. *Physiol Rev* 2001; 81: 1031-1064.
15. Cherbut C, Ferrier L, Roze C, Anini Y, Blottiere H, Lecannu G, Galimiche JP. Short-chain fatty acids modify colonic motility through nerves and polypeptide YY release in the rat. *Am J Physiol* 1998; 38: G1414-G1422.
16. Fernandez ML. Guinea pig as models for cholesterol and lipoprotein metabolism. *J Nutr* 2001; 131: 10-21.
17. Massimino SP, McBurney MI, Field CJ, Thomson ABR, Keelan M, Hayek MG, Sunvold GD. Fermentable dietary fibre increases GLP-1 and improves glucose homeostasis despite increased intestinal glucose transport capacity in healthy dogs. *J Nutr* 1998; 128: 1786-1793.
18. Huerkamp MJ, Murray KA, Orosz SE. Guinea Pigs. In: Laber-Laird K, Swindle MM, Flecknell P, eds. *Handbook of rodent and rabbit medicine*, first edition. Oxford, UK: Elsevier Science, 1996; 91-149.
19. American Institute of Nutrition (1993) AIN-93 purified diets for laboratory rodents: final report on the American Institute of Nutrition ad hoc writing committee on the reformulation of AIN-76A rodent diet. *J Nutr* 1993; 123: 1939-1951.
20. Patten GS, Head RJ, Abeywardena MY, McMurchie EJ. An apparatus to assay opioid activity in the infused lumen of the isolated guinea pig ileum. *J Pharmacol Toxicol* 2001; 44: 1-8.
21. Sutherland SD, Festing MFW. The guinea pig. In: Poole TB, ed. *The UFAW Handbook on care & management of laboratory animals*, sixth edition. Essex, England: Longman Scientific & Technical, 1987; 393-410.
22. Schafer KH, Hansgen A, Mestres P. Morphological changes of the myenteric plexus during early postnatal development in the rat. *Anat Record* 1999; 256: 20-28.
23. Lee HM, Udupi V, Englander EW, Rajaraman S, Coffey RJ, Greeley GH. Stimulatory actions of insulin-like growth factor-1 and transforming growth factor-alpha on intestinal neurotensin and peptide YY. *Endocrinol* 1999; 140: 4065-4069.
24. Shimizu J, Wada M, Takita T, Innami S. Curdlan and gellan gum, bacterial gel-forming polysaccharides, exhibit different effects on lipid metabolism, cecal fermentation and fecal bile acid secretion in rats. *J Nutr Sci Vitaminol* 1999; 45: 251-262.
25. Glitso LV, Brunsgaard G, Hojsgaard S, Sandstrom B, Bach Knudsen KE. Intestinal degradation in pigs of rye dietary fibre with different structural characteristics. *Br J Nutr* 1997; 80: 457-468.
26. Lewis SJ, Heaton KW. The metabolic consequences of slow colonic transit. *Am J Gastroenterol* 1999; 4: 2010-2016.
27. Bird AR, Brown IL, Topping DL. Starches, resistant starches, the gut microflora and human health. *Curr Issues Intest Microbiol* 2000; 1: 25-37.
28. Kendall PE, McLeay LM. Excitatory effects of volatile fatty acids on the in vitro motility of the rumen of the sheep. *Res Vet Sci* 1996; 61: 1-6.
29. Cucho G, Malbert CH. Ileal short-chain fatty acids inhibit transpyloric flow in pigs. *Scand J Gastroenterol* 1999; 34: 149-155.
30. Cucho G, Malbert CH. Short-chain fatty acids present in the ileum fasting gastrointestinal motility in conscious dogs. *Neurogastroenterol Motility* 1999; 11: 219-555.
31. Cherbut C, Ferrier L, Roze C, Anini Y, Blottiere H, Lecannu G, Galimiche JP. Short-chain fatty acids modify colonic motility through nerves and polypeptide YY release in the rat. *Am J Physiol* 1998; 38: G1414-G1422.
32. Topping DL, Illman RJ, Clarke JM, Trimble RP, Jackson KA, Marsona Y. Dietary fat and fiber alter large bowel and portal venous volatile fatty acids and plasma cholesterol but not biliary steroids in pigs. *J Nutr* 1993; 123: 133-143.
33. Costa NM, Walker AF, Low AG. The effect of graded inclusion of baked beans (*Phaseolus vulgaris*) on plasma and liver lipids in hypercholesterolaemic pigs given a Western-type diet. *Br J Nutr* 1993; 70: 515-524.

34. Abdul-Hamid A, Luan YS. Functional properties of dietary fibre prepared from defatted rice bran. *Food Chem* 2000; 68: 15-19.
35. Dvorak B, Williams CS, McWilliam DL, Shinohara H, Dominguez JA, McCuskey RS, Phillips AF, Koldovsky O. Milk-borne epidermal growth factor modulates intestinal transforming growth factor- $\alpha$  levels in neonatal rats. *Pediatr Res* 2000; 47: 194-200.
36. Le Poul E, Loison C, Struyf S, Spingael J-Y, Lannoy V, Decobecq M-E, Brezillon S, Dupriez V, Vassart G, Van Damme J, Parmentier M, Detheux M. Functional characterization of human receptors for short chain fatty acids and their role in polymorphonuclear cell activation. *J Biol Chem* 2003; 278: 25481-25489.
37. Hazama H, Nakajima T, Asano M, Iwasawa K, Morita T, Igarashi K, Nagata T, Horiuchi T, Suzuki J, Soma M, Okuda Y. Omega-3 polyunsaturated fatty acids - modulation of voltage-dependent L-type  $Ca^{2+}$  current in guinea-pig tracheal smooth muscle cells. *Eur J Pharmacol* 1998; 355: 257-266.
38. Hirafuji M, Ebihara T, Kawahara F, Minami M. Effect of docosahexaenoic acid on smooth muscle cell function. *Life Sci* 1998; 62: 1689-1693.
39. Visi ES. Acetylcholine release from guinea-pig ileum by parasympathetic ganglion stimulants and gastrin-like polypeptides. *Br J Pharmacol* 1973; 47: 765-777.
40. Bennett A. Correspondence: polypeptides, acetylcholine release, and gut motility. *Gastroenterology* 1973; 65: 992-994.
41. Sjoqvist A, Cassuto J, Jodal M. Actions of serotonin antagonists on cholera toxin-induced intestinal fluid secretion. *Acta Physiol Scand* 1992; 145: 229-237.
42. Plaisanchie P, Barcelo A, Moro F, Claustre J, Chayvialle JA, Cuber JC. Effects of neurotransmitters, gut hormones, and inflammatory mediators on mucus discharge in rat colon. *Am J Physiol* 1998; 38: G1073-G1084.
43. Fozard J. 5-HT<sub>3</sub> receptors and cytotoxic drug-induced vomiting. *Trends Pharmacol Sci* 1987; 8: 44-454.
44. Hasler WL. Serotonin receptor physiology - relation to emesis. *Dig Dis Sci* 1999; 44: 108S-113S.
45. Gui XY. Mast cells - a possible link between psychological stress, enteric infection, food allergy and gut hypersensitivity in the irritable bowel syndrome. *J Gastroenterol Hepatol* 1998; 13: 980-989.
46. Raithel M, Schneider HT, Hahn EG. Effect of substance P on histamine secretion from gut mucosa in inflammatory bowel disease. *Scand J Gastroenterol* 1999; 34: 496-503.
47. Gershon MD. 5-HT (serotonin) physiology and related drugs. *Curr Opin Gastroenterol* 2000; 16: 113-120.
48. Gershon MD. Review article: roles played by 5-hydroxytryptamine in the physiology of the bowel. *Aliment Pharmacol Ther* 1999; 13 Suppl 2: 15-30.
49. Nagakura Y, Kiso T, Ito H, Miyata K, Yamaguchi T. The role of 5-hydroxytryptamine(3) and 5-hydroxytryptamine(4) receptors in the regulation of gut motility in the ferret. *Life Sci* 2000; 66: PL331-PL338.
50. Eberhart CE, DuBois RN. Eicosanoids and the gastrointestinal tract. *Gastroenterology* 1995; 109: 285-301.
51. McQueen S, Hutton D, Allen A, Garner A. Gastric and duodenal surface mucus gel thickness in rat: effects of prostaglandins and damaging agents. *Am J Physiol (Gastrointest Liver Physiol)* 1983; 245: G388-G393.
52. Ferreira SH, Herman A, Vane JR. Prostaglandin generation maintains the smooth muscle tone of the rabbit isolated jejunum. *Br J Pharmacol* 1972; 44: 328P-330P.
53. Mohajer B, Ma TY. Eicosanoids and the small intestine. *Prostaglandins Other Lipid Mediat* 2000; 61: 125-143.
54. Bennett A, Eley KG, Stockley HL. The effects of prostaglandins on guinea-pig isolated intestine and their possible contribution to muscle activity and tone. *Br J Pharmacol* 1975; 54: 197-204.
55. Reilly MP, Lawson JA, FitzGerald GA. Eicosanoids and iso-eicosanoids: indices of cellular function and oxidant stress. *J Nutr* 1998; 128: 434S-438S.
56. Stern LT, Roberts LJ, Morrow JD. The isoprostanes: novel prostaglandin-like products of the free radical-catalysed peroxidation of arachidonic acid. *J Biomed Sci* 1999; 6: 226-235.
57. Morrow JD, Chen Y, Brame CJ, Yang J, Sanchez SC, Xu J, Zackert WE, Awad JA, Roberts LJ. The isoprostanes: unique prostaglandin-like products of free-radical-initiated lipid peroxidation. *Drug Metab Rev* 1999; 31: 117-139.
58. Patten GS, Bird AR, Topping DL, Abeywardena MY. Dietary fish oil alters the sensitivity of guinea pig ileum to electrically driven contractions and 8-*iso*-PGE<sub>2</sub>. *Nutr Res* 2002; 22: 1413-1426.
59. Patten GS, Abeywardena MY, McMurchie EJ, Jahangiri A. Dietary fish oil increases acetylcholine- and eicosanoid-induced contractility of isolated rat ileum. *J Nutr* 2002; 132: 2506-2513.
60. Paton WDM, Vizi ES. The inhibitory action of noradrenaline and adrenaline on acetylcholine output by guinea-pig ileum longitudinal muscle strip. *Br J Pharmacol* 1969; 35: 10-28.