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# Specific protein supplementation using soya, casein or whey differentially affects regional gut growth and luminal growth factor bioactivity in rats; implications for the treatment of gut injury and stimulating repair

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1 **Specific protein supplementation using soya, casein or whey differentially affects**  
2 **regional gut growth and luminal growth factor bioactivity in rats; implications**  
3 **for the treatment of gut injury and stimulating repair**

4

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20 **Running title:** Specific dietary protein supplementation and regional gut

21 growth

22 **Abbreviations:** ANOVA; analysis of variance, C; casein, ED; elemental diet, EGF;

23 epidermal growth factor, GLP-2; glucagon-like peptide-2, SCFAs; short chain fatty

24 acids, SI; small intestine, SP; soya protein, W; whey protein,

25

25

26 **ABSTRACT**

27 Modulation of regional growth within specific segments of the bowel may have  
28 clinical value for several gastrointestinal conditions. We therefore examined the  
29 effects of different dietary protein sources on regional gut growth and luminal growth  
30 factor bioactivity as potential therapies. Rats were fed for 14 days on isonitrogenous  
31 and isocaloric diets comprising elemental diet (ED) alone (which is known to cause  
32 gut atrophy), ED supplemented with casein or whey or a soya protein-rich feed.  
33 Effects on regional gut growth and intraluminal growth factor activity were then  
34 determined. Despite calorie intake being similar in all groups, soya rich feed caused  
35 20% extra total body weight gain. Stomach weight was highest on soya and casein  
36 diets. Soya enhanced diet caused greatest increase in small intestinal weight and  
37 preserved luminal growth factor activity at levels sufficient to increase proliferation *in*  
38 *vitro*. Regional small intestinal proliferation was highest in proximal segment in ED  
39 fed animals whereas distal small intestine proliferation was greater in soya fed  
40 animals. Colonic weight and proliferation throughout the colon was higher in animals  
41 receiving soya or whey supplemented feeds. We conclude that specific protein  
42 supplementation with either soya, casein or whey may be beneficial to rest or increase  
43 growth in different regions of the bowel through mechanisms that include  
44 differentially affecting luminal growth factor bioactivity. These results have  
45 implications for targeting specific regions of the bowel for conditions such as Crohn's  
46 disease and chemotherapy.

47

47

48 **INTRODUCTION**

49 Many intrinsic inflammatory and ingested injurious agents cause regional damage to  
50 the gut. These include Crohn's disease (mainly affecting distal small intestine),  
51 ulcerative colitis (affecting colon) and non-steroidal inflammatory drugs (mainly  
52 affecting stomach and small intestine). Therapeutic targeting of drugs may occur  
53 through site specific release formulations or topical therapy (such as colonic enemas).  
54 As an alternative to pharmacological approaches, there is currently interest in using  
55 nutritional manipulation to both reduce the risk of relapse and for treatment when an  
56 injury is present. For example, elemental diets (ED), consisting of amino acids,  
57 mono/disaccharides and short/medium chain fatty acids, are of proven value in the  
58 treatment of inflammatory bowel disease, especially in children.<sup>1,2</sup> The mechanism(s)  
59 of action of EDs are unclear but might include reducing systemic antigenic load and  
60 "resting" the bowel. More recently, polymeric diets have been shown to be equally  
61 efficacious to ED<sup>3</sup>, establishing the principal that selective additions to feeds may  
62 enhance efficacy without losing the beneficial effects of ED. Prolonged use of ED  
63 causes intestinal atrophy, particularly of the distal bowel, potentially increasing the  
64 risk of bacterial translocation and bacteraemia.<sup>4</sup> Dietary formulations which reduce  
65 gut luminal antigenic load whilst minimizing the associated gut atrophy could,  
66 therefore, have therapeutic advantages. Similarly, because the gut is second only to  
67 the bone marrow in cell turnover, damage to the intestine is a common issue in  
68 patients undergoing chemotherapy (chemotherapy induced mucositis) and diets which  
69 reduce turnover while chemotherapy is administered or enhancing growth after  
70 treatment may also be useful.

71 The physiological control of gut growth is complex and poorly understood. Possible  
72 controlling mechanisms include alteration in circulating trophic hormones, local  
73 nutrition and luminal growth factors. We have previously suggested that ingestion of  
74 specific food proteins could influence gut growth by reducing the destruction of  
75 luminal growth factors, such as epidermal growth factor (EGF), through acting as  
76 competitive substrates for pancreatic proteases.<sup>5</sup> In this model, the efficacy of the  
77 various proteins to stimulate gut growth would be dependent on their ability to act as  
78 substrate for pancreatic proteases, relative to the affinity of the luminal growth factors  
79 for the same enzymes. Support for this idea comes from an *in vitro* study that showed  
80 soya bean trypsin inhibitor or casein were better substrates than whey protein  
81 (“lactalbumin”) for human pancreatic proteases. Furthermore, the addition of soya  
82 protein or casein to human duodenal juice reduced its ability to digest EGF, whereas  
83 addition of whey protein or elemental diets did not.<sup>5</sup> Soya protein or casein  
84 supplementation of feeds such as elemental diets might, therefore, be a clinically  
85 useful method of decreasing gut hypoplasia, while having a relatively minor effect on  
86 “antigenic load”.

87

88 We, therefore, examined the effect of isocaloric and isonitrogenous diets comprising  
89 standard elemental diet (E028, Nutricia, Liverpool, UK), E028 supplemented with  
90 either casein (ED+C) or whey protein (ED+W), and a commercially available soya-  
91 protein rich diet (SP, containing human food grade soya bean concentrate) on regional  
92 gut growth in rats and also assessed total “growth factor” bioactivity within the lumen  
93 of the small intestine.

94

95 **MATERIALS AND METHODS**

96

97 Diets

98 Three of the four test diets comprised standard elemental E028 ('ED', Nutricia  
99 Liverpool, UK) or variants of E028 containing whey protein ('ED+W', 12.5g/100g,  
100 the two major components of whey being  $\alpha$ -lactoglobulin and  $\beta$ -lactoglobulin.)<sup>6</sup>  
101 or casein ('ED+C', 10.5g/100g). Both protein supplemented elemental diets were  
102 specifically produced for the studies by Nutricia Liverpool. The fourth group  
103 consisted of a commercially available soya protein rich diet ('SP', produced by SDS,  
104 Essex, UK). All four diets were similar in terms of digestible protein equivalence and  
105 amino-acid content but the SP diet had a higher carbohydrate content, lower added fat  
106 and contained some crude fibre (5.3%). Details of the various diets are shown in  
107 Supplemental Table 1.

108

109 Protease inhibitory activity of feeds

110 To determine trypsin inhibitory activity within the feeds, a standard BAPNA substrate  
111 assay<sup>7</sup> was undertaken with concentrations of trypsin (0 - 50  $\mu$ g) added to generate a  
112 standard curve. Additional tubes, each containing 20  $\mu$ g trypsin had varying  
113 concentrations of the feeds added and any effect on reduction of tryptic activity (due  
114 to competitive inhibition) determined. In addition, to reproduce the situation found *in*  
115 *vivo*, where the feeds would be exposed to gastric juice prior to entering the small  
116 intestine, additional samples were pre-incubated with pepsin (1mg/ml) for 1 h. at pH 2,  
117 37°C before neutralization and subsequent BAPNA analyses.

118

119 **Animal Experiments**

120 All experiments were performed in compliance with relevant United Kingdom laws

121 and guidelines. All animal experiments were approved by the Local Institutional  
122 Animals Ethics Committees (Imperial College and Cancer Research UK) and  
123 performed under institutional guidelines. Studies were covered by the appropriate  
124 licences under the United Kingdom Home Office Animals Procedures Acts, 1986 and  
125 performed under the UK home office Animal Research: Reporting of In Vivo  
126 Experiments) guidelines (ARRIVE).

127

128 Pilot Study: We initially examined the caloric intake of rats (5 per group) fed on the  
129 various diets if allowed access *ad libitum*. Dietary intake was similar with all of the  
130 ED groups but was 10% greater in SP diet fed animals (data not show). Dietary  
131 restriction of SP fed animals was, therefore, used for the main study to ensure equal  
132 caloric intake between the various groups.

133

134 Main study: Forty adult male, Sprague-Dawley rats (Charles River, UK, 180-225g)  
135 were coded, weighed and randomized into the four diet groups (n=10 per group) and  
136 fed exclusively on these diets for 14 days. Animals remained in their wire bottomed  
137 cages in groups of 5 throughout the study.

138 Food was given in 2-ring concentric aluminium containers designed to minimize food  
139 spillage. The food consumption of each cage with determined each day by back  
140 weighing, including the small amount of spilled food beneath the cages.

141

142 Autopsy procedure and collection of samples.

143 At the end of the 14 day study, rats were given vincristine sulphate by intraperitoneal  
144 injection (1mg/kg, Eli Lilly, Basingstoke, UK) and killed 2 h later. Analyses of tissue  
145 was performed using the methods published by our group previously.<sup>8</sup> The weight and

146 unstretched length of the various portions of the gastrointestinal tract were measured.  
147 To assess the effect of these various diets on luminal growth factor activity, 1 ml of  
148 iced, normal saline was then lavaged through the small intestine, collected into  
149 Eppendorf tubes, snap frozen using frozen CO<sub>2</sub> (dry ice) and stored at -70 °C until  
150 assay for growth factor activity.

151 The stomach and samples of the small intestine and colon were then fixed in Carnoy's  
152 fluid, and stored in 70% (volume/volume) ethanol. For the intestinal tissues, the  
153 position of the various samples was defined by expressing its harvest site as a  
154 percentage of that organs total length at 1.5, 50 and 90% small intestinal distance and  
155 can be considered as equivalent to duodenum, jejunum and ileum respectively.

156 Similarly, 10, 50 and 90% colon can be considered as proximal, mid and distal colon.

157

#### 158 Analysis of tissue samples.

159 Assessment of metaphase accumulation was performed on micro-dissected tissue  
160 using our well validated, previously published methods<sup>8</sup> by a person blinded to the  
161 experiment. Briefly, tissues were sequentially rehydrated, hydrolyzed and stained with  
162 Schiff's reagent (the Feulgen reaction) and transferred to 45% (volume/volume) acetic  
163 acid. The crypts were then teased apart under a dissecting microscope, transferred to a  
164 glass microscope slide, flattened gently beneath a coverslip and examined using a  
165 compound microscope. The number of blocked mitoses per crypt was counted and the  
166 mean for 20 crypts per animal per site determined and used in the subsequent one way  
167 analysis of variance (ANOVA).

168

#### 169 Analysis of luminal growth factor activity.



170 Analyses of luminal growth factor activity were determined using thymidine  
171 incorporation into primary rat hepatocytes as a bioassay and an EGF-standard curve  
172 as described previously by us.<sup>9,10</sup> Briefly, to prepare primary rat hepatocytes for the *in*  
173 *vitro* assay of luminal growth factor activity, male Sprague-Dawley rats were  
174 anaesthetized using Hypnorm (Janssen Pharmaceutica-Crown Chemicals Ltd,  
175 Lamberhurst, Kent, UK) and hepatocytes isolated by *in situ* collagenase perfusion.  
176 The basic protocol consists of a two-step perfusion of the liver *in situ*, via the portal  
177 vein, first with calcium-free buffer followed by a calcium supplemented buffer  
178 containing collagenases. The digested liver was removed, the cells dispersed, filtered,  
179 collected by centrifugation and resuspended in a plating medium. For all studies,  
180 hepatocytes were grown in Williams E medium without L-glutamine (Gibco BRL,  
181 Paisley, UK) containing 5% fetal calf serum. Cell viability, determined by the ability  
182 to exclude 0.2% trypan blue, was greater than 80% in all experiments.

183

184 Assay of growth factor activity: Various volumes (50 to 200  $\mu$ l) of luminal perfusate  
185 was added to the wells. To assess the percentage of cells entering DNA synthesis,  
186 [<sup>3</sup>H] thymidine (2 mCi/well, 10 ml, Amersham International, Bucks, UK) was  
187 included in the cultures eight hours after the addition of test samples. The amount of  
188 [<sup>3</sup>H] thymidine incorporated was assessed biochemically, 18 hours after the addition  
189 of [<sup>3</sup>H] thymidine. Cells were washed for 15 seconds with water using a Dynatech  
190 multimash automatic self-harvester and solubilized by incubation at 37°C for 1 h in  
191 200  $\mu$ l of 1 M KOH. 50  $\mu$ l of the cell extract was counted in a  $\beta$ -counter in 1 ml of  
192 scintillant (Optiphase safe, LKB-Pharmacia Wallac).

193

194 The increase in thymidine uptake was then expressed in terms of EGF-like bioactivity  
195 by determining the equivalent amount of EGF that was needed to be added to the  
196 hepatocytes, to stimulate a similar increase in thymidine uptake. Measurements of  
197 EGF-like bioactivity in intestinal juice samples were performed in quadruplicate in 4  
198 separate wells.

199

## 200 **Statistical Analysis.**

201 Data were analysed using one-way ANOVA with the different diets as the factor.  
202 Where a significant effect of diet was seen, individual comparisons were performed  
203 comparing against ED, using t-tests based on the residual and degrees of freedom  
204 obtained from the ANOVA, a method equivalent to repeated measures analysis. All  
205 data is expressed as mean +/- SEM unless stated.

206

## 207 **RESULTS**

### 208 Protease inhibitory activity of feeds

209 *In vitro* assay of feeds showed higher trypsin inhibitory activity (136 mg trypsin  
210 inhibition per 100g feed) in SP feed, moderate amount in ED+C (42 mg trypsin  
211 inhibition per 100g feed) but much lower amounts in ED (13 mg/100g) or ED+W (15  
212 mg/100g). Samples which had been pre-incubated with pepsin at pH 2 to reproduce  
213 prior exposure to gastric contents gave similar results (SP 120 mg trypsin inhibition  
214 per 100g feed, ED+C 38 mg/100g, ED 13 mg/100g and ED+W 12 mg/100g).

215

### 216 **Animal study**

217 Dietary intake: Daily caloric consumption per cage for each of the groups were as  
218 follows ED; 696, 666 - 726 cal. (mean, range), ED+W; 718, 682 - 759 cal., ED+C;

219 714, 695 - 742 cal., SP; 705 cal (no range as all food eaten each day). This gave an  
220 average daily calorie consumption of 139 per animal in the ED group, 144 in ED+W  
221 group, 144 in ED+C group and 141 in SP fed animals. The caloric consumption of all  
222 groups were, therefore, within 3% of each other ( $p = 0.085$ ) although because of the  
223 different constituents of the SP diet, this resulted in SP fed animals receiving  
224 approximately 18% greater protein equivalent intake.

225

226 Total body weight gain: All animals increased weight over the two-week test period.  
227 with ED group showing an average of  $54.5 \pm 4.4$  g increase. Compared to the ED  
228 diet, weight gain in ED+C and ED+W ( $48.5 \pm 3.2$  g and  $58.5 \pm 2.1$  g, respectively)  
229 was not significantly different but was 20% greater in SP group ( $65.5 \pm 3.7$  g,  
230  $p < 0.01$ ).

231

232 Stomach weight: Absolute stomach weights were similar in ED and ED+W groups  
233 but about 10% greater in the ED+C and SP groups ( $p < 0.05$  and  $0.01$  vs. ED,  
234 respectively, Figure 1A).

235

236 Small intestine (SI) weight, proliferation and intraluminal growth factor assay:

237 Animals fed on the elemental diets all had similar SI weight whereas SP group small  
238 intestinal weight was 17% heavier ( $p < 0.01$ , Figure 1B). Correspondingly, luminal  
239 growth factor activity equivalent to  $3.9 \pm 0.4$  ng of EGF was present in SP group but  
240 unrecordable in all other groups.

241 Small intestinal regional proliferation varied dependent on site with the different diets.

242 Proximal small intestinal proliferation was greatest in the ED, with the other three  
243 groups being significantly lower (Figure 2A). In contrast, SP group had the highest

244 proliferation rate in the mid small intestine (significantly greater than ED+W and  
245 ED+C, Figure 3B) and this difference was particularly marked in the distal small  
246 intestine with SP having proliferation rate of 150% of the other diets (Fig 2C).

247

248 Colon weight and proliferation: Compared to ED fed animals, total colonic weight  
249 was 15% higher in ED+W animals and 50% higher in SP animals (both  $p < 0.01$ ,  
250 Figure 1C). Regional proliferation data was similar throughout the colon with ED+W  
251 and SP animals having 50% (proximal colon), 80% (mid colon) and 120% (distal  
252 colon) higher proliferation compared to ED or ED+C groups ( $p < 0.01$ , Figure 3).

253

## 254 **DISCUSSION**

255 We have shown different dietary protein sources cause differential regional effects on  
256 gut growth and proliferation and that diets containing soya protein result in increased  
257 small intestinal luminal growth factor activity.

258

259 Care was taken to ensure caloric intake of the groups were comparable to remove a  
260 potential confounding factor. We used fourteen days dietary intervention to reflect  
261 what would likely be used in a clinical setting in situations such as pre- or post-  
262 chemotherapy intervention. We used a rat hepatocyte system to assay intraluminal  
263 growth factor activity as this is a robust, reproducible method to determine relative  
264 bioactivity of EGF-R type ligands in the gut lumen and has the advantage over most  
265 cell lines of maintaining viability in the presence of bile and other luminal contents.<sup>9</sup>  
266 10 The *in vitro* trypsin (inhibition) assay is a simple robust system to examine  
267 differential effects of food substances and builds on our earlier *in vitro* work<sup>5</sup> showing  
268 that soya-bean trypsin inhibitor causes marked trypsin inhibition, casein causes

269 moderate trypsin inhibition (probably acting as a competitive substrate), whereas ED  
270 or whey have very limited inhibitory activity.

271

272 The trophic effects of the various diets differed dependent on which region of the gut  
273 was analyzed. Compared to ED diet, stomach weight was better maintained in ED+C  
274 and SP rats. The effect of ED+C may be due to casein being precipitated in stomach  
275 acid, delaying gastric emptying, whereas rapid transit into the small intestine is seen  
276 in ED or ED+W animals.<sup>11</sup>

277

278 The most notable finding of the ED diet was the much higher proliferation rate seen in  
279 the proximal small intestine compared to the other diets. This is probably as a result of  
280 rapid transit into the proximal intestine, where the ED constituents are rapidly  
281 adsorbed. This finding could be explained by the luminal workload hypothesis that  
282 suggests that regions actively involved in digestion/absorption have increased  
283 proliferation. This would also explain the low mitotic level seen in more distal regions  
284 of the small bowel as the majority of the feed would have been adsorbed more  
285 proximally.

286

287 The most notable effects of ED+W were the increased weight and proliferation of the  
288 colon. Several potential mechanisms might have relevance in explaining these  
289 findings. Changes in circulating trophic factors such as glucagon-like peptide-2  
290 (GLP-2) may have relevance although it is noteworthy that the trophic effect was  
291 restricted to the colon and not the small intestine. GLP-2 is produced by the ileum and  
292 colon, is trophic to the small intestine and colon and is rapidly degraded by tissue  
293 DPP-IV (dipeptidyl peptidase 4). Liu and co-workers showed oral whey

294 supplementation decreased colonic tissue DPP-IV in rats receiving continuous  
295 intravenous GLP-2, possibly increasing local colonic tissue GLP-2 levels.<sup>12</sup> Therefore,  
296 even if circulating levels of GLP-2 remain stable, local changes in DPP-IV as a result  
297 of dietary manipulation may alter local concentrations. A further mechanism that  
298 would explain our results is our finding that whey proteins are poor substrates for  
299 luminal proteases when compared against casein or soya. The amount of nutrient  
300 reaching the terminal bowel is, therefore, likely to be higher in the ED+W compared  
301 to the other groups. Support for this idea comes from recent studies showing whey  
302 supplementation increased colonic short chain fatty acids (SCFAs) concentrations, a  
303 known substrate for colonocytes.<sup>13</sup> Further studies to determine the importance of  
304 colonic bacteria in maintaining the growth of the colon in these animals could include  
305 measurement of colonic SCFAs or use of 'germ free' animals.

306

307 The most notable effects of the SP diet in the current studies were the increased total  
308 body weight gain, the increased small and large bowel growth and the finding that  
309 bioactive growth factor activity was present in small intestinal washings. The  
310 additional increase in body weight in the SP-fed group was not due to differences in  
311 total calorie intake as these were well matched across the groups although it should be  
312 noted that the SP group received a slightly higher percentage of their calories from  
313 protein. We have previously suggested that control of intestinal growth might be  
314 mediated by changes in intraluminal growth factor concentrations, with the trophic  
315 effect dependent on the relative affinity of the luminal proteases for the ingested food  
316 and the luminal growth factors.<sup>5</sup> This idea was supported by our findings that  
317 pancreatic diversion away from the gut lumen increases luminal growth factor  
318 concentrations and caused associated regional hypertrophy.<sup>5</sup> The current studies

319 extend these findings showing that ingestion of soy based proteins (which includes  
320 soya bean trypsin inhibitor) are effective competitive substrates for luminal proteases  
321 *in vivo*, allowing luminal growth factors such as EGF to survive better than when  
322 animals are fed whey or casein. These findings may have relevance to the trophic  
323 effect on the small and large intestine although it must be noted that the SP diet  
324 contained some fibre which may have relevance for colonic growth through acting as  
325 a fuel source for colonic bacteria, increasing colonic SCFAs levels in a similar way to  
326 that discussed for the whey fed animals. Further studies examining ED supplemented  
327 with pure soya protein which has had the fibre further reduced, combined with  
328 measurement of colonic SCFAs and possibly the use of germ-free animals could help  
329 address this question.

330

331 There is currently much interest in the use of dietary proteins for the prevention and  
332 treatment of ill health.<sup>14</sup> Our findings that dietary manipulation can cause regional  
333 changes in gut growth and also modulates luminal growth factor levels has potential  
334 clinical relevance. ED or polymeric diets are of proven benefit for Crohn's disease, a  
335 condition that predominantly affects the small intestine. Specific protein  
336 supplementation of ED with whey may have advantages in "resting" the small  
337 intestine while preserving colonic growth, which among other advantages should  
338 reduce the risk of bacterial translocation. Supplementation of ED with soy protein  
339 may have the advantage of locally increasing luminal EGF levels, facilitating repair in  
340 a similar way to that shown by us in treating ulcerative colitis with topical therapy.<sup>15</sup>  
341 Further studies examining the effect of these proteins on intraluminal growth factor  
342 concentrations and injury and repair in animal models of inflammatory bowel disease  
343 appear warranted but go beyond the scope of this paper. Similarly, chemotherapy

344 induced injury to the gut is a common side effect of high dose chemotherapy and  
345 systemic administration of several different growth factors to promote repair have  
346 been tested or are being assessed.<sup>16</sup> Dietary manipulation to reduce gut cellular  
347 turnover prior to treatment of non-gut tumours using systemic chemotherapy (to  
348 reduce gut side effects) and/or to increase gut proliferation post treatment, therefore,  
349 has potential therapeutic advantage by locally increasing luminal growth factor  
350 concentrations without the risk of systemic administration. Further studies appear  
351 justified.



352

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#### 408 **FIGURE LEGENDS**

##### 409 **Figure 1. Effect of diets on percentage change in gastrointestinal weight**

410 Animals were fed for two weeks on isonitrogenous feeds comprising elemental diet  
411 (ED) alone, ED supplemented with milk whey (ED+W), ED supplemented with  
412 casein (ED+C) or fed a commercial soya protein rich diet (SP). All groups received  
413 similar total calorie intake. Changes in weight of the stomach (**A**), small intestine (**B**)  
414 and colon (**C**) after the two-week period were expressed as % change compared to  
415 that of animals fed ED diet. \* and \*\* signifies  $p < 0.05$  and  $p < 0.01$ , respectively  
416 compared with ED.

417

##### 418 **Figure 2. Effects of diets on small intestinal mitosis.**

419 All animals on the various diets were injected with vincristine two hours prior to  
420 killing to allow assessment of rate of mitosis. Tissue was microdissected and data  
421 expressed as number of mitosis per crypt. The three regions analyzed were located  
422 according their position as a percentage of total small intestinal length; proximal small  
423 intestine (1.5%, duodenum, **A**), mid small intestine (50%, jejunum, **B**) and distal  
424 small intestine (90%, ileum, **C**). \* and \*\* signifies  $p < 0.05$  and  $p < 0.01$ , respectively

425 compared with ED.

426

427 **Figure 3. Effects of diets on colon mitosis.**

428 Colonic tissue from the same animals as Fig 3 was collected and the number of

429 mitosis per crypt assessed. The regions analyzed were located according their

430 position as a percentage of total colonic length; 5% site of colon (proximal colon, **A**),

431 50% site of colon (mid-colon, **B**), 90% site of colon (distal colon, **C**). \* and \*\*

432 signifies  $p < 0.05$  and  $p < 0.01$ , respectively compared with ED.

433

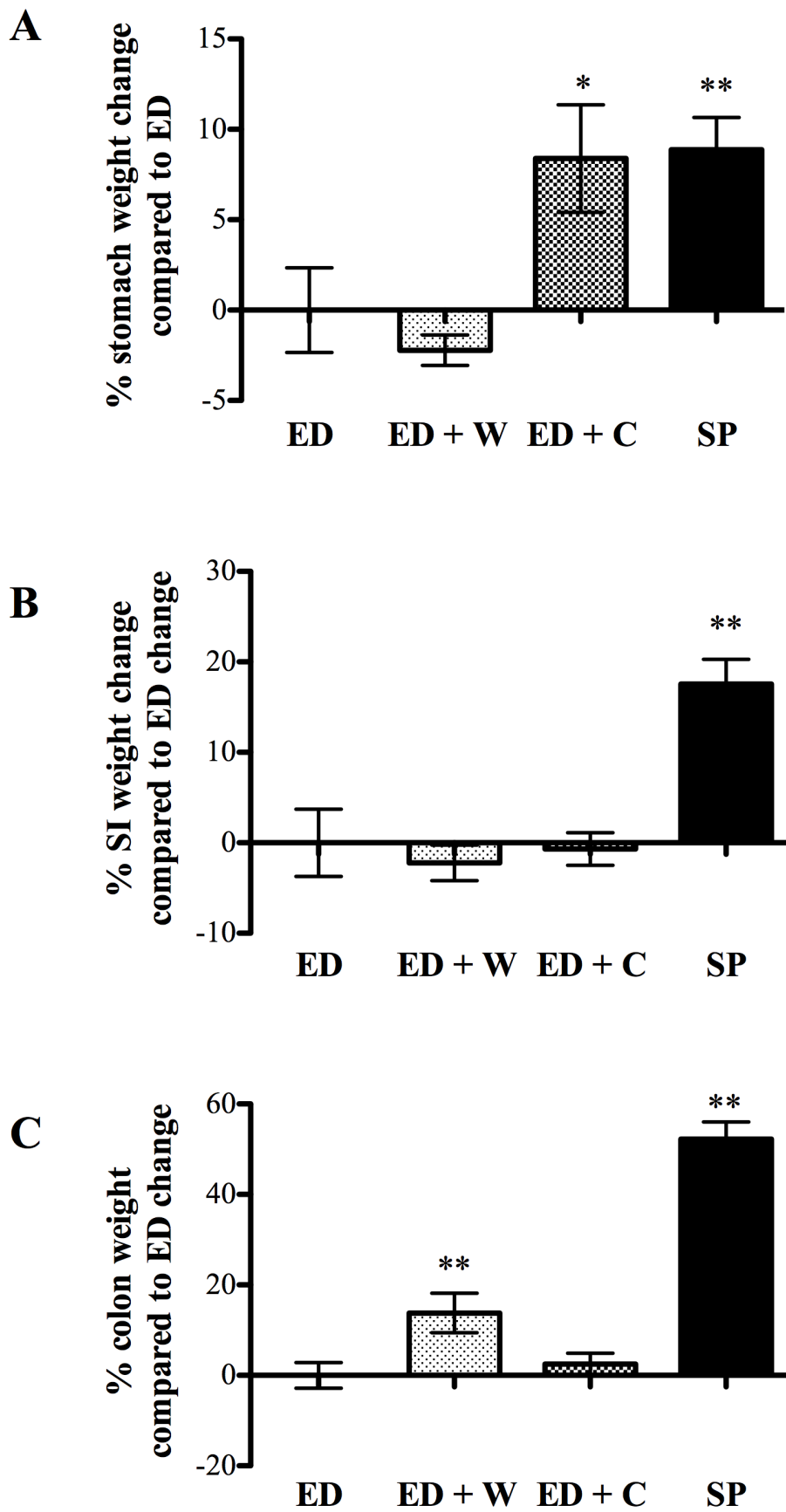


Fig 1

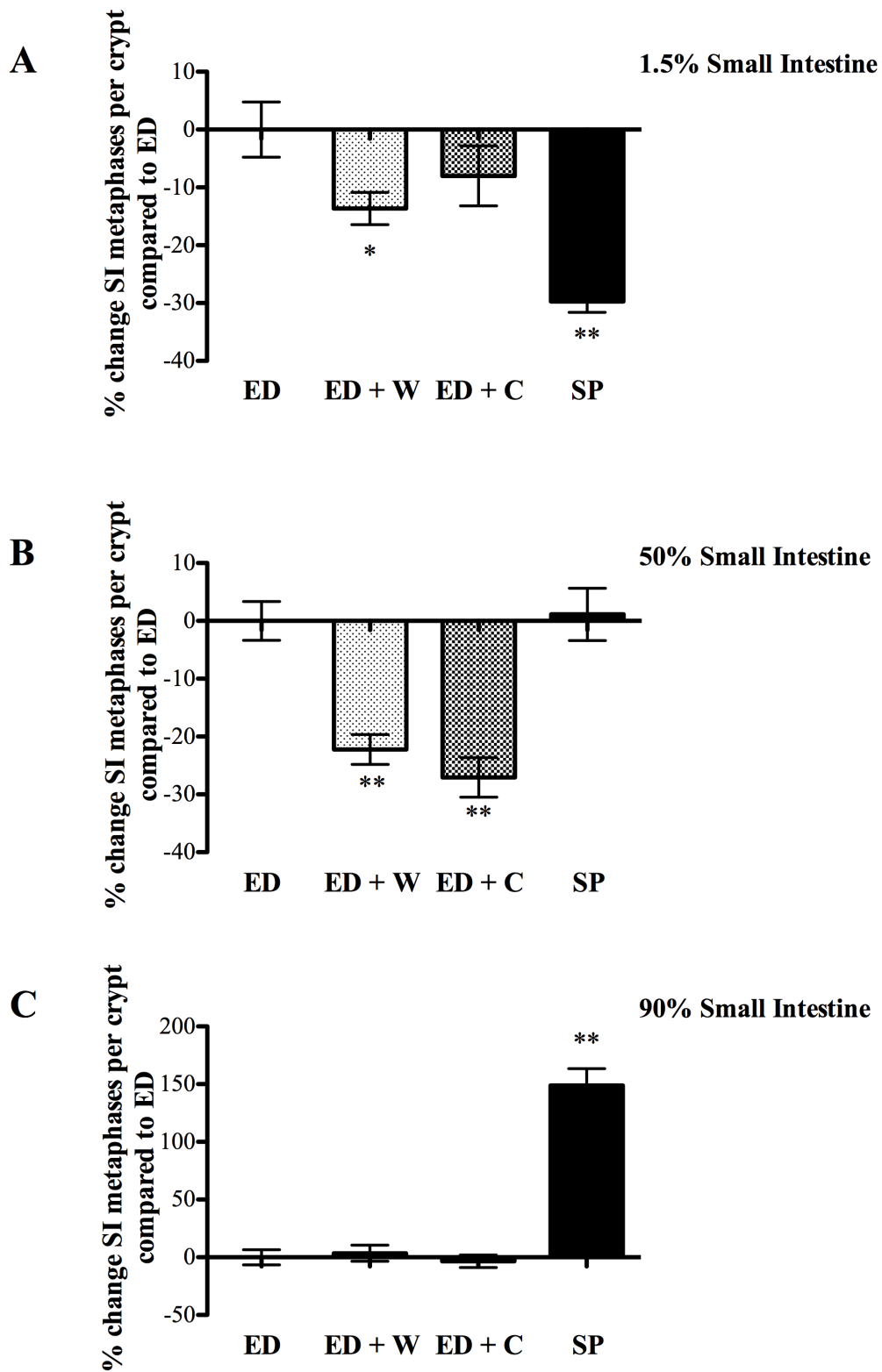


Fig 2

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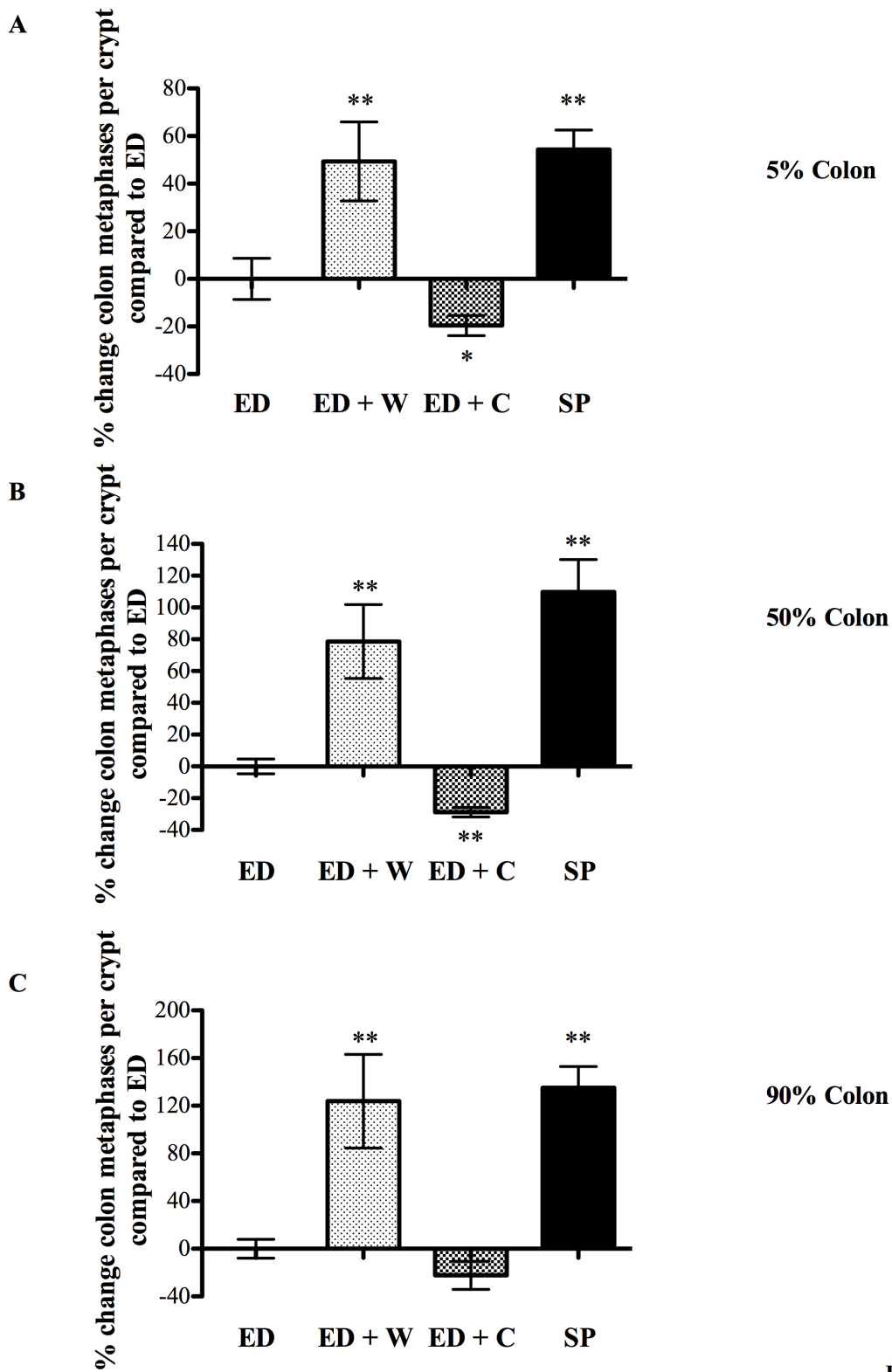


Fig 3

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441 **Supplemental Table 1. Composition of feeds used for study.**

	E028 (ED)	E028 and casein 400g cans (ED + C)	E028 and whey protein 400g cans (ED + W)	Soya protein enhanced diet (SP)
Digestible protein equivalent (g/100g)	12.5	13.8	13.8	12.9
Energy content (kcal/100g)	443	447	447	354
Total amino acid content (g)	15	15	15	14.22
Fat content (g)	17.45	17.45	17.45	2.6
Carbohydrate source	Dried glucose syrup	Dried glucose syrup	Dried glucose syrup	Wheat, barley,
Nitrogen source	Crystalline amino acids	Crystalline amino acids +  casein proteins	Crystalline amino acids + whey proteins	Soya protein, soya protein concentrate, wheat and barley proteins
Fat source	Coconut, safflower and canola oil	Coconut, safflower and canola oil	Coconut, safflower and canola oil	Soya oil
Nitrogen content (%)	2.2	2	2.1	2.35

442

443 1. ED – elemental diet

444 2. ED + C - elemental diet + casein

445 3. ED + W - elemental diet + whey protein

446 4. SP-soya protein enhanced diet

447

448