Metabolism of the soyabean isoflavone glycoside genistin *in vitro* by human gut bacteria and the effect of prebiotics

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The isoflavone genistein is found predominantly in soyabeans and is thought to possess various potent biological properties, including anti-carcinogenic effects. Studies have shown that genistein is extensively degraded by the human gut microflora, presumably with a loss of its anti-carcinogenic action. The aim of the present study was to investigate the potential of a prebiotic to divert bacterial metabolism away from genistein breakdown: this may be of benefit to the host. Faecal samples were obtained from healthy volunteers and fermented in the presence of a source of soyabean isoflavones (Novasoy™ (10 g/l); ADM Neutraceuticals, Erith, Kent, UK). Bacterial genera of the human gut were enumerated using selective agars and genistein was quantified by HPLC. The experiment was repeated with the addition of glucose (10 g/l) or fructo-oligosaccharide (10 g/l; FOS) to the fermentation medium. The results showed most notably that counts of *Bifidobacterium* spp. and *Lactobacillus* spp. were significantly increased ($P < 0.05$ and $P < 0.01$ respectively) under steady-state conditions in the presence of FOS. Counts of *Bacteroides* spp. and *Clostridium* spp. were, however, both significantly reduced ($P < 0.05$) during the fermentation. A decline in genistein concentration by about 52 and 56 % over the 120 h culture period was observed with the addition of glucose or FOS to the basal medium ($P < 0.01$), compared with about 91 % loss of genistein in the vessels containing Novasoy™ (ADM Neutraceuticals) only. Similar trends were obtained using a three-stage chemostat (gut model), in which once again the degradation of genistein was about 22 % in vessel one, about 24 % in vessel two and about 26 % in vessel three in the presence of FOS, compared with a degradation of genistein of about 67 % in vessel one, about 95 % in vessel two and about 93 % in vessel three in the gut model containing Novasoy™ (ADM Neutraceuticals) only. The present study has shown that the addition of excess substrate appeared to preserve genistein *in vitro*. In particular, the use of FOS not only augmented this effect, but also conferred an additional benefit in selectively increasing numbers of purportedly beneficial bacteria such as bifidobacteria and lactobacilli.

Genistein: Human gut microflora: Metabolism: Fructo-oligosaccharide

The isoflavone genistein belongs to a class of hormone-like diphenolic phyto-oestrogens and it possesses a similar structure to the female oestrogen, oestradiol 17β (Adlercreutz, 1995; Cassidy, 1999). Scientific interest in soyabean isoflavones, and in particular genistein, has increased dramatically over the past few years. Initially, this interest stemmed from epidemiological observations that the incidence of hormone-related diseases, and some cancers in particular, were much lower in Japan and China where soyabeans are commonly consumed in the diet (Setchell, 1998).

Genistein is predominantly present in plants and therefore in the diet as the glycoside, genistin (Murphy *et al.* 1997). Following ingestion, it is hydrolysed to the aglycone, genistein, by β-glucosidases. Enzymes capable of carrying out this deglycosylation step are found at the small intestinal brush border (lactase phloridzin hydrolase; Day *et al.* 2000) and within enterocytes (cytosolic β-glucosidases; Day *et al.* 1998). In addition, several major groups of colonic bacteria possess β-glucosidase activity, including *Lactobacillus* spp., *Bacteroides* spp. and *Bifidobacterium* spp. (Hawksworth *et al.* 1971; Friend & Chang, 1984).

Pharmacokinetic studies confirm that healthy adults absorb genistein rapidly and efficiently. In most adults, peak plasma concentrations of genistein following ingestion of the aglycone are between 4 and 7 h, whereas following consumption of the β-glucoside, the plasma concentration peaks at about 8–11 h. The latter result suggests that bio-availability of genistin is dependent upon deglycosylation by intestinal bacteria (Setchell *et al.* 2001). Excretion of the aglycone occurs mainly in urine and to some extent in faeces; however, in a feeding study with human subjects, where the subjects were given known doses of genistein ranging from 3·4 to 10·3 μmol/kg body weight, an incomplete recovery of intact genistein ranging from 14 to 43 % seems to suggest that isoflavone aglycones are extensively degraded, probably by human intestinal bacteria (Xu & Thomas, 1995).

Genistein undergoes a reduction of the C-ring at C_2 and C_3 to form an intermediary molecule dihydrogenistein. At this point, the metabolism may proceed in two different ways: cleavage of the C-ring at C_3 and C_4 to produce 6'-hydroxy-O-desmethylagolensin or complete cleavage of the C-ring to produce 2,4,6-trihydroxybenzoic acid and *p*-ethyl phenol, with both these endproducts being metabolically inactive. Both Kim *et al.* (1998) and Chang & Nair (1995) have demonstrated the conversion of genistein to dihydrogenistein by human faecal bacteria, but neither study identified the microbial genera involved. Hur *et al.* (2000) isolated *E. coli* and an unidentified Gram-positive strain capable of degrading genistein, whilst Schoefer *et al.* (2002) demonstrated that *Eubacterium ramulus* isolated from human faeces was also capable of degrading genistein.

De Boever *et al.* (2000) used a colonic model to investigate the prebiotic effect of soyagerm powder. They reported an overall increase in all bacterial groups compared with controls, with a $\log_{10} 2$ increase in *Lactobacillus* spp. However, several major bacterial groups were isolated, including *Bacteroides* spp. and *Bifidobacterium* spp., and only the breakdown of the glycoside to the aglycone genistein was investigated.

The aim of the present study was to use anaerobic fermentation techniques and HPLC analyses to confirm that human gut bacteria are capable of breaking down the aglycone isoflavone genistein and to investigate the potential of a prebiotic to reduce this function.

Materials and methods

Single-stage continuous cultures

Continuous cultures to study the bacterial metabolism of genistein in the presence and absence of excess substrate were prepared as follows. Basal medium (150 ml; containing (per litre): peptone water 2 g, yeast extract 2 g, NaCl 0·10 g, K_2HPO_4 0·04 g, KH_2PO_4 0·04 g, $Mg_2SO_4.7H_2O$ 0·01 g, $CaCl_2.2H_2O$ 0·01 g, Tween 80 2 ml, hemin 0·005 g, phylloquinone 10 μl (10 μg/ml), cysteine-HCl 0·50 g, bile salts 0·50 g, resazurin 0·001 g) was placed in each of three separate reaction vessels (working volume 300 ml) and sparged with N_2 gas (15 ml/min). The temperature of the growth medium was maintained at 37°C and the culture pH was maintained at 6·5 through automatic addition of 1 M-NaOH or 1 M-HCl.

Faecal samples were obtained from three healthy volunteers (one male, two female; average age 32·7 (SD 10·9) years). None of the volunteers had been prescribed antibiotics for at least 3 months prior to donating samples or had any history of gastrointestinal disorders. The reaction vessels were inoculated with faecal slurry (100 g/l) prepared in anaerobic 0·1 M-phosphate buffer, pH 7·0, to give a final concentration of 50 g/l in the culture vessel. After inoculation, chemostats were left for 24 h to equilibrate before the medium pump was switched on. Sterile medium was added from a 5 litre reservoir vessel using a Watson-Marlow pump (Watson Marlow, Falmouth, UK) to regulate the flow rate. The dilution rate was set at 0·03 litres/h.

Reservoir vessels contained either basal medium with Novasoy™ (200 g genistin/kg, 10 g Novasoy™/l; ADM Nutraceuticals, Erith, Kent, UK) or the same medium containing either 10 g glucose or 10 g fructo-oligosaccharide (FOS)/l. Samples (1 ml) were removed from each reaction vessel at 0 h (*t* 0), 24 h and at steady state (approximately seven culture turnovers). Under anaerobic conditions (CO_2–H_2–N_2 (10:10:80, by vol.)), tenfold serial dilutions (from 10^1 and 10^7) were prepared using sterile, pre-reduced half-strength peptone water (9 ml); portions, placed into glass bottles, contained (per litre): peptone 1 g, cysteine-HCl 0·5 g, adjusted to pH 7·0 using 4 M-NaOH. A 20 μl sample was taken from each dilution series and plated in triplicate onto selective agars for the enumeration of predominant genera of gut bacteria. Eight purportedly selective agars were used for quantification (Table 1). In addition, duplicate samples (250 μl) were taken at 0, 6 and every 24 h thereafter for HPLC analysis of genistin and genistein. Samples were mixed with 250 μl methanol and 500 μl dimethyl sulfoxide, vortexed and stored at −20°C for analysis by HPLC. The experiment was repeated in triplicate using a faecal sample from a different subject each time.

Three-stage continuous culture (gut model)

A three-stage continuous culture system, representing conditions present in the proximal, transverse and descending colon, was used to evaluate the fermentation of genistein in the human large intestine. The system comprised a pumped cascade of three reaction vessels (working volumes 280, 300 and 300 ml). Culture pH in the three fermentation vessels was automatically adjusted to 5·5, 6·2 and 7·0

Table 1. Selective agars and their modifications as used for determining counts of major genera resident in the human large intestine

Agar*	Selectivity for genera	Additions to agar
Wilkins−Chalgren agar	Total anaerobes	−
Columbia agar	*Bifidobacterium* spp.	Glucose and agar (5 g/l), cysteine-HCl (0·5 g/l), propionic acid (5 ml/l). Adjusted to pH 5·0 using 4 M-NaOH
Brucella blood-base agar	*Bacteroides* spp.	Hemin (5 μg/ml), phylloquinone (10 μg/ml), kanamycin (75 μg/ml), vancomycin (7·5 μg/ml), laked horse blood (50 ml/l)
Rogosa agar	*Lactobacillus* spp.	Glacial acetic acid (1·32 ml/l)
Reinforced Clostridial agar	*Clostridium* spp.	Novobiocin (8 mg/l), cholistin (8 mg/l)
Azide agar	Gram-positive cocci	−
Nutrient agar	Total aerobes	−
MacConkey agar	Coliforms	−

* All agars used were purchased from Oxoid (Basingstoke, Hants., UK) and prepared according to the supplier's instructions.

respectively through the automatic addition of 1 M-NaOH or 1 M-HCl as appropriate. Each vessel was magnetically stirred and sparged with N_2 gas (15 ml/min) to maintain anaerobic conditions. The temperature of each vessel was maintained at 37°C using a circulating water-bath. The reaction vessels were inoculated with faecal slurry (100 g/l, 0·1 M-phosphate buffer, pH 7·0) to give a final concentration of 50 g/l. After inoculation, 1 ml faecal slurry was taken for the enumeration of bacteria as described earlier. The three-vessel system was left for 24 h to equilibrate before the medium pump was switched on. Sterile basal medium containing Novasoy™ (10 g/l; ADM Neutraceuticals) was added from a 5 litre reservoir vessel using a Watson-Marlow pump to regulate flow rate. The system had an overall retention time of 70 h. After steady-state conditions had been established (at least seven culture turnovers, about 490 h), samples (1 ml) were taken from the three reaction vessels for the enumeration of bacteria as described earlier. In addition, duplicate samples (250 µl) were taken from each vessel for HPLC-based analysis of genistin and genistein.

The gut model experiment was repeated as described earlier, but with the addition of FOS (10 g/l) in the basal medium to study the metabolism of genistein by human intestinal bacteria in the presence of a prebiotic. Samples were taken as described earlier for the enumeration of major intestinal bacterial genera on selective agars and for analysis of genistin and genistein by HPLC.

HPLC analysis of isoflavones

Samples from the fermentation experiments were analysed for genistin and genistein by HPLC using a method adapted from that of Wang & Murphy (1994). A Waters 600E system controller and pump coupled to a Waters 484 u.v. detector was used (Waters Ltd, Elstree, UK). The column (5 µm, 250·0 × 4·6 mm) was packed with Prodigy 5 µm optical density spheres (3) 100A (Phenomenex, Macclesfield, Ches., UK). Solvents A (1 ml glacial acetic acid/l HPLC water) and B (undiluted acetonitrile) were run using a linear gradient of 15 % B (6 min), increasing to 29 % B (30 min), 35 % B (8 min), 50 % B (5 min) and then to 90 % B (4 min). Both solvents A and B were continually gassed with He. The flow rate was 1·0 ml/min for the first 5 min; this was increased to 1·5 ml/min over 1 min, maintained for 50 min at 1·5 ml/min then returned to 1·0 ml/min for the remaining 15 min of the total run time. Each sample was spiked with an internal standard (2 mmol quercetin/l) to check for recovery and injected (20 µl) onto the column. The eluate was monitored for absorbance at 260 nm. U.v. spectra and peak areas were integrated using a Hewlett Packard 3396A integrator (Hewlett Packard, Bracknell, UK). Calibration curves for genistin and genistein (2 mmol to 125 µmol/l) were prepared, and the content of samples expressed as µmol/l fermentation medium.

Statistical analysis

Bacterial counts from single-stage chemostats were analysed using a two-tailed paired t test for differences

between initial counts and steady-state counts and differences between each treatment. Bacterial counts from the gut model were not analysed statistically as this experiment was carried out using only one subject.
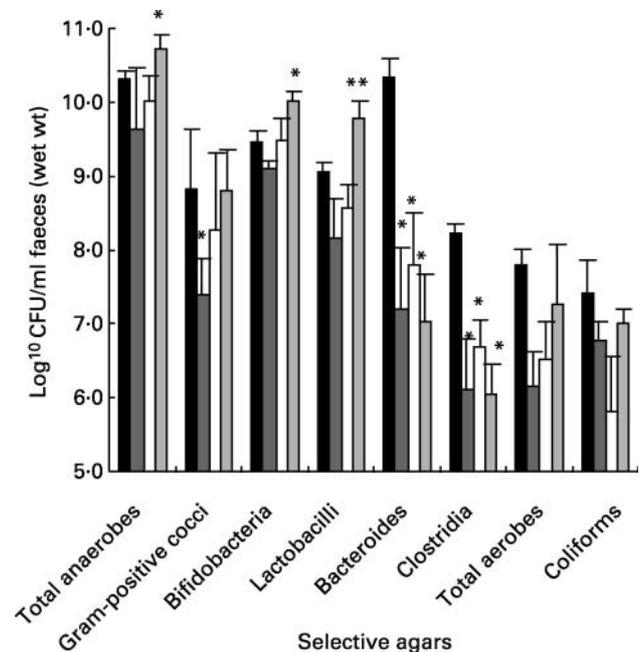
Results

### Single-stage continuous cultures with Novasoy™

Bacterial counts of major genera of human faecal bacteria, initially and at steady state, in single-stage continuous cultures using media without and with glucose or FOS, are shown in Fig. 1. Counts represent data from three separate experiments using faecal samples from three separate individuals with the standard deviation representing the variation between the experiments. Counts of total anaerobic bacteria significantly increased ($P < 0.05$) at steady state in the presence of FOS (10 g/l). Numbers of *Bifidobacterium* spp. and *Lactobacillus* spp. were also significantly higher ($P < 0.05$ and $P < 0.01$ respectively) at steady state in the presence of FOS. However, *Bacteroides* spp. and *Clostridium* spp. both showed a statistically significant ($P < 0.05$) decrease in counts under all substrate conditions tested when compared with initial counts.

HPLC analysis of isoflavones from continuous cultures

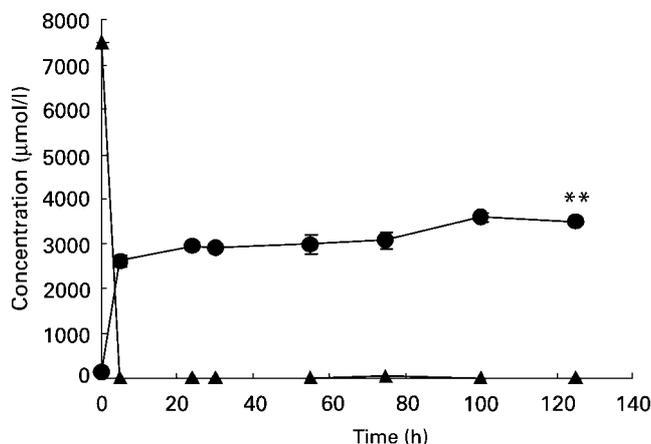Baseline analyses of each batch of basal medium with Novasoy™ (10 g/l; ADM Neutraceuticals) showed that

Fig. 1. Colony counts of major genera of human faecal bacteria in the presence of Novasoy™ (200 g genistin/kg, 10 g Novasoy™/l; ADM Neutraceuticals, Erith, Kent, UK) in a single-stage continuous culture in the presence and absence of excess substrate (either 10 g glucose or 10 g fructo-oligosaccharide (FOS)/l). CFU, colony-forming units. ■, t 0; ■, steady-state Novasoy™; □, steady-state Novasoy™ + glucose; ▨, steady-state Novasoy™ + FOS. For details of procedures, see p. 636. Values are means and standard deviations for three experiments using faecal samples from three subjects. Mean values were significantly different from initial counts at t 0: *$P < 0.05$, **$P < 0.01$.
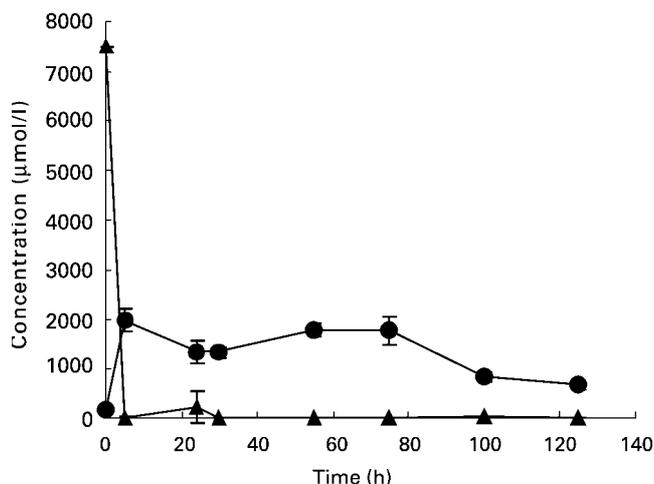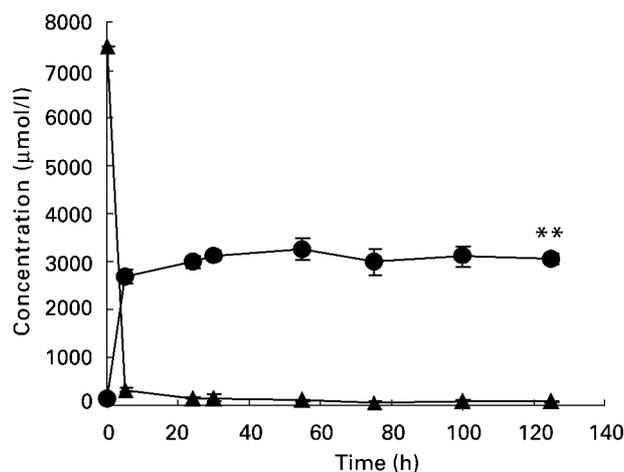
Fig. 2. Metabolism of genistin (▲) and genistein (●) from Nova-soy™ (200 g genistin/kg, 10 g Novasoy™/l; ADM Neutraceuticals, Erith, Kent, UK) in a basal medium by human faecal bacteria in pH-controlled (6·5) continuous culture over 125 h. For details of procedures, see p. 636. Values are means and standard deviations for three fermentation experiments using faecal samples from three subjects.

initial levels of genistin and genistein were 7480 (SD 250) and 145 (SD 29) μmol/l respectively. Fig. 2 shows the metabolism of genistin and genistein by human faecal bacteria in pH-controlled (6·5) continuous culture in the presence of basal medium containing Novasoy™ (10 g/l; ADM Neutraceuticals). Genistin was rapidly metabolised within the first 5 h of fermentation and was reduced to undetectable levels for the remainder of the experiment, suggesting efficient deglycosylation. Correspondingly, gen-istein increased rapidly during the first 5 h of fermentation, reaching a peak level of 1875 (SD 131) μmol/l 55 h after initial inoculation and decreased thereafter to 652 (SD 72) μmol/l 125 h after inoculation.

Fig. 3 shows the metabolism of genistin and genistein by human faecal bacteria in pH-controlled (6·5) continuous culture in the presence of basal medium containing Nova-soy™ (10 g/l; ADM Neutraceuticals) and glucose (10 g/l). As in the unsupplemented medium, genistin decreased to undetectable levels within the first 5 h after inoculation, with a corresponding increase in genistein. A peak level of 3592 (SD 90) μmol/l was reached 100 h after inocu-lation. Genistein formation was significantly higher ($P<0.01$) at all time points under these culture conditions when compared with the unsupplemented controls.

When human faecal bacteria were incubated under these conditions using media supplemented with FOS (10 g/l; Fig. 4), genistin again decreased to undetectable levels within the first 5 h. Genistein concentrations were similar to those seen with the glucose-supplemented media, and were significantly higher than those from the unsupplemen-ted control.

Three-stage continuous culture (gut model) using Novasoy™

Counts of human faecal bacteria in a pH-controlled three-stage continuous culture system with basal medium con-taining Novasoy™ (10 g/l; ADM Neutraceuticals) are shown in Fig. 5. No increase in bacterial numbers was observed in any of the three vessels compared with initial counts. In vessel one, *Bacteroides* spp. and *Clostridium* spp. decreased by 5·51 and 4·93 log counts respectively. In vessel two, counts of *Clostridium* spp. remained low at 5·68 (SD 0·61) log counts, whilst total anaerobes and coliforms were detected at 5·80 (SD 0·03) and 5·21 (SD 0·03) log counts respectively. In vessel three, *Lactobacillus* spp. showed the greatest decrease of 3·50 log counts, whilst counts of *Clostridium* spp. recovered to 7·90 (SD 0·08).

Fig. 3. Metabolism of genistin (▲) and genistein (●) from Nova-soy™ (200 g genistin/kg, 10 g Novasoy™/l; ADM Neutraceuticals, Erith, Kent, UK) with glucose (10 g/l) in a basal medium by human faecal bacteria in pH-controlled (6·5) continuous culture over 125 h. For details of procedures, see p. 636. Values are means and stan-dard deviations for three fermentation experiments using faecal samples from three subjects. Mean value was significantly different from genistein concentration shown in Fig. 2: **$P<0.01$.
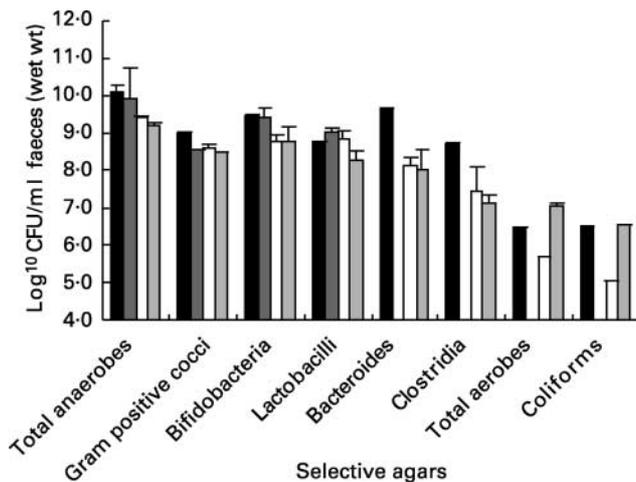

Fig. 4. Metabolism of genistin (▲) and genistein (●) from Nova-soy™ (200 g genistin/kg, 10 g Novasoy™/l; ADM Neutraceuticals, Erith, Kent, UK) with fructo-oligosaccharide (10 g/l) in a basal med-ium by human faecal bacteria in pH-controlled (6·5) continuous cul-ture over 125 h. For details of procedures, see p. 636. Values are means and standard deviations for three fermentation experiments using faecal samples from three subjects. Mean value was signifi-cantly different from genistein concentration shown in Fig. 2: **$P<0.01$.
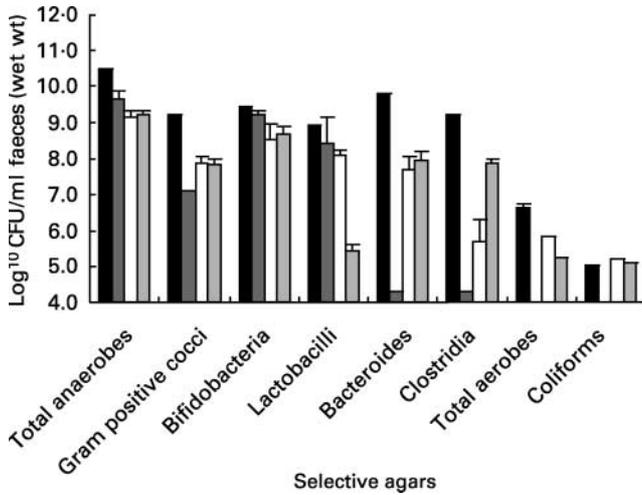
Fig. 5. Colony counts of major genera of human faecal bacteria in the presence of Novasoy™ (200 g genistin/kg, 10 g Novasoy™/l; ADM Neutraceuticals, Erith, Kent, UK) in a pH-controlled three-stage continuous culture model. CFU, colony-forming units. ■, Initial; ■, steady-state vessel one; □, steady-state vessel two; ▨, steady-state vessel three. For details of procedures, see p. 636. Values are means and standard deviations for triplicate samples using a faecal sample from one subject.

When the experiment was repeated using media supplemented with FOS (Fig. 6), the only genus to increase in counts in vessel one was *Lactobacillus* spp. (0·26 log counts). *Bacteroides* spp., *Clostridium* spp., total anaerobes and coliforms all decreased below the level of detection. Both *Bacteroides* spp. and *Clostridium* spp. recovered their counts in vessel two and vessel three, but did not increase to levels observed initially.

Fig. 6. Colony counts of major genera of human faecal bacteria in the presence of Novasoy™ (200 g genistin/kg, 10 g Novasoy™/l; ADM Neutraceuticals, Erith, Kent, UK) with fructo-oligosaccharide (10 g/l) in a pH-controlled three-stage continuous culture model. CFU, colony-forming units. ■, Initial; ■, steady-state vessel one; □, steady-state vessel two; ▨, steady-state vessel three. For details of procedures, see p. 636. Values are means and standard deviations for triplicate samples using a faecal sample from one subject.

HPLC analysis of isoflavones from the gut model

Fig. 7 shows the metabolism of genistin and genistein by human faecal bacteria in a three-stage continuous culture using basal (unsupplemented) media. In all three vessels, genistin was deglycosylated to form genistein within the
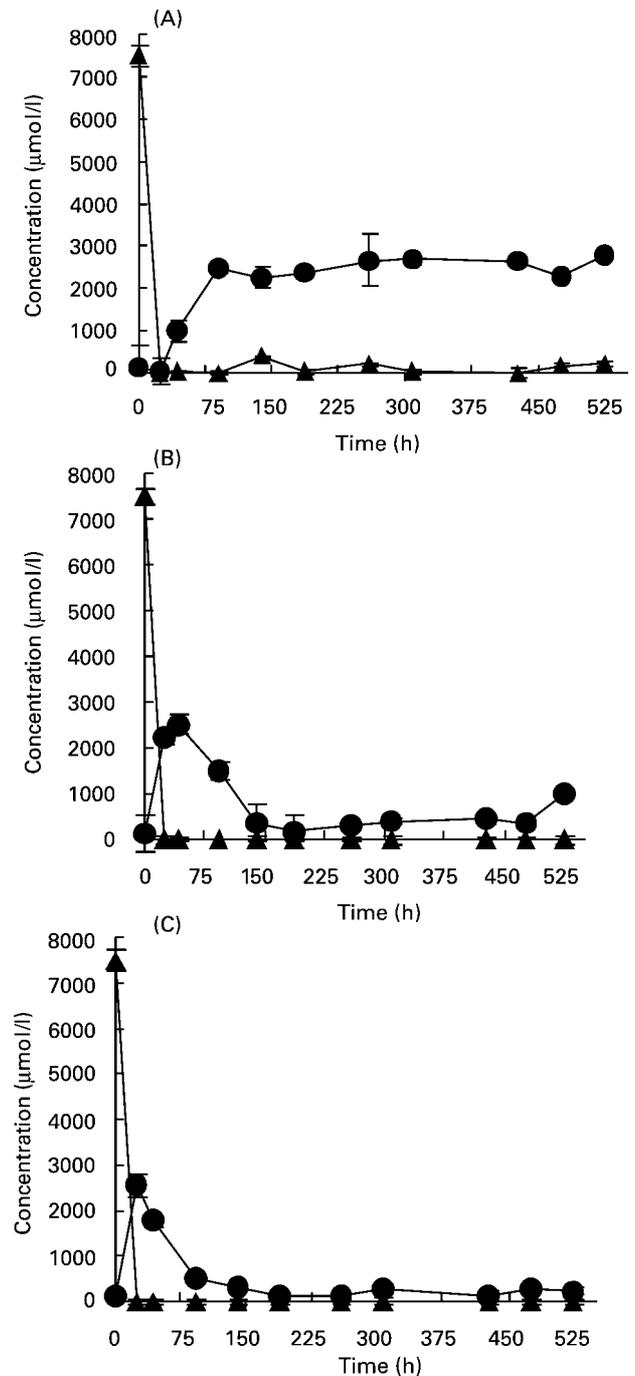
Fig. 7. Metabolism of genistin (▲) and genistein (●) from Novasoy™ (200 g genistin/kg, 10 g Novasoy™/l; ADM Neutraceuticals, Erith, Kent, UK) in a basal medium by human faecal bacteria in pH-controlled three-stage continuous culture over 525 h. (A), vessel one; (B), vessel two; (C), vessel three. For details of precedures, see p. 636. Values are means and standard deviations for triplicate samples using a faecal sample from one subject.

first 24 h of fermentation. Genistein levels in vessels one, two and three increased over the first 48 h of fermentation to 2470 (SD 110), 2522 (SD 189) and 2568 (SD 41) μmol/l respectively. In contrast to vessel one, where genistein levels remained constant between 48 and 140 h after inoculation, in vessel two they had declined to 374 (SD 29) μmol/l and in vessel three to 511 (SD 89) μmol/l.
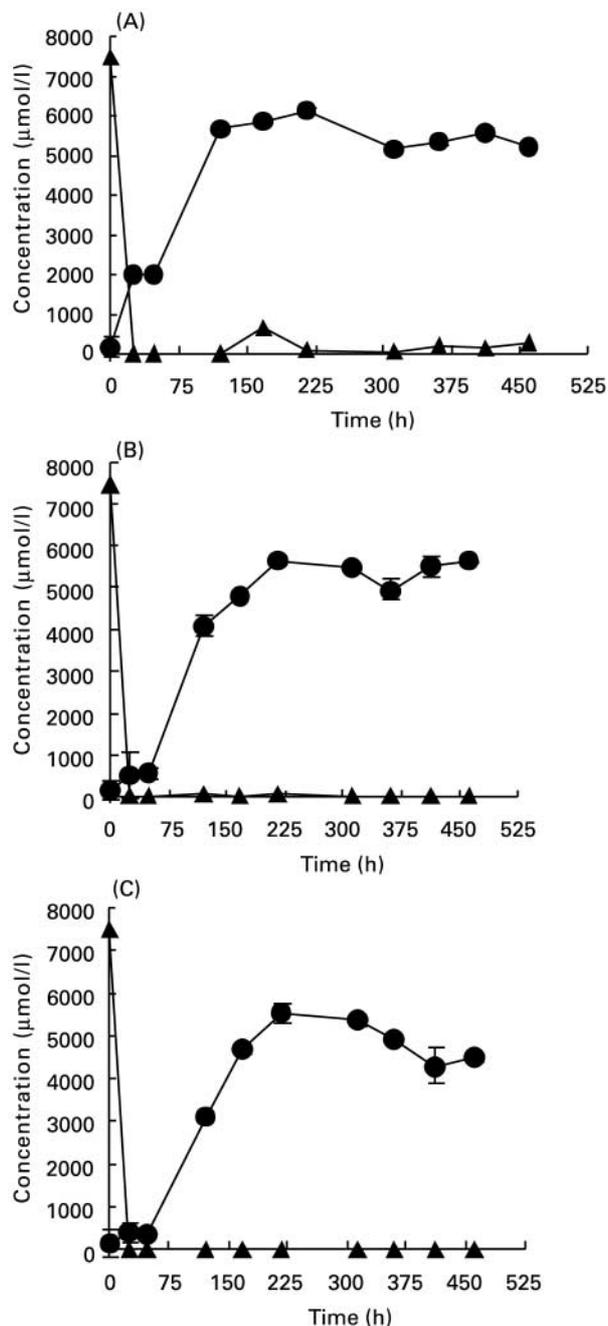
Fig. 8 shows the metabolism of genistin and genistein by human faecal bacteria in a three-stage continuous culture in the presence of FOS (10 g/l). Similar to the previous gut model experiments, conjugated forms of genistein disappeared to negligible levels within the first 24 h of fermentation in all three vessels. In vessel one, genistein maintained a much higher level during the fermentation period compared with those levels seen previously in vessel one. Genistein increased during the fermentation period to reach a maximum level of 6130 (SD 201) μmol/l 125 h after inoculation. A similar level of genistein was also observed in vessel two and vessel three, where genistein increased to 5656 (SD 43) and 5510 (SD 79) μmol/l 225 h after inoculation respectively and remained relatively stable.

Discussion

The aim of the present study was to determine the effect of prebiotics to preserve genistein in the presence of human faecal bacteria in order to maintain its purported health benefits. Novasoy™ (ADM Neutraceuticals) was used as a source of genistein in the fermentation experiments. It contained a complex mix of conjugated isoflavones (200 g genistin, 154 g daidzein and 40 g glycitin/kg), other unspecified phyto-components (400 g/kg), protein (120 g/kg) and fat (70 g/kg). Whilst the use of this substrate complicated bacterial analysis, the cost of pure genistein precluded use in such experiments.

A generally consistent pattern of bacterial counts was observed in all the fermentation experiments carried out. Populations of *Bifidobacterium* spp. and *Lactobacillus* spp. were maintained in single-stage chemostats and in the first vessel of the gut model system, whilst *Bacteroides* spp. and *Clostridium* spp. decreased. In fermentation experiments where FOS was used, an increase in *Bifidobacterium* spp. and *Lactobacillus* spp. was observed, although this was only significant in the single-stage chemostat. Such an increase was expected, since it has been well documented that FOS is selectively metabolised by such genera (Williams *et al.* 1994; Gibson *et al.* 1995; Buddington *et al.* 1996).

The rapid metabolism of genistin over the first 24 h in all fermentation experiments was not surprising. Several major groups of intestinal bacteria possess β-glucosidase activity, including *Lactobacillus* spp., *Bacteroides* spp. and *Bifidobacterium* spp. (Hawksworth *et al.* 1971; Friend & Chang, 1984). Glucose liberation during deglucosylation would also have enriched the medium for bacterial growth. In all cases, a decrease in the concentration of the glycoside (genistin) was accompanied by a concomitant rapid rise in aglycone (genistein) levels. In the single-stage continuous cultures using a basal medium, genistein concentrations then declined slowly. A similar profile

Fig. 8. Metabolism of genistin (▲) and genistein (●) from Novasoy™ (200 g genistin/kg, 10 g Novasoy™/l; ADM Neutraceuticals, Erith, Kent, UK) with fructo-oligosaccharide (10 g/l) in a basal medium by human faecal bacteria in pH-controlled three-stage continuous culture over 525 h. (A), vessel one; (B), vessel two; (C), vessel three. For details of procedures, see p. 636. Values are means and standard deviations for triplicate samples using a faecal sample from one subject.

was observed in the first vessel of the gut model chemostats, except that genistein was maintained at a slightly higher level. These results suggest two possible scenarios: either strains unable to metabolise this compound were washed out of the system whilst strains able to metabolise genistein were enriched, or the balance between genistin deglycosylation by certain genera was

slightly outweighed by degradation by the same or perhaps by other genera. In contrast, however, the genistein profiles of vessels two and three in the gut model were very different and genistein concentrations in the culture medium were rapidly reduced to reach very low levels. Initially, this may suggest that the breakdown of genistein was favoured by a higher pH. However, this is not entirely consistent with the observation that genistein levels were maintained in the single-stage chemostats at pH 6·5, and that bacterial ecology suggests that the metabolism of genistein was predominated by bacterial genera that tended to survive at a lower pH. The consistently high levels of genistein maintained in single-stage chemostats, and in vessel one of the more complex gut model, were indicative of an equilibrium being reached between the supply of isoflavonoid, deglycosylation and further metabolism.

FOS was incorporated into the growth medium for continuous cultures to assess whether isoflavone breakdown might be reduced in an environment where *Bifidobacterium* spp. and *Lactobacillus* spp. counts were elevated. Incorporation of either glucose or FOS into the basal medium significantly increased the level by which genistein was maintained in the single- and multi-stage continuous cultures, indicative of a preserving function of both glucose and FOS. There was no significant difference between the two substrates with regard to this effect. However, Xu & Thomas (1995) have suggested that due to its structure, genistein is more vulnerable to degradation than other isoflavones such as daidzein. This is supported by the observations of Griffiths & Smith (1972) who, using a rat model, demonstrated that isoflavones possessing a 5-OH group (such as genistein but not daidzein) were much more susceptible to C-ring cleavage.

Furthermore the preservation of genistein in the presence of FOS was seen in all three vessels. In vessel one, peak levels were reached 125 h after inoculation, whilst peak levels in vessels two and three were attained 225 h after inoculation. This suggests that these levels in vessels two and three were dependent upon the supply of medium from vessel one. Indeed, this would fit with the turnover time of 70 h and would indicate that FOS was not completely utilised in vessel one, but persisted through to vessels two and three, maintaining counts of *Bifidobacterium* spp. and *Lactobacillus* spp. in these vessels, whilst at the same time diverting metabolism away from genistein degradation. It might be reasonable to assume some persistence of FOS through the gut model, since several trials with human subjects (Hidaka *et al.* 1986; Mitsuoka *et al.* 1987; Gibson *et al.* 1995; Buddington *et al.* 1996) have shown an increase in faecal *Bifidobacterium* spp. when the prebiotic has been fed to human volunteers.

It is acknowledged that in the fermentation experiments carried out here, Novasoy™ (ADM Neutraceuticals) was present in excess, the bacterial exposure levels being approximately fourteen times the reported daily total isoflavone intake where soyabean products are regularly consumed in the diet (Cassidy, 1999). This could have created a stressed environment (Rumney & Rowland, 1992) and extrapolation of the results to the *in vivo* situation should be carried out with caution. However, the results suggest that *Bifidobacterium* spp. and *Lactobacillus*

spp. may be very effective deglycosylators, whereas *Bacteroides* spp. and *Clostridium* spp may play a role in degrading the aglycone further. The addition of excess substrate appeared to preserve genistein *in vitro*. In particular, the use of FOS as a substrate not only preserved genistein to a greater extent, but also conferred an extra benefit through a selective increase of beneficial bacterial genera such as *Bifidobacterium* spp. and *Lactobacillus* spp.

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