Impact of soy isoflavone treatment during menopause in the intestinal microbiota and metabolism of women. By L. Guadamuro[1], B. Mayo[1]*, A. Suárez[2], and A. B. Flórez[1], S. Delgado[1], *[1]Departmento de Microbiología y Bioquímica de Productos Lácteos, Instituto de Productos Lácteos de Asturias (IPLA-CSIC), Paseo Río Linares, s/n, 33300-Villaviciosa, Asturias, Spain, [2]Servicio de Digestivo, Hospital Universitario Central de Asturias, C/Celestino Villamil, s/n, 33006-Oviedo, Asturias, Spain*

Introduction

It is well recognized that dietary isoflavones require the action of components of the intestinal microbiota for transformation and activation (Crozier *et al.*, 2009; Kemperman *et al.*, 2010). On the other hand, as polyphenolics, isoflavones may also have an inhibitory effect on certain intestinal microbial groups. Identifying the microbial species involved in isoflavone activation and metabolism will help in designing strategies aimed to increase desirable populations, enhancing isoflavone activation and minimizing isoflavone degradation. Little is also known on the evolution of metabolic markers of (intestinal) health during isoflavone interventions. These aspects are critical for understanding the mechanisms through which their intake has beneficial effects in menopause symptoms (Bolaños *et al.*, 2010; Messina, 2010) and other diseases (Virk-Baker *et al.*, 2010; Bhupathy *et al.*, 2010).
In this study, we evaluated the effect of isoflavones on faecal microbiota composition of 18 climacteric women receiving a daily treatment for six months with a dietary supplement rich in soy isoflavones (Fisiogen, Zambon). Identification and quantification of equol, an isoflavone metabolite, in urine during the treatment was also monitored.

Material and Methods

The analysis of the microbial populations was conducted using culturing techniques (using selective and differential culture media for majority and representative bacterial groups) and the culture-independent method of denaturing gradient gel electrophoresis (DGGE). The presence of equol in urine, the most estrogenic compound derived from the metabolism of the daidzein isoflavone, was scored by an ultra performance liquid chromatography (UPLC) method.

Results and Discussion

As shown in Figure 1, culturing microbiological analysis revealed a decrease along the treatment on enterobacteria counts in most women. Furthermore, all studied bacterial populations of faeces showed a decreasing tendency in some women throughout the treatment, while no effect on bacterial population numbers was observed in samples from some others.
Molecular microbial analysis of the faecal populations by the DGGE technique using universal primers for the bacterial 16S rRNA gene showed individual and characteristic electrophoretic profiles for each woman. Figure 2 shows the DGGE profiles of two different individuals.

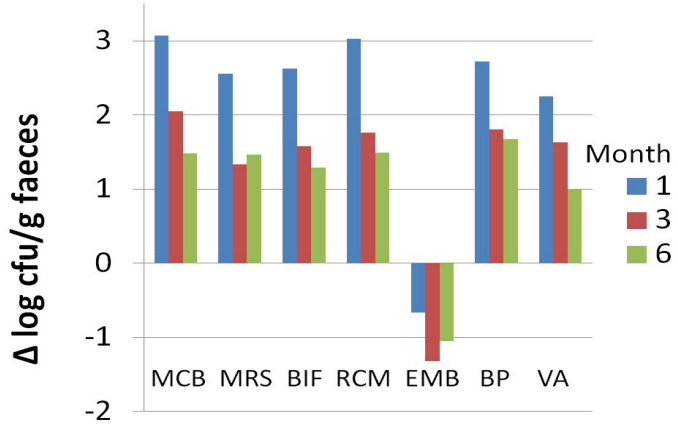

Figure 1. Changes in faecal counts (log cfu/g) of some microbial groups after 1, 3 and 6 months of isoflavone treatment. Key of media and bacterial groups: MCB, colonic microbiota; MRS, lactic acid bacteria; BIF, bifidobacteria; RCM, clostridia; EMB, enterobacteria; BP, *Bacteroides-Prevotella* group; and VA; *Veillonella*.

Intensification and/or appearance of new bands in some patients during isoflavone intake was also observed (Figure 2). Reamplification and sequencing proved these bands to belong to species such as *Ruminococcus flavefaciens* (Figure 2A), *Bifidobacterium adolescentis/ruminantium* (Figure 2B), and *Lactonifactor longoviformis* (not shown). These microorganisms might be favoured somehow by the presence of high levels of isoflavones into the gastrointestinal tract.

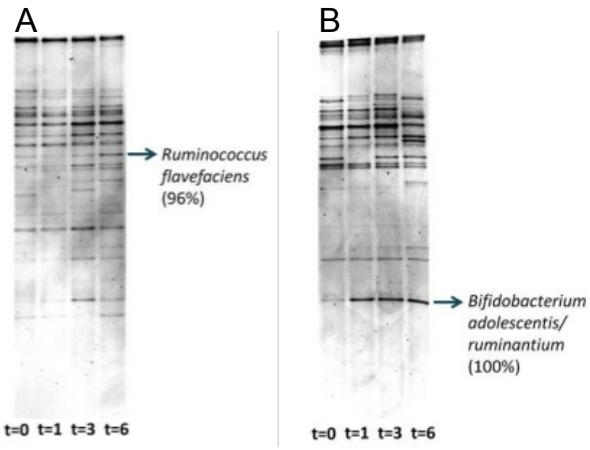

Figure 2. DGGE profiles of 16S rRNA gene amplicons from feacal samples of two different women during isoflavone treatment at time 0, and after 1, 3 and 6 months of isoflavone intake.

On the other hand, in most women it appears that the bacterial diversity decreased during isoflavone treatment. This reduction in diversity was estimated on the basis of the number of bands (assuming to be equivalent to the number of species) and their relative intensity by using the index of Shannon-Weaver (H index) (Figure 3).

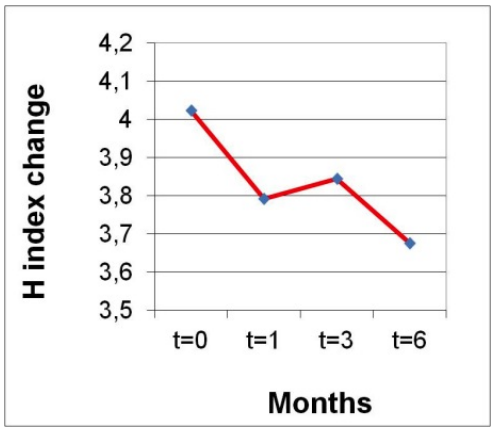

Figure 3. Variation of the Shannon diversity index (H index) in the DGGE profiles of faecal samples throughout the treatment from a volunteer, as calculated using the GeneTools software (Syngene).

By UPLC, equol was detected in the urine of 12 women. The variation of this compound in three of the women during the treatment is depicted in Figure 4. In eight out of the 12 women a low and constant equol concentration through the treatment was observed, as shown in the figure for Woman 15. However, in four women equol concentration increases significantly in urine after isoflavone intake, as compared to the basal levels (see Woman 9 and 26 in Figure 4).

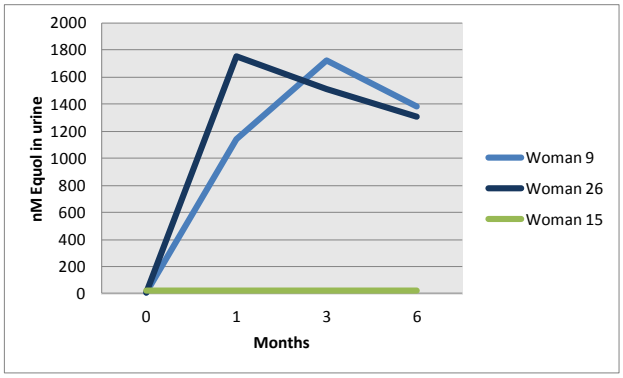

Figure 4. Variation in the level of equol in urine in three different women during isoflavone intake.

The results suggest that isoflavone intake significantly affects the numbers of some intestinal microbial populations and the level of equol in some equol-producing women. These effects might ultimately be related to the beneficial health properties of isoflavones consumption.

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