CPQ Nutrition (2021) 4:2
Review Article

CIENT PERIODIQUE

Impact of Soy Isoflavones on Gut Microbiota Dysbiosis

Cíntia Rabelo e Paiva Caria* & Gabriela Alves Macedo

Department of Food and Nutrition, Faculty of Food Engineering, University of Campinas, Brazil

***Correspondence to:** Dr. Cíntia Rabelo e Paiva Caria, Department of Food and Nutrition, Faculty of Food Engineering, University of Campinas, Brazil.

Copyright

Received: 11 December 2021

Published: 21 December 2021

Keywords: *Soy Isoflavones; Gut Microbiota and Dysbiosis*

Abstract

The impact of soy isoflavones on the intestinal microbiota has been investigated in different situations of dysbiosis, such as the type of diet adopted, medications taken, intestinal mucosa, immune system and the microbiota itself. Thus, we aim to describe, through a literature review, the modulation of the intestinal microbiota after consumption and/or treatment with soy isoflavones in situations of dysbiosis. Through the studies selected in this review, we could observe that soy isoflavones are able to modulate the intestinal microbiota, but the effects observed in dysbiosis are not fully understood.

Abbreviations

FSBM: fermented soybean meal
FSM: fermented soymilk
HFD: high-fat diet
LcS: *Lactobacillus casei* Shirota
LPS: lipopolysarides
NAFLD: non-alcoholic fatty liver disease
NOD: non-obese diabetic mouse model

O-DMA: O-desmethylangolensin
SBM: soybean meal

Introduction

Isoflavones are mostly found in soybeans, characterizing them as soy isoflavones [1]. When consumed they can be rapidly hydrolyzed by gut bacteria into aglycones that are partially absorbed by the gut epithelium [2]. Epidemiological studies show that a proper level of isoflavones consumed in the diet can prevent diseases like cancer or diabetes type II [3], although there are some controversies along these findings [1]. Moreover, isoflavones bioavailability is important to reach the benefits and food processing can change isoflavones profile interfering in their absorption [4]. Also, the quantity and quality of gut microbiota can result in a dysbiosis state and it is not known how and how much dysbiosis participate in the xenobiosis in the presence of soy isoflavones [5].

Materials and Methods

This study has a descriptive character and was carried out through a literature review, which aims to describe the modulation of intestinal microbiota after treatment and/or consumption of soy isoflavones in different situations of dysbiosis. A search was performed in the National Library of Medicine using the descriptors "soy isoflavones" and "gut microbiota". All articles considered relevant to the topic discussed were selected for discussion in this review.

Results and Discussion

Isoflavones Metabolism and Bioavailability

It is known that the human gut microbiota is involved in the degradation of several polyphenolic compounds that are consumed in the diet, including flavonoids, flavanones, flavan-3-ools, anthocyanidins, isoflavones, flavones, tannins, lignans and chlorogenic acids. The level of biotransformation of these compounds depends on their structural specificity, as well as on the interindividual richness of the gut microbiota [6]. Flavonoids and their derivatives, for example, are most absorbed by the intestine [6], while conversion of isoflavones, which have large hydrophilic structures, are not easily absorbed by intestinal absorptive cells [7].

For this (Figure 1), the primary metabolites of isoflavones are metabolized by the liver, while the unabsorbed portion is hydrogenated by glucosidases, hydrolytic enzymes produced by the colonic gut microbiota. Then, the complex isoflavone molecules, that are glycosidic forms (daidzine, genistin and glycite) are broke down into aglycone forms (daidzein, genistein and glycitein), that is, in smaller molecules that can be absorbed in the intestine and, finally, metabolized into their final active metabolites [2,8]. Then, these products are absorbed through the portal vein and travel to other tissues and organs, providing several metabolic actions, such as, for example, the estrogenic [9,10], anti-androgenic and hypolipidemic activity of aglycone equol [7].

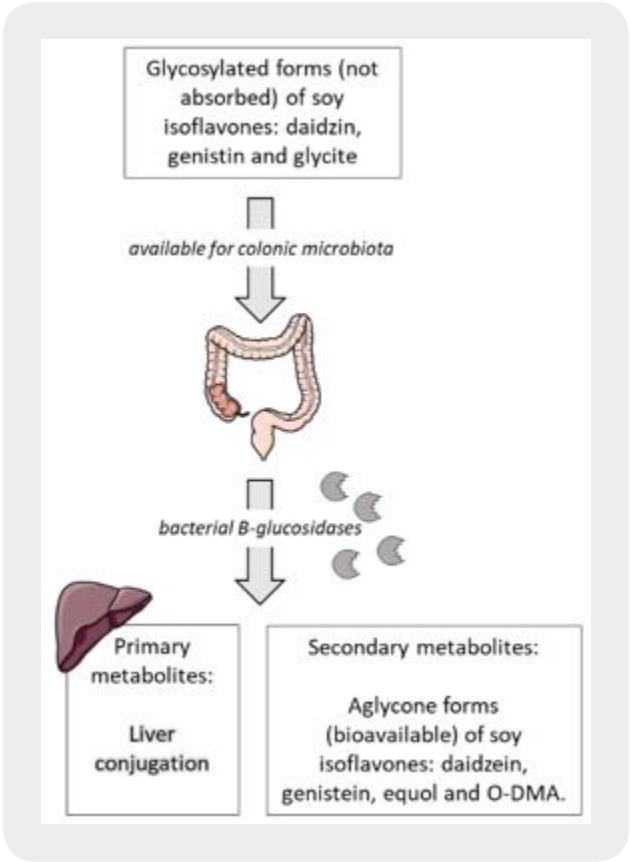

Figure 1: *Metabolism of isoflavones*

It is estimated that there are trillions of microorganisms inhabiting the human body [11], with approximately one thousand different species found in the human large intestine [12]. Through fermentation of the gut microbiota, the relationship between beneficial bacteria (*Bifidobacterium spp.* and *Lactobacillus spp.* for example) and harmful bacteria (*Clostridium* for example) produces a variety of products that can have positive or negative effects on the intestine, as well as to influence various systemic responses [8]. Beneficial effects for health promotion can be observed as a result of a symbiotic relationship between the intestinal microbiota and its host, while a dysbiotic relationship can result in the development of inflammatory diseases and even cancers [11].

Regarding aglycones isoflavone's metabolism, several microorganisms have been implicated, such as lactic acid bacteria and *Bifidobacterium*, due to strong β-glucosidase activity. However, other species also are able to improve the bioavailability and conversion rate of isoflavones by the action of the β-glucosidase, including *Lactobacillus acidophilus, Lactobacillus casei, Lactobacillus fermentum, Lactobacillus rhamnosus, Lactobacillus plantarum, Lactobacillus bulgaricus, Bifidobacterium breve*, and *Bifidobacterium bifidum* [2].

Impact of Soy Isoflavones on Gut Microbiota

The gut microbiota maintains a symbiotic relationship with its host, interfering in the function of the metabolism of nutrients, xenobiotics and drugs, maintaining the structural integrity of the intestinal mucosa barrier, immunomodulation and protection against pathogens [7]. For the best performance of the gut microbiota functions, a stable cellular composition is required, consisting mainly of bacteria from the phyla Bacteroidetes, Firmicutes, Actinobacteria and to a lesser extent, Proteobacteria [13].

However, many factors can interfere negatively in bacterial population presents in the gut, as example, stress conditions, type of diet adopted, ingested drugs, the intestinal mucosa, the immune system, and the microbiota itself. The combination of these factors, in addition to the presence of some diseases, such as inflammatory bowel diseases and metabolic disorders, are associated with intestinal dysbiosis [13]. Dysbiosis of gut microbiota is characterized by the reduction of microbial diversity and beneficial bacteria, beyond the growth of harmful bacteria [5], leading to a disease-promoting imbalance [13]. Thus, identifying the bacterial profile in different situations can contribute to a better understanding of the impact of the intestinal microbiota on health and disease.

Regarding soy isoflavones, the gut microbiota is important in the bioavailabity and biological activities of isoflavones and influences the host's metabolic profile after consumption [6]. In healthy volunteers, a single-dose of soybean meal (SBM) and fermented soybean meal (FSBM) biscuits, containing isoflavones (0.44 mg/kg of body weight), increased aglycones metabolites in urine in both treatments, being FSBM 54% higher than SBM biscuits. The FSBM biscuits were also able to excret all metabolites more rapidly than SBM and urinary recovery of isoflavones was 67% higher after FSBM biscuits consumption. From an improvement in the bioavailability of isoflavones, it was possible to suggest a reduction in the impact of the intestinal microbiota on the metabolism of isoflavones [14], (Table 1). Additionaly, healthy young volunteers that received a single dose of 30 mg of genistein in tablet, containing low fat milk and soy milk, resulted in presence of free genistein in the plasma, 7 more of its phase-II metabolites, 15 gut-derived metabolites, and a new metabolite, genistein-4 glucuronide-7-sulfate (G-4 G-7S). Despite the lower bioavailability of genistein tablet used in this study, after intake of genistein or genistin formats, it was observed the presence of dihydrogenistein and 2 (4-hydroxy phenyl) propionic acid, both considered the major phase II metabolite and gut-derived metabolite [15], (Table 1). The dihydrogenistein is an intermediate compound in the production of 5-hydroxy-equol from genistein by certain intestinal bacteria, which was not demonstrated in the Smith and collaborators` study. On the other hand, 2-(4-hydroxyphenyl) propionic has been described as a degradation compound of genistein metabolism by *Eubacterium ramulus*, through the transformation of 6-hydroxy-O-DMA into floroglucinol and 2-(4-hydroxyphenyl) propionic acid [16].

Table 1: *Findings summary from metabolism isoflavones and microbiota changes*

Type of study	Population	Control and Soy intervention groups	Duration	Main results	Microbiota changes	REF
Healthy						
Randomized, double-blind, crossover trial	18 volunteers aged 20-45 years, body mass index (BMI) between 18.5 and 25.0kg/m2 (normoweight).	Soybean meal (SBM) and fermented soybean meal (FSBM) biscuits (containing isoflavones 0.44 mg/kg of body weight)	Single-dose of soybean meal or biscuits	SBM and FSBM: ↑ Aglycones metabolites in urine, being FSBM 54% higher than SBM biscuits.FSBM biscuits: ↑ Urinary recovery of isoflavones was higher	Suggestion: Since fermentation has improved the bioavailability of isoflavones, it possibly also reduces the impact of the intestinal microbiota on the metabolism of isoflavones.	[14]
Randomized cross-over trial	12 healthy young volunteers	30 mg genistein tablet (low fat milk, and soy milk containing genistein glycosides).	Single oral dose	Presence in the plasma: Free genistein (Intact isoflavone) 7 phase-II metabolites 15 gut-derived metabolites New metabolite: genistein-4 glucuronide-7-sulfate (G-4 G-7S).	Not applicable	[15]
Experimental	Intestinal bacteria isolated from stool samples of 3 equol-producers women	Not applicable	Not applicable	73 fecal isolates were considered to harbor equol-related genes. However, genome sequences suggested a deletion in the former involving a large part of the equol operon.	W18.34a strain, identified as *Adlercreutzia equolifaciens*, as well as other strains known to produce equol, showed deletions within the equol operon	[17]

Experimental	37 strains dominant and representative bacterial populations from human gut	Isoflavone glycosides (daidzin and genistin), their derived aglycones (daidzein and genistein), and equol, against bacterial strains.	Not applicable	Isoflavone-derived compounds could modify numbers of key bacterial species in the gut	Genistein: ↓ *Bacteroides fragilis, Lactococcus lactis subsp. lactis,* and *Slackia equolifaciens* Genistein and Equol: ↑ *Lactobacillus rhamnosus* and *Faecalibacterium prausnitzii.*	[18]
Pre and Post-Menopause						
Placebo-controlled intervention trial	58 postmenopausal Japanese women aged 48 to 69 years	Self-administered questionnaires assessed their recent and remote food intake histories and lifestyle habits	Not applicable	Women equol-producing bacteria (97% of women) Equol-producers (22% of women): Greater diversity of microflora and more than 50% of equol producers were constant consumers of soy-based foods and other foods.	Equol producers: ↑ Firmicutes ↓ Proteobacteria	[19]
One-group pre-post treatment study	Eleven Tai postmenopausal women, aged more than 45 years	Single oral dose of 375 mL UHT soy milk (SOY phase) 250 mg oral Ciprofoxacin (CIPRO/SOY phase)	SOY phase (Day 0) CIPRO/SOY phase (ciprofoxacin orally after breakfast and dinner for three consecutive days)	↓ bioavailability of daidzein and genistein	Not applicable	[20]

Randomized clinical trial	60 postmenopausal women, aged 40 to 60 years.	Oral isoflavone (150 mg dry extract of glycine max) alone or isoflavone plus probiotic (*Lactobacillus acidophilus, L. casei, Lactococcus lactis, Bifidobacterium bifidum, and B. lactis*) or hormone therapy (1 mg estradiol and 0.5 mg norethisterone acetate).	16 weeks	Probiotics improved the metabolism of isoflavones, but were unable to relieve vulvovaginal symptoms in menopausal women.	Not applicable	[21]
Cross-sectional study	137 peri- and 218 post-menopausal women, aged 44–55 years	Consuming servings of soy, and were either in the menopausal transition or were postmenopausal.	At least three or more servings of soy per week	ODMA non-producer phenotype is associated with obesity in peri- and post-menopausal women Equol non-producer phenotype was not associated with obesity	Not applicable	[22]
Randomised, double-blind, placebo-controlled trial	60 healthy premenopausal Japanese women, aged 18-55 years	Fermented soymilk (FSM) with or without *Lactobacillus casei* Shirota (LcS)	4-week pre-intake period, 8-week intake period (twice a day for 8 weeks) 4-week post-intake period.	Improved skin condition ↑ levels of urinary isoflavones Changes of gut microbiota	FMS + LcS *(intake period)* ↑ Lactobacillaceae and Bifidobacteriacea FSM *(intake period)*: ↓ Enterobacteriaceae and Porphyromonadaceae	[23]

Menopausal women	18 menopausal women under treatment of menopause symptoms	80 mg of isoflavones containing genistin/daidzin in the range of 55–72%.	one tablet a day for 6 months	Equol synthesis from two primers developed: dihydrodaidzein reductase (ddr) and tetrahydrodaidzein reductase (tdr) genes tdr: all equol producers (n=3) ddr: only 2 equol producers Some non-equol producers also was detected both genes.	Not applicable	[24]
Postmenopausal women	17 postmenopausal women	Revival soy bar (160 mg of soy isoflavones and 1 g saponin)	two one-week sessions: 1ª regular diet daily excluding soy products from their diet 2ª regular diet daily with a soy diet-supplementation	Changes in fecal bacteria composition in postmenopausal women after a week of soy diet-supplementation.	After soy intake: ↑ Bifidobacterium ↓ Lactobacillus and unclassified Clostridiaceae S-(-)equol producers: ↑ Bifidobacterium, Rothia, other Bifidobacteriaceae and Actinobacteria ↓ Roseburia	[25]
Experimental	Faecal slurries from menopausal women with an equol producer phenotype	80 mg/day of an isoflavone concentrate	6 months	Bioconversion of isoflavones into equol	Enrichment of some bacterial gut, such as Collinsella, Faecalibacterium and members of the Clostridium clusters IV and XIVa.	[8]

Obesity and its complications						
Cross-sectional study	Adults aged 18–65 years in Beijing	Questionnaire about dietary intake. Isoflavones and other nutrient intakes were calculated according to China Food Composition, 2009	Information was collected by a 3-day, 24-h dietary record and food frequency questionnaire.	Equol producers showed lower prevalences of dyslipidemia, which suggests the important role that equol might play in lipid metabolism by gut microbiota.	It was identified 32 species of bacteria associated with equol production, including Adlercreutzia equolifaciens and Bifdobacterium bifidum.	[26]
Randomized, controlled, crossover design	Adults (n=17) with cardiometabolic risk	Soy nuts or control food.	four-weeks, separated by a twoweek washout.	The equol+ODMA producer phenotype reflects the composite of biochemical markers, intermediary metabolism and secondary metabolites influenced by gut microbiota profiles and does not shift markedly following soy consumption.	Not applicable	[27]
Double-blind randomized controlled trial	Healthy men aged 50–75 y who were screened to be at a 10-20% 10-y absolute risk of CVD	Soy isoflavones (80-mg aglycone equivalents) on arterial stiffness [carotid-femoral pulse-wave velocity (cfPWV)],	a soy challenge was consumed daily over 3 consecutive days	Acute soy intake improved cfPWV, equating to an 11-12% reduced risk of cardiovascular disease if sustained.	Not applicable	[28]
Obese individuals	Obese, overweight, and normal weight individuals, aged 18-95 years (n=297).	Commercial soy bar (Revival, Kernersville, NC) or one-third of a bag of soy nuts (Genisoy, San Francisco, CA)	Once per day for three days	ODMA-producer phenotype, but not equol-producer phenotype, is associated with obesity in adults.	Not applicable	[29]

Experimental	NAFLD in high-fat diet obese male Sprague Dawley rats	Fermented soy paste	9 weeks	2% Fermented soy paste improved HFD-induced lipid accumulation in the liver by activating fatty acid oxidation and modulating gut microbiota.	↑ Bacteroidetes and Proteobacteria ↓ Firmicutes	[30]
Experimental	High-fat diet obese male Sprague Dawley rats	Negative: chow diet Positive: High-fat diet for nine weeks to induce obesity Test: Obese mice + Soy isoflavone extract (150 or 450mg/kg)	4 weeks		↑ Bacteroidetes and Proteobacteria ↓ Firmicutes and Firmicutes/Bacteroidetes ratio	[31]
Experimental	High-fat diet (HFD)-induced hyperglycemic (male Sprague–Dawley (SD) rats)	Normal chow diet with 13.5% kcal fat High-fat diet (coconut oil 25%, cholesterol 2%, feed powder 73%) cooked soybean (40 mg/kg body weight/day), or probiotic fermented tempeh (40 mg/kg body weight/day)	14 weeks	The modulation of serum glucose and lipid levels occurs via alterations in the internal microbiota, leading to the inhibition of cholesterol synthesis and promotion of lipolysis.	Cooked soybean: Dominant bacteria Bacteroides changed to Prevotella	[32]
Experimental	Adult non-obese diabetic (NOD) mouse model (male and female)	soy-free diet vehicle control (VH; 25 mM sodium carbonate soy-based diet genistein (GE) at 20 mg/kg body weight by gavage daily.	5 months	↓ blood glucose level and improves glucose tolerance Produces anti-inflammatory responses Produces a perturbed gut microbiota in both males and females	GE+ NOD males: ↑ Prevotella ↓ Alistipes and Blautia GE+ NOD females: ↑ Erysipelotrichaceae ↓ Escherichia, Lachnoslira, Firmicutes and Enterococcus	[33]

Experimental	Offspring of non-obese diabetic mice (male and female)	Vehicle (0.1 ml/10 g body weight sodium carbonate) Isoflavone genistein (20 mg/kg body weight) Isoflavone genistein (20 mg/kg body weight)	Embryonic day 7 to postnatal day 21	Gut Microbiota was perturbed toward a pro-inflammatory response among female offspring, But toward anti-inflammatory response among male offspring	Female offspring: ↑ Enterobacteriales ↓ Bacteroides/Firmicutes ratio	[34]
Experimental	*In vitro* assays with RAW 264.7 murine macrophages and 3T3-L1 murine preadipocytes	Biotransformed soymilk with tannase and β-glycosidase enzymes	Not applicable	Improvement of antioxidant capacity and anti-inflammatory responses	Not applicable	[35]
Inflammatory bowel disease (IBD)						
Experimental	*In vitro* assays with Caco-2	Biotransformed soymilk with tannase and probiotic strains	Not applicable	Biotransformation: Improvement in aglycone content and antioxidant capacity Caco-2: anti-inflammatory activity	Not applicable	[36]
Cancer						
Subjects undergoing colonoscopy	40 consecutive subjects undergoing colonoscopy, of both sexes aged 50-75 years 20 with sporadic colorectal adenomas (SCA/P group) 20 without proliferative lesions (control group)	Soy derived product challenge: 4 capsules of a food supplement based on standardized soy extract in daidzein-rich isoflavones (11 mg daidzein and 4.38 mg genistein/capsule)	1 month after colonoscopy	The genus Bacteroides in the fecal samples of the control groups seems to be involved in the transformation of daidzein into equol.	Parabacteroides distasonis, Clostridium clostridioforme and Pediococcus pentasaceus only in control group Bacteroides fragilis and Prevotella melaningenica only in SCA/P fecal samples.	[37]

Experimental	Transgenic adenocarcinoma of mouse prostate Transgenic adenocarcinoma of mouse prostate (TRAMP) model	Control diet (16% of calories from fat) Control and high-fat diet (40% of calories from fat) Daidzein (3 mg of daidzein dissolved in 0.2ml of DMSO for the first 3 days, and 6mg of daidzein dissolved in 0.2 ml of DMSO on the fourth day)	22 weeks of HFD ou control diet 4 days of intervention	dietary high fat intake may promote prostate carcinogenesis via adversely affecting the gut microbiota and microbiota-mediated equol metabolism.	21 microbial phylotypes were increased and 11 phylotypes were decreased in abundance in HFD group Correlations between equol and Adlercreutzia, Ruminococcus and Rikenella.	[38]
Antibiotics						
Experimental	Antibiotics cocktail-induced pseudo germ-free in male BALB/c mice	Dietary of soymilk or fermented soymilk from *Lactobacillus rhamnosus* strain with or without antibiotic treatments	3 days of antibiotics + 28 days of antibioticsassociated with experimental diet	↑ isoflavone metabolites in urine Fermented soymilk group: - Improved fecal enzyme activity - Kept the balance of the gut microbiota when antibiotics were used.	↑ Odoribacter, Lactobacillus and Alistipes (soymilk) ↑ Bacteroides and Lactobacillus (soymilk from *L. rhamnosus* strain) ↓ in bacterial taxa (antibiotics were concomitantly administered with soymilk)	[39]
Experimental	Dinitro- fluorobenzene (DNFB)-induced contact hypersensitivity (CHS) in female BALB/c mice.	Negative: non-CHS Positive: CHS Dietary soy isoflavones with or without antibiotic treatments	3 weeks before the first DNFB challenge.	↓ contact hypersensitivity Gut microbiota influenced their suppressive activities on contact hypersensitivity.	Antibiotic-treated mice: ↑ Proteobacteria ↓ Bacteroidetes and Actinobacteria ↑ Firmicutes/ Bacteroidetes ratio	[40]
Experimental	Dinitro- fluorobenzene (DNFB)-induced contact hypersensitivity (CHS) in female BALB/c mice.	Negative: non-CHS Positive: CHS Soymilk-treated non-CHS and soymilk-treated CHS	3 weeks before the first DNFB challenge and continuing for 29 days.	↓ contact hypersensitivity ↓ changes in the gut microbiota caused by CHS	soymilk-treated non-CHS: ↑ Lactobacillaceae Turicibacteraceae levels are not affected by soymilk consumption	[41]

As previously mentioned, the positive effects of isoflavones consumption on health are highly dependent on isoflavones bioavailability, which is influenced by several factors, including dose and frequency of consumption, isoflavone food source, sex, age, intestinal microbiota composition, and gut transit time [14].

Soy isoflavones act as compounds of phytoestrogens, as they are structurally and functionally similar to estradiol, and thus bind to estrogen receptors and perform various biological activities, including the relief of symptoms associated with menopause and associated diseases [9]. In this context, as soy isoflavones interact with the signaling pathways of estrogen, the main female sex hormone, there is concern that these compounds feminize men. A recent meta-analysis examined clinical data published over the past two decades on the effects of soy intake on estrogen levels in men, demonstrating that soy intake or its isoflavones do not affect the levels of total testosterone, free testosterone, estradiol or estrone, even when exposure occurs in the long term and exceeds the amount of isoflavones ingested by Asian populations. Despite the lack of effect of soy intake and exposure to isoflavone in these reproductive hormones in men, it cannot be concluded that its ingestion has no hormonal effect, but isoflavones can also have independent biological effects on hormone levels [42].

However, the effectiveness of isoflavones is related to an individual's ability to convert their glycosylate forms into equol [8,9]. Equol is a metabolite of daidzein produced exclusively by the intestinal microbiota of only a few individuals, resulting in a wide range of beneficial health effects due to its superior nutraceutical effect compared to soy isoflavones [9,43].

A randomized clinical trial with 60 postmenopausal women showing symptoms of genitourinary syndrome, was administrated isoflavones (150mg of dry glycine extract max) in association with the use of probiotics (*Lactobacillus acidophilus, L. casei, Lactococcus lactis, Bifidobacterium bifidum* and *B. lactis*) for 16 weeks, resulted in an increase in daidzein, glycitein, equol intermediate and O-dimethylangolensin content. However, the increased content of isoflavones and their metabolites did not alleviate vulvovaginal symptoms [21], Table 1. The relationship between the gut microbiota and lifestyle habits in postmenopausal Japanese women were investigated from self-administered questionnaires, fecal microbiome analysis and urinary equol concentration. This study showed that 97% of women had equol-producing bacteria, but only 22% had equol-producers, while more than 50% of subjects with equol-producers were constant consumers of soy-based foods among other foods and showed significantly higher microbiota diversity compared with non-equol producers women, with higher percentage of Firmicutes and lower of Proteobacteria [19], (Table 1). In another study with postmenopausal women, a daily soy diet-supplementation (containing 160mg of soy isoflavones and 1g saponin) for one week, resulted in changes of fecal bacteria composition, increasing *Bifidobacterium* and decreasing *Lactobacillus* and unclassified Clostridiaceae. For equol producers women there was an increase of *Bifidobacterium, Rothia,* other Bifidobacteriaceae and Actinobacteria, and reduction of *Roseburia*, supporting the hypothesis that equol production is dependent on gut microbiota composition [25], (Table 1).

In order to detect and quantify equol-producing bacteria, a study used two sets of targeted primers involved in equol synthesis, the dihydrodaidzein reductase (ddr) and tetrahydrodaidzein reductase (tdr) genes. Both primers showed high specificity when used in the DNA of control bacteria, such as *Slackia isoflavoniconvertens, Slackia equolifaciens, Asaccharobacter celatus, Adlercreutzia equolifaciens and Enterorhabdus mucosicola*. In fecal

samples from menopausal women treated with soy isoflavone concentrate (80mg genistin / daidzine) for 6 months, the tdr gene was detected in the feces of all women producing equol, while the ddr gene was detected only in two of the three women considered to be previously detected equol producers of the molecule in urine. However, no significant increase in the number of copies of equol-related genes was observed during treatment with soy isoflavone concentrate and, moreover, in fecal samples from women, tdr and ddr genes not producing equol, suggesting that the genes used may be non-functional or possibly daidzein has been metabolized to other compounds in samples from these two women producing equol [24], (Table 1). Although this work did not relate the genes used to the treatment with the soy isoflavone concentrate, the species of bacteria *Adlercreutzia equolifaciens, Slakia isoflavoniconvertens* and *Slakia equolifaciens*, used as control of the PCR assays, as well as other species *Streptococcus intermedius, B. ovatus* , *Ruminococcus products, Eggerthella sp. Julong732* are known for the conversion of dadzein to equol by the minority of individuals, while the conversion of dadzein to O-DMA occurs from the involvement of a species of *Clostridium* by the majority of individuals [44].

In order to assess the impact of isoflavone supplementation on intestinal bacterial composition and its associated transformations, fecal samples from menopausal women with an equol-producing phenotype treated with an isoflavone concentrate (80mg/day) for 6 months were added to a model of anaerobic culture *in vitro*, resulting in the bioconversion of isoflavones into equol, as well as in the enrichment of some bacterial strains, such as *Collinsella, Faecalibacterium* and members of *Clostridium* clusters IV and XIVa [8], Table 1.

In another study involving faecal samples from three women producing equol, several bacterial strains capable of producing dihydrodaidzein and O-DMA from daidzein and dihydrogenistein from genistein were detected. However, none of the strains tested produced equol from daidzein, although the isolate W18.34a was identified as *A. equolifaciens*, a well-described equol-producing species. In this study, the authors suggested deletions within the operon equol for the strain W18.34a, among other strains that could produce equol, contributing to the argument of the coexistence of bacterial strains that produce and do not produce equol in the human intestine [17], Table 1. On the other hand, a study used 37 strains of dominant and representative bacterial populations of isoflavone glycosides from the human intestine (daidzine and genistin), their aglycone derivatives (daidzein and genistein) and equol, against bacterial strains, resulting in the modulation of the number of species essential bacterial in the intestine, such as reduction of *Bacteroides fragilis, Lactococcus lactis subsp. lactis* and *Slackia equolifaciens* from genistein, in addition to the increase in Lactobacillus *rhamnosus* and *Faecalibacterium prausnitzii* from genistein and equol [18], Table 1.

Some dietary strategies have been used in order to modulate the gut microbiota, bringing improvements to health. Probiotics, defined as bacterial food supplements, are known to modify the composition of intestinal bacterial populations and their products, maintaining, or improving health or even for therapeutic purposes [45].

The combination of probiotics to any substrate that is capable of resisting the upper gastrointestinal environment and being made available to the intestinal microbiota, stimulating the fermentation of beneficial bacteria [45], act synergistically, stimulating proliferation and/or activating the metabolism of health-promoting bacteria [46]. The consecutive association of fermented soy milk (FSM) with *Lactobacillus casei* Shirota (LcS), a known probiotic, resulted in increased levels of isoflavones in the urine and the levels

of Lactobacillaceae increased significantly and those of Bifidobacteriaceae tended to increase in healthy Japanese women in pre- menopause. In contrast, the levels of Enterobacteriaceae and Porphyromonadaceae decreased significantly during the period of ingestion in the FSM group. From these results, it was suggested that only the FSM beneficially modifies the intestinal microbiota in healthy pre-menopausal women [23], (Table 1).

On the other hand, some drugs may adversely affect the intestinal microbiota. Oral ciprofloxacin for example, commonly used to treat acute cystitis among postmenopausal women. In this context, a study investigated the effect of short-acting oral ciprofloxacin (250mg twice a day for three consecutive days) on the pharmacokinetics of isoflavone (single oral - 375mL of UHT soybeans) in healthy postmenopausal Thai women, resulting a significant decrease of dadzein and genistein. In this study, the reduction in the bioavailability of isoflavones after the use of ciprofloxacin, was associated with a presumed decrease in the amount of intestinal microbiota. However, a direct quantification of the fecal microbiota has not been performed, which could determine the impact of oral ciprofloxacin on the intestinal microbiota [20], Table 1.

The consumption of a high-fat diet or Western diet is also considered responsible for changing the intestinal microbiota generating dysbiosis, which triggers local inflammation and causes an increase in intestinal permeability, thus allowing the absorption of lipopolysarides (LPS) [47], and mucosal immune responses, contributing to the development of obesity and chronic inflammation [31]. The increase in intestinal permeability is also associated with a reduction in *Bifidobacteria spp*, bacteria known to reduce LPS levels and also improve intestinal barrier function. In addition, the high-fat diet is able to induce the proliferation of gram-negative bacteria, such as Enterobacteriaceae, which is also associated with an increase in plasma LPS [47].

A cross-sectional study with peri- and post-menopausal women, consuming at least three or more servings of soy per week, related O-DMA non-producer phenotype with obesity, however equol non-producer phenotype was not associated with obesity [32], Table 1. In Chinese adults, equol producers showed lower prevalences of dyslipidemia, which was associated with presence of 32 species of bacteria, including *Adlercreutzia equolifaciens* and *Bifdobacterium bifidum*. However, equol production was not determined by intake of soy isoflavones, suggesting that gut microbiota is critical in the equol production process [26], Table 1. ODMA-producer phenotype was also related with obesity, even after ingestion commercial soy bar for three days [29]. In addition, soy isoflavones (80mg aglycone equivalents) daily ingestion over three consecutive days, was able to reduce arterial stiffness, reducing risk of cardiovascular disease in healthy men [28]. In contrast, adults with cardiometabolic risk factors that received soy nuts for four weeks did not present any healthy benefit, but equol+ODMA producers were associated with gut microbiota profiles through of composite of biochemical markers, intermediary metabolism and secondary metabolites [27].

From animal studies, non-alcoholic fatty liver disease (NAFLD) in high-fat diet (HFD) obese male Sprague Dawley rats, 2% of fermented soy paste for 9 weeks improved HFD-induced lipid accumulation in the liver by activating fatty acid oxidation and modulating gut microbiota, increasing Bacteroidetes and Proteobacteria, and reducing Firmicutes [30], Table 1. Another study with HFD obese male Sprague Dawley rats, the treatment with soy isoflavone extract (150 or 450mg/kg) for 4 weeks also resulted in an increase of

Bacteroidetes and Proteobacteria and reduction of Firmicutes and Firmicutes/Bacteroidetes ratio [31], Table 1. Besides that, the treatment with cooked soybean (40mg/kg body weight/day) or probiotic fermented tempeh (40mg/kg body weight/day) for 14 weeks promoted lipolysis, inhibiting cholesterol synthesis and modulated gut microbiota, increasing Bacteroides and changed to Prevotella [32]. In adult non-obese diabetic (NOD) mouse model (male and female), genistein (20mg/kg body weight) daily for 5 months reduced blood glucose levels, improved glucose tolerance, produced anti-inflammatory responses and also perturbed gut microbiota in both males and females, increasing Prevotella and reducing *Alistipes* and *Blautia*. In NOD females also increased Erysipelotrichaceae and reduced Escherichia, *Lachnospira*, Firmicutes and *Enterococcus* [33]. Another study with NOD model treat with genistein (20mg/kg body weight) during embryonic day 7 to postnatal day 21, gut microbiota was perturbed toward a pro-inflammatory response among female offspring, increasing Enterobacteriales and reducing Bacteroides/Firmicutes ratio, but toward anti-inflammatory response among male offspring [34].

In an *in vitro* study, biotransformed soymilk with tannase and β-glycosidase enzymes showed antioxidant and anti-inflammatory activity in RAW 264.7 murine macrophages and 3T3-L1 murine preadipocytes. Although no reduction in lipid accumulation was observed by the 3T3-L1 adipocyte assay, biotransformed soymilk was able to reduce the expression of inflammatory cytokines, such as TNFα and IL-6, protecting cells from oxidative damage and helping to maintain health under stress inflammatory conditions [35].

In another *in vitro* study using the Caco-2 cell line, biotransformed soy milk with tanase in association with probiotic strains, resulted in an improvement in aglycones content, reduced intracellular reactive oxygen species production, as well as anti-inflammatory responses, demonstrating a positive impact of soy isoflavones present in biotransformed soy extract on antioxidant defense systems and modulation of intestinal inflammation [36].

The microorganisms present in the host can be permanent or transient residents, as well as their by-products, including toxic metabolites that can contribute to the appearance or progression of cancer in places far from where a specific microorganism resides, but can also migrate to other parts of the organism and become associated with the development of tumors. Therefore, an organism's resident microbiota plays an essential role in activating, training and modulating the host's immune response [11].

In this context, some studies have shown an association between different types of cancers being influenced by soy isoflavones consumption and gut microbiota composition. Subjects of both sexes with or without precancerous lesions, undergoing colonoscopy received four capsules of soy extract containing 11mg of daidzein and 4.38 mg genistein per capsule. Seventeen bacterial species were present in the fecal samples, while ten species belong to the *Bacteroides* genus. *Parabacteroides distasonis, Clostridium clostridioforme* and *Pediococcus pentasaceus* were identified only in control group, while *Bacteroides fragilis* and *Prevotella melaningenica* were identified only in colorectal adenomas group. Regarding the daidzein and genistein levels, no significant differences were observed between the groups, however, urinary equol were lower in colorectal adenomas subjects compared to controls [37], Table 1. The results obtained in this study suggest that the presence of *Bacteroides* genus in the fecal samples of control subjects seems to be involved in the daidzein transformation to equol, an antioxidative, anti-carcinogenic molecule, which shows the most active estrogenic properties of soy isoflavones. In the transgenic adenocarcinoma of mouse prostate model fed with

control or HFD and treat with 3mg of daidzein for the first three days and 6 mg of dadzein on the fouth day, resulted in the increase of 21 microbial phylotypes and reduction of 11 phylotypes, including decreased of equol-producing bacterium *Adlercreutzia*. This study, also revealed lower levels of equol in HFD group, suggesting that HFD may promote prostate carcinogenesis through adversely affecting equol-producing bacterium [38], Table 1. In another experimental study, the humanization of germ-free RAG2-/- athymic female mice were carried out by transplanting fecal samples from breast cancer patients before and after chemotherapy in order to investigate whether the intestinal microbiota of breast cancer patients could be modulated by genistein. For that, humanized mice were treat with genistein supplemented (special corn oil customized diet) for four weeks, resulting in the increase of *Lactococcus* and *Eubacterium* genus, confirming the hypothesis that genistein modulates the gut microbiota and may contribute on increasing the latency of breast tumor and reducing tumor growth [48], Table 1.

The use of antibiotics also dramatically affects bacterial composition and consequently isoflavone absorption and metabolism [4]. A fermented soymilk from *Lactobacillus rhamnosus* strain diet with antibiotic treatment improved fecal enzyme activity and kept the balance of the gut microbiota, increasing isoflavone metabolites in urine, besides increasing *Bacteroides* and *Lactobacillus*, and reducing in bacterial taxa in germ-free in male BALB/c mice [39], Table 1. In another study using dinitro- fluorobenzene-induced contact hypersensitivity in female BALB/c mice, dietary with soy isoflavones associated with antibiotics treatments resulted in the increase of Proteobacteria and Firmicutes/Bacteroidetes ratio, and reduction of Bacteroidetes and Actinobacteria [40], Table 1. In the early study, the same researchers investigaded changes in the gut microbiota caused by dinitro- fluorobenzene-induced contact hypersensitivity in female BALB/c mice in association of soymilk, improving contact hypersensitivity and changing the gut microbiota caused by contact hypersensitivity, increasing mainly Lactobacillaceae [41], Table 1.

Conclusions

It was possible to confirm that isoflavones can modulate the intestinal microbiota, but the effects seen on dysbiosis are not fully understood, because the amount of species that are found according both to a preexistent diet and/or after a supplementation with soy isoflavones and its subproducts. We could observe that many studies concerning the Asian diet, which is notably rich in soy products, may call our attention to the importance of isoflavones, but they are not a good parameter for studies regarding the difficulties in keeping studies patterns beyond isoflavones. A clinical randomized trial was not able to measure microbiota changes and more studies with equol producers are needed in order to characterize the responsible populations in microbiota. Experimental studies show a better controlled diet and soy isoflavones intake, thus an increase in Prevotella and a decrease in Firmicutes populations were observed in studies which verified changes in microbiota due to obesity and NAFLD. Further studies are necessary regarding cancer and inflammatory bowel disease. The use of antibiotics has a drastic effect on microbiota with or without isoflavones intake, however the presence of isoflavones decreases contact hipersensivity and keeps the balance of gut microbiota during antibiotics use.

Acknowledgements

This work was supported by Fundação de Amparo à Pesquisa do Estado de São Paulo - FAPESP, Brazil, (2019/10325-0).

Conflicts of Interests

The authors declare that there is no conflict of interest.

Bibliography

1. Setchell, K. D. R. (2017). The history and basic science development of soy isoflavones. *Menopause, 24*(12), 1338-1350.

2. Monteiro, N. E. S., Queirós, L. D., Lopes, D. B., Pedro, A. O. & Macedo, G. (2018). Impact of microbiota on the use and effects of isoflavones in the relief of climacteric symptoms in menopausal women - A review. *Journal of Functional Foods, 41,* 100-111.

3. Zhou, T., Meng, C. & He, P. (2019). Soy Isoflavones and their Effects on Xenobiotic Metabolism. Curr Drug Metab, 20(1), 46-53.

4. Larkin, T., Price, W. E. & Astheimer, L. (2008). The key importance of soy isoflavone bioavailability to understanding health benefits. *Crit Rev Food Sci Nutr, 48*(6), 538-552.

5. Freedman, S. N., Shahi, S. K. & Mangalam, A. K. (2018). The "Gut Feeling": breaking down the role of gut microbiome in multiple sclerosis. *Neurotherapeutics, 15*(1), 109-125.

6. Rafii, F. (2015). The role of colonic bacteria in the metabolism of the natural isoflavone daidzin to equol. *Metabolites, 5*(1), 56-73.

7. Jandhyala, S. M., Talukdar, R., Subramanyam, C., Vuyyuru, H, Sasikala, M. & Reddy, D. N. (2015). Role of the normal gut microbiota. *World J Gastroenterol, 21*(29), 8787-8803.

8. Guadamuro, L., Dohrmann, A. B., Tebbe, C. C., Mayo, B. & Delgado, S. (2017). Bacterial communities and metabolic activity of faecal cultures from equol producer and non-producer menopausal women under treatment with soy isoflavones. *BMC Microbiol, 17*(1), 93-104.

9. Queirós, L. D., Macedo, J. A. & Macedo, G. A. (2016). A new biotechnological process to enhance the soymilk bioactivity. *Food Sci Biotechnol, 25*(3), 763-770.

10. Ávila, A. R. A., Queirós, L. D., Lopes, D. B., Barina, C. G., Ueta, T. M., Ruiz, A. L. T. G., *et al.* (2018). Enhanced estrogenic effects of biotransformed soy extracts. *Journal of Functional Foods, 48*(3), 117-124.

11. Rajagopala, S. V., Vashee, S., Oldfield, L. M., Suzuki, Y., Venter, J. C., Telenti, A., *et al.* (2017). The human microbiome and cancer. *Cancer Prev Res (Phila), 10*(4), 226-234.

12. Hobden, M. R., Guérin-Deremaux, L., Commane, D. M., Rowland, I., Gibson, G. R. & Kennedy, O. B. (2017). A pilot investigation to optimise methods for a future satiety preload study. *Pilot Feasibility Stud, 17*(3), 61.

13. Weiss, G. A. & Hennet, T. (2017). Mechanisms and consequences of intestinal dysbiosis. *Cell Mol Life Sci, 74*(16), 2959-2977.

14. Silva, F. O., Lemos, T. C., Sandôra, D., Monteiro, M. & Perrone, D. (2020). Fermentation of soybean meal improves isoflavone metabolism after soy biscuit consumption by adults. *J Sci Food Agric, 100*(7), 2991-2998.

15. Smit, S., Szymańska, E., Kunz, I., Roldan, V. G., Tilborg, M. W. E. M., Weber, P., *et al.* (2014). Nutrikinetic modeling reveals order of genistein phase II metabolites appearance in human plasma. *Mol Nutr Food Res, 58*(11), 2111-2121.

16. Peirotén, A., Gaya, P., Álvarez, I. & Landete, J. (2020). Production of O-desmethylangolensin, tetrahydrodaidzein, 6'-hydroxy-O-desmethylangolensin and 2-(4-hydroxyphenyl)-propionic acid in fermented soy beverage by lactic acid bacteria and Bifidobacterium strains. *Food Chem, 15*(318), 126521.

17. Vázquez, L., Flórez, A. B., Redruello, B. & Mayo, B. (2020). Metabolism of soy isoflavones by intestinal bacteria: genome analysis of an adlercreutzia equolifaciens strain that does not produce equol. *Biomolecules, 10*(6), 950.

18. Vázquez, L., Guadamuro, L., Giganto, F., Mayo, B. & Flórez, A. B. (2017). Development and use of a real-time quantitative pcr method for detecting and quantifying equol-producing bacteria in human faecal samples and slurry cultures. *Front Microbiol, 8,* 1155.

19. Yoshikata, R., Myint, K. Z., Ohta, H. & Ishigaki, Y. (2019). Inter-relationship between diet, lifestyle habits, gut microflora, and the equol-producer phenotype: baseline findings from a placebo-controlled intervention trial. *Menopause, 26*(3), 273-285.

20. Temyingyong, N., Koonrungsesomboon, N., Hanprasertpong, N., Takuathung, M. N. & Teekachunhatean, S. (2019). Effect of short-course oral ciprofloxacin on isoflavone pharmacokinetics following soy milk ingestion in healthy postmenopausal women. *Evid Based Complement Alternat Med.,* 7192326.

21. Ribeiro, A. E., Monteiro, N. E. S., Moraes, A. V. G., Costa-Paiva, L. H. & Pedro, A. O. (2018). Can the use of probiotics in association with isoflavone improve the symptoms of genitourinary syndrome of menopause? Results from a randomized controlled trial. *Menopause, 26*(6), 643-652.

22. Miller, L. M., Lampe, J. W., Newton, K. M., Gundersen, G., Fuller, S., Reed, S. D. & Frankenfeld, C. L. (2017). Being overweight or obese is associated with harboring a gut microbial community not capable of metabolizing the soy isoflavone daidzein to O-desmethylangolensin in peri- and post-menopausal women. *Maturitas, 99,* 37-42.

23. Nagino, T., Kaga, C., Kano, M., Masuoka, N., Anbe, M., Moriyama, K., *et al.* (2018). Effects of fermented soymilk with Lactobacillus casei Shirota on skin condition and the gut microbiota: a randomised clinical pilot trial. *Benef Microbes, 9*(2), 209-218.

24. Vázquez, L., Flórez, A. B., Guadamuro, L. & Mayo, B. (2017). Effect of soy isoflavones on growth of representative bacterial species from the human gut. *Nutrients, 9*(7), 727.

25. Nakatsu. C. H., Armstrong, A., Clavijo, A. P., Martin, B. R., Barnes, S. & Weaver, C. M. (2014). Fecal bacterial community changes associated with isoflavone metabolites in postmenopausal women after soy bar consumption. *PLoS One, 9*(10), e108924.

26. Zheng, W., Ma, Y., Zhao, A., He, T., Lyu, N., Pan, Z., *et al.* (2019). Compositional and functional differences in human gut microbiome with respect to equol production and its association with blood lipid level: a cross-sectional study. *Gut Pathog, 11,* 20.

27. Reverri, E. J., Slupsky, C. M., Mishchuk, D. O. & Steinberg, F. M. (2017). Metabolomics reveals differences between three daidzein metabolizing phenotypes in adults with cardiometabolic risk factors. *Mol Nutr Food Res, 61*(1), 01.

28. Hazim, S., Curtis, P. J., Schar, M. Y., Ostertag, L. M., Kay, C. D., Minihane, M., *et al.* (2016). Acute benefits of the microbial-derived isoflavone metabolite equol on arterial stiffness in men prospectively recruited according to equol producer phenotype: a double-blind randomized controlled trial. *Am J Clin Nutr, 103*(3), 694-702.

29. Frankenfeld, C. L., Atkinson, C., Wahala, K. & Lampe, J. W. (2014). Obesity prevalence in relation to gut microbial environments capable of producing equol or O-desmethylangolensin from the isoflavone daidzein. *Eur J Clin Nutr, 68*(4), 526-530.

30. Tung, Y. C., Liang, Z. R., Chou, S. F., Ho, C. T., Kuo, Y. L., Cheng, K. C., *et al.* (2020). Fermented soy paste alleviates lipid accumulation in the liver by regulating the ampk pathway and modulating gut microbiota in high-fat-diet-fed rats. *Journal of Agricultural and Food Chemistry, 68*(35), 9345-9357.

31. Luo, Q., Cheng, D., Huang, C., Li, Y., Lao, C. & Xia, Y. (2019). Improvement of colonic immune function with soy isoflavones in high-fat diet-induced obese rats. *Molecules, 24*(6), 1139.

32. Huang, Y. C., Wu, B. H., Chu, Y. L., Chang, W. C. & Wu, M. C. (2018). Effects of tempeh fermentation with lactobacillus plantarum and rhizopus oligosporus on streptozotocin-induced type ii diabetes mellitus in rats. *Nutrients, 10*(9), 1143.

33. Huang, G., Xu, J., Lefever, D. E., Glenn, T. C., Nagy, T. & Guo, T. L. (2017). Genistein prevention of hyperglycemia and improvement of glucose tolerance in adult non-obese diabetic mice are associated with alterations of gut microbiome and immune homeostasis. *Toxicol Appl Pharmacol, 332,* 138-148.

34. Huang, G., Xu, J., Cai, D., Chen, S-Y., Nagy, T. & Guo, T. L. (2018). Exacerbation of type 1 diabetes in perinatally genistein exposed female non-obese diabetic (nod) mouse is associated with alterations of gut microbiota and immune homeostasis. *Toxicol Sci, 165*(2), 291-301.

35. Ávila, A. R. A., Queirós, L. D., Ueta, T. M., Macedo, G. A. & Macedo, J. A. (2019). Exploring *in vitro* effects of biotransformed isoflavones extracts: Antioxidant, antiinflammatory, and antilipogenic. *J Food Biochem, 43*(7), e12850.

36. Hiramatsu, E. Y., Ávila, A. R. A., Gênova, V. M., Queirós, L. D., Macedo, G. A. & Martins, I. M. (2020). Biotransformation processes in soymilk isoflavones to enhance anti-inflammatory potential in intestinal cellular model. *J Food Biochem, 44*(3), e13149.

37. Polimeno, L., Barone, M., Mosca, A., Viggiani, M. T., Joukar, F., Mansour-Ghanaei, F., *et al.* (2020). Soy metabolism by gut microbiota from patients with precancerous intestinal lesions. *Microorganisms, 8*(4), 469.

38. Liu, Y., Wu, X. & Jiang, H. (2019). High dietary fat intake lowers serum equol concentration and promotes prostate carcinogenesis in a transgenic mouse prostate model. *Nutr Metab (Lond), 16,* 24.

39. Dai, S., Pan, M., El-Nezami, H. S., Wan, J. M. F., Wang, M. F., Habimana, O., *et al.* (2019). Effects of lactic acid bacteria-fermented soymilk on isoflavone metabolites and short-chain fatty acids excretion and their modulating effects on gut microbiota. *J Food Sci, 84*(7), 1854-1863.

40. Nagano, T., Katase, M. & Tsumura, K. (2019). Impact of soymilk consumption on 2,4-dinitrofluorobenzene-induced contact hypersensitivity and gut microbiota in mice. *Int J Food Sci Nutr, 70*(5), 579-584.

41. Nagano, T., Katase, M. & Tsumura, K. (2019). Inhibitory effects of dietary soy isoflavone and gut microbiota on contact hypersensitivity in mice. *Food Chem, 272,* 33-38.

42. Reed, K. E., Camargo, J., Hamilton-Reeves, J., Kurzer, M. & Messina, M. (2021). Neither soy nor isoflavone intake affects male reproductive hormones: An expanded and updated meta-analysis of clinical studies. Reproductive Toxicology, 100, 60-67.

43. Lopes, D. B., Avila, A. R. A., Queiros, L. D., Macedo, J. A. & Macedo, G. A. (2016). Bioconversion of isoflavones into bioactive equol: state of the art. *Recent Pat Food Nutr Agric, 8*(2), 91-98.

44. Rowland, I., Gibson, G., Heinken, A., Scott, K., Swann, J., Thiele, I., *et al.* (2018). Gut microbiota functions: metabolism of nutrients and other food components. *Eur J Nutr, 57*(1), 1-24.

45. Gibson, G. R., Hutkins, R., Sanders, M. E., Prescott, S. L., Reimer, R. A., Salminen, S. J., *et al.* (2017). Expert consensus document: The International Scientific Association for Probiotics and Prebiotics (ISAPP) consensus statement on the definition and scope of prebiotics. *Nat Rev Gastroenterol Hepatol, 14,* 491-502.

46. Kolida, S. & Gibson, G. R. (2011). Synbiotics in health and disease. *Annu Rev Food Sci Technol, 2,* 373-393.

47. Bibbò, S., Ianiro, G., Giorgio, V., Scaldaferri, F., Masucci, L. & Gasbarrini, A. (2016). The role of diet on gut microbiota composition. *Eur Rev Med Pharmacol Sci., 20*(22), 4742-4749.

48. Paul, B., Royston, K. J., Li, Y., Stoll, M. L., Skibola, C. F., Wilson, L. S., *et al.* (2017). Impact of genistein on the gut microbiome of humanized mice and its role in breast tumor inhibition. *PLoS One, 12*(12), e0189756.