Scientific Evidence for the Beneficial Effects of Dietary Blueberries on Gut Health: A Systematic Review

Ceres Mattos Della Lucia[1,2], **Livya Alves Oliveira**[2], **Kelly Aparecida Dias**[2], **Stephanie Michelin Santana Pereira**[2], **Aline Rosignoli da Conceição**[2], **Pon Velayutham Anandh Babu**[1,*]

[1]Department of Nutrition and Integrative Physiology, College of Health, University of Utah, Salt Lake City, Utah 84112, USA.

[2]Department of Nutrition and Health, Universidade Federal de Viçosa, Viçosa, Minas Gerais, Brazil.

Abstract

Emerging evidence indicates the association between an unhealthy gut and chronic diseases. A healthy gut comprises an intact gut epithelium and balanced gut microbes. Diet is one of the critical factors that modulate gut health by positively or negatively affecting the intestinal barrier and gut microbes. Blueberries are an excellent source of health-promoting bioactive components, and this systematic review was conducted to evaluate the effect of dietary blueberries on gut health. A literature search was conducted on PubMed/MEDLINE, Scopus, Web of Science, and Embase databases to review relevant studies published between 2011–2022 according to the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines. The Systematic Review Centre for Laboratory Animal Experimentation's (SYRCLE) risk-of-bias tool was used for methodological quality assessments. Sixteen studies included from four countries were reviewed and the results were synthesized narratively. Our data analysis indicates that blueberry supplementation improves gut health by improving intestinal morphology, reducing gut permeability, suppressing oxidative stress, ameliorating gut inflammation, and modulating the composition and function of gut microbes. However, there are significant knowledge gaps in this field. These findings indicate that further studies are needed to establish the beneficial effects of blueberries on gut health.

*Correspondence to: Pon Velayutham Anandh Babu, Department of Nutrition and Integrative Physiology, College of Health, University of Utah, Salt Lake City, Utah 84112. Tel: +1-801-581-8376, anandh.velayutham@utah.edu.

1. Introduction

Emerging evidence indicates the importance of gut health in preventing several chronic diseases such as Alzheimer's, cardiovascular disease, diabetes, inflammatory bowel disease, and colon cancer [1]. A healthy gut is characterized by an intact gut epithelium with a thick mucus layer [formed by tightly connected intestinal epithelial cells (IEC)] and balanced gut microbes [1, 2]. The intestinal barrier regulates barrier permeability (by tight-junctional proteins and formation of a mucus layer), acts as a defense barrier against foreign substances (bacteria, toxins, and allergens), and is a modulator of the intestinal immune and inflammatory response (through secretion of cytokines, chemokines, antimicrobial peptides, and mucins) [2]. Any disruption in these factors leads to reduced tight junctions, a thinner mucus layer, gut inflammation, and dysbiosis (a shift in gut microbial balance) that are reported in the pathogenesis of intestinal disorders [1, 2]. Hence, targeting gut health may be a potential strategy to prevent and/or treat many chronic diseases.

Diet is one of the critical factors capable of modulating gut health. Different food patterns are associated with physical and biochemical changes in the gut, including gut inflammation and permeability [3]. For example, consumption of a high-fat diet (HFD) changes the gut microbial composition that subsequently alters the production of gut microbial metabolites such as short-chain fatty acids (SCFA), lipopolysaccharide (LPS), and bile acids [4]. These changes affect satiety, food efficiency, lipid metabolism, intestinal permeability, and low-grade inflammation of the host leading to an increased risk of developing various chronic diseases [5]. But consuming fruits and vegetables beneficially modulates the gut microbes and reduces the risk of chronic diseases [4–6]. Blueberries contribute significantly to the human diet and are an excellent source of health-promoting bioactive components such as anthocyanins. Anthocyanins have been widely reported for their benefits against endotoxemia, inflammation, and obesity through modulating the intestinal microbiota [7, 8].

Anthocyanins are glycosidic compounds made up of a glycan component (monosaccharide) and a non-glycan component (anthocyanidin) [9]. Cyanidin, malvidin, peonidin, and delphinidin are the major anthocyanidins found in blueberries [10]. The metabolism of anthocyanins in the gastrointestinal tract starts from the oral cavity [11]. Anthocyanins are partially hydrolyzed into aglycons in the oral cavity, a portion of anthocyanins is absorbed in the stomach (by specific transporters), and intact anthocyanins are absorbed in the small intestine (by the action of enzymes and transporters) [11]. Gut microbes play a critical role in the metabolism of anthocyanins in the large intestine [12]. Microbial glycosidases act on the glycosidic bond of anthocyanins to release the anthocyanidin from the carbohydrate component [13]. The gut microbiota uses these monosaccharides as an energy source, and the anthocyanins act as a prebiotic [14]. Additionally, microbial hydrolases act on the anthocyanidins to produce metabolites such as phenolic acids which are more easily

absorbable [15]. Blueberry bioactive components are extensively metabolized by intestinal microbiota in humans suggesting that microbiota-induced metabolites might mediate the health beneficial effects of dietary blueberry [15]. Studies from our lab and others indicate that dietary blueberries impact gut health by improving gut dysbiosis [15, 16].

The present systematic review highlights the recent developments in our understanding of the effects of dietary blueberries on gut health with special emphasis on the current knowledge on the effect of dietary blueberries on intestinal morphology, gut permeability, oxidative stress, gut inflammation, and gut microbes. In addition, knowledge gaps and challenges in this field, and future direction will be discussed.

2. Methods

2.1. Study selection

Two research questions guided this systematic review: (1) "What is the impact of dietary blueberries on gut health in preclinical models?". (2) "What are the possible molecular mechanisms involved in the beneficial effects of dietary blueberries on gut health?". This review was developed according to the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines [17].

2.2. Eligibility criteria

The present review article was developed according to the Preferred Reporting Items for Systematic Reviews and Meta-Analysis (PRISMA) guidelines, which guide studies' selection, screening, and eligibility. The participants, intervention comparators, outcomes, and study design (PICOS) criteria adopted in this study are shown in Table 1. Duplicate studies were excluded, and the search and screening for titles and abstracts were carried out by the authors, in pairs, according to the inclusion criteria (Table 1) [17].

2.3. Search strategy

Five authors (CMDL, ARC, KAD, LFM, and SMSP) independently searched for original articles using the following electronic databases: PubMed/MEDLINE (https://www.ncbi.nlm.nih.gov/pubmed), Scopus (https://www.scopus.com/home.uri), Web of Science (https://www.webofknowledge.com), and Embase (https://www.embase.com). The descriptors were structured based on search filters built for four domains: (i) blueberry, (ii) intestine, (iii) microbiota, and (iv) gut inflammation. The PubMed/MEDLINE platform filters were constructed using a hierarchical distribution of the MeSH terms (Medical Subject Headings) and by the algorithm TIAB (Title and Abstract). These filters were adapted for research in the Scopus platform, Web of Science, and Embase; however, the filter for the original article was provided by the Scopus platform (Supplementary Table S1). The search strategies were limited by the last ten years (2011 – 2022). Only articles published in English were considered in this review. The bibliographic search was performed on January 25th, 2023. The authors (CMDL, ARC, KAD, LFM, and SMSP) selected eligible studies following the analysis based on titles and abstracts. The level of agreement among these reviewers was assessed using *kappa* (0.925). The information was extracted independently and analyzed separately. Letters, reviews, book chapters,

abstracts, unpublished articles, case studies, *in vitro* studies, human interventions, studies that focused on organs other than the intestine, studies conducted with other types of berries or other associate compounds (example: green tea), and studies that evaluated the use of blueberries on diseases (example: cancer, diabetes, colitis) were excluded. Selections were then compared, and differences were resolved in consultation with another reviewer (PVAB).

2.4. Data extraction and quality assessment

After selecting the studies, the data of the publications were extracted using standardized information such as an author's name, year of publication, the country where the study was conducted, animal model (age, sex, number of animals per group), study protocol, follow-up, intervention characteristics (dosage and form of blueberries), and outcomes (primary and secondary). After the data extraction step, the researchers compared the data to ensure integrity and reliability.

2.5. Study risk-of-bias assessment

The methodological quality of the included studies was assessed. The risk of bias was verified using the Systematic Review Center for Laboratory Animal Experimentation Risk of Bias (SYRCLE RoB) tool, which helped to identify study quality and measure the bias in research involving animal studies [18]. The SYRCLE RoB toll considers ten entries that are related to six types of bias: selection bias, performance bias, detection bias, attrition bias, reporting bias, and others. For each included study, the six bias types were classified as "high" [+], "low" [−] or "unclear" [?] (Figure 2).

3. Results

3.1. Study characteristics

The flowchart with the number of selected and excluded articles in each stage was built according to the PRISMA guidelines (Figure 1). After searching PubMed, Scopus, Web of Science, and Embase, we have identified 1017 articles. We have posteriorly excluded 438 duplicates and then excluded 552 articles after reading the titles and abstracts. We also excluded studies that involve inflammatory mouse disease mouse model mdr1a−/− or induced colitis model (2 manuscripts), did not assess parameters of interest (3 manuscripts) and do not have full texts (6 manuscripts). We have performed the citation search to identify other relevant studies but none met the eligibility criteria. Finally, we have included 16 articles for this systematic review.

3.2. Description of included studies

The studies included in this article [n=16] were performed in four different countries that include China [n=7] [19–25], USA [n=4] [16, 26–28], Canada [n=3] [29–31], Ukraine [n=1] [32] and Sweden [n=1] [33]. Regarding the animal model used in the studies, 11 of them were performed with mice [19–23, 25, 28–31, 33] and 5 were performed with rats [16, 24, 26, 27, 32]. Most studies used male animals [n=14] [16, 19–33] and two used both male and female animals [24, 32]. The initial age of the animals ranged from 1 week to 24 months old, although one study did not mention this information [16]. The studies' main

characteristics were chronologically organized by the publication year, starting with the first published and shown in Table 2.

3.3. Key findings on the impact of dietary blueberries on gut health

In this systematic review, the impact of dietary blueberries on gut health was assessed based on (i) intestinal morphology and permeability, (ii) intestinal inflammation and oxidative stress, and (iii) gut microbiota (microbial composition, short-chain fatty acids, and microbial metabolic pathways) which are summarized in Table 3.

3.3.1. Effect of dietary blueberries on Intestinal morphology and gut permeability—Two studies [n=2; 12.5%] evaluated the effect of dietary blueberries on the morphology of intestinal crypts and villi. Blueberry supplementation restored gastrointestinal integrity and increased ileal villus length in HFD-fed mice [16]. Consistent with this study, fermented blueberry pomace (FBP) supplementation was shown to improve the integrity and orderly arrangements of villi in the HFD-fed mice that is associated with an increase in the villus length and the ratio of the villus to the crypt [21]. In addition, two studies [n=2; 12.5%] evaluated the crypt's depth [30, 31]. However, no difference in crypt depth was found between HFD-fed animals supplemented with blueberries and the HFD control group in these studies.

Four studies [n = 4; 25%] indicated an alteration in intestinal permeability with dietary blueberry supplementation [19, 21, 23, 30]. Among these, three studies showed positive effects [19, 21, 23]. Guo *et al.* (2019) showed that blueberry supplementation increases mRNA expression of occludin and tight junction protein 1 (TJP1) both in the ileum and colon alleviating the increased permeability of the colon and distal ileum. Cheng *et al.* (2020) also observed that blueberry supplementation increases the mRNA expression of structural tight junctional proteins (ZO-1, claudin-1, claudin-4 and occludin), adherens junction protein (E-cadherin), and mucins as well as increased gut barrier function as compared to HFD-fed control group. Similarly, Si *et al.* (2021) observed that the administration of blueberry anthocyanin-rich extracts improves HFD-induced damage in colonic mucosal structure as shown by neatly arranged colonic epithelial cells and increased mucosal layer thickness. However, Rodríguez-Daza, Daoust, *et al.* (2020) did not observe a difference in the expression of ZO-1 and occludin mRNA in high-fat and high sucralose diet (HFHS) fed mice supplemented with or without blueberry. Cheng *et al.* (2020) showed an increased barrier function through NFκB and Myosin light-chain kinase (MLCK) signaling pathways with dietary supplementation of fermented blueberry pomace.

Three studies evaluated goblet cells [n = 3; 18.75%] [16, 30, 31]. Two indicated an increase in the number of goblet cells with blueberry supplementation [16, 30]. Lee *et al.* (2018) showed that the goblet cell number per crypt was significantly higher in the HFD-fed rats supplemented with blueberry compared to the HFD-fed rats. Similarly, Rodríguez-Daza *et al.* (2020a) showed an increased number of total mucin-secreting goblet cells in mice that received the blueberry fraction rich in polymeric (PACs) compared to the HFD control group. However, Rodríguez-Daza *et al.* (2020b) did not find differences in the number of goblet cells with blueberry supplementation.

Four studies [n=4; 25%] investigated mucus protein levels and mucus layer in the gut. Two showed that blueberry extract supplementation increases mucus protein (MUC2) levels in HFD-fed mice [16, 19]. Lee *et al.* (2018) reported an increase in gene expression of MUC2 in the ileum whereas Guo *et al.* (2019) reported an increase in MUC2 levels in the colon. Furthermore, two other studies observed that mice treated with blueberry polyphenols extract had a thicker and restored mucus layer as compared to the HFD group [n=2; 12.5%] [30, 31]. Finally, one study [n=1; 6.25%] used lipopolysaccharide (LPS)-binding protein (LBP) as a proxy to assess circulating LPS concentrations [16]. This study showed that blueberry supplementation significantly reduces circulating LBP in HFD-fed mice.

3.3.2. Effect of dietary blueberries on intestinal inflammation and oxidative stress—The markers related to intestinal inflammation or oxidative stress are reported in five studies [n=6; 37.5%] included in this review [16, 19, 21, 24, 25]. Two studies [n=2; 12.5%] found a significant increase in mRNA levels of inflammation-related genes in the gut of HFD-fed animals, which was normalized by the blueberry supplementation [16, 19]. Importantly, blueberries reduced the expression of tumor necrosis factor-α (TNF-α) in the colon and ileum. On the other hand, one study [n=1; 6.25%] did not find such an effect [24]. One study [n=1; 6.25%] found a reduction in the mRNA levels of interleukin 6 (IL-6) in animals that received blueberries [19], however, two other studies [n=2; 12.5%] did not find such an effect [16, 24]. Myeloperoxidase (MPO) at inflammatory sites is released into the extracellular space after phagocyte activation and induces damage to the host tissue. Cheng *et al.* (2020) found that mice MPO activity was significantly inhibited by fermented blueberry pomace supplementation.

To assess the effects of dietary blueberries on intestinal antioxidant status, two studies [n=2; 12.5%] evaluated oxidative enzymes in the ileum and jejunum [21, 24]. Both studies found catalase (CAT) and total antioxidant capacity (T-AOC) increase in the ileum of the experimental animals with blueberry supplementation. One study observed an increase in SOD with a reduction in ileal MDA [21] though another study did not observe this effect [24]. Zhao *et al.* (2021) further evaluated the antioxidant status in the jejunum and found that blueberry treatment increased SOD and T-AOC, whereas it reduced jejunal MDA. Further, blueberry supplementation markedly down-regulated Keap1 gene transcription and up-regulated the mRNA abundance of Nrf2, mTOR, S6K1 and 4EBP1 in the jejunum and ileum compared with the control group. Furthermore, one study [n=1; 6.25%] assessed the colonic antioxidant status through levels of T-AOC, GSH, T-SOD, CAT, GSH-PX, and MDA [25]. This study observed an increase in the levels of SOD and AOC in the colon compared with the control group following the intake of blueberry extract.

3.3.3. Effect of dietary blueberries on gut microbiota

3.3.3.1. Microbial composition: The reviewed experimental studies overall demonstrated an improved gut microbiota in animals fed blueberry. One study [n=1; 6.25%] showed a significant reduction in the abundance of the genera *Lactobacillus* and *Enterococcus* in mice fed dietary blueberries [27]. However, another study [n=1; 6.25%] showed an increase in the abundance of *Lactobacillus, Roseburia, Faecalibaculum*, and *Parabacteroides* with blueberry supplementation [23]. Three studies [n=3; 18.75%] observed an expansion of

Bifidobacterium in mice fed blueberries [19, 20, 23]. Guo *et al.* (2019) observed an increase in the abundance of *Akkermansia* along with a significant decrease in the *Desulfovibrio* and *Bilophila* in the blueberry group. Further, blueberry extract administration increased the diversity of the gut microbiota and decreased the abundance of *Proteobacteria*. Huang et al. (2022) [n=1; 6.25%] also observed a decrease in *Proteobacteria* in the blueberry powder group, as well as a significant decrease in the unclassified genera from Desulfovibrionaceae and Ruminococcaceae families, and an increase in the unclassified genera from Clostridiales and Lachnospiraceae families. The abundance of *Klebsiella pneumoniae* and *Morganella morganii* decreased significantly with 0.5 mL/day blueberry juice treatment for 12 weeks in one study [n=1; 6.25%] [32]. Additionally, the abundance of *Actinomyces naeslundii* and *Bacteroides* significantly decreased whereas *E. faecalis* and *staphylococci* were increased under the influence of a blueberry juice-based diet. Two studies [n=2; 12.5%] showed a decreased abundance of *Streptococcaceae* after blueberry supplementation [22, 32]. One study [n=1; 6.25%] showed that the prebiotic-type selective effects on *Akkermansiaceae* and *Coriobacteriales_Incertae_Sedis* were enhanced by blueberry powder supplementation, but this result was not triggered by their fibrous fractions [31]. While one study [n=1; 6.25%] found a significant increase in the relative abundance of *Actinobacteria* [27], another study [n=1; 6.25%] showed that the supplementation with blueberries decreased the level of this phylum [20]. A favored in the family *Coriobacteriaceae*, *S24–7*, *Verrucomicrobia* and the order *Clostridiales* in the groups fed with wild blueberry polyphenols extract (WBE) and blueberry polyphenolic fractions (BPFs), and also an increase proportion of *Adlercreutzia equolifacens* in the mice treated with WBE and oligomeric PACs fractions (BPF 2), compared to the group that received the HFHS diet, was observed in one study [30] [n=1; 6.25%]. Finally, one study [29] [n= 1; 6.25%] showed that a high-fat, high-sucrose diet supplemented with whole blueberry powder modestly affects the gut microbiota with an increase in the abundance of *Turicibacter sp. H121*. Another study [n=1; 6.25] demonstrated that oral gavage with extract of blueberry positively regulated bacteria that were SCFAs-producing, including *Prevotella* and *Barnesiella*. Unexpectedly, the growth of *Blautia* and *Clostridium_XIVa* was inhibited in the blueberry group compared with the control group [25].

3.3.3.2.   Microbial metabolic pathways: Three studies reported the modulation of microbial metabolic pathways with blueberry supplementation. In one study [n=1; 6.25%], deep whole genome sequencing (WGS) showed that dietary blueberries increase the abundance of microbial protein families that are involved in amino acid metabolism, metal ion binding, and REDOX homeostasis [26]. Further, protein families involved with integrase/recombinase, reverse transcriptase, and transposon/transposase were at a lower abundance in the blueberry group. Two studies [n=2; 12.5%] evaluated the Kyoto Encyclopedia of Gene and Genome (KEGG) pathways to understand the effect of blueberries on microbial function [22, 27]. Lacombe *et al.* (2013) showed that the blueberry diet impacts ~9% of the KEGG metabolic pathways. Specifically, xenobiotic metabolism, benzoate degradation, and glycosaminoglycan degradation were increased, whereas bacterial invasion of epithelial cells was decreased (8-fold) with dietary blueberry. In addition, another study showed an increase in basic metabolism with blueberry extract supplementation. One study [n=1; 6.25%] determined the microbial protein functions

using Clusters of Orthologous Groups of proteins (COG) analysis [22]. In this study, 1% blueberry extract feeding showed a difference in lipid transport and metabolism, cell motility, and RNA processing and modification. Further, the 2% blueberry extract feeding group showed differences in functions, including inorganic ion transport and metabolism, translation, ribosomal structure and biogenesis, amino acids transport, and energy production and conversion. In one study [n=1; 6.25%], the metagenomic analysis revealed that the pathway related to β-lactam resistance was increased in mice fed high-fat and high-sugar diets supplemented with blueberries. However, the pathways related to pantothenate and CoA biosynthesis and the pool of carbon per folate were increased in mice fed a high-fat and high-sugar diet [29]. Further, pantothenate and CoA biosynthesis and one carbon pool by folate were associated with *Akkermansia muciniphila* and *Bacteroides thetaiotaomicron* respectively. The pathway related to β-lactam was associated with *Eubacterium plexicaudatum*. In this study, an increase in alanine, aspartate, and glutamate metabolism was associated with *Adlercreutzia* in HFHS-fed mice and an increase in oxidative phosphorylation for those fed proanthocyanidin-HFHS. However, such a difference was detected when pathway analysis was performed between HFHS-fed mice and anthocyanidin-HFHS-fed mice.

3.3.3.3.   Microbial metabolite short chain fatty acids (SCFA): Animals fed a high-fat diet decrease the production of total SCFA compared to the low-fat diet fed mice. Two studies [n=2; 12.5%] indicated that the supplementation of blueberry anthocyanin extracts reverses this decrease in SCFA [22, 23]. In addition, one study [n=1; 6.25%] showed that blueberry supplementation was associated with higher concentrations of acetate and propionate, whereas the lower concentration of butyrate as compared to the control group [16]. This is also associated with a 3-fold increase in the gene expression of SCFA receptor *Gpr43*. However, another study [n=1; 6.25%] demonstrated that mice fed an HFHS diet supplemented with blueberry for 12 weeks had lower fecal concentrations of acetic and propionic acids than the HFHS control without changes in butyrate concentration [29]. Furthermore, the concentration of valeric acid was lower in the feces of mice fed HFHS containing whole blueberry powder or anthocyanidin-rich extract. In contrast, the levels of the BCFAs isobutyric acid and isovaleric acid were decreased in the feces of mice receiving whole blueberry powder, anthocyanidin or proanthocyanidin-rich extract compared to control HFHS [29].

3.4. Risk of bias

In all the studies that were included in this systematic review [n=16], the baseline characteristics (sex, age, and initial weight of animals) were completed in five studies [n=7; 43.75%]. In none of the studies, the allocation of animals was described in detail. Three studies did not mention if the animal allocation to treatment groups was performed randomly [n=3, 18.75%]. Furthermore, none of the studies reported blinding the investigators involved in the research (Figure 2).

4. Discussion

The present systematic review evaluates the effects of blueberries on gut health focusing on studies conducted in murine models. Evidence shows that dietary blueberries improve gut health by acting on multiple targets such as (i) improving intestinal morphology, (ii) reducing gut permeability, (iii) modulating oxidative stress, (iv) suppressing gut inflammation, and (v) modulating composition and function of gut microbes (Fig. 3).

The effect of dietary blueberries on intestinal epithelial morphology was determined by assessing intestinal crypts and villi. A compartmentalized crypt-villus structure is a key factor for maintaining intestinal homeostasis and the functional capacity of the tissue. Hence, a compromised crypt-villus negatively impacts gut health [34]. Dietary blueberry was shown to increase ileal villus length, improve villi arrangement, increase the ratio of the villus to the crypt, and restore gastrointestinal integrity in mice fed HFD without altering the crypt's depth [16, 30, 31]. These intestinal morphology changes induced by blueberry anthocyanins could result in a better absorptive function, improving absorption surface, brush border enzyme expression, and nutrient transport [35].

The intestinal barrier depends on interactions among barrier components, including the mucus layer, immunoglobulin A, antibacterial peptides, and intercellular tight junctions [36]. The tight junctions complex are cells attached by a protein complex and constitute the major determinant of the intestinal physical barrier, regulating the paracellular permeability of water, ions, and macromolecules [37]. The tight junctions structure consists of transmembrane proteins, such as claudins, occluding, and zonula occludens. Damage to these proteins increases the paracellular permeability resulting in the permeation of pro-inflammatory molecules, forcible activation of immune cells and inflammation [36–38]. The beneficial effect of blueberries on intestinal permeability was reported in HFD-fed mice [19, 21, 23]. These studies demonstrate an increase in the expression of structural tight junctional proteins, adherens junction protein, and mucins which are associated with an increase in mucosal thickness and gut barrier function. However, blueberry supplementation was ineffective in improving the expression of junctional proteins such as ZO-1 and occludin in mice fed high fat and high sucralose diet (HFHS) [30]. The discrepancy could be due to the difference in the dietary model (HFD vs. HFHS) used to induce damage to the colon. The possible mechanism suggested for the effect of blueberry on gut barrier function is nuclear factor-kappa B (NFκB) and myosin light-chain kinase (MLCK) signaling pathways [21]. Anthocyanins exhibit anti-inflammatory as shown by a decrease in the gene expression of pro-inflammatory cytokines that damage intestinal barriers such as TNF-α. This effect is mediated through the inhibition of NFκB via regulation of the I-Kappa-B-alpha (IκBα) phosphorylation [35, 39].

Goblet cells in the IEC are responsible for the synthesis and secretion of mucins which is an important component of the mucus layer of ICE [40]. Mucins are highly glycosylated proteins that provide luminal protection of the gastrointestinal tract [16]. Hence, qualitative and quantitative changes in mucin are associated with several gastrointestinal conditions including inflammatory bowel disease, colorectal cancer, and enteric infections [40]. Blueberry increased goblet cells in animals fed HFD [16, 30]. Blueberry also increased

mucus protein MUC2 (the primary glycoprotein of the gastrointestinal mucus layer) and established a thicker mucus layer in animals fed HFD [16, 19, 30, 31]. These studies indicate the beneficial effects of blueberries on the mucus layer.

The gut is a key resource of reactive oxygen species as the intestinal tract is frequently exposed to foreign substances and microbial pathogens. The gut is vulnerable to damage from oxidative stress, which can lead to intestinal dysfunction and subsequent impairment of nutrient metabolism [41]. Thus, maintaining the proper functioning of the antioxidant defense system in the gut is essential. Indeed, pathogen sensing and intestinal homeostasis are important physiological functions of the intestine. An imbalanced reactive oxygen species and the resulting oxidative stress play a critical role in intestinal injury that activates intestinal barrier dysfunction leading to gut inflammation. This is implicated in several complications including inflammatory bowel diseases, enteric infections, and colorectal cancer [42]. The effect of dietary blueberries on intestinal antioxidant status was determined by assessing antioxidative enzymes, Nrf, and antioxidant capacity in the ileum and jejunum. Blueberry supplementation increases antioxidant enzymes (catalase and superoxide dismutase), Nrf2, and total antioxidant capacity in experimental animals [21, 24]. Malondialdehyde (MDA) is one of the lipid peroxidation products widely used as an indicator of cellular injury and a biomarker of oxidative stress [43]. Blueberry supplementation reduces MDA in jejunum indicating that blueberry can suppress lipid peroxidation [21, 24]. Myeloperoxidase (MPO) catalyzes the production of a number of reactive oxidant species. MPO at the inflammatory sites is released into the extracellular space after phagocyte activation and induces damage to the host tissue. Blueberry was shown to inhibit MPO [21]. Nrf2 is considered an important nuclear transcription factor that accelerates the transcription of endogenous antioxidant enzyme genes by promoting the binding of the antioxidant response element in the promoter region of the antioxidant enzyme genes [44]. Blueberry supplementation was shown to upregulate Nrf2 transcription in the jejunum and ileum [24]. This finding indicates that blueberry supplementation can increase jejunal and ileal antioxidant capacity by regulating the Nrf2-Keap1 signaling pathway. The beneficial effects of the blueberry on antioxidant enzymes may be partially attributable to the upregulation of Nrf2 signaling. These studies indicate the beneficial effects of dietary blueberries on oxidative stress in the gut.

Cytokines play important roles in the regulation of intestinal epithelial homeostasis during gut inflammation. IL-6, interferon-γ (IFN-γ) and TNF-α are known to negatively regulate the barrier and self-renewal properties of the intestinal epithelium, thus modulating epithelial homeostasis and exacerbating mucosal inflammation [45]. Blueberry supplementation was shown to decrease the pro-inflammatory factors such as TNF-α, IL-6, and IFN-y in the colon and ileum of HFD-fed animals [19]. These findings suggest that blueberry supplementation may protect the intestine from oxidative stress and associated inflammation. However, another study did not report such an effect of blueberry on oxidative stress and gut inflammation [24].

The human microbiota is a complex ecosystem that colonizes the gastrointestinal tract with a greater distribution in the colon (10^{14} cells per gram of feces). This ecosystem consists of bacteria (more than 1000 identified species), archaea, fungi, and viruses that

are 1.3 times larger than the number of eukaryotic cells in the human body [46]. Diet influences the development, composition, and function of the gut microbiota [15, 47, 48]. Studies included in this systematic review have demonstrated that blueberry polyphenols modulate the composition of gut microbiota at different taxonomic levels in HFD-fed animals. Among the bacterial phyla known for being most prevalent and responsible for more than 160 species are Actinobacteria, Akkermansia, Bacteroidetes, Firmicutes, and Proteobacteria [49]. Actinobacteria play a key role in maintaining gut homeostasis despite it representing a small percentage of gut microbes [50]. An increase in Proteobacteria indicates gut dysbiosis and an unstable microbial community [51]. Blueberry supplementation increases the diversity of gut microbes with an increased abundance of Akkermansia and decreased abundance of Proteobacteria indicating dietary blueberries may improve gut dysbiosis [19, 27]. Blueberry supplementation also alters the gut microbial composition at the genus and species levels. Blueberry supplementation increases the abundance of *Bifidobacterium, Faecalibaculum, Lactobacillus, Parabacteroides,* and *Roseburia* whereas it reduces the abundance of *Desulfovibrio* and *Bilophila* in experimental animals [19, 20, 23]. *Bifidobacterium* and *Lactobacillus* are commensal bacteria associated with beneficial effects for the host such as reduced low-grade inflammation and improved gut barrier function [15]. The improved intestinal environment with dietary blueberries may be attributed to the increased *Bifidobacterium* and *Lactobacillus* [23]. *Faecalibaculum, Parabacteroides,* and *Roseburia* are involved in producing SFCA [23]. Specifically, *Roseburia* produces butyrate and is considered the main reason for significantly enhanced butyrate in blueberries-treated mice [23]. Further, blueberries reduce the relative abundance of *Enterococcus faecalis* [27]. *Enterococcus faecalis* was shown to induce irritable bowel syndrome (IBS) in IL-10 gene-deficient mice suggesting that certain enteric microbes tend to be more opportunistic and induce colon inflammation [52]. *Desulfovibrio* is an H2S-producing gut bacteria [19, 53]. Excess H_2S can reduce the disulfide bonds in the mucous network and increase the permeability of the intestine, thus contributing to the transposition of bacteria and their metabolites such as LPS that can trigger a systematic inflammatory response [54, 55]. Blueberry supplementation significantly reduced *Desulfovibrio* in HFD-fed mice [19].

The impact of dietary blueberries on microbial metabolite SCFA is also reported in several studies included in this systematic review. Blueberry anthocyanin was shown to reverse the decrease in SCFA caused by HFD feeding [22, 23]. The alterations in gut microbiota and SCFA production revealed the role of blueberry in improving the intestinal barrier action and the healthy colonic environment, which further benefits the lipid metabolism and the defensive capability of the whole body [23]. The gut microbiota can increase glucose uptake from the small intestine and directly produce SCFA such as acetate, propionate, and butyrate by energy metabolism following the fermentation of carbohydrates in the colon [56]. The increased SCFAs maintained the stable colon environment (such as pH) and further improved the colonic integrity and mucosa functions, preventing harmful bacteria and toxins from entering the bloodstream. Additionally, SCFAs could also participate in lipid metabolism and appetite regulation in peripheral organs and hypothalamus regions [57, 58]. However, modulation of the microbiota can modify the type and amount of SCFA produced. Blueberry supplementation was associated with higher concentrations of acetate and propionate in HFD-fed mice which is also associated with an increased expression

of SCFA receptor Gpr43 [16]. However, blueberry supplementation reduced acetate and propionate in HFHS-fed mice [29]. The difference in dietary composition (HFD vs HFHS) may have influenced the observed results. In the case of obesity, butyrate and propionate have been classified as predominantly anti-obesogenic, while acetate exerts the opposite effect. This highlights the importance of analyzing the type of SCFA produced and not just the total SCFA [59]. These studies indicate that dietary blueberries may improve gut barrier integrity by modulating SCFA production.

Diet can modify not only the composition of gut microbes but also their metabolic potential [60]. The consumption of foods rich in polyphenols can cause a bioprotective action on the microbiota since it can increase beneficial microorganisms and act on catabolic processes. This will contribute to the bioavailability of nutrients that will act on DNA repair, chromosomal maintenance, reduction of formation of free radicals, and elimination of free radicals [61]. Indeed, these changes were observed in a study where the blueberry-enriched diet increased protein families involved in biosynthetic and metabolic processes of amino acids and redox homeostasis [26]. Further, dietary blueberry was able to modify metabolic pathways related to the metabolism of amino acids, carbohydrates, and xenobiotics, demonstrating that blueberries are a good source of energy collection for the intestinal microbiota, since this comes from the host`s food consumption [22, 27]. Blueberry consumption can affect important metabolic pathways through the activity of certain gut microbes [29]. Gut microbes ferment the fiber in blueberries, which can alter the activity of bacteria responsible for the degradation of polyphenols [6]. In addition, the metabolism of polyphenols can be reduced by the binding of fibers with proanthocyanidins, resulting in a decrease in the production of bioactive anti-inflammatory metabolites [62]. Thus, gut microbes play a key role in mediating the beneficial effects of dietary polyphenols. These studies suggest that the beneficial effects of dietary blueberries on gut health could possibly be mediated by modulating gut microbial metabolism.

5. Knowledge gaps, challenges and future direction

The studies included in this systematic review indicate the beneficial effects of dietary blueberries on gut health in murine models. However, there is a significant knowledge gap in this field, and further studies are needed to establish a scientific rationale for recommending dietary blueberries to improve gut health. (1) The bioactive components of blueberries, including anthocyanins, are greatly metabolized by gut microbes, and the bioactivities are possibly mediated through their circulating metabolites. Hence, studies are needed to identify the causal association between dietary blueberries, gut microbes, blueberry metabolites, and gut health. (2) Dose and time-dependent effects of dietary blueberries are needed to identify the optimum dosage and duration to improve gut health. (3) Dietary composition has a major impact on the metabolism of anthocyanins, and understanding the role of the food matrix on the bioactivities of anthocyanins is needed. (4) Future studies using shotgun metagenomics will help to identify the influence of dietary blueberries on gut microbial metabolism and its impact on the host's gut health. (5) The transcriptomic, proteomic, and metabolomic studies will also help to demonstrate host-microbiome interaction in improving gut health. (6) Efforts toward personalized medicine will be helpful to promote the understanding of the gut microbiota's functional metabolic

capacity as a tool to design individualized treatments. (7) Murine models are a valuable tool to assess the role of diet on the gut microbiome and its impact on health. However, several factors, such as vendor and environment, affect the composition and functional potential of the gut microbes in animals [63]. This is one of the major challenges associated with the translatability of microbiome studies conducted in murine models.

6. Conclusion

Together, the studies discussed in this systematic review indicate that blueberries improve gut health possibly through modulating intestinal morphology, reducing gut permeability, modulating oxidative stress, ameliorating gut inflammation, and improving gut microbes. However, there are significant knowledge gaps in this field. Hence further studies are needed to establish the beneficial effects of blueberries on gut health.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Funding

Dr. Ceres Mattos Della Lucia

Dr. Ceres Mattos Della Lucia is an Adjunct Professor in the Department of Nutrition and Health at the University of Viçosa, Brazil. Dr. Della Lucia's research is focused on the nutritional and functional value of foods and diets, working mainly on the following topics: development and validation of methods for analyzing vitamins and other bioactive compounds in foods, control of vitamin losses in fruits and vegetables, the impact of food fortification on population groups, new products development, and the association between phenolic compounds and gut microbiota.

Dr. Anandh Babu Pon Velayutham is an Associate Professor in the Department of Nutrition and Integrative Physiology at the University of Utah, USA. Dr. Velayutham's research is focused on two main projects. (1) Identifying the vascular effects of dietary

berries with special emphasis on the diet-derived microbial metabolites and the molecular mechanisms involved. These studies will provide key insights toward understanding the causal association between dietary berries, gut microbiome, berry-derived microbial metabolites, and vascular health. (2) Identifying the gut microbial taxonomic and functional aspects in adolescents with type 1 diabetes and their association with dietary and clinical factors.

ABBREVIATIONS

BPFs	blueberry polyphenolic fractions
CAT	catalase
GSH-PX	glutathione peroxidase
GSH	glutathione
HFD	high-fat diet
HFHS	high fat high sucrose diet
ICE	intestinal epithelial cells
IFN-γ	interferon-γ
IL-6	interleukin-6
IκBα	I-Kappa-B-alpha
LBP	lipopolysaccharide (LPS)-binding protein
LPS	lipopolysaccharide
MDA	Malondialdehyde
MLCK	Myosin light-chain kinase
MPO	Myeloperoxidase
MUC2	Mucin 2
PICOS	participants, intervention comparators, outcomes, and study design
PRISMA	Preferred Reporting Items for Systematic Reviews and Meta-Analyses
SCFA	short chain fatty acids
SOD	Superoxide dismutase
SYRCLE-RoB	Systematic Review Centre for Laboratory Animal Experimentation Risk of Bias

SYRCLE	Systematic Review Centre for Laboratory Animal Experimentation
T-AOC	Total Antioxidant Capacity
TJP1	tight junctional protein 1
TNF-α	Tumor necrosis factor alpha
WBE	wild blueberry polyphenols extract
ZO-1	Zonula occludens - 1

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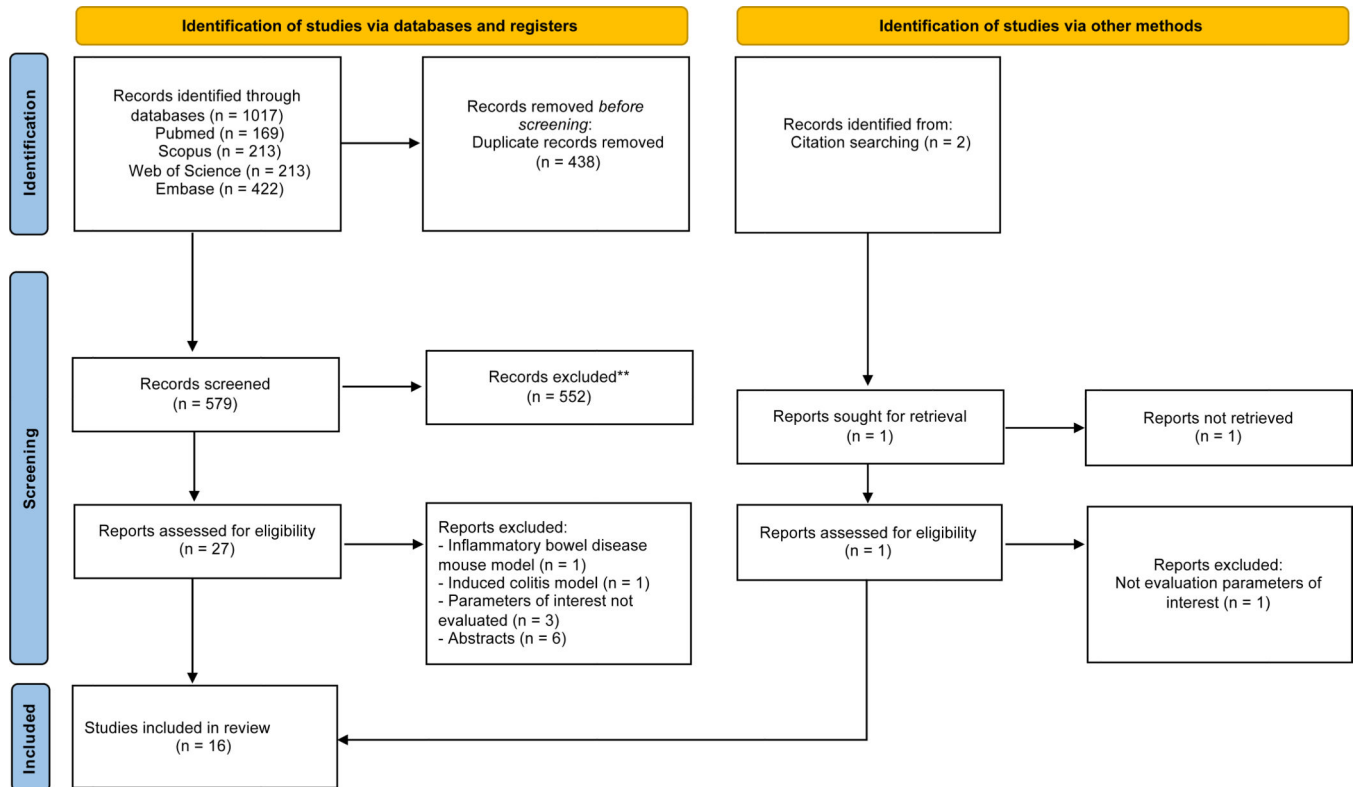**Identification of studies via databases and registers**

Identification of studies via other methods

Identification

Records identified through databases (n = 1017)
Pubmed (n = 169)
Scopus (n = 213)
Web of Science (n = 213)
Embase (n = 422)

Records removed *before screening*:
Duplicate records removed (n = 438)

Records identified from:
Citation searching (n = 2)

Screening

Records screened
(n = 579)

Records excluded**
(n = 552)

Reports sought for retrieval
(n = 1)

Reports not retrieved
(n = 1)

Reports assessed for eligibility
(n = 27)

Reports excluded:
- Inflammatory bowel disease mouse model (n = 1)
- Induced colitis model (n = 1)
- Parameters of interest not evaluated (n = 3)
- Abstracts (n = 6)

Reports assessed for eligibility
(n = 1)

Reports excluded:
Not evaluation parameters of interest (n = 1)

Included

Studies included in review
(n = 16)

Fig. 1.
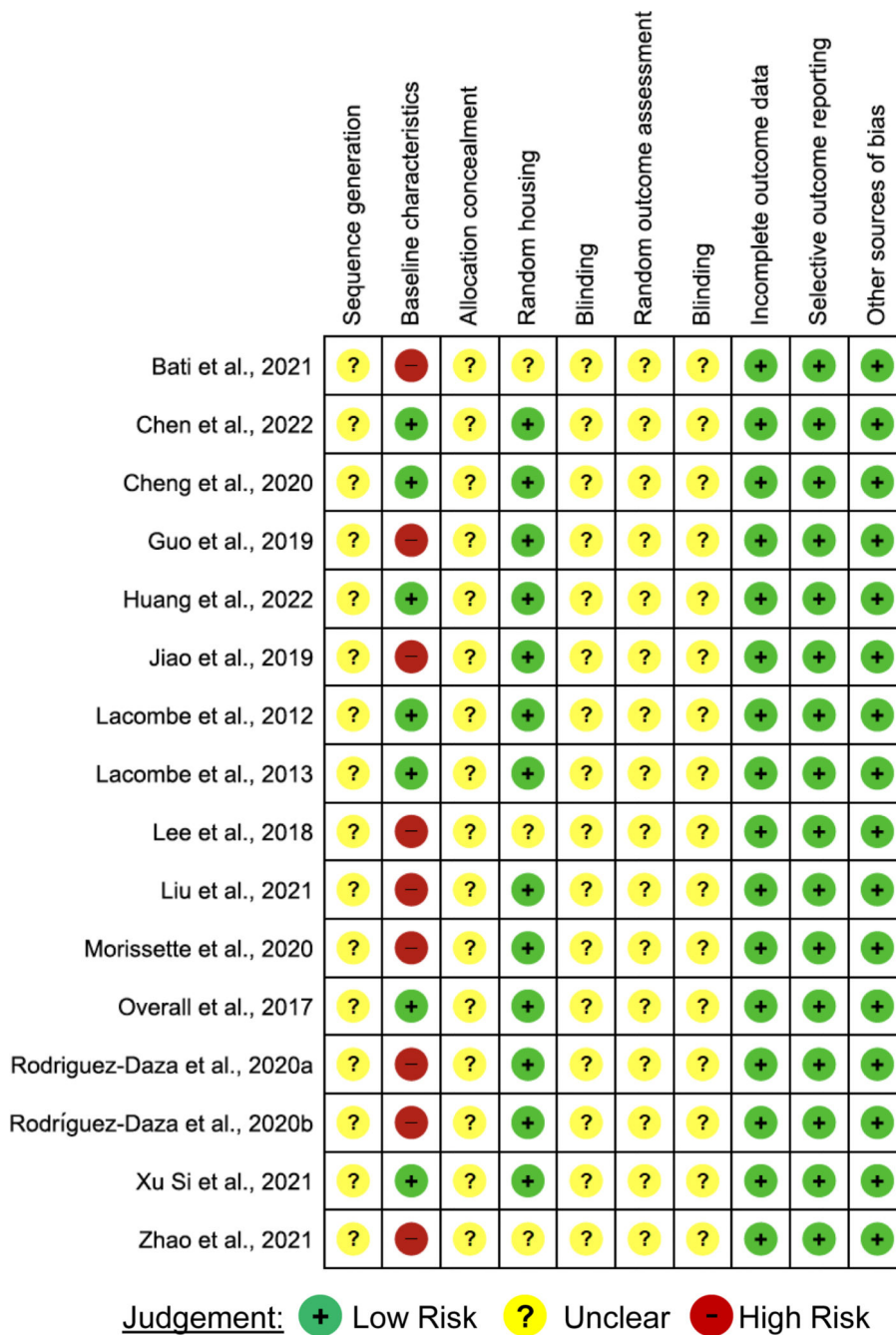PRISMA Flowchart Depicting Study Search and Selection Process.

Fig. 2.
Risk of Bias Assessment Evaluated according to the SYstematic Review Centre for Laboratory Animal Experimentation (SYRCLE) Risk of Bias Tool.
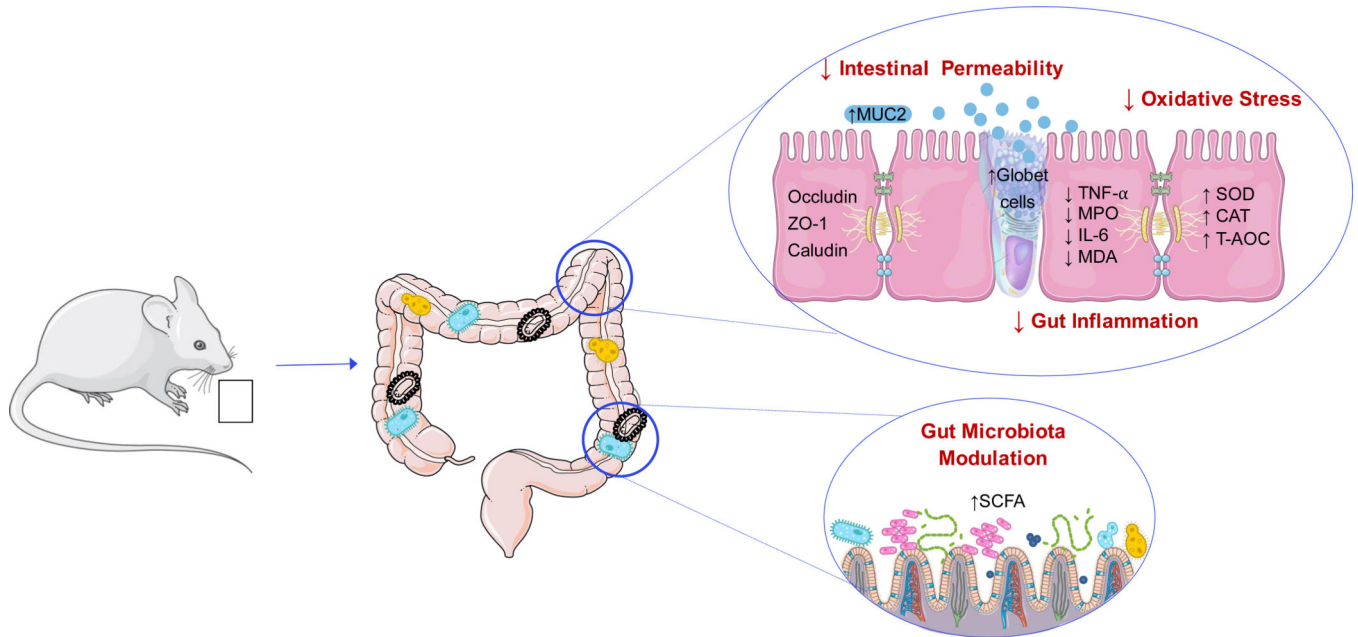
Fig. 3.
Possible Mechanisms for the Beneficial Effects of Blueberries on Gut Health.[1]
[1]CAT: catalase; IL-6: interleukin-6; MDA: Malondialdehyde; MPO: Myeloperoxidase;
MUC2: Mucin 2; SCFA: short chain fatty acids; SOD: Superoxide dismutase; T-AOC: Total
Antioxidant Capacity; TNF-α: Tumor necrosis factor alpha; ZO-1: Zonula occludens.

Table 1.

PICOS criteria for study inclusion

Parameter	Inclusion criteria
Model	Preclinical model (mice and rat)
Intervention	Blueberry supplemented diet
Comparison	Negative control (without the intervention)
Outcomes	Gut inflammation and oxidative stress, gut permeability, composition of gut microbes, short chain fatty acids and histological parameters of gut.
Study design	Experimental controlled studies

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Table 2.

Characteristics of animal studies

Country	Intervention Group	Control Group	Form of Blueberry	Blueberry dosage	Mode of Administration	Ref
USA	Diet AIN93 + LWB powder	AIN-93	Powder	8% w/w LWB	Blueberry supplemented diet	[26]
USA	Diet AIN93 + LWB powder	AIN-93	Powder	8% w/w LWB	Blueberry supplemented diet	[64]
USA	○ LFD (10% fat) ○ HFD (60% fat) ○ Blackberry (BB) ○ Black currant (BC) ○ Black raspberry (BR) ○ Blueberry (BL) ○ Concord grape (CG) ○ Maqui berry (MB)	LFD and HFD	Powder	400 mg/g Total anthocyanins	Blueberry supplemented diet	[28]
USA	○ Blue berry powder + LFD (10% fat) ○ Blueberry powder + HFD (45% fat)	LFD and HFD	Powder	10 g Freeze-dried blueberry powder/100 g. The blueberry powder was a Tifblue/Rubel 50/50 blend with 38.39 mg phenolics/g and 21.34 mg anthocyanins/g. Selection of 10% blueberry concentration was based on previously published studies	Blueberry supplemented diet	[16]
China	Blueberry polyphenol extract + HFD	HFD	Extract	200 mg/kg	Blueberry in drinking water	[20]
China	○ Study 1: Blueberry extract + HFD ○ Study 2: Blueberry extract + Control diet ○ Study 3: Blueberry extract + HFD and Blueberry extract + HFD + antibiotic	Study 1: Control diet and HFD Study 2: Control diet Study 3: Control diet, HFD, HFD + antibiotic	Extract	5 gL^{-1} BE in drinking water (219.24mg of anthocyanin/g BE)	Blueberry in drinking water	[19]
Canada	○ WBE + HFHS ○ Anthocyanins and phenolic of WBE + HFHS ○ PACs oligomers, phenolic acids and flavonols of WBE + HFHS ○ PACs polymers of WBE + HFHS	CHOW and HFHS (65% fat)	Extract and fraction	WBE: 200 mg/kg – 17 mg polyphenols/day (1.68 mg/kg of anthocyanin) Anthocyanins and phenolic acids: 32 mg/kg PACs oligomers, phenolic acids and flavonols: 53 mg/kg PACs polymers: 37 mg/kg	Oral gavage	[30]
China	○ Fermented blueberry pomace (FBP) + Control diet ○ Fermented blueberry pomace (FBP) + HFD	Control diet and HFD	Pomace	4% or 8% (w/w) FBP-supplementation	Oral gavage	[21]
Canada	Blueberry powder (BB) + HFHS Blueberry anthocyanin-rich fraction (ANT) + HFHS Proanthocyanidin-rich fraction (PAC) + HFHS	HFHS	Powder	4% (w/w)	Blueberry supplemented diet	[29]
Canada	Blueberry powder or blueberry fibrous residue + HFHS	HFHS	Powder and fibrous residue	200 mg/kg BW (1.94 mg/kg BW of anthocyanin)	Blueberry supplemented diet	[31]

Country	Intervention Group	Control Group	Form of Blueberry	Blueberry dosage	Blueberry Administration	Mode of Ref
China	Blueberry polyphenol extract + Standard diet	Standard diet	Extract	200 mg/kg	Blueberry supplemented diet	[24]
China	Blueberry anthocyanin-rich extract + HFD	HFD	Extract	100 and 200 mg/kg	Blueberry supplemented diet	[23]
Ukraine	Blueberry juice + Control group	Standard vivarium diet	Juice	0.5mL/day	Oral gavage	[32]
China	Blueberry extract + HFD	HFD	Extract	1% or 2% blueberry extract	Blueberry supplemented diet	[22]
China	○ Blueberry (BLUB) ○ Black berry (BLAB) ○ Black goji berry (BGJB) Mulberry (MULB) ○ Red Chinese bayberry (RCBB) ○ Raspberry (RASB) ○ Red goji berry (RGJB) Strawberry (STRB) ○ White Chinese bayberry (WCBB)	Distilled water	Extract	50 mg/kg^{-1}	Oral gavage	[25]
Sweden	○ Lingonberry + HFD ○ Bilberry + HFD ○ Cloudberry + HFD ○ Blackcurrant + HFD ○ Sea buckthorn + HFD ○ Blueberry + HFD	LFD and HFD	Fine powder	6% (w/w) on a dry weight basis	Rodent diet pellets	[33]

BE: blueberry extract; BW: body weight; CHOW: standard chow diet; HFD: high fat diet; HFHS: high-fat high-sucrose diet; LFD: low fat diet; LWB: lowbush wild blueberry; PACs: oligomeric proanthocyanidins; WBE: blueberry extract.

Table 3.

Key findings on the effects of blueberries on gut health in preclinical models

Animal Model	Study Protocol	Duration of Intervention	Effect of blueberries on gut health		Ref
			Primary Outcome	**Secondary Outcome**	
Sprague-Dawley rats Male Age: 3 Weeks	AIN-93 Diet (n=4) AIN93 Diet supplemented with 8% lowbush wild blueberry powder (w/w) (n=5)	6 Weeks	↑ Protein families involved with amino acid metabolism, 2,4-dienoyl-CoA reductase, metal ion binding, glutamate synthase, REDOX homeostasis, and aryl transferases. ↓ Protein families involved with integrase/recombinase, reverse transcriptase, and transposon/transposase.		[26]
Sprague-Dawley rats Male Age: 3 Weeks	AIN-93 Diet (n=4) AIN93 Diet supplemented with 8% lowbush wild blueberry powder (w/w) (n=5)	6 Weeks	↑ Abundance of the phylum Actinobacteria, the order Actinomycetales and family *Bifidobacteriaceae* and *Coriobacteriaceae*, ORFs assigned to KEGG category xenobiotics biodegradation and metabolism. ↓ Abundance of the genera *Lactobacillus* and *Enterococcus*, number of ORFs assigned to the bacterial invasion of epithelial cells.		[64]
C57BL/6J mice Male Age: 6 Weeks	Low fat diet (n=12) High fat diet (n=56) Obese mice were further randomized to control high fat diet (HFD, n=8) or berry-supplemented treatment groups normalized to 400 mg/g total anthocyanins.	12 Weeks	↑ Firmicutes, Bacteriodetes, Actinobacteria ↓ Anthocyanin content of the feces	↑ Lean body and water mass, insulin sensitivity ↓ Body weight, fat body mass ↔ Food intake, blood glucose levels	[28]
Wistar rats Male	Low fat diet (LF) (n=8) High fat diet (HF) (n=8) High fat diet supplemented with 10% blueberry powder (w/w) (HF_BB) (n=8)	8 Weeks	↔ Abundance and ratio of the main phyla *Firmicutes* and *Bacteriodetes* (HF vs. LF), IL-1B (ileum), IL-6 (ileum), CD11d (ileum), CD6 (ileum) ↑ *Firmicutes* and *Bacteriodetes* abundance (HF_BB vs. HF and LF) ↑ Proteobacteria (HF_BB vs. HF and LF) ↑ Fusobacteria (HF_BB vs. HF and LF) ↑ Bacilli (class) (HF_BB vs. HF and LF) ↑ *Porphyromonadaceae* (family) (HF_BB vs. HF and LF) ↓ Butyrate (HF_BB vs. LF) ↓ TNF-A (ileum) (HF_BB and LF vs. HF) ↑ Acetate (HF_BB vs. HF and LF) ↑ Propionate (HF_BB vs. LF) ↑ Gpr43 and Defb2 gene expression (HF_BB vs. LF) ↑ Villus length (HF_BB and LF vs. HF) ↑ Goblet cells/crypt (HF_BB vs. HF) ↑ Muc2 gene expression (HF_BB vs. HF) ↑ Glp1 gene expression (LF and HF_BB vs. HF)	↔ Body weight, mesenteric fat, epididymal fat, AUC, IL-6 (adipose tissue), CD68 (adipose tissue), phosphorylation of NF-κB p65 in adipose tissue ↑ Retroperitoneal fat (HF_BB vs. LF) ↑ Gene expression of PPARG (HF_BB vs. HF) ↑ Gene expression of PPARD (HF_BB and LF vs. HF) ↑ Urine F2-isoprostanes (HF_BB and LF vs. HF) ↑ Liver fat droplets (HF_BB and HF vs. LF) ↓ Blood glucose (min. 15) (HF_BB vs. LF) ↓ Serum insulin (min. 30) (HF_BB and LF vs. HF) ↓ TNF-A and IL-1B (adipose tissue) (HF_BB and LF vs. HF) ↑ Hepatic p-IRS1 (Ser307) to IRS1 ratio (HF_BB and LF vs. HF) ↓ CD11D (adipose tissue) (HF_BB vs. LF and HF) ↓ Serum LBP in adipose tissue (HF_BB vs. HF) ↓ Liver MDA (HF_BB vs. HF)	[16]

Effect of blueberries on gut health

Animal Model	Study Protocol	Duration of Intervention	Primary Outcome	Secondary Outcome	Ref
C57BL/6J Mice Male Age: 4 Weeks	Normal fat diet (n=6) High fat diet (HFD) (n=6) HFD supplemented with blueberry polyphenol extract (200 mg/kg body weight/day) (HFD + PPE) (n=6)	12 Weeks	↔OTUs and species richness (HFD vs. HFD+PPE) ↓ The Simpson diversity (HFD+PPE vs. HFD) ↑ *Bifidobacterium, Desulfovibrio, Adlercreutzia, Helicobacter,* and *Flexispira* (HFD+PPE vs. HFD) ↓ *Adlercreutzia* and *Prevotella* (HFD+PPE vs. HFD) ↔ Bacterial taxa	↓Body weight, weight gain and food intake (HFD+PPE vs. HFD) ↓FER compared to HFD ↔ TG, AST, ALT, and leptin (serum) ↓ LDL-C (HFD+PPE vs. HFD) ↑ HDL-C (HFD+PPE vs. HFD) ↓T-CHO in (HFD+PPE vs. HFD) ↓Hepatic PPARγ, FAS, SREBP-1 (HFD+PPE vs. HFD) ↑ Hepatic CPT1 and PPARα (HFD+PPE vs. HFD) ↓EWAT PPARγ, FAS, SREBP-1, aP2, GAPDH, GLUT4 (HFD+PPE vs. HFD) ↑Hepatic and EWAT p-AMP/total AMPK ratio (HFD+PPE vs. HFD)	[20]
Study 1: C57BL/6 mice Male Age: 1–3 Weeks *Study 2:* C57BL/ KsJ *db/db* mice Male Age: 21 Days *Study 3:* C57BL/6 mice Male Age: 8 Weeks	*Study 1:* (n=9–12/group) G1: CHOW 1 (standard chow diet) G2: HFD 1 (high fat diet, 60% fat) G3: CBE (CHOW + 5 gL^{-1} blueberry extract in drinking water) G4: BE 1 (HFD + 5 gL^{-1} blueberry extract in drinking water) *Study 2:* (n=10/group) G1: CHOW 2 (standard chow diet) G2: BE 2 (CHOW + 5 gL^{-1} blueberry extract in drinking water) *Study 3:* (n=10–12/group) G1: CHOW 3 (standard chow diet) G2: HFD 3 (high fat diet, 60% fat) G3: BE 3 (HFD + 5 gL^{-1} blueberry extract in drinking water) G4: Abx (HFD + 200uL PBS containing antibiotic) G5: ABE (HFD + 5 gL^{-1} blueberry	*Study 1:* 15 Weeks *Study 2:* 10 Weeks *Study 3:* 15 Weeks	*Study 1:* ↑ mRNA expression of occludin, TJP1, MUC2 levels ↓ mRNA levels TLR4, IL-6, TNF-α in the colon and ileum ↑ *Akkermansia* and *Bifidobacterium* ↓ *Desulfovibrio* and *Bilophila* genera ↑ Abundance of the significant taxa ↑ Ratio Bacteroidetes to Firmicutes *Study 2:* ↑ Diversity of the GM ↓ Abundance of *Proteobacteria* ↑ Ratio *Bacteroidetes* to *Firmicutes* *Study 3:* ↓ Fecal anthocyanin content (BE vs. ABE)	*Study 1:* ↓ Weight gain (BE1 vs. HFD 1) ↔ Energy intake, water intake ↓ Body fat, liver fat induced by HFD ↓ Hepatic and plasmatic TG ↓ ALT and HDL ↑ Energy expenditure, core body temperature ↓ mRNA levels LPS, IL-6, TNFα in plasma ↓ Plasma leptin ↑ Glucose tolerance and insulin sensitivity ↓ Expression of CCAAT in the iWAT and mRNA of proteins linked to lipolysis, FXR, SHP, TGR5 ↓ Plasma BAs *Study 2:* ↓ Weight gain ↔ Energy intake ↓ TG, colesterol, leptin ↑ Energy expenditure ↑ Hepatic steatosis, systemic inflammation, fat deposition ↑ Glucose and lipid metabolism ↓ Plasma BAs *Study 3:* ↔ Weight gain ↔ TG, hepatic fat, cholesterol ↔ Metabolic disease, systemic inflammation and glucose metabolism ↔ Plasma BA pool size and composition, FXR, TGR5	[19]

extract in drinking water + 200uL PBS containing antibiotic)

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Animal Model	Study Protocol	Duration of Intervention	Primary Outcome	Secondary Outcome	Ref
C57BL/6J mice Male Age: 6 Weeks	(n=12/ Group) Standard chow diet (n=12) High-fat high-sucrose diet (HFHS) (n=12) HFHS diet supplemented with wild blueberry extract (200 mg/kg/day equivalent to 17 mg polyphenols) (WBE) HFHS diet supplemented with anthocyanins and phenolic acids (32 mg/kg) (F1) HFHS diet supplemented with anthocyanins and phenolic acids (32 mg/kg) (F1) G5: F2 (PACs oligomers, phenolic acids and flavonols, 53mg/kg) + HFHS G6: F3 (PACs polymers, 37mg/kg) + HFHS oligomeric proanthocyanidins (PACs)	8 Weeks	↑ Mucus layer thickness (colon) (WBE and BPFs) ↔ Crypt's depth (WBE, BPF) and total goblet cells (WBE, F1 and F2) ↔ Firmicutes/ Bacteroidetes ratio ↑ Number of total goblet cells (GC) (at F3 group) ↑ Adlercreutzia equolifaciens (WBE e F2) ↑ Mix of neutral and acidic mucins (BPFs) ↑ Neutral mucin-filled GC proportion (WBE) ↓ Proportion of mucin-unfilled GC (WBE and BPFs) ↔ mRNA of ZO-1 and occludin ↔ α-diversity index, species abundance and Shannon's diversity index, β-diversity of the gut microbiota ↑ Family Coriobacteriaceae, S24–7, Verrucomicrobia and the order Clostridiales (WBE and BPFs) ↓ Unassigned genus of the family S24–7 (WBE) ↓ A. muciniphila at feces (F2 group) ↑ A. equolifaciens at feces (F2, WBE)	↔ Total energy intake ↔ Visceral mass, fasting glycemia, HOMA-IR index ↓ AUC of the OGTT (WBE e F3)	[30]
C57BL/6 mice Male Age: 5 weeks	(n=7/ Group) G1: C (control) G2: CL (C with 4% fermented blueberry pomace (FBP) supplementation) G3: CH (C with 8% FBP-supplementation) G4: HFD (high fat diet) G5: HFDL (HFD with 4% FBP-supplementation) G6: HFDH (HFD with 8% FBP-supplementation)	5 Weeks	↑ Villus length (HFDL and HFDH vs. HFD) ↑ Ratio of villus length to crypt depth (HFDL and HFDH vs. HFD) ↑ Claudin-4 mRNA level (HFDL and HFDH vs. HFD) ↑ Occludin mRNA level (HFDL and HFDH vs. HFD) ↑ Goblet cells (HFDL and HFDH vs. HFD) ↑ mRNA expression of ZO-1 (HFDL and HFDH vs. HFD) ↑ Claudin-1 mRNA level (HFDL vs. HFDL and HFD) ↑ E-cadherin mRNA level (HFDH vs. HFDL vs. HFD) ↑ Muc 2 mRNA level (HFDH vs. HFDL vs. HFD) ↓ Crypt depth (CL vs. C) ↑ T-AOC (ileum) (HFDH vs. HFD) ↑ CAT (ileum) (HFDH vs. HFDL vs. HFD) ↑ SOD (ileum) (HFDH vs. HFDL) ↓ MDA (ileum) (HFDH vs. HFD)	↓ TNF-α (serum) (HFDL vs. HFD) ↑ IL-10 level (serum) (HFDH vs. HFDL vs. HFD) ↓NF-κB mRNA level (HFDH vs. HFDL and HFD) ↓ protein level of NF-κB p-P65 (HFDH vs. HFD and HFDL) ↓ MPO activity in small intestine tissue (HFDL and HFDH vs. HFD) ↓ MLCK mRNA level (HFDH vs. HFDL and HFD) ↓Protein level of p-MLC (HFDH vs. HFDL vs. HFD) ↓ Final body weight (HFDL and HFDH vs. HFD) ↓ Abdominal fat index (HFDL and HFDH vs. HFD) ↑ GSH (liver) (HFDH vs. HFD and HFDL) ↑ T-AOC (liver) (HFDH and HFDL vs. HFD) ↑ CAT (liver) (HFDH vs. HFDL and HFD) ↑ SOD (liver) (HFDH and HFDL vs. HFD)	[21]
C57BL/6J mice Male Age: 8 weeks	G1: Chow (n=14); G2: HFHS: High-fat.high-sucrose diet (n=13); G3: BB-HFHS: High-fat, high-sucrose	20 Weeks	↓ Lachnospiraceae bacterium Choco86, Ruminococcus, Blautia hansenii and Blautia sp. N6H1–15 (BB-HFHS and ANT-HFHS vs. HFHS). ↑Turicibacter sp. H121 abundance (BB-HFHS vs. HFHS) ↑ Muribaculum intestinale abundance (PAC-HFHS vs. HFSH)	↓ Weight gain (PAC-HFHS VC HFHS) ↔ Food intake, IWAT, MWAT, lean mass, energy expenditure, glycaemia, insulinemia 6h fasting, C-Peptide,	[29]

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Animal Model	Study Protocol	Duration of Intervention	Primary Outcome	Secondary Outcome	Ref
	+ whole blueberry powder (n=14); G4: ANT-HFHS: High-fat, high-sucrose diet + blueberry anthocyanin-rich fraction (n=14); G5: PAC-HFHS: High-fat, high-sucrose diet + proanthocyanidin-rich fraction (n =13)		↓Acetic and propionic acids (BB-HFHS vs. HFHS) ↓Valeric acid (BB-HFHS and ANT-HFHS vs. HFHS) ↓Isobutyric and isovaleric acid BB-HFHS, ANT-HFHS and PAC vs. HFHS) ↔ Fecal Butyric Acid	↓liver weight, TBARS, liver TG, liver cholesterol, AST, ALT ↔ IL-2, IL-6, TNF-a, MCP-1, INFy ↓HOMA-IR (PAC-HFHS vs. HFHS) ↓Insulin (ANT-HFHS and PAC-HFHS vs. HFHS)	[31]
C57BL/6J Male mice Age: 6 weeks	(n=12/ Group) G1: CT (standard chow diet) G2: HFHS (high-fat high-sucrose diet) G3: HFHS + BP (high-fat high-sucrose diet + blueberry powder) G4: HFHS + BF (high-fat high-sucrose diet + blueberry fibrous residue)	8 Weeks	↑Mucus thickness (BP vs. HFHS) ↔ Crypt depth, number of Goblet cells, types of mucin ↑Microbial richness relative ↑Gut microbiota diversity ↑Verrucomicrobia (BP group) ↑Firmicutes (BP group) ↔ Abundance of pathobionts, taxa at the family level (BP group) ↑Akkermansiaceae (BP group) ↑Eggerthellaceae and Coriobacteriales_Incertae_Sedis (BP group) ↑A. muciniphila (BP group) ↑Romboutsia, Ruminiclostridium, and Oscillibacter ↑Raxa Lachnospiraceae_NK4A136_group e Acetatifactor ↑Polysaccharide-degrading taxa such as Clostridium_senso_stricto1, Muribaculaceae, and Roseburia ↑Pathways of metabolism of cofactors and vitamins, lipid metabolism and DNA replication and repair (BP group)	↔ Body weight ↑EWAT (BF group) ↑TG (BF vs. HFHS) ↔ Glycemia, HOMA-IR ↑Fasting insulin (BF vs. HFHS) ↔ Cecum weight	
Sprague-Dawley rats Age: 21 days	(n=48/Group) G1: Standard diet (AIN-93G) G2: Standard diet + 200 mg/kg blueberry polyphenol extract	2 Weeks	↑SOD jejunal, CAT ileal, T-AOC jejunal and ileal ↓MDA jejunal ↔ SOD and MDA ileum, CAT jejunum ↓IL-1 and IFN-y (jejunum and ileum) ↔ IL-6 and TNF-a (jejunum and ileum) ↑Keap1 jejunal and ileal ↑Nrf2 jejunal and ileal ↑mRNA of mTOR, S6K1, 4EBP1 jejunal and ileal ↔ HO-1 jejunal and ileal	↔ Growth performance	[24]
C57BL/6 mice Male Age: 4 weeks	(n=6/ Group) G1: ND (normal diet) G2: HFD (high fat diet, 53.8% basic feed, 21% lard oil, 20% saccharose, 5% cholesterol, and 0.2% sodium cholate) G3: BAE100 (HFD and 100 mg/kg body weight of blueberry anthocyanin-rich extract) G4: BAE200 (HFD and 200 mg/kg body weight of blueberry anthocyanin-rich extract)	8 Weeks	↓Staphylococcus (BAE100 and BAE200) ↓Ruminiclostridium (BAE100 and BAE200) ↑Bacteroidetes/Firmicutes (BAE100) ↑Bifidobacterium (BAE200) ↑Lactobacillus (BAE200) ↑Roseburia (BAE200) ↑Faecalibaculum (BAE200) ↑Parabacteroides (BAE200) ↑Acetate (BAE200 vs. BAE100 vs. HFD) ↑Butyrate (BAE200 vs. BAE100 vs. HFD) ↑Propionate (BAE200 vs. BAE100 vs. HFD)	↓Serum concentrations of phospholipids with PUFA (BAE100 and BAE200 vs. HFD) ↑Liver T-AOC (BAE200 vs. BAE100 vs. HFD) ↑Liver SOD activity (BAE200 vs. BAE100 vs. HFD) ↑Liver GSH-Px (BAE200 vs. BAE100 vs. HFD) ↑USFA/SFA (BAE200 vs. BAE100 vs. HFD)	[23]
Outbred laboratory rats Male and female Age: 22–24 months	(n=12/ Group) G2: blueberry juice; G9: control group (standard vivarium diet food)	12 Weeks	↓Klebsiella pneumoniae, Morganella morganii, E. coli, Actinomyces naeslundii; and Bacteroides ↔ B. subitillis ↓Streptococcus parvulus ↑E. faecalis and staphylococci	↓Body weight, total lipid, cholesterol, glucose, calcium levels ↔ LDL, triglycerides, blood urea	[32]

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Animal Model	Study Protocol	Duration of Intervention	Primary Outcome	Secondary Outcome	Ref
C57BL/6J mice Male Age: 5 weeks	(n= 9/ Group) G1: LFD (Low fat diet - 10% kcal FAT) G2: HFD (High fat diet - 60% kcal FAT) G3: HFD + BL (High fat + 1% blueberry extract) G4: HFD + BH (High fat + 2% blueberry extract)	24 Weeks	↑ SCFA (BL and BH vs. HFD) ↔ *Firmicutes/Bacteroidetes* ratio ↑ *Bacteroidetes* (BH group) ↑ Abundance of *Rikenellaceae* (BL and BH vs. HFD) ↓ Abundance of *Streptococcaceae* (BH group) ↓ Relative abundance of *Allobaculum, Anaerotruncus, Intestinimonas, Oscillibacter, Ruminiclostridium,* and *norank_f_Bacteroidales_S24–7_group* ↑ Abundance of *Rikenella* ↑ Abundance of *Peptoclostridium* ↑ Functions such as general function prediction only, lipid transport and metabolism, cell motility, RNA processing and modification ↑ Abundance of metabolic pathways related of basic metabolism (BH group)	↓ Weight gain induced by HFD ↔ energy intake ↑ Accumulation of white adipose tissue ↓ Plasmatic TC ↑ HDL/TC ratio (BL group) ↓ Plasma LPS (BH group) ↔ TNFα, MCP-1, IL-1β ↓ Liver weight, hepatic total lipids ↔ Hepatic cholesterol	[22]
Male mice Age: 6 weeks	(n=7/ Group) G1: CON (distilled water) G4: BLUB (blueberry)	2 Weeks	↑ SOD activity e AOC (colon) ↔ MDA e GSH content, CAT e GSH-Px activity (colon) ↑ *Prevotella, Clostridium_III, Clostridium_XVIII, Intestinimonas, Ruminococcus* and *Barnesiella* ↓ *Escherichia, Klebsiella, Proteus, Blautia, Enterococcus, Staphylococcus, Mucispirillum, Acinetobacter* and *Clostridium_XIVa*	↓ Weight gain, food intake and liver index ↓ CAT activity (serum) ↔ AOC, MDA and GSH content (serum) ↔ SOD (liver) ↑ SOD activity (serum)	[25]
C57BL/6J mice Male Age: 22 weeks	(n= 15/Group) G1: LFD (Low fat diet - 10% kcal FAT) G2: HFD (High fat diet - 60% kcal FAT) G3: HFD + Blueberry (High fat + 6% blueberry powder)	16 Weeks	↑ *Shannon diversity (G3 vs. G2)* ↑ *Total OTUs richness (G3 vs. G2)* ↑ *Firmicutes (G3 vs. G2)* ↑ *Proteobacteria (G3 vs. G2)* ↑ Unclassified genus from *Clostridiales, Lachnospiraceae (G3 vs. G2)* ↓ Unclassified genus from *Ruminococcaceae, Desulfovibrionaceae (G3 vs. G2)* ↔ *Akkermansia muciniphila*	↔ Body weight, energy intake, fat pad weight	[33]

4EBP1: eukaryotic initiation factor 4E-binding protein 1; AIN93: nutritional standard; ALT: alanine aminotransferase; AMP: adenosine mono phosphate; AMPK: AMP-activated protein kinase; aP2: adipocyte-specific acid binding protein; AST: aspartate transaminase; AUC: area under curve; CAT: catalase; iWAT: epididymal white adipose tissue; CPT1: Carnitine palmitoyl transferase I; Defb2: defensin beta 2; eWAT: epididymal white adipose tissue; FAS: fatty acid synthase; FER: food efficiency ratio; GAPDH: Glyceraldehyde-3-Phosphate Dehydrogenase; Glp1: Glucagon-like peptide-1; GLUT4: glucose transporter 4; Gpr43: G-protein-coupled receptor 43; GSH-Px: liver glutathione peroxidase; HDL-C: high-density lipoprotein cholesterol; HFD: high fat diet; HO-1: heme oxygenase-1; HOMA-IR: Homeostasis Model Assessment-Insulin Resistance; IFN-γ: interferon-γ; IL-1: interleukin-1; IL-10: interleukin-10; IL-2: interleukin-2; IL-6: interleukin-6; iWAT: inguinal white adipose tissue; Keap1: Kelch-like ECH-associated protein 1; KEGG: Kyoto Encyclopaedia of Gene and Genome; LBP: Adipocyte lipopolysaccharide-binding protein; LDH: lactate dehydrogenase; BA: bile acids; LDL-C: low-density lipoprotein cholesterol; LWB: lowbush wild blueberry; MCP-1: monocyte chemoattractant protein-1; MDA: malondialdehyde; MLCK: myosin light chain kinase; MPO: myeloperoxidase; mTOR: mammalian target of rapamycin; Muc2: mucin 2; mWAT: mesenteric white adipose tissue; NFκB: nuclear factor kappa B; Nrf2: nuclear factor-E2-related factor 2; ORFs: open reading frames; OTUs: operational taxonomic units; p-IRS1: Phospho-insulin receptor substrate 1; p-MLC: phospho-myosin light chain 2; PPAR-d: peroxisome proliferator-activated receptor delta; PPARα: Peroxisome proliferator-activated receptor α; PPARγ: peroxisome proliferator-activated receptor γ; PUFA: Polyunsaturated fatty acids; S6K1: ribosomal p70 S6 kinase; SFA: saturated fatty acids; SOD: superoxide dismutase; SREBP-1: sterol regulatory element-binding protein 1; T-AOC: total antioxidant capacity; T-CHO: serum total cholesterol; TBARS: thiobarbituric acid reactive substances; TG: triglycerides; TNF-α: tumor necrosis factor-α; USFA: unsaturated fatty acid; ZO-1: Zonula occludens-1.