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A systematic review of the effect of dietary pulses on microbial populations inhabiting the human gut

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REVIEW ARTICLE

Abstract

Pulses are dry leguminous crops consisting of beans, lentils, chickpeas, and peas. They are a broad category of food that are often aggregated when their contribution to healthy dietary patterns are disseminated. However, the different genera and varieties of pulses vary in composition and are consumed in different amounts, largely dictated by geographic region and ethnicity. Given the number of pulse-derived components, including fibre, that have the capacity to alter the composition of the gut microbiome, the objective of this study was to systematically review dietary pulses and pulse-derived ingredients as a broader food group, to determine their effect on gut microbiota in humans. Major scientific databases were used to conduct the search, which spanned from 1990 until February 2019. The search strategy identified 2,444 articles and five studies were included in this analysis. Two studies used whole pulses (chickpeas and pinto beans), one study used cooked navy bean powder, and the two remaining studies used pulse-derived fibre (lupin or yellow pea hulls). Although inconsistent, some studies demonstrated that whole pulses (pinto beans and chickpeas), cooked navy bean powder, and pulse-derived fibre (lupin kernel fibre), did impose changes to the microbiota that inhabit the human large intestine. However, there was considerable variability concerning the methodologies and endpoints used to decipher the observed effects on the abundance, diversity, and/or richness of specific microbiota or the microbiome. More extensive human studies that directly link the effects of specific types of pulses on the gastrointestinal microbial environment to health outcomes in the host are required.

Keywords: pulses, legumes, fibre, gut, microbiome, prebiotic, systematic review

1. Introduction

Dietary constituents that affect specific intestinal bacteria or modulate the richness and diversity of the mammalian gut microbial environment can facilitate short-and long-term health benefits to the host (Gibson *et al.*, 2010, 2017; Mohajeri *et al.*, 2018). Although the mechanisms are not fully understood, immune system modulation, short-chain-fatty acid (SCFA) production and subsequent metabolism, and interactions with the gut-brain axis are plausible mechanisms through which an advantageous symbiotic relationship between the microbiome and humans is achieved (Hills *et al.*, 2019; Jeffery *et al.*, 2013; Vallianou *et al.*, 2019).

Pulses are a broad grouping of leguminous crops consisting of a variety of dry beans, lentils, chickpeas, and peas. They are nutritionally dense foods that have been collectively shown to reduce risk factors for chronic disease including low-density lipoprotein cholesterol (Ha *et al.*, 2014), blood pressure (Jayalath *et al.*, 2014), body weight and obesity (Kim *et al.*, 2016; Li *et al.*, 2014), and fasting and post-prandial blood sugar management (Sievenpiper *et al.*, 2009). In addition, specific pulse-derived constituents including dietary fibre, resistant starch, protein fractions, and phenolic compounds (Campos-Vega *et al.*, 2010; Marinangeli *et al.*, 2017; Singh *et al.*, 2017a; Zhang *et al.*, 2015), may also modulate the abundance or functionality of the microbiome (Cardona *et al.*, 2013; Mohajeri *et al.*,

2018). Dry pulses contain between 14-30 g/100 g dietary fibre (soluble and insoluble) (Tosh and Yada, 2010), which escapes digestion in the small intestine and is subsequently fermented by the microbiota in the large intestine. Cell wall fibres of the cotyledon and seed coat are primarily celluloses, hemicelluloses, and pectin-type fibres, while the storage polysaccharides are highly fermentable α-galactooligosaccharides, including raffinose, stachyose, and verbascose (Tosh and Yada, 2010). Additionally, pulses are a source of plant-based protein, containing approximately 7-9 g protein/100 g cooked (Marinangeli *et al.*, 2017). Recent evidence also suggest that diets with higher levels of plant-proteins are associated with an increase in microbiome richness and diversity of humans compared to animal-based protein (Singh *et al.*, 2017b). Also, pulses with higher levels of pigments have greater concentrations of phenolic acids (Campos-Vega *et al.*, 2010). Phenolic compounds that escape absorption in small intestine can be metabolised by gut microbiota, potentially modulating intestinal bacterial populations in humans and animals (Cardona *et al.*, 2013).

Although the nutritional attributes of pulses are similar across genera and variety, such as a source of dietary fibre and plant-based protein, the composition and levels of these and other dietary factors can differ between pulse types (Marinangeli *et al.*, 2017; Tosh and Yada, 2010). Furthermore, while consumption data tends to group legumes and pulses as a single food item, the types and quantities of each pulse crop consumed can be dictated by geographic location and ethnic factors. Therefore, the objective of this study was to systematically review dietary pulses (including pulse-derived ingredients) as a single broad group of foods and determine what effect pulses have on the abundance, richness, and diversity of gut microbes in humans. Results from this review will identify knowledge gaps to guide future research initiatives that investigate the microbiome-mediated effects of pulses on human health.

2. Materials and methods

Eligibility criteria

Literature search study selection

Pubmed, EMBase, Scopus, Web of Science, Proquest, and the Cochrane Library were used to search the scientific literature. The timelines for the search strategy were from 1990 until February 2017. A second search from February 2017 until February 2019, was commenced in March 2019. Results from both strategies were pooled together for reporting in the present review.

Study selection

Literature was searched using the search terms (Pulse* OR dietary pulse* OR lentil* OR lens culinaris OR peas OR pisum sativum OR chickpea* OR cicer arietinum OR lupin OR lupine OR lupinus OR bean* OR dry bean* OR phaseolus OR vigna OR kidney bean* OR black bean* OR pinto bean* OR fava OR faba OR vicia OR white bean* OR legum* OR fabaceae) AND (microbiome OR prebiotic OR gut microflora OR gut microbiota OR microbiota OR microflora OR faecal yeast OR fecal yeast OR yeast OR faecal microbiota OR fecal microbiota OR fecal microflora OR faecal microflora OR faecal bacteria OR fecal bacteria OR firmicutes OR lactobacillus OR bifidobacteria OR saccharomyces boulardii OR saccharomyces).

The strategy included research articles, abstracts, and student theses that were published in English. The systematic review focused on human interventions with a control or placebo group, randomised control trials (cross-over or parallel arm), and could be blinded or un-blinded. The search strategy included both acute and medium duration studies with no restrictions on sex, age, ethnicity, or educational status of subjects. Both healthy and disease-state humans, including those with cardiovascular disease, diabetes, and obesity were included. Furthermore, only control/placebo and pulse treatments of studies were considered, summarised, and compared. Additional non-pulse treatment arms were not included in the analysis.

Studies were excluded if treatments contained multiple active components (e.g. pulses + dietary fibre, or pulses + probiotics). Studies that included non-pulse legumes (e.g. soybean) as treatment interventions were excluded during the data extraction stage of the systematic review.

Data extraction

Studies were independently screened by CPFM and TCR. Titles and abstracts were reviewed and studies unrelated to study objectives were removed. Differences were settled by SVH. Full-text reports were obtained for the remaining studies and screened against the inclusion criteria.

3. Results

The flow and results of the systematic search are summarised in Figure 1. In total, the search generated 2,444 reports. Duplicate reports (n=623) were eliminated. Following the removal of 1,810 citations based on titles and abstracts, 11 reports underwent full-text review. Of these, 6 were excluded: one was a case-control study, one was a thesis where relevant results were subsequently published in a study undergoing full text review, two had an unsuitable endpoint(s), and were two were non-pulse studies. The five remaining studies were included in the analysis.

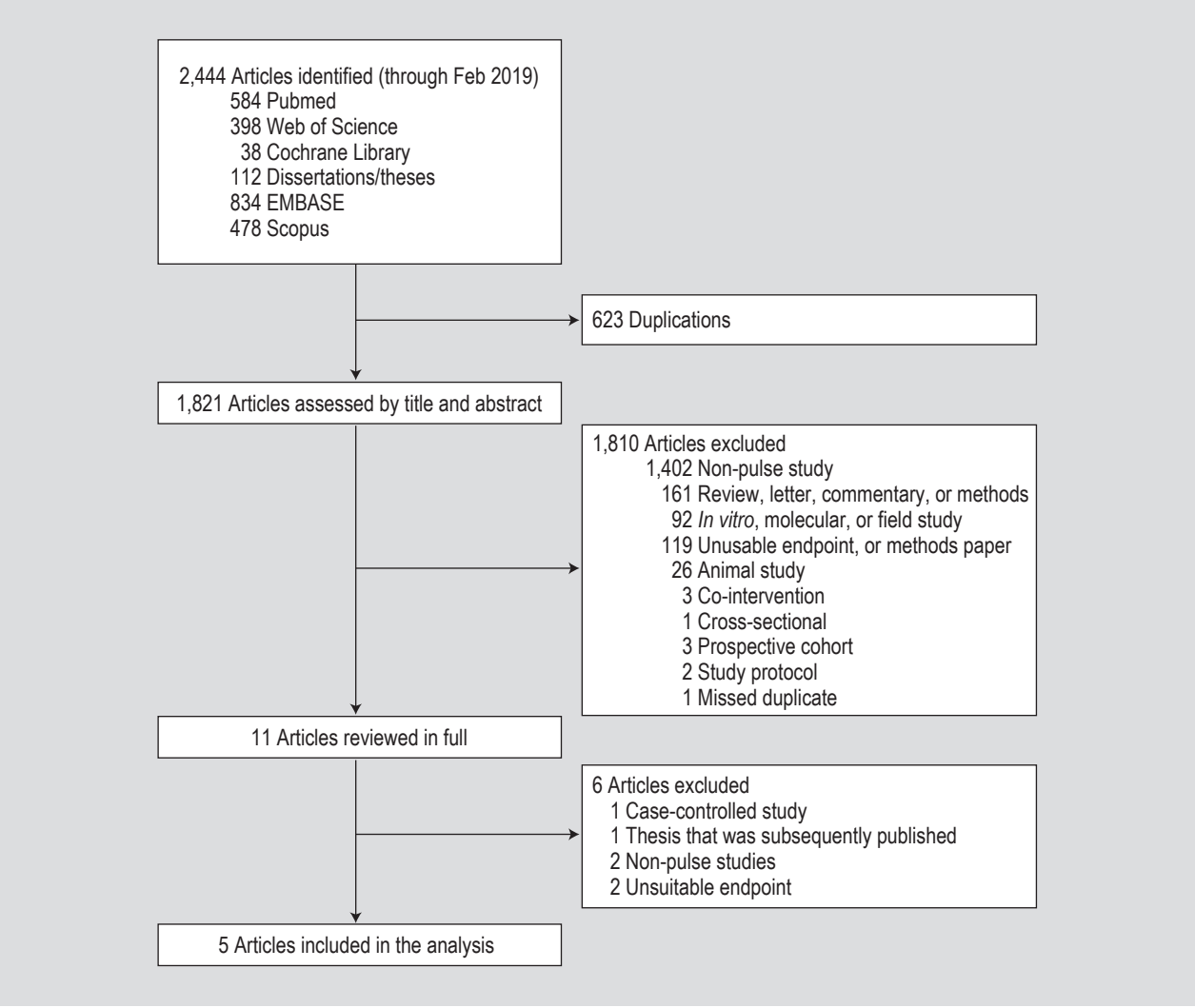

Figure 1. The flow and results of the systematic search.

Study population

A summary of studies that met the inclusion criteria is summarised in Table 1. Over half the studies (n=3) utilised healthy volunteers (Fernando *et al.*, 2010; Finley *et al.*, 2007; Smith *et al.*, 2006). The study by Finley *et al.* (2007) also recruited age and sex-matched volunteers with pre-metabolic syndrome. The remaining studies, also used unhealthy populations with volunteers characterised as overweight and obese (BMI 25-38) (Lambert *et al.*, 2017; Sheflin *et al.*, 2017), and as colorectal cancer survivors (Sheflin *et al.*, 2017). All studies recruited both males and females, with the exception of Smith *et al.* (2006), who recruited only male subjects. All studies were 4 weeks or 28 days in duration, except for Fernando *et al.* (2010) (3 weeks), and Finley *et al.* (2007) (12 weeks + 4 week run-in). The study by Sheflin *et al.* (2017) used faecal samples from the Beans/Bran Enriching Nutritional Eating For Intestinal Health Trial (Borresen *et al.*, 2016).

Types of pulses

Two studies used whole cooked pulses as chickpeas (200 g/day) (Fernando *et al.*, 2010) and canned pinto beans (130 g day) (Finley *et al.*, 2007). While Sheflin *et al.* (2017) used cooked navy bean powder (35 g/day) incorporated into meals and snacks that were compared to the same control foods that were matched for macronutrient and energy levels. Cooked navy bean powder was characterised as navy beans that were cooked, and subsequently dried and milled. The 35 g/day dose of navy bean powder used by Sheflin *et al.* (2017) was equivalent to 0.5 cup whole cooked navy beans (Borresen *et al.*, 2014). The two remaining studies (Lambert *et al.*, 2017; Smith *et al.*, 2006), used pulse-derived high fibre ingredients, including: (1) fibre from lupin kernels (total fibre: 88%; soluble fibre: 44%) (Hall *et al.*, 2005; Smith *et al.*, 2006), and (2) milled yellow pea hulls (total fibre: 92%; soluble fibre: 8%) (Lambert *et al.*, 2017). Lupin fibre was incorporated into bread, muffins, chocolate brownie,

Table 1. Characteristics of studies that underwent full-text review.[1]

Fernando *et al.* (2010)	
Pulses	Whole pulses
Subjects	healthy
n (gender)	12 (M:7; F:5)
Age (Mean ± SD)	25.6±8.7 years
Design	randomised crossover intervention; subjects were blinded to the control, but not the chickpea treatment
Treatment and dose	control diet; control diet + 200 g/day chickpeas
Treatment matrix	soup and dessert containing chickpeas
Treatment duration	3 weeks
Method comments	faecal samples collected over 3 days during the 3rd week of each intervention; T-RFLP (16sRNA) and restriction enzyme digestion with *Msp1*, *HaeIII*, and *Hha1* were used to identify microbial populations; Shannon-Weiner diversity index determined using T-RFLPs; qPCR used to quantify specific microbial populations (16srRNA gene copies/g faeces): *Clostridium coccoidal – Eubacterium rectale* group; *Clostridium leptum* subgroup; *Bifidobacterium; Bacteroidetes; Lactobacilli*
Finley *et al.* (2007)[2]	
Pulses	Whole pulses
Subjects	healthy and pre-MetSyn
n (gender)	healthy control: 40 (M: 20; F: 20); pre-MetSyn: 40 (M: 20; F: 20)
Age (Mean ± SD)	M Healthy: control = 30.7±12.3 yrs; beans = 33.7±12.3 yrs; M pre-MetSyn: control = 40.4±11.6 yrs; beans = 39.1±10.0 yrs; F Healthy: control = 42.2±8.7 yrs; beans = 43.1±7.1 yrs; F pre-MetSyn: control = 44.3±12.1 yrs; beans = 45.8±5.5 yrs
Design	2×2 factorial design randomised parallel-arm design; subjects and without pre-MetSyn received pinto beans or an isocaloric control; healthy subjects were age and sex matched to pre-MetSyn subjects
Treatment and dose	control = no beans; cooked pinto beans (canned) =130 g/day
Treatment matrix	control = isocaloric chicken soup entrée; treatment= 130 g cooked pinto beans
Treatment duration	12 weeks + 4-week run-in
Method comments	subjects started treatment after a 4-wk run-in; a single faecal sample collected at the end of the run-in period and the end of the treatment period. values reported as means ± SEM and are expressed as ΔΔCt (the threshold cycle (ΔCt) of the treatment (beans) minus the ΔCt of the control); microbiota analysis by qPCR: *Bifidobacterium longum; Peptostreptococcus productus; Bacteroides vulgatus; Clostridium clostridiiforme; Eubacterium limosum; Methanobrevibacter smithii*
Sheflin *et al.* (2017)[3]	
Pulses	Pulse flour (powder)
Subjects	colorectal cancer survivors; overweight and obese; (control BMI: 27.3±3.3; navy bean powder BMI: 28.5±7.9
n (gender)	control: 10 (M: 4; F: 6); cooked navy bean powder: 10 (M: 4; F: 6)
Age (Mean ± SD)	Control: 64±14 years; navy bean powder: 59±12 years
Design	single-blinded randomised control trial; samples from the Beans/Bran Enriching Nutritional Eating for Intestinal health Trial (ClincalTrials.gov: NCT01929122)
Treatment and dose	control; cooked navy bean powder: 35 g/day (equivalent to 1/2 cup cooked navy beans) (Borresen *et al.*, 2014)
Treatment matrix	meals and snacks formulated with cooked navy bean powder, or no treatment
Treatment duration	28 days
Method comments	stool samples collected on day 0, 14, and 28; DNA extracted from faecal samples; 16srRNA was pyrosequenced to classify and quantify the microbiome; ecological analysis by determining observed species richness (Sobs), estimated species richness (Chao1) and the Simpson diversity index

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Table 1. Continued.

Smith *et al.* (2006)	
Pulses	Pulse fibre
Subjects	healthy
n (gender)	18 (M)
Age (Mean ± SD)	42.8±11.9 years
Design	single-blinded, randomised, crossover design; free living; 28-day washout between treatments
Treatment and dose	control: low fibre; lupin kernel fibre
Treatment matrix	bread, muffin, chocolate brownie, chocolate milk drink, toasted muesli, pasta, and instant mashed potato
Treatment duration	28 days
Method comments	foods that contained treatment (lupin fibre or no lupin fibre) was provided (bread, muffin, chocolate brownie, chocolate milk drink, toasted muesli, pasta, and instant mashed potato); lupin fibre treatment period was designed to provide 17-30 g additional fibre compared to the control diet; subjects told to avoid legumes, high-fat foods, lipid-modifying foods, and fermented foods (e.g. yogurt); faecal collection on day 24, 25, and 26; 3-day faecal collection was pooled and analysed using 16sRNA analysis FISH: total bacteria; *Bifidobacterium*; *Lactobacillus* (and *Enterococci*); *Bacteroides – Prevotella; E. rectale* and *Clostridium coccoides* (*Clostridium* clusters XIVa and XIVb); *Enterobacteriaceae* (including *Escherichia coli*); *Clostridium lituseburense* (*Clostridium* cluster XI) and the *Clostridium histolyticum* group (*Clostridium* clusters I and II); *Clostridium ramosum, Clostridium spiroforme,* and *Clostridium cocleatum*
Lambert *et al.* (2017)	
Pulses	Pulse fibre
Subjects	overweight and obese (placebo BMI: 33.3±5.3; pea fibre BMI: 33.1±6.1)
n (gender)	control: 22 (M: 4; F: 18); pea fibre: 22 (M: 4, F: 18)
Age (Mean ± SD)	18-70 years
Design	double-blinded, randomised, placebo-controlled trial
Treatment and dose	placebo; yellow pea hull fibre 15 g/day
Treatment matrix	wafers formulated with and without yellow pea hull fibre
Treatment duration	12 weeks
Method comments	wafers consumed 30 min before largest meals; doses were increased over time: wk 1 = 5 g/d; wk 2 = 10 g/d; wk 3 = 15 g/day; stool samples collected at week 0 and week 12; 16sRNA analysis qPCR was used to quantify specific groups of microbiota (16srRNA gene copies/20 ng total genomic DNA): Total bacteria; *Bacteroides/Prevotella* spp.*; Bifidobacterium* spp.*; Enterobacteriaceae; Methanobrevibacter* spp.*; Firmicutes; Lactobacillus* spp.; *C. leptum* (cluster IV); *C. coccoides* (cluster XIVa); *Clostridium cluster* I; *Clostridium cluster* XI; *Roseburia* spp.

[1] BMI = body mass index; FISH = fluorescent in situ hybridisation; SEM = standard error of the mean; pre-MetSyn = pre-metabolic syndrome.
[2] Finley *et al.* (2007): Study indicates that n=80 (n=10 per group). However, methods section indicates that n=79.
[3] BMI and age data for treatment groups reported for Sheflin *et al.* (2017) was from Borresen *et al.* (2016).

chocolate milk drink, toasted muesli, pasta, and instant mashed potato (Smith *et al.*, 2006), while yellow pea hull fibre was incorporated into wafers (Lambert *et al.*, 2017).

Analysis of the changes in gut microbiota

In all studies, faecal samples were used to sample the gut microbiota from human subjects. Various methods used 16sRNA to estimate abundance, diversity, and/or richness including T-RFLP analysis (Fernando *et al.*, 2010), quantitative polymerase chain reaction (qPCR) (Fernando *et al.*, 2010; Finley *et al.*, 2007; Lambert *et al.*, 2017), fluorescent *in situ* hybridisation (FISH) (Smith *et al.*, 2006), and pyrosequencing (Sheflin *et al.*, 2017). Furthermore, Fernando *et al.* (2010) and Sheflin *et al.* (2017) used results from qPCR and pyrosequencing of 16sRNA sequences to apply ecological indices of diversity and richness: Shannon-Weiner diversity index, and Chao1 and the Simpson diversity index, respectively.

Effects of pulses on microbial abundance, diversity, and richness

Given the differences in treatments and methods applied across the five included studies, the effects of whole pulses and pulse-derived ingredients on various indices of changes in microbial abundance, diversity, and/or richness were mixed. Results are summarised in Table 2.

Table 2. Summary of results from studies that underwent full-text review.[1]

Study	Treatment and dose	Results
Whole pulses		
Fernando *et al.* (2010)	• control diet • control diet + 200 g/day chickpeas	Pooled phylogenetic analysis: • *Clostridium* cluster IV was most abundant bacterial group (control, 47% of sequences; chickpea, 40% of sequences) • other abundant groups: – Within *Clostridium* cluster IV: ▪ *Faecalibacterium* (control, 17% of sequences; chickpea, 24% of sequences) ▪ *Subdoligranulum* (control, 29% of sequences; chickpea, 10% of sequences) – *Clostridium* cluster XIVa (control, 40% of sequences; and chickpea, 35% of sequences) – Within *Actinobacteria*, *Bifidobacterium* were detected in the chickpea library • no differences in the taxonomic composition of pooled libraries across treatment groups. T-RFLP and qPCR analysis: • the number of individuals that were positive for *MspI* TRFs associated with *Clostridium* cluster XI and cluster I/II was lower for chickpeas (7.24±1.3) than the control (10.8±3.4) (P<0.05) • qPCR demonstrated no differences in 16sRNA gene copies/g faeces between bacterial groups Diversity • Shannon Diversity Index: no differences between diets
Finley *et al.* (2007)	• control: no beans • cooked pinto beans (canned): 130 g/day	qPCR analysis: • no effect of beans on measured bacterial populations (ΔΔCt), except f*or Eubacterium limosum* • pre vs post-intervention abundance was lower for *E. limosum* in healthy and pre-MetSyn bean groups compared to pre vs post levels in non-bean groups • *Peptostreptococcus productus* was increased in pre-MetSyn group compared to healthy controls groups with no effect of beans
Pulse flour (powder)		
Sheflin *et al.* (2017)	• control • cooked navy bean powder: 35 g/day (equivalent to 0.5 cup cooked navy beans) (Borresen *et al.*, 2014)	Cooked navy bean powder increased species richness (Chao1index) by day 28, but had no effect on observed species richness (Sobs), and diversity (Simpson Diversity Index); the control treatment had no effect on richness and diversity; cooked navy bean powder and the control had no effect of phyla abundance Pyrosequencing analysis: • Cooked navy bean powder – day 14: The abundance *Bacteroides fragilis* OTU ↓0.393× and the abundance of *Lachnobacterium* sp. OTU ↑9× compared to baseline – day 28: The abundance of *B. fragilis* OTU remained ↓0.35× and *Anaerostipes* sp. ↓0.08× vs baseline. *Lachnospira* sp. OTU (↑3.38×), *Coprococcus* sp. OTU (↑1.91×), and *Clostridium* sp. (↑6.86×) increased compared to baseline • Control – no difference in OTUs at day 14 and 28 compared to baseline
Pulse fibre		
Smith *et al.* (2006)	• control: low fibre • lupin kernel fibre: 17-30 g/day	No difference in total protein and carbohydrate intakes between groups; fibre intake was increased (P<0.05) in lupin kernel fibre group (~20 g/day) compared to the control diet; no effect of diet on faecal bacterial cell numbers (cells/g dry weight faeces) FISH analysis – effect of treatments on specific bacterial groups: • lupin fibre increased the abundance of *Bifidobacterium* spp. compared to the control diet (P=0.001) (lupin fibre: 1.7×10^9 cells/g dry weight faeces vs control 0.8×10^9 cells/g dry weight faeces) • lupin fibre decreased the abundance of combined *Clostridium ramosum*, *Clostridium cocleatum*, and *Clostridium spiroforme* (P=0.039) relative to the control diet (lupin fibre: 6.0×10^7 cells/g dry weight faeces vs control: 7.9×10^7 cells/g dry weight faeces) • of the 18 subjects, 15 had higher numbers (cells/g dry weight faeces) of *Bifidobacterium* with lupin kernel fibre, and 13 demonstrated lower *C. ramosum*, *C. cocleatum*, and *C. spiroforme* vs control • lupin fibre consumption tended (P=0.053) to decrease the abundance of bacteria in the genus *Bacteroides-Prevotella* (lupin:1.3×10^{10} cells/g dry weight faeces vs control: 2.5×10^{10} cells/g dry weight faeces)

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Table 2. Continued.

Study	Treatment and dose	Results
Lambert *et al.* (2017)	• placebo • yellow pea hull fibre: 15 g /day	qPCR analysis: • no specific effect on pea fibre wafers on abundance of microbiota (16srRNA copes/20 ng total genomic DNA from faeces) • *Clostridium leptum* (cluster IV) (P=0.023), *Clostridium* cluster I (P<0.001), and *Roseburia spp*. (P=0.047) increased in both groups compared to baseline

[1] FISH = fluorescent *in situ* hybridisation; OTU = operational taxonomic unit; pre-MetSyn = pre-metabolic syndrome.

Whole pulses

In regard to effects of canned pinto beans (130 g/day), qPCR demonstrated that, after 12 weeks, healthy and pre-metabolic syndrome subjects had a lower abundance (ΔΔCt) of *Eubacterium limosum* compared to corresponding groups not consuming beans (Finley *et al.*, 2007). In the study by Fernando *et al.* (2010), pooled phylogenetic analysis showed that, for the control and chickpea treatment groups, *Clostridium* cluster IV was the most abundant group. Moreover, pooled analysis of the taxonomic libraries did not show any major differences in microbiota composition. However, unpooled analysis of faecal samples by T-RFLP demonstrated that, compared to controls, the percentage of individuals positive for *Clostridium* cluster *XI* and *Clostridium* cluster I/II was decreased with chickpea consumption. When TRFs produced from *Msp*I digestion were used to measure diversity using the Shannon-Weiner Diversity index, chickpeas did not alter diversity compared to the control. Finally, qPCR analysis (16srRNA copies/g faeces) of specific groups demonstrated no differences between any of the treatment groups. It is acknowledged that the study by Fernando *et al.* (2010) had a third active treatment arm as raffinose (5/day) that did not modulate the percentage individuals positive for targeted microbiota, 16srRNA copes/g faeces, or diversity.

Pulse flour: cooked navy bean powder

Sheflin *et al.* (2017) analysed samples from colorectal cancer survivors enrolled in the Beans/Bran Enriching Nutritional Eating For Intestinal Health Trial described by Borresen *et al.* (2016). Analysis of pyrosequenced 16sRNA transcripts from faecal samples demonstrated that navy bean powder increased microbial richness compared baseline, but had no effect on diversity (Sheflin *et al.*, 2017). Also, compared to baseline, principle component analysis revealed that navy bean powder did not affect the community structure of the microbiome. Although navy bean powder did not affect the abundance of phyla, changes at the genus and species level were observed. After 14 days, navy bean powder increased the number of operational taxonomic units (OTUs) of the genus *Lachnobacterium* sp. by 9×, and decreased the number of OTUs for *Bacteroides fragilis* (0.393×). After 28 days, *B. fragilis* remained lower than baseline (0.35×), while *Anaerostipes* also decreased (0.08×). OTUs for genera *Clostridium* sp., *Lachnospira* sp., *and Coprococcus* sp. were increased 6.86×, 3.38× and 1.91×, respectively, compared to baseline. Functional analysis of 16sRNA transcripts demonstrated no differences in the functional categories of microbiome with navy bean powder at days 14 and 28, compared to baseline. Although not included in Tables 1 and 2, it is worthwhile to note that rice bran, a third treatment arm, was shown to modulate both richness and diversity at day 28. In addition, on days 14 and 28, considerably more OTUs (23 OTUs and 69 OTUs, respectively) differed compared to baseline with rice bran than with what was observed with cooked navy bean powder (see above).

Pulse-derived fibre

In the study by Smith *et al.* (2006), 17-30 g lupin fibre/day was incorporated into a range of food products and significantly increased daily fibre intakes by ~20 g/day compared to the control group. Consumption of lupin fibre had no effect on the abundance of total bacteria (5.0×10^{10} cells/g dry weight faeces) compared to controls (5.1×10^{10} cells/g dry weight faeces). Lupin fibre increased the abundance of *Bifidobacterium* spp. (lupin fibre: 1.7×10^{9} cells/g dry weight faeces vs control 0.8×10^{9} cells/g dry weight faeces) and decreased the abundance of the aggregated group of *Clostridium ramosum, Clostridium cocleatum*, and *Clostridium spiroforme* (lupin fibre: 6.0×10^{7} cells/g dry weight faeces vs control: 7.9×10^{7} cells/g dry weight faeces) relative to the control diet. Lupin fibre consumption tended (P=0.053) to decrease the abundance of *Bacteroides-Prevotella* genera (lupin:1.3×10^{10} cells/g dry weight faeces vs control: 2.5×10^{10} cells/g dry weight faeces). Analysis of individual responses demonstrated that increases in *Bifidobacterium* spp. and reductions in the group encompassing *C. ramosum, C. spiroforme, and C. cocleatum* were driven by directional responses in 15 and 13 of the 18 subjects, respectively.

In the second study by Lambert *et al.* (2017), yellow pea hulls failed to facilitate any changes in the abundance (16sRNA copies/20 ng total genomic DNA extracted from faecal samples) of specific family, genera, and species of bacteria analysed by qPCR (total bacteria, *Bacteroides/ Prevotella* spp., *Bifidobacterium* spp., *Enterobacteriaceae*, *Methanobrevibacter* spp., *Lactobacillus* spp., *Clostridium leptum* (cluster IV), *Clostridium coccoides* (cluster XIVa), *Clostridium* cluster I, *Clostridium* cluster XI, and *Roseburia* spp.). There was an increase 16sRNA copies for *C. leptum* (cluster IV) (P=0.023), *Clostridium* cluster I (P<0.001), and *Roseburia* spp. (P=0.047) in the placebo and yellow pea fibre group compared to baseline.

4. Discussion

The results of this systematic review demonstrate that a limited number of human interventions have investigated the effects of a small number of pulse types and pulse ingredients on the microbial populations that inhabit the human gut. Some studies demonstrated that whole pulses (pinto beans and chickpeas), cooked navy bean powder, and pulse-derived fibre (lupin kernel fibre), did affect the abundance, diversity, and/or richness of gut microbiota. However, the methodologies used to decipher the observed effects varied between studies, making it difficult to assert a consistent effect. At the present time, more studies are required to ascertain the effects of specific pulses and their derivatives on microbes that inhabit the human gut.

In general, legumes, including pulses, are promoted as nutrient dense foods in healthy dietary patterns (Marinangeli *et al.*, 2017). The nutrient composition of pulses, including high levels of dietary fibre and plant-based protein, underpins hypotheses on their positive effects on human gut microbiota. Pulses are increasingly being used as a dietary strategy to fill a protein gap as dietary patterns are being recommended to incorporate more plant-based foods for reducing risk for chronic disease and to address the impact of food consumption on climate change (Springmann *et al.*, 2018; Willett *et al.*, 2019). Plant-based dietary patterns that contain less animal-based protein have been associated with higher microbiome diversity (Singh *et al.*, 2017b). It has been demonstrated that a microbiome with extensive diversity and abundance is associated with better health (Lloyd-Price *et al.*, 2016). This could be due to the displacement of animal-based protein for plant-based protein, such as pulses, that contain fibre and other various substrates that can stimulate growth of intestinal microbiota (Tomova *et al.*, 2019).

However, results from this review demonstrate that a limited number of human studies have evaluated the *in vivo* effects of pulses and pulse ingredients on microflora that inhabit the human gut. Furthermore, studies have failed to expand their analysis to investigate how pulse-derived changes to gut microbial populations affect indices of health. Despite demonstrating changes in abundance and diversity, using dietary pulses to modify microbial diversity and function is only relevant if it elicits a positive health benefit. In fact, a 'prebiotic' is defined as 'a substrate that is selectively utilised by host microorganisms conferring a health benefit (Gibson *et al.*, 2017).' Nevertheless, it is this ability to identify a measured cause-and-effect benefit to the host that arises from changes in the abundance, richness, or functionality of microbiota from specific foods that is particularly challenging.

In this regard, animal models are useful as a proxy for deciphering the association between diet, including pulses, and microbiota-related changes in health outcomes. For example, rodent studies have shown that lentil-based diets, diets with added chickpea alpha-galactooligosaccharide or white kidney bean extracts (3% phaseolamin) increased or decreased the abundance of gut *Firmicutes* (Graf *et al.*, 2019; Siva *et al.*, 2018), and increased the abundance of *Lactobacillus* and *Bifidobacterium* (Dai *et al.*, 2017; Song *et al.*, 2016). These microbiological effects were associated with reductions in lipidemia, body fat accumulation, and an improved metabolic profile. A higher abundance of *Firmicutes* and lower *Bacteroidetes* has been shown to be associated with excesses body weight and an unhealthy metabolic profile (Ley *et al.*, 2006; Turnbaugh *et al.*, 2008). In the present review however, targeted and genome sequencing studies produced mixed results on effects on the abundance of genera belonging to *Firmicutes*, with reductions (Fernando *et al.*, 2010; Finley *et al.*, 2007; Sheflin *et al.*, 2017; Smith *et al.*, 2006), increases (Lambert *et al.*, 2017; Sheflin *et al.*, 2017), or null effects (Fernando *et al.*, 2010; Finley *et al.*, 2007; Lambert *et al.*, 2017; Sheflin *et al.*, 2017; Smith *et al.*, 2006) of treatments being reported. Also, one cannot dismiss the complexities of translating rodent models to delineate the interplay between human gut microbiota and health, including evolutionary and ecological (genotype, phenotype, diet, behaviour, physiology, and environment) differences (Arrieta *et al.*, 2016). Regardless, given that foods, on their own, elicit physiological and metabolic responses, it is inherently challenging to separate the direct and indirect (i.e. via the microbiome) effects of food on metabolic health responses.

Of the studies included in this review, only one by Lambert *et al.* (2017) included physiological endpoints and showed that pea hull fibre elicited reductions in body weight (-0.87 kg), body fat (-0.74 kg), energy intake (-16%), and post-prandial glucose responses. In spite of this, the pea hull fibre failed to show differential effects on the microbial populations analysed compared to subjects receiving the control treatment. However, targeted genomic analysis used in this study may have excluded genera of microbiota responsible for the physiological changes observed. In a follow-up study to the Lambert *et al.* (2017) that was

published after this systematic search, bioinformatic analysis of the microbiome demonstrated that wafers enriched with yellow pea fibre induced additional changes in the abundance of gut microbiota with a decrease in the family *Actinomycetaceae* and an increase in the family *Barnesiellaceae* compared to baseline (Mayengbam *et al.*, 2019). The overall structure of the microbial community did not differ between groups. However, changes in body weight were negatively associated with changes in the abundance of *Lachnospira* (Mayengbam *et al.*, 2019). Further analysis also showed correlations between glucose area under the curve, metabolites, such as SCFA, and specific genera of bacteria (Mayengbam *et al.*, 2019). Nevertheless, recent analyses suggest that the association between gut microbial communities and physiological outcomes, such as obesity, can be highly confounded (Sze and Schloss, 2016).

It has been hypothesised that functional characteristics of the microbes could be more important for fostering a healthy symbiotic relationship with the host (Shafquat *et al.*, 2014). SCFA production is an indicator of metabolically active microbiota, but human studies demonstrating strong links between SCFA and health outcomes are limited (Gill *et al.*, 2018; Hernandez *et al.*, 2019). In this review, Finley *et al.* (2007) showed that faecal samples from healthy, but not subjects with pre-metabolic syndrome, inoculated with dried bean powder supported higher production of total SCFA and propanoic acid. Sheflin *et al.* (2017) and Fernando *et al.* (2010) also demonstrated no effects of navy bean powder or whole chickpeas on faecal SCFA levels, respectively, despite changes in microbial abundance and richness. In a companion study, Baxter *et al.* (2018) analysed the same faecal samples as Sheflin *et al.* (2017) for metabolic differences arising from microbial changes. Using the pathway enrichment score, they evaluated the effects of cooked navy bean powder on the gut metabolome and observed significant functional changes in metabolic pathways corresponding to carbohydrate, amino acid, lipid, nucleotide, cofactor, and vitamin metabolism (Baxter *et al.*, 2018). Studies that broaden their analyses to incorporate metabolomic and functional outcomes may also provide mechanistic insights that bridge the microbiome to clinical endpoints.

The present review has several limitations. First, between-person heterogeneity is a limitation for deciphering the effects of pulses and other dietary factors on the human microbiome. It also cannot be ignored that a 'healthy' microbiome has yet be identified in humans (Lloyd-Price *et al.*, 2016); which, in and of itself, is a challenge. Sheflin *et al.* (2017) identified inter-individual variation as a possible factor for not demonstrating effects of navy bean powder on SCFA production. Moreover, the studies by Smith *et al.* (2006) and Fernando *et al.* (2010) observed that a subset of subjects receiving pulse treatments demonstrated changes in *Bifidobacerium* and *Clostridium,* or were positive for specific genera (*Clostridium* clusters I/II and XI) of bacteria after receiving treatments, respectively.

Second, most studies investigating the effects of pulses on human intestinal bacterial populations and/or metabolism have used targeted analyses that evaluates specific phyla, genera, and/or species of microbiota. Conversely, the study by Sheflin *et al.* (2017) was the only study to use a genome-wide method, pyrosequencing, to evaluate the effects of a pulse-based intervention on the human intestinal microbiome. Given the complexity of microbiological gut environment, targeted analyses may be insufficient to identify shifts in diversity, richness, and abundance of specific microbiota.

Third, this review investigated the quantitative microbial population changes in the human gut, without links to clinically relevant health indices. For these linkages to be made, more research in humans is required. Future studies should give careful consideration to study design including, the health status of the study population (healthy vs unhealthy), treatment dose, frequency of consumption, and study duration. Also, one cannot dismiss the fact that, while similar, different types of pulses or the ingredients from which they are derived can differ in composition. Each of these factors can affect responses of the microbiome to treatments and whether changes in the microbiome further facilitate a health benefit. For example, individuals with hypercholesterolemia are more likely to demonstrate reductions in circulating cholesterol levels in response to a treatment, versus those with healthy cholesterol concentrations. Methods that are able to establish whether changes in health outcomes are due to diet-mediated changes in the microbiome also require further development. Increased focus on the effects of whole pulses on the microbiome should also be emphasised, as whole pulses are promoted in dietary guidelines, are already associated with health outcomes, and more accessible than supplements.

5. Conclusions

This systematic review demonstrated that specific types of whole pulses and pulse ingredients can modulate microbial populations in the human gut. However, these reported changes are inconsistent between studies and cannot be extrapolated to an effect for all pulses. More extensive human studies that employ comprehensive approaches to examine the effects of dietary pulses on the microbiome and health outcomes are required. The utilisation of pulses within the context of healthy dietary patterns that facilitate the establishment of a highly diverse and abundant microbiome could be the most useful for demonstrating the utility of pulses for facilitating health via the symbiotic human relationship with intestinal microflora. Future studies that take a more comprehensive approach

to investigating the effects of pulses on the microbiome are required.

Conflicts of interest

CPFM is employed by Pulse Canada and was formally employed by Kellogg Canada. TCR, SVH, and MZ have no conflicts to declare.

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