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Short title: Protein metabolism by human gut bacteria

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Abstract

The composition and metabolic activity of the bacteria that inhabit the large intestine can have a major impact on health. Despite considerable inter-individual variation across bacterial species, the dominant phyla are generally highly conserved. There are several exogenous and gut environmental factors that play a role in modulating the composition and activities of colonic bacteria including diet with intakes of different macronutrients, including protein, accounting for approximately 20% of microbial variation. Certain bacterial species tend to be considered as generalists and can metabolise a broad range of substrates, including both carbohydrate and protein derived substrates, whist other species are specialists with a rather limited metabolic capacity. Metabolism of peptides and amino acids by gut bacteria can result in the formation of a wide range of metabolites several of which are considered deleterious to health including nitrosamines, heterocyclic amines and hydrogen sulphide as some of these products are genotoxic and have been linked to colonic disease. Beneficial metabolites however include short chain fatty acids and certain species can use amino acids to form butyrate which is the major energy source for colonocytes. The impact on health may however depend on the source of these products. In this review we consider the impact of diet, particularly protein diets, on modulating the composition of the gut microbiota and likely health consequences and the potential impact of climate change and food security.

General Introduction

The human colon is an anoxic and dynamic environment which constantly interacts with the hosts immune system. The colon harbours a dense collection of bacteria that inhabit the large intestine that mainly belong to five different phyla. These phyla are comprised of many hundreds of different species and most of these bacteria are anaerobes. Given the advances in the molecular methods available to profile the gut microbiota[1] there is currently a good understanding of composition. Moreover, affordable complete genome sequencing of gut bacteria has meant that it is feasible to mine the genomes of many human gut bacterial species for traits of interest. For example, certain bacterial species, often with large genomes, such as some *Bacteroides* species, are considered are generalists as these have a remarkable repertoire of enzymes[2] which allows these bacterial cells to metabolise a broad range of substrates as carbon, nitrogen and energy sources.

Numerous factors influence gut microbial composition such as host genetics, general health status, exposure to microbes during early life and consumption of antibiotics. Diet however is a major contributor to microbial structure and the main dietary macronutrients: carbohydrate, proteins and fat will influence gut microbial activities and metabolic outputs. Also, the types of foods consumed, cooking processes used and balance of macronutrients and micronutrients are likely to be important drivers of health. The major products of fermentation include the short chain fatty acids and butyrate, in particular, has a special role for the host and it is the major energy source for colonocytes[3]. Other bacterial metabolites formed mainly from the metabolism of proteins can result in the formation of less beneficial products including N-nitrosamines and heterocyclic amines that can be deleterious to health[4]. The overall balance of benefit and detriment for the host will therefore depend on the status of the microbial community in terms of its distribution, diversity, species composition and metabolic outputs[5].

Increasingly there is considerable concerns around the types of foods we eat and the global impact of both climate change and the SARS-Cov-2 pandemic has highlighted the urgency to maintain food security. Moreover, this pandemic has highlighted the need for many countries, including the UK, to consider more sustainable food chains and reducing transportation of imported foods. The UK currently imports around half of the food consumed. Consideration therefore needs to be given to agricultural practices and food production systems with an additional aim of reducing greenhouse gas emissions[6]. The food we eat impacts on the overall metabolic activities of colonic microbes and dietary changes will have an impact on in health

and disease. In this review we consider the complex relationship between diet, particularly protein content, and the gut microbiota and its metabolism and how this may impact on health whilst outlining the impact of different protein diets on the environment.

The human gut microbial ecosystem

The human large intestine performs several key functions including degradation of dietary substrates, nutrient absorption, excretion of waste and is the major site of salt and water absorption. Other than diet, the composition of the gut microbiota may be influenced by many external factors including host genetics which has been estimated to explain approximately 9% of the variation[7]. Other factors that impact on microbial variation may include age, geographical location and antibiotic usage[8].

Moreover, some host factors may also impact on gut microbial composition including gut transit which can be very variable. The mean gut transit time across the complete length of the intestinal tract in healthy adults has been estimated to be between 26 and 35 hours[9,10], but can be up to several days thereby allowing for the establishment of an abundance of microorganisms[11]. There are many discrete physiological environments within the human gastrointestinal tract, which includes the highly acidic conditions in the stomach[10], to the more alkaline pH in the small intestine with changes in pH along the large intestine.

Importantly, diet is thought to explain approximately 20% of the variation in gut microbial composition[12]. Undigested dietary material that escapes digestion by host enzymes enter the colon and are rapidly fermented by the resident microbiota. This results in rapid microbial growth and production of short chain fatty acids and other metabolites, which in turn lowers the pH[13]. As the digesta moves towards the distal colon, carbohydrate sources become depleted therefore microbial growth and carbohydrate fermentation decreases whilst peptide fermentation can increase, depending on dietary intake, resulting on the formation of a range of nitrogenous products including ammonia which is one of the products that drives an increase in pH towards neutrality[13].

Colonic microbial composition

The human large intestine harbours viruses many of which are bacteriophages[14,15], fungi including the dimorphic *Candida* species[16] and bacteria. The latter is comprised of thousands of different bacterial species that reach their highest density in the large intestine. The composition and metabolic activities of these microbes are likely to strongly influenced by diet which will impact on health and disease[5,12].

The adult microbial community usually contains around 10^{11} bacterial cells/g of faeces[17]. The gut microbiome also contains many more genes (approximately 150-times more) than the human genome, which is currently estimated to possess around 24 000 genes, providing the host with greatly expanded functionality, particularly with regard to complex carbohydrate metabolism[18] and although gut bacterial composition can be decidedly variable, functionally it somewhat more highly conserved[19].

Despite inter-individual variability at the genus and species level, in the composition of the gut microbiota, there are core species that are found in most healthy individuals. At the phylum level, Firmicutes and Bacteroidetes are the most dominant. The less abundant phyla are the Proteobacteria, Actinobacteria and Verrucomicrobia[20]. A key species of the latter is the mucin-degrading bacterium *Akkermansia muciniphila*[21] which is considered to be health protective.

Bacteroidetes usually comprise around 30 % of the total microbiota, although this can be quite variable, and it is becoming increasingly apparent that there is a divergence with some individuals tending to be either *Bacteroides* or *Prevotella* dominant[8]. *Bacteroides* and *Prevotella* species can utilise carbohydrate or protein derived substrates[22]. Commonly occurring species include *B. vulgatus, B. fragilis, B. distasonis, B. uniformis, B. thetaiotaomicron* and *B. eggerthii*[23].

The Firmicutes are members of the clostridia class and the predominant human colonic species mainly belong to two phylogenetic groups. One group is the Lachnospiraceae that includes genera such as *Eubacterium, Roseburia, Butyrivibrio*, *Coprococcus* and *Lachnospira* and the second is the Ruminococcaceae that encompasses *Faecalibacterium* and *Ruminococcus* species. Other commonly reported genera found in lower abundance include *Bifidobacterium* and *Veillonella* species[20,24,25].

Facultative anaerobes are usually much less dominant in the healthy colon but their abundance may be elevated in certain diseases[26] and includes Enterobacteriaceae species. A number of other, specialised groups may exist at lower levels including the sulphate-reducing bacterial species *Desulfovibrio*[27]. Proteobacteria are usually in low abundance in the healthy gut but are often more prevalent in frail elderly[28]. Archaeal methanogens may also be present in approximately 50 % of adults with *Methanobrevibacter smithii* as the predominant species[29] and methane which is a major end product that may slow gut transit.

Role of gut environmental factors including anaerobiosis, pH and bile on microbiota composition

There are many factors that are likely to impact on the composition of the gut microbiota. This includes host factors as well as gut environmental factors such as anaerobicity, pH and bile salt levels.

Anaerobic ecosystem

The two dominant phyla that inhabit the large intestine are the Firmicutes and Bacteroidetes and their niche in the colon may partly be driven by redox potential (Eh) and gas phase. Gut microorganisms persist in an environment with low partial oxygen pressures and this anaerobic ecosystem has an Eh value of around -250 mV. Anaerobes generally lack electron transport chains found in facultatively anaerobic bacteria to regenerate the reduced co-factors and therefore do not gain further energy by electron transport level phosphorylation. Instead, metabolic intermediates are reduced mainly to acidic fermentation products and gases. Some gut bacteria including Proteobacteria perform anaerobic respiration involving electron transport chains by using electron acceptors such as sulphate or carbon dioxide[20].

Bacteroides species have been described as 'nanaerobes' as many species can survive for several hours in the presence of oxygen but require anoxic conditions to grow. By comparison, many gut bacterial species belonging to the Firmicutes are considered as strict anaerobes and are unable to survive for even a few minutes upon exposure to air[5]. Interestingly, one of the most abundant Firmicutes species in the large intestine, *Faecalibacterium prausnitzii*, has adapted to using an electron shuttle of thiols and flavins to transfer electrons to oxygen[30].

Gastrointestinal pH

As much of the metabolism of gut anaerobes is given over to fermenting dietary macronutrients, particularly non-digestible carbohydrates to short chain fatty acids, the pH of

the proximal colon which is the most active site of fermentation in healthy subjects is mildly acidic (around pH 5.5-6.0). There are usually less carbohydrates available in the distal colon, bacteria that are resident in this section are also reliant on the metabolism of peptides and amino acids as sources of carbon and nitrogen. This is likely to result in the formation of higher levels of nitrogenous products including ammonia and will contribute to driving pH values closer to neutrality. Many of the Gram-positive Firmicutes species are more tolerant of the mildly acidic conditions in the proximal colon which is likely to provide a competitive advantage for these bacterial species whereas the growth of Bacteroidetes species are likely to be restricted here but are likely to be more active in the distal colon where the pH is closer to neutrality[27], This has been supported by studies that revealed these the major changes in species composition and metabolic products when comparing the impact of pH 5.5 and 6.5 in model colonic *in vitro* fermentor systems[31] whereby mildly acidic conditions (around pH 5.5) is favoured by, for example, butyrate *Roseburia* species. When the pH is closer to neutrality (pH 6.5) *Bacteroides* spp. that have a role in peptide metabolism tend to be favoured[32].

Bile salts

Enzymes in the liver convert cholesterol to bile acids which are secreted into the intestine from the gall bladder. More cholesterol is formed when diets are high in saturated fats and consequently the secretion of bile increases when consuming these diets. The bile acids made in the liver are known as primary bile acids and in humans there are two major types are cholic acid and deoxycholic acid. Within the liver these are usually conjugated to two amino acids, either glycine or taurine. The latter is biosynthesised from methionine and cysteine whilst glycine is from serine.

Interestingly, it is the type of dietary macronutrients that largely dictates whether the bile acids are conjugated with glycine which is likely to be largely plant based or alternatively to taurine which is most likely to occur when diets are high in animal protein and fat. When these conjugated bile acids reach the large intestine, certain gut bacterial species that possess bile salt hydrolases can cleave the linkages that bond the bile acids to the amino acids. In the case of taurocholic acid the deconjugation process results in the release of taurine and choline into the intestine. Certain bacterial species such as *Bilophila wadsworthii* can metabolise taurine to form ammonia, acetate and hydrogen sulphide and the latter product is genotoxic. *Clostridium scindens* can remove the hydroxyl group from choline to form deoxy choline which is a tumour

promoting agent[33]. The activities of these bacterial species in the colon are therefore considered likely to contribute to the promotion of colorectal cancer[34].

Moreover, habitual intakes of a typical Western-style diet which is usually considered to be low in fibre, but high in refined sugar and fat, has been associated with increased levels of endotoxin-producing bacteria such as *Escherichia coli.* This may be due to high fat resulting in increased bile formation and that these species may be more tolerant of bile than other bacteria that are dominant in the large intestine.

Impact of diet on modulating the gut microbiota

Recent major advances in molecular profiling technologies has progressed our understanding of the composition of the gut microbiota and how it changes through life stages from birth[35] to the elderly[36]. In adults, short-term dietary interventions have demonstrated that these shifts occur rapidly[37] however these changes may be transient.

Dietary influences on the gut microbiota of infants, adults and elderly

Diet is a factor that shapes the composition of the gut microbiota across all the differing life stages. The GI tract of a foetus is usually considered sterile then following birth the gut microbiota of babies is likely to depend on mode of delivery[35], bile acids[38] and feeding regime, including whether babies are breast or formula fed[39]. The intestinal tract of breast-fed babies is largely dominated by members of the *Bifidobacterium* genus, which appear to be exquisitely adapted to utilise human milk oligosaccharides[40]. Breast milk is a rich source of peptides, from casein and whey, in addition to non-digestible sugars, usually referred to as human milk oligosaccharides (HMOs) is likely to drive bifidobacterial population establishment in the colon. HMOs are amongst the most abundant components of human milk after water and lactose. These HMOs have a degree of polymerization from three to 32 with around 50 of these different carbohydrates present in mother's milk. Major HMOs include lacto-N-tetraose, lacto-N-neotetraose and lacto-N-hexaose along with fucosylated molecules. Formula-fed babies, in contrast, usually possess a more complex gut microbiota that is more adult-like in composition[41]. The introduction of solid foods at weaning results in completely altered substrate availability in the colon and triggers the expansion of obligately anaerobic

bacterial groups such as the Bacteroidetes and Firmicutes, which are able to breakdown and metabolise more complex polysaccharide sources[42].

Following weaning and up to around three years of age the microbiota of infants tends to become more diverse with a high rate of microbial instability and therefore this is a crucial period for the development of the gut microbiota which may impact on long term health. Beyond three years of age the gut microbiota tends to stabilise although dietary intakes will influence the microbiota composition. Changes in the composition of the gut microbiota may however undergo more prolonged development than previously suspected with the microbial diversity of children having perhaps greater diversity than that of healthy adults.

Despite a tendency for microbial stability in adulthood with habitual diet providing a constant source of nutrients, the gut microbiota is in a constant state of flux. A diverse diet that includes a number of different types of plant foods has been associated with greater bacterial diversity and (12) observed a positive relationship between dietary diversity and microbial stability with *F. prausnitzii* being increased in individuals that consumed more than 30 plant types per week compared to those that consumed less than 10 per week[43].

In the elderly, previous studies have compared the differences in the microbiota in elderly within community-dwellers to those who are staying in care homes as the latter tend to have more health problems[44,45]. The study found that care home dwellers had a higher proportion of Bacteroidetes than that found in community dwellers. *Roseburia* species that are known butyrate producers were also present in lower abundance in care home residents and *F. prausnitzii* was lower in abundance in frail elderly. Moreover, the elderly population (> 65 years old) has been found to have a gut microbiota that is less diverse than in healthy young adults which may be due to the reduction in diet variation and also deterioration in dentition, salivary function and gut transit[36].

Ageing may also affect the ileal microbiota, as has been suggested by examinations of the ileal contents of sudden death elderly patients, which revealed that their microbial community contained high proportional abundances of Proteobacteria, *Bacillus, Streptococcus* and *Lactobacillus* species when compared to that of adult ileostomy patients with were observed to have lower proportional abundance of Proteobacteria and higher abundance of species belongs to Firmicutes[46].

Dietary macronutrients

Diet has a key role in modulating the composition of the human intestinal microbiota which impacts on health. The balance, type and amount of dietary macronutrients, including carbohydrates and protein, can have a major impact on the composition of the intestinal microbiota whilst micronutrient status including vitamin availability, some of which is derived from the certain species of the microbial community, is also important for health. Many of the dominant bacteria that reside in the human colon may be auxotrophic and therefore unable to synthesis all vitamins required for growth. These species are therefore dependent on the host or other bacteria for certain vitamins to facilitate growth[47].

Dietary carbohydrates

Complex dietary fibre is the most commonly accepted nutrient to exert a beneficial effect on microbial composition[5]. The main carbohydrate consumed by adults and available for utilization by intestinal microbes include resistant starches followed by non-starch polysaccharides and oligosaccharides[13]. The amounts of these macronutrients consumed per day can be highly variable with intakes of resistant starch ranging from less than 10 to more than 40 grams per day. Resistant starch is defined as dietary starch that escapes digestion by host enzymes in the upper gastrointestinal tract because of protection provided by other polymers (RS1), particle structure (RS2), retrogradation (RS3), or chemical cross-linking (RS4). The starch-degrading enzyme systems from human gut symbionts that have been studied in detail for *Bacteroides thetaiotaomicron*, *Eubacterium rectale* and more recently *Ruminococcus bromii* which is a keystone species for the breakdown of resistant starch in the human large intestine[48]. Unlike starch, pectins found in plan cell walls are structurally highly complex and are classified into homo-polygalacturonan (PG), rhamnogalacturonan I (RG I) and rhamnogalacturonan II[49]. Pectin degradation requires glycoside hydrolases (GH), polysaccharide lyases (PL) and carbohydrate esterases (CE) bacterial activities[42]. Pectin may be degraded by Gram-negative *Bacteroides* species[23] and a few Gram-positive bacterial species have also been reported to ferment pectin or pectin breakdown products including *Eubacterium eligens*[26,50] which possesses anti-inflammatory activity by promoting the production of IL-10 by epithelial cells[51].

Edible plants can contain several hundreds of phenolic compounds derived by the phenylpropanoid pathway and based on their structures these are classified predominantly into phenolic acids, flavonoids, stilbenes, lignans and tannins. On average around one gram of plant

phenolics may be consumed per day depending on dietary intakes[52]. Phenolic compounds can exert dual effects on the gut microbiota as they can inhibit the growth of specific taxa whilst enhancing the growth of others whereby, they can be metabolised into bioavailable substrates for the host. Food rich in phenolics such as fruits, vegetables, cereals, tea and coffee are associated with a range of health promoting activities with a reduced risk of chronic disease[53]. Aromatic amino acids such as tyrosine and phenylalanine are fermented to further phenolic compounds including cresol and phenol derivatives whilst tryptophan is fermented to indoles[54]. Further studies revealed that two abundant phenylpropanoid-derived compounds found in human faecal samples are phenylacetic acid (PAA) and 4-hydroxylphenylacetic acid (4-hydroxyPAA) and although they have the potential to be derived from diets rich in plant-based foods these compounds can also be derived from the microbial fermentation of aromatic amino acids in the colon and is a likely to be a major source of phenylpropanoid-derived metabolites in the colon[53].

Dietary protein

Approximately 3-18 grams of dietary protein enters the human large intestine every day[54] which is diet dependent. On a very low protein diet this can range from 3g/day to 16g/d on a vegan diet high in unprocessed cereals and grains. This can increase to 18/g/day on meat rich diets[55]. High protein and low carbohydrate diets may aid weight loss given their impact on satiety[56]. Undigested protein reaching the large intestine may however lead to an increase of pathogenic microorganisms with associated higher risk of metabolic diseases. High consumption of red meat, which in addition of being rich in protein also contains heme and has been associated with an elevated risk of developing colorectal cancer[57].

Dietary proteins are hydrolyzed into peptides and amino acids by both host- and bacterial-derived proteases and peptidases[58,59]. The released peptides and amino acids can be further utilized by both gut bacteria and the host. Bacterial metabolism of extracellular amino acids is likely however to require specific transporters. Peptide and amino acid-fermenting bacteria include species that belong to the following genera, *Bacteroides, Prevotella, Clostridium*, *Veillonella, Megasphaera*, *Acidaminococcus* and *Selenomonas* (**Fig. 1**). Certain species possess highly active dipeptidyl peptidase and dipeptidase activities, suggesting that these bacteria might be important for protein digestion and amino absorption in the mammalian digestive tract. Most gut bacteria utilize amino acids and ammonia as their preferred nitrogen

source although for others such as certain *Prevotella* species peptides are the preferred nitrogen source[60]. *Bacteroides* species can secrete proteases with presumed activity near the brush border of absorptive cells and a high abundance of *Bacteroides* species may result in an excess of proteases, which may degrade maltase and surcase enzymes in the brush borders of enterocytes[61].

The levels of proteins, peptides, and amino acids are relatively high in the proximal colon and reduced in the distal colon. Regarding the large intestine, it appears that amino acids are not significantly absorbed by the colonic mucosa, but rather are intensively metabolized by the large intestinal microbiota[62]. This higher rate of bacterial protein fermentation has been related to high pH and low carbohydrate availability in the large intestine[63] resulting in the generation of a complex combination of metabolic end products including short chain fatty acids and the major acids in the colon are acetate, propionate, and butyrate and the branched-chain fatty acids valerate, iso-butyrate, and iso-valerate. In addition, microbial metabolism of amino acids will also result in the formation of ammonia and amines and the latter is produced by decarboxylation of amino acids. The amines mainly produced by the resident microbiota include cadaverine (a decarboxylation product of lysine) and agmatine (a decarboxylation product of arginine)[64]. These amines can have significant physiological effects and agmatine has been shown to influence metabolic functions including elevating tissue cAMP levels, ultimately replicating the effects of caloric restriction with respect to metabolic reprogramming and leading to reduced diet-induced weight gain[65].

Microbial metabolites

Although microbial cells are usually prevented from breaching barriers allowing access to host cells in the large intestine, smaller molecular weight microbial metabolites can cross this barrier by diffusion and active transport. The gut microbiota forms an array of primary and secondary metabolites which can be transported into colonocytes and exert beneficial or deleterious effects on these epithelial cells depending on their concentrations in the lumen. Certain metabolites have been postulated to have a role in a wide range of health conditions, including diabetes, atherosclerosis, kidney disease, inflammatory bowel disease and cancer[66]. The gut anaerobes ferment dietary nutrients to form short chain fatty acids which include acetate, propionate, butyrate and gases including carbon dioxide and hydrogen. Some of the weak acidic metabolites including propionate and butyrate are likely to provide health benefits

including appetite control, dampen inflammation, maintain gut and systemic health and modulate disease progression. Conversely, lactate which is generally considered as an intermediate fermentation product can result in acidosis unless this product is removed by bacterial cross feeding[67]. Specialist gut microbial species can release and transform dietary plant phenolics and the spectrum of products formed may provide potent antioxidant and anti-inflammatory activities[53]. On the other hand, consumption of high animal protein and fat diets may lead to the formation of damaging microbial products including elevated levels of nitroso-compounds, hydrogen sulphide and trimethylamine[4].

Short chain fatty acids

Microbial fermentation in the large intestine results in the formation of a range of short chain fatty acids and the main acids detected in the large intestine are acetate, propionate and butyrate that make up around 90% of acids in the colon and are usually detected in molar proportions of around 3:1:1 but this is dependent on diet and the composition of each individual's microbiota. Some minor SCFA including iso-butyrate and iso valerate are formed by bacterial fermentation of branched chain amino acids. The total level of short chain fatty acids are usually in the region of 60 mM to 180 mM depending on factors such as diet and gut transit[37,68]. The majority of intestinal bacteria use the glycolytic pathway and the pentose phosphate pathway to harvest energy from carbohydrates, both pathways lead to the formation of pyruvate which is a key intermediate in SCFA formation[11]. Although acetate reaches the highest concentration of any of the SCFA in faeces, it is known that many human faecal bacteria are net consumers of acetate in pure culture[69] including the dominant butyrate producers *Faecalibacterium prausnitzii*, *Roseburia* species and *Eubacterium rectale*[70]. Butyrate is generally believed to be synthesised via two main routes namely butyrate kinase or butyryl CoA:acetate CoA transferase routes[71]. Butyrate is generally considered to provide a number of health benefits and is the preferred energy source for the colonocytes[3,72,73]. Increased levels of butyrate have been associated with increased intestinal transit[74].

Propionate can stimulate the gut hormones, peptide YY and glucagon like peptide-1, which increase satiety and thereby reducing energy intake and body weight gain in adults[75,76]. A large group of the gut bacteria can generate propionate including the abundant Bacteroidetes phylum[77]. Propionate can be formed via three different metabolic routes and these are the acrylate, succinate and the propanediol pathways[78].

Amino acids utilized by gut anaerobes that can be metabolized to acetate include glycine, threonine, glutamate, lysine, ornithine, and aspartate[61]. Threonine can give rise to all three major SCFAs and with propionate mainly being produced from threonine[61]. Butyrate can be generated from the metabolism of threonine, glutamate, and lysine. The latter can be used by species of *Intestinimonas* to form butyrate[79]. The branched chain amino acids namely valine, leucine, and isoleucine give rise to the formation of the branched chain fatty acids, iso-leucine, iso-valine and valine as has been reported for *Anaerotignum* species[80].

Hydrogen sulphide

Hydrogen can be formed by fermentative bacteria in the large intestine and in turn can be consumed by methanogens, acetogens and sulphate reducing bacteria. These bacteria are likely to compete for hydrogen. The end product of sulphate reduction, hydrogen sulphide can be formed by bacterial species such as *Desulfovibrio piger*[27] and can inhibit butyrate metabolism and is therefore highly toxic to the colonic mucosa[81]. This bacterial metabolic product can also inhibit colonic smooth muscle contractility[55]. Hydrogen sulfide is produced by fermentation of sulfur-containing amino acids, such as methionine and cysteine which is also derived from the reduction of inorganic sulfate and sulfite additives, and the catabolism of intestinal sulfomucins.

Ammonia and other nitrogenous bacterial metabolites

Peptides and amino acids are metabolised by gut bacteria following deamination and decarboxylation to several metabolites including ammonia, polyamines, phenols, indoles. Ammonia is generally found at millimolar concentrations in the large intestine and concentrations increase from the ascending to the descending colon, which is consistent with a higher rate of protein metabolism in the distal compared to the proximal colon. The ammonia concentration in the large intestine is mainly a microbial metabolite associated with amino acid deamination and urea hydrolysis. Intestinal microbiota can use ammonia, and ammonia can also be absorbed by the epithelial cells. Urea hydrolysis in the intestinal lumen is performed via bacteria urease activities which is better understood in ruminants than in humans[82]. A reduction in urease activity will result in a reduction in blood ammonia levels which is beneficial to health as high levels of ammonia have been linked to encephalopathy[83]. Nitrosamines are known carcinogens and can be detected in human faeces. Gastric formation

of nitrosamines has been well described in humans and the involvement of the microbiota has been demonstrated by comparing germ free and conventional rats[84]. Several bacterial species are capable of nitrosamine production including species belong to Proteobacteria[85,86].

The bacterial deamination of aromatic amino acids leads to the production of phenolic compounds and tyrosine deamination mainly yields phenol and *p*-cresol. The main food sources of tyrosine are egg, cod, seaweed and cheese and an increase of the nutritional protein load in healthy individuals and principally results in greater urinary excretion of *p*-cresol. An example of possible health impacts is that the tryptophan metabolite indole-3-propionic acid, which has been shown to be a potent anti-non-alcoholic steatohepatitis (NASH) microbial metabolite in preclinical models[87].

Polyamines are biogenic amines involved in host cell growth and differentiation and are produced by bacterial metabolism of species belonging to several genera including *Bacteroides*, *Lactobacillus*, *Veillonella*, *Bifidobacterium* and *Clostridium.* These bacteria can produce polyamines including putrescine, cadaverine, tyramine and histamine following the metabolism of amino acids including arginine, ornithine, lysine, tyrosine, histidine methionine[88]. Preclinical studies have shown that putrescine and spermidine in the colon is dependent on colonic microbiota[89] and that pectin fermentation by *Bacteroides thetaiotaomicron* and *Fusobacterium varium* stimulated polyamine production. Microbial synthesis of polyamines is considered as a therapeutic target[90], but there is limited information from human intervention studies on the impact of diet.

Regular consumption of cooked or processed meat can increase the risk of colon cancer and heterocyclic amines, such as 2-amino-1-methyl-6-phenylimidazo [4,5-b] pyridine, are considered to be a contributing factor. There is some evidence that the gut microbiota and in particular, the most abundant carcinogenic HCA; PhIP can be transformed by representatives of the phyla Firmicutes, Bacteroidetes, and Proteobacteria[91]. Similarly, the genotoxicity of mutagen 2-amino-3-methylimidazo[4,5-f]quinoline (IQ) was impacted on by the gut microbiota[92].

Human dietary protein studies

High levels of proteins and peptides in the large intestine could lead to increased production of deleterious metabolites. Magee *et al.*[93] reported that when subjects were fed a high-protein diet, levels of sulfide were elevated due to the bacterial fermentation of sulfur containing amino

acids. Butyrate concentrations and numbers of butyrate-producing bacteria are decreased in the large intestine as well in the feces[20]. It is widely regarded that butyrate is the main energy source for colonic epithelial cell, thus, a decrease in butyrate concentration and an increase in concentrations of ammonia and sulfide may explain the detrimental effect of high protein diet on the large intestine (e.g., increased incidence of colon cancer).

Consumption of red meat is often considered to have negative health outcomes however it is perhaps important to take into consideration, intakes, levels of processing and other dietary factors. The consumption of high-quality red meat is poorly associated with diabetes risk and coronary heart disease for a serving of 100 g of red meat per day. In contrast, higher risks were observed for processed meat consumption with an increased incidence of colorectal cancer (by 22%), heart disease (by 42%) and type 2 diabetes (by 19%)[94,95]. Moreover, there were no associations with stroke for any of the meat type products. Processed meats tend to contain higher sodium levels which may worsen cardiovascular conditions over habitual intake. The links between red meat and poorer health outcomes may therefore be confounded by the effects of processing. Higher plant protein intake and lower intake of some animal-based protein sources may contribute to the lower risk of disease associated with vegetarian diets. It maybe however that that the benefits of high plant protein intakes are linked to other nutrients.

The protein intake of children in western countries is very high and the average protein intake in children between 4-6 years old is around 55g/day. In infants where energy, protein and amino acid requirements are high, protein requirements are primarily met by intakes of human milk and infant formula. It is not clear if the protein requirements of older adults are higher than that of younger adults or is only higher in the frail elderly who are at risk of malnutrition because of acute or chronic illness.

Vegetarians exclude meat and fish from their diets and therefore there is a gradient of protein intake from meat eaters to vegans in western countries. In general, the adult population in western countries have a protein intake of around 1.3/kg/day which about twice the estimated average requirement of 0.66g/kg/day although a proportion of lacto-ovo-vegetarians may have protein intakes that do not meet their individual requirements.

It is often considered that amino acids may be inadequate in vegetarian diets although almost all plant-based foods contain all 20 amino acids, including the nine indispensable amino acids. The distribution profile of the amino acids however is less optimal in plant foods than in animal foods with lysine often being present in much lower than optimal proportions for human needs

in grains. Also, the sulphur containing amino acids, methionine and cysteine, are proportionally slightly lower in legumes than would be optimal for human needs. Mixing complementary protein sources within the same meal however may simply be a practical way to secure long-term adequacy when total protein intake is low.

Meat consumers were found to have the lowest fibre intake of less than 10 g per day and lower poly-unsaturated fatty acid consumption. Inadequacies for folate intake was also reported. However, the corresponding intake of micronutrients were found to be the highest for this group for zinc, phosphorus, and vitamin B_{12}. Iron intake was found to be inadequate in the case of women. Vegetarians were found to comply with most dietary requirements and among the three groups and have a fibre intake of approximately 33% greater than meat-eaters. Vegans however were found to have a fibre intake which was 75% greater than the meat-eating group and had mineral and micronutrient intake values similar to those observed in vegetarians. For people consuming plant-based diets further scientific evidence is required to determine if the protein intakes of vegetarian and vegan diets are adequate[96]. The amino acid requirements are therefore considered to be adequately met for vegetarian diets although some inadequacies were observed in the Adventist study population data which may have likely risen from over dependence on a few protein sources[97].

Ten different diets were compared and based on average consumption of various plant and animal products consumed, in a Swiss study. Diets high in animal products were found to be detrimental to health and the environment although this model failed to consider micronutrient intake which may affect long term population health.

Across the Nutri-Santé and Oxford EPIC studies[98,99], protein intake followed the expected trend of Meat Eaters > Pescatarians > Vegetarians > Vegans. Furthermore, the drop in protein intake across each group was approximately 0.1 g / Kg body weight (translating to ~1.2% protein with meat eaters having an intake of ~1.2 g/ Kg body weight). It is common in high income countries to have diets with a protein contribution greater than 15% in the daily caloric requirement. Vegans who form the baseline for an animal-free diet; were found to consume 0.99 g protein / Kg body weight in previously published studies. Given the minimum protein requirement to initiate anabolism is 30 g per day for a 70 Kg person (translating to ~10% of caloric intake or 0.8 g / Kg body weight), protein intake appears to be adequate across all diets.

The average contribution to calories from protein in across various food groups is shown in **Fig. 2.**

Food security

There is considerable interest on the impact of plant based diets on the environment[100–102]. Current insecurities around food production stems from the inefficiencies of food distribution, poor intensification strategies, water use and waste management[103–105]. The allocation of resources towards protein production is currently centred towards animal husbandry as the relative price of animal produce is about ten times higher than most plant products. The Scottish land mass is predominantly marginal owing to its hilly terrain. Consequently, the nature of agriculture favours animal rearing rather than high intensity cropping (**Fig. 3**). For sustaining a given population, about 0.2 ha of land is required per individual[106], which implies a land mass of 1.1 x 10^6 Ha capable of high intensity cropping. Existing capable land in Scotland is about 6 x 10^5 ha which is about 45% lower than required. Animal husbandry is therefore important to ensure sustained food supply. Moreover, climatic conditions make it difficult to cultivate a variety of vegetables which are often imported from countries with favourable conditions.

The emissions associated with agriculture in Scotland is shown in **Fig. 4**. Greenhouse gas emissions of food production as a share of anthropogenic emissions is comparable to global averages. However, satisfying indigenous nutritional requirement is mostly dependent on meat. In terms of carbon efficiency in Scotland, a kilogram of protein produced from animal sources results in an emission of 102.4 Kg. CO_2 eq. while plant protein results in 13.5 Kg. CO_2 eq. The average food prices in Scotland are therefore relatively higher than global averages[107] diversity in diet is low, dependent on imports is high, and consequently, food security and environmental impact of food production is significant.

The WHO healthy plate guideline aims to recommend foods which meet nutritional requirements as well as ensures low greenhouse gas emissions. These recommendations do not account for real-world wastage of food in common households, which was estimated to contribute around 9% of total household dietary emissions[108]. Furthermore, the single largest contributor to dietary emissions comes from animal products and in particular red meat. In India, for example, the contribution from red meat consumption among non-vegetarians is negligible, and consequently leading to a dietary per capita emission of 757 Kg CO_2 eq. per annuum compared to the WHO healthy diet (1288 Kg CO_2 eq.)[109]. This can be explained by

the unique reliance on pulses, fish, and poultry to obtain nutrition which saved about 20% of emissions. Current research is aimed at meat replacements[110,111] and reducing enteric emissions by altering diet and gut composition of ruminants[112,113], but sustainable long term solutions are mostly directed towards locally sourced, low red-meat high plant diets[114]. Climate change affects food availability either by directly disrupting crop growth through unfavourable conditions or through altering crop quality due to increased atmospheric CO_2 levels[115]. Smith *et al.*[116] established a close linear relation between protein and mineral content in plant-based diets in low-income countries which is not observed in animal-based products where mineral composition is relatively independent of the protein content. Paradoxically, WHO recommended diets to mitigate nutrient deficiency relies on supplementation using animal products which of course adds to emissions[117].

Conclusions

Dietary protein is metabolized by proteases and peptidases in the human small intestine, and the released amino acids from dietary protein can be used for protein synthesis by gut microbes. This contributes to the nitrogen cycling and utilization between the microbiota and host. Moreover, the undigested protein and amino acids are mainly fermented into various bacterial metabolites, such as SCFA, hydrogen sulfide, ammonia and other nitrogenous and aromatic metabolites. Some of these bacterial metabolites can be transported inside colonocytes and exert beneficial or deleterious effects. These effects might be attributed to modulation of the intestinal barrier function and immune defense by the altered gut microbiota. Further studies will be necessary to elucidate the relationship between dietary protein and gut microbiota as well as the interaction of microbial function and host health. This becomes increasingly important with a changing agricultural landscape addressing sustainability in our food system and transition to a more circular economy.

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Conflict of interest

None.

Authorship

The authors had joint responsibility for the preparation of this manuscript.

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931 **Figure legends**

932 **Figure 1.** Protein metabolism by colonic bacteria.

933 **Figure 2.** Relative contribution of protein to calories for a range of food group from
934 FAOSTAT Database (2020)[118]. Lines refer to the mean caloric contribution of protein in
935 plant and animal derived foods.

936 **Figure 3.** Protein production from Scottish agriculture [119–120]. Left panel accounts for protein
937 from major animal and plant-based produce. The right panel compares the land allocation for
938 the animal and plant based agriculture and the corresponding production of protein from
939 these sources respectively.

940 **Figure 4.** Scottish anthropogenic emissions [121].

Peptide/amino acid utilisers

Bacterial genera include:
Bacteroides, Prevotella, Clostridium, Veillonella, Megasphaera, Acidaminococcus, Selenomonas, Intestinimonas

↓ Bacterial proteases and peptidases

Amino acids and peptides

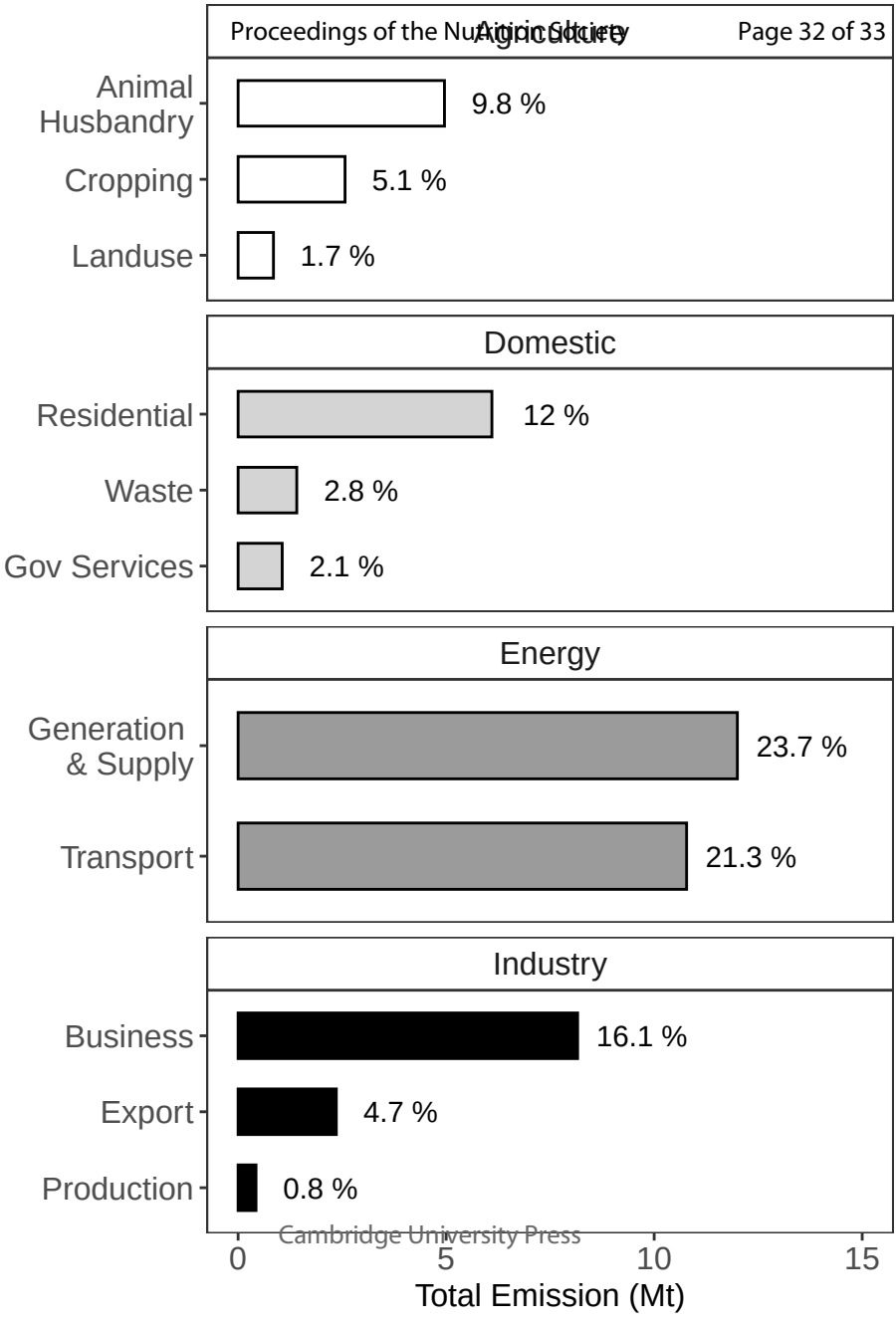
↓ Bacterial fermentation

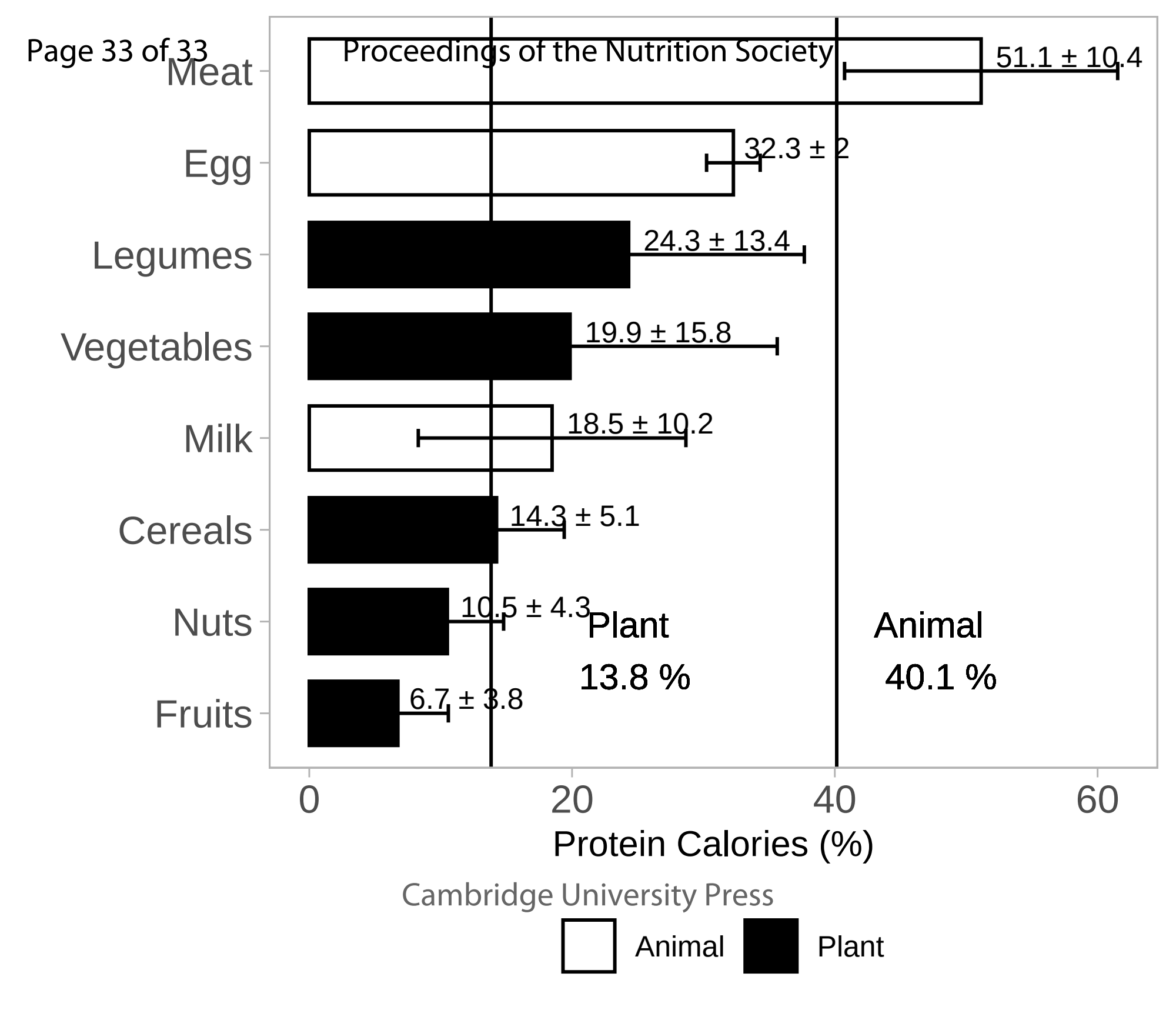
Bacterial metabolites including short chain fatty acids, nitrosamines, indoles, phenols and cresol

↓ Metabolites impact on host cells

LUMEN

EPITHELIAL CELLS




Meat
Egg
Legumes
Vegetables
Milk
Cereals
Nuts
Fruits
51.1 ± 10.4
32.3 ± 2
24.3 ± 13.4
19.9 ± 15.8
18.5 ± 10.2
14.3 ± 5.1
10.5 ± 4.3
6.7 ± 3.8
Plant
13.8 %
Animal
40.1 %
0
20
40
60
Protein Calories (%)
Cambridge University Press
Animal
Plant