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Symposium four: Protein sources: impact on environment and sustainability

Role of novel protein sources in sustainably meeting future global requirements

Andrew M. Salter* D and Carlos Lopez-Viso D

Division of Food, Nutrition and Dietetics, School of Biosciences & Future Food Beacon, University of Nottingham, Sutton Bonington Campus, Loughborough LE12 5RD, UK

> Global population growth, increased life expectancy and climate change are all impacting world's food systems. In industrialised countries, many individuals are consuming significantly more protein than needed to maintain health, with the majority being obtained from animal products, including meat, dairy, fish and other aquatic animals. Current animal production systems are responsible for a large proportion of land and fresh-water use, and directly contributing to climate change through the production of greenhouse gases. Overall, approximately 60% of the global protein produced is used for animal and fish feed. Concerns about their impact on both human, and planetary health, have led to calls to dramatically curb our consumption of animal products. Underutilised plants, insects and singlecell organisms are all actively being considered as alternative protein sources. Each present challenges that need to be met before they can become economically viable and safe alternatives for food or feed. Many plant species contain anti-nutritional factors that impair the digestion and absorption of protein and micronutrients. Insects represent a potentially rich source of high-quality protein although, questions remain relating to digestibility, allergenicity and biosecurity. Algae, fungi and bacteria are also a rich source of protein and there is growing interest in the development of 'cultured meat' using stem cell technology. For the foreseeable future, it appears likely that the 'protein-economy' will remain mixed. The present paper reviews progress and future opportunities in the development of novel protein sources as food and animal feed.

Protein: Food: Climate change: Animal feed: Aquaculture

As the global population continues to rise, there is increasing concern over our ability to sustainably meet the nutritional demands of the most vulnerable individuals. In most Western, industrialised countries, overt malnutrition is relatively rare and recent decades have seen dramatic rises in obesity, and related diseases such as diabetes and CVD, associated with consumption of diets rich in saturated fat⁽¹⁾ and refined carbohydrate⁽²⁾. By contrast, in large parts of Africa and Asia, malnutrition and micronutrient deficiency are still common. Dietary protein, containing appropriate amounts of indispensable amino acids (IAA) is essential to maintain health and support pregnancy and growth⁽³⁾. The WHO has estimated the average protein requirement for an adult is 0.66 g/kg bodyweight/d with a population safe level set at 0.83 g/kg bodyweight/d⁽³⁾. Additional amounts are required to maintain growth in children and to support pregnancy and lactation.

Actual protein requirements are dependent on quality, as well as quantity, of protein consumed. The afore-mentioned WHO requirements assume a protein digestibility-corrected amino score of $1.0^{(3)}$. Protein digestibility-corrected amino

Abbreviations: FM, fishmeal; IAA, indispensable amino acid. *Corresponding author: Andrew M. Salter, email Andrew.salter@nottingham.ac.uk

score represents both a measure of amino acid composition and protein digestibility and essentially compares a dietary protein source with an 'ideal' protein⁽³⁾. Although values approaching 1 are commonly associated with animals sources, such as meat, milk and eggs, values associated with plant-based foods can be much more variable, both as a result of lower IAA contents and poorer digestibility, often associated with the presence of anti-nutritional factors such as trypsin inhibitors, phytates, saponins and tannins⁽⁴⁾. In more recent years, the value of the protein digestibility-corrected amino score has been questioned, primarily because it does not adequately take into account the bioavailability of individual amino acids⁽⁵⁾. In 2013, the FAO of the UN recommended that a new measure of protein quality, digestible indispensable amino acid score, should be used instead⁽⁶⁾. The digestible indispensable amino acid score is based on determining the ileal digestibility of each individual IAA and basing the score on the least available one. However, as yet, only limited data are available on ileal digestibility of amino acids in human subjects, and although significant progress has been made in developing in vitro techniques for determining protein digestibility⁽⁷⁾, in vivo measurements in the pig are currently regarded as the best surrogate⁽⁸⁾.

When protein is being consumed from a variety of different sources, both omnivorous and vegetarian diets can usually supply protein in sufficient quantity, and of appropriate quality, to meet these needs. FAO Food Balance Sheets show that in most of the Western industrialised countries the amount of protein available for human consumption significantly exceeds requirements⁽⁹⁾. In populations that have access to less variety of food types, and often obtain most of their protein from a single cereal crop, deficiency is still relatively common⁽⁹⁾. This is particularly the case in Africa where the majority of protein-deficient countries are located⁽¹⁰⁾.

Fig. 1 is based on data presented by Berners-Lee et $al.^{(11)}$ and demonstrates that, on a global scale, approximately five times more protein is produced than is required to feed the world. However, 60% of this is not consumed directly by human subjects but by farmed animals. Ultimately, only 34% protein produced (animal + plant) is directly consumed by people. Thus, the major concern relating to protein is not the overall amount currently produced, but the unequal distribution between different populations and the direct and indirect impacts of animal production on the environment (12-14). Protein intake in Western countries has also been influenced by perceived health benefits of high-protein diets. Replacing refined carbohydrate with protein in the diet has been increasingly recommended as a potential way of reducing obesity, due to high satiating effects of protein⁽¹⁵⁾. Furthermore, high-protein diets are frequently recommended to improve athletic performance⁽¹⁶⁾. However, both protein production and the excretion of nitrogen associated with excessive intake may negatively impact the environment⁽¹⁷⁾.

The impact of animal production on the environment has come under intense scrutiny in recent years^(12–14). Production of feed for animals is responsible for

significant use of agricultural land. Animal agriculture is also responsible for a significant proportion of global fresh-water use. Effluent from animal production is a major source of pollution of waterways and ruminant animals, particularly cattle, contribute to global warming through the production of methane. Such concerns have led to calls for a major shift in dietary patterns across much of the developed world with much more emphasis on plant-based foods. A recent report⁽¹⁸⁾ suggests that this may be achieved in the UK by greater adherence to the national dietary guidelines (the Eatwell Guide) and this represents the basis of the One Blue Dot diet proposed by the British Dietetic Association⁽¹⁹⁾. However, others suggest more radical solutions are required to address both the impact of diets on our health and that of the planet. One such example is the recent Planetary Diet proposed by the Eat Lancet Commission⁽²⁰⁾ which describes major reduction in consumption of animalbased foods, particularly red meat, and suggests they should be replaced by a combination of protein-rich plant foods, including legumes, nuts and pulses. There is also increasing interest in the sustainable production of novel sources of high-quality protein that can be used both as human food and ingredients for animal feed. This includes currently underutilised plant sources, insects and single-celled organisms, including bacteria. The potential value of such sources is discussed next.

Alternative sources of protein for human consumption

Fig. 2 shows the sources of protein consumed across the world and is based on data presented by Gorissen and Witard⁽²¹⁾. Overall, approximately 60% of total protein consumed by human subjects is derived from plants. This does, however, vary substantially between different continents, from 75% in Africa down to only 36% in Oceania. The primary sources of plant protein consumed globally are the staple cereals (largely wheat, rice and maize). However, reliance on these can lead to deficiency of IAA, particularly lysine⁽²²⁾. By contrast, legumes, pulses and nuts provide only 11% protein consumed by human subjects across the world. This does not, however, reflect their true contribution to human protein intake as 75% soyabean crop produced actually goes into animal feed^(21,23). The Eat Lancet report⁽²⁰⁾ suggests that such sources should represent a much larger proportion of our daily protein intake, replacing a large proportion of the red meat currently consumed. Soyabeans certainly represent a protein-rich plant-based food which also contains high concentration of IAA. Similar to other legumes, they also contain a range of anti-nutritional factors that can impact the digestibility and bio-accessibility of the protein, although processing and cooking can often minimise their effects⁽⁴⁾. In the West, soyabeans have frequently been used to produce 'meat analogues', products which have been specifically designed to look like, and have the same sensory properties as, meat products such as burgers and sausages⁽²⁴⁾. There has been a</sup> dramatic rise in popularity of such products in recent years⁽²⁵⁾ and it could thus be argued that much of the

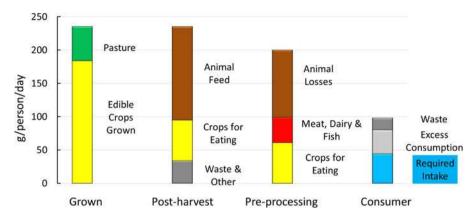


Fig. 1. (Colour online) Global protein food chain, indicating the amount of protein (g/person/d), produced, harvested and consumed, indicating losses from the human food chain. Based on data presented by Berners-Lee *et al*.⁽¹¹⁾.

soyabeans currently grown for animal feed could be repurposed for direct human consumption. However, there is increasing concern about the environmental impact of clearance of wild habitat, particularly in South America, for soyabean production on our ecosystems⁽²¹⁾. Other plant proteins sources commonly used in the production of meat analogues include wheat gluten⁽²⁶⁾, although this does have an inferior amino acid composition, and most recently, pea protein, which has the advantage of being produced in more moderate climates⁽²⁷⁾.

Increasing attention is being turned to alternative plant sources of protein which until recently have remained underutilised as sources of human food, with particular emphasis on tropical legumes which may help to meet the rising demands for protein in Africa and Asia⁽²⁸⁾. Two such potential legumes are bambara groundnut and winged bean. Bambara groundnut is a drought-resistant, nitrogen-fixing legume which is commonly consumed by subsistence farmers in Western Africa⁽²⁹⁾. However, for a variety of reasons, including the physical properties of the seeds, water absorption and further hardening during prolonged storage under hot and humid conditions, bambara is considered 'hard to cook'⁽³⁰⁾. Similar to many legumes, the nutritional value of bambara is further impacted upon by the presence of anti-nutritional factors including trypsin inhibitors, tannins and phytic acid⁽²⁹⁾. In many respects, the physical and chemical factors associated with reducing the nutritional value of bambara are not dissimilar to those associated with soyabean, which, as already mentioned, is now widely consumed across the world by both human subjects and animals. A number of processing methods, including soaking, fermentation or treatment with exogenous enzymes have been shown to reduce the anti-nutrient content of such legumes^(29,30). This clearly shows that with appropriate investment in breeding and processing techniques, bambara has the potential to make an important contribution to the protein intake of some of the world's poorest populations, while having a relatively low impact on the environment and the capacity to grow in relatively arid conditions.

Winged bean represents another underutilised tropical legume that may have the potential to contribute to the protein requirements of future generations. It is a crop which grows under hot and humid conditions and is one of the richest sources of plant protein at almost 30 g/100 g. Similar to sovabean, and other legumes, it is also a relatively good source of IAA. Its low requirements for water and other external inputs make it an attractive source of sustainable protein, although further research is required to try and improve its relatively poor and variable yields⁽³¹⁾. However, similar to bambara, with appropriate investment, winged beans may help to meet the future demands of the growing global population, particularly in tropical regions, while minimising the impact of protein production on the environment. As will be further discussed next, such underutilised crops may also help to replace soyabean meal commonly used as animal feed.

Insects as human food

In many parts of the world, including Africa, Asia and Central/Southern America, insects are commonly consumed as a traditional part of the human diet⁽³²⁾. It has been estimated that over 2000 species of insect are consumed across 113 countries worldwide⁽³³⁾. In most cases, the contribution of such insects to the overall nutritional intake of such populations is likely to be relatively small. However, in recent years there has been increasing interest in insects as a sustainable and healthy source of protein, and other nutrients for both people and farmed animals. Compared to conventional crops and animalbased foods, insects require less space and reduced input (of water and feed) and are reported to have a significantly reduced carbon footprint⁽³⁴⁾. Many insect species also have the potential capacity to be reared on substrates that would not be appropriate for consumption by either people or animals. In terms of protein content, they frequently exceed the 55 % (of DM) associated with soyabean meals and exhibit often superior IAA composition⁽³⁴⁾. A number of products have appeared

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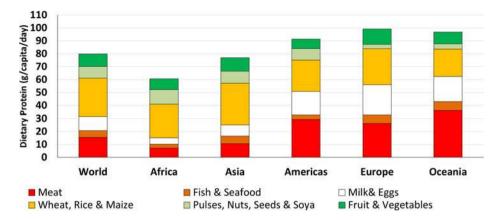


Fig. 2. (Colour online) Sources of protein consumed in each content of the world (g/capita/d). Based on data presented by Gorissen and Witard⁽²¹⁾.

within the Western market including protein bars, flours and cookies prepared from crickets. Currently, this remains a relatively niche market and there remain some safety concerns including allergenicity and the ability of insect to accumulate toxins or to host biopathogens⁽³⁴⁾. However, it appears that insect protein will be increasingly seen as a sustainable source of protein for direct human consumption and, as discussed next, as an ingredient of animal feed.

Single-cell sources of protein

Single-cell, or microbial, sources of protein include algae, fungi and bacteria. Some are already established sources of human food, such as mushrooms, and yeast and bacteria as components of various fermented foods $^{(35)}$. In more recent years, Fusarium venenatum, a filamentous microfungus has been used to produce mycoprotein, the protein-rich ingredient of the meat analogue brand Quorn⁽³⁶⁾. Quorn products are now widely available in over seventeen countries as a range of both red meat and poultry analogues. Mycoprotein is a highly digest-ible source of protein⁽³⁷⁾ which is relatively rich in IAA and has the advantage of being grown on a carbohydrate substrate with no need for an exogenous protein input $^{(36)}$. Microalgae also represent a potential source of high value protein, with Spirulina platensis being one of the most promising candidates. Spirulina contains up to 630 g/protein per kg DM and has been reported to have a IAA composition close to that of animal protein and which exceeds most plant sources⁽³⁸⁾. Despite its potential as a source of high-quality protein, as yet limited use has been made of it as a component of the human diet, which may be associated with relatively high production costs and poor sensory properties. As already alluded to the use of bacteria to ferment foods has been an established part of human nutrition for many years. However, bacteria themselves also represent a rich source of high-quality protein. They have a very high-protein content (approximately 80%) and are a good source of IAA⁽³⁵⁾. However, they are also rich in nucleic acids, the consumption of which may be

associated with raised serum uric acid and development of gout⁽³⁹⁾. As such, if bacterial protein were to be considered as a significant component of the human diet, nucleic acid content would have to be reduced to acceptable levels. As with many of the novel protein sources described for potential human consumption, there is considerable interest in the development of bacteria as a source on animal/fish feed.

Cultured meat

One alternative to conventional animal products, that has received considerable attention in recent years, is cultured meat⁽⁴⁰⁾. Cultured meat is derived from stem cells isolated from living animals. The cells are grown under laboratory conditions and can be differentiated into muscle or adipose cells. By growing them on a 'biomaterial scaffold' the cells can be assembled into complexes that look like meat. Potentially, a product can be made which has the nutritional value of meat but without negative environmental impacts of livestock production. Although still in relatively earlier stages, there has been considerable private investment in developing this technology as a more ethical and environmentally friendly alternative to livestock production. Should the industry overcome the challenges of producing material on an economically viable and commercial scale, such technology could potentially transform the world's agricultural and food industries. However, there are also a range of consumer concerns, which vary considerably between different demographics, that would also need to be addressed⁽⁴¹⁾.

Alternative protein sources for animal feed and aquaculture

With an increasing global population, demand for animal-derived foods is increasing at an unsustainable rate^(13,42). One of the major issues for animal production is the amount of protein needed, with feed representing 60-70% total livestock production costs⁽⁴³⁾. This is

even more dramatic for the aquaculture industry, which also depends on fishmeal (FM) and fish oil to meet the nutritional requirements of farmed fish⁽⁴⁴⁾. Finding novel, sustainable and affordable alternative sources of protein has become a major priority for the animal feed industry. Diverse alternatives, including insects, non-human-edible plants and single-cell organisms (fungi, bacteria and algae) are all currently under consideration^(24,45).

The main protein source for feeding monogastricfarmed animals (poultry and pigs) is soyabean meal, with almost 250 million tonnes being produced in 2020⁽⁴⁶⁾. The majority of this was produced in China (30%), United States (19%), Argentina (14%) and Brazil (13%). The vast majority of the sovabean crop is processed for oil, directed towards human consumptions, with the remaining protein-rich cake being used in animal feed⁽²³⁾. As already eluded to, there is considerable concern about clearance of natural habitat to grow soyabeans, and in particular its impact on deforestation in Argentina and Brazil⁽²³⁾. There are also concerns relating to the environmental impact of soyabean production on greenhouse emissions, carbon footprint and residues (45,47). As a result, there is increasing interest in finding alternative sources of protein of, at least, similar quality to soyabean meal.

The aquaculture industry produces about 50% of the total fish consumed in the world and it is expected to keep increasing⁽⁴⁸⁾. Although there are similarities between feeds for farmed terrestrial animals and fish, aquafeeds have specific requirements which are often met by the inclusion of FM and fish oil, especially for carnivorous fish⁽⁴⁹⁾. As these are obtained from wild-fish captures, as wild populations decline, this is increasingly being seen as environmentally and economically unsustainable⁽⁵⁰⁻⁵²⁾. Although, in several species, at least part of the FM can be replaced with soyabean meal, the lower protein and higher carbohydrate content, together with the presence of a range of anti-nutritional factors, has limited its use, particularly in carnivorous species $^{(53)}$. In addition, other, non-human-edible, plants have also been tried as an alternative to FM, although these often show limited digestibility, with high concentrations of anti-nutritional factors⁽⁴⁹⁾. Furthermore, gut inflammation and other pathologies have been reported as negative effects in fish fed on plant-based diets^(54,55). In some countries, processed animal proteins such as meat and bone, blood, feather and by-products derived from poultry have been used as a source of protein for aquaculture⁽⁴⁸⁾, although the high variability of raw materials and biosecurity are significant issues.

Insects as a source of protein for animal and fish feed

Considerable interest has been shown in the use of insects as a protein source for monogastric animals, including poultry^(56–60) and pigs^(61,62). In general, most research has focused on insect larvae, predominantly mealworm (*Tenebrio molitor*) or black soldier fly larvae (*Hermetia illucens*). Most of the published studies suggest that at least a proportion of soyameal can be replaced in the diets of poultry and pigs without negative impacts on growth⁽³⁴⁾. However, although significant progress is being made, establishing sustainable and economically-viable large scale insect production systems remains challenging.

Insects have also been actively considered as a protein source in aquaculture. The use of insects as an alternative source of protein has shown promising results by partial or total FM replacement in diets of different fish species such as rainbow trout $(Oncorhynchus mykiss)^{(63)}$, European seabass $(Dicentrarchus labrax)^{(64)}$, barramundi (*Lates calcarifer*)⁽⁶⁵⁾, Atlantic salmon (*Salmo salar*)⁽⁶⁶⁾, sea bream (*Sparus aurata*)⁽⁶⁷⁾, and Nile tilapia (*Oreochromis niloticus*)⁽⁶⁸⁾. The most utilised insects in aquafeeds are mealworms, black soldier fly larvae and housefly (Musca domestica) larvae, which have been approved as feeds by the European Union regulation (Commission Regulation (EU) 2017/893 on 24 May 2017)⁽⁴⁸⁾. Moreover, social perception and acceptance, which are important for the market, is positive, due to their natural relationship of predator-prey in the trophic chain⁽⁶⁹⁾. To provide insect meal as a cost-efficient and sustainable protein source, they should, ideally, be reared on economically viable feedstuffs, which are not suitable for human or animal consumption⁽⁷⁰⁻⁷²⁾. As such, there is considerable interest in growing them on by-products from the food industry^(73,74), which would allow a circular economy based on sustainable, waste revalorisation. Insects generally have a relatively high fat content (15-50 % DM) which may not be suitable as a feed ingredient for fish and may thus require a defatted process. However, the extracted fat may have further economic value as a feed ingredient for other animal species, or as a biodiesel substrate (69,75-77). As with their use as human foods, there are potential safety concerns over the use of insects as feed, including possible heavy metals accumulation and allergenicity⁽⁷⁸⁾. There is still research required on production scalability^(79,80) before the widespread commercial use of insects as a major source of protein for animal and fish agriculture.

Single-celled organisms as a source of protein for animal and fish feed

Further alternative protein sources are single-cell organisms including algae, fungi and bacteria, all of which are at various stages of research⁽⁸¹⁾. Currently, two species of microalgae (unicellular) are produced in Europe, *Spirulina* and *Chlorella* spp., and have been targeted for their high long-chain *n*-3 fatty acid content. At present, 30 % of the total algae produced is used for animal feed⁽⁸²⁾ and it has been estimated that they could replace one-third of the soyabean meal used for chicken and pig feeding^(83,84). Additionally, marine macroalgae (multicellular organisms) have high growth rates, the ability to grow in saltwater, and no need of cultivable land. Moreover, macroalgae can provide monogastric animals with bioactive compounds. For example, red seaweed (*Porphyra* sp.) has shown potential as a feed ingredient for sea bream^(45,85,86). However, as an alternative source of protein, such sources frequently need to be processed and bio-refined, due to low total protein and IAA⁽⁸⁷⁾.

Yeast and yeast-derived ingredients have shown some positive properties as a component of animal feed, including probiotic activity, and enhanced immune and stress response in both fish and terrestrial animals. Yeast also has a similar protein content compared to oilseed plants⁽⁸⁸⁾. Moreover, as a by-product of the brewery industry, yeast potentially represent a sustainable feed ingredient⁽⁸⁹⁾.

There is growing interest in the production of bacteria as an alternative source of protein. Photosynthetic bacteria, such as Rhodopseudomonas faecalis, are reported to provide high-protein yield and are relatively rich in IAA, vitamins, carotenoids, lipids and PUFA^(90,91). Furthermore, they can grow on wastewater, thus providing, a sustainable environmental solution for both animal feed requirements and waste management⁽⁹²⁾. Other bacteria of interest include Methylophilus methylotrophus⁽⁹³⁾. methanotrophic bacteria⁽⁸¹⁾, Bacillus licheniformis⁽⁹⁴⁾ and Bacillus subtilis⁽⁹⁵⁾. A further advantage of single-cell organisms is their potential genetic modification in order to improve properties of interest, but, safety, regulations and social concerns are delaying their application and work is still required to produce economically viable, industrial scale production plants⁽⁸¹⁾.

Conclusions

Currently, enough human-edible protein is produced as is required to meet the nutritional requirements of the global population. However, this is inequitably distributed around the world, and large proportions are used to feed livestock. In the face of global population growth, and the uncertain impacts of climate change on our food systems, there is an urgent need to re-assess the 'global protein balance sheet'. For most high-income countries, this should include an overall reduction in the consumption of animal products, particularly red meat, which would have major benefits for human health, as well as the environment. For many, this could simply mean reducing the excessive amount of protein they consume. For others, animal protein should be replaced with high-quality plant sources and, perhaps, other more novel sources including insects and single-cell organisms. In the future, 'meat' cultured from animal cells could also make a significant contribution. However, we must acknowledge that, for the foreseeable future, animal products will remain part of the human diet and in many low- and middle-income countries they represent a 'safety net' against malnutrition. However, we must try to replace traditional agricultural practices of feeding farm animals with human-edible protein sources (including soyabean meal) and the use of wild-caught FM in aquaculture. Insects and single-cell organisms, including bacteria, represent exciting, and with continued research and investment, economically and environmentally favourable alternatives. An overall reduction in the excessive amount of protein produced and, in richer countries, consumed could have a major impact on reducing greenhouse gas emissions, land and water pollution and help to preserve natural landscapes and biodiversity.

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Conflict of Interest

None.

Authorship

Both authors wrote and approved the manuscript.

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