



# Protein sources in human and animal diet

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## ABSTRACT

Current estimates predict that due to population growth by 2050, the world will need about 70% more food than is currently being produced. With the growth of the population whose diet also requires protein intake, livestock production will continue to grow, and proteins of animal origin will remain an important part of the population's diet. The production of proteins of animal origin will increase in the future mainly due to the increased production of pork and poultry meat. Therefore, the demand for protein in feed will increase, as pigs and poultry have a greater need for protein in feed compared to ruminants. Therefore, the needs and challenges for nowadays are to find those sources of protein that will satisfy the nutritional needs of the largest part of the population and animals, and for which production will be cheaper and more accessible than for the sources of protein that are currently used in human and animal nutrition. To date, several new sources of protein for human and animal nutrition are already in use, and their importance will be even greater in the future. Insects, proteins from single-celled organisms and algae are increasingly used as alternative protein supplements. In addition, microbial technology and biological fermentation can improve the digestibility and, thus, the utility value of protein supplements.

## 1. Introduction

By 2050, the world population is expected to reach 10 billion. Current estimates predict that the world will, therefore, need about 70% more food than is currently being produced (Liu *et al.*, 2022). The structure of the world's population is changing as the consumption power of a large number of people increases, primarily in Asia. Namely, the world has already reached a turning point where more than half of the population is now considered middle class or richer and where the majority lives in cities. Regardless of the increase in the number of people belonging to the middle class, stratification among the population will exist also in the future, so a large part of the world's population will not have enough food, especially that of high value, which is food of animal origin. The latest data indicate that almost 690 mil-

lion people in the world are malnourished, and predictions are that by 2030, that number will reach 840 million. The areas most affected by hunger are Africa, Asia and partly Latin America (WHO, 2020). That is why the needs and challenges in today's time are to find those types of food that will meet the nutritional needs of the majority of the population, and for which production will be cheaper and more accessible than for production of food of animal origin.

Another problem we face is the lack of variety of foods used in people's diets. The population uses a small number of foods in their diet, so 75% of the world's food production is obtained from only 12 types of plants and 5 types of animals (FAO, 1999). Almost 60% of the total calories obtained from plants come from just three types of plants, rice, corn and wheat. A lack of variety in human diet can

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have a negative impact on health. Although middle- and upper-class people may consume enough calories, energy-dense diets lead to obesity (Rouhani *et al.*, 2016). In addition, a monotonous diet cannot provide enough vitamins and minerals, resulting in health problems (Aiking and de Boer, 2020). There is an array of untapped food species that can provide diverse nutrients while also providing unique tastes and textures for discerning consumers (Colgrave *et al.*, 2021). One of the ways to offer the population diversity in diet, and on the other hand to meet the nutritional needs of a large part of the poor population, is to use in the diet more proteins obtained from plants and other sources (Aiking and de Boer, 2020).

## 2. Sources of protein in human and animal nutrition

Proteins are important macronutrients in human and animal nutrition, because they play a key role in growth and physiological processes in the body (Boye *et al.*, 2012). All amino acids are important in the synthesis and functioning of muscles and organs, as well as enzymes, hormones and the immune system (Wu, 2009). Amino acids are classified as essential and non-essential, based on whether the body can synthesize a particular amino acid. Non-essential amino acids can be synthesized *de novo* by the human body, while essential amino acids cannot be synthesized, so the only source of essential amino acids is protein in food. Therefore, it is important to ensure their adequate dietary intake (FAO, 2013). The amount of protein that the population over the age of 18 should consume every day (recommended daily food intake) has been determined and amounts to 0.83 g of protein per kg of body weight. Protein intake for children, pregnant women, lactating women and the elderly population is higher than recommended for adults (EFSA, 2012).

Dietary proteins differ in chemical, biological, functional and nutritional characteristics depending on their source and molecular structure (Day *et al.*, 2022). It is generally known that animal proteins have a higher nutritional value than plant proteins due to their amino acid composition, digestibility and ability to transport other important nutrients, such as calcium and iron. In addition, their technological properties, such as gelling, emulsifying and foaming, give food an attractive texture and sensory properties, so they are considered more valuable than plant-based proteins (Kim *et al.*, 2020). Although proteins of plant origin are being increasingly represented in the human diet due to their sus-

tainability, as well as health and ethical reasons, their nutritional value is lower compared to proteins of animal origin. Namely, proteins of plant origin have an unbalanced amino acid composition and reduced or slower digestibility due to their molecular structure (Day *et al.*, 2022). Additionally, there are a number of arguments why an exclusively plant-based diet can lead to nutrient deficiencies, as some essential nutrients are absent or present in small amounts in plant-based foods, such as vitamin B12 or iodine. Other essential micronutrients may be present in sufficient quantities in plant foods, such as calcium (Ca) or zinc (Zn), but the low availability of these minerals in many plants due to the presence of phytates or oxalates may lead to their deficiencies in humans (Adhikari *et al.*, 2022).

## 3. Proteins of animal origin

The use of food of animal origin in human nutrition has a long history. There are assumptions that meat was used during human evolution 5–7 million years ago. The use of meat went through four periods during evolution: 1) random hunting and eating the remains of dead animals, 2) real hunting that began about 2 million years ago, 3) then the domestication and breeding of animals, as well as the cultivation of agricultural crops that occurred before 10,000 years ago, 4) and, nowadays, the use of food of animal origin with an unfavourable composition of fatty acids that are harmful to human health (Larsen, 2003). About 2.5 million years ago, human ancestors used stone tools to remove meat from animals. Although human ancestors used meat in their diet, it was not a significant part of their nutrition until organized hunting began about 2 million years ago (Shipman, 1986). At that time, groups of hunters joined together and caught large prey, which allowed a greater amount of food of animal origin to be eaten. It is very possible that hunting contributed to the improvement of the health of human ancestors, so about 2.0–1.7 million years ago there was a significant size increase (by 33% in men and by 37% in women) and an increase in body weight (by 44% in men and by 55% in women) (McHenry and Coffing, 2000). The development of agriculture approximately 10,000 years ago was a great improvement for humans, because life then changed from surviving day to day by hunting and gathering to organized and safer food production. However, with the development of agriculture, a change in human nutrition occurred, so the consumption of meat decreased,

and the intake of cereals increased. Archaeological evidence confirms that during this period, there was a deterioration in human health, as the frequency of caries, anaemia due to iron deficiency, infections and the development of osteoporosis increased. With the development of agriculture, people began to gather around arable land, and this created ideal conditions for disease transmission (Larsen, 2003). Nowadays, the consumption of meat has increased, and with it the frequency of cardiovascular diseases. Although the diet of modern man is in many ways similar to the diet of people more than 10,000 years ago who were hunter gatherers, the deterioration of human health today can be explained by the lower intake of monounsaturated and polyunsaturated fatty acids, as well as lack of physical activity (Cordain et al., 2002).

Meat, eggs and dairy products have exceptional nutritional value, as they contain high-value proteins, fats (n-3 fatty acids), carbohydrates and micro-nutrients, such as various minerals (calcium, iron and zinc) and vitamins. Food of animal origin produces 18% of the total calories and 25% of the total protein produced in the world. Malnutrition, which occurs due to insufficient intake of protein of animal origin, is a constant problem in poor countries. Adding animal protein to the diet of people who do not consume enough high-quality protein could prevent the development of sarcopenia, osteoporosis and anaemia (Colgrave et al., 2021). With the growth of the population whose diet also requires protein intake, livestock production will continue to grow, and animal protein will remain an important part of the population's diet (Tilman and Clark, 2014). Predictions are that from 2005 to 2050, meat production will increase by 57%, and milk by 48%. Therefore, livestock production will increase by 21% from 2010 to 2025 (Kim et al., 2019). Of the total produced food of animal origin, cattle make up 45%, poultry 31%, and pigs 20% (Mottet et al., 2017). The production of protein of animal origin will grow in the future, mostly thanks to the increased production of pork and poultry meat. Therefore, the demand for protein in feed will increase, as pigs and poultry have a greater need for protein in feed compared to ruminants (Kim et al., 2019). In addition, the use of fish in the human diet provides high-value proteins, but also various necessary micronutrients, including vitamins (A, B and D), minerals (calcium, iodine, zinc, iron and selenium) and polyunsaturated n-3 fatty acids, such as docosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA). With pop-

ulation growth and increased consumer awareness of the health benefits of using fish in the human diet, the average world fish consumption has increased to 20 kg per year per capita. This demand for fish now far exceeds the sustainable yield from the ocean, with aquaculture supplying more than half of total fish production. However, in order to increase the production of fish from aquaculture, several challenges need to be overcome, primarily insufficient quantity of feed for fish nutrition, environmental pollution, poor water quality, fish diseases, as well as a lack of regulations regarding this branch of agriculture (Colgrave et al., 2021).

In pigs, the need for protein in the diet differs according to the age and physiological state of the animal (pregnancy, lactation), and is often determined according to the weight of the animal. Daily requirements for lysine in pigs increase with growth from 4.0 g/day to 18.3 g/day when animals reach 100 kg of body weight. So far, several essential amino acids are added to pig diets, such as lysine, threonine, methionine, tryptophan, valine, arginine and isoleucine. Adding these essential amino acids to pig diets can reduce the amount of legume and animal protein required in pig nutrition (Kim et al., 2019). Proteins of animal origin, such as blood cells, blood meal, blood plasma, milk powder, fish meal, meat and bone meal, poultry by-products, dried eggs and whey protein concentrate are used as feed for piglets. These feeds of animal origin have high nutrient digestibility and contain functional proteins that improve the health of weaned piglets (Weaver et al., 2014).

Protein and amino acid requirements in poultry nutrition depend on their productivity. Turkeys and broilers require high levels of protein and amino acids in their diets to meet their rapid growth needs (National Research Council, 1994). Breed, housing (cage or floor system, stocking density), egg yield, environmental temperature and diseases affect the protein needs of laying hens and ducks (Kim et al., 2019). Dietary protein requirements of poultry are often met by providing 17–22% vegetable protein feed, 1–2% animal protein feed and 0.2–0.5% synthetic amino acids (National Research Council, 1994). Young, fast-growing, heavy-line turkeys require 28% crude protein in their diet, so turkey meal contains more than 50% protein nutrients (soybean meal and fish meal). For feeding geese and ducks, cottonseed meal and rapeseed meal can be used as substitutes for soybean meal, while fishmeal is rarely used (Kim et al., 2019).

In the past, feed for carnivorous fish was largely produced from fishmeal and fish oil derived from fish caught in the open sea. Therefore, fishmeal, as an excellent source of protein and essential nutrients for fish, is a limited natural resource and its annual production is about 5 million tons. Fish oil is also a highly sought product due to its richness in n-3 fatty acids. Over the years, aquaculture has redistributed the use of these limited resources, so today instead of fishmeal and fish oil, some plants (soybean, corn, oilseed rape and wheat flour) and by-products of animal origin (poultry meal, blood flour, feather meal and meat and bone meal) are used (Naylor *et al.*, 2000). However, one of the biggest challenges today is the current restrictions placed by many countries on the use of animal by-products as a source of protein in feed. Moreover, the availability and price of plant nutrients often vary, so proteins from algae and insects are increasingly used for fish nutrition (Colgrave *et al.*, 2021).

#### 4. Proteins of vegetable origin

Plants in human nutrition are important, because they are a source of carbohydrates, fats, proteins, fibre, as well as short-chain n-3 fatty acids. Soybeans, beans and to a lesser extent peas are the main sources of protein of vegetable origin. In the near future, soybean is likely to become a main source of plant-based protein because of its desirable processing properties (e.g. texture, taste, and appearance) and nutritional quality. Compared to other types of plants, soybean is very similar to beef and dairy products in terms of amino acid composition and protein digestibility (Colgrave *et al.*, 2021). Today, new sources of plant-based protein that can be used in human nutrition are being researched, while ensuring that consumers get all the necessary essential amino acids through their diet, because many plants lack certain essential amino acids. Additional research should include improving the quality and quantity of protein in grains (e.g. buckwheat and quinoa) and legumes (e.g. chickpeas, fava beans and lentils) that are underutilized in our diet. There will also be a need to develop new plant varieties that could accommodate different climate regions and/or soils. In addition, technological innovation will lead to the development of new products rich in plant-based proteins, such as bakery products, pasta, breakfast cereals and snacks, or substitutes for meat or dairy products (Colgrave *et al.*, 2021).

In animal nutrition, the main sources of plant protein are proteins obtained from oilseeds. Of the total oilseeds produced, soybean meal is in first place

with 226 million tons (70%), followed by rapeseed meal with 39 million tons (12%) (American Soybean Association, 2017). Animals have a unique ability to incorporate inedible or low-value plants for humans into high-value proteins, increasing the amount of protein available for human consumption (Mottet *et al.*, 2017). Protein supplements are one of the most expensive and limiting food ingredients. Until now, soybean meal, as a by-product of soybean oil extraction, was the main source of high-quality protein for animal nutrition. Namely, 85% of the world's soybean production is annually processed into soybean cake and oil, of which approximately 97% is used as animal feed (Kim *et al.*, 2019). However, due to the large number of hungry people in the world, the justification of feeding animals with soy protein, as well as the use of other oilseeds and cereals in the diet of animals that are also food for humans, is increasingly being considered. Therefore, there is an increasing need to find new nutrients and new sources of protein for animals and to replace the existing ones (FAOSTAT, 2016). The supply of nutrients in the future will be affected by many factors, including the availability of arable land and water, climate change, energy costs and the possibility of improved yields (Kim *et al.*, 2019). In addition, the need for protein in feed can be reduced by improving the efficiency of animal nutrition and the nutritional value of feed ingredients (Mottet *et al.*, 2017).

Although leguminous products are most often added to the meal for pigs as the main source of protein, by-products of the milling and brewing industry can also be used as protein feeds of vegetable origin (up to 30%). Anti-nutritional substances found in protein feeds, including trypsin inhibitors, allergens (such as glycinin and  $\beta$ -conglycinin) and flatulence-producing compounds (oligosaccharides such as raffinose), limit their use in diet for piglets. With certain processing procedures, e.g. fermentation or the use of enzymes, the anti-nutritive substances in these nutrients can be inactivated, so legumes can also be used in the diet for piglets (Kim *et al.*, 2019). Since antimicrobial growth promoters are not desirable in piglet diet, it should be formulated to be low in crude protein to reduce the incidence of diarrhoea (Heo *et al.*, 2009). In addition, decreasing the crude protein content by adding synthetic amino acids to diet for piglets can reduce faecal nitrogen excretion and ammonia emissions (Kim *et al.*, 2019).

Adding larger amounts of protein nutrients of plant origin to poultry meals is not possible due to the presence of indigestible ingredients and/or due

to the presence of anti-nutritive or toxic substances (Kim et al., 2019). Similar to pigs, adding synthetic amino acids to poultry feed can reduce the use of animal protein sources in these feeds, and reduce nitrogen pollution of the environment (Bregendahl et al., 2008). In order to produce sufficient quantities of poultry feed in the future, it is important to predict the increase in poultry meat consumption in the coming decades. New protein feeds, such as insects, proteins from single-celled organisms and algae will be of great importance in the future as protein supplements in poultry nutrition (Makkar, 2017). In addition, microbial technology and biological fermentation can be used to improve the digestibility and, therefore, the use of protein supplements (Verstraete and De Vrieze, 2017).

Unlike pigs and poultry, ruminants meet their needs for amino acids through two different sources: proteins from feed that pass through the rumen undegraded and proteins synthesized by microorganisms in the rumen (microbial protein). These protein sources are then digested and absorbed in the lower parts of the digestive tract, where the absorbed amino acids are defined as metabolic protein. Microbial protein is an essential protein source for cattle and sheep and can provide approximately 70% of total metabolic protein requirements (Sok et al., 2017). In addition, microbial protein is an excellent source of essential amino acids, and its optimal production requires the simultaneous use of protein nutrients with degradable proteins, as well as fermentable carbohydrate nutrients that provide the necessary energy for protein synthesis in the rumen (Schwab and Broderick, 2017). More than 60% of crude protein can be broken down in the rumen to provide ammonia for the growth of microorganisms. While a large amount of ammonia can be used by microorganisms in the rumen for protein synthesis, excess ammonia can be absorbed into the animal's bloodstream and excreted through urine and faeces (Kim et al., 2019). However, ruminants have a lower utilization of nitrogen taken through feed, so in dairy cows 72%, beef cattle 78% and sheep 81% of the nitrogen taken in is excreted through faeces and urine. For now, the most acceptable approach is to feed ruminants with lower protein rations to reduce nitrogen excretion. One study found that feeding dairy cows with diets containing 169 g/kg dry matter of crude protein instead of 183 g/kg of dry matter had no adverse effect on milk production, but nitrogen excretion in faeces and urine decreased by 11% (Hynes et al., 2016).

Because an insufficient quantity of protein feed is being produced, researchers and the feed industry have taken various approaches to improve the supply of protein feed. Firstly, alternative protein sources, such as insect protein and algal protein, have been developed to replace conventional protein feeds for poultry, pigs and fish. Then, genetic selection has reduced the content of anti-nutritional substances (soy with lower oligosaccharide content and corn with lower phytate content) or improved the content of desirable nutrients (soy with higher methionine content, corn with higher lysine content and higher phytase activity). Furthermore, the processing and treatment of protein supplements, such as the removal of oligosaccharides or trypsin inhibitors from soy, has improved their usability. Finally, synthetic amino acids as well as enzymes are added to animal feed to improve protein digestibility and reduce the amount of added crude protein (Kim et al., 2019).

## 5. Sources of protein from the sea: algae and seaweed

Algae can be divided into microalgae and macroalgae (seaweed). Algae have been part of the human diet for thousands of years and provide a variety of nutrients essential to human health, including vitamins, minerals, fibre and protein. Microalgae and seaweed consume carbon dioxide in their metabolism by absorbing it directly from the sea together with nitrogen and phosphorus. The importance of algae in human nutrition is reflected in the fact that their large-scale production will be possible in closed production systems or bioreactors that use recycled water and carbon dioxide produced by other industrial activities (Caporgno and Mathys, 2018).

The majority of algal protein production is expected to come from two freshwater algae species, the filamentous cyanobacterium *Arthrospira platensis* (spirulina) and the unicellular green alga *Chlorella*. These two algae are recognized as sources of high-value proteins, as they contain up to 70% protein in dry matter, as well as all essential amino acids (although smaller amounts of cysteine and lysine). In addition, algae are rich in minerals, such as calcium, iron and copper, and n-3 fatty acids, but they are also one of the few non-animal sources of vitamin B12 (Caporgno and Mathys, 2018). However, the possibilities of their use in human nutrition are limited by intense pigment and taste (Colgrave et al., 2021). One of the disadvantages of algal proteins is that the proteins are often bound to carbohydrates in the cell

wall, thus limiting the availability of the protein to digestive enzymes. Phenolic compounds, found in various types of algae, bind amino acids, forming insoluble compounds and reducing the digestibility of proteins. However, improved digestibility of algae proteins is possible after the application of enzymes, temperature or pressure in order to break the bonds between proteins and other compounds (Kadam *et al.*, 2013). Pigments (e.g. carotenes, chlorophylls and phycobiliproteins) and flavours of algae currently limit the amounts that can be added to many food products. As mentioned earlier, processing technologies can isolate proteins or remove unwanted colour or taste and thus allow for wider application of algal proteins (Colgrave *et al.*, 2021).

Although the nutritional value of microalgae was investigated in the fifties of the last century, recent research has established their potential as a third-generation raw material for the production of biofuels, whereby the produced biomass can be used as the main ingredient of animal feed (Lum *et al.*, 2013). Defatted microalgae contain approximately 20–45% crude protein with a desirable amino acid composition. In addition, defatted microalgae had a positive effect on protein synthesis in the liver and muscles. Defatted microalgae are not only an excellent source of protein, but are a source of n-3 polyunsaturated fatty acids (PUFA) and iron to enrich their content in meat and eggs. However, although the addition of microalgae to beef diet increased the PUFA content of beef, their addition negatively affected the taste and colour of the meat (Kim *et al.*, 2019).

## 6. Insects as a source of protein

Entomophagy, or the practice of using insects as food, has been part of human history for thousands of years, during which it has played an important role in some cultural and religious rituals around the world. During the past, the ancestors of *Homo sapiens* and early human communities used insects in their diet as a source of nutrients. Before humans invented tools for hunting and gathering food, insects played a significant role in human nutrition, which was confirmed by analysing the composition of coprolites – the fossilized remains of ancient human faeces (Hardy *et al.*, 2017). According to Yi *et al.* (2010), insects were part of the diet in China as early as 3200 years ago. The earliest written evidence of the use of insects in human food in Europe is described in the work of Aristotle (384–322 BC), *Historia Animalium*, in which he described how

female crickets tasted best after mating due to the presence of ripe eggs. In the holy books of the Christian, Jewish and Islamic religions, there are sections related to entomophagy (Govorushko, 2019).

Insects have a high nutritional value, because they are rich in proteins that contain essential amino acids, and their digestibility is similar or slightly lower compared to egg or beef proteins (van Huis, 2013). Insects are a good source of proteins, minerals, vitamins and energy, they can cost less than animal protein, and their use can prevent many cases of malnutrition in the poor population. The advantages of raising insects for human and animal consumption are that they can provide or supplement the increasing demand for protein (Alexander *et al.*, 2017). Compared to other food sources, the advantage of growing insects are that they require less space and water, have a short life cycle and better feed conversion, are more nutritious for humans compared to many types of food, the products obtained from them can be used in diet for people and animals, they are easily transported, and as far as insect breeding is concerned, the return on invested funds is quick, the earnings are high and no extensive training is required (Govorushko, 2019).

Edible insects have a higher protein content than other sources of protein, such as beef, chicken, fish and soybeans (Teffo *et al.*, 2007). The nutritional value of insects can vary significantly depending on the type of insect, growth stage and feeding method. Thus, adult mealworms are an excellent source of iron, iodine, manganese, magnesium and zinc, while their larvae are rich in B group vitamins. Edible crickets are a rich source of macronutrients, proteins (about 70%), lipids (7–25%) and carbohydrates, as well as micronutrients (vitamins). Due to their nutritional value, insects are suitable as food for both humans and animals (Imathiu, 2020).

A large number of different species of insects (over 2000) are consumed by about 2 billion people every day in more than 100 countries. Despite the long history of their use in food in some parts of the world, insects have not found their place in the cuisine of Western civilization. In developed countries, consumers hardly accept insects as a source of protein, so by processing insects and extracting high-value protein in the form of powder, this reluctance can be overcome (Colgrave *et al.*, 2021). The acceptance of insects in human nutrition can be boosted for three reasons, namely the positive impacts on human health and environmental protection and for economic reasons. The advantage for human health

is reflected in the fact that insects are a good substitute for proteins of animal origin, many types of insects have a high protein content and enough calcium, iron and zinc, and in addition, insects are already part of the diet of many peoples. The advantages for the environment are that insects release much smaller amounts of harmful greenhouse gases compared to other animals, raising insects requires far less land and water than raising livestock and insects are cold-blooded animals, so their efficiency of converting feed into protein is very high. The economic and social reasons that make the breeding of insects more desirable than the breeding of livestock are that their breeding does not require high technology and large investment, and the breeding of insects provides opportunities for both urban and rural populations (Govorushko, 2019).

For animal nutrition, insects that use food waste in their diet can provide a significant amount of protein for feeding fish and livestock. By the way, insects are natural feed for fish and poultry. Five main insect species have been investigated for the production of protein feeds and they are: the common housefly (*Musca domestica*), the black soldier fly (*Hermetia illucens*), the large yellow mealworm (*Tenebrio molitor*), grasshoppers (*Locusta migratoria*, *Schistocerca gregaria*, *Okia specosis*) and silkworm (*Bombyx mori*) (Makkar et al., 2014). The use of insects as a source of protein for animal feed is considered promising and sustainable (Allegretti et al., 2018).

While the benefits of entomophagy are many, one of the biggest obstacles to using insects in human and animal nutrition is food and feed safety. Eating insects carries the risk of chemical, physical and biological hazards or the occurrence of allergies. Cases of histamine poisoning have been described after eating fried insects that have a higher histidine content (Govorushko, 2019). Possible risks of disease occurrence after the use of insects in human and animal nutrition can be overcome by introducing hygienic practices in the entire chain of production of insects as food. Further research is needed to determine the possible presence of toxins or allergens in insects used in human and animal diets (Colgrave et al., 2021). In addition, there are legal obstacles that will have to be overcome in the future in order to enable a wider application of insects in human and animal nutrition. Recently, EFSA approved the use of mealworms for human consumption, so this insect could be found in a snack, as an ingredient of certain feeds or served as a main dish (Colgrave et al., 2021).

## 7. Single-cell protein

For more than four decades single-cell protein has been recognized as a possible protein source for livestock, especially for monogastric animals (Taylor and Senior, 1978). Single-cell proteins are isolated from the cells of microorganisms, as dried cells and/or as purified proteins. Single-cell proteins have a high protein content (60–82% of dry matter weight), desirable amino acid composition, low fat content, and a higher protein content than carbohydrates (Srividya et al., 2013; Bajpai, 2017). In addition, single-cell proteins contain vitamins (e.g. thiamin, riboflavin, pyridoxine, nicotinic acid, pantothenic acid, folic acid, biotin, cyanocobalamin, ascorbic acid,  $\beta$ -carotene and  $\alpha$ -tocopherol), essential amino acids, minerals, nucleic acids and lipids (Suman et al., 2015). So far, single-cell protein has been widely used in human food as a carrier of flavours and vitamins, in animal feeds (for pigs, poultry, cattle, fish), and in paper and lead industries (Bratosin et al., 2021). Although single-cell proteins can be produced by many microorganisms, including bacteria, yeasts and fungi, only a small number of organisms are used commercially. Yeasts are probably the most widely accepted and used source of single-cell protein. The most commonly used yeast species are *Candida*, *Hansenula*, *Pichia*, *Torulopsis* and *Saccharomyces*. In addition, microorganisms can use as a substrate for fermentation agricultural waste, such as rice straw, rice husks, manure and starch residue, converting them into high value protein (Oshoma and Eguakun-Owie, 2018). There are two main advantages of using waste for the production of single-cell protein, namely the conversion of cheap organic waste into a useful product and the reduction of environmental pollution. Cellulose, hemicellulose and lignin are natural wastes from wood and can be a substrate for microorganisms, but they must first be chemically (acidic hydrolysis) or enzymatically (cellulases) broken down into sugars that can be fermented by microorganisms. Agricultural waste can be an excellent substrate for the economic production of single-cell protein, resulting in a product rich in good quality protein and suitable for animal feed. After processing, single-cell protein can also be used by humans in food (Yunus et al., 2015).

Currently, single-cell protein is commercially used as a feed supplement for feeding laying hens, fattening poultry, beef, pigs and fish, then as a food additive (carriers of vitamins and aromas and emulsifiers), to improve the nutritional value of food

(ready-to-eat meals, soups), as cultures for fermentation (baker's, brewer's and wine yeast) and in industrial processes, as a foam stabilizer and in paper and leather processing. Single-cell protein is primarily used as a protein supplement in the diet of humans and animals and a substitute for high-value proteins of animal origin, due to its low production cost, easy production methods and high nutritional value (Bratosin *et al.*, 2021).

Various substrates can be used for the production of microorganisms, such as energy-rich raw materials (gas oil, natural gas, ethanol, methanol, *n*-alkanes and acetic acid), raw materials of plant origin (starch, sugar and cellulose), waste of different origins (sulphite waste, molasses, whey, milk and fruit waste) and carbon dioxide (Bratosin *et al.*, 2021). The choice of substrate depends on its price, availability, oxygen required for fermentation, the amount of heat produced and the possibility of cooling the fermenter, but also the costs related to post-treatment processing (Suman *et al.*, 2015). Selected substrates are used for the growth of microorganisms in order to increase their mass. Fermentation is the main process responsible for single-cell protein production. The available biomass is harvested when the fermentation process is complete and the biomass is further processed by purification, cell disruption, washing and protein extraction to ensure high protein content (John *et al.*, 2011).

The nutritional and dietary values of single-cell protein depend on the microorganisms used. The amino acid composition of bacteria-derived proteins is similar to fish proteins, and yeast proteins are similar to soy proteins. Microorganisms used for single-cell protein production must not be pathogenic, must not synthesize toxins, must be fast-growing and produce a large amount of biomass, easy to handle and easily separated from the substrate (Bratosin *et al.*, 2021).

Although single-cell proteins have very desirable properties, there are limitations to their application because some microbes can produce toxic compounds, their cell wall is indigestible, and/or they have a high concentration of nucleic acids that cause certain health problems (Kim *et al.*, 2019). Single-cell protein contains up to 16% nucleic acids, which

can be a problem if it is intended for human consumption, as the recommended content of nucleic acids in human nutrition is up to 2% (Nangul and Bhatia, 2013). Purines produced by the breakdown of nucleic acids during metabolic processes are responsible for increasing the level of uric acid in the blood, which can lead to the formation of gout and kidney stones (Nasseri *et al.*, 2011). The process of preparing a single-cell protein for human consumption also involves developing the desired aroma and taste of the product, which makes the process less profitable. In addition, single-cell protein can cause allergic reactions in some people with sensitive digestive systems. Waste materials used as a substrate for single-cell protein production can contain unknown substances that could cause other health problems (Spalvins *et al.*, 2018). In addition, single-cell proteins are deficient in sulphur-containing amino acids (methionine and cysteine), so supplementation of these amino acids is necessary. However, if attention is paid to these shortcomings and they are removed, single-cell proteins represent an excellent protein supplement for human and animal nutrition due to their nutritional value, and their use will be greater in the future.

## 8. Conclusion

Insufficient protein production for human and animal nutrition is already a problem. With the increase in the population in the future, the need for protein in human and animal nutrition will increase significantly. Current academic research is aimed at finding as many new sources of protein for human and animal nutrition as possible, such as algae, insects and single-cell proteins. However, finding new sources of protein will not satisfy the protein needs of humans and animals unless new ways of processing these proteins are applied to increase their digestibility and safety. In addition, new protein production technologies should enable the use of by-products from various industries in order to reduce the amount of waste and environmental pollution, and make the products cheap and affordable for everyone.



# Izvori proteina u ishrani ljudi i životinja

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## INFORMACIJE O RADU

### Ključne reči:

Proteini  
Insekti  
Proteini jednoćelijskih organizama  
Alge

## APSTRAKT

Trenutne procene predviđaju da će zbog rasta stanovništva do 2050. godine svetu biti potrebno oko 70% više hrane nego što se trenutno proizvodi. Sa rastom stanovništva čija ishrana zahteva i unos proteina, stočarska proizvodnja će nastaviti da raste, a proteini životinjskog porekla će ostati važan deo ishrane stanovništva. Proizvodnja proteina životinjskog porekla će se u budućnosti povećati uglavnom zbog povećane proizvodnje svinjskog i živinskog mesa. Shodno tome, potražnja za proteinima u stočnoj hrani će se povećati, jer svinje i živina imaju veću potrebu za proteinima u hrani u odnosu na preživare. Stoga su potrebe i izazovi današnjice da se pronađu oni izvori proteina koji će zadovoljiti nutritivne potrebe najvećeg dela populacije i životinja, a čija će proizvodnja biti jeftinija i pristupačnija nego za izvore proteina koji su trenutno dostupni i koristi se u ishrani ljudi i životinja. Do danas se u upotrebi pojavilo nekoliko novih izvora proteina za ishranu ljudi i životinja, a njihov značaj će u budućnosti biti još veći. Insekti, proteini iz jednoćelijskih organizama i alge se sve više koriste kao alternativni proteinski dodaci. Pored toga, mikroba tehnologija i biološka fermentacija mogu poboljšati svarljivost i, samim tim, upotrebnu vrednost proteinskih suplemenata.

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## References

- Adhikari, S., Schop, M., de Boer, I. J. M. & Huppertz, T. (2022). Protein quality in perspective: A review of protein quality metrics and their applications. *Nutrients*, 14, 947.
- Aiking, H. & de Boer, J. (2020). The next protein transition. *Trends in Food Science & Technology*, 105, 515–522.
- Alexander, P., Brown, C., Arneith, A., Finnigan, J., Moran, D. & Rounsevell, M. D. (2017). Losses, inefficiencies and waste in the global food system. *Agricultural Systems*, 153, 190–200.
- Allegretti, C., Talamini, E., Schmidt, V., Bogomi, P.C. & Ortega, E. (2018). Insect as feed: an energy assessment of insect meal as a sustainable protein source for the Brazilian poultry industry. *Journal of Cleaner Production*, 171, 403–412.
- American Soybean Association (2017). Soy Stats 2017: A Reference Guide to Important Soybean Facts and Figures. St. Louis, MO: Am. Soybean Assoc. 36 pp.
- Bajpai, P. (2017). Single Cell Protein Production from Lignocellulosic Biomass. Singapore, Springer.
- Boye, J., Wijesinha-Bettoni, R. & Burlingame, B. (2012). Protein quality evaluation twenty years after the introduction of the protein digestibility corrected amino acid score method. *British Journal of Nutrition*, 108, S183–S211.
- Bratosin, B. C., Darjan, S. & Vodnar, D. C. (2021). Single cell protein: A potential substitute in human and animal nutrition. *Sustainability*, 13, 9284.
- Bregendahl, K., Roberts, S. A., Kerr, B. & Hoehler D. (2008). Ideal ratios of isoleucine, methionine, methionine plus cystine, threonine, tryptophan, and valine relative to lysine for White Leghorn-type laying hens of twenty-eight to thirty-four weeks of age. *Poultry Science*, 87, 744–758.
- Caporgno, M. P. & Mathys, A. (2018). Trends in microalgae incorporation into innovative food products with potential health benefits. *Frontiers in Nutrition*, 5, 58.
- Colgrave, M. L., Dominik, S., Tobin, A.B., Stockmann, R., Simon, C., Howitt, C. A., Belobrajdic, D. P., Paull, C. & Vanhercke, T. (2021). Perspectives on future protein production. *Journal of Agricultural and Food Chemistry*, 69, 15076–15083.
- Cordain, L., Eaton, S. B., Brand Miller, J., Mann, N. & Hill, K. (2002). The paradoxical nature of hunter-gatherer diets: meat-based, yet nonatherogenic. *European Journal of Clinical Nutrition*, 56, S1–S11.
- Day, L., Cakebread, J.A. & Loveday, S.M. (2022). Food proteins from animals and plants: Differences in the nutritional and functional properties. *Trends in Food Science & Technology*, 119, 428–442.
- EFSA, (2012). Panel on Dietetic Products; Nutrition and Allergies (NDA). Scientific Opinion on Dietary Reference Values for protein, 10, 2557.
- FAO, (1999). Women: Users, Preservers and Managers of Agrobiodiversity; Rome, Italy.

- FAO, (2013). Dietary Protein Quality Evaluation in Human Nutrition: Report of an FAO Expert Consultation; FAO: Auckland, New Zealand.
- FAOSTAT, (2016). Food and Agriculture Data 2016. Rome: Food Agric. Organ.
- Govorushko, S. (2019). Global status of insects as food and feed source: A review. *Trends in Food Science & Technology*, 91, 436–445.
- Hardy, K., Radini, A., Buckley, S., Blasco, R., Copeland, L., Burjachs, F., Girbal, J., Yll, R., Carbonell, E. & Bermúdez de Castro, J. M. (2017). Diet and environment 1.2 million years ago revealed through analysis of dental calculus from Europe's oldest hominin at Sima del Elefante, Spain. *Naturwissenschaften*, 104 (1–2), 2.
- Heo, J. M., Kim, J. C., Hansen, C. F., Mullan, B. P., Hampson, D. J. & Pluske, J. R. (2008). Effects of feeding low protein diets to piglets on plasma urea nitrogen, faecal ammonia nitrogen, the incidence of diarrhoea and performance after weaning. *Archives of Animal Nutrition*, 62, 343–358.
- Hynes, D. N., Stergiadis, S., Gordon, A. & Yan, T. (2016). Effects of nitrogen levels in concentrate supplements on animal performance and nitrogen utilization of lactating dairy cows fed fresh-cut perennial grass. *Journal of Dairy Science*, 99, 8111–8120.
- Imathiu, S. (2020). Benefits and food safety concerns associated with consumption of edible insects. *NFS Journal*, 18, 1–11.
- John, R. P., Anisha, G. S., Nampoothiri, K. M. & Pandey, A. (2011). Micro and macroalgal biomass: A renewable source for bioethanol. *Bioresource Technology*, 102, 186–193.
- Kadam, S. U., Tiwari, B. K. & O'Donnell, C. P. (2013). Application of novel extraction technologies for bioactives from marine algae. *Journal of Agricultural and Food Chemistry*, 61, 4667–4675.
- Kim, W., Wang, Y. & Selomulya, C. (2020). Dairy and plant proteins as natural food emulsifiers. *Trends in Food Science & Technology*, 105, 261–272.
- Larsen, C. S. (2003). Animal source foods and human health during evolution. *The Journal of Nutrition*, 133 (11), 3893S–3897S.
- Liu, F., Li, M., Wang, Q., Yan, J., Han, S, Ma, C., Ma, P., Liu, X. & McClements D. J. (2022). Future foods: Alternative proteins, food architecture, sustainable packaging, and precision nutrition. *Critical Reviews in Food Science and Nutrition*, 25, 1–22.
- Lum, K. K., Kim, J. G. & Lei, X. G. (2013). Dual potential of microalgae as a sustainable biofuel feedstock and animal feed. *Journal of Animal Science and Biotechnology*, 4, 53.
- Makkar, H. P. S. (2017). Review: feed demand landscape and implications of food-not-feed strategy for food security and climate change. *Animal*, 12 (8), 1744–1754.
- Makkar, H. P. S., Tran, G., Heuze, V. & Ankers P. (2014). State-of-the-art on use of insects as animal feed. *Animal Feed Science and Technology*, 97, 1–33.
- McHenry, H. M. & Coffing, K. (2000). *Australopithecus to Homo*: transformations in body and mind. *The Annual Review of Anthropology*, 29, 125–146.
- Mottet, A., de Haan, C., Falcucci, A., Tempio, G., Opio, C. & Gerber, P. (2017). Livestock: On our plates or eating at our table? A new analysis of the feed/food debate. *Global Food Security*, 14, 1–8.
- Nangul, A. & Bhatia, R. (2013). Microorganisms: A marvelous source of single cell proteins. *Journal of Microbiology, Biotechnology and Food Sciences*, 3, 15–18.
- Nasseri, A. T., Rasoul-Amini, S., Morowvat, M. H. & Younes, G. (2011). Single cell protein: Production and process. *American Journal of Food Technology*, 6 (2), 2, 103–116.
- National Research Council (1994). Nutrient Requirements of Poultry. National Academy Press, Washington DC, 9<sup>th</sup> rev. ed.
- Naylor, R., Goldburg, R., Primavera, J., Kautsky, N., Beveridge, M. C. M., Clay, J., Folke, C., Lubchenco, J., Mooney, H. & Troell, M. (2000). Effect of aquaculture on world fish supplies. *Nature*, 405, 1017–1024.
- Oshoma, C. E. & Eguakun-Owie, S. O. (2018). Conversion of food waste to single cell protein using *Aspergillus niger*. *Journal of Applied Sciences and Environmental Management*, 22 (3), 350–355.
- Rouhani, M. H., Haghghatdoost, F., Surkan, P. J. & Azadbakht, L. (2016). Associations between dietary energy density and obesity: A systematic review and meta-analysis of observational studies. *Nutrition*, 32, 1037–1047.
- Schwab, C. G. & Broderick, G. A. (2017). A 100-year review: protein and amino acid nutrition in dairy cows. *Journal of Dairy Science*, 100, 10094–10112.
- Shipman, P. (1986). Scavenging or hunting in early hominids: theoretical framework and tests. *American Anthropologist*, 88, 27–43.
- Spalvins, K., Zihare, L. & Blumberga, D. (2018). Single cell protein production from waste biomass: Comparison of various industrial by-products. *Energy Procedia*, 147, 409–418.
- Srividya, A. R., Vishnuvarthan, V. J., Murugappan, M. & Dakhake, P. G. (2013). Single cell protein — a review. *International Journal of Pharmaceutical Research*, 2, 472–485.
- Suman, G., Nupur, M., Anuradha, S. & Pradeep B. (2015). Single cell protein production: A review. *International Journal of Current Microbiology and Applied Sciences*, 4, 251–262.
- Taylor I. J., Senior P. J. (1978). Single cell proteins: a new source of animal feeds. *Endeavour*, 2, 31–34.
- Teffo, L. S., Toms, R. B. & Eloff, J. N. (2007). Preliminary data on the nutritional composition of the edible stink-bug, *Encosternum delegorguei* Spinola, consumed in Limpopo province, South Africa. *South African Journal of Science*, 103, 434–436.
- Tilman, D. & Clark, M. (2014). Global diets link environmental sustainability and human health. *Nature*, 515, 518–522.
- van Huis, A. (2013). Potential of insects as food and feed in assuring food security. *The Annual Review of Entomology*, 58, 563–583.
- Verstraete, W. & De Vrieze, J. (2017). Microbial technology with major potentials for the urgent environmental needs of the next decades. *Microbial Biotechnology*, 10, 988–994.
- Weaver, A. C., Campbell, J. M., Crenshaw, J. D., Polo, J., Kim, S. W. (2014). Efficacy of dietary spray dried plasma protein to mitigate the negative effects on performance of pigs fed diets with corn naturally contaminated with multiple mycotoxins. *Journal of Animal Science*, 92, 3878–3886.
- WHO (2020). The State of Food Security and Nutrition in the World 2020: Transforming Food Systems for Affordable Healthy Diets; Food and Agriculture Organization: Rome, Italy.
- Wu, G. (2009). Amino acids: Metabolism, functions, and nutrition. *Amino Acids*, 37, 1–17.
- Yi, C., He, Q., Wang, L. & Kuang, R. (2010). The utilization of insect-resources in Chinese rural area. *Journal of Agricultural Science*, 2, 146–154.
- Yunus, F. u.-N., Nadeem, M. & Rashid, F. (2015). Single-cell protein production through microbial conversion of lignocellulosic residue (wheat bran) for animal feed. *The Journal of the Institute of Brewing*, 121, 553–557.