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**Nutritional profile of plant-based meat
alternatives**

BACHELOR'S THESIS

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Declaration

I hereby declare that I have done this thesis entitled Nutritional profile of plant-based meat alternatives independently, all texts in this thesis are original, and all the sources have been quoted and acknowledged by means of complete references and according to Citation rules of the FTA.

In Prague 26.3.2024

.....

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Abstract

This bachelor's thesis deals with examining the nutritional profile of plant-based meat alternatives, which come mainly from tropical and subtropical regions, are universally applicable and represent a suitable protein source for athletes. Using a comparative methodology, various indices for assessing protein quality were meticulously examined with the aim of summarizing and differentiating the advantages and disadvantages of these indices. Additionally, the thesis addressed the environmental impact of the animal industry, intending to provide context regarding the necessity of sustainable food sources. One of the key parts of this work is a comparative analysis of protein consumption in tropical and temperate climate zones, serving for illustration. The work also illustrates the dependence of a country's income on meat consumption and the percentage distribution of protein intake in countries divided according to the new FAO guidelines. The results emphasize the potential of plant-based proteins as a sustainable and optimal protein source. They also highlight the environmental benefits of transitioning away from traditional animal farming practices. This study contributes significantly to the field of nutritional science by emphasizing the importance of sustainable dietary habits for human health and nature conservation.

Key words: plant-based meat alternatives, needs of athletes, quality of protein, impact, ultra-processed food, environment, nutritional analysis

Abstrakt

Tato bakalářská práce se zabývá zkoumáním nutričního profilu rostlinných masných alternativ, které pocházejí převážně z tropických a subtropických oblastí a rovněž jsou vhodným zdrojem bílkovin pro sportovce. Za využívání komparativní metodologie byly detailně zkoumány různé indexy pro hodnocení bílkovinné kvality s cílem sumarizovat a rozlišit výhody a nevýhody těchto indexů. Kromě toho se práce zabývá environmentálním dopadem živočišného průmyslu, s úmyslem poskytnout kontext ohledně nezbytnosti udržitelných zdrojů potravin. Jednou z klíčových částí této práce je komparativní analýza konzumace bílkovin v tropickém a mírném podnebném pásu sloužící pro ilustraci. Rovněž je v práci ilustrována závislost příjmu zemí na konzumaci masa a procentuální rozdělení příjmu bílkovin v zemích rozdělených dle nové směrnice FAO. Výsledky zdůrazňují potenciál rostlinných bílkovin jako udržitelného a optimálního zdroje bílkovin. Rovněž podtrhují environmentální výhody přechodu od tradičních praktik živočišného chovu. Tato studie je cenným přínosem pro oblast nutriční vědy, díky zdůraznění významu udržitelných stravovacích návyků pro lidské zdraví a ochranu přírody.

Klíčová slova: rostlinné alternativy k masu, potřeby sportovců, kvalita bílkovin, dopad, ultrazpracované potraviny, životní prostředí, nutriční analýza

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List of the abbreviations used in the thesis

CED – Chronic Energy Deficiency

DIAAS – Digestible Indispensable Amino Acid Score

EAA – Essential Amino Acid

FAO – Food and Agriculture Organization

HELENA – Healthy Lifestyle in Europe by Nutrition in Adolescence

IAA – Indispensable Amino Acid

IARC – International Agency for Research on Cancer

NOVA – A system to classify food based on the extent and purpose of food processing

NPU – Net Protein Utilization

PBMAs – Plant-Based Meat Alternatives

PDCAAS – Protein Digestibility Corrected Amino Acid Score

PER – Protein Efficiency Ratio

Pro KG BW¹den⁷¹ – Protein per kilogram of Body Weight per day for a 71 kg individual

RDA – Recommended Daily Allowance

RNA – Ribonucleic Acid

TID – True and Ileal Digestibility

UK – United Kingdom

UNU – United Nations University

UPF – Ultra-Processed Food

USDA – United States Department of Agriculture

WHO – World Health Organization

1. Introduction

There has been a significant increase in the popularity and consumption of plant-based meat alternatives in recent years. Many consumers are opting for these alternatives and considering them as a viable replacement for traditional meat products due to concerns about environmental sustainability, animal health and welfare. The shift towards plant-based diets has initiated a comprehensive examination of meat-based alternatives and their nutritional profile. The goal of these investigations is to provide adequate nutrition, that is balanced and comparable to animal products.

The nutritional quality of plant-based meat alternatives is a multifaceted topic. Protein quality is one of the critical aspects to be considered. Protein quality plays a key role in determining the overall nutritional value of these products. Protein is composed of amino acids and the composition and digestibility of these amino acids varies depending on the protein source. Various indices or scoring systems have been developed to assess the protein quality of these alternatives. The aim of these tools is to assess the amino acid profile, digestibility or bioavailability of proteins. This assessment can then provide information on their nutritional adequacy.

To gain a comprehensive understanding of plant-based meat alternatives and their nutritional environment, this thesis deals with a detailed analysis of popular plant-based meat alternatives that are good sources of protein and are grown or sourced in tropical and subtropical climates that are suitable for athletes. It also deals with the critical evaluation of selected indices for assessing protein quality and their advantages or disadvantages. Last but not least, the work has a graphical representation of the diversity and abundance of protein sources in tropical/subtropical and temperate climates, offering insight into regional differences in protein availability and consumption overall, as well as the consumption and availability of plant and animal protein sources in individual countries for illustration. The work also includes an examination of protein intake in countries divided according to FAO guidelines and also illustrates the dependence of a country's income on meat consumption and the environmental impact of the livestock industry.

By exploring these interrelated topics, this thesis aims to contribute to the already ongoing debate on plant-based diets as appropriate sources of food intake for athletes and to offer valuable insights into the nutritional quality of plant-based meat alternatives as well as factors influencing their nutritional adequacy. Using mostly international peer-reviewed scientific articles as sources, this thesis intends to inform primarily athletic consumers or researchers about advances in sustainable and nutritionally sound plant-based food alternatives.

2. Aims of the Thesis

The aim of this thesis is to analyse and summarize available data about plant proteins from tropical and subtropical areas with a high protein content suitable for athletes. The specific aim is to compile for analyse information on randomly selected countries in tropical and temperate climate zones and their daily protein intake. Also to illustrate the dependence of income on protein consumption. Additionally, to assemble from scientific literature the advantages and disadvantages of indices for calculating protein quality, and last but not least, to highlight the issues associated with the animal industry.

3. Methodology

Data focused on plant-based protein sources suitable for athletes, predominantly originating from tropical or subtropical regions, the environmental impact associated with the animal industry, plant proteins and athletes' protein needs were obtained from scientific databases (Web of Science, Google Scholar, PubMed, ScienceDirect) using keywords such as: high protein content, tropical, athletes' protein requirements. Information regarding the protein content was sourced from the FoodData Central website.

Data on protein quality indices (definitions, advantages, disadvantages, calculation methods) were obtained from scientific databases (Web of Science, PubMed, and Cambridge) using keywords such as: PDCAAS, DIAAS, PER, Biological Value.

4. Literature Review

4.1. Proteins

Proteins are composed of L-amino acids, which are formed by the process of proteosynthesis. They contain more than 100 amino acids per molecule. These amino acids are linked together by peptide bonds to form linear chains (Dostál 2003). Proteins are particularly important as a building block of muscle fibres in our body. It is also the basis of hormones, enzymes, and the entire immune system. For the development of tissues and individual organs of our body, their intake and sufficiency is essential (Konopka 2004). Increased protein loss occurs in sweat or urine due to physical activity. If the body lacks glycogen, proteins also serve as a source of energy from diet and muscles. If we perform a performance lasting several hours, the breakdown of proteins can replace up to 15 % of the energy needed (Kumstát 2022).

Our body is unable to store protein. Unlike carbohydrates and lipids, the body cannot store excess protein in a usable form. The excess is excreted in the form of urea or can be used to rebuild carbohydrates. It is therefore necessary to supply the body with dietary protein on a regular basis (Konopka 2004). However, a higher intake of only one amino acid is undesirable for a person who is healthy. Therefore, it cannot be said, that only one amino acid can significantly increase muscle growth. If we do not provide our body with sufficient protein intake, it can lead to loss of performance, fatigue or in rare cases poor immune system function (Fořt 2001; Brazier 2007a).

Although insufficient protein intake is detrimental to the body, we can also say that an excess carries certain health risks. These are health issues primarily for women. Bones are an important reservoir of calcium and amino acids that the body absorbs, and it is essential to bind them with calcium. If the recipient has problems, for example, with the pancreas or stomach, excessive protein consumption is more dangerous for them. In the long term, protein intake triggers amino acid imbalance, obesity, fatigue, increased blood pressure, elevated cholesterol levels, or may strain the liver (Fořt 2002). For successful obesity management, the daily protein intake recommended to the individual is crucial (Maughan & Burke 2006). Athletes who consume 30-60 % of their energy

intake in the form of proteins before performance should also be cautious. If we aim for body shaping, excessive protein intake at the expense of carbohydrates and fats can thwart the expected outcome. According to the nutrition pyramid, proteins should thus constitute around 10-15 % of the total athlete's energy intake (Fořt 2001).

4.1.1. Division of proteins

Proteins can be divided according to their origin into animal, plant, and microbial proteins. From a compositional standpoint, animal proteins are more favourable, mainly due to the content of essential amino acids. Leading animal proteins include eggs, milk, meat, poultry, and fish. Proteins from eggs are divided into egg white and yolk. They also have a higher content of fat and cholesterol, which has led to decreased consumption over the past decades among older individuals (Abeyrathne et al. 2013). Milk proteins are divided into two main groups: caseins and whey proteins. Both of these groups are also complete proteins with mineral content such as phosphorus and calcium (Bär et al. 2019). Meat contains proteins associated with fatty acids. These predominantly saturated fatty acids can be a cause of various health risks. These risks include type 2 diabetes, high fat content, and cholesterol (Picot et al. 2006). Fish, rich in proteins, also have a high content of vitamins, omega-3 fatty acids, and minerals such as iron, calcium, and zinc. However, their bioaccumulation of heavy metals such as lead, nickel, and mercury raises concerns about human health (Maurya et al. 2019).

Plant proteins have a lower content of essential amino acids compared to animal proteins. The diet typically contains 20 primary amino acids. The body can synthesize 12 on its own and these are mentioned in the second column in table 1. In the diet, we must then supply the remaining 8 essential (indispensable) amino acids, which are mentioned in first column in this table. Many peptides and proteins, which are inherent to the body, can be formed through combinations and varying proportions of individual amino acids in the protein diet (Konopka 2004).

Table 1 Essential and Nonessential Amino Acids

Essential	Nonessential
Histidine	Alanine
Isoleucine	Asparagine
Lysine	Aspartic acid
Methionine	Cysteine
Phenylalanine	Glutamic acid
Threonine	Glutamine
Tryptophan	Glycine
Valine	Proline
	Serine
	Tyrosine
	Arginine
	Histidine

(Konopka 2004)

4.1.2. Plant proteins

Since the beginning of life, plants have been an important source for humanity, providing food, wood, medicines, and fibres. Plants have many uses and have long been considered as a bioproduction system for their valuable substances. In addition to therapeutic effects, plants provide primary and secondary metabolites. Primary metabolites, often referred to as the building blocks of life, consist of proteins, fats, carbohydrates, and nucleic acids. Plants produce secondary metabolites for their protection against pathogens and predators. They can also protect them from attracting pollinators and can handle environmental stress (van Vliet et al. 2021).

Plant proteins make up 65 % of all edible protein sources in the world (Young & Pellet 1994). Among the main sources are grains, legumes, oilseeds, and also nuts. In high-income countries, the intake of plant proteins is lower than that of animal proteins, but in upper-middle income, lower-middle income, and low-income countries, they are the primary source compared to animal proteins as we can see in figure 1. Cereals dominate, with wheat accounting for 43 %, rice 39 %, and maize representing 12 % (Rosegrant 1999). Plant protein sources may differ from animal sources primarily in digestibility, amino acid composition, and the presence of antinutritional factors, which negatively affect safety and digestibility. They also differ in the presence of phytoprotectants, which can be effectively used as indirect protection against diseases. These and many other factors have led to increased consumption of plant-based foods as a healthy diet and support the perception associated with the need to reduce animal production (Health Education Authority 1996).

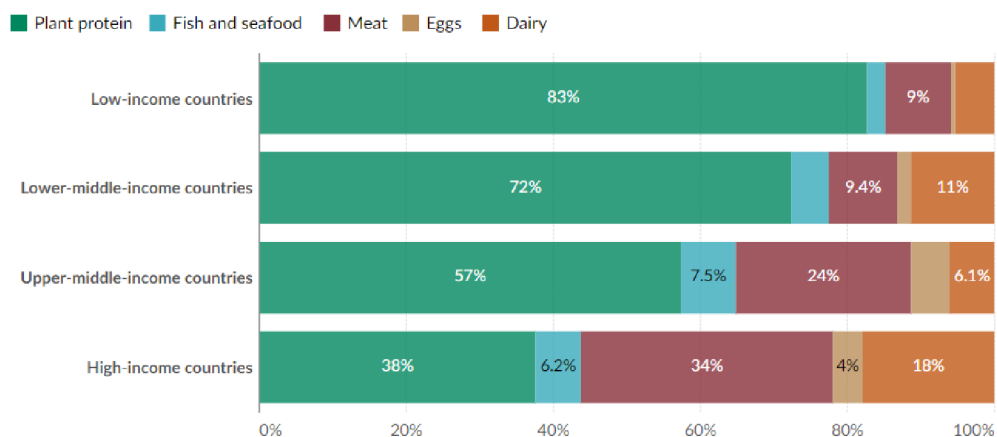


Figure 1 Percentage representation of proteins according to FAO classification, 2020

(Food and Agriculture Organization of the United Nations 2023a)

There is a misconception worldwide that plant proteins are nutritionally inferior to animal proteins, even though it is often reiterated that plants can provide proteins that meet all human needs. The consequence of these misconceptions is primarily a complex cultural and societal attitude towards animal production, as well as scientific traditions that focus on scientific findings and the assessment of protein quality in animals. We already know that plant proteins could cover all human amino acid requirements across all age groups. Therefore, it remains a crucial question whether this is a practical task achievable only with very expensive and carefully selected raw materials, mostly consumed by wealthier vegetarians, or whether it can be achieved with relatively inexpensive grains or other basic raw materials that are accessible to poorer developing communities (Millward 1999).

With a few exceptions, animal and plant proteins have very similar digestibility (Young & Pellet 1994). However, this statement does not necessarily mean that a single plant source is sufficient for sports nutrition and its balance. Lysine plays a very important role. Its adequate intake can be easily ensured by consuming a variety of plant proteins. A suitable combination of protein sources can provide a sufficient supply of all amino acids (Babinská 2009).

Quality sources of plant proteins include nuts, seeds, legumes, whole grains, soy, peas, and beans. Ingredients originating from tropical and subtropical regions of the world, such as seitan, tempeh, tofu, etc., contain quality proteins and the aforementioned small amounts of fat (Wang 1984; Marcinčáková et al. 2004; Brazier 2007).

4.1.3. Comparison of protein intake in different countries

Figure 2 illustrates the overall economic performance of a country, highlighting that countries with higher incomes consume more animal-based protein. Conversely, countries with the lowest economic performance have a lower intake of animal protein. Protein intake in figure 2 is calculated as the grams of protein consumed per person daily, encompassing protein from meat, dairy, eggs and seafood. Gross domestic product per capita is adjusted to account for inflation and variants in prices across countries.

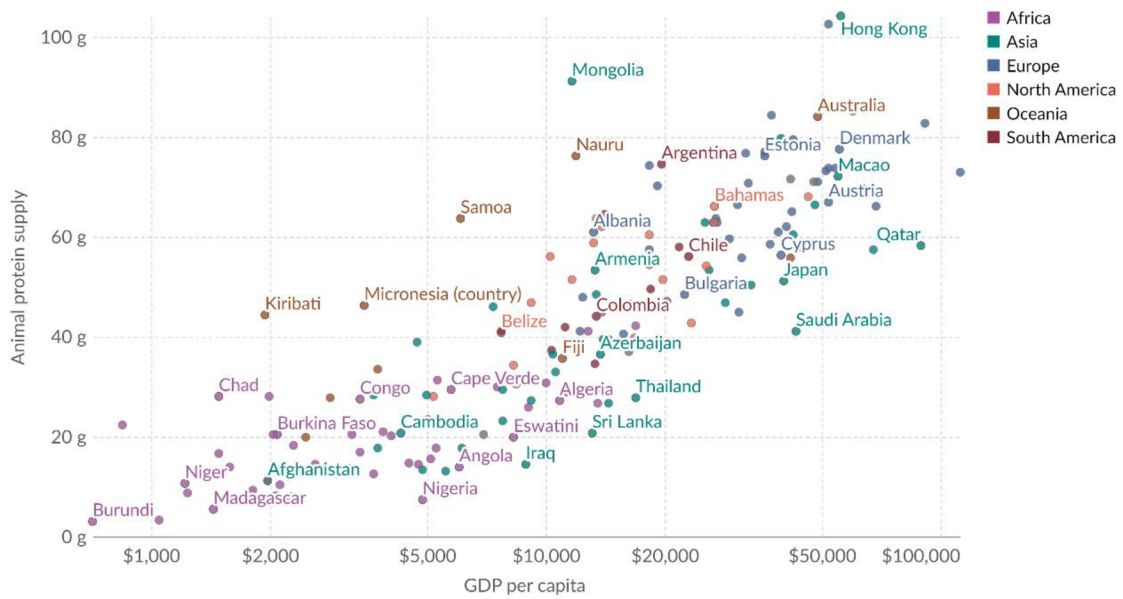


Figure 2 Protein intake from animal sources compared to GDP per capita, 2020

(Food and Agriculture Organization of the United Nations 2023b)

Figure 3 shows an overview of the average daily protein consumption per person for the year 2020 in randomly selected countries within the tropical zone, used for illustration purposes. Countries from Africa, South America, and South Asia were chosen. According to the FAO, animal sources in this case include meat, eggs, dairy products, fish, and seafood. The table also indicates that in some countries, overall protein consumption is critical.

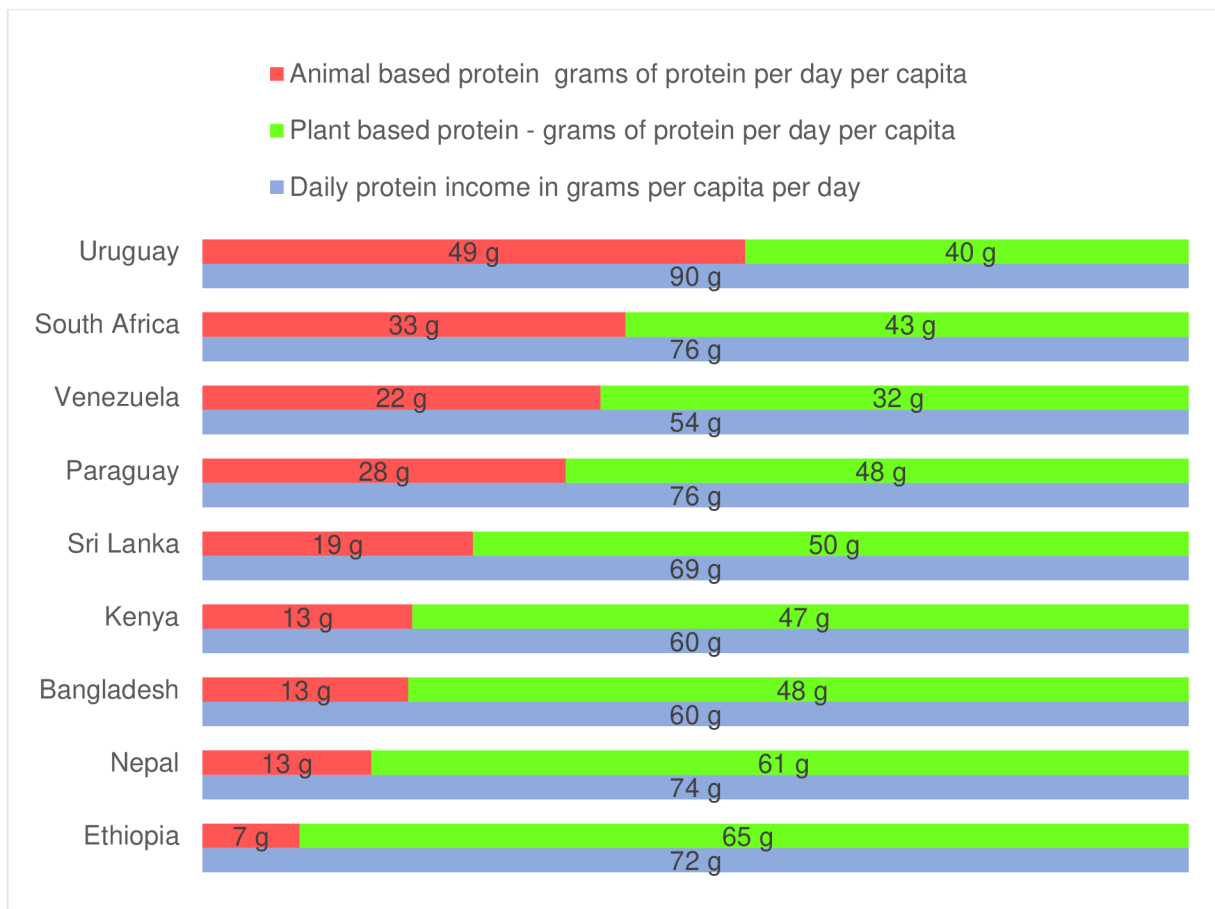


Figure 3 Sources of protein per capita per day in selected countries situated in the tropical zone

(Food and Agriculture Organization of the United Nations 2010, 2013, 2023c)

Figure 4 shows an overview of the average daily protein consumption per person for the year 2020 in randomly selected countries within the temperate zone, used for illustration purposes. Countries from Europe, North America, and East Asia were chosen. According to the FAO, animal sources in this case include meat, eggs, dairy products, fish, and seafood.

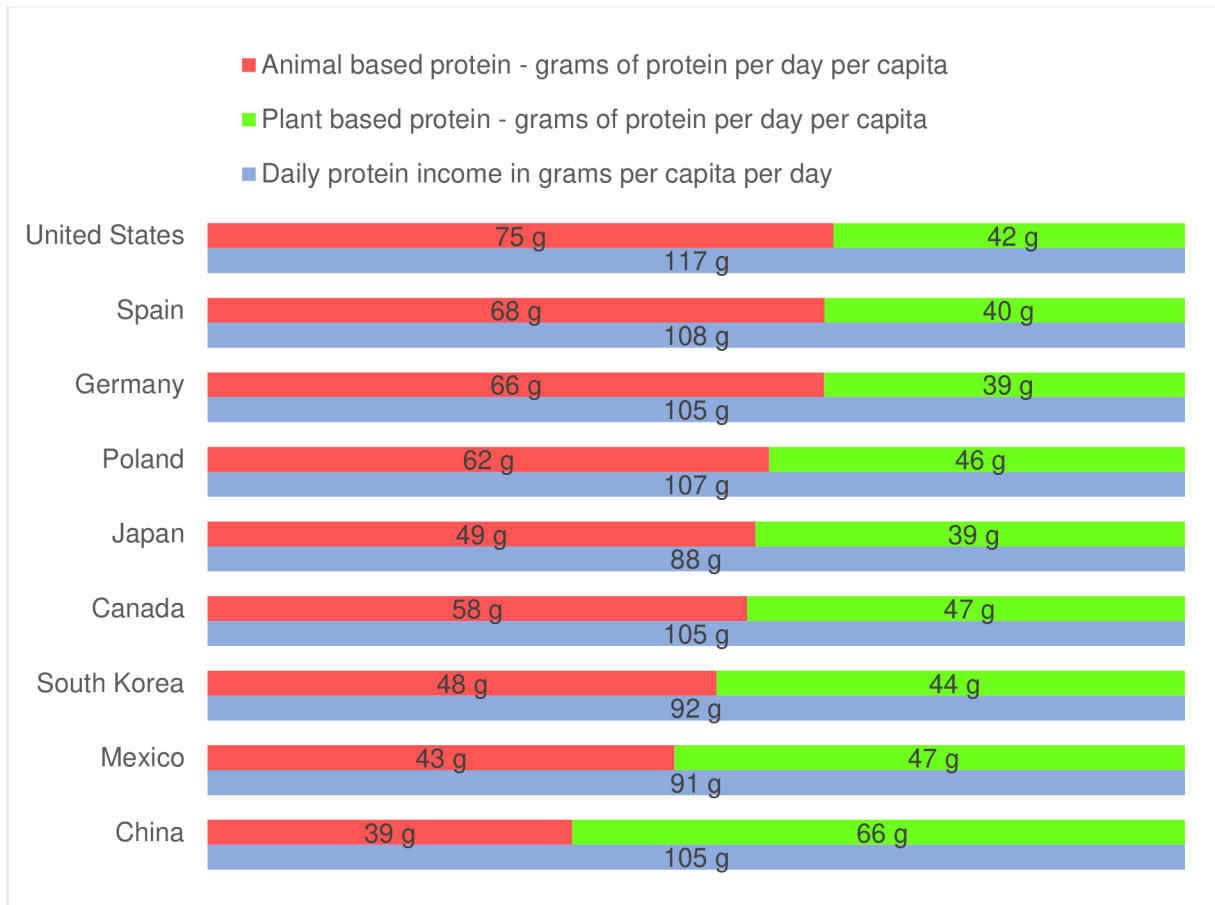


Figure 4 Sources of protein per capita per day in selected countries situated in temperate zone

(Food and Agriculture Organization of the United Nations 2010, 2013, 2023c)

4.1.4. Mycoprotein

Mycoprotein is a food created through continuous fermentation of a carbohydrate substrate by the filamentous fungus *Fusarium venenatum*. This fermentation process results in a food ingredient with low fat content and, most importantly, high protein content. It can be flavoured or textured to closely resemble meat. Mycoprotein is available on the market under the trade name "Quorn". Its availability has expanded to Europe and the United States, where it serves as the primary protein content in various products containing mycoprotein, such as ground meat substitutes, ready meals, chicken pieces, stuffings, and pies.

The main reason for the development of this ingredient was the concern of nutritionists in the 1960s and 1970s about future global protein and food shortages. Their goal was to find new food sources capable of meeting the needs of humanity in the following years. Initially, the focus was on single-celled organisms, specifically yeasts and bacteria (Kihlberg 1972; Kharatyan 1978). Unfortunately, during trials, concerning side effects were discovered, such as rashes, increased concentration of uric acid in urine and blood, or gastrointestinal symptoms (Udall et al. 1984). After several years, fibrous microfungi, commonly found in soil, became the new subject of research. In 1967, an organism called *Fusarium venenatum* was discovered in the UK, which was later used as the main source for producing mycoprotein.

Edward (1986) extensively described the production of mycoprotein. It involves continuous production through the fermentation of *Fusarium venenatum* on a glucose substrate. *Fusarium venenatum* is continually supplied with essential vitamins and minerals necessary for nutrient growth. Meanwhile, to maintain a constant volume of the fermentation medium, a portion of the cultivation medium is periodically removed. This results in the replacement of the entire volume of the cultivation medium every 5-6 hours in the fermenter. After harvesting, the cultivation medium undergoes rapid heat treatment. The aim of this treatment is to reduce the ribonucleic acid (RNA) content to less than 2 % from the original 10 %. This is achieved by thermally activating endogenous RNase enzymes. This reduces the purine content to a minimal amount and prevents a high concentration of uric acid in the body. Mycoprotein is obtained in a paste form after removing most of the water through centrifugation of the heat-treated cultivation medium.

Finally, the mycoprotein is mixed with egg albumin, obtained from free-range chickens, as a binder (Trinci 1992).

Mycoprotein is a protein and fibre-rich ingredient that also has a very low-fat content, making it a suitable candidate for inclusion in a healthy diet. Quorn products are also suitable for vegetarians, but not for vegans due to the use of egg albumin as a binder. Mycoprotein also has a favourable fatty acid profile. However, its high fibre content is noteworthy and comparable to other plant-based protein sources (Denny et al. 2008).

Table 2 Meat and meat alternatives nutritional composition chart per 100 g

Food / per 100g	Energy (kcal)	Protein (g)	Total carbohydrate (g)	total fat (g)	saturates (g)	fibre (NSP) (g)
Mycoprotein food ingredient (wet weight basis)	86	11.5	1.7	2.9	0.6	6
Tofu, steamed	73	8.1	0.7	4.2	0.5	1.3
Soya beans, dried, boiled in unsalted water	141	14	5.1	7.3	0.9	6.1
Red kidney beans, dried, boiled in unsalted water	103	8.4	17.4	0.5	0.1	6.7
Hazelnuts	650	14.1	6.0	63.5	4.7	6.5
Eggs, raw	151	12.5	Trace	11.2	3.2	0
Milk, semi-skimmed, average	46	3.4	4.7	1.7	1.1	0
Lean beef, average, raw	129	22.5	0	4.3	1.7	0
Lean lamb, average, raw	153	20.2	0	8.0	3.5	0
Lean pork, average, raw	123	21.8	0	4.0	1.4	0
Chicken, light meat, average	106	24.0	0	1.1	0.3	0

1 (Food Standards Agency 2002; Ujvary 2009) 2 (USDA 2019)

Table 2 displays the nutritional composition of mycoprotein and various animal/plant protein sources. The nutritional composition in the table reflects the positive attributes of mycoprotein, including its low-fat content and high protein content. Mycoprotein food ingredients have a relatively low energy value of 86 kcal per 100 grams. In terms of nutritional composition, mycoprotein, with 11.5 grams of protein per 100 grams, is protein-rich and comparable, for example, to chicken meat. It also has a low content of both carbohydrates and saturated fatty acids. Mycoprotein is also rich in fibre, with its fibre content being comparable to that of soy foods. Overall, it is evident that ingredients made from mycoprotein are a healthier alternative rich in protein and fibre. Due to its low carbohydrate and fat content, mycoprotein can be an excellent food source for individuals aiming for a healthy and balanced diet.

4.1.5. Protein quality

High-quality proteins are those that contain a large amount of all essential amino acids. They can include both animal-derived foods and some plant-based foods. Among plant sources, these include soy, quinoa, and amaranth. Other plant proteins usually contain all essential amino acids, but the quantity of one or two amino acids may be lower (Marsh et al. 2012).

Animal-based foods generally have a higher protein content. However, plant-based proteins can also be an excellent source of protein. A key aspect to consider is that the quantity of protein consumed in the diet, primarily from plant sources, plays an important role. Compared to animal-based proteins, plant proteins can contribute to an overall increase in protein content and essential amino acids in the diet. Plant proteins have a lower energy value than those of animal origin. It is necessary to increase the overall intake to maintain energy efficiency and meet the need for essential amino acids. The availability of various types of proteins in the food industry can ensure the availability of these raw materials, such as isolates or concentrates of plant proteins on the market. Therefore, it is easier for consumers to increase their intake of plant-based proteins (Hertzler et al. 2020).

However, it is important to correctly interpret the term "quality" as this term is always associated with necessity. Therefore, it is highly desirable in today's world to properly revise this term to prevent incorrect interpretations in context (Katz et al. 2019). There are several approaches to assessing quality of protein. The most traditional approach considers the positive biochemical impact on protein synthesis and nitrogen balance in the diet (WHO/FAO 2007). An alternative approach considers the influence of proteins in the diet on the metabolism and function of specific organs or hormones. This can involve body microbiome, glucose homeostasis, satiety, bone health, body composition and its regulation, and gastrointestinal function (Millward et al. 2008). A more recent approach evaluates the direct effects of diet on the environment as well as human health (Katz et al. 2019).

A) Traditional approach to evaluating protein quality

This approach relies on two main factors and can be defined by its effective capability to meet nitrogen requirements, including essential amino acids. The first factor is the specific amino acid composition, where essential amino acids play a crucial role. They are key in determining the internal protein quality, which is determined by nine essential amino acids and their combination. If a single essential amino acid has a dietary content lower than an individual's reference requirement, a deficiency arises, limiting the use of any other amino acid. In this case, even if the overall nitrogen intake is sufficient, the limited amino acid becomes the factor preventing protein synthesis at a normal rate and also dictates the value of the overall nutritional content of nitrogen and protein in the diet (Institute of Medicine 2005). Assessing diet on a daily basis, however, is much more complicated. Therefore, this is a simplified explanation that can bypass the limiting amino acid problem by supplementing with a precise number of protein sources (Adhikari et al. 2022). The aforementioned limiting amino acids led scientists to research the quality and evaluation of individual amino acids, which later brought about the amino acid reference pattern used for evaluation. Although this system is currently associated with some uncertainties, it is suitable for comparing different protein sources and their quality (Institute of Medicine 2005; WHO/FAO 2007).

When evaluating the external protein quality, two processes need to be considered: absorption and protein digestion, as well as the utilization of absorbed amino acids. In the first mentioned process, digestibility is defined by the difference between excreted and ingested nitrogen, expressed as a proportion of the ingested nitrogen. It is more appropriate to consider ileal digestibility rather than fecal digestibility due to the protein intestinal microbiota and its metabolic processes. Therefore, digestibility is more accurately measured as true and ileal digestibility (TID), which takes into account both specific and basal protein endogenous losses (Hodgkinson & Darragh 2000). In the second process, availability is primarily important for utilizing the already absorbed amino acids to support overall protein synthesis throughout the body.

Together, these two processes are essential for the so-called biological availability (or metabolic availability) of nutrients, which in this case are amino acids from sources of dietary proteins. For the combination of these mentioned components and the subsequent quantitative assessment of quality, a corresponding score was proposed in 1991. It is the Protein Digestibility-Corrected Amino Acid Score (PDCAAS) (FAO/WHO 1991). The formula for this score is:

$$PDCAAS (\%) = \frac{100 \times [mg \text{ of limiting amino acid } 1g \text{ test protein}]}{mg \text{ of same amino acid in } 1g \text{ reference protein}} \times [true \text{ digestibility } (D_F)(\%)]$$

The PDCAAS score also has certain limiting factors, including the truncation of the score to a maximum value of 100 %, which then leads to the inability to compare proteins with high scores among themselves. Moreover, it predominantly uses fecal digestibility rather than TID, and there is a limitation to considering only the first limiting amino acid. The last problem is the insufficient consideration of each individual essential amino acid and its biological availability (Wolfe et al. 2016). This issue has gained importance mainly due to the recently understood significance of individual nutrients in amino acids. In response to this problem, another index was introduced in 2011 during an FAO expert consultation on protein quality evaluation in human diets. It is the Digestible Indispensable Amino Acid Score (DIAAS), calculated as follows (FAO 2013):

$$DIAAS (\%) = \frac{100 \times [\text{mg of digestible dietary IAA in 1g test protein}]}{\text{mg of same amino acid in 1g reference protein}} \times [\text{true digestibility } (D_F)(\%)]$$

The DIAAS score provides us with a categorization of proteins into three categories: 1) Excellent sources, where DIAAS > 100 %, 2) Good sources, where 100 % > DIAAS > 75 %, and 3) Sources with no claim, where DIAAS < 75 %.

B) A new concept in protein quality assesment

Regarding newer approaches, it is necessary to expand the concept of protein quality to include new elements in quantitative evaluation. The expansion is needed due to additional functions of proteins in the diet beyond just maintaining protein body mass. For effective application of these new methods, it is crucial to primarily consider values exceeding the RDA in countries with high incomes as the norm (Millward et al. 2008). These levels were determined exclusively based on maintaining nitrogen balance in almost all healthy individuals (97.5 %) (Office of Dietary Supplements (NIH) 2022).

4.1.6. Protein isolation

For individuals, it might be challenging to consume larger amounts of protein from whole plant foods due to their low protein content. However, this can be easily addressed through isolation. Plant protein concentrates and isolates often contain more than 80 % protein by weight. In a single serving of a drink or powder, one can intake 10-20 grams of plant proteins in one serving (Hertzler et al. 2020).

Isolation, quantification, and purification of proteins occur depending on their physicochemical properties. An important part of suitable isolation methods includes the biological, chemical, and physical properties of sources and also the type of protein (Zhu & Fang 2013). Parameters such as temperature, type of solvent, and pH are carefully controlled when using these techniques (Hadnadjev et al. 2017). After isolation, protein purification follows to separate the non-protein part. Various techniques are used for purification, such as dialysis, ultrafiltration, isoelectric precipitation, or micellar precipitation techniques. These techniques are primarily used for the extraction of proteins. During extraction, a combination of the non-protein part and proteins is

obtained. Therefore, it is necessary to purify the protein itself using additional methods (Moure et al. 2006).

Extraction using ultrasound is one of the currently used methods. Its advantage was described years ago on wheat sprouts. It was found that ultrasound combined with micelles can be an effective way to increase protein yield. Not only did the extraction increase, but the overall time was also reduced. Soybean ground mash was subjected to ultrasonic extraction using a laboratory probe. The treatment occurred during various time intervals, namely 0, 0.5, 1.5, and 15 minutes. From the treatment for one minute, it was observed that the protein yield increased by approximately 10 %. Further studies were conducted using a confocal laser. The effect of ultrasound was revealed by this scanning, showing the presence of intact whole cells. It was found that the improvement in soy protein extraction was due to improved solubility. These studies confirmed that ultrasound-assisted extraction is a reliable method that results in higher yield in a shorter time frame with lower energy requirements (Chemat et al. 2011).

Peanuts have a high protein and oil content, but their isolation and separation can be a more complex process. To separate proteins from the seed, a protein extraction technique using enzymes can be employed. Using different proteases, this method has confirmed a significant increase in protein yield (Zhu & Fang 2013). In addition to enzyme-assisted methods, other studies confirm the use of an electroactive method where proteins were isolated from various plant sources such as rapeseed. With this method, it is possible to extract more protein with better protein quality. The use of these highly advanced methods should be highly effective specifically for protein isolation from plant sources.

4.2. Needs of athletes

If we consider the recommended daily protein intake, athletes who are highly trained and undergo periods of high and intense load consume a maximum of 1.7 g PRO/kg BW/day which mean protein per kilogram of body weight per day for a 71 kg individual (Tarnopolsky 2004). These values should meet the needs of both male and female athletes. Female athletes who participate in combined events have a daily dietary protein intake of around 1.4 g PRO/kg BW/day (Mullins et al. 2001). Regarding the transition period, where the off-season workload and training duration is reduced, protein intake can be approximated to the needs of recreational athletes (0.8 - 1.0 g PRO/kg BW/day (American College of Sports Medicine 2000; Burke 2007).

It is questionable to recommend an exact amount of protein to athletes because, based on recent research, it is unrealistic to recommend a specific amount of protein due to many factors. A general recommendation for protein intake in athletes cannot be given. Mainly since the needs will be different for each sport and each athlete at the same time and then in each period (Tipton & Witard 2007). To make individual recommendations for protein intake, two factors need to be looked at. The first is to cover the total energy value so that we can ingest protein that will continue to be used for muscle growth, repair, and maintenance (Tipton & Wolfe 2004; Tipton & Witard 2007). Second is the timing of protein intake. During the period when athletes are trying to build strength and improve performance, the timing of protein intake is crucial. It is important to focus on adequate energy intake during this period while consuming sufficient protein at the beginning and/or end of the training unit. During rest days athletes also need to maintain adequate protein and energy intake (Tipton & Wolfe 2004). High protein intake is common in athletes, but care must be taken to ensure that protein consumption does not compromise the consumption of other nutrients, such as carbohydrates, which are also essential for athletic success (Tipton & Wolfe 2004; Tipton & Witard 2007).

Safety concerning protein intake exceeding RDA

From the media, we can hear sometimes misleading informations about excessive protein intake and its harmful effects on metabolic health, especially concerning kidney strain, which can lead to complete kidney damage and dysfunction. Among other reported problems is an increased risk of osteoporosis with diets high in protein content, which increase calcium excretion. However, these concerns are unfounded due to the lack of evidence. There is no evidence suggesting that protein intake would have a negative impact on healthy and exercising individuals in these cases (Campbell et al. 2007).

No statistically significant differences are found in age, weight, gender, and kidney function between non-vegetarians and vegetarians, which is a group with a demonstrated lower protein intake (Blum 1989; Bedford & Barr 2005). These two groups exhibited similar kidney function with the same rate of progressive deterioration with age in renal physiology (Blum 1989). Although it may seem that protein intake exceeding RDA is harmless for physically active individuals, for those with mild renal insufficiency, it is necessary to carefully monitor their intake. This monitoring is important due to epidemiological studies and their evidence on dietary protein intake, which may correlate with the progression of kidney disease (Lentine & Wrone 2004; Martin et al. 2005). The relationship between bone metabolism and high food intake has also led to some disputes, such as kidney function. There is a specific concern that believes that if the dietary protein intake is high, it leads to leaching of calcium from bones, which is a possible cause of osteopenia and may also predispose certain individuals to osteoporosis. This assumption is based on recent studies that have indicated increased urine acidity due to increased protein intake in the diet. This should involve buffering of acid load by drawing calcium from bones. The mentioned effect, however, is limited by a small number and size of samples, methodological errors, or the use of large doses of purified protein forms (Ginty 2003).

Table 3 provides the 20 most suitable sources of plant proteins. The criteria for this table were that the foods originated or were cultivated in tropical or subtropical climatic zones and that they were good sources of protein - meaning 10 g of protein or more per 100 g serving. The selected foods can be consumed individually as a 100 g serving, or they can be mixed as desired. Plant proteins are a suitable alternative for athletes who want to diversify their diet.

Table 3 Plant foods from tropical/subtropical regions suitable for normal consumption by athletes

Latine name (family)	Name	Proteins/100 g	References
<i>Amaranthus caudatus</i> (Amaranthaceae)	Amaranth	13.5	1
<i>Anacardium occidentale L.</i> (Anacardiaceae)	Cashew nut	18.2	1
<i>Arthospira platensis</i> (Spirulinaceae)	Spirulina (dried)	57.5	1
<i>Based on glycine max</i> (Fabaceae)	Tempeh	20.3	1
<i>Based on glycine max</i> (Fabaceae)	Tofu	23	1
<i>Canavalia ensiformis (L.) D.C.</i> (Fabaceae)	Jack bean	23-34	2, 3
<i>Cannabis sativa</i> (Cannabaceae)	Hemp seed	31.6	1
<i>Eisenia bicyclis</i> (Lessoniaceae)	Arame (seaweed)	10	1
<i>Glycine max</i> (Fabaceae)	Soya / soybean	39-51.1	1

Table 3 - Continued

Latine name (family)	Name	Proteins/100 g	References
<i>Chenopodium quinoa</i> (Amaranthaceae)	Quinoa	11.9	1
<i>Chlorella vulgaris</i> (Chlorellaceae)	Chlorella	60	1
<i>Lentinula edodes</i> (Marasmiaceae)	Shitake mushrooms	20	1
<i>Lycium chinense</i> (Solanaceae)	Goji berries	14.3	1
<i>Moringa oleifera</i> (Moringaceae)	Moringa powder	50	1
<i>Persea americana Mill.</i> (Lauraceae)	Avocado	15.5	4
<i>Pinus pines</i> (Pinaceae)	Pine nuts	10	1
<i>Rhododymenia palmata</i> (Palmariaceae)	Dulse (seaweed)	28.6	1
<i>Salvia hispanica</i> (Palmariaceae)	Chia seeds	16.5	1
<i>Undaria pinnatifida</i> (Palmariaceae)	Wakame	25	1
<i>Vigna radiata</i> (Fabaceae)	Mung beans	25.2	1

1 (USDA 2019), 2 (Food and Agriculture Organization of the United Nations 1995) 3 (Akpapunam & Sefa-Dedeh 1997) 4 (Ejiofor et al. 2018)

4.3. Comparison chart of quality protein

Table 4 shows four selected, well-known indices for evaluating protein quality. Sources from both animal and plant origins were chosen for the evaluation. From the values, it is evident that each index assesses protein quality quite differently and comes with its own advantages and disadvantages.

Table 4 Comparison chart of different indexes of protein quality

Protein Type	Protein Digestibility Corrected Amino Acid Score (PDCAAS)	Digestible Indispensable Amino Acid Score	Protein Efficiency Ratio (PER)	Biological Value	References
Whey Protein	1.00	133	3.2	104	1,2
Whole Egg	1.00	122	3.9	100	1,3
Casein	1.00	93	2.5	77	1,4
Soy Protein Concentrate	1.00	98	2.2	74	1,2
Beef Protein	0.92	94	2.9	80	1,5
Wheat Gluten	0.25	47	0.8	64	1,5

1(Sarwar 1997; U.S Dairy Export Council 1999) 2 (Mathai et al. 2017) 3 (Heo et al. 2012) 4 (Sarwar 1984) 5 (FAO/WHO Expert Consultation 1990)

4.3.1. Protein digestibility corrected amino acid score

The most well-known index is the PDCAAS (Protein Digestibility Corrected Amino Acid Score). Using the PDCAAS index, proteins with a score of 1.0 or close to it can be labelled as "high quality." Proteins of animal origin are considered to be of very high quality. These include milk and casein variants such as S32S, proteins found in milk, eggs, and especially most types of meat. After removing antinutritional components, isolated soy proteins also have a PDCAAS score of 1.00 (Phillips & van Loon 2011).

If we aim for the ideal value of 1.00 according to the PDCAAS index and want to create a combination of plant-based ingredients that would meet this value, it is not complicated. Grains often have lysine as the limiting factor; however, they do contain sulphur, which is a rich source of amino acids. Legumes, on the other hand, lack sulphur-containing amino acids but are a good source of lysine. By combining these two complementary ingredients, we can meet the human body's requirements for both types of essential amino acids. Pea and rice protein can serve as an example. Using the FAO's reference model from 2011 for adult individuals, blends of pea and rice protein can achieve a maximum PDCAAS score of 1.00 (Food and Agricultural Organization of the United Nations 2013).

According to the general formula, PDCAAS is based on two principles. The first is the fixed principle, based on the assumption that a protein can meet nutritional requirements only when amino acids can be obtained from the diet. Therefore, it is desirable to consider the digestibility of proteins. The second principle is the ability of a given protein to fully meet all amino acid requirements, for which the content of the first limiting essential amino acid in the protein is a critical factor. Properly reflected amino acid availability is a key prerequisite for the digestibility of proteins in feces and also for the composition of the reference protein being valid. This method has also faced criticism. Since its introduction, it has been criticized, for example, during a conference held in San Francisco in 1999. The main subject of this discussion was that PDCAAS is an important means for routine protein quality assessment. However, there is a need to work on improvements and address several imperfections. It is necessary to consider the reference proteins that are currently recommended and their appropriate composition of essential amino acids. Furthermore, it is essential to cap PDCAAS values exceeding 100 % at

100%. It is also necessary to use amino acid availability as a measure of the actual digestibility of proteins in feces, anti-nutritional factors and their influence, and last but not least, the biological efficiency of added amino acids in improving protein quality (Schaafsma 2005).

4.3.1.1. Digestible Indispensable Amino Acid Score

It is important to remember that not all proteins have the same nutritional value. The digestibility and the content of essential amino acids (IAAs) in the diet is essential because of the nutrients needed to synthesize body proteins (Leroy et al. 2023). The Indispensable Amino Acid Score (DIAAS) (Moughan 2021) is used to describe protein quality. By breaking down the term "protein quality" we can increase the efficiency of food as a protein source in practice. The calculation of the index should help us to estimate the actual digestibility. It requires information about the IAAs in the food, which then assigns a score of 1 or higher if all absorbed IAAs are usable. Thus, a lower score means that only part of the IAAs contained in the food are available for utilization. For meat, DIAAS values take on values of 0.8-1.4, while for most plant proteins they are clearly lower (Marinangeli & House 2017). For nuts (0.4-0.9), the values are like those for pulses, where they range from 0.4- 1.1. For cereals, the values are even lower, at 0.1-0.8. Many plant foods do not meet the "good protein source" because of the limiting nature of some essential amino acids (IAAs). Due to the complex structures of plant cells, anti-nutritional factors and the presence of fibre, their digestibility is reduced (Marinangeli & House 2017).

For protein sources, it is not enough to consider only the amount of protein contained if we want to assess nutritional values. Protein that is of poor quality cannot be fully utilised unless it is combined with other protein sources. Translated into means that even if an individual reaches the recommended crude protein intake, they may be deficient in essential amino acids if their dietary DIAAs score is <1. Meat is a great complement to plant-based protein sources due to its high DIAAS value (Leroy et al. 2023).

RDAs (Recommended Dietary Allowance) are the recommended values for protein, which are higher in global analyses in almost all countries. Despite this, RDAs may be below ideal intake levels for a large proportion of the population. Certainly, the

quality of protein is not considered in these approaches. However, the conclusions change when we consider the bioavailability of IAAs as well as bioavailability itself. Even when considering bioavailability, Moughan (2021) showed us that more than 100 countries face insufficient protein supplies for their populations. These situations apply mainly to lower-income countries, where less bioavailability counts towards low dietary diversity. We can certainly factor in limited access to animal foods (Leroy et al. 2023).

In high-income countries, it is often claimed that protein is overconsumed, thus above the recommended daily allowance (RDA) of 0.83 g per kg of human weight. However, it must be understood that this claim overlooks the quality of protein and thus only looks at the basic need to prevent muscle loss in healthy people. However, it may not be the appropriate amount. Some populations may benefit from a larger amount (e.g., double the recommended daily allowance), especially when related to building muscle mass, breastfeeding, pregnancy, healthy aging, or when faced with acute or chronic illness (Leroy et al. 2022). With plant-based alternatives, it is possible to achieve the same goal in protein intake, where DIAAS values are usually lower, but some specific dietary strategies will be needed. Outside of those who are protein deficient, protein quality and its effect are particularly important for individuals who do not have a high enough daily energy intake and a set daily protein intake goal higher than the recommended RDA values (Leroy et al. 2023).

4.3.2. Protein Efficiency Ratio (PER)

The aforementioned indexes are not the only ones that can be used to assess protein quality. For example, in Canada, they use the Protein Efficiency Ratio (PER) method, which is based on the ratio of protein efficiency. This method was the very first to evaluate the quality of food proteins. It is used in rats, where the young are typically fed either a control diet or a test diet containing casein for 4 weeks. The PER value is determined as the weight gain (g) per gram of consumed protein. This index can be calculated as a multiple of the PDCAAS by the number 2.5 (Health Canada 2020). However, the amino acid requirements are not the same for rats and humans. Therefore, PER is disadvantageous in terms of amino acid requirements. Another difference between humans and rats is that rats have a 50 % higher requirement for sulphur-containing amino acids. They need these amino acids to support fur development. Advances in analytical

technology, combined with these disadvantages, led to an expert consultation by FAO and WHO in 1989 (Food and Agriculture Organization & World Health Organization 1991). Together, they concluded that the value of proteins could be equally assessed by expressing the content of the first limiting essential amino acid in the tested protein as a % of the content of that amino acid in a reference prototype of the essential amino acid. The resulting percentage is then adjusted to the true digestibility of the protein being tested. This led to the creation of the PDCAAS method, which is now accepted as the standard method for determining protein quality.

Table 5 The data demonstrate the inability of the PDCAAS index to recognize the additional value of high-quality proteins

Product	PER (casein = 2.5)	PDCAAS (%)
Casein + Methionine	3.1	100
Whey protein concentrate	3.0	100
Egg - white solids	3.0	100
Lactalbumin	2.8	100
Skim milk powder	2.8	100
Milk protein isolate	2.8	100
Minced beef	2.7	100
Beef salami	2.6	100
Tuna	2.6	100

(Gilani 2012)

In table 5, the difference between the PER index and PDCAAS can be seen. While the PDCAAS index value is always 100 %, with the PER index we see different values, which help us determine the protein quality more precisely. Therefore, the PDCAAS index is not able to accurately record high-quality proteins due to its inability to have a value higher than 100 %.

4.3.3. Biological Value

The last mentioned index is based on the determination of Biological Value. Biological Value, or in other words, protein quality, depends on the amount of essential amino acids consumed in the diet (Ohdachi et al. 1999). Protein Biological Value can be defined as the number of amino acids present in specific protein sources that can be absorbed, digested, and utilized by the body for the synthesis of new proteins (Moore & Soeters 2015). High-quality proteins are those with a high content of essential amino acids, thus having a high Biological Value. Proteins with low quality, due to one or more missing amino acids, have a low Biological Value (Friedman 1996). The Biological Value can be calculated from the estimated amount of nitrogen consumed and simultaneously excreted by the body. This method is used because the primary component of amino acids is nitrogen. The determination is carried out on two groups of experimental animals with different dietary conditions. The first group is fed a nitrogen-free diet, while the second group is fed a protein diet. In both groups, the amount of nitrogen lost in feces and urine is then calculated. The Biological Value represents the number of retained and absorbed amino acids in the body.

$$\text{Biological Value (BV)} = \frac{\text{retained N}}{\text{absorbed N}} = \frac{(N \text{ intake}) - (\Delta \text{ fecal N}) - (\Delta \text{ urinary N})}{(N \text{ intake}) - (\Delta \text{ fecal N})}$$

(where N = nitrogen content)

The Biological Value is closely related to the effective utilization of proteins, making it useful for assessing protein requirements from qualitatively different foods. Thus, the concept of Biological Value becomes advantageous. However, there are also significant risks associated with using this method. One of the risks is that it ignores the severity of factors affecting the interaction and digestion of proteins with other dietary circumstances before absorption. For example, if a protein had a requirement to meet a Biological Value of 100, it would be only half as demanding as a requirement for a protein value of 50. For methodological reasons, it is not straightforward to use Biological Value data for human protein requirements. From nitrogen (N) data, Net Protein Utilization (NPU) can be calculated, expressing the proportion of ingested proteins that the body retains. Typically, NPU and Biological Value are determined using a single protein level. In quality, it is important to maximize existing differences, which is why measurements

are made at a protein content in the diet that is less than the required amount. However, these differences can be minimized or almost masked. Essential Amino Acids (EAA) have requirements that can be met even by proteins considered of low quality if an individual consumes a sufficient amount of the amino acid that is most limiting to their needs. Thus, the maximum possible protein capability is measured by NPU and Biological Value (FAO/WHO/UNU 1981).

Many years ago, it was demonstrated and subsequently confirmed that the percentage of retained proteins and their utilization decreases with their increasing concentration in the diet (Barnes et al. 1946). At lower intakes, the Biological Value may differ by a factor of two, up to 90 % (100 mg N/kg), and at higher intakes around 40 % (500 mg/kg) (Bressani n.d.). In young men, a reduction in the Biological Value of wheat gluten from 100 to 45, and then to 25, was determined when the intake was 100 mg/kg, 400 mg/kg, and 1.09 mg/kg, respectively, with increasing intake (Inoue 1974). A similar trend was observed for egg protein. There was a drop from a value of 100 when the intake was 200 mg/kg to approximately 60-70 when the intake increased to 400 and 500 mg/kg (Young 1973). From the values, it is evident that the relationship between dose and response was not linear across all intake intervals but was curvilinear. This curvature was also observed at intakes approaching the requirements. At quantities not exceeding 200 mg/kg for gluten and eggs, the differences in Biological Value were negligible. Differences became apparent only at higher doses. As the amount of protein increased, the differences between values also increased (Inoue 1973). The degree of curvature depends on the most limiting amino acid.

The Biological Value is significantly lower than the maximum when intake is aimed to meet requirements. When calculating protein requirements using data derived under conditions designed to evaluate maximum potential, a high degree of imprecision should be considered. This statement also applies to the Biological Value data of two proteins obtained at levels set to demonstrate maximum differences in quality. Therefore, they serve to determine the requirements for these two proteins because at higher levels, the proportional difference between proteins will not be preserved compared to observations at lower levels. Extrapolating from the curve does not allow for estimating the requirements for any proteins when the dose-response was determined using low protein levels. If we aim to obtain values using the Biological Value to calculate human

protein requirements, measurements should be conducted at several intake levels close to the required levels (FAO/WHO/UNU 1981).

When using the Biological Value, another problem arises when this index is used to indicate the quality of proteins. Proteins lacking one complete essential amino acid can still achieve a value of 40 in the mentioned index because the body has the ability to store and also recycle essential amino acids as an adaptation to insufficient intake of a specific amino acid. It is also necessary to mention that the requirement for growth and maintenance of essential amino acids varies. No individuals consume proteins solely from one food source. Proteins originate from both animal and plant sources or combinations of plant-based foods. When combining two or more proteins, the quality result can be as follows:

- 1) The value of the overall mixture lies between the individual values, which is predetermined by changes in the content of essential amino acids (EAA).
- 2) The value is identical or very close to the component which is better.
- 3) The value is higher than the value of the better component.

No case has been recorded where the value fell below the value of the worst component. An important clarification is that determining the Biological Value of a single protein has limited value, and the Biological Value should be determined when combining proteins contained in a typical diet. The determination should also be based on nitrogen balance, that is, at the level of protein intake for adults and for the growth of children. It would be appropriate to assess the quality of proteins in the diet as they are actually consumed. While the mentioned approach could provide the best estimate of protein quality, it still carries some risks. These studies are usually conducted in metabolic departments where conditions are strictly controlled. We also have evidence that certain conditions influencing the results during these studies may not reflect real-life situations (FAO/WHO/UNU 1981).

The index similar to Biological Value is Net Protein Utilization (NPU). The method of determining quality is similar to Biological Value. However, the difference lies in the absorbed retained nitrogen, which is measured directly (Hoffman & Falvo 2004).

4.3.4. Advantages and disadvantages of protein quality indexes

From the description of individual indices for evaluating protein quality, certain advantages and disadvantages flowed smoothly. Table 6 shows us a summary and analysis of these advantages and disadvantages based on the description of individual indices.

Table 6 Comparison of protein quality assesment indexes

Index	Advantages	Disadvantages	References
PDCAAS	<ul style="list-style-type: none"> - comprehensive view of protein quality - takes into account digestibility and content of essential amino acids - precisely determines high-quality proteins 	<ul style="list-style-type: none"> - values are overestimated - reference proteins have deficiencies - does not consider factors affecting protein digestion under real conditions 	1,2
DIAAS	<ul style="list-style-type: none"> - genuinely assesses protein quality - high content of essential amino acids - distinction between plant-based and animal-based proteins 	<ul style="list-style-type: none"> - plant-based proteins have lower values - to achieve optimal values, it requires a strategy for balancing food - requires detailed information about amino acids in food 	3
Biological Value	<ul style="list-style-type: none"> - evaluates the efficiency of protein utilization by the body - provides a practical insight into its quality - uses typical protein combinations in the diet 	<ul style="list-style-type: none"> - influences by many factors (individual digestive capabilities, anti-nutritional factors...) - does not consider all factors affecting protein quality - the methodology is complex and can lead to inaccuracies in calculating protein requirements 	4,5
PER Index (Protein Efficiency Ratio)	<ul style="list-style-type: none"> - the very first method for evaluating protein quality - easy and quick calculation - allows easy differentiation between high and low-quality protein sources 	<ul style="list-style-type: none"> - not suitable for humans - overlooks the complexity of digestion, does not take into account factors that influence protein digestion along with other dietary circumstances 	6,7

1 (Phillips & van Loon 2011), 2 (Schaafsma 2005), 3 (Marinangeli & House 2017), 4 (Ohdachi et al. 1999), 5 (FAO/WHO/UNU 1981), 6 (Health Canada 2020), 7 (Food and Agriculture Organization & World Health Organization 1991)

4.4. Plant-based proteins and their impact on health

Plant proteins have become a topic of interest in recent years due to their positive effects on long-term health. The studies mentioned below are not intended to serve as a comprehensive list of plant proteins and their health effects. They merely highlight key studies on specific health issues in recent years. Meat also plays a useful role. Due to its high cholesterol and saturated fatty acid content, meat is associated with several chronic diseases. Diseases such as cancer, Type 2 diabetes, obesity, and cardiovascular diseases are consequences of diets high in red meat and consumption of processed meat products. After reviewing epidemiological evidence, the World Health Organization (IARC) classified processed meats. Products like bacon or sausages are carcinogenic for colorectal cancer. Unprocessed red meats like beef or pork are classified as probably carcinogenic (Bouvard et al. 2015).

1) Cardiovascular disease

In 2017, one of the first published studies examined the potential impact of plant protein intake on reducing cardio-metabolic risk factors. Compared to the consumption of animal-derived proteins, positive effects of consuming plant proteins on reducing cardiovascular disease markers were demonstrated. This reduction was evidenced in a meta-analysis of 112 randomized clinical trials involving adults without hyperlipidemia (Li et al. 2017). The authors reported not only lower blood lipid levels but also a reduction in cholesterol with lower-density lipoproteins and many other benefits. The studies confirm plant proteins as a functional substitute for animal proteins in the diet, primarily as an effective means to reduce cardiovascular diseases and their risk factors in adults.

One of the latest studies was conducted in the form of a meta-analysis across 32 intervention studies in individuals treated for hypercholesterolemia, examining the impact of animal proteins compared to plant proteins (Zhao et al. 2020). In this analysis, soy products were studied as interventions compared with animal proteins from various sources. Although there is clear evidence favoring plant proteins in reducing lipid profiles, it is challenging to draw conclusions about all plant proteins. It is possible that the results might be biased due to other bioactive properties of soy products based on limited types of plant proteins.

2) Adolescence

Several studies have also examined the potential benefits of plant proteins in combating obesity, metabolic syndrome, and weight management. Obesity is a rapidly growing problem worldwide. A cross-sectional study in this field was the Healthy Lifestyle in Europe by Nutrition in Adolescence (HELENA), which focused on European adolescents. It was found that obese adolescents had a higher intake of animal proteins. Adolescents who consumed more plant proteins had a lower percentage of body fat and BMI compared to those who consumed animal proteins. Protein and its adequate intake are important factors for maintaining proper physiological functions and developmental aspects. The study suggests that incorporating plant proteins could be an effective tool in helping combat obesity in adolescents. The benefits of increasing plant protein intake in the diet of adolescents instead of animal proteins could positively influence cardio-metabolic factors and also aid in obesity management (Lin et al. 2015).

3) Appearance of cancer

The risk of developing cancer is influenced by many factors, including genetic predisposition, dietary habits, environment, and lifestyle. Through analyses of interactions between environment and genes, one group specialized in studying the risks associated with colorectal cancer in individuals. This study encompassed several genetic and lifestyle factors, including the risk of cancer development. The authors reported changes associated with high meat intake in their research. They found an association between colorectal cancer and specific genetic polymorphisms related to metabolism. Individuals who consumed smaller amounts of meat had the same genetic polymorphism as those whose diet included larger amounts of meat, and this intake was associated with a high risk of colorectal cancer (Andersen et al. 2019). A strategy to reduce the risks associated with colorectal cancer in individuals with specific genetic variations might be replacing animal proteins in the diet with plant proteins.

Overall results regarding the benefits of transitioning from animal proteins to plant proteins are diverse. Therefore, it is not possible to precisely determine whether increased intake of plant proteins will reduce the risk of colorectal cancer. In the literature review used, there is evidence suggesting the benefits of plant proteins over animal proteins, but it is inconclusive in determining clear effects on reducing cancer risks. It is expected that interest in research will grow in the future to gain a better understanding of whether better health outcomes can be achieved by increasing plant proteins in the diet (Hertzler et al. 2020).

Interest among people is gradually shifting from animal meat to plant-based meat. Their concerns include animal welfare, positive environmental impact, and health (van Vliet et al. 2021). Globally, there is an increasing demand for plant proteins. The goal is to replace animal products with plant-based alternatives that will not be subject to health concerns and environmental impact (Tso & Forde 2021b). Over time, more people are choosing foods based on nutritional composition and the health implications associated with meat. Meat alternatives, according to study results, have been confirmed to have lower fat and saturated fat content, total energy value, and cholesterol levels compared to meat products. They also have a significant fiber content (Curtain & Grafenauer 2019).

4.5. Ultra-processed foods

With ultra-processing, problems arise related to harmful health consequences, even though there is not enough precise evidence about plant-based meat alternatives (PBMA) or their nutritional value, as well as the ability of these alternatives to replicate the nutritional profile of meat substitutes. Thus, even though a plant-based diet is primarily associated with health benefits, there is a compelling argument to increase consumer literacy regarding PBMA. The nutritional value and knowledge about PBMA need to be expanded among consumers and thus support informed choices. Understanding these factors can influence the subsequent engagement of target consumer groups, which can ultimately facilitate the production of better and healthier PBMA. Such evidence-based approach in food production could positively influence both planetary and individual health in the future (Flint et al. 2023). When substituting 1 kg of beef protein

with beans, the replacement can offer up to an 18-fold reduction in land use (Sabaté et al. 2015).

Ultra-processed foods can be classified as so-called Ultra-Processed Foods, abbreviated as UPF. These foods are known for having a reduced amount of taste-regulating substances, such as proteins and fiber. Other concerns arise with these foods. They include a higher salt content, free sugars, and saturated fats. Risks also include artificial colors, preservatives, and flavors added as ingredients (Elizabeth et al. 2020a; Srouf & Touvier 2020a; Wickramasinghe et al. 2021; Monteiro & Astrup 2022). Authors in the NutriNet-Santé cohort in France registered increased consumption of ultra-processed foods in diets that aim to limit meat compared to omnivorous diets. Although new foods containing a high level of processing facilitate the reduction of meat consumption, the opinion is supported to continue investigating their health value (Alae-Carew et al. 2022). However, not all UPFs can be labeled as unhealthy. About 50 products were categorized as healthy due to the characterization system NOVA, between 2011 and 2018. This finding led to criticism of the NOVA system, and it was labeled as an unclear classification system (Gibney et al. 2017; Drewnowski et al. 2020; Braesco et al. 2022; Rego 2022; Visioli et al. 2023). The term "ultra-processed" can also be misleading since it is used as one general term and doesn't distinguish the variety of different processing techniques that have different functions (Rego 2022). However, the evidence regarding harmful health effects is very limited, especially concerning new food products (Elizabeth et al. 2020b; Srouf & Touvier 2020b; Aschemann-Witzel et al. 2021).

Authors Graufenauer and Curtain in their survey in Australian supermarkets (Curtain & Grafenauer 2019) claim that PBMA has a healthier profile than meat alternatives. In energy density, saturated fat, and total fat, PBMA values were lower, while the fiber content was higher. Regarding sodium content, PBMA was very high, and only in 4 % of products was it classified as low. Plant-based meat substitutes actually had up to six times higher sodium content compared to meat sausages.

4.6. Environmental impact

Influence of food consumption to the environmental impacts is dominantly caused by livestock consumption (Weidema et al. n.d.). The food industry is very important with an impact on the environment. It is associated with water consumption, biodiversity loss and high greenhouse gas emissions (Hallström n.d.). We are urged to consume less meat because meat production is one of the biggest contributors to environmental degradation (Hedenus 2014). Rather than eating simply more meat that is sustainably produced is necessary to decrease in the Western world meat consumption (Smith & Gregory 2013). Unfortunately, meat plays a major role in the diet of many people and cultures (Fiddes 1992). Carbon sequestration in soils and nitrogen utilization efficiency are the one way to enhancing our production methods. First method is concerns with minimize environmental impacts and waste with the help of optimizing the use of nitrogen-based fertilizers. Additionally, crucial role can also play a soil carbon sequestration when we are adopting practices. Implementing techniques like agroforestry, cover cropping, or reduced tillage can help improve the storage and enhance of carbon in the soil. If we implement these strategies, production activities on the environment gets better and we can also reduce the negative consequences (Smith & Gregory 2013). Overall research affirms that the Western world needs to decrease the consumption of livestock products (Garnett 2011). Sustainable consumption can be a divide between ‘weak’ and ‘strong’ (Fuchs & Lorek 2005) promotes that their “strong sustainable consumption”, concretely “changes in consumption patterns and reductions in consumption levels in industrialized countries” is more necessary and sufficient than increased efficiency of consumption through technological improvements (Heath & Chatzidakis 2012) dealing with this problem too, but from marketing perspective.

Mainly in recent times, the evaluation of the environmental impact when using proteins has become an important consulted topic. The main systems related to food can be measured from all different factors using the footprint methodology. These systems can also be classified into several categories (Vanham et al. 2019):

- 1) **Carbon footprint** – greenhouse gases and their emissions
- 2) **Utilization of arable land** – ecological larger footprint or soil footprint
- 3) **Utilization of freshwater** – water footprint or use of blue water
- 4) **Loss of biological diversity** – biodiversity footprint
- 5) **Comulative energy consumption** – carbon component of the ecological footprint, CED
- 6) **Chemical substances and their use** – fertilizers, pesticides (for pesticides, a specific component of the chemical footprint, and for fertilizers, a specific component of the material footprint)

Katz et al. (2019) came up with the first proposal for these measurements using the definition of two distinct sample metrics.

The environment and the health of the population are threatened by current food systems. In alignment with priorities related to climate change and health support, increasing the consumption of plant-based foods and reducing meat consumption is highly relevant. Due to consumer demand, the market for plant-based foods is constantly growing. Plant-based diets offer several advantages, including low energy density, low saturated fat content, and richness in nutrients. However, the issue arises with the over-processing of food, where modern lifestyles favor meat-mimicking foods. The aim of processing is to improve the taste and safety of products, enhance or enrich nutritional values (Flint et al. 2023).

The outline of the food system is a key aspect when considering future sustainability. It is necessary to understand the factors influencing fish and meat consumption when building a system that will be sustainable in food distribution and production. Specifically for sustainability, dietary habits such as fish or meat consumption are serious consequences (Goodland 1997; Gerbens-Leenes & Nonhebel 2002).

A study by Springmann (Springmann et al. 2018), becomes relevant, emphasizing the importance of dietary changes in reducing environmental pressure and its four factors. These factors include the use of fertilizers and water, soil, and carbon footprints. An analysis of current dietary habits compared to actual adherence to nutritional guidelines and a more plant-based diet (flexitarianism) was also conducted. Flexitarianism has recently become a significant element in reducing the human-caused carbon footprint. Even with current significant technological improvements and efforts to reduce or manage food losses and waste, flexitarianism is crucial for maintaining certain planetary boundaries. resulted in reduced premature mortality. There was also a reduced environmental impact seen in flexitarian diets, as well as other dietary patterns divided into three distinct groups for the research method. The first group consisted of a diet including only plant sources with an environmental goal, replacing 25 % animal-based food with 100 % plant-based food in the initial phase. The replacement was done with isocaloric profile foods, which were purely plant-based. The composition was 75 % solid legume mix and 25 % fruits and vegetables. The second group included patterns that were irrelevant to this research. These were food security and its goals, which managed to improve the energy imbalance progressively by 25, 50, 75, and 100 %. There was also a reduction in overweight, obesity, and underweight individuals. The third group contained vegetarian patterns focusing on public health, hence on vegetarianism, veganism, and flexitarianism. Regarding the diets of the first group, very important results were obtained, including reduced mortality and greenhouse gas emissions.

In a recently published overview, which is currently the most relevant source, by Fresán and Sabaté (Fresán & Sabaté 2019), several conclusions were drawn. While the authors of the aforementioned article mentioned only vegetarian and vegan diets, the results of this study demonstrated a smaller reduction in greenhouse gas emissions but a more extensive reduction in land use and water usage. All mentioned data depict a similar trend in greenhouse gases as in the AHS-2 and EPIC-Oxford vegetarian cohorts analyses (Soret et al. 2014; Scarborough et al. 2014). In conclusion, it is important to note that a smaller negative impact of vegetarian diet than carnivorous diet was partially proven. If we could change the dietary system of the population, the above-mentioned trilemma could be a suitable solution (Sabaté & Soret 2014).

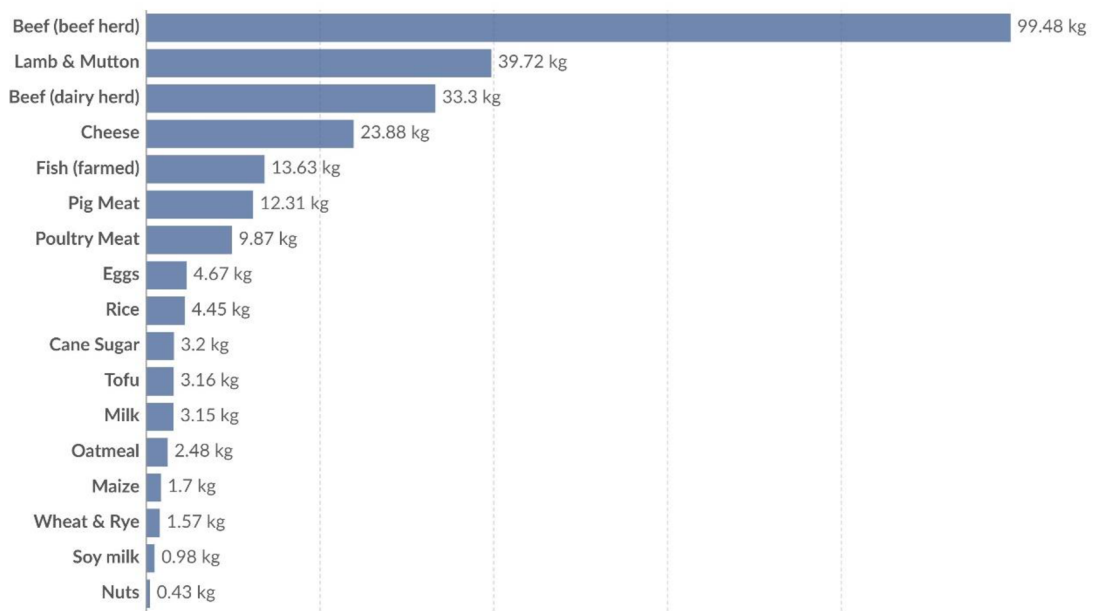


Figure 5 View of the amount of greenhouse gas emissions of food product per kilogram
(Poore & Nemecek 2018)

Figure 5 showing greenhouse gas emissions quantified in kilograms of carbon dioxide-equivalents. This means that gases other than CO₂ are evaluated based on their ability to cause warming over a 100-year timeframe. This figure displays greenhouse gas emissions difference between animal and plant sources. As can be seen, animal sources are the largest contributors to pollution, while plant sources are responsible for pollution to a lesser extent.

Food systems account for a quarter (26 %) of global greenhouse gas emissions. These emissions include production on farms, land use changes, transportation, processing, packaging, and retail. Overall, food system emissions can be divided into 4 categories:

- A) **Livestock farming and fishing** – a full 30 % of food emissions come from this category. Ruminants, especially cattle, produce methane through their digestive processes. This category also includes pasture management and fertilization. 1 % is attributed to wild fishing – primarily due to the fuel consumption of fishing vessels.
- B) **Crop production** – a quarter of emissions come from crop production, which includes crops for human consumption and animal feed.
- C) **Land use** – 24 % of food emissions come from land use, with 16 % allocated to livestock farming and the remaining 8 % for crops for human consumption.
- D) **Supply chains** – they account for 18 % of food emissions and include food processing, transportation, packaging, distribution, and retail.

Another study suggests that an even larger portion (one-third) of global greenhouse gases comes from food production (Crippa et al. 2021). This difference is reflected in the classification of non-food agricultural products, such as biofuels, various industrial crops, textiles, and uncertainties related to emissions from food and land use.

Consumers began to shift towards plant-based products after realizing that the environmental burden created by the production of animal proteins contributes to global warming, animal welfare concerns, and resource consumption (Siegrist & Hartmann 2019; Hartmann & Siegrist 2020). Plants, such as legumes, have a positive impact on preserving biodiversity. Their beneficial effects are also reflected in agriculture and in maintaining soil fertility. This assessment has been concluded by groups focusing on environmental and life cycle research (Boukid 2021).

Animal protein and their impact on the environment

The global environmental impact of meat production varies due to a wide range of practices used in agriculture. Water and land consumption, environmental pollution through the use of fossil fuels, are some of the effects associated with meat production (Petrovic et al. 2015) Dairy products and meat require more water and land than plant-based products, which may result in biodiversity loss and deforestation (Fiorentini et al. 2020; McClements & Grossmann 2021; Singh et al. 2021; Szenderák et al. 2022). Although meat has historically been considered an essential part of the diet due to its content of vitamin B12, calcium, and iron, it is now considered harmful when consumed excessively, and particularly processed meat carries certain health consequences (Rust et al. 2020; Malek & Umberger 2021; Tso & Forde 2021a). The current consumption of animal-based protein is unsustainable for the future (Wellesley et al. 2015) The global production of beef is increasing by approximately 1 % annually. This growth is partly due to population growth, but a significant role is also played by increased demand in many countries per capita (Jorgenson & Birkholz 2010). The total meat consumption worldwide was 328 million tons in 2021. An enormous increase is expected by 2050, with consumption projected to be about 70 % higher than in 2021 (Our World in Data 2017; Choudhury et al. 2020; Gastaldello et al. 2022; Statista 2022). The WHO has classified red meat as a Group 2A carcinogen (probable cause of cancer) and processed meat as a Group 1 carcinogen (known cause of cancer) (Bouvard et al. 2015). Additionally, the World Cancer Research Fund recommended limiting red meat consumption to three or fewer portions per week and avoiding processed meat consumption altogether (World Cancer Research Fund 2022). Complete exclusion of meat is not recommended, mainly because it is a key source of nutrition and energy (MacDiarmid 2021; Tso & Forde 2021a). To feed 10 billion people, estimates suggest that meat consumption needs to be drastically reduced by approximately 50–75 % (Hartmann & Siegrist 2017; Willett et al. 2019; Gastaldello et al. 2022). It is also mentioned that replacing 3 % of processed red meat from the total daily energy intake with plant-based sources could reduce the risk of mortality by 12 %, regardless of the cause of death (Song et al. 2016).

The main source of the greenhouse effect in beef production is the loss of grass, trees, or other vegetation that naturally absorbs CO₂ in the soil where feed crops are grown and subsequently harvested. As a second and very significant factor, there is CH₄, which is

released by animals during food digestion and also from animal waste (Jorgenson & Birkholz 2010).

Within the human impact, cattle and milk production are the main methane producers (Lasseby 2007). Thus, the impact on global climate change due to the ruminant production system and its generated methane has become a major concern for the global population (Martin et al. 2010). 14 % of all emissions in the United States in 2007 were methane. Half of this was caused by agriculture (U.S. Environmental Protection Agency 2013). As mentioned earlier, changing dietary habits could trigger a gradual cascade of effects. By reducing manure and cattle production, there would be a reduced demand for feed, which would then lead to lower greenhouse gas and nitrogen emissions, while agricultural land would also be freed up for different purposes (Steinfeld et al. 2006). Cultivated meat refers to meat that has been produced using in vitro tissue engineering techniques. This meat could be a more efficient and healthier alternative to traditional meat. Cultivated meat has a lower energy demand by 7-45 % compared to meat produced conventionally in Europe. Only poultry has a lower energy demand. However, it also has lower greenhouse gas emissions by 78-96 %, reduced water consumption by 82-96 %, and uses 99 % less land compared to the compared product. In conclusion, despite existing significant uncertainties, it can be said that all environmental impacts related to production are smaller for cultivated meat than for conventionally produced meat (Tuomisto & de Mattos 2011).

5. Conclusions

In conclusion of this work, it is clear that plant-based meat alternatives offer significant potential as a functional replacement for traditional meat products, whether it concerns nutritional value. Based on the analysis and summarization conducted, it can be stated that plant alternatives can provide an adequate amount of protein, which is essential for athletes and also necessary for a healthy diet.

Although indexes for determining protein quality provide a useful framework for evaluating the nutritional profiles of proteins, it is also essential to recognize their limitations. When overall assessing the nutritional quality of plant-based meat alternatives, it is crucial to consider the overall context of the diet, including the bioavailability of individual products.

The analysis of sources with high protein content from tropical and subtropical climate zones demonstrates the overall diversity and abundance of available plant-based protein sources worldwide. It also highlights the importance of a country's income, which significantly influences their consumption of plant-based or animal-based proteins. Lastly, it emphasizes the concept of protein consumption, divided according to FAO guidelines, and features a graphical representation of countries to raise awareness of protein intake in tropical/subtropical and temperate zones, designed for illustration. This information can serve as an important resource for the development of new products or strategies in the field of sports nutrition. Overall, it can be said that to achieve a healthy and sustainable food system, it is very important to support innovation and development in the field of plant alternatives that also consider local resources, needs, and cultures, which respect environmental sustainability. Understanding the nutritional profiles of these plant alternatives can contribute to strengthening the transition to more sustainable and healthier habits on a global scale.

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