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Single-Cell protein for feed and food

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Abstract

As global demand for protein sources continues to rise due to increasing populations and changing dietary preferences, the shortage of conventional protein for feed and food poses significant challenges for food security. Single-cell protein (SCP), derived from microorganisms such as yeasts and bacteria, represents a promising alternative to traditional protein sources. Among these, methanotropic bacteria such as *Methylococcus* sp. and *Methyocystis* sp. can provide protein from methane as their sole carbon and energy source. Oleaginous yeasts like *Yarrowia lipolytica* are gaining attention in animal nutrition, particularly chicken and aquaculture, since they not only contain protein but also lipids. *Yarrowia lipolytica*, which comprises approximately 20% lipids by cell weight, can effectively supplement protein in animal diets, improving feed efficiency and average daily gain (ADG). Incorporating 3% of this yeast instead of soybean meal can enhance growth performance, while higher inclusion rates may lead to adverse effects such as diarrhea in animals like piglets due to increased lipid content and reduced nutrient digestibility. The thick cell wall of *Yarrowia lipolytica* can limit nutrient absorption, indicating that lysis of the yeast cell walls may be necessary to optimize nutrient release. Additionally, the use of another oleaginous yeast, *Lipomyces starkeyi*, has demonstrated potential as a viable substitute for vegetable oils in fish diets, maintaining growth and meat quality without negative impacts. Research indicates that SCP can constitute significant portions of nitrogen intake in livestock, supporting performance without inducing adverse heat production. These findings underscore the potential of SCP and oleaginous yeasts in addressing protein shortages while promoting sustainable practices in animal nutrition. However, further studies are essential to optimize their utilization in various dietary formulations.

Keywords: Single-cell protein (SCP), Single-cell oils (SCO), Nutrient digestibility, Animal nutrition, Sustainability

Introduction

Global food production is consistently experiencing exponential growth, paralleling the geometric exponential increase in human and animal populations. Consequently, food scarcity and food security (García Martínez et al., 2022) have emerged as a critical issue that numerous societies are striving to address. As the global population continues to rise, the demand for food, particularly protein, escalates daily. This situation is exacerbated by the persistent challenge of meeting the protein requirements of human populations, which is fundamentally linked to the nutritional needs of livestock, poultry, and aquatic species (Daszkiewicz, 2022). On top of these challenges, there is the need to produce feed and food in a more sustainable way than currently. A significant contributing factor to these challenges is the scarcity of water resources worldwide, which has resulted in drought conditions and the depletion of natural resources. This scarcity has led to a shortage of fodder and animal feed, as well as the degradation of various habitats, thereby becoming a paramount concern for the livestock, poultry, and fisheries sectors (Mancosu et al., 2015).

2. Overview of SCP

Given the aforementioned challenges and the classification of proteins as one of the three primary macronutrients, alongside carbohydrates and fats, the exploration of alternative and novel protein sources has become a vibrant environment for research. Protein is the costliest of these macro-ingredients. This has motivated scientists to investigate cost-effective sources of protein for both human and animal consumption (Kumar et al., 2022).

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In recent years, extensive studies have been conducted to use microorganisms such as yeasts and bacteria as sources of microbial protein for the production of human and livestock nutrition (instead of common protein sources). Animals often receive fish meal as protein source, or soy protein; Alternative protein (alt protein) for food comes as insect protein, non-traditional plant protein, lab-grown meat (cultured meat) as well as SCP. There are only a hand-full of producers of SCP for feed and even less for food today.

Yet many countries and market incumbents are considering how to convert abundant and low-cost waste and side streams into valuable protein sources. The use of cellulose waste, whey, urban sewage, molasses, bagasse, methanol, natural gas, synthesis gas, ethanol, even CO_2 + H_2, as substrates for the production of microbial proteins has been investigated. One of the most affordable and safest raw materials is waste from agricultural products and food industries (Sekoai et al., 2024). The bioeconomy today is largely based on sugars for fermentation processes, and for scalability, other feedstocks are preferred.

The history of using microorganisms such as yeasts and bacteria in human food goes back thousands of years (e.g. baker's yeast for bread production, beer and wine manufacturing, cheese production), but in recent decades new methods have been invented to use microbial fermentation products in human food and animal feed. The designation "single cell protein" pertains to the desiccated cells of microorganisms, including bacteria, yeasts, archaea, algae, or fungi, which are produced on an extensive scale and utilized as protein sources for both livestock and human consumption. In this context, the term SCP can refer to both the isolated cellular protein and the entirety of the cellular biomass (Ritala et al., 2017; Bajić et al., 2023).

The specific microorganism utilized in SCP production is of significant importance, as both the quantity and quality of protein are heavily influenced by the chosen microorganism and the fermentation process employed. A diverse array of microorganisms possesses the capability to generate SCP from various substrates (Li et al., 2024), some of which have already attained approval, e.g. under the EU feed regulation EU 68/2013 (e.g. bacterial SCP from *Methylococcus capsulatus*) or the EU's Novel Food legislation (e.g. Fermotein™, while others have been on the market before that date, such as Quorn™, a mycoprotein made from sugar by *Fusarium venenatum*, a soil mould. Algae, fungi, and bacteria are the primary sources of microbial protein that can be employed as SCP (Anupama and Ravindra, 2000). Also, protein-rich side streams from fermentation process such as MSG (monosodium glutamate) residue have been used as SCP in aquaculture (Behera et al., 2024) and chicken (Edea, 2024) feeding.

Bacteria exhibit a notably high growth and reproduction rate under optimal conditions, making them ideal hosts for large-scale, low-cost protein production. Also, many bacteria are extremophiles, demonstrating the ability to thrive at elevated temperatures or at extreme pH conditions compared to yeasts and filamentous fungi, making monoculture feasible under non-sterile conditions. Additionally, bacteria typically possess a higher protein content, with SCP derived from this group of microorganisms reported to contain typically approximately 50-70% protein, including certain essential amino acids. Furthermore, bacteria are distinguished by their elevated levels of nucleic acids, which exceed the acceptable limits for human consumption. This excess can result in the accumulation of uric acid in the bloodstream, potentially leading to the onset of gout. Consequently, bacteria are preferably utilized as feed for poultry and aquaculture, as these organisms do not present complications associated with the urea cycle. Conversely, for human consumption, the nucleic acid (NA) content of bacteria needs to be reduced, e.g. through enzymatic or chemical methods, which subsequently raises the final cost of the resulting product (Li et al., 2024). Heat treatment can be used for fungal SCP to reduce the NA level. It is also possible to break the cell walls and purify the protein by removing (non-soluble) cell wall debris and NA (Wang and Tannenbaum, 1975). Figure 1 gives an overview of SCP.

SCPs derived from fungi exhibit a rich composition of B-complex vitamins, including thiamin, riboflavin, biotin, niacin, pantothenic acid, pyridoxine, choline, glutathione, and folic acid. Additionally, these SCPs are typically characterized by a high content of sulfur-rich amino acids and a protein concentration ranging from 30% to 40%. Numerous filamentous fungi possess the capability to decompose a diverse array of substrates and demonstrate resilience to low pH environments. In recent years, there has been a notable advancement in the production of mycoprotein, or mushroom protein, which possesses appealing nutritional, physical, and chemical attributes, thereby establishing its viability as a food source for human consumption (Li et al., 2024; Onyeaka et al., 2022). Quorn™ and Marmite™ are well-known products on the market in this respect.

SCPs derived from algal microorganisms are composed of approximately 40-60% protein, alongside significant quantities of fats and vitamins, including B, C, D, A, and E. Additionally, these SCPs contain around 7% mineral salts, chlorophyll, and fiber. Notably, they exhibit a relatively low concentration of nucleic acids (Li et al., 2024).

The production of single-cell protein (SCP) utilizing waste materials as substrates presents a cost-effective path (EPA, 1977). SCP can be beneficial for both animal feed and human consumption, as it can satisfy dietary protein requirements (Kuhad et al., 1997) at acceptable costs. Generally, the high production rates and protein yields, along with the ease of production management, render SCP a more appealing protein source in comparison to traditional plant and animal sources (Paraskevopoulou et al., 2003), but also in comparison to other alternative protein sources such as insects or lab-grown meat. Lab-grown meat has a high specific sugar demand, and its production is based on costly growth media. Unicellular microorganisms, by contrast, tend to be less demanding.

SCP has the potential to serve as a sustainable alternative

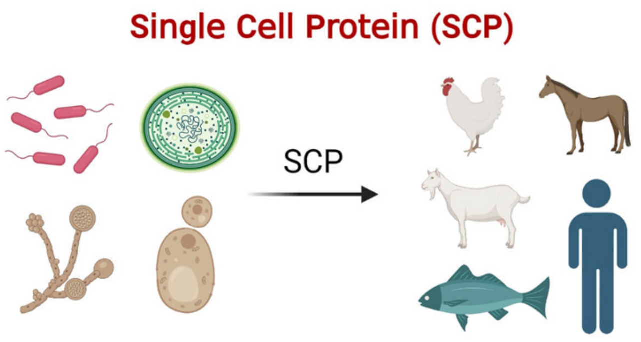

Figure 1. Single Cell Protein (SCP). Source: (Li et al., 2024).

protein source in animal nutrition, potentially replacing traditional ingredients such as fish meal, meat and bone meal, and soybean meal (Hellwing et al., 2007b). Research indicates that certain proportions of conventional protein sources can be substituted with SCP without negatively impacting the performance of swine (Braude et al., 1977; Hanssen and Farstad, 1980). Prior investigations have demonstrated that dietary nitrogen can be replaced by SCP at levels of up to 40% for chickens and 50% for pigs, without adverse effects on nitrogen retention, heat production, or energy retention (Hellwing et al., 2005; 2006; 2007a) Tables 1 and 2 summarize the merits of several fungal and algal SCPs and oils, respectively.

Oleaginous yeasts have been employed in a range of biotechnological applications, as detailed in Table 2 (Groenewald et al., 2014; Khot et al., 2012). Among these, *Yarrowia lipolytica* is particularly distinguished for its ability to produce valuable proteins, lipids, lipolytic enzymes, and organic acids, which have significant applications in the food industry (den Haan et al., 2015). This yeast not only serves as an alternative protein source in animal feed but also provides a lipid source, constituting approximately 20% of its cell weight (Hatlen et al., 2012). Research has indicated that the inclusion of 3% dried *Yarrowia lipolytica* as a protein supplement in lieu of soybean meal can enhance average daily gain (ADG) and feed efficiency (Czech et al., 2016). However, a higher inclusion rate of 6% *Yarrowia lipolytica*, attributed to its increased lipid content, has been linked to diarrhea in piglets and a reduction in growth performance among nursery pigs (Czech et al., 2016). Furthermore, Cheng et al. (2022) demonstrated that replacing poultry fat with 1.5% *Yarrowia lipolytica* as an energy feed maintained both intestinal health and growth performance in nursery pigs, although the thick cell wall structure may impede nutrient digestibility when 3% *Yarrowia lipolytica* is included. Hatlen et al. (2012) found that incorporating 10 to 30% *Yarrowia lipolytica* into fish diets improved feed efficiency and the retention of protein and energy; however, the digestibility of both protein and energy was reduced due to the presence of the indigestible yeast cell wall. These observations suggest that the lysis of yeast cell walls may be essential for releasing nutrients and enhancing digestibility in dietary formulations. Berge et al.

Table 1. Selected fungi and their documented benefits for application as single-cell protein (SCP).

Fungi	Advantages	References
Candida utilis	The subject exhibits a high protein content, with a notable presence of amino acids, particularly lysine, as well as a comprehensive vitamin B complex and ergosterol. Additionally, it possesses the capability to chelate metal ions and demonstrates a reduced generation time.	Munawar et al., 2010; Błazejak et al., 2004; Miura et al., 1998
Kluyveromyces marxianus	exhibits a substantial protein content of 59% and demonstrates the capability to utilize a variety of substrates	Aggelopoulos et al., 2014
Cryptococcus aureus G7a	The production of inulinase, along with the generation of beneficial protein content	Sheng et al., 2007
Dipodascus spp.	The protein content is beneficial, ranging from 30% to 36%, and exhibits a favorable amino acid profile.	Brown et al., 1996
Geotrichum candidum	The subject exhibits a high protein content of 40%, demonstrates favorable digestibility characteristics, and employs a cost-effective recovery method.	Ziino et al., 1999
Pleurotus florida	High protein content (63%)	Nassimi et al., 2006
Wickerhamomyces anomalus	high protein content of 56%, facilitates the synthesis of numerous essential amino acids, and produces a killer toxin that is effective against contaminating yeast species	Lombard et al., 2016

Table 2. Algal SCP and single-cell oils (SCO) and their effects on growth performance.

Microorganism	Species	Animal Model	Effect	Reference
Microalgae	*Schizochytrium* sp.	Growing-finishing pigs	Improved average daily gain	Sardi et al., 2006
Microalgae	*Schizochytrium* sp.	Growing-finishing pigs	Decreased average daily gain	De Tonnac et al., 2016
Microalgae	*Aurantiochytrium Acetophilum*	Broilers	Decreased average daily gain	Sun et al., 2022
Microalgae	*Schizochytrium* sp.	Nursery pigs	Decreased average daily gain	Lee et al., 2019
Microalgae	*Schizochytrium* sp.	Growing-finishing pigs	Decreased average daily gain	De Tonnac et al., 2018

(2013) reported that disrupted *Yarrowia lipolytica* resulted in a greater release of lipids and improved nutrient digestibility. Additionally, another oleaginous yeast, *Lipomyces starkeyi*, has been recognized as a suitable alternative to vegetable oils in aquaculture, exhibiting no adverse effects on fish growth or meat quality (Blomqvist et al., 2018; Cheng and Kim, 2022).

3. Nutritional Advantages of Single Cell Protein (SCP)

The principal objective of SCP production is to serve as a protein substitute, particularly meat, in order to mitigate food scarcity and hunger in the foreseeable future. For SCP to be suitable for human consumption or as animal feed, it must fulfill specific nutritional criteria, including adequate protein content, appropriate amino acid composition, and digestibility of the protein produced. Obviously SCP must be manufactured to the highest standards to ensure its safety and efficacy for use in food and feed applications (Linder et al., 2019). According to Finnigan et al. (2017), SCP is characterized by its provision of various macro- and micronutrients, which include proteins, lipids, carbohydrates, β-carotene, vitamin A precursors, biotin, folic acid, niacin, pantothenic acid, pyridoxine, riboflavin, thiamine, vitamin B12 (cyanocobalamin), as well as vitamins C and E (Finnigan et al., 2017).

The nutritional value of SCP is contingent upon its chemical composition, which includes amino acids, nucleic acids, minerals, enzymes, and vitamins. Notably, SCP is relatively cost-effective in comparison to other sources derived from plants and animals. Research indicates that dried cells of *Pseudomonas spp*., cultivated on petroleum-based liquid paraffin, can contain up to 69% protein. In contrast, SCP derived from algal processing typically comprises approximately 40% protein. Although microbial proteins encompass all essential amino acids, their specific composition is influenced by the type of substrate utilized and the particular microorganism cultivated in a given medium.

It is essential that the microorganisms selected for SCP production are non-pathogenic, free from toxins, manageable, and easily separable from the substrate, while also demonstrating resilience during the scaling-up of production processes. They need to be non-genetically modified. The use of rapidly proliferating microorganisms is critical for achieving substantial biomass output within a given timeframe. However, increased biomass production correlates with elevated levels of ribonucleic acid (RNA) within the cells, which is considered an anti-nutritional factor in the final product. Additionally, the techniques employed for harvesting, drying, and processing significantly impact the nutritional quality of the end product (Bhalla et al., 2007; Jamal et al., 2008; Mahajan and Dua, 1995; McEvoy et al., 1996; Olvera-Novoaet al., 2002). The potential of SCP to address food scarcity in developing nations has attracted considerable attention from both the scientific community and industry stakeholders. For SCP to achieve future success, it is imperative to address food technology challenges to enhance consumer acceptance, while ensuring that its production remains competitive with alternative protein sources (Bratosin et al., 2021).

4. Utilization of Single Cell Proteins in Animal Nutrition

The incorporation of SCP into the diets of livestock and poultry has a longstanding history aimed at mitigating the expenses associated with the import of protein sources. Numerous studies have evaluated the digestibility, biological value, and other quality metrics of SCP, demonstrating the acceptability of various forms of microbial proteins (Shanon, 1992). It has been established that the inclusion of microbial proteins in poultry diets at levels of 6-8% is deemed suitable, with some practitioners reporting successful utilization of even higher percentages without adversely affecting poultry performance (Al-shadeedi, 1998). Chirwa and Lebitso (2011) demonstrated that the protein content and amino acid profile of microbial protein derived from sludge processing closely resemble that of fish meal supplements. Their findings indicated that broiler chickens, when fed varying levels of sludge-derived single-cell protein during the 25-35 day rearing period, exhibited greater weight gain compared to those on a conventional diet. Furthermore, the cost associated with raising broiler chickens on sludge protein to achieve the target weight was reduced by 23% in comparison to those on a standard diet (Chirwa, 2010; Esonu et al., 2006; Chirwa and Lebitso, 2011). This suggests a significant nutritional advantage of sludge-derived protein (Chirwa and Lebitso, 1999).

Vriens et al. (1989) established that the lysine to methionine ratio, a critical nutritional parameter in poultry diets, is comparable to that found in fish meal when utilizing SCP. Additionally, Ravindra et al. (2000) reported that SCP sourced from algae is abundant in protein, fats, and vitamins D, C, B, and E (Ravindra, 2000). The incorporation of SCP derived from yeast has also been shown to positively influence the performance of broiler chickens (Haldar et al., 2011; Pelicia et al., 2010; Silva et al., 2009).

Helwing et al. (2007) noted that the apparent and standard digestibility of raw protein and certain amino acids, including arginine, histidine, isoleucine, leucine, phenylalanine, and tryptophan, in SCP is lower than in soy. Conversely, the digestibility of lysine, methionine, and threonine was unaffected. The reduced digestibility of SCP relative to soy may be attributed to differences in nucleotide content, as well as the structural characteristics of microbial protein compared to plant proteins like soy, or it may be influenced by the production source of SCP and its effects on animal physiology. In their study involving piglets, these researchers concluded that up to 550 grams of SCP could be substituted for soybean meal in each feeding without adversely affecting growth (Helwing et al., 2007). Daghir and Sell (1982) identified methionine and arginine as the first and second limiting amino acids in SCP. They further reported that the use of granulated SCP, as opposed to its powdered form, enhanced the performance of broiler chickens. Their research indicated that microbial protein, when supplemented with the amino acids methionine and arginine, could be utilized independently of soy (Daghir and Sell, 1982).

Ashraf (1981) demonstrated that the mortality rate in chickens rose with increasing levels of dietary single-cell protein. He hypothesized that this phenomenon was attributable to a deficiency in selenium. Al-Awadhi et al. (1995) reported that there were no significant abnormalities observed in the weights or histopathological characteristics of the organs in rats that were administered a diet containing SCP. Sternberg et al. (1975) indicated that lysinoalanine, identified as a renal toxic agent in rats, has been detected in the proteins of both home-cooked and commercially prepared foods and ingredients.

White and Balloun (1977) conducted an evaluation of SCP as a replacement for soybean meal in broiler diets, with substitution levels reaching up to 15%. Their findings indicated that the powdery texture of SCP was associated with a reduction in body weight gain and feed conversion efficiency when included at levels of 9% or higher in the mash diet. Notably, the adverse effects observed were mitigated when the diets were pelleted. Specifically, when pelleting was employed, the substitution of SCP for soybean meal, on a weight-for-weight basis, at levels up to 15% resulted in enhanced and more efficient weight gains. Furthermore, Anonymous (1989) noted that free lysinoalanine exhibits greater toxicity compared to covalently bound lysinoalanine in food proteins. This discrepancy in toxicity may be attributed to variations in absorption and the copper-binding affinity between the dietary L-lysinoalanine and D-lysinoalanine isomers.

Manal and Abou El Nagha (2012) documented an increase in body weight gain among broilers that were administered a diet containing 0.3%, 0.5%, and 0.7% SCP. Similarly, Shareef and Al-Dabbagh (2009) observed enhanced body weight gain in broilers when yeast was incorporated as SCP at a concentration of 2% in their diet. The findings of Pourelmi et al. (2018) suggest that incorporating up to 5% (SCP) into broiler diets does not adversely affect the growth performance of chickens, and therefore, such supplementation may be considered acceptable in their dietary formulations.Skrede et al. (1998) speculated that the digestibility of nitrogen is influenced by the digestibility of nucleic acids and other non-protein constituents found in single-cell protein (SCP). The diminished digestibility of SCP may be linked to its high concentrations of non-protein nitrogen, such as nucleotides, which are not effectively utilized by swine (Wang et al., 2013). SCP comprises approximately 10% nucleic acids, primarily in the form of ribonucleic acid (RNA) (Skrede et al., 1998). The concentrations of RNA and deoxyribonucleic acid (DNA) in SCP are significantly higher than those found in fish meal, as observed by Greife (1984). Consequently, the increased nucleic acid content may play a role in the reduced amino acid digestibility associated with SCP. Øverland et al. (2004) indicated that replacing soybean meal with 5%, 10%, or 15% SCP in diets designed to maintain equivalent metabolizable energy (ME) and consistent levels of available lysine, methionine, and threonine resulted in a linear decrease in growth performance during the piglet stage. However, this substitution did not adversely affect the growth performance of pigs during the growing-finishing phase or throughout the overall growth period. Furthermore, Øverland et al. (2001) demonstrated that diets in which SCP replaced 50% of the lysine content in soybean meal (with low SCP diets containing either 30 or 52 g/kg SCP) did not significantly influence growth performance during the growing phase. This finding suggests that a higher proportion of protein can be substituted with SCP in the diets of growing or finishing pigs compared to those of weaned piglets. Therefore, the incorporation of a specific level of SCP into the diets of weaned piglets is a feasible option. Zhao et al. (2012) reported that the nitrogen metabolism in growing-finishing pigs remained stable when 25% of dietary soybean protein was replaced with SCP. However, in the group where 50% of the soybean protein was substituted, a reduction in nitrogen digestibility was noted, with a decrease of 5% during the growing phase and 3% during the finishing phase in comparison to the control group. Similarly, Aas et al. (2006) found that the apparent digestibility of nitrogen and the total amino acid content decreased as the proportion of dietary SCP increased. Furthermore, Zhang et al. (2013) indicated that although Prosin and Protide (two types of

SCP, obtained from fermented cornstarch by *Corynebacterium glutamicum*) had lower metabolizable energy (ME) content and standardized ileal digestibility of amino acids (AA) than fish meal, replacing 50% of fish meal in the diet with Prosin and Protide, while maintaining equivalent nutrient levels, resulted in comparable performance, nutrient digestibility, and intestinal morphology in weaned pigs.

The existing body of research on the utilization of fungal, algal, and bacterial biomasses as protein sources in piglet diets is relatively sparse. Nevertheless, it can be deduced that SCP represents a promising protein source for these animals. Current data suggest that approximately 30-60% of the total crude protein in piglet diets may be sourced from yeast protein, 25-33% from algal protein, and up to 40% from bacterial protein, all without adversely affecting performance. Some studies have even indicated improved performance in piglets when SCP is incorporated into starter diets. However, due to the significant variability in the composition and characteristics of various SCP sources, it is essential to determine appropriate inclusion levels for each specific biomass source. At present, there is a notable deficiency in information regarding the ileal digestibility of protein and amino acids derived from SCP, which is critical for assessing the nutritional value of these protein sources for piglets. Therefore, additional research and pilot studies are necessary before undertaking cost-benefit analyses related to the integration of SCP into sustainable agricultural practices.

Ikurior (1995) and Miyada et al. (1997) established that the addition of dietary yeast, which constituted approximately 30-60% of the total crude protein, did not negatively influence the performance of weanling pigs. In fact, the integration of yeast was observed to improve feed efficiency (Ikurior, 1995; Miyada et al., 1997). In contrast, Landell et al. (1994) reported that centrifuged vinasse yeast had an adverse effect on the performance of weanling pigs. The specific reasons for this negative outcome remain uncertain; however, it is plausible that the yeast product contained an excessive level of potassium. Slagle and Zimmerman (1979) conducted feeding trials using an unspecified "yeast single-cell protein (SCP) product." Pigs, with an initial weight of 5.6 kg, were fed diets containing 18% crude protein sourced from either SCP or dehulled soybean meal, with both diets supplemented with methionine. The findings revealed that the apparent digestibility of dry matter and nitrogen was significantly higher in the SCP diet compared to the soybean meal diet. In a subsequent experiment, SCP was incorporated into the diets of weanling pigs, replacing up to 100% of the soybean meal. The inclusion of SCP did not significantly affect average daily gain, average daily feed intake, or the gain-to-feed ratio.

Landell et al. (1994) conducted a study to investigate the effects of centrifuged vinasse yeast (*Saccharomyces cerevisiae*), a byproduct of sugarcane alcohol production, on the dietary intake of Landrace piglets. The subjects had an average initial weight of 11.3 kg. The experimental diets, which were isoproteic formulations based on corn and soybean, included up to 20% dried vinasse yeast, which accounted for 36% of the total crude protein content. The results revealed that an increase in the proportion of centrifuged vinasse yeast in the diet was correlated with a linear decrease in the performance of the pigs. Ikurior (1995) conducted a study in which up to 60% of the total crude protein in a diet consisting of soybean meal and maize for crossbred weanling pigs was replaced with brewers yeast slurry (*Saccharomyces carlsbergensis*). The subjects of this study were Large White and Landrace breed pigs, aged 8 weeks and with an average live weight of 11.8 kg. The findings revealed no significant differences in feed intake, weight gain, or feed-to-gain ratio among the pigs. Importantly, the incorporation of brewers yeast slurry resulted in a substantial decrease in feed costs per kilogram of live weight and improved feed utilization, thereby enhancing overall production efficiency in the pigs.

In a distinct study conducted by Miyada et al. (1997), dried yeast was incorporated into a pelleted starter diet for crossbred weanling pigs, with the inclusion level reaching up to 20%, which accounted for 30% of the total crude protein. The subjects, with an initial body weight of 10.3 kg, were provided with five different isonitrogenous and isocaloric diets ad libitum over a 28-day experimental period. The results indicated that while the increased inclusion of dried yeast did not significantly affect average weight gain, it led to a reduction in daily feed intake and an enhancement in the feed-to-gain ratio. Furthermore, the study observed an elevation in hematocrit, hemoglobin, and uric acid levels correlated with the dietary inclusion of yeast. Specifically, plasma uric acid levels increased from 0.01 mg/dl at 0% yeast to 0.31 mg/dl at 20% yeast. In contrast, levels of urea, albumin, cholesterol, and the albumin/globulin ratio were diminished, whereas plasma concentrations of total protein, globulin, and triglycerides remained unchanged. Grinstead et al. (2000) conducted feeding trials involving dehydrated *Spirulina platensis* (cyanobacteria, microalgae) with weanling pigs (PIC, L326 X C22), which had an initial weight of 3.7 ± 0.85 kg and were aged between 11 to 12 days. From day 0 to day 14 post-weaning, the pigs were provided with either a control diet or pelleted diets that contained 0.2%, 0.5%, or 2% Spirulina platensis, replacing soybean meal on an equivalent lysine basis. It is noteworthy that the incorporation of 2% *Spirulina platensis* led to only a 3.2 to 3.4% substitution of the total dietary lysine. All experimental diets were supplemented with a growth-promoting agent (carbadox)1 and zinc oxide. Throughout the duration of the experiment, no significant differences in pig performance, as measured by average daily feed intake and weight gain, were observed. In contrast to the pelleted diets, the meal diets displayed variable responses to the addition of *Spirulina platensis*.

Newport and Keal (1980) undertook feeding trials with pigs that were weaned at 2 days of age, utilizing a single-cell protein (SCP) product named PRUTEEN™ and formerly produced by ICI (UK). This product is derived from the methanol-grown bacterium *Methylophilus methylotrophum*, which is processed through harvesting and spray-drying to produce a finely ground powder. The experimental diets were formulated to include up to 80% of total crude protein sourced from SCP, alongside skimmed milk, whey, and soybean oil. These diets were administered to the pigs at hourly intervals, calibrated according to their live weight, over a 28-day period, a practice referred to as "artificial rearing." The findings indicated that the performance of pigs receiving a diet with 40% of total protein replaced by PRUTEEN™ was comparable to that of the control group, which was fed an all-milk diet. However, higher levels of milk protein replacement with SCP resulted in decreased performance. Newport and Keal (1980) posited that this decline in performance at SCP levels exceeding 40% could be linked to insufficient enzyme adaptation and a reduction in gastrointestinal transit time. Additionally, analyses of the digesta conducted after the 28-day period demonstrated that SCP facilitated an increase in the secretion of pepsin and chymotrypsin, while concurrently lowering the pH in the stomach digesta.

Wu et al. (1999) conducted a series of experiments to investigate the effects of fermented soybean meal. The fermentation process resulted in increased concentrations of crude protein, lysine, and methionine. When fermented soybean meal was utilized as a substitute for concentrated soy protein or as a replacement for both concentrated soy protein and soybean meal in the pre-starter diet, no statistically significant differences were observed in average daily feed intake, average daily gain, or feed conversion ratios during the periods of 0 to 14 days and 0 to 28 days post-weaning. However, a modest improvement in average daily gain and feed conversion was noted when fermented soybean meal replaced soybean meal. Additionally, the incorporation of fermented soybean meal resulted in a decrease in anti-soy antibody titres and exhibited enhanced digestive utilization and growth performance compared to soybean meal in weaned pigs.

Growth trials suggest that SCP may represent a promising alternative protein source; however, the results have been inconsistent and occasionally contradictory (Øverland et al., 2001 and 2004; Skrede et al., 2003; Storebakken et al., 2004). Research indicates that dietary SCP, which can account for up to one-third of nitrogen (N) intake, effectively supports the performance and health of slaughter chickens (Skrede et al., 2003) and blue foxes (Skrede and Ahlstrøm, 2002). Additionally, when SCP constituted approximately 50% of the dietary N, no adverse effects were noted in growing-finishing pigs (Øverland et al., 2001) or Atlantic salmon (Storebakken et al., 2004). In contrast, studies involving piglets revealed that when 40 to 50% of dietary N was sourced from bacterial protein meal (BPM), a decline in performance during the piglet stage was observed in certain trials (Øverland et al., 2001 and 2004).

A thorough assessment of feedstuffs should not be limited to performance metrics (Øverland et al., 2001 and 2004); it is essential to also consider the effects on protein and fat deposition. An optimal protein source should promote significant nitrogen retention without leading to excessive heat production. Research on the incorporation of SCP into diets and its influence on protein and energy deposition is currently sparse, with existing studies predominantly involving chickens and minks. These investigations revealed that retained nitrogen (RN) remained unchanged when SCP comprised 20 to 60% of the digestible nitrogen in the mink diet (Hellwing et al., 2005) and 6.5 to 20% in the chicken diet (Hellwing et al., 2006). Additionally, heat production in both minks (Hellwing et al., 2005) and chickens (Hellwing et al., 2006) was found to be similar between the control diet and those supplemented with SCP.

Conclusion

In conclusion, the increasing global demand for protein and the limitations of traditional protein sources necessitate the exploration of alternative options, such as single-cell proteins (SCP). These innovative protein sources, particularly bacteria such as methanotrophs (*Methylococcus* sp., *Methylocysis* sp.) which can use CH_4 as their sole carbon and energy source (Lackner et al., 2022a and Lackner et al., 2022b), or yeasts like *Yarrowia lipolytica* and *Lipomyces starkeyi*, which can ferment sugars to proteins and lipids, have demonstrated significant potential in improving animal nutrition and performance while addressing sustainability concerns in food production. Algae can also be used to obtain single-cell oil (SCO). Research indicates that SCP can effectively contribute to dietary nitrogen intake in various livestock species without adversely affecting growth performance or health, as long as inclusion rates are carefully managed. Additionally, the modifications of yeast cell walls to enhance nutrient digestibility further bolster the feasibility of these alternatives in animal diets. The findings highlight the importance of continued research to optimize the incorporation of SCP in feed and food formulations. This will ensure the development of efficient, sustainable feeding strategies that can support animal growth, health, and overall welfare, ultimately contributing to addressing the ongoing protein shortages worldwide. Emphasizing innovation in feed technology can play a crucial role in enhancing food security (García Martínez et al., 2022) while minimizing the environmental impact of animal agriculture.

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