

Single Cell Proteins in diets for weanling pigs

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1. Samenvatting

Single Cell Protein (SCP) omvat een zeer brede range van verschillende producten die bestaan uit micro-organismen als fungi, algen of bacteriën. De hoge groeisnelheid van micro-organismen, de hoge efficiëntie van hun substraatconversie en eiwitsynthese en goedkope substraten vormen de commerciële basis voor de productie van SCP. De productie van SCP is een efficiënte manier om koolhydraatrijke overschotten of afvallen door micro-organismen naar eiwitrijke grondstoffen voor de diervoeding om te zetten. Fungi en bacteriën kunnen koolhydraten zoals hemicellulose of lignocellulose als koolstofbron benutten, terwijl eiwitrijke eendekroos of algen CO₂ kunnen fixeren met behulp van lichtenergie.

Zowel in de biologische als in de duurzame landbouw heerst een groot tekort aan voereiwit. SCP is een veelbelovende eiwitbron voor de diervoeding. Een vergelijking van de eiwitgehalten van verschillende organismen toont dat lagere organismen in het algemeen een hoger eiwitgehalte hebben dan hogere organismen. Ook wat betreft de eiwitopbrengst per hectare en jaar zijn lagere organismen zoals algen en fungi productiever. Een verder voordeel van SCP ten opzichte van conventionele eiwitbronnen is de mogelijkheid van continu oogsten. Net als leguminosen kunnen sommige fungi gemeenschappen vormen met stikstoffixerende bacteriën. Op die manier kan naast organische stikstof ook atmosferische stikstof (N₂) in vorm van eiwit worden vastgelegd. De hoge efficiëntie van de vastlegging van stikstof en fosfor door micro-organismen zou eveneens een substantiële bijdrage kunnen leveren aan een vermindering van het verlies van deze elementen in de agrarische nutriëntenkringloop.

Tegenwoordig zijn veel minder bedrijven betrokken bij de ontwikkeling van technologieën en de productie van SCP dan het geval was in de jaren 1960 tot 1980, toen biotechnologen enthousiast waren over de mogelijkheden om goedkope grondstoffen of organische afvalstoffen om te zetten in eiwitrijke producten. Hun onderzoek werd gestimuleerd door de voorspelling dat in de nabije toekomst de groeiende behoefte aan eiwit van de wereldbevolking niet meer door conventionele productie gedekt kan worden. De meeste SCP technologieën die toen ontwikkeld werden bleken echter niet in staat om de economische concurrentie met geïmporteerde eiwitrijke grondstoffen (sojabonen, vismeel) aan te kunnen. Aanvankelijk werden SCP vooral geproduceerd op fossiele substraten. Een duurzame productie vraagt echter om recycling. Duurzaamheid zou kunnen worden gewaarborgd door SCP op reststromen uit de landbouw en de voedselproductie te kweken of fototrofe organismen met behulp van lichtenergie te kweken, en dierlijke uitscheidingsproducten als stikstof- en fosforbron te gebruiken.

De schaal aan mogelijkheden om SCP te produceren loopt van high-tech bioreactor technologieën tot low-input systemen voor de recycling van afvalmaterialen. De meest prominente organismen die tegenwoordig voor de SCP productie gebruikt worden zijn algen (b.v. *Spirulina*, *Chlorella*) en gisten (b.v. *Saccharomyces cerevisiae*). Alhoewel het aantal studies waarin biomassa van fungi, algen en bacteriën geëvalueerd werden als eiwitbron voor gespeende biggen beperkt is, kan geconcludeerd worden dat SCP een potentieel geschikte eiwitbron voor deze dieren is. Uit toegankelijke gegevensbronnen blijkt dat ongeveer 30 – 60 % van het ruw eiwit in het voer vervangen kan worden door gisteiwit, 25 – 33 % door eiwit uit algen of tot 40 % door bacterieel eiwit zonder dat de zoötechnische prestaties (groei, voeropname, voederconversie) achteruit gaan. Sommige onderzoekers vonden zelfs betere technische resultaten met SCP in het startvoer. Vanwege de grote variatie wat betreft de samenstelling en de kenmerken van de individuele SCP, kunnen maximale verwerkingspercentages alleen per eiwitbron worden vastgesteld. Er was geen informatie verkrijgbaar over de ileale verteerbaarheid van eiwit en aminozuren in SCP voor biggen. Gegevens over de ileale verteerbaarheid zijn echter essentieel voor de inschatting van de nutritionele waarde van deze eiwitbronnen voor biggen. Voordat een inschatting kan worden

gemaakt van de kosten en baten van het gebruik van SCP in een duurzame landbouw is dus nader onderzoek op dit terrein noodzakelijk.

De aanwezigheid van toxinen, pathogene organismen, niet verteerbare celwanden of een hoog nucleïnezuurgehalte kunnen de veiligheid en de verteerbaarheid van SCP beperken. Men dient dus te voorkomen dat er toxinen gevormd worden of besmetting optreedt tijdens de productie van SCP. Om de enzymatische afbreekbaarheid van SCP te verbeteren zijn verschillende technieken beschreven, waarbij eukaryotische cellen worden geopend of hun celwanden beschadigd. In tegenstelling tot de mens hebben varkens geen problemen met verhoogde nucleïnezuurgehaltes in het rantsoen. Bij de mens veroorzaakt het weinig oplosbare metabooliet urinezuur nierenstenen en jicht. Varkens kunnen urinezuur echter omzetten naar allantoïne door middel van het enzym uraatoxidase. Bij de mens is dit enzym tijdens de evolutie non-functioneel geworden. Allantoïne is beter wateroplosbaar dan urinezuur en wordt via de urine uitgescheiden.

Om de veiligheid van diervoeder en voedsel te waarborgen heeft de Europese Gemeenschap voorschriften uitgebracht betreffende de producten die in diervoeders gebruikt mogen worden. De 'European Communities (Protein Feedingstuffs) (Amendment) Regulations' van 1996 geven in de bijlage een lijst van toegelaten producten. Om te worden opgenomen in deze bijlage dienen de producten (a) een nutritionele waarde te hebben, (b) geen nadelig effect op de menselijke en dierlijke gezondheid of het milieu te hebben en (c) te analyseren te zijn in een voer. De huidige lijst bevat bacteriën (*Methylophilus methylotrophus*, *Methylococcus capsulatus*, *Alcaligenes acidovorans*, *Bacillus brevis*, *Bacillus firmis*), gisten (*Saccharomyces cerevisiae*, *Saccharomyces carlsbergiensis*, *Kluyveromyces lactis*, *Kluyveromyces fragilis*), algen (alleen als categorie) en lagere fungi (*Penicillium chrysogenum*¹).

¹ Er bestaan behoorlijke discrepanties in de classificatie van fungi; *Penicillium chrysogenum* wordt meestal als een hogere fungus beschouwd.

2. Summary

The term Single Cell Protein (SCP) comprises a broad range of very diverse products, originating from micro-organisms as fungi, algae, or bacteria. The high growth rates of micro-organisms, their high efficiency of substrate conversion and protein synthesis, and use of inexpensive raw materials form the commercial basis for the production of SCP. SCP production is an efficient way of converting any surplus or waste carbohydrate into protein-rich feedstuff. Fungi and bacteria can use carbohydrates like hemi- or lignocellulose as carbon source, for example, while protein-rich duckweed or algae can fix CO₂ using light energy.

There is a severe shortage of feed protein in both organic and sustainable farming. SCP has great potentials as protein source in animal feed. A comparison of protein contents of different organisms shows that lower organisms generally contain more protein than higher organisms. The yield that can be obtained per hectare and year also shows the superiority of lower organisms as protein suppliers. Another advantage of SCP over conventional protein sources is the possibility of a continuous harvest. Like legumes, some fungi can live in symbiosis with nitrogen-fixing bacteria. In this way, in addition to organic nitrogen sources, atmospheric nitrogen (N₂) can be used for protein production. The high efficiency of nitrogen and phosphorus fixation by micro-organisms could, moreover, substantially contribute to a minimisation of the loss of these elements in the agricultural nutrient cycle.

Currently, much less companies are involved in the development of technologies and the production of SCP than were from the 1960s till the 1980s, when biotechnologists were enthusiastic over the possibilities of transforming cheap raw materials or organic wastes into protein. Their research was driven by the prediction that in the not too distant future, the growing demands of the world population cannot be met any more by conventional protein production. Most SCP technologies developed during these years, however, proved unable to compete economically with imported protein-rich raw materials (soybean and fish meal). While in the early stages particularly fossil substrates were employed for cultivation of SCP, sustainable SCP production necessitates recycling. Sustainability could be realized by cultivating SCP on carbohydrate-rich waste materials from agriculture and food production or growing phototrophic micro-organisms, and by employing animal excreta as source of nitrogen and phosphorus.

The scale of possibilities of SCP production range from high-tech bioreactor technologies to low-input systems for recycling of waste materials. The most prominent organisms used for SCP production are algae (e.g. *Spirulina*, *Chlorella*) and yeasts (e.g. *Saccharomyces cerevisiae*). Although the number of studies in which fungal, algal, and bacterial biomasses were evaluated as protein source in piglet diets are limited, it can be concluded that for these animals SCP seems to be a suitable protein source. Available data suggest that about 30 – 60 % of total crude protein in the diet can be included in form of yeast protein, 25 – 33 % in form of algal protein, or up to 40 % in form of bacterial protein, without adverse effects on performance. Some researchers even registered an improvement of performance with SCP in the starter diet. Because of the rather large variation in composition and characteristics of individual SCPs, suitable inclusion levels should always be determined per source of biomass. No information was available on the ileal digestibility of protein and amino acids of SCP. Data on ileal digestibility are essential for an assessment of the nutritional value of these protein sources for piglets. Therefore, further research and pilot studies seem necessary before cost benefit analyses can be made about the application of SCP in sustainable agriculture.

The presence of toxins or pathogenic organisms, non-digestible cell walls, or a high nucleic acid content can possibly impede feed safety and digestibility of SCP. Hence, it must be ensured that no toxins are formed and

no contamination can take place in the SCP production line. For crushing cells of eukaryotic SCP or rupturing cell walls, which restrict protein accessibility and digestibility, several techniques have been described. In contrast to human beings, pigs evidently have no problems with elevated nucleic acid contents in the diet. While humans develop stones in the urinary system or gout from the barely soluble degradation product uric acid, pigs can transform uric acid into allantoin. This reaction is catalysed by the enzyme urate oxidase, which is non-functional in man. Allantoin has a higher water solubility than uric acid and is excreted via urine.

To guarantee feed and food safety, the European Communities brought out regulations concerning products used in animal nutrition. The European Communities Protein Feedingstuffs (Amendment) Regulations, 1996, give an annex of allowed products. Prerequisites for being added to the annex are that products (i) have nutritional value, (ii) have no detrimental effect on human and animal health or the environment, and (iii) can be monitored. The current list contains bacteria (*Methylophilus methylotrophus*, *Methylococcus capsulatus*, *Alcaligenes acidovorans*, *Bacillus brevis*, *Bacillus firmis*), yeasts (*Saccharomyces cerevisiae*, *Saccharomyces carlsbergiensis*, *Kluyveromyces lactis*, *Kluyveromyces fragilis*), algae (no entry), and lower fungi (*Penicillium chrysogenum*²).

² There is a considerable discrepancy in the classification of fungi; *Penicillium chrysogenum* is mostly classified as a higher fungus.

3. Definition and introduction

According to Israelidis (2003), “the term single cell protein (SCP) refers to dead, dry cells of micro-organisms such as bacteria, fungi and algae. The name “single cell protein” was used for the first time by the M.I.T. professor Carol Wilson to give a better image than “microbial protein” (Ware, 1977).”

Consumption of microbial cells is no new idea. Apparently, the Aztecs already ate algae (*Spirulina*). *Spirulina* is still being used in Chad (Africa), where locals traditionally consume 9 - 13 grams per meal (Delpeuch et al., 1976). Apart from algae, fungi and bacteria also play a classical role in human nutrition, like in cheese, yoghurt, and fermented sausages.

The high growth rates of micro-organisms, the high efficiency of their substrate conversion, and inexpensive raw materials form the commercial basis for the production of SCP. Other advantages of SCP over conventional protein sources are high protein yields and the possibility of a continuous harvest.

SCP production technology started in Germany during World War I, when the yeasts *Saccharomyces cerevisiae* and *Candida utilis* were grown on molasses or sulphite waste liquors for use as a protein supplement (Rose, 1979). In the 1960s, the growing petrochemical industry offered prospects for cultivation of micro-organisms on oil fractions. The thus gained SCP was intended to replace fishmeal and soybean meal as protein sources for pigs and cattle. Due to concerns about the presence of carcinogenic residues, however, research on hydrocarbon-grown SCP stopped (WU, 2003) and SCP got a bad name.

Nevertheless, this setback did not stop further developments of the SCP technology. In 1967, the development of a new biotechnological SCP process started. This time, the bacterium *Methylophilus methylotrophus* (nomenclature subjected to changes) was grown on methanol, ammonia, oxygen, and trace elements. Methanol was produced from North Sea gas. The product, PRUTEEN, can be added to animal feed as a supplement. PRUTEEN contains 72 % crude protein and has a high content of vitamins. Unfortunately, the production of PRUTEEN by Imperial Chemical Industries was unable to compete economically with soya and fish meal (Israelidis 2003; WU, 2003). The same was the case with TORUTEIN, a product of the Amoco Company in the US, which utilised the food grade yeast *Torula (Candida utilis)* grown on ethanol. Other productions seem to be more promising. In Finland, the PEKILÖ process is operating since 1974 for the manufacture of animal feed ingredients from the microfungus *Paecilomyces variotii*, using the spent liquor of sulphite pulp mills as growth substrate (Moresi, 1994; Romantschuk, 2003). A SCP product, which is commercially available for human consumption since 1985, is QUORN myco-protein, made from the filamentous fungus *Fusarium graminearum* A3/5 by Ranks Hovis McDougall (Trinci, 1994).

In the EEC, the majority of the protein required by the animal feed industry must be imported. According to Moresi (1994), SCP production from agro-industrial wastes or by-products appears to be potentially competitive and capable of supplying a part of the EEC protein needs. Focusing on “leftovers” and waste materials from agriculture and food production as growth substrates for SCP should enable a sustainable protein production. After all, recycling is a basic principle in organic farming. Besides, the high efficiency of mineral fixation by micro-organisms can possibly contribute to a minimisation of the loss of nitrogen and phosphorus in the agricultural nutrient cycle. By culturing SCP in a closed system, emission to the environment can probably be further reduced.

3.1. Objective of this study

This study was performed in the framework of the LNV programme PO-34 “Ontwikkeling van duurzame biologische Veehouderij”, project “Voeding biologische varkens”, to give some insight into production methods and nutritional aspects of SCP for weanling pigs. For some SCP sources more detailed information was available than for others. Cost benefit analyses were not included in this study. The intention of this study was to show some promising facets of the up to now little utilised protein from micro-organisms in sustainable agriculture.

3.2. References

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4. Comparison of protein yields, amino acid contents, and digestibilities

4.1. Protein yields

The protein yield that can be obtained per hectare on a yearly basis impressively reflects the superiority of lower organisms as algae and fungi as protein sources (Table 1).

Table 1. Kilograms of protein produced per hectare per year

PROTEIN SOURCE	PROTEIN YIELD (kg dry wt ha ⁻¹ y ⁻¹)
<i>Spirulina platensis</i> (prokaryotic alga)	24,300 ^a
<i>Chlorella pyrenoidosa</i> (eukaryotic alga)	15,700 ^a
Mushrooms	8,000 ^b
Soybeans	716 ^c
Peanuts	470 ^a
Peas	395 ^a
Wheat	300 ^a
Milk from cattle on grassland	100 ^a
Meat from cattle on grassland	60 ^a

^aKay (1991), ^bDibben (2002), ^cSchang and Azcona (1999)

4.2. Amino acid contents

Research on the use of water plants as protein sources is being done mainly for and in developing countries (for example by the University of Tropical Agriculture Foundation, UTA). Water plants that are rich in protein as duckweed (*Lemnaceae*) or azolla are known and applied in animal feeding since about 20 years (Culley et al., 1981; Becerra et al., 1995; Leng et al. 1995; Ly et al. 2002). Apparently, products derived from single celled algae as *Chlorella* (green alga; see 7.), *Aphanizomenon* and *Spirulina* (blue-green algae; see 7.) contain even higher protein contents (on a dry matter basis) than more complex water plants. Algae, on a dry matter basis, evidently can yield up to double the protein content detected in the seeds of legumes (Table 2).

Table 2. Amino acid contents of different products (in % dry matter; rounded)

SOURCE	PRODUCT	SUM AMINO ACIDS %	LYSINE %	METHIONINE %	CYSTINE %	THREONINE %	TRYPTOPHANE %	ARGININE* %	
Animal	Meat meal ^a	50.1 - 55.2	2.7 - 3.4	0.7 - 0.8	0.5 - 0.6	1.8 - 2.2	0.4 - 0.5	3.2 - 4.1	
	Fish meal ^a	51.9 - 65.0	4.3 - 5.4	1.6 - 2.0	0.5 - 0.6	2.4 - 3.0	0.6 - 0.8	3.3 - 4.2	
	Milk powder ^a	28.8 - 36.1	2.2 - 2.7	0.8 - 0.9	0.2 - 0.3	1.2 - 1.5	0.4 - 0.5	1.0 - 1.2	
	Whey powder ^a	10.9 - 23.6	0.9 - 2.0	0.2 - 0.4	0.2 - 0.5	0.7 - 1.5	0.2 - 0.4	0.3 - 0.7	
Plant	Soybean meal ^a	42.0 - 46.2	2.6	0.6	0.6	1.6 - 1.7	0.6	3.1	
	Soybeans (heat-treated) ^a	35.3	2.2	0.5	0.5	1.4	0.5	2.6	
	Lupine seed ^a	30.0 - 34.9	1.5 - 1.8	0.2 - 0.3	0.5 - 0.6	1.1 - 1.3	0.3	3.4 - 4.0	
	Horse beans ^a	23.1 - 27.1	1.6 - 1.9	0.2	0.3 - 0.4	0.9 - 1.0	0.2 - 0.3	2.3 - 2.7	
	Peas ^a	20.2	1.5	0.2	0.3	0.8	0.2	1.9	
	Grass meal (dehydrated) ^a	11.2 - 16.5	0.6 - 0.8	0.2 - 0.3	0.1 - 0.2	0.6 - 0.9	0.2 - 0.3	0.6 - 0.9	
	Potatoes (dehydrated) ^a	8.5	0.5	0.2	0.1	0.4	0.1	0.5	
	Sugarbeet pulp (dehydrated) ^a	7.0 - 7.7	0.4 - 0.5	0.1 - 0.2	0.1	0.4 - 0.5	0.1	0.3 - 0.4	
	Maize ^a	8.4	0.3	0.2	0.2	0.3	0.1	0.4	
	<i>Lemna</i> , <i>Spirodela</i> , <i>Wolffia</i> , <i>Wolffella</i> (duckweed) ^b	37.5 - 44.7 [#]	1.5 - 1.8	0.3 - 0.5	n.s.	1.1 - 1.4	n.s.	1.6 - 2.1	
	Green alga	<i>Chlorella</i> ^c	58	3.1	1.3	n.s.	2.4	0.5	3.3
	Blue-green alga	<i>Aphanizomenon flos-aquae</i> ^c	63	3.4	0.7	0.2	3.2	0.7	3.8
		<i>Spirulina platensis</i> ^{d, e, f}	58.5 - 67.0 [#]	1.6 - 2.9	0.8 - 1.2	0.2 - 0.5	1.7 - 2.4	0.5	2.6 - 3.7
<i>Spirulina maxima</i> ^{g, h, i}		59 - 71 [#]	2.3 - 3.1	0.8 - 1.7	0.2 - 0.7	2.5 - 3.5	0.9 - 1.1	3.9 - 4.5	
Fungus	<i>Agaricus</i> (Button Mushroom) ^k	17.6 - 33.2	1.2 - 2.0	0.3 - 0.4	0.3 - 0.7	0.8 - 1.1	n.s.	0.9 - 3.4	
	<i>Russula</i> ^k	13.0 - 17.8	0.9 - 1.1	0.1 - 0.4	0.1 - 1.2	0.7 - 1.0	n.s.	0.3 - 1.0	
	<i>Lentinus edodes</i> (Shi-Take) cap ⁿ	15.2	0.8	0.3	0.4	0.6	n.s.	0.9	
	<i>Paecilomyces variotii</i> (microf.) ^o	55 - 60 [#]	2.5 - 2.8	0.8 - 0.9	0.6 - 0.7	2.5 - 2.8	0.7	n.s.	
Bacterium	<i>Pseudomonas</i> sp. ^p	76 [#]	5.3	2.1	0.6	3.8	0.8	4.0	
	<i>Pseudomonas</i> (CH ₃ OH-grown) ^q	63.9	4.8	3.0 (met + cys)		3.4	n.s.	3.7	

^aCVB (2000), ^bRusoff et al. (1980), ^cKay (1991), ^dHugh et al. (1985), ^eNarasimha et al. (1982), ^fRoss and Dominy (1989), ^gClément et al. (1967), ^hFévrier and Séve (1976), ⁱWu and Pond (1981), ^kVetter (1993), ⁿVetter (1995), ^oRomatschuk (2003); ^pMauron (1975)
[#]Essential for young pigs; [#] crude protein (N x 6.25); n.s. = not specified

Table 3. Ileal/fecal digestibility of crude protein and ileal digestibility of amino acids in different products for pigs

PRODUCT	SUM AMINO ACIDS %	ILEAL/FECAL DIGEST. OF CP %	LYSINE %	METHIONINE %	CYSTINE %	THREONINE %	TRYPTOPHANE %	ARGININE* %
Meat meal ^a	50.1 - 55.2	72/83	76	75	48	71 - 72	70	84
Fish meal ^a	51.9 - 65.0	83/87	89	88	71	86	84 - 85	91
Milk powder ^a	28.8 - 36.1	88/93 - 94	95	96	84	89	88	94
Whey powder ^a	10.9 - 23.6	81 - 87/80 - 91	88 - 91	86 - 90	84 - 89	82 - 88	80 - 87	78 - 85
Soybean meal ^a	42.0 - 46.2	83 - 85/89 - 93	86 - 89	87 - 90	80 - 82	81 - 84	84 - 87	91 - 94
Soybeans (heat-treated) ^a	35.3	80/88	81	80	72	75	79	86
Lupine seed ^a	30.0 - 34.9	84/84	86 - 87	78 - 79	83 - 84	81 - 82	82	94
Horse beans ^a	23.1 - 27.1	73 - 82/79 - 83	80 - 87	61 - 82	53 - 67	71 - 78	63 - 71	87 - 93
Peas ^a	20.2	74/85	79	69	63	69	64	87
Grass meal (dehydrated) ^a	11.2 - 16.5	40 - 43/35 - 57	41 - 44	58 - 60	19 - 24	38 - 41	41 - 44	42 - 44
Potatoes (dehydrated) ^a	8.5	n.s./45	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
Sugarbeet pulp (dehydrated) ^a	7.0 - 7.7	35 - 36/45 - 47	45 - 48	50 - 52	27 - 31	14 - 17	30 - 36	42 - 45
Maize ^a	8.4	71/75	62	82	72	62	55	79
<i>Spirulina maxima</i> ^b	57.5	n.s./67-84	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
Brewers' yeast (dehydrated) ^a	40.1	n.s./82	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
Yeast (species not given) ^c	47.2	n.s./90	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
Yeast (species not given) ^d	61.8 [#]	n.s./94	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
Hydrocarbon-grown yeast ^e	62.0 [#]	n.s./81	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
TOPRINA (<i>Candida lipolytica</i> on n-paraffin; BP Ltd.) ^f	56.0	n.s./91-93	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
<i>Torula (Candida utilis)</i> ^g	49.2 [#]	n.s./81	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
<i>Paecilomyces variotii</i> ^g	58.3 [#]	n.s./83	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
Bacterial SCP ^h	73.1 [#]	n.s./81	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
<i>Pseudomonas</i> (on CH ₃ OH) ⁱ	63.9	n.s./84	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.

^aCVB (2000; pigs of 40 - 100 kg), ^bFévrier and Seve (1976; pigs of 12 - 42 days of age), ^cTegbe and Zimmerman (1977; pigs of initially 5.6 kg, 27 days of age), ^dSlagle and Zimmerman (1979; pigs of initially 5.3 - 5.7 kg, 25 - 33 days of age), ^eBarber et al. (1971; pigs of 20 - 60 kg), ^fVan Weerden (1970; cited in Schulz and Oslage, 1976), ^gSalo and Pekkarinen (1981, pigs of 35 - 50 kg), ^hZimmerman and Tegbe (1977; pigs of initially 5.1 kg, 25 days of age), ⁱSchulz and Oslage (1976)

*Essential for young pigs; CP = crude protein; [#]crude protein (N x 6.25); n.s. = not specified

Micro-organisms as bacteria and fungi, like algae and plants, are able to transform different nitrogen sources (e.g. from animal excreta) to essential and non-essential amino acids, which are the building blocks for protein synthesis. Essential amino acids are those amino acids that cannot be synthesised by humans and animals; they rely on dietary intake from micro-organisms and plants that do produce them. In contrast to algae and plants, bacteria and fungi cannot exploit light energy. They hence need an organic carbon and energy source (see 4.1.).

Mushrooms score higher than most field plants and only slightly lower than legume seeds in their protein content (Table 1). Yet, in contrast to legumes, of which only the seeds are protein-rich, with mushrooms the whole fruit body is rich in protein and they are seemingly more efficient in protein formation than legumes (Table 1). With the microfungus *Paecilomyces variotii*, a higher fungus, amino acid contents even comparable to meat or fish meal can be obtained (Table 2).

With the exception of meat, fish meal, and seeds of legumes, it seems that the lower the organism, the higher its protein content. The bacterium *Pseudomonas* shows the highest crude protein content of all organisms referred to in Table 2.

For some organisms, no specifications were found as regards their pure protein content (Table 2). In these cases, the protein content was given as crude protein. Crude protein is determined by multiplying the nitrogen content by 6.25. Fast-growing organisms as micro-organisms, however, contain high concentrations of nitrogen-rich nucleic acids, which makes the crude protein approach less accurate. According to Roth and Kirchgessner (1980), algae contain about 55 % crude protein and 50 % pure protein in dry matter, yeasts 65 % and 55 %, and bacteria 80% and 65 %, respectively.

4.3. Protein digestibility for pigs

Table 3 gives an overview of the digestibility of crude protein and different amino acids in feedstuff ingredients for pigs. For common feedstuff ingredients (CVB, 2000), most details are available. Information about amino acid or protein digestibilities of SCP for pigs, on the other hand, is very scarce (Table 3). Nevertheless, the data available suggest that the alga *Spirulina maxima*, yeasts, and bacteria have very good digestible crude protein (Table 3). Their protein digestibilities are comparable to those of animal products and legume seeds. In contrast to the aforementioned products, crude protein from grass meal, potatoes, and sugar beet pulp is poorly digestible. Only about half of their crude protein can actually be digested (Table 3).

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5. Nitrogen fixation for higher protein yields

5.1. Nitrogen-fixation

The availability of nitrogen in the soil is a limiting factor in agriculture, despite the fact that the atmosphere consists of about 80% of molecular nitrogen (N₂). The reason lies in the fact that in contrast to certain micro-organisms, plants and animals are unable to use atmospheric nitrogen. N₂ must first be “fixed” (combined with oxygen or hydrogen) into compounds as nitrate or ammonia to become an accessible nitrogen source for the synthesis of amino acids and protein in higher organisms. Some plants, as legumes, fill this gap by living in a symbiosis with nitrogen-fixing or “diazotrophic” bacteria, which support the plant with nitrogenous compounds. However, legumes are not the only higher organisms profiting from the metabolic abilities of bacteria. Some fungi also benefit from nitrogen-fixing bacteria.

5.2. Nitrogen-fixing bacteria

All nitrogen-fixing organisms are micro-organisms (BTO, 2003). Some of them live independently of other organisms - the so-called free-living nitrogen-fixing bacteria. Others live in symbiotic associations with plants or other organisms. Examples are shown in Table 4.

Table 4. Examples of nitrogen-fixing bacteria (from BTO, 2003)

FREE LIVING		SYMBIOTIC WITH PLANTS	
AEROBIC	ANAEROBIC	LEGUMES	OTHER PLANTS
<i>Azotobacter</i> <i>Beijerinckia</i> <i>Klebsiella</i> (some) Cyanobacteria (some)*	<i>Clostridium</i> (some) <i>Desulfovibrio</i> Purple sulphur bacteria* Purple non-sulphur bacteria* Green sulphur bacteria*	<i>Rhizobium</i>	<i>Frankia</i> <i>Azospirillum</i>

*denotes a photosynthetic bacterium

5.3. Consortia of nitrogen-fixing bacteria and fungi

Although there are several authors claiming that certain fungi are able to fix atmospheric nitrogen by themselves (Millbank 1969, 1970; Ginterová, 1973; Kvasnikov et al., 1974; Ginterová and Maxianová, 1975; Rangaswami et al., 1975; Dijkstra, 1976; Thapar and Pokriyal, 1993), an absolute proof, as for example the detection of the nif gene, is still missing. Some yeasts evidently cannot fix nitrogen from air (Millbank, 1969; Bab'eva et al., 1977).

Fungi, like legumes, can however live in cross-feed symbioses with nitrogen-fixing bacteria (Bab'eva et al., 1977; Cojho et al., 1993; Halsall, 1993; Hurek et al., 1997; Bianciotto et al., 2000; Minerdi et al., 2001, 2002). Some bacteria are endosymbiotic, for example to mycorrhizal fungi. Mycorrhizae are units of fungi and nitrogen-fixing bacteria attached to the roots of plants. Plant and fungus benefit from the nitrogen made available by bacteria, fungus and bacteria get assimilation products from CO₂ by the plant, and the fungus stimulates an increased plant root production and thus allows for more water and nutrient uptake. In such a system, nitrogen-

fixing bacteria can adhere quite close to fungi, as was shown by Dörr et al. (1998). Other fungus-bacteria communities consist of separate organisms, which nevertheless support each other. Bab'eva et al. (1977), Hurek et al. (1997), Cojho et al. (1993), and Halsall (1993) gave examples of consortia of free-living nitrogen-fixing bacteria with different classes of fungi. Examples are shown in Table 5.

Table 5. Cross-feeding consortia of nitrogen-fixing bacteria and fungi

FUNGUS	BACTERIUM	AUTHORS
<i>Cyathus stercoreus</i> (basidiomycete)	<i>Beijerinckia indica</i> B15	Hasall (1993)
<i>Ustilagomyces</i> -related fungus (basidiomycete)	<i>Azoarcus</i> spp.	Hurek et al. (1997)
<i>Fusarium oxysporum</i> (ascomycete)	<i>Azospirillum</i> sp. DN64	Hasall (1993)
<i>Gigaspora margarita</i> (zygomycete; arbuscular mycorrhizal fungus)	<i>Burkholderia</i> sp. (endosymbiont)	Bianciotto et al. (2000), Minerdi et al. (2001, 2002)
<i>Lipomyces kononenkoae</i> (yeast)	<i>Acetobacter diazotrophicus</i>	Cojho et al. (1993)
<i>Lipomyces lipofer</i> (yeast)	<i>Pseudomonas</i> sp. 5	Bab'eva et al. (1977)

The benefit in a consortium of a diazotrophic bacterium with a fungus is mutual. Many fungi can degrade resistant compounds as cellulose and lignin, but are unable to access N₂. Hence, they evidently exchange soluble carbon compounds against nitrogen compounds synthesised by the bacterium.

5.4. References

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6. Fungi

The name “fungi” is derived from their most obvious representatives, the mushrooms (Latin, *fungus*). Fungi are eukaryotes, i.e. in contrast to bacteria, but like animals and plants, their genetic information is situated in a cell core. Fungi share many features with plants. However, they obtain their energy not from light as plants do, but by oxidation of organic compounds.

The main groups of fungi are the higher fungi (mushrooms, yeasts), the lower fungi (many aquatic fungi, parasitic fungi, and fungi growing on rotting materials), and the true slime moulds. For SCP production, higher fungi are the most promising group. Basidiomycetes (e.g. Button Mushroom), together with Ascomycetes (e.g. truffle), form the higher fungi. The Basidiomycetes are the most highly developed group of fungi. Yeasts, or budding fungi, belong to the Protoascomycetes among the Ascomycetes.

6.1. Cultivation of fungi on agricultural and industrial (by-)products

6.1.1. Basidiomycetes and Ascomycetes

The most abundant and renewable agricultural substrates for the production of SCP seem to be lignocellulosic wastes (Israelidis en Coduonis, 1982). Lignocellulosic wastes are mainly used for mushroom production. Not only straw, but many by-products from agricultural production and food processing can be used as growing media in mushroom production. The materials remaining after harvest can be composted and applied directly to the soil as an organic ameliorant (Beetz and Greer, 2002). Peter Oei (1991) describes in some detail alternative mushroom production systems successfully used in developing countries. Many ideas for low-input systems are included in his book.

Mushrooms are cultured on solid media. However, some higher fungi can also be grown submerged in a bioreactor. In contrast to the highly organised fruiting bodies of some fungi that are known as mushrooms, the reproductive structures of other fungi are too tiny for harvest and consumption. Submerge culturing allows to harvest the vegetative body every fungus possesses, the mycelium. Table 6 gives an overview of possible growth substrates for higher fungi.

6.1.2. Protoascomycetes (yeasts)

Yeast are unicellular fungi. The most well-known and commercially significant yeasts are species and strains related to *Saccharomyces cerevisiae*. *Saccharomyces cerevisiae* is commonly used as baker's yeast. The yeast's function in baking is to ferment sugars present in the flour or added to the dough. This fermentation results in the formation of carbon dioxide and ethanol. The carbon dioxide is trapped within tiny bubbles and results in the dough rising; ethanol evaporates during baking. Apart from baking, yeasts are used to ferment the sugars of rice, wheat, barley, and corn to produce alcoholic beverages.

Due to their high protein contents, yeasts also play a prominent role in SCP production. Table 7 gives an overview of possible growth substrates for SCP production from yeasts.

Table 6. Examples of growth media for Basidiomycetes and Ascomycetes

GROWTH MEDIUM	MUSHROOM SPECIES	AUTHORS
Bean straw	Oyster (<i>Pleurotus</i>)	Beetz and Greer (2002)
Wheat straw	Oyster (<i>Pleurotus</i>), Common (<i>Agaricus</i>), <i>Stropharia</i> , Straw (<i>Volvariella</i>), <i>Chaetomium cellulolyticum</i> , <i>Penicillium funiculosum</i> , <i>Trichoderma viride</i>	Moo-Young et al. (1981), Oei (1991), Somani et al. (1991), Beetz and Greer (2002),
Sawdust	Shiitake (<i>Lentinus</i>), Oyster (<i>Pleurotus</i>), <i>Hericium</i> , Ear (<i>Auricularis</i>), Reishi (<i>Ganoderma</i>), Winter (<i>Flammulina</i>) <i>Penicillium funiculosum</i> , <i>Trichoderma viride</i>	Somani et al. (1991), Beetz and Greer (2002)
Sawdust-straw	Oyster (<i>Pleurotus</i>), <i>Stropharia</i> ,	Beetz and Greer (2002)
Lignocellulosic materials (bark)	<i>Chrysonilia sitophila</i>	Durán et al. (1994); Rodriguez et al. (1997)
Logs	Nameko (<i>Pholiota</i>), Shiitake (<i>Lentinus</i>), White jelly (<i>Tremella</i>),	Beetz and Greer (2002)
Paper	Oyster (<i>Pleurotus</i>), <i>Stropharia</i> ,	Beetz and Greer (2002)
Horse manure (fresh or composted)	Common (<i>Agaricus</i>)	Oei (1991), Beetz and Greer (2002)
Corn cobs	Oyster (<i>Pleurotus</i>), <i>Hericium</i> , Shiitake (<i>Lentinus</i>), <i>Aspergillus</i> sp.	Oei (1991), Nazarenko et al. (1993), Beetz and Greer (2002)
Lignocellulosic plant residues from <i>Brassica napus</i>	<i>Pleurotus ostreatus</i>	Sarikaya and Ladisch (1999)
Crushed bagasse and molasses wastes from sugar industry	Oyster (<i>Pleurotus</i>)	Oei (1991), Beetz and Greer (2002)
Sugar-cane vinasse	<i>Aspergillus niger</i> and <i>Cryptococcus laurentii</i>	Ceccato and Tauk (1994)
Distillers grain waste	<i>Hericium</i>	Oei (1991), Beetz and Greer (2002)
Sulphite pulp waste liquor	<i>Paecilomyces variotii</i>	Romantschuk (2003)

Table 7. Examples of growth media for yeasts

GROWTH MEDIUM	YEAST SPECIES	AUTHORS
Wheat straw, saw dust (hydrolysate)	<i>Candida utilis</i>	Somani et al. (1991)
Wood hydrolysate (treated with cellulases, β -glucosidase)	<i>Candida utilis</i> NRRL Y-900	Parajo et al. (1995)
Apple pomace	<i>Kloeckera apiculata</i> , <i>Candida utilis</i>	Bhalla and Joshi (1994); Rahmat et al. (1995)
Leaf juices from turnip (<i>Brassica campestris</i> L.), mustard (<i>Brassica nigra</i> Koch.), radish (<i>Raphanus sativus</i> L.), cauliflower (<i>Brassica oleracea</i> L. var. <i>botrytis</i>)	<i>Saccharomyces cerevisiae</i> , <i>Candida utilis</i> , <i>Candida lipolytica</i>	Chanda and Chakrabarti (1996)
Molasses-enriched vinasse	<i>Candida utilis</i>	Cibis et al. (1992)
Sugar-cane vinasse	<i>Cryptococcus laurentii</i> (co-culture with <i>Aspergillus niger</i>), <i>Rhodotorula gracilis</i>	Jakob (1991), Ceccato and Tauk (1994)
Sugar beet stillage from alcohol factory	<i>Hansenula</i>	Shojaosadati et al. (1999)
Wastewater from grains washing	<i>Candida tropicalis</i>	Zhang and Wang (2000)
Wastewater from poultry processing plant (rich in starch and fat from breasting and frying)	<i>Saccharomycopsis fibuligera</i> (untreated starch), <i>Saccharomyces cervisiae</i> (hydrolysate)	Najafpour et al. (1994)
Whey	<i>Kluyveromyces</i> sp., <i>Candida intermedia</i> , <i>Candida utilis</i> , <i>Candida krusei</i>	Tusé (1984); Moresi (1994); Ghaly and Ben-Hassan (1995); Kar and Misra (1998a,b)
Chicken waste	Yeast	Abo (1993a,b)

6.2. Yeasts in diets for weanling pigs

Summary: All publications found about fungi in diets for weanling pigs refer to yeasts. Ikurior (1995) and Miyada et al. (1997) found that the performance of weanling pigs was unimpeded by about 30 - 60 % of total crude protein from dietary yeast. Yeast even improved feed utilisation (Ikurior, 1995; Miyada et al., 1997). Landell et al. (1994) observed a negative effect of centrifuged vinasse yeast on the performance of weanling pigs. The reason for this result is not clear, maybe the yeast product contained too much potassium.

Slagle and Zimmerman (1979) performed feeding experiments with an unspecified "yeast SCP product". 18 % crude protein from either SCP or dehulled soybean meal were fed to pigs that weighed 5.6 kg at the beginning of the experiments. Both diets were supplemented with methionine. Apparent dry matter and N digestibility were

higher for the SCP than for the soybean meal diet. In another experiment, SCP replaced up to the entire soybean meal in the diet of weanling pigs. SCP levels did not affect average daily gain, average daily feed intake, and gain/feed ratio.

Landell et al. (1994) studied the effect of centrifuged vinasse yeast (*Saccharomyces cerevisiae*) from sugar-cane alcohol production in the diet of Landrace piglets. The pigs had an average initial weight of 11.3 kg. Up to 20 % of dried vinasse yeast (36 % of total crude protein) was included in isoproteic corn/soybean diets. An increasing level of centrifuged vinasse yeast in the diet resulted in a linear diminution of pig performance.

Ikurior (1995) replaced up to 60 % of total crude protein in a soybean meal/maize diet of crossbred weanling pigs with brewers yeast slurry (*Saccharomyces carlsbergensis*). The pigs were Large White and Landrace breeds, 8 weeks of age, and averaging 11.8 kg liveweight. There were no significant differences in feed intake, weight gain or feed/gain ratio of the pigs. Brewers yeast slurry reduced feed cost per kilogram liveweight significantly and improved feed utilisation, thus also improving pig production efficiency.

Miyada et al. (1997) incorporated up to 20 % dried yeast (30 % of total crude protein) into a pelleted starter diet for crossbred weanling pigs (10.3 kg initial body weight). Five different isonitrogenous and isocaloric diets were supplied *ad libitum* during a 28-day experimental period. An increased level of dried yeast in the diet did not affect average weight gain, but depressed daily feed intake and improved feed/gain ratio. Moreover, an increase of hematocrit, hemoglobin, and uric acid was registered with yeast in the diet. Plasma uric acid increased from 0.01 mg/dl with 0 % yeast to 0.31 mg/dl with 20 % yeast. Urea, albumin, cholesterol, and the albumin/globulin ratio were reduced. Plasma contents of total protein, globulin and triglycerides were not affected.

6.3. References

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7. Algae

Aphanizomenon flos-aquae (blue-green alga), *Spirulina* (blue-green alga), and *Chlorella* (green alga) are the most prominent protein-rich algae, which are commercially produced on large scales.

Blue-green algae (also called cyanobacteria) are micro-organisms, because of their simple cellular structure. Some *Aphanizomenon* and *Spirulina* are toxic (Gentile and Maloney, 1969; Alam et al., 1973; Pereira et al., 2000). However, the strains cultured for consumption do not contain toxins. While *Spirulina* is not able to fix nitrogen, *Aphanizomenon flos-aquae* possesses this ability. Apart from their high protein contents, health promoting qualities are attributed to blue-green algae, such as the supply of unsaturated fatty acids (Nichols and Wood, 1968; Pascaud, 1993), stimulation of the immune system (Pascaud, 1993), and protection from cancer (Schwartz and Shklar, 1987; Schwartz et al., 1988; Qishen et al., 1989).

Chlorella consists of small spherical cells with a cell nucleus, and appeared a billion years after blue-green algae. *Chlorella* is hence no micro-organism, but a higher organism. Intake of *Chlorella* extracts is associated with enhanced immune responses and antitumor effects (Tanaka et al., 1984, 1986; Konishi et al., 1985; Hasegawa et al., 1997; publications of Research Laboratories, Chlorella Industry Co., Japan).

7.1. Cultivation of algae

7.1.1. Aphanizomenon

Aphanizomenon flos - aquae grows naturally in large amounts in the Upper Klamath Lake. The lake is situated within the Cascade Mountains in the centre of Oregon. The *Aphanizomenon flos - aquae* product harvested from the lake is sold as Klamath Lake Algae™ by Desert Lake Technologies, LLC. (DLT). Since 1984, Rossha Enterprises harvested, processed, and marketed *Aphanizomenon flos-aquae* from Klamath Lake. In March, 2000, DLT, a leading harvester and worldwide distributor of zooplankton to the aquaculture industry, acquired Rossha Enterprises.

7.1.2. Spirulina

Jourdan (2003) gives detailed information about how to culture *Spirulina*. The following is an excerpt of his recommendations:

Growth of *Spirulina* only takes place in light (photosynthesis). Although light is an important factor, full sunlight may not be the best rate of illumination: 30% of full sun light is actually better, except that more may be required to quickly heat up the culture in the morning. Another important factor, which influences the rate of growth of *Spirulina*, is temperature. Below 20°C, growth is practically nil, but *Spirulina* does not die. The optimum temperature for growth is 35°C, above 38°C *Spirulina* is in danger. *Spirulina* thrives in alkaline, brackish water. Any watertight, open container can be used to grow *Spirulina*, provided it will resist corrosion and be non-toxic. Its dimensions are only limited by the necessity of accessing for agitation and cleaning. A greenhouse over the ponds offers many advantages. *Spirulina* can live in a wide range of compositions of water; Table 8 shows a convenient analysis. In addition, the solution contains traces of all micronutrients necessary to support plant life. Such a solution can be obtained by dissolving various combinations of chemicals (Table 9).

Table 8. Example of suitable water composition for *Spirulina*

FACTOR	CONCENTRATION
Carbonate	2800 mg/l
Bicarbonate	720 mg/l
Nitrate	614 mg/l
Phosphate	80 mg/l
Sulphate	350 mg/l
Chloride	3030 mg/l
Sodium	4380 mg/l
Potassium	642 mg/l
Magnesium	10 mg/l
Calcium	10 mg/l
Iron	0.8 mg/l
Urea	< 50 mg/l
Total dissolved solids	12.847 g/l
Density at 20°C	1010 g/l
Alkalinity	0.105 N (moles strong base/litre)

Table 9. Defined growth medium for *Spirulina*

COMPOUND	g/l
Sodium carbonate (soda ash)	5
Sodium chloride, crude	5
Potassium nitrate	2
Sodium bicarbonate	1
Potassium sulphate, crystallised	1
Urea	0.02
Monoammonium phosphate, crystallised	0.1
Magnesium sulphate, crystallised, (MgSO ₄ x 7 H ₂ O)	0.2
Lime	0.02
Ferrous sulphate, crystallised (FeSO ₄ x 7 H ₂ O)	0.005

The water used should be clean or filtered to avoid foreign algae. The culture medium described above (Table 9) is used to start new cultures. In case of necessity ("survival" type situations), nitrogen, phosphate, sulphate, sodium, potassium and magnesium can all be brought by urine (from persons or animals in good health, not consuming drugs) at 5 ml/l and iron by a saturated solution of iron in vinegar (use about 0.1 ml/l) (Jourdan, 2003).

Wu and Pond (1981) cultured *Spirulina maxima* on swine, cattle, and poultry faeces and sewage sludge. Each material was first lyophilised, then tenfold diluted and anaerobically fermented for 14 days at 25°C. After that, the supernatant liquor was filtered through cotton cloth and paper for use as algae nutrient source. The resulting liquor was continuously pumped into the culture tanks to final concentrations of 3 - 7 % (w/v). There were no important differences among the amino acid compositions of algae grown on different nutrient sources. However, yeast, fungi, and spore-forming bacteria were present in significant numbers in algae grown on all fermented wastes. In the algae cultivated in synthetic medium, the accompanying microflora remained at low levels. The authors concluded that many bacteria might have survived lyophilisation and anaerobic fermentation. An effective hygienisation of the sludge together with a controlled high pH might restrict microbiological contamination. Hygienic problems arising from the use of animal excreta for growth of *Spirulina* are not solved yet, and there is no generally approved technology for biomass production using faeces, urine, or manure as sources of nitrogen and phosphorus. Olguín et al. (1994) showed that *Spirulina* removed 99 % of total phosphates and all ammonia-N from anaerobically treated pig slurry when at the same time a maximum of protein (71 %, w/w) was accumulated. Such a system would not only allow low-cost protein production, but could simultaneously control pollution. However, up to now, the only generally accepted application for wastewater and *Spirulina* is in wastewater purification (Canizares et al., 1993).

7.1.3. *Chlorella*

Chlorella is found worldwide in all aquatic environments. As their natural habitats differ, the optimum temperatures of *Chlorella* strains differ. *Chlorella* has a high growth rate ($12 - 35 \text{ g day}^{-1} \text{ m}^{-2}$) in batch, continuous and open pond culture (Shubert, 1988). Mahasneh (1997) showed that the performance of *Chlorella* strains appears to be related to the environment they are isolated from. Biomass and SCP yields can hence be manipulated by adapting the culture conditions.

According to Henrikson (2003), a propagator of *Spirulina*, three drawbacks limit the potential of *Chlorella*. First, *Chlorella* culture is easily contaminated by undesirable weed algae. Unlike *Spirulina*, which flourishes in highly alkaline water unfriendly to other algae, *Chlorella* grows in normal water conditions where many algae grow. *Chlorella* is grown in individual batches, started in a sterile test tube, moved to indoor tanks and then outside to larger ponds. When it achieves maximum density, the entire batch is harvested. Controls required for batch cultivation can be difficult for small farms. Second, unlike *Spirulina*, tiny *Chlorella* cells cannot be harvested by a screen. Centrifuges are required to separate the cells from pond water. Third, *Chlorella*'s hard cellulose cell wall protects the cell, but resists digestion by the human body, and nutrients cannot be fully absorbed. Commercial farms crack open this hard cell wall in the drying process, or mechanically crush it.

Nature's Balance (2003), a promoter of *Chlorella*, emphasises the advantages of *Chlorella* compared to *Aphanizomenon* and *Spirulina*. *Chlorella*, which is at least 100 times smaller than either *Aphanizomenon* or *Spirulina*, is harvested by centrifugation and filtration. In this way, a very pure and clean product is obtained.

According to Nature's Balance (2003), the cellulose cell-wall structure of *Chlorella* has been shown to be valuable in adsorbing and expelling environmental toxins from the gastrointestinal tract.

Chlorella is currently commercially produced and processed mainly in Japan (e.g. by Chlorella Industry Co., Ltd., and Chlorella Center Co., Ltd.) for application as a nutritional supplement for humans.

7.2. Algae in diets for weanling pigs

Summary: Février and Sève (1976), Yap et al. (1982), and Hugh et al. (1985) found that the performance of weanling pigs was unimpeded by 25 - 33 % of total crude protein replaced by algal protein in the diet. Février and Sève (1976) even reported an improvement of metabolic feed utilisation with algae in the feed.

Février and Sève (1976) incorporated dehydrated *Spirulina* (*Spirulina maxima*) in the diets of 12-day-old weaned pigs. From 12 to 21 days of age, *Spirulina* was incorporated at a level of 12 % (25 % of total crude protein) in the ration, replacing dried skim milk. From 21 to 42 days of age, *Spirulina* was fed at a level of 8 % of the diet, replacing soybean meal. Although there was some reduction in digestibility of the diet when *Spirulina* was incorporated, growth was satisfactory and equivalent in all groups. Février and Sève (1976) concluded that the metabolic utilisation of the fraction of feed absorbed was better for the *Spirulina* group than for the control group, notably during the period between 12 and 21 days, although the supply of lysine in the *Spirulina* group was 12 % lower.

Yap et al. (1982) replaced one-half of soybean meal (33 % of total dietary protein) in a corn-soybean meal/dried skim milk starter diet with algal proteins (*Spirulina maxima*, *Spirulina platensis*, and *Chlorella* sp.). The trial was performed with Yorkshire pigs weaned to a dry diet at 4 to 8 days of age. There was no significant difference between control and algal diets during the 15- and 26-day trial periods in growth, diarrhoea, loss of appetite, or toxicity. The researchers concluded that at least one-half of the protein supplied by soybean meal (one-third of the dietary protein) could be replaced by algal protein without adverse effects.

Hugh et al. (1985) included up to 9 % dehydrated *Spirulina* (*Spirulina platensis*) as protein replacement in swine starter diets, which comprised up to 32 % of the total dietary crude protein. Satisfactory live animal performance was obtained with the 3- to 4-week-old crossbred weanling pigs. No apparent toxicity problems arose with any *Spirulina* level used.

Grinstead et al. (2000) also performed feeding experiments with dehydrated *Spirulina platensis* and weanling pigs (PIC, L326 X C22; initially 3.7 ± 0.85 kg and 11 - 12 days of age). From days 0 to 14 after weaning, pigs were fed a control diet or pelleted diets containing 0.2, 0.5, or 2 % *Spirulina platensis* replacing soybean meal on an equal lysine basis. With 2 % *Spirulina platensis*, only 3.2 to 3.4 % of total dietary lysine was replaced. All diets contained a medication (carbodox) and zinc oxide for growth promotion. No differences in pig performance, measured as average daily feed intake and gain, were observed during this interval. In contrast to pelleted diets, meal diets resulted in inconsistent responses to *Spirulina platensis*.

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8. Bacteria

Bacteria are very diverse in their physiological characteristics. Some are pathogenic, others can be applied as probiotics. Apart from an efficient fixation and transformation of phosphorus and nitrogen, bacteria (and some fungi) can excrete siderophores to make iron available, when the supply of iron becomes growth limiting. In the presence of oxygen, iron forms ferric hydroxide, which is practically insoluble. This might be of interest in organic farming, where elements have to be made available in a natural way.

8.1. Cultivation of bacteria

For cultivation of bacteria, specialised knowledge is necessary. Bacteria are cultured submersely in bioreactors under controlled conditions as regards composition of the growth medium, temperature, and gas atmosphere. To prevent contamination, growth conditions that exclude unwanted organisms or sterile working conditions are obligatory. Bacteria hence seem unsuitable for low-input systems. An exception might be fermentations to improve product quality. Lactic acid fermentations as performed in ensiling or for preservation of vegetables can increase the protein content of the raw materials (Sabaeny, 1996; Wu et al., 1999). Bacteria that are used for SCP production are i.a. hydrocarbon-assimilating strains (Tusé, 1984), methane and methanol utilising bacteria (Bewersdorff and Dostalek, 1971; PRUTEEN), lactic acid bacteria (Sabaeny, 1996), and propionibacteria (Skupin et al., 1978).

8.2. Bacteria in diets of weanling pigs

Summary: Whittemore and Hinks (1976) and Newport and Keal (1980) performed feeding experiments with PRUTEEN, the methanol-grown bacterium *Methylophilus methylotrophus* produced by ICI, which was commercially available in the 1970s and 80s. A replacement of up to 40 % of total crude protein in the diet by PRUTEEN SCP did not impair the performance of weanling pigs. In a totally different approach, Wu et al. (1999) improved the protein content and quality of soybean meal by fermentation. In this case, the growth substrate for SCP is not a waste product, but a high-value feedstuff.

Whittemore and Hinks (1976) fed diets containing 20 % dried cells of *Methylomonas methylotropha* (PRUTEEN, ICI) to weaned piglets from 21 to 42 days of age. They registered higher live-weight gains and efficiencies of feed use for pigs pair-fed the SCP diet in comparison to a fish meal diet. In an *ad libitum* feeding trial, differences were not so clear-cut; the SCP group showed only slightly higher live-weight gains. The composition of the gain was similar for pigs fed SCP or fish meal at 21 days and 42 days of age.

Zimmerman and Tegbe (1977) evaluated “an experimental bacterial SCP made by batch process” for young pigs. Unfortunately, they did not mention the organism used and if it was a pure culture or not. They used isonitrogenous corn/soybean meal/dried whey-based diets with 12, 18, or 20 % crude protein in separate experiments. Either half or total crude protein was replaced by SCP in the starter diets. The diets were individually or *ad libitum* supplied to crossbred pigs of 5.1 – 5.4 kg and about 25 days of age for 21 - 27 days. The 20 % “all SCP” diet was somewhat refused. When reducing the SCP content of the diet, still less feed was consumed, less weight was gained, and feed efficiency was reduced compared to the control diet.

Newport and Keal (1980) performed feeding experiments with pigs weaned at 2 days of age and PRUTEEN (ICI). PRUTEEN was prepared by harvesting and spray-drying to a finely ground powder the cells of the methanol-

grown bacterium *Methylophilus methylotrophum*. Up to 80 % of total crude protein from SCP was included in liquid skim-milk/whey/soybean oil diets. Pigs were fed the diets at hourly intervals on a scale based on live-weight for 28 days (“artificial rearing”). Performance of pigs fed the diet in which 40 % of total protein was replaced by PRUTEEN was as good as with the all-milk diet used as control. Greater levels of milk protein replacements by SCP reduced performance. According to Newport and Keal (1980), insufficient enzyme adaptation and a decrease of transit time may have accounted for the poorer performance of the pigs with SCP levels above 40 % in the diet. Analyses of digesta performed after 28 days indicated that SCP stimulated secretion of pepsin and chymotrypsin, and reduced the pH value in digesta in the stomach.

Wu et al. (1999) conducted experiments with fermented soybean meal. Crude protein, lysine, and methionine contents increased by fermentation. When fermented soybean meal was used as a replacement for concentrated soy protein or both concentrated soy protein and soybean meal in the pre-starter diet, no significant differences in average daily feed intake, average daily gain or feed conversion were observed from 0 to 14 and 0 to 28 days post-weaning. However, average daily gain and feed conversion increased slightly when fermented soybean meal was used as a replacement for soybean meal. Fermented soybean meal decreased anti-soya antibody titres and improved digestive utilisation and growth performance better than did soybean meal in weaned pigs.

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9. Feed and food safety

9.1. Feed safety and protein accessibility

Safety studies for the consumption of SCP by animals were mainly performed for existing SCP technologies:

Algae

Independent feeding tests in France, Mexico and Japan showed no undesirable results and no toxic side effects of *Spirulina* on humans, rats, pigs, chickens, fish and oysters (Henrikson, 2003b). The performance of weanling pigs was unimpeded by 25 - 33 % of total crude protein replaced by algal protein in the diet (Février and Sève, 1976; Yap et al., 1982; Hugh et al., 1985).

Yeast

Pigs fed *Candida* G or L SCP (grown on alkanes, process developed by BP) at weanling age as well as at breeding and lactating stages showed no ill effects. Growth results showed no differences between 15 % yeast rations and standard diets of fish meal, skim milk, whey, and barley (Tusé, 1984). Ikurior (1995) and Miyada et al. (1997) found that the performance of weanling pigs was unimpeded by about 30 - 60 % of total crude protein from dietary yeast.

Paecilomyces variotii

Feeding trials at the Finnish Agricultural Research Centre with various animals, mainly pigs, calves and chickens proved that PEKILÖ protein (*Paecilomyces variotii*) could be used as a partial substitute for soybean meal, fish meal or skim milk powder (Romantschuk, 2003). In studies where PEKILÖ replaced up to 60 % of total protein in the diet of pigs, there was no evidence of damages caused by lignosulfonic acids, which *Paecilomyces variotii* may pick up from the substrate sulphite liquor waste (Tusé, 1984).

There are four main factors that might impair the usefulness of SCP for consumption by animals:

- a. presence of toxins;
- b. presence of pathogenic organisms;
- c. high nucleic acid content;
- d. non-digestible cell wall (mainly higher algae and yeasts).

9.1.1. Toxins

Algal toxins are capable of causing widespread poisoning of animals and humans. Most kinds of the blue-green algae *Microcystis*, *Anabaena*, *Aphanizomenon*, and *Spirulina* are toxic (Carmichael, 1992). However, for SCP production, selected non-toxic species are used. It must nevertheless be made sure that no other cyanotoxin-containing algae are inadvertently harvested. This is especially a risk when harvesting algae from natural bodies of water with mixed cultures of microscopic algae.

In 1995-96, a group of leading microalgae producers sponsored research conducted by algal toxicologists (Henrikson, 2003b). The result was a Technical Booklet for the Microalgae Biomass Industry as a guide to the use of a very sensitive enzyme linked immunosorbant assay (ELISA) and a protein phosphate inhibition assay (PPIA) for the detection of toxic microcystins and nodularins. These methods can detect, monitor and control cyanotoxins, so producers can assure a safe, nutritious product for human and animal food supplements (An and Carmichael, 1996).

Some fungi also produce potent toxins that are dangerous when eaten. Fungal toxins are called mycotoxins. An example of a mycotoxin is aflatoxin, which is synthesised by *Aspergillus flavus* and *Aspergillus parasiticus*. For production of SCP, it must hence be made sure that the organism employed is non-toxic.

9.1.2. Pathogenic contaminants

If the SCP technology employed is non-aseptic, there is a risk that pathogenic contaminants can enter the production line. Especially animal excreta, as a promising source of nutrients for the production of SCP (Moo-Young et al., 1981; Wu and Pond, 1981; Abo, 1993), are a possible source of pathogens (Wu and Pond, 1981; Bartak et al., 1977).

9.1.3. Nucleic acids

Fast-growing organisms as micro-organisms contain high concentrations of nucleic acids. Table 10 gives an overview of the nucleic acid and protein contents in micro-organisms. High nucleic acid contents in food results in high uric acid levels in the blood of humans, which can cause gout (Waslien et al., 1970). While humans as well as New World monkeys lost the ability to degrade uric acid, pigs possess the enzyme urate oxidase, which allows them to transform uric acid into allantoin (Wu et al., 1989).

Table 10. Nucleic acid and protein contents in micro-organisms (% dry matter; from Roth and Kirchgessner, 1980)

	CRUDE PROTEIN	NUCLEIC ACIDS IN % CRUDE PROTEIN	PURE PROTEIN
Algae	~ 55	6 - 13	~ 50
Yeasts	~ 65	13 - 20	~ 55
Bacteria	~ 80	15 - 25	~ 65

Pigs digest about 95 % of the nucleic acid nitrogen, but only less than one third of it is retained in the body, the rest is excreted in the urine (Roth and Kirchgessner, 1977, 1980). Miyada et al. (1997) found that the plasma uric acid level in weanling pigs (10 - 28 kg) increased from 0.01 mg/dl with 0 % yeast to 0.31 mg/dl with 20 % yeast in the diet. The plasma urea level, on the other hand, decreased from 30 mg/dl to 22.6 mg/dl (Miyada et al., 1997). Roth and Kirchgessner (1980) calculated for the urine of pigs (10 - 12 kg) that per g ingested nucleic acid-N 210 mg allantoin-N and 420 mg urea-N + NH₃-N, but only 9 mg uric acid-N was formed. They hence concluded that the possible impact of uric acid from SCP on young pigs is negligible.

9.1.4. Cell walls

The presence of whole eukaryotic cells decreases the availability and digestibility of SCP (Giec and Skupin, 1988). This is mainly due to protection of the cells by a cell wall. The digestion of yeast cell wall polysaccharides in the small intestine of calves is very low (Gaillard and Weerden, 1976). However, growing pigs proved to digest the carbohydrates of PEKIL0 and torula rather well – with the assistance of the bacterial flora of their large intestine (Salo and Pekkarinen, 1981). Like yeasts, *Chlorella* also possesses a hard cell wall, which protects the cell, but resists digestion by the human body, and nutrients cannot be fully absorbed. According to Henrikson (2003a),

commercial farms crack open this hard cellulose cell wall in the drying process, or mechanically crush it. In this way, SCP is made accessible to organism, which cannot handle the cell walls themselves. Giec and Skupin (1988) present an overview of different methods of cell wall destruction.

9.2. Food Safety

To ensure food safety in pig production, the European Communities have banned Antibiotic Growth Promoters, *Salmonella* surveillance and control systems have been established, and the use of veterinary medicines is being controlled. The risk of SCP in animal feed consists in the transfer of possible harmful substances via the food chain.

To guarantee feed and food safety, the European Communities brought out regulations concerning products used in animal nutrition. The European Communities Protein Feedingstuffs (Amendment) Regulations, 1996, give an annex of allowed products. Prerequisites for being added to the annex are that products (i) have nutritional value, (ii) have no detrimental effect on human and animal health or the environment, and (iii) can be monitored. Table 11 shows a list of those protein feedstuffs from micro-organisms which are allowed by the European Communities Regulations. Quite strikingly, there are no specifications in the category “algae”; “mushrooms” are not even mentioned in the list.

Table 11. Excerpt from the European Communities (Protein Feedingstuffs) (Amendment) Regulations, 1996, giving effect to Commission Directive 95/33/EC of 10 July 1995 and to Council Directive 82/471/EEC of 30 June, 1982.

1	2	3	4	5	6	7
NAME OF PRODUCT GROUP	NAME OF PRODUCT	DESIGNATION OF NUTRITIVE PRINCIPLE OR IDENTITY OF MICRO-ORGANISM	CULTURE SUBSTRATE (SPECIFICATIONS IF ANY)	COMPOSITION CHARACTERISTICS OF PRODUCT ¹	ANIMAL SPECIES	SPECIAL PROVISIONS ¹
1. PROTEINS OBTAINED FROM THE FOLLOWING GROUPS OF MICRO-ORGANISMS.						
1.1 BACTERIA						
1.1.1 Bacteria cultivated on methanol	1.1.1.1 Protein product of fermentation obtained by culture of <i>Methylophilus methylotrophus</i> on methanol	<i>Methylophilus methylotrophus</i> NCIB strain 10.515	Methanol	Crude protein: minimum 68% Reflectance index: at least 50	Pigs Calves Poultry Fish	Declarations to be made on the label or packaging of the product: - name of the product; - crude protein; - crude ash; - crude fat; - moisture content; - instructions for use: - declaration of the words "avoid inhalation". Declarations to be made on the label or packaging of compound feedingstuffs: - amount of the product contained in the feedingstuff.

1	2	3	4	5	6	7
NAME OF PRODUCT GROUP	NAME OF PRODUCT	DESIGNATION OF NUTRITIVE PRINCIPLE OR IDENTITY OF MICRO-ORGANISM	CULTURE SUBSTRATE (SPECIFICATIONS IF ANY)	COMPOSITION CHARACTERISTICS OF PRODUCT ¹	ANIMAL SPECIES	SPECIAL PROVISIONS ¹
1.1.2 Bacteria cultivated on natural gas	1.1.2.1 Protein product of fermentation from natural gas obtained by culture of: <i>Methylococcus capsulatus</i> (Bath), <i>Alcaligenes acidovorans</i> , <i>Bacillus brevis</i> and <i>Bacillus firmis</i> , and the cells of which have been killed	<i>Methylococcus capsulatus</i> (Bath) NCIMB strain 11132 <i>Alcaligenes acidovorans</i> NCIMB strain 12387 <i>Bacillus brevis</i> NCIMB strain 13288 <i>Bacillus firmis</i> NCIMB strain 13280	Natural gas: (approx. 91% methane 5% ethane 2% propane 0.5% isobutane, 1% other components), ammonia, mineral salts	Crude protein: 65 %	- Pigs for fattening from 25 to 60kg - Calves from 80kg on - Salmon	Declarations to be made on the label or the packaging of the product: - the name "Protein product of fermentation from natural gas obtained by culture of <i>Methylococcus capsulatus</i> (Bath), <i>Alcaligenes acidovorans</i> , <i>Bacillus brevis</i> and <i>Bacillus firmis</i> ," - crude protein - crude ash - crude fat - moisture content - instructions for use - maximum incorporation rate in the feed: - 8% pigs for fattening - 8% calves - 19% salmon (freshwater) - 33% salmon (seawater) - declaration of the words "avoid inhalation" - Declarations to be made on the label or packaging of compound feedingstuffs: - the name: "Protein product obtained by bacterial fermentation of natural gas" - amount of the product contained in the feedingstuffs
1.2 YEASTS						
1.2.1 Yeasts cultivated on substrates of animal or vegetable origin	All yeasts: - obtained from the micro-organisms and substrates listed in columns 3 and 4 respectively - the cells of which have been killed	<i>Saccharomyces cerevisiae</i> , <i>Saccharomyces carlsbergiensis</i> , <i>Kluyveromyces lactis</i> , <i>Kluyveromyces fragilis</i>	Molasses, distillery residues, cereals and products containing starch, fruit juice, whey, lactic acid, hydrolysed vegetable fibres	-	All animal species	-
1.2.2 Yeasts cultivated on substrates other than those given in 1.2.1						

1	2	3	4	5	6	7
NAME OF PRODUCT GROUP	NAME OF PRODUCT	DESIGNATION OF NUTRITIVE PRINCIPLE OR IDENTITY OF MICRO-ORGANISM	CULTURE SUBSTRATE (SPECIFICATIONS IF ANY)	COMPOSITION CHARACTERISTICS OF PRODUCT ¹	ANIMAL SPECIES	SPECIAL PROVISIONS ¹
1.3 ALGAE						
1.4 LOWER FUNGI						
1.4.1 Products from the production of antibiotics by fermentation	1.4.1.1 Mycelium, wet by-product from the production of penicillin ensiled by means of <i>Lactobacillus brevis</i> , <i>plantarum</i> , <i>sake</i> , collenoid and <i>Streptococcus lactisto</i> inactivate the penicillin and heat treated	Nitrogenous compound. <i>Penicillium chrysogenum</i> ATCC 48271	Different sources of carbohydrates and their hydrolysates	Nitrogen expressed as crude protein: min. 7%	Ruminants Pigs	Declaration to be made on the label or packaging of the product: - the name: "Mycelium silage from the production of penicillin" - nitrogen expressed as crude protein - crude ash - moisture - animal species or category Declaration to be made on the label or packaging of the compound feedingstuff: - the name: "Mycelium silage from the production of penicillin"
2. NON-PROTEIN NITROGENOUS COMPOUNDS						
2.3 By-products from the production of amino acids by fermentation	2.3.1 Concentrated liquid by-products from the production of L-glutamic acid by fermentation with <i>Corynebacterium melassecola</i>	Ammonium salts and other nitrogenous compounds	Sucrose, molasses, starch products and their hydrolysates	Nitrogen expressed as crude protein: minimum 48% Moisture: maximum 28%	Ruminants from the beginning of rumination	Declarations to be made on the label or packaging of the product: - the name "By-products from the production of L-glutamic acid", - nitrogen, expressed as crude protein, - crude ash, - moisture, - animal species or category. Declarations to be made on the label or packaging of compound feedingstuffs: - percentage of the total crude protein provided by non-protein nitrogen, - indication, in the instructions for use, of the level of total non-protein nitrogen which should not be exceeded in the daily ration of each animal species or category

1	2	3	4	5	6	7
NAME OF PRODUCT GROUP	NAME OF PRODUCT	DESIGNATION OF NUTRITIVE PRINCIPLE OR IDENTITY OF MICRO-ORGANISM	CULTURE SUBSTRATE (SPECIFICATIONS IF ANY)	COMPOSITION CHARACTERISTICS OF PRODUCT ¹	ANIMAL SPECIES	SPECIAL PROVISIONS ¹
	2.3.2 Concentrated liquid by-products from the production of L-lysine monohydrochloride by fermentation with <i>Brevibacterium lactofermentum</i>	Ammonium salts and other nitrogenous compounds	Sucrose, molasses, starch products and their hydrolysates	Nitrogen expressed as crude protein: minimum 45%	Ruminants from the beginning of rumination	Declarations to be made on the label or packaging of the product: - the name "By-products from the production of L-lysine", - nitrogen, expressed as crude protein, - crude ash, - moisture, - animal species or category. Declarations to be made on the label or packaging of compound feedingstuffs: - percentage of the total crude protein provided by non-protein nitrogen, - indication, in the instructions for use, of the level of total non-protein nitrogen which should not be exceeded in the daily ration of each animal species or category.

¹The contents laid down or to be declared, in accordance with columns 5 and 7, refer to the product as such

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