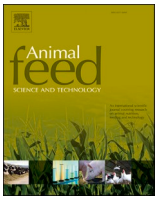
Animal Feed Science and Technology 294 (2022) 115498

Contents lists available at ScienceDirect

Animal Feed Science and Technology

journal homepage: www.elsevier.com/locate/anifeedsci



Evaluation of nutritional quality for weaner piglets of a new methanotrophic microbial cell-derived protein feed

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ARTICLE INFO

Keywords:
Fish meal
Microbial cell protein
Piglet performance
Potato protein
Soy protein concentrate
Standardized intestinal digestible

ABSTRACT

This study aimed to test the hypothesis that a new methanotrophic microbial cell protein (MCP) product, Uniprotein®, has a protein value comparable to other high-quality feeds for weaner piglets. Two experiments were conducted. Experiment 1 was a balanced 6 × 6 Latin square design experiment with six ileal cannulated castrated pigs (start weight 30.2 ± 1.4 kg). They were fed six different diets (one diet did not belong to this study) over six periods, each of one week duration. Four of the diets contained 180 g crude protein (CP)/kg as-fed with either MCP, potato protein (PP), fishmeal (FM), or soy protein concentrate as the sole N-source. The fifth diet was N-free. Experiment 2 was a dose-response trial, where piglets during the first 14 days post-weaning were fed diets including increasing amounts of MCP (0, 30.0, 60.0, or 100.0 g MCP/kg as-fed, equivalent to 0, 112, 221 and 359 g microbial-derived CP/kg diet CP) at the expense of FM and PP. Crystaline CP, Lys, Met, Thr, Trp and Val were added to minimize differences across diets, and no medicinal zinc was added to any of the diets. In experiment 1, MCP had the second highest content of essential amino acids (EAA), but the lowest coefficient of standardized ileal digestibility for all EAA except Lys ($P < 0.001$). However, when contents of ileal digestible EAA were expressed relative to contents of ileal digestible Lys, MCP complied with Danish nutrient recommendations for weaner piglets for 7 out of 9 EAA, and with particularly high levels of digestible Met and Trp. Microbial cell protein had a superior profile as compared to FM for digestible EAA relative to digestible Lys. In experiment 2, MCP could constitute up to 221 g CP/kg diet without any addition of medicinal zinc during the first 14 days post-weaning without any negative impacts on daily weight gain or feed efficiency (gain:feed) compared to the control diet (0 MCP). In conclusion, MCP fulfills Danish recommendations for EAA composition for most EAA and can replace other high quality protein sources to constitute up to 22% of diet CP for weaner piglets without compromising piglet performance.

Abbreviations: CP, crude protein; EAA, essential amino acids; FM, fish meal; MCP, microbial cell-derived protein; PP, potato protein.

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https://doi.org/10.1016/j.anifeedsci.2022.115498
Received 18 December 2021; Received in revised form 19 October 2022; Accepted 23 October 2022
Available online 29 October 2022
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1. Introduction

The global demand for protein for food and feed is projected to increase over the coming decades. In Europe, there is an increased focus to develop more sustainable livestock production systems. This encourages the search for new sustainable protein ingredients with high security of supply and high nutritive value and palatability, particularly when it is intended for feeding of young fast growing animals in the early stages of life (van der Heide et al., 2021). It has become an increasing focus, when looking for new protein ingredients for animal production, to search for ingredients that are not directly suitable for human consumption (Øverland et al., 2010), where production does not rely on access to arable land to reduce competition between humans and animals for food and feed production or both.

Bacteria are known for their rapid growth and high content of protein (Anupama, 2000). A non-genetically modified bacterial culture consisting of the methanotrophic *Methylococcus capsulatus* (Bath) (NCIMB strain 11132), *Alcaligenes acidovorans* (NCIMB strain 13287, former taxonomic name *Ralstonia sp.*), *Bacillus brevis* (NCIMB strain 13288, former taxonomic name *Aneurinibacillus danicus*) and *Bacillus firmus* (NCIMB strain 13289, former taxonomic name *Brevibacillus sp.*) is capable of converting the greenhouse gas, methane, into a protein-rich bacterial biomass, when a suitable non-protein nitrogen source is also available, and with minimum dependence on land-use and water (Øverland et al., 2010). This biomass can then be harvested and converted into a microbial cell protein (MCP).

In 1995, MCP was first approved for use in animal diets by the European Union, but with some restrictions on the upper inclusion rate. These restrictions have since been removed (Regulation (EU) No. 575/2011). A relatively high content of nucleic acids has previously been suggested as an issue regarding the use of bacterial protein for human consumption (Kuhad et al., 1997), which called for a few studies to investigate the nutritive value of MCP for slaughter pigs (Skrede et al., 1998; Øverland et al., 2004; Hellwing et al., 2007a). Microbial cell protein contains approximately 70% crude protein (CP), and the amino acid (AA) profile has been reported to resemble that of fish meal (FM), except for lower Lys and higher Trp concentrations (Skrede et al., 1998; Storebakken et al., 2004). Hellwing et al. (2007a) found that the overall protein and energy metabolism in growing pigs were unaffected, when up to 50% of dietary N was derived from MCP. However, in the late 1990 s and early 2000 s, when the major part of research on MCP was done, the production cost was high, and a low digestibility of intracellular membrane-bound proteins caused low competitiveness of MCP compared to other high-priced protein sources that are commonly used in diets for weaner piglets (Øverland et al., 2010).

Uniprotein® (Unibio Group, Roskilde, Denmark) is a MCP product produced by a new technology for upscaled and low-cost MCP production, where the culture conditions for bacterial growth have been optimized. A downstream process developed to increase availability of membrane-bound nutrients, is believed to have overcome the problem of low digestibility of membrane-bound proteins.

It was hypothesized that MCP produced by the new technology would have improved small intestinal digestibility of essential amino acids (EAA) to levels similar to those of other high-value protein sources used in commercial diets for newly weaned piglets. Two experiments were conducted to test this hypothesis. In experiment 1, the aim was to determine the coefficients of standardized ileal digestibility (CSID) and contents of standardized ileal digestible (in the following referred to only as digestible) dry matter (DM), CP and individual AA in the improved MCP product using ileal-cannulated pigs (start weight appr. 30 kg). In experiment 1, the MCP product was compared to three other commonly used high-quality protein feeds; FM, potato protein (PP), and a refined soy protein concentrate (SPC) with low contents of anti-nutritional factors. Experiment 2 was a dose-response feeding trial with newly weaned piglets to assess their growth performance during the first 14 days post-weaning, when MCP was included in increasing amounts in the diet (0, 30.0, 60.0 or 100.0 g/kg as-fed, equivalent to 0, 112, 221 and 359 g microbial-derived CP/kg diet CP). The diets were formulated to have the same contents of digestible CP, Lys, Met, Thr, Val and Trp, and no medicinal zinc was added to any of the diets.

2. Materials and methods

2.1. Production of the MCP

The cultivation of bacteria takes place in a closed patented U-Loop® technology fermentation process (PCT No. WO 00/70014), where methane is used as the sole carbon source and ammonia as the sole nitrogen source to produce a protein-rich microbial biomass consisting of 90% *Methylococcus capsulatus* (Bath) (NCIMB strain 11132), 8% *Alcaligenes acidovorans* (NCIMB strain 13287, former taxonomic name *Ralstonia sp.*), 1% *Bacillus brevis* (NCIMB strain 13288, former taxonic name *Aneurinibacillus danicus*), and 1% *Bacillus firmus* (NCIMB strain 13289, former taxonic name *Brevibacillus sp.*). The biomass is harvested and upconcentrated in DM by the use of centrifugation, and thereafter homogenized and heat-treated by ultra-high temperature (UHT) processing to make the protein accessible and to inactivate the bacteria. Finally, the UHT-treated biomass is spray-dried with an integrated fluid bed to produce a protein-rich powder with a content of minimum 700 g CP/kg DM.

2.2. Experiment 1: digestibility trial with ileal-cannulated pigs

This experiment was conducted in the intensive care unit in the experimental animal facilities at Aarhus University, Foulum, Denmark. The experiment was conducted under a license issued by the Danish Animal Experiments Inspectorate, the Ministry of Food, Agriculture, and Fisheries, Danish Veterinary and Drug Administration, Denmark, and was in accordance with the Danish Ministry of Justice, law number 253 of 8 March 2013, concerning experiments with and care of experimental animals.

2.2.1. Animals and diets

A randomized 6 × 6 Latin square design experiment was conducted with six castrated male pigs [(Landrace x Yorkshire) x (Hampshire x Duroc)] with an initial weight of 30.2 ± 1.4 kg. The pigs were fed six different diets over six periods, each with a duration of one week. One diet was not part of this study. Results from all six diets were included in the statistical analyses, but results will only be reported for the five diets pertaining to this study.

Before the start of the experiment, the pigs were surgically fitted with a T-cannula (outer diameter 2.7 cm, inner diameter 2 cm) placed 15 cm proximal to the ileocaecal valve, as previously described by Decuypere et al. (1977). The pigs were purchased from a commercial herd, and before surgery, they were acclimatized for 5–6 days in the intensive care unit in individual straw-bedded pens. After surgery, the pigs were allowed another 5–6 days of recovery. During the recovery period, they were fed a standard commercial diet.

The four experimental diets contained either Uniprotein® (Unibio Group, Roskilde, Denmark; Diet MCP), a PP product (Protastar; Scagro A/S, Gesten, Denmark; Diet PP), FM (FF Classic; FF Skagen, Skagen, Denmark; Diet FM) or SPC (Vilosoy; Vilomix, Mørke, Denmark; Diet SPC) as their sole N source. The fifth diet was an N-free diet (Diet N-free). The diet compositions and contents of CP and AA are shown in Tables 1 and 2, respectively. The feed was produced at the experimental feed factory at Aarhus University, Foulum, Denmark.

Table 1
Formulation of experimental diets used in Experiment 1 containing either microbial cell protein (MCP), refined potato protein (PP), fishmeal (FM) or soy protein concentrate (SPC), and composition of the pure protein feeds.

	Experimental diet:				
	N-free	MCP	PP	FM	SPC
Diet ingredient (g/kg as-fed):					
Microbial cell protein	0	234	0	0	0
Refined potato protein	0	0	218	0	0
Fish meal	0	0	0	246	0
Soy protein concentrate	0	0	0	0	333
Wheat starch	794	642	631	654	541
Sucrose	100	51.6	52.0	52.0	52.0
Cellulose	35.0	32.7	32.8	35.0	24.0
Soy bean oil	30.0	9.9	26.0	3.0	22.0
Sodium chloride	2.6	0.0	2.6	0	2.5
Monocalcium phosphate	24.7	10.5	22.4	0.3	14.3
Calcium carbonate	4.4	10.0	5.2	0	6.4
Vitamin-mineral mix[a]	2.0	2.0	2.0	2.0	2.0
Titaniumdioxide	2.8	2.8	2.8	2.8	2.8
Colour microgrits	5.0	5.0	5.0	5.0	0.0
Calculated nutrient composition (g/kg as-fed):					
Crude protein	0	174[b]	180	180	180
Crude fat	30	30	29	30	30
Sugar	96	50	50	50	50
Starch	714	582	568	589	489
Crude fibre+cellulose	33	33	33	33	33
Ca	6.6	6.6	6.6	7.6	6.6
P	5.6	5.5	5.6	5.6	5.6
Na	1.0	1.0	1.0	1.9	1.0
Nutrient contents of the protein ingredients used in diets (g/kg as-fed)[c]:					
		MCP	PP	FM	SPC
DM		975	923	941	926
Crude protein		737	825	732	540
Crude fat		81.0	20.3	109	25.0
Sugar		0.0	*0.0*	*0.0*	*0.0*
Starch		29.1	*0.0*	*0.0*	*0.0*
Crude fibre		6.8	*6.5*	*0.0*	*31*
Ca		2.7	*0.65*	*30.8*	*4.0*
P		13.1	*2.4*	*22.5*	*7.1*
Na		4.4	*0.11*	*7.8*	*0.18*

[a] Content in diet per kg (as fed): 4200 IU vitamin A; 420 IU vitamin D_3; 42.9 IU vitamin E/DL-alpha-tocopherol; 3.8 mg vitamin K_3; 2.1 mg vitamin B_1; 2.1 mg vitamin B_2; 3.2 mg vitamin B_6; 0.021 mg vitamin B_{12}; 10.5 mg D-pantothenic acid; 21 mg nicotinic acid; 0.053 mg biotin; 42 mg Mn-(II)-oxide; 84 mg Fe-(II)-oxide, monohydrate; 10 mg Cu-(II)-sulphate, pentahydrate; 55 mg Zn-(II)- oxide; 0.22 mg CaI, 0.32 mg sodiumselenite.

[b] Amount of MCP delivered was insufficient to reach the desired content of 18% CP in this diet.

[c] Protein ingredients in the diets MCP, PP, FM and SPC were: microbial cell protein product, Uniprotein® (Unibio Group, Roskilde, Denmark); refined potato protein product, Protastar (Scagro A/S, Gesten Denmark); fish meal product, FF Classic (FF Skagen, Skagen, Denmark); and soy protein concentrate product, Vilosoy (Vilomix, Mørke, Denmark). Values in italics were extracted from the Danish feedstuff table for pigs (https://svineproduktion.dk/Viden/Paa-kontoret/Oekonomi_ledelse/Beregningsvaerktoejer/Fodervaerktoejer).

Experimental protein diets, except for the N-free diet, were formulated to contain per kg as-fed: 180 g CP (from either of the feed protein sources), 50 g sugar, 30 g fat, 33 g crude fibre including cellulose, approimately 570 g starch (not possible to achieve in the SPC diet, where the content became 485 g), 6.6 g Ca, 5.6 g P, and 1.0 g Na using sucrose, corn oil, cellulose, wheat starch, $Ca(H_2PO4)_2$, $CaCO_3$ and NaCl to balance the diets. The N-free diet was included to be able to estimate the endogenous N loss (Adeola et al., 2016), and the macronutrient composition in this diet was formulated according to Stein et al. (2007), except that sucrose was used instead of dextrose. A vitamin-mineral premix (Trouw Nutrition, Amersfoort, Netherlands) was added to all diets to meet the minimum requirements for microminerals and vitamins (NRC, 2012). Titanium dioxide (TiO_2) was included in all diets as an indigestible solid phase marker for calculation of CSID. For visual identification of the individual diets, microgrits (Jadis Additive, Haarlem, the Netherlands) of different colours were added to four of the diets.

At the beginning of each experimental week, the body weight (BW) of the pigs was recorded, and the daily feed ration was adjusted according to the individual BW of the pig (40 g feed per kg BW) at the start of each period. The pigs were fed three times daily at 0730, 1500 and 2200 h, with their daily feed ration evenly distributed across meals. The pigs were gradually adapted to a new experimental diet over the first six feedings in each period. During the entire experiment, the pigs were housed in individual pens with slatted plastic floor without bedding material and had free access to water from drinking nipples and access to toys.

2.2.2. Sample collection

Ileal samples were quantitatively collected from 0730 to 1530 h on day 5 and 7 of each experimental week. A plastic bag with 2–3 drops of antimicrobial 0.2% sodium azide solution was fastened on the barrel of the cannula. The digesta flowing into the plastic bag was collected. The bags were changed at least every 0.5 h or whenever full. Digesta collected during each collection day were pooled.

Table 2
Contents of crude protein (CP) and amino acids (AA) in the experimental diets used in Experiment 1, containing either microbial cell protein (MCP), refined potato protein (PP), fishmeal (FM) or soy protein concentrate (SPC) as the sole protein source.

	Experimental diet:			
	MCP	PP	FM	SPC
Dry matter, g/kg as-fed	920	915	913	914
CP, g/kg dry matter	196	190	199	187
CP, g/kg as-fed	180	174	182	171
Total AA[a], g/kg as-fed	147	175	159	162
Total AA[a], % of CP	81.8	101	87.1	94.6
Essential AA, weight-% of total AA				
His	2.5	2.1	2.8	2.7
Ile	5.2	5.7	5.1	5.0
Leu	8.4	9.9	8.0	7.8
Lys	7.2	7.5	8.8	6.1
Met	3.1	2.2	3.3	1.4
Phe	5.0	6.1	4.4	5.1
Thr	5.0	5.7	4.7	4.1
Trp	2.8	1.4	1.4	1.3
Val	6.8	6.8	6.1	5.3
In total	45.8	47.3	44.5	38.6
Semi-essential AA, weight-% of total AA				
Cys	0.6	1.5	1.1	1.5
Tyr	3.9	5.0	3.4	3.5
In total	4.5	6.5	4.5	5.0
Semi-essential AA and precursor, weight-% of total AA:				
Met+Cys	3.7	3.7	4.4	2.8
Phe+Tyr	8.8	11.1	7.7	8.6
Non-essential AA, weight-% of total AA	49.7	46.1	51.0	56.4
Essential AA, g/kg as-fed				
His	3.68	3.79	4.56	4.46
Ile	7.72	10.37	8.29	8.37
Leu	12.6	17.9	13.01	13.01
Lys	10.9	13.6	14.2	10.2
Met	4.59	3.95	5.31	2.24
Phe	7.41	11.07	6.99	8.52
Thr	7.47	10.36	7.56	6.82
Trp	4.14	2.52	2.17	2.22
Val	10.2	12.3	9.81	8.90
Semi-essential AA, g/kg as-fed				
Cys	0.88	2.74	1.74	2.49
Tyr	5.80	9.05	5.46	5.81
Semi-essential AA and precursor, g/kg as-fed				
Met+Cys	5.47	6.69	7.04	4.73
Phe+Tyr	13.2	20.1	12.5	14.3

[a] Sum of 18 analyzed AA.

The pooled samples from the two collection days were combined and a representative sample was taken. The samples were stored at −20 °C until they were chemically analysed. Switch to the next experimental diet was commenced immediately after finishing the collection of digesta on day 7.

2.2.3. Chemical analyses

The protein feed sources were analysed for contents of DM, ash and N, and experimental diets and ileal samples were analysed for DM, ash, TiO_2, N, and individual AA. The DM was analysed in feed by oven drying the samples at 103°C for 24 h (AOAC International, 2000), whereas ileal samples were freeze dried until a stable weight was achieved. Ash was determined by combustion at 525°C for 6 h

Table 3
Ingredients, analysed nutrient composition and derived energy content of experimental diets used in Experiment 2 containing increasing amounts of microbial cell protein (MCP) at the expense of fishmeal (FM) and refined potato protein (PP).

	Dietary inclusion of MCP (g/kg as-fed)[a]:			
	0	30	60	100
Fixed ingredients (g/kg as-fed)				
Wheat, heat treated	500.0	500.0	500.0	500.0
Whey powder	80.0	80.0	80.0	80.0
Dextrose	49.2	49.2	49.2	49.2
Unrefined potato protein	15.0	15.0	15.0	15.0
Formic and benzoic acids (2:1)	15.0	15.0	15.0	15.0
Yeast products	11.0	11.0	11.0	11.0
Sugar beet molasses	10.0	10.0	10.0	10.0
Vitamin-mineral premix[b]	4.0	4.0	4.0	4.0
Flavors	2.7	2.7	2.7	2.7
Phytase	0.40	0.40	0.40	0.40
Variable ingredients (g/kg as-fed)				
MCP	0.0	30.0	60.0	100.0
PP	63.2	74.4	75.3	52.0
FM	50.0	20.0	0.0	0.0
Barley	84.3	50.0	20.0	20.0
Oats	30.0	30.0	20.0	30.0
Wheat	14.8	31.8	57.1	26.9
Fatty acid distillate (palm fat)	25.0	27.8	28.8	32.9
Monocalcium phosphate	14.7	16.3	17.0	15.3
Calcium carbonate	0.0	1.25	2.54	2.90
Sodium chloride	6.14	6.79	7.16	6.93
Amino acids and keto acid	24.6	24.4	24.8	25.9
Lysine sulfate 80%	11.8	12.1	12.6	13.2
Methionine DL 98%	2.64	2.70	2.82	2.96
Threonine 98%	4.60	4.47	4.49	4.79
Tryptophan 99%	1.90	1.68	1.49	1.28
Valine L 96.5%	2.66	2.47	2.44	2.71
Nutritional content (per kg as-fed)				
Energy, MJ NE	9.15	9.22	9.22	9.22
Dry matter (g)	899	904	909	909
Crude protein (g)	183	195	200	200
Crude fat (g)	38	39	43	43
Crude ash (g)	45	45	47	46
Crude fiber (g)	19	16	17	16
Lys (g)	15.4	15.4	16.1	15.9
Met (g)	5.7	5.7	6.3	6.4
Cys (g)	2.7	2.9	2.8	2.5
Thr (g)	11.5	11.7	12.8	12.3
Trp (g)	3.7	4.0	4.1	4.1
Val (g)	10.3	10.3	11.4	11.3
Ile (g)	7.3	7.6	8.1	7.8
Leu (g)	12.8	13.4	14.2	13.8
Phe (g)	7.7	8.3	8.6	8.8
Tyr (g)	5.0	5.9	6.2	5.8
His (g)	3.33	3.39	3.32	3.25
Ca (g)	6.7	6.6	7.7	7.3
P (g)	6.1	6.5	6.5	6.7
Fe (mg)	343	349	395	410
Cu (mg)	111	141	138	142

[a] 0, 30, 60 and 100 g MCP/kg diet as-fed corresponded to 0, 112, 221 and 359 g microbial-derived CP/g diet CP.

[b] Mineral and vitamins provided in all diets per kg as-fed according to manufacturers information: Na: 3.1 g, Mg: 1.2 g, Mn: 50 mg, Zn: 100 mg, I: 0.25 mg, Se: 0.3 mg, A.vitamin: 12,500 IU, D_3-vitamin 1500 IU, DL-alphatocopherol: 163 mg, B_1-vitamin: 6.5 mg, B_2-vitamin: 16.3 mg, B_6-vitamin: 9.8 mg, B_{12}-vitamin: 0.06 mg, D-panthothenic acid: 38.8 mg, niacin: 65 mg, folic acid: 3.0 mg, K_3-vitamin: 5.0 mg, biotin: 0.90 mg, antioxidant: 50 mg, phosphatase: 2000 FYT, xylanase: 4000 U.

(AOAC International, 2000). Nitrogen was analysed to determine CP content (N × 6.25) using the Dumas method (Ebeling, 1968) with a Vario MAX CN analyser (Elementar Analysensysteme GmbH, Langenselbold, Germany). Samples for AA analysis were hydrolysed for 23 h at 110 °C with (Cys and Met) or without (Ala, Arg, Asp, Glu, Gly, His, Ile, Leu, Lys, Phe, Pro, Ser, Thr, and Val) performic acid oxidation. Afterwards, AA were separated by ion-exchange chromatography and quantified by photometric detection after a ninhydrin reaction (Commission Directive (EC) 98/64). To analyse Trp, samples were hydrolysed with a saturated barium hydroxide solution under alkaline conditions and heated to 110°C for 20 h. An internal Trp standard was added, and subsequently, Trp was determined by high-performance liquid chromatography with fluorescence detection (Commission Directive 2000/45/EC). Titanium dioxide was analysed as described by Short et al. (1996). Furthermore, all protein feed sources were analysed for crude fat by a commercial laboratory (AgroLab LUFA GmbH, Kiel, Germany).

Values for contents of sugar, starch, crude fibre, Ca, P, and Na in FM, PP and SPC used to balance nutrient composition, when formulating the experimental diets, were extracted from the official Danish feedstuff table for pigs (Fodervaerktoejer, 2021).

2.3. Experiment 2: dose-response feeding trial with increasing dietary concentrations of MCP

A dose-response feeding trial was conducted under on-farm conditions at a large scale facility (TestPig, Danish Pig Advisory Center, Herning, Denmark) with nine sections for weaner piglets and managed by the all-in/all-out principle. Each section had space for 750 piglets divided in 24 individual pens with partly slatted floor, and two pens shared one dry feed dispenser for automatic feeding ad libitum with feed registration per double pen (for detailed outline of the facility, see http://www.testgris.dk/). Water was available ad libitum via drinking nipples.

The feeding trial included a total of 3575 piglets. Piglets were during the first 15 days after weaning fed pelleted diets ad libitum containing 0, 30.0, 60.0 or 100.0 g MCP/kg as-fed. The hypothesis was that inclusion of up to 60 g MCP/kg diet as-fed would be possible without negative consequences for piglet performance despite the high content of non-protein N compared to the other protein feeds. Inclusion of MCP was done at the expense of first FM and then PP. The diets were formulated using the WinOpti feed optimisation program (Agrovision, Apeldoorn, Netherlands) to have roughly the same contents per kg DM of net energy, starch, fibre, and digestible CP, Lys, Met, Thr, Trp and Val, based on CSID values determined in Experiment 1 for MCP, and for the other ingredients, CSID values from the the Danish feedstuff table for pigs (Fodervaerktoejer, 2021) were used. Micromineral and vitamins were provided in a premix (Danish Agro a.m.b.a, Karise, Denmark). Medicinal zinc was not added to any of the diets. Dietary compositions are shown in Table 3, and the four diets were produced at a commercial feed factory (Danish Agro, Karise, Denmark).

In each of seven consecutive calendar weeks, piglets were weaned (minimum 21 days of age), divided into four different weight groups (excluding runts), and inserted into eight double pens in an empty, clean section, and each double pen was holding piglets from the same weight group. Thus, there were four repetitions (i.e., four individual pens) for growth rate determination and two repetitions (i.e., two double pens) for feed intake recordings for each diet per insertion week, amounting to 28 or 14, respectively, repetitions per diet in total over the seven insertion weeks. On average 31.9 ± 3.2 piglets were inserted into individual pens.

The total weight of piglets from each individual pen was determined at weaning (day 1; i.e., the day of insertion into the weaner unit) and day 15 post-weaning. Feed distribution recordings were made daily for each double pen. Daily recordings were also made during inspections of each individual pen for any signs of diseased or dead piglets, and the cause was noted, when it could be determined. In case of diarrhea, antibiotic treatments were given individually to the first three cases in a pen as prescribed by the herd veterinarian, and the number of days each piglet needed treatment was recorded. The only exception to this was during the first experimental week (first insertion of piglets), where all pens received a diarrea preventive antibiotic treatment following the default herd management routines due to a miscommunication.

2.3.1. Chemical analyses

Representative samples of all diets used in the experiment were taken at the time of delivery to the farm, and additional representative sub-samples of each type of diet was taken each week new batches of piglets were inserted in the weaner unit. The samples were subsequently pooled, before analysed for chemical composition using NIR analyses at a commercial laboratory (AgroLab) by DIN EN ISO/IEC 17025:2018 accredited procedures.

2.4. Calculations and statistical analysis

In the experiment 1, CSID and contents of ileal digestible CP and AA were calculated according to Stein et al. (2007). Data were analysed by the mixed procedure of SAS (SAS Inst., Inc., Cary, NC, USA) with pig and experimental week as random effects and diet as a fixed effect.

In the experiment 2, data for daily weight gain, feed consumption and feed efficiency (gain:feed) during the 14 day post-weaning period were analysed by ANOVA using the lme procedure of R (R Core Team, Vienna, Austria) and a model, where dietary treatment, average weight of piglets in an inserted group and number of piglets inserted in either individual pens (weight gain) or double pens (feed provided and feed efficiency) were fixed effects, and repetition numbers within an insertion week were random effects. Data were analysed for normality using Shapiro-Wilk normality test. Results are presented as least squares means and variance in the data is expressed as standard error of the mean (SEM). For pairwise comparisons, the Tukey-Kramer multiple comparisons test was used to separate treatment means (Littell et al., 2002). In addition, to analyse for linear or quadratic effects of dose of MCP, additional contrasts reflecting linear or quadratic relationships were generated post-hoc after model selection within the emmeans package of R (Lenth, 2021). Significance was considered at $P < 0.05$ and tendencies between $0.05 < P < 0.10$.

3. Results

3.1. Experiment 1

3.1.1. Composition of the protein sources and experimental diets

The MCP contained 737 g CP/kg as-fed, which was close to that of FM (732 g/kg as-fed), higher than in SPC (540 g/kg as-fed), but lower than PP (825 g/kg as-fed; Table 1). The MCP and FM had higher contents of crude fat (81.0 and 109 g/kg as-fed, respectively) than the protein extracts PP and SPC (20.3 and 25.0 g/kg as-fed, respectively).

The CP levels in the experimental diets ranged from 171 to 182 g/kg as-fed (Table 2), SPC being the lowest and FM the highest. The total content of AA (18 analysed) in % of CP was lowest in MCP (81.8%), slightly higher in FM (87.1%) and close to 100% in the PP and SPC diets. To assess the quality of the protein sources, the AA profiles were expressed both as the content in g of each AA per kg as-fed as well as the relative proportion of each AA in % of total AA. The MCP had almost twice as high content of Trp (% of total AA and g/kg as-fed) and it also had the highest proportion of Val (together with PP) in % of total AA, but the lowest Cys content among all the studied protein feeds. Compared to SPC, MCP had higher proportions of all EAA and semi-essential AA in % of total AA, except for His, Phe and Cys. The SPC generally had the lowest amount of CP (g/kg DM) and proportion of EAA (except for His and Phe) in % of total AA among all the protein sources. Compared to FM, MCP had higher or similar contents (% of total AA) of most essential and semi-essential AA, with the exception of His, Lys, and Met, and had higher Tyr and Phe+Tyr. The PP had the highest total content of AA (g/kg as-fed and % of CP), and highest content of EAA and semi-essential AA, except for His, Lys, Met, and Trp, among all protein sources. However, expressed as % of total AA, MCP was superior or similar to PP for His, Met, Trp, Val, and Met+Cys.

3.1.2. Coefficients of standardized ileal digestibility

One pig was euthanized and replaced in week five due to inflammation around the inserted cannula, and data for this pig has been included until the time it became sick. No other clinical health problems were encountered during the experimental period.

Coefficients of standardized ileal digestibility (CSID) for CP and AA are provided in Table 4, and for apparent ileal digestibility coefficients in Supplementary Table 1. The average endogenous loss of CP was estimated from the N-free diet to be 22.6 g CP/kg DM intake.

The PP (0.968) and FM (0.953) had the highest CSID for DM, followed by MCP (0.909) and lowest for SPC (0.883; $P < 0.001$). The PP was also found to have the highest CSID for CP (0.901; $P < 0.001$) and for all individual essential and semi-essential AA (except for Trp) with values for individual AA ranging from 0.796 to 0.920 ($P < 0.001$).

The MCP had the lowest CSID for CP (0.807) and for all EAA (except Lys) and semi-essential AA, with values ranging from 0.570 (Trp) to 0.866 (Lys; $P < 0.001$). When significant differences were found between FM and SPC, they were consistently in favour of SPC.

The content of digestible Lys in MCP was 40.2 g/kg as-fed (Table 5), which was markedly higher than in SPC (25.6 g digestible Lys/kg as-fed), but lower than in PP and FM (56.9 and 50.2 g digestible Lys/kg as-fed, respectively).

3.2. Experiment 2

Results for average daily weight gain, feed intake and feed efficiency (kg feed provided/kg weight gain) of piglets fed diets with different concentrations of MCP the first 14 days after weaning are shown in Table 6. Feed intake was unaffected by the dietary inclusion rate of MCP. There was a tendency for a difference in daily weight gain across treatments ($P = 0.088$), which could be ascribed

Table 4
Coefficients of standardized ileal digestibility (%) for dry matter (DM) (n = 6), crude protein (CP) and individual amino acid (AA) in microbial cell protein (MCP), refined potato protein (PP), fishmeal (FM) and soy protein concentrate (SPC) in pigs.

	Protein source:					
	MCP	PP	FM	SPC	SEM	*P*-value
DM	0.909[b]	0.968[a]	0.953[a]	0.883[c]	0.006	< 0.001
CP	0.807[c]	0.901[a]	0.826[bc]	0.860[b]	0.015	< 0.001
Essential AA						
Lys	0.866[b]	0.911[a]	0.866[b]	0.842[b]	0.011	< 0.001
Met	0.762[c]	0.913[a]	0.858[b]	0.894[a]	0.009	< 0.001
Thr	0.741[c]	0.904[a]	0.815[b]	0.799[b]	0.013	< 0.001
Trp	0.570[c]	0.796[ab]	0.761[b]	0.824[a]	0.014	< 0.001
Val	0.759[c]	0.889[a]	0.827[b]	0.845[b]	0.012	< 0.001
Ile	0.735[c]	0.892[a]	0.839[b]	0.866[ab]	0.012	< 0.001
Leu	0.735[c]	0.919[a]	0.854[b]	0.862[b]	0.012	< 0.001
Phe	0.649[c]	0.918[a]	0.820[b]	0.881[a]	0.013	< 0.001
His	0.789[c]	0.899[a]	0.835[b]	0.881[a]	0.011	< 0.001
Semi-essential AA						
Cys	0.618[c]	0.907[a]	0.673[bc]	0.733[b]	0.027	< 0.001
Tyr	0.663[c]	0.920[a]	0.807[b]	0.876[a]	0.014	< 0.001

[a, b, c] Values within row are different if superscript differs ($P < 0.05$).
Values are least squares means and standard error of the means (SEM).

Table 5
Contents of standardized ileal digestible essential amino acid (AA) in microbial cell protein (MCP), refined potato protein (PP), fishmeal (FM) and soy protein concentrate (SPC) expressed as % of digestible lysine. Danish recommendations (i.e., composition of the ideal protein for piglets) are shown for comparison.

	MCP	PP	FM	SPC	Ideal[a]
Digestible Lys, g/kg as-fed	40.2	56.9	50.2	25.6	
Digestible AA, % of digestible Lys					
Lys	100	100	100	100	100
Met	37.3	28.9	36.8	23.9	32
Thr	58.7	75.3	50.1	64.1	62
Trp	25.1	16.4	13.5	21.2	21
Val	82.4	87.8	65.5	87.7	62–64
Ile	60.4	74.3	56.5	85.2	46–48
Leu	98.5	132.7	89.8	131.0	86–90
Phe	51.4	81.8	46.7	87.7	54
His	30.9	27.2	30.9	46.2	28–29
Met+Cys	43.3	49.0	46.3	45.1	54
Phe+Tyr	92.3	148.9	82.6	147.7	95

[a] Danish recommendations for ideal protein composition for piglets (6–15 kg) indicated as the content of digestible amounts of the individual AA as % of digestible lysine in the protein (Tybirk et al., 2021).

Table 6
Impact of increasing the dietary inclusion of microbial cell protein (MCP) on initial and final body weight (BW) of piglets, average daily weight gain, average daily feed intake and feed efficiency (gain:intake) of piglets during the first 2 weeks after weaning.

	Dietary inclusion of MCP (g/kg as-fed)[1]:					*P*-value:		
	0	30	60	100	SEM	Treatment	Linear	Qadratic
Initial BW[2] (kg)	6.38 ± 0.8	6.37 ± 0.8	6.35 ± 0.9	6.37 ± 0.9				
Final BW[2] (kg)	8.71 ± 0.9	8.76 ± 1.0	8.64 ± 1.1	8.55 ± 0.9				
Weight gain (kg/d)	0.154	0.159	0.152	0.145	0.005	0.088	0.580	0.126
Feed intake (kg/d)	0.216	0.219	0.219	0.214	0.004	0.733	0.962	0.293
Feed efficiency	0.713[ab]	0.724[b]	0.696[ab]	0.678[a]	0.016	0.005	0.222	0.134

[1]0, 30, 60 and 100 g MCP/kg diet as-fed corresponded to 0, 112, 221 and 359 g microbial-derived protein/kg diet crude protein.
[2]Values are means±standard deviations.
[a, b]Values within row are different if superscript differs ($P < 0.05$).
For BW measures and weight gain: n = 28. For Feed intake and Feed efficiency: n = 14.
Values are least squares means with standard error of the means (SEM).

to a slight reduction in weight gain in the piglets on diets with the highest inclusion rate of MCP (100 g/kg as-fed, equivalent to 359 g MCP/kg diet CP). This resulted in a decrease in feed efficiency (P = 0.005) from 0.724 for piglets on the diet containing 30 g MCP/kg as-fed to 0.678 for piglets on the diet with the highest inclusion level of MCP (100 g/kg as-fed), but feed efficiency was not different from that achieved on a diet without MCP. Diarrhea incidence or piglet mortality during the first two weeks post-partum were unaffected by dietary inclusion level of MCP in this experiment (results not shown), where no medicinal zinc was added to any of the diets.

4. Discussion

It is generally recognised that MCP produced by methanotrophic bacteria has a higher proportion of non-protein N in CP than other (plant derived) feed protein sources (Skrede et al., 1998). It was therefore an expected finding that MCP had the lowest content of AA in CP compared to the other protein sources in this study. The higher content of non-protein N can be ascribed to high contents of nucleic acids in bacteria, where DNA and RNA can account for 9.5% of MCP (Øverland et al., 2001). With this number in mind, the actual difference with respect to the proportion of AA in MCP (83% of CP) as compared to the high valued FM (89% of CP) was less than expected.

Nucleic acids cannot be used directly for protein synthesis, which has given rise to speculations regarding the suitability of MCP as a protein source for growing pigs. Nevertheless, previous pig experiments have shown that up to 40% of adenine, 15% of guanine and 20% of pyrimidine bases in diets containing MCP could be retained in the body, indicating that part of the nucleic acid N can be used for non-essential AA synthesis (Greife and Molnar, 1984). In recent studies with pigs, positive effects were even seen on performance and immune system function by supplementing small amounts of nucleotides (Jang and Kim, 2019; Jiao and Kim, 2019).

Furthermore, it was shown in studies with growing pigs that MCP could constitute up to 50% of protein in the diet without negative effects on growth performance or protein turnover (Hellwing et al., 2007a, 2007b). The content of non-protein N in MCP of the magnitude observed in this study, is therefore not likely to have any practical implications on performance of weanling or growing pigs, unless included in very high amounts. This was also confirmed by the conducted feeding trial, where inclusion in diets for newly

weaned piglets of MCP with up to 221 g microbial-derived CP/kg diet CP at the expense of FM and PP did not have negative impact on neither daily weight gain nor feed intake. Only at the highest inclusion of 356 g microbial-derived CP/kg diet CP was a certain decrease in weight gain and hence poorer feed efficiency observed.

In terms of EAA composition, MCP was a particularly rich source of Trp, having almost twice as high a content of Trp (in % of total AA) compared to the other protein sources, and for most other EAA, only PP had higher EAA contents per kg. Overall, the AA profile in MCP was thereby comparable to the other high quality protein sources used in this study and superior particularly to SPC. The high amount of Trp in MCP is of special interest, as it tends to become second, third or fourth limiting EAA in diets for monogastric animals (Warnants et al., 2003). The most deficient AA in MCP was the semi-essential Cys.

The CSID (and coefficients of apparent ileal digestibility, CAID) for all AA were lower in MCP than in any of the other protein sources, except for Lys, where the CSID was as high as in FM, and the lowest CSID for Lys was found in SPC. Based on previous studies with other types of MCP, it has been discussed that peptidoglycans in the cell wall of bacteria could be resistant to proteases (Schøyen et al., 2005), and the proportion of AA associated with the cell membrane will then negatively affect the AA digestibility. Furthermore, *M. capsulatus* has proven to contain a complex system of poorly digestible internal membranes, and removal of these cell membranes improved digestibility (Schøyen et al., 2005). Cell rupture is, therefore, a necessary part of the production of MCP products (Øverland et al., 2010) to increase the protein digestibility, and lysing (cell rupture) is already part of the production process for the Uniprotein® MCP product. In one former study with another MCP product, higher CAIDs were found (Skrede et al., 1998). It can therefore not be ruled out, that further optimization of the lysing and potentially also drying processes could improve availability of protease-resistant proteins to improve the AA digestibility of MCP, as the production process is still under development.

The nutritional value of a protein feed depends on both the actual amounts of digested EAA as well as the profile of absorbed EAA to sustain protein synthesis. Hence in any feed, a low CSID for a given EAA can be compensated by a high total content of that EAA, whereby MCP achieved a higher content of digestible Lys (g/kg as-fed) than SPC. A deficiency of digestible amounts of an EAA in one feed, can be compensated by a surplus of that EAA from another source in the final diet. The Danish protein recommendations (Tybirk et al., 2021) are based on the definition of a protein with an ideal EAA composition, and in the Danish recommendations, the value of a protein is expressed as the digestible amounts of each EAA (including Met+Cys and Phe+Tyr) in % of digestible Lys. In Table 5, such ratios of digestible EAA:Lys have been provided for the protein feeds and compared to the composition of the defined ideal protein for weaner piglets.

Despite low CSID for EAA, MCP met the Danish recommendations for most EAA, but not quite for Thr (94.7%) and Phe (95.2%). Due to low digestible amounts of also the semi-essential Cys, the combined recommendations for the two semi-essential AA and their precursors were not met (Met+Cys: 80%, Phe+Tyr: 92%).

The MCP was superior to all the other three protein feeds with respect to ratios of digestible Met:Lys and Trp:Lys. The MCP was superior and for His equivalent to FM with respect to all the Danish protein recommendations, except Met+Cys, and FM only fulfilled recommendations for 5 EAA (His, Ile, Leu, Met and Val) and was the poorest in digestible Trp:Lys. The SPC had the lowest content of digestible Lys/kg as-fed, but complied with Danish recommendations for all EAA, except Met (poorest of the protein feeds) and hence also not for Met+Cys. The highly digestible PP had the highest content of digestible Lys/kg as-fed, but did not fulfill recommendations for His (poorest of protein feeds), Met (and hence Met+Cys) and Trp. Microbial cell protein was in fact superior to PP for these 3 EAA.

Thus, despite the low CSID for EAA, it was demonstrated in Experiment 2 that performance of newly weaned piglets can be sustained, when up to 60 g MCP is included per kg diet as-fed (221 g MCP/kg diet CP) at the expense of FM, even in the situation, where the gut-health promoter medicinal zinc is not added to the diets. If the inclusion rate is increased beyond that, a decrease in feed efficiency and possibly also growth rates can be anticipated.

Our results are supported by Øverland et al. (2004), who suggested that the protein quality of MCP was better than that of soybean meal, since increasing inclusion levels of MCP at the expense of soybean meal resulted in better AA and Lys utilization in growing pigs. This improvement might be associated with the high Trp content in MCP, which was the richest source of digestible Trp and Met relative to digestible Lys. An increase in digestible Trp:Lys above 20% will generally not improve piglet performance (Nørgaard et al., 2015), but for newly weaned and immune-challenged piglets, Trp can act as a modulator for controlling immune response and maintaining health in challenged pigs (Le Floc'h, Seve, 2007), and therefore the high digestible Trp content in MCP could add value in weaner diets without high concentrations of medicinal zinc.

Because of the high environmental and climate impact associated with the use of soybean meal in pig feeding, opportunities to replace soy products in pig production are pursued. The high digestibility of PP makes it a highly suited replacement for soy products, and it can reduce unnecessary N excretions (Nørgaard et al., 2014) and N emissions (Hansen et al., 2014) by lowering the dietary CP concentration. However, PP is not available in abundant quantities. The MCP, on the other hand, can complement the Met and Trp content of PP, and has been found in this experiment to be a suitable alternative protein source, and it can be included in balanced diets for weaner piglets with at least 60 g/kg as-fed without any signs of negative effects on piglet performance or apparently gut health. Implementation of this new protein in practical pig feeding will obviously depend on aspects such as availability of different protein feeds and their relative cost.

5. Conclusion

This study demonstrated that MCP can be considered a suitable and high-quality protein source for weaner piglets. Performance and health of newly weaned piglets were sustained, when MCP was included in balanced diets with up to 60 g/kg as-fed (221 g/kg diet CP), even without addition of medicinal zinc to the diets. At the highest inclusion rate (100 g MCP/kg as-fed), feed efficiency was reduced. The MCP has a high content of digestible Lys. The profile of digestible EAA relative to Lys fulfilled most of the Danish

recommendations for protein, was comparable to the other studied high-quality protein sources commonly used in pig production, and had a better profile of digestible EAA:Lys than FM, except for Cys and Met+Cys. The MCP had a particularly high content of digestible Met and Trp compared to the other protein feeds in this study, which may be of particular benefit to immunologically compromised newly weaned piglets. Cys was the only AA, for which the recommendations could not be met by any of the studied protein feeds.

CRediT authorship contribution statement

Marie Rønn: Methodology, Validation, Formal analysis, Investigation, Writing – original draft, Writing – review & editing, Visualization. **Mirka Thorsteinsson:** Methodology, Validation, Investigation, Supervision, Writing – original draft, Writing – review & editing. **Jakob Christoffer Johannsen:** Formal analysis, Writing – review & editing. **Jan Værum Nørgaard:** Methodology, Writing – review & editing. **Ina Karlshøj Julegaard:** Funding acquisition, Resources, Writing – review & editing. **Mette Olaf Nielsen:** Conceptualization, Methodology, Validation, Supervision, Writing – review & editing, Reviewing, Editing, Project administration.

Conflict of Interest

When the 2 experiments in the present study were conducted, Ina Karlshøj Julegaard held a position as technical product manager at Unibio A/S, Roskilde, Denmark, and Unibio provided the MCP product for the two experiments. Ina Karlshøj Julegaard was not involved in the design, practical conduct or sample analyses in the study, but contributed to manuscript writing with description of the MCP production process and general proof-reading. Ina Karlshøj Julegaard is now employed as optimization consultant at Danish Agro a.m.b.a., Køge, Denmark, which is the commercial feed company that formulated and produced the four diets for Experiment 2. Otherwise, the authors declare that there are no conflicts of interest.

Acknowledgements

The present study was part of a larger project financially supported by the National Green Development and Demonstration Programme (GUDP grant no.: 34009–19-1544 of 17 June 2019), Denmark. Two private companies supplied the protein sources used in this study: The MCP product was supplied by Unibio A/S, Roskilde, Denmark, and the FM, PP and SPC products were supplied by Danish Agro a.m.b.a., Køge, Denmark. Danish Agro formulated and produced the four diets used in Experiment 2. Special thanks are due to the following personnel at the Department of Animal and Veterinary Science, Aarhus University, Denmark: Senior Researcher Nuria Canibe for surgical preparation of the pigs, and to Ms Karin K. Jensen, Ms. Lene Nørskov, Ms. Mette K. Christensen and Ms. Mette L. Pedersen for expert care of the animals and help with experimental procedures in Experiment 1. Special thanks are also due to TestGris project leaders Niels Ove Nielsen and Dorthe Carlsson, the Danish Pig Advisory Center, Herning, Denmark, and all staff at the pig facility Skjoldborg, Herning, Denmark, for expert care of the animal and assistance in Experiment 2, and to post doc Marleen Elise van der Heidi, Department of Animal and Veterinary Science, Aarhus University, Denmark, for analysing data from this trial for linear and quadratic effects of dietary doses of MCP analyses.

Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.anifeedsci.2022.115498.

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