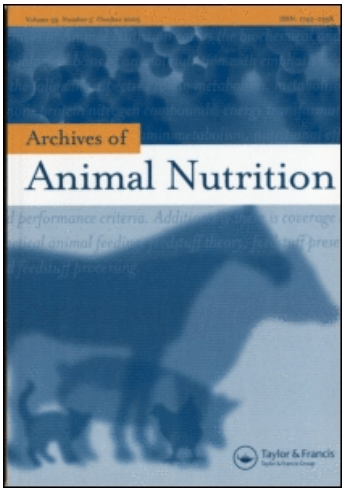

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REVIEW

Evaluation of methane-utilising bacteria products as feed ingredients for monogastric animals

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Bacterial proteins represent a potential future nutrient source for monogastric animal production because they can be grown rapidly on substrates with minimum dependence on soil, water, and climate conditions. This review summarises the current knowledge on methane-utilising bacteria as feed ingredients for animals. We present results from earlier work and recent findings concerning bacterial protein, including the production process, chemical composition, effects on nutrient digestibility, metabolism, and growth performance in several monogastric species, including pigs, broiler chickens, mink (*Mustela vison*), fox (*Alopex lagopus*), Atlantic salmon (*Salmo salar*), rainbow trout (*Oncorhynchus mykiss*), and Atlantic halibut (*Hippoglossus hippoglossus*). It is concluded that bacterial meal (BM) derived from natural gas fermentation, utilising a bacteria culture containing mainly the methanotroph *Methylococcus capsulatus* (Bath), is a promising source of protein based on criteria such as amino acid composition, digestibility, and animal performance and health. Future research challenges include modified downstream processing to produce value-added products, and improved understanding of factors contributing to nutrient availability and animal performance.

Keywords: bacterial products; chemical composition; microbial protein; natural gas; non-ruminants; nutritive value

1. Introduction

Rapid growth and high protein content are well known properties of bacteria in protein production (Stringer 1982; Kuhad et al. 1997; Anupama and Ravindra 2000). In the 1960s and 1970s, considerable research and industrial development were devoted to production of microbial protein (single cell protein, SCP) from hydrocarbon substrates such as methanol and methane, with the aim of supplying protein for human and animal nutrition (Roth 1980; Stringer 1982). Imperial Chemical Industries Ltd (ICI) have produced a commercially available product (PRUTEEN®) from methanol, using the methanol-obligate bacteria *Methylophilus methylotrophus*. The production of bacterial protein from methanol was a major biotechnological breakthrough, and the results of feed evaluation studies, with a number of species, were generally encouraging (Waldroup and Payne 1974; D'Mello

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and Acamovic 1976; D'Mello et al. 1976; Roth and Kirchgessner 1976; Whittemore et al. 1976; Braude and Rhodes 1977; Braude et al. 1977; White and Balloun 1977; Kaushik and Luquet 1980). However, commercial production was terminated, due mainly to economical considerations related to increasing oil prices and the low price of conventional protein sources.

In recent years, the increasing global demand for sustainable protein sources, independent of marine origin, agricultural land use and climatic changes, has led to renewed attention on the potential of microbial protein for use in animal production. Focus has been on methane, the main component of natural gas, which is found widely in nature (Hanson and Hanson 1996; Dalton 2005), as an attractive substrate for bacterial protein production. The abundant supply, cheap transportation, and reasonable cost of natural gas, indicate that protein production from natural gas could be realistic on a large scale. Using methane-oxidising bacteria as an amino acid source in animal nutrition may spare over-exploited sources of protein suitable for direct human consumption. Methanotrophic bacteria are obligatory aerobic respiratory bacteria that utilise methane as their sole source of carbon and energy for growth. Oxidation of methane to methanol is the first step of their metabolic pathway (Hanson and Hanson 1996; Dianou and Adachi 1999). The biology and distribution of methane-oxidising bacteria were first reported by Dworkin and Foster (1956) and Leadbetter and Foster (1958). The methanotrophs are ubiquitously distributed and play an important role in the global methane budget reducing the impact of methane on global warming (Hanson and Hanson 1996). The biochemistry of methane oxidation was recently reviewed by Hakemian and Rosenzweig (2007).

Childress et al. (1986) discovered that a mussel in the Gulf of Mexico gets its required energy and protein through symbiosis with a methane-oxidising bacterium living in gill cells. Thus, the conversion of methane to produce protein-rich biomass by methanotrophic bacteria may be an important process in the natural nutritional chain. Methane contains carbon in a reduced and energy efficient form, and can support a high yield of microbial cells in the bioconversion of the substrate (Hanson and Hanson 1996). In the early studies of D'Mello (1972) two strains of continuously grown methane-utilising bacteria were found to have a nutritionally favourable profile of essential amino acids. Subsequent results from studies with young chickens suggested that the methane-utilising bacteria were useful sources of protein for monogastric animals (D'Mello 1973).

The naturally occurring methanotroph *Methylococcus capsulatus* (Bath) has shown high efficiency in production of bacterial protein from methane (Bothe et al. 2002). The genomic sequence of *Methylococcus capsulatus* (Bath) was reported by Ward et al. (2004). Considerable research has been carried out on the bacterial meal "BioProtein®" (BM), produced from mainly methane by natural gas fermentation, as a protein source for a number of animal species, including pigs, chickens, mink (*Mustela vison)*, fox (*Alopex lagopus*), dogs, Atlantic salmon (*Salmo salar*), rainbow trout (*Oncorhynchus mykiss*), and Atlantic halibut (*Hippoglossus hippoglossus*) (Skrede et al. 1998, 2003; Øverland et al. 2001, 2005, 2006a; Skrede and Ahlstrøm 2002; Hellwing et al. 2005, 2006, 2007a, 2007b; Schøyen et al. 2005, 2007a; Aas et al. 2006a, 2006b, 2006c). It was suggested from the feeding experiments with target species that bacterial protein derived from natural gas could be utilised as a sustainable future protein source for animal production. The high content of nucleic acids may make bacteria unsuitable for human food unless the nucleic acids are

partly removed (Kuhad et al. 1997; Anupama and Ravindra 2000). Although the main focus on nucleic acids has been directed towards the constraints on their use directly as human food, their potential usefulness should be part of the evaluation as animal feed ingredients (Rumsey et al. 1992; Lòpez-Navarro et al. 1996; Mydland et al. 2008).

Bacterial protein grown on natural gas was approved by the European Union in 1995 for use in diets for pigs (max. 8%), veal calves (max. 8%) and salmon (max. 33% for salmon in salt water and 19% in fresh water) (Council Directive No. 82/471/EEC). Revision of the EU regulations concerning microbial protein sources (Regulation (EC) No 767/2009) may facilitate further development and use of such products as feed ingredients. The aim of this review is to evaluate, from a current perspective, the future potential of bacteria grown on natural gas as a source of nutrients for monogastric animals.

2. Production technology and products

The technology involved in production of bacterial protein from methanol by fermentation was reviewed by Davis (1974) and Roth (1980). Although literature suggests that methane may be economically more attractive than methanol, due to lower substrate cost and higher protein yield (Cooney et al. 1980), there are few published reports on large-scale production technology. The current technology in the production of BM by bacterial fermentation of natural gas was developed by modification of the procedure reported by Bothe et al. (2002). The BM was grown by continuous aerobe fermentation in a specially designed and patented loop fermentor, utilising 2 m^3 of methane as carbon and energy source per kg of biomass dry matter, corresponding to approximately 1.7 kg methane per kg crude protein, and the naturally occurring aerobe methanotroph *Methylococcus capsulatus* (Bath) as the main bacteria in the culture. Natural gas contains different concentrations of ethane and propane in addition to methane and use as fermentation substrate requires use of *Methylococcus capsulatus* in a co-culture with the heterogenic bacteria *Ralstonia* sp., *Brevibacillus agri* and *Aneurinibacillus* sp. to enable growth in a continuous system (Bothe et al. 2002). Ammonia was used as a nitrogen source although this may reduce growth in a continuous culture (Carlsen et al. 1991). Also, oxygen and a mineral solution were added to the fermentor, and mass transfer was about 4 $kg/m^3/h$. Cells were continuously harvested from the fermentor, centrifuged, ultrafiltered, heat inactivated, and finally spray-dried.

Further development of the BM technology has resulted in a number of products with specific characteristics. These include autolysates and autolysate extracts produced under high pressure and catalysed by endogenous enzymes, containing high levels of free amino acids and low molecular weight peptides (Schøyen et al. 2005; Mydland et al. 2008). The contents of RNA and DNA can be reduced by endogenous nucleases (Larsen and Joergensen 1996).

3. Nutritional value

3.1. Chemical characterisation

The chemical composition of bacterial biomass depends on factors such as substrate and conditions of fermentation, type of bacteria, and processing after fermentation.

The nutritional value of biomass produced from methane or methanol is mainly determined by the contents of protein and to a lesser extent lipids.

In Table 1 and 2 the proximate composition and amino acid profile of bacterial protein grown on natural gas (BM) are compared with average data on methanol-derived bacterial protein (PRUTEEN®, *Methylophilus methylotrophus*). The average crude protein content of BM was lower than that of PRUTEEN®, partly due to a lower content of nucleic acids. According to Stringer (1982) the amino acid-N in PRUTEEN® accounted for about 71% and nucleic acid-N 19% of total N, the remainder contributed by glucosamine and ethanolamine as constituents of the cell wall. The studies by D'Mello (1973) revealed a nutritionally favourable amino acid composition in the continuously grown methane-utilising bacteria *Methylococcus capsulatus* and *Methylomonas albus*. Also the amino acid composition of BM is well balanced and similar to that of PRUTEEN®, but contents of arginine and tryptophan are higher in BM (Table 2). For most indispensable amino acids there are minor differences between BM and average data for fishmeal and soybean meal, but lysine content is lower and tryptophan higher in BM than in fishmeal, and the BM protein contains more methionine and tryptohan than soybean meal. By compiling data from nine different published studies on BM, Schøyen (2007) showed that the batch variation in the amino acid composition of BM appeared to be similar to the corresponding variation in fishmeal and soybean meal. Recent studies with the BM culture have shown no difference in amino acid composition between biomass grown on methanol or natural gas as substrates (Skrede et al. 2009).

The slightly higher content of lipids in BM, compared to previous data for bacterial cells grown on methanol, may be due to analytical differences. In bacterial biomass grown on methanol, the main fatty acids are hexadecanoic acid and cis-9-hexadecanoic acid, which comprise 85% of total fatty acids (Stringer 1982). Also the lipids in BM contain predominantly 16:0 and 16:1 fatty acids, representing about 85% of total fatty acids (Table 3), and a high proportion of phospholipids (Müller et al. 2004). The phospholipids are mainly found in the cell wall and membranes of the BM bacteria, and consist mainly of phosphatidylethanolamine (74%) and phosphatidylglycerol (13%), and minor amounts of phosphatidylcholine (8%) and

Table 1. Concentration of crude protein (N · 6.25), lipids, ash and nucleic acids of bacterial meal (BM) grown on methanol or methane [g/100 g DM].

Reference	Bacteria culture	Crude protein	Lipids	Ash	Nucleic acids
Methanol					
Gow et al. (1974), Roth and Kirchgessner (1976)*	*Methylophilus methylotrophus*	81.3	7.2[†]	9.1	15.9
Methane (Natural gas, BM)					
Skrede et al. (1998)	BM culture[#]	73.2	10.7[§]	8.5	9.9
Storebakken et al. (2004)	BM culture[#]	68.1	10.4[§]	8.0	ND
Hellwing et al. (2005)	BM culture[#]	68.7	8.0[‡]	8.0	ND
Vhile et al. (2005)	BM culture[#]	73.4	8.4[‡]	7.7	ND
Øverland et al. (2006a)	BM culture[#]	71.9	8.3[‡]	6.7	ND
Aas et al. (2006a)	BM culture[#]	69.5	8.1[+]	6.2	11.1
Aas et al. (2006b)	BM culture[#]	67.0	9.9[+]	6.4	ND

Notes: *Average data; [#]*Methylococcus capsulatus, Ralstonia* sp., *Brevibacillus agri, Aneurinibacillus* sp.; [†]Ether extract; [§]HCl/ethyl ether extract; [‡]HCl/petroleum ether extract; [+]Crude fat, Soxtec.

Table 2. Amino acid composition of bacterial meal grown on natural gas (BM) compared with soybean meal and fishmeal (from Schøyen 2007), and average data on bacterial protein grown on methanol* [g/16 g N ± standard deviation of free, hydrated amino acids].

	BM	Soybean meal	Fishmeal	Methanol-grown bacterial protein
Indispensable amino acids				
Arginine	6.3 ± 0.3	7.4 ± 0.5	6.2 ± 0.6	4.6
Histidine	2.2 ± 0.2	2.7 ± 0.2	2.5 ± 0.4	1.9
Isoleucine	4.4 ± 0.4	4.7 ± 0.3	4.7 ± 0.3	4.3
Leucine	7.5 ± 0.2	7.5 ± 0.5	7.9 ± 0.4	7.0
Lysine	5.6 ± 0.4	6.1 ± 0.3	8.2 ± 0.3	6.0
Methionine	2.6 ± 0.2	1.3 ± 0.1	3.0 ± 0.1	2.4
Phenylalanine	4.2 ± 0.2	5.0 ± 0.3	4.1 ± 0.2	4.1
Threonine	4.3 ± 0.3	3.9 ± 0.3	4.0 ± 0.6	4.6
Tryptophan	2.2 ± 0.8	1.4[#]	0.9[#]	0.9
Valine	5.8 ± 0.3	4.8 ± 0.4	5.3 ± 0.1	5.6
Dispensable amino acids				
Alanine	7.1 ± 0.4	4.2 ± 0.4	6.1 ± 0.1	7.1
Aspartic acid	8.5 ± 0.4	11.2 ± 0.7	9.9 ± 1.2	8.8
Cysteine + cystine	0.7 ± 0.1	1.5 ± 0.2	0.9 ± 0.2	0.7
Glutamic acid	10.6 ± 0.6	18.2 ± 1.4	12.6 ± 1.0	10.6
Glycine	4.9 ± 0.3	4.2 ± 0.3	6.0 ± 0.8	5.7
Proline	3.8 ± 0.3	5.0 ± 0.3	4.3 ± 0.6	2.9
Serine	3.6 ± 0.2	5.2 ± 0.3	4.1 ± 0.3	3.3
Tyrosine	3.6 ± 0.3	3.8 ± 0.3	3.2 ± 0.1	3.4

Notes: *Average data on PRUTEEN® (*Methylophilus methylotrophus*) from Gow et al. (1974), D'Mello et al. (1976), and Braude et al. (1977); [#]Standard deviation not available.

Table 3. Fatty acid composition and contents of minerals and vitamins of bacterial protein grown on natural gas (BM).

Nutrient	Mean	Range	Nutrient	Mean	Range
Minerals (in DM)[#]			Fatty acids [% of total fatty acids] ($n = 4$)*		
Phosphorus [g/kg]	14.8	13.7–15.9	C12:0	0.1	0.0–0.1
Calcium [g/kg]	2.8		C13:0	0.1	0.1–0.1
Magnesium [g/kg]	2.9		C14:0	4.2	3.9–4.4
Potassium [g/kg]	6.4		C14:1n-5	0.5	0.1–1.0
Sodium [g/kg]	2.9		C15:0	0.7	0.6–0.9
Iron [mg/kg]	317	293–341	C16:0	49.2	48.1–51.1
Manganese [mg/kg]	2.7	2.4–3.0	C16:1	36.0	32.4–39.5
Zinc [mg/kg]	22.4	22.1–22.7	C17:0	0.5	0.2–1.1
Copper [mg/kg]	83.9	79.9–87.9	C18:0	0.3	0.3–0.4
			C18:1n-9	0.2	0.1–0.5
Vitamins (in DM)*			C18:1n-7	0.2	0.2–0.3
Vitamin A [IU/g]	$<1^{\dagger}$		C18:2n-6	0.1	0.0–0.2
Vitamin E [mg/kg]	$<5^{\dagger}$		C18:3n-3	0.3	0.1–0.5
Thiamine [mg/kg]	12.1		C20:1n-11,n-9	0.1	0.0–0.2
Riboflavin [mg/kg]	73		Unidentified	7.5	4.0–12.1
Niacin [mg/kg]	130				
Inositol [mg/kg]	30				

Notes: *Unpublished data; [#]Data from Aas et al. (2006a, 2006b); [†]Below detection limit.

cardiolipin (5%) (Makula 1978; Müller et al. 2004, 2005). The cell wall of methanotrophic bacteria contains lipopolysaccharides and peptidoglycans, and the linkages between sugar derivatives in the peptidoglycan layer are β (1,4) glycosidic bonds (Schøyen et al. 2007a).

The content of minerals in bacterial biomass is partly determined by composition of the fermentation substrate (Roth 1980). The BM contained about 15 g/kg phosphorus in DM, mainly derived from nucleic acids and phospholipids, and high levels of iron and copper (Table 3). While BM is a rich source of some B vitamins, especially riboflavin and niacin, vitamin A and vitamin E seem to be virtually absent (Table 3).

Fast growing bacteria may contain up to 16% nucleic acids on a DM basis (Kuhad et al. 1997; Anupama and Ravindra 2000). Thus, the nucleic acid content in BM grown on natural gas may be lower than in many other bacterial proteins. According to studies where DNA and RNA have been analysed separately, the BM contains 7–8% RNA and about 2% DNA (Larsen and Joergensen 1996; Skrede et al. 1998), but this may depend on growth rate.

3.2. Digestibility

Digestibility of amino acids in BM determined in several terrestrial species and fish is shown in Table 4. The digestibility of individual amino acids in BM varied considerably: high digestibility was found for lysine and arginine, while digestibility of cysteine was low. There were highly significant correlations between ileal amino

Table 4. Apparent digestibility of crude protein and amino acids of bacterial meal (BM) in different species.*

	Mink	Pigs		Broiler chicken	Atlantic salmon
		Total tract	Ileal		
Crude protein	79.0	85.4	78.1		81.9
Indispensable amino acids					
Arginine	89.4	89.8	88.0	86.2	91.8
Histidine	83.0	86.2	82.5	80.3	80.8
Isoleucine	84.3	83.5	81.4	80.4	83.1
Leucine	82.8	83.4	81.2	80.3	83.5
Lysine	88.7	89.2	84.5	82.6	91.7
Methionine	84.5	82.5	83.8	80.9	83.4
Phenylalanine	75.8	77.3	76.1	73.8	77.1
Threonine	76.2	83.5	77.6	75.8	82.2
Tryptophan	75.3			75.4	
Valine	84.3	85.5	82.5	81.8	84.7
Dispensable amino acids					
Alanine	81.9	87.2	82.3	82.5	84.6
Aspartic acid	81.5	85.1	81.2	79.4	82.2
Cysteine	47.2	77.0	54.9	44.7	51.9
Glutamic acid	84.4	88.9	83.7	83.3	86.4
Glycine	78.4	85.5	77.8	76.3	79.8
Proline	81.0	86.8	78.3	78.2	83.6
Serine	75.2	80.7	73.1	72.3	79.9
Tyrosine	79.9	79.9	77.6	76.7	73.9

Note: *Skrede et al. (1998).

acid digestibility in pigs and total tract digestibility in mink ($r = 0.985$), chickens ($r = 0.987$) and salmon ($r = 0.944$) (Skrede et al. 1998). The digestibility of crude fat in BM was estimated at 87.2% in salmon and 90.5% in mink (unpublished data). When BM was added to diets for grower pigs, replacing soybean meal, there was a decrease in the digestibility of N, but digestibility of energy was not affected (Hellwing et al. 2007a). When BM was added to diets for broiler chicks, replacing soybean meal or fishmeal, the digestibility of amino acids were either unaffected or increased (Schøyen et al. 2007b). In both salmon and rainbow trout, the digestibility of most amino acids, fat and energy decreased with increasing dietary levels of BM replacing fishmeal (Aas et al. 2006a, 2006b). The reduction in digestibility of amino acids in diets containing BM compared to soybean meal as observed in pigs, and compared to fishmeal as observed in salmon and rainbow trout, could be due to a negative effect of microbial membrane and cell wall components (Rumsey et al. 1991). Also, the peptidoglycans in the cell wall of bacteria may be resistant to proteases due to the presence of D-amino acids (Voet D and Voet J 1995). The differences in the apparent digestibility among the different amino acids in BM may be due the differences in the proportion of these amino acids that are associated with the cell membrane, or by differences in the proportions of these amino acids in the endogenous secretions.

Downstream processing of BM by autolysis was shown to give a slightly higher amino acid digestibility compared to diets containing BM in mink, but not in rainbow trout (Schøyen et al. 2005; Aas et al. 2006b; Øverland et al. 2006a). Autolysis and hydrolysis followed by filtration to remove the membrane fraction resulted in increased apparent digestibility of most amino acids (Schøyen et al. 2005). The results indicate that downstream processing of BM affects the availability of nutrients, but the effect is dependent upon the methodology applied: applying high pressure followed by autolysis to disrupt the cell wall did not improve nutrient digestibility, while subsequent ultrafiltration did. Microbial cells may contain poorly digestible cell walls and membranes (Gaillard and van Weerden 1976; Rumsey et al. 1991), and cell rupture may be a necessary part of the production process. A complex system of internal cell membranes is formed when *Methylococcus capsulatus* is grown on methane, especially if the medium contains high concentrations of copper (Prior and Dalton 1985). In a recent study, Skrede et al. (2009) reported a higher apparent digestibility of amino acids in bacterial protein meal produced from *Methylococcus capsulatus* grown on methanol compared to a bacterial meal grown on natural gas. The results indicated that growth media affects the availability of nutrients in bacterial meal, where methanol results in a lower content of cell membranes and a lower proportion of intracellular membrane-bound protein than natural gas. Studies by Braude et al. (1977) and D'Mello et al. (1976) have also shown high amino acid digestibility in pigs fed bacterial protein meal produced from methanol. Dietary RNA and DNA are decomposed into nucleic acids in the intestinal lumen, and further decomposed into nucleosides and free purine and pyrimidine bases by nucleoside phosphatase enzymes in the mucosa (Privat de Garilhe 1967). The nucleotides in microbial meals were well digested by mink (95%, Mydland et al. 2008), chickens (87–95%, Greife and Molnar 1980), and pigs (98–100%, Roth and Kirchgessner 1980).

3.3. *Quantitative energy, protein and nucleic acid metabolism*

Quantitative protein and energy metabolism has been studied in balance and respiration experiments with BM fed to pigs, broiler chickens and mink (Table 5).

Table 5. Quantitative protein and energy metabolism of terrestrial animals fed diets with bacterial meal (BM).

	Mink (Hellwing et al. 2005)				Growing pigs (Hellwing et al. 2007a)				Chickens (Hellwing et al. 2006)			
Bacterial meal [% of CP]	0	18	37	56	0	18	35	52	0	6.5	13	20
Ingredients replaced	–	FM[+]	FM	FM	–	SBM[$]	SBM	SBM	–	FM	FM	FM
Initial age	9.5 weeks				Weanling piglets				Day-old			
Duration of experiment	19 weeks				100 d				35 d			
No. of observations	18	18	20	20	15	16	16	16	15/10*	15/10*	14/10*	15/10*
Nitrogen metabolism												
Ingested N [$g \cdot kg^{-0.75} \cdot d^{-1}$]	2.82	2.82	2.86	2.48	3.21	3.40	3.18	3.38	4.35[a]	4.07[b]	4.10[b]	4.14[b]
Digested N (DN) [$g \cdot kg^{-0.75} \cdot d^{-1}$]	2.34[a]	2.29[a]	2.26[a]	1.92[b]	2.51[ab]	2.62[a]	2.37[b]	2.55[ab]	n.d.[‡]	n.d.	n.d.	n.d.
Retained N (RN) [$g \cdot kg^{-0.75} \cdot d^{-1}$]	0.45	0.54	0.52	0.40	1.50	1.53	1.33	1.46	1.59	1.44	1.52	1.50
RN/DN [%]	19	24	23	21	60	58	54	57	37[#]	35[#]	37[#]	36[#]
N excretion in faeces [%]	20[c]	23[b]	25[a]	27[a]	43	44	45	45	n.d.	n.d.	n.d.	n.d.
N excretion in urine [%]	80[a]	77[b]	75[c]	73[c]	57	56	55	55	n.d.	n.d.	n.d.	n.d.
Energy metabolism												
ME intake [$kJ \cdot kg^{-0.75} \cdot d^{-1}$]	816[a]	797[a]	814[a]	652[b]	1362	1426	1360	1442	1523	1513	1565	1578
Heat production [$kJ \cdot kg^{-0.75} \cdot d^{-1}$]	665	645	652	663	741	730	749	777	795	860	877	792
Retained energy [$kJ \cdot kg^{-0.75} \cdot d^{-1}$]	150[a]	152[a]	162[a]	−11[b]	620	696	613	664	728	653	705	786
In protein [$kJ \cdot kg^{-0.75} \cdot d^{-1}$]	68	81	78	59	220	226	195	217	408	384	380	383
In fat [$kJ \cdot kg^{-0.75} \cdot d^{-1}$]	82	71	84	−70	400	470	419	446	317	261	316	394
$RQ_{np}/RQ^{\dagger}$	0.77[bc]	0.79[ab]	0.80[a]	0.75[c]	1.07	1.06	1.04	1.05	0.92	0.94	0.94	0.93

Notes: [+]FM, Fish meal; [$]SBM, Soybean meal; *Number of observations in balance and respiration experiments, respectively; [‡]n.d., Not determined; [#]Retained N/ingested N; [†]RQ_{np}, Non-protein respiratory quotient (for minks and pigs); RQ, Respiratory quotient (for chickens); [abc]Values within line and species which share no common superscript differ significantly at $p < 0.05$.

Balance data normalised to metabolic body size, revealed that, despite being a strict carnivore, the mink had the lowest intake of N, and the chicken the highest. The retention of N was not affected by BM levels in any species. Expressed in terms of efficiency of utilisation of digested N for retention, the mink retained 19–24%, and the pig 54–60%. The chicken retained 35–37% of intake. These data demonstrate that the N in BM was equally well utilised as N in the other protein sources, and results also suggested that nucleic acid N was well utilised. With increasing dietary BM, N excretion was repartitioned towards more N in faeces, reflecting decreasing N digestibility, but maintained, or even improved, utilisation of digested N (Hellwing et al. 2005). In previous studies feeding methanol-grown bacterial meal to pigs, N-balance has either been similar to that of diets based on fishmeal (Braude et al. 1977) or soybean meal (Greife et al. 1984), or N-retention and utilisation as well as net protein utilisation were poorer than for diets based on soybean meal (Roth and Kirchgessner 1977). Similarly, studies with chickens showed that N utilisation was marginally improved up to an inclusion level of close to 10%, but at higher inclusion levels adverse effects were found (D'Mello and Acamovic 1976). Protein turnover studies showed that in the pig all traits were similar, independent of dietary BM level, but in the mink protein synthesis and breakdown rates were highest on the highest BM inclusion level (Hellwing et al. 2007b).

Adding BM, at levels up to 36% to diets for Atlantic salmon at the expense of fishmeal, resulted in higher retention of N and energy (Aas et al. 2006a). The N and energy budgets showed that increased dietary BM levels reduced the branchial and/or renal N and energy losses and the energy spent on activity and maintenance, when calculated per kg gain in the salmon. In rainbow trout, minor differences in utilisation of N and energy were found when feeding increasing levels of BM at the expense of fishmeal (Aas et al. 2006b).

In pigs and chickens intake of metabolisable energy (ME), heat production and retention of energy were similar among diets (Table 5). Also the efficiency of utilisation of ME for retention was independent of BM inclusion, ranging from 45–49% in pigs, and 43–50% in chickens. In the mink though, animals on the highest BM inclusion level were in negative energy balance and mobilised fat from the body during the balance periods, caused by low intake of ME. The data on heat production and energy retention showed that the energy provided by the BM containing diets was utilised as well as that of the control diets.

The ability of farm animals to utilise the nucleotides in BM has been investigated in mink, pigs, chickens, and salmon (Ahlstrøm et al. 2006; Andersen et al. 2006; Hellwing et al. 2005, 2007a, 2007b, 2007c, 2007d; Mydland et al. 2008). High dietary levels of free adenine, but not nucleotides or nucleosides, may affect feed intake and animal performance negatively as found in rats (Brulé et al. 1988). In chickens 0.1% free adenine supported normal feed intake and body weight gain whereas these traits were negatively affected when BM made up 1% of the diet (Baker and Molitoris 1974; D'Mello 1986). In mink and pigs, the intake of nucleotides increased with dietary level of BM, but the urinary excretion pattern of the purine base derivatives differed somewhat between species; allantoin increased in both species whereas uric acid, xanthine and hypoxanthine were not significantly affected in pigs. In mink, the daily excretion of xanthine and hypoxanthine decreased with increasing dietary BM. Allantoin made up between 95 (0% BM diet) and 97% (highest BM inclusion) of the purine base excretion in mink and between 95% (0% BM diet) and 91% (highest BM inclusion) in pigs, suggesting that the purine bases were more completely

decomposed into their end-product allantoin in the mink than in the pig (Hellwing et al. 2007b). Furthermore, plasma concentrations of xanthine and hypoxanthine in pigs were not significantly affected by dietary BM (Hellwing et al. 2007b, 2007c), whereas uric acid was lowest on the highest BM inclusion (Hellwing et al. 2007c, 2007d), data corroborating that pigs metabolise nucleotides in BM efficiently. Urinary excretion of allantoin and xanthine decreased, but hypoxanthine and uric acid were not consistently affected by increasing dietary BM (Ahlstrøm et al. 2006). Collectively, these data indicate that dietary purines from BM have been partially salvaged and utilised by the animals as reported by D'Mello (1982).

3.4. Growth performance and health

Effects of BM on growth performance in pigs, broiler chicken, fur animals, and farmed fish are shown in Table 6. In general, results from the pig experiments suggest that BM can at least constitute up to 41% of the dietary protein for piglets and up to 44% for growing-finishing pigs without impairing growth performance. Studies with broiler chickens suggest similar feed intakes at levels of up to 14–17% of the dietary protein from BM and improved feed efficiency at levels up to 33%, but decreased weight gain at inclusion levels above 30% of the dietary protein, when replacing soybean meal. The studies also show that BM can at least constitute up to 20% of the dietary protein in diets for the strictly carnivorous mink and up to 30% of the dietary protein for the facultative carnivorous blue fox without negative effects on growth. The results also demonstrated that BM can constitute up to 52% of the dietary protein replacing high-quality fishmeal in diets for carnivorous fish species like Atlantic salmon without adversely affecting growth, while rainbow trout and Atlantic halibut seemed to perform better at the lower inclusion levels of 38 and 13% of dietary protein from BM, respectively. The results confirm earlier work with bacterial protein meal produced from methanol fed to pigs (Whittemore et al. 1976; Braude and Rhodes 1977). Other studies with bacterial protein meal produced from methanol have shown varied effects on feed intake, feed conversion, and growth rates in broiler chicks (D'Mello 1973; Waldroup and Payne 1974; Bornstein et al. 1981; Plavnik et al. 1981). Also, the results confirm earlier work with rainbow trout where 25% (Perera et al. 1995) and 80% (Kaushik and Luquet 1980) of the fishmeal were replaced by bacterial protein meal produced from methanol.

The increase in weight gain in salmon fed BM was a result of improved feed conversion ratio, rather than increased feed intake. The improvement in feed conversion ratio and the increase in N-retention in salmon occurred despite a slightly lower nutrient digestibility. The nucleic acids in BM may have an N-sparing effect in the animals, which may partially explain the improvement in feed conversion ratio and the increase in N-retention in salmon fed BM. Similar results were also observed in chickens. Salmon and rainbow trout adapted faster to BM than halibut and broiler chickens. A reduction in feed intake in mink was observed when feeding a diet containing 56% of digestible crude protein from BM, possibly due to the sticky consistency of the wet feed (Hellwing et al. 2005). Other causes of reduced feed intake may include increased amounts of dust and changes in the physical quality of the pelleted feed (Øverland et al. 2006b). High levels of dietary free adenine (reviewed by Hellwing et al. 2006) have been associated with reduced feed intake in chickens and rats. Because the nucleic acids in BM mainly exist as nucleoprotein and

Table 6. Growth performance of terrestrial animals and farmed fish species fed diets with bacterial meal (BM).

	Ingredients replaced*	Duration	Protein‡ [%]	CP from BM [max %]	Response§ [in relation to % of CP from BM] ADFI†	FCR+	ADG#	Comments	Authors
Pigs									
Growing-finishing	SBM	106 d	LP HP	32% 44%	44%→	44%→	21%→; 44%↓ grower; 44%→ d 106	44% CP from BM reduced growth during grower period	Øverland et al. (2001)
Growing-finishing	SBM	101 d		44%	44%→	44%→	44%→	No differences in growth performance	Øverland et al. (2001)
Piglets	SBM, FM, MBM	28 d		41%	26%→ 41%↑	41%→	41%→	41% CP from BM improved feed intake	Øverland et al. (2001)
Chicken (broilers)									
Day-old	SBM	35 d		33%	ND$	>14%↑	20%→; >27%↓	Improved feed efficiency from 14 to 33% CP from BM	Skrede et al. (2003)
Day-old	SBM	35 d	LP HP	30% 27%	10%↓ 30%↓	10%↑ 30%↑	20%→ 30%↓	30% CP from BM reduced feed intake and weight, but improved feed efficiency	Skrede et al. (2003)
Day-old	SBM	35 d	HP	27%	ND	27%→	27%→	No differences in growth performance	Skrede et al. (2003)
Day-old	SBM	35 d		17%	17%→	17%↑	17%→	17% CP from BM improved feed efficiency	Schøyen et al. (2007a)
Day-old	SBM	35 d		21%	14%→; 21%↓; 21%\	>14%↑	21%→	Linear decrease in feed intake with BM. 14 and 21% CP from BM improved feed efficiency	Schøyen et al. (2007c)
	FM	35 d		20%	20%→	20%→	20%→	No differences in growth performance	Schøyen et al. (2007c)

(*continued*)

Table 6. (*Continued*).

	Ingredients replaced*	Duration	Protein‡ [%]	CP from BM [max %]	Response§ [in relation to % of CP from BM] ADFI†	FCR+	ADG#	Comments	Authors
Fur animals									
Blue fox	FM, SBM, MM	114 d		30%	ND	ND	30%→	No difference in growth	Skrede and Ahlstrøm (2002)
Mink	FM	5 months		20%	ND	ND	20%→	No differences in growth	Ahlstrøm et al. (2006)
Farmed fish									
Atlantic salmon	FM	5 months		35%	35%→	35%→	35%→; 35%\	Linear decrease in growth with BM	Berge et al. (2005)
Atlantic salmon	FM	48 d		52%	52%→	52%↑	>27%↑	Up to 52% of CP from BM improved growth and feed efficiency	Aas et al. (2006a)
Rainbow trout	FM			38%	38%→	38%→	38%→	No differences in growth performance	Aas et al. (2006b)
Halibut	FM	62 d		26%	13%→; 26%↓	13%→; 26%↓	13%→; 26%↓	13% CP from BM did not affect performance, while 26% CP from BM reduced growth performance	Aas et al. (2006c)

Notes: *SBM, Soybean meal; FM, Fish meal; MBM, Meat and bone meal; MM, Meat meal; ‡LP, Low protein; HP, High protein; §↑Increased/improved ($p < 0.05$); ↓Decreased/worse ($p < 0.05$); →;No significant change compared with control; \Decreased (linear, $p < 0.05$); †ADFI, Average daily feed intake; +FCR, Feed conversion ratio; #ADG, Average daily gain; $ND, Not determined.

not as free purines, components other than nucleic acids might be responsible for a reduction in feed intake in some experiments.

No indications of any negative effects on clinical health in pigs, broiler chickens, or fur animals have been observed in studies with BM (Table 6). Neither pig plasma concentrations of metabolites nor enzymes related to protein and fat metabolism were affected by dietary BM inclusion (Hellwing et al. 2007d). Studies by Berge et al. (2005) and Aas et al. (2006a) revealed no mortality or health problems in response to feeding BM to salmon. Also, histological evaluation in trout gave no indication of adverse morphological changes in the gastro-intestinal tract in response to feeding BM (Aas et al. 2006b).

The phospholipid components in BM may produce beneficial health effects. It has been suggested that dietary phospholipids lower plasma lipoprotein levels, but conflicting results exist in the published literature. Studies have also suggested that bioactive components in BM lipids can lower blood cholesterol. In adult mink, a reduction in total cholesterol, low density lipoprotein (LDL), and to a lesser extent, high density lipoprotein (HDL) were found when feeding high levels of lipids extracted from BM, replacing 17 and 67% of fat from soybean oil in the diet (Müller et al. 2004, 2005). These authors concluded that the decreases in plasma cholesterol and LDL:HDL cholesterol ratio were mainly caused by the specific mixture of phospholipids in BM, characterised by a high level of phosphatidylethanolamine, and not by the dietary fatty acid composition. In growing pigs, however, no effect was found on total cholesterol or LDL cholesterol with an inclusion of up to 15% BM, replacing soybean meal and corresponding to 35.7% of the total dietary lipids from BM (Hellwing et al. 2007d). The differences in response to BM on blood lipid profile in mink and pigs could be due to differences in the lipid level used or age differences between the species, as mature animals may be more prone to high plasma cholesterol levels than young growing animals. Also, the mink were fed much higher levels of BM lipids compared to the pigs.

When evaluating bacterial protein meal as a source of protein for animals, safety aspects should be considered. Based on growth performance and clinical health status of the animals, however, BM seems to be well tolerated across the species tested. Also, the bacteria used in the production of BM are non-pathogenic, and the downstream processing of the bacteria during manufacture includes heat treatment to kill all bacteria. The high content of nucleic acids in bacterial meal has been associated with potential health hazards due to elevated plasma uric acid levels. However, the results indicate that the nucleic acids in BM were well utilised by the animals and might also have contributed to the increase in N-retention in salmon (Aas et al. 2006a). No increase in plasma uric acids levels were reported in salmon (Andersen et al. 2006) or pigs (Hellwing et al. 2007c) while uric acid levels increased with high BM inclusions in rainbow trout (Aas et al. 2006b). In salmon, an up-regulation of the enzymes in the uricolytic pathway with increasing dietary levels of BM has been reported (Andersen et al. 2006). Also, in chickens a reduced activity of uricase has been reported (Andersen et al. 2006), suggesting that they might be more prone to elevated uric acids levels than other species. A nucleic acid reduced bacteria product derived from BM by the heat-shock method (Larsen and Joergensen 1996) caused activation of the immune system and induction of a systemic antibody response towards this product in Wistar rats (Mølck et al. 2002). In mice, feeding of BM has been shown to induce systemic and mucosal immune responses (Christensen et al. 2003). The systemic response could be avoided by feeding a product free of

whole cells, indicating the importance of adequate processing. There is also evidence that BM dust might represent a certain hazard to the factory workers (Sikkeland et al. 2008). Pelleting will reduce dust exposure to the workers during feed manufacture.

4. Bacterial meal and product quality

It has been shown that BM can increase the lean-fat ratio in broiler chickens, but discrepancies among results exist. Skrede et al. (2003) and Schøyen et al. (2007b) reported a reduction in abdominal fat in broiler chickens when feeding BM at the expense of soybean meal. Hellwing et al. (2006), however, reported no differences in carcass composition of broiler chickens fed BM at the expense of fishmeal. Also, Schøyen et al. (2007b) reported no significant effect on abdominal fat level in chickens when BM replaced fishmeal.

Several studies have shown that BM may improve the quality of meat from monogastric farm animals. When adding BM to diets for growing-finishing pigs and broiler chickens, either at the expense of fishmeal or soybean meal, an improvement in storage stability and sensory quality of frozen-stored meat has been observed. Inclusion of BM in diets for broiler chickens has been shown to reduce the intensity of sensory attributes associated with lipid oxidation (Skrede et al. 2003; Schøyen et al. 2007b). When adding BM to growing-finishing pig diets, improved fat quality (Øverland et al. 2001, 2004) and sensory quality of the pork (Øverland et al. 2001, 2005) and reduced lipid oxidation of frozen-stored pork have been reported (Øverland et al. 2005). Feeding bacterial protein meal produced from methanol to growing pigs at the expense of fishmeal resulted in an increase in saturated fatty acids, but a reduction in monounsaturated fatty acids (oleic acid) and unsaturated fatty acids in the back fat (Braude and Rhodes 1977). In the latter study, no differences were found in carcass or meat quality of the pork determined as odour intensity of cooked fat or flavour intensity of cooked pork from pigs fed bacteria protein meal or fishmeal. Also, one study reported that feeding up to 20% BM to salmon, replacing high quality fishmeal, gave no differences in sensory attributes related to lipid oxidation of fresh fish flesh (Berge et al. 2005), but no information exists on the effect of BM on sensory quality of stored fish flesh. The underlying mechanism for the improvement in sensory quality of broiler chicken meat and pork when feeding BM is not known, but may be partially associated with changes in fatty acid composition (Øverland et al. 2005; Schøyen et al. 2007b). Other mechanisms might be involved such as increased antioxidant activity due to feeding of BM.

5. Conclusions and future perspectives

There is increasing awareness of the potential of microbial protein sources in the exploitation of sustainable supply of feed protein for monogastric animals. Bacterial protein can be grown rapidly and may relieve the pressure on limited and expensive high quality protein sources like fishmeal. Furthermore, whereas production of microbial protein requires a small physical footprint, there are constraints on the production of plant proteins including limited land area, water and fertiliser supply, and environmental challenges.

The nutritional value of bacterial protein sources depends on the growth media, the process of manufacture, and downstream processing. Production of bacterial

protein for animal feed purposes should be based on consistent substrates and processing conditions, and standardised to yield uniform products. This review addresses current knowledge of bacterial meal grown on natural gas as energy and carbon source as an alternative feed ingredient for a number of monogastric species including terrestrial animals as well as fish. Natural gas contains mainly methane and the methanotrophic bacteria can be grown directly on natural gas or after conversion of methane to methanol. Methane has a certain advantage over methanol as a growth substrate because of the reduced form and higher energy efficiency in the production, and lower cost of the product. Moreover, natural gas can be easily transported by pipeline, and the supply of methane is essentially unlimited since it may be obtained from other sources than fossil natural gas. The chemical composition of bacterial meal produced on methane and methanol are similar, but use of methanol seems to support higher amino acid digestibility than methane.

A number of recent studies have confirmed the nutritional value of bacterial meal (BM), based on criteria such as chemical composition, effects on protein and energy metabolism, and growth performance and animal health in feeding experiments. These studies have been carried out with a number of batches of BM, produced during a period from 1992–2006, partly at a pilot plant and partly at a large-scale commercial plant. Most data on the nutritional value of BM indicate only minor differences among batches, but slight differences due to processing modifications may have occurred. It is possible to conclude that BM manufactured during a 15-year period has been shown to be a promising protein source for monogastric animals. However, we are still facing a number of challenges that require further research such as addressing efficiency of production to reduce cost, downstream processing to improve nutritional quality, and manufacture of value-added products adapted to the requirements and preferences of different animals. An improved understanding of factors that contribute to animal performance and health is also needed, and the mechanisms whereby product quality can be improved by adding BM to diets for poultry and pigs still remain unclear. We hope that this review will serve to inspire further research with the purpose of providing high quality bacterial feed ingredients for monogastric nutrition.

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