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# Effects of growth substrate and partial removal of nucleic acids in the production of bacterial protein meal on amino acid profile and digestibility in mink*

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ABSTRACT

Effects of growth substrate and nucleic acid reduction in the production of bacterial protein meal (BPM) were studied using a bacteria culture containing mainly *Methylococcus capsulatus* (Bath). BPM were produced on natural gas (>90% methane) (BPMG), methanol (BPMM), or methanol followed by nucleic acid reduction (BPMM-NAR). BPMG and BPMM had similar crude protein (CP) and amino acid contents, whereas BPMM-NAR had slightly higher CP and amino acid contents. Digestibility of CP and amino acids in mink was higher for BPMM than for BPMG. Tryptophan revealed the greatest difference in true digestibility (70.5% for BPMG vs 89.5% for BPMM) and lysine the least difference (93.2% for BPMG and 95.7% for BPMM). There were no differences in CP or amino acid digestibility between BPMM and BPMM-NAR. It was concluded that BPM grown on methanol gave higher digestibility of CP and amino acids than natural gas, probably due to lower proportion of bacterial intracellular membrane protein.

KEY WORDS: bacterial protein, natural gas, methanol, amino acids, nucleic acids, digestibility, mink

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## INTRODUCTION

BioProtein® is a bacterial protein meal (BPM) grown by continuous aerobe fermentation of natural gas (>90% methane). The final product is a reddish/brownish meal, containing about, %: dry matter (DM) 96, crude protein (CP) 70, lipids 10 and ash 7. The BPM has been reported to have a nutritionally favourable amino acid composition (Skrede et al., 1998), and to be a suitable protein source for pigs (Øverland et al., 2001), broiler chickens (Skrede et al., 2003; Schøyen et al., 2007), mink (Ahlstrøm et al., 2006) and salmonid fish species (Aas et al., 2006).

The bacterial culture used for BPM production consists of mainly *Methylococcus capsulatus* (Bath) and the heterotrophic bacteria *Ralstonia* sp., *Aneurinibacillus* sp., and *Brevibacillus agri* (Bothe et al., 2002). The methanotrophic bacteria *M. capsulatus* are found in nature where methane and oxygen are available, growing only on methane or methanol as the sole sources of carbon and energy. Oxidation of methane to methanol, catalysed by methane monooxygenase, is the first step in methane conversion (Hakemian and Rosenzweig, 2007). The BPM grown on natural gas has slightly lower digestibility of protein than high-quality fish meal, characteristically high digestibility of lysine and arginine and relatively low digestibility of cysteine and tryptophan (Skrede et al., 1998). In the studies by Kaushik and Luquet (1980), protein digestibility increased with increasing substitution of fish meal protein with bacterial protein produced on methanol as growth substrate. Thus, it seems interesting to study whether the use of methanol rather than methane in BPM production would improve nutrient digestibility.

The BPM contains about 7.6% RNA and 2.3% DNA on a dry matter basis (Skrede et al., 1998). The individual nucleobases in BPM have been shown to be highly digestible (Mydland et al., 2008). Nucleic acids are normal constituents of animal feed ingredients and nucleic acids from BPM seem to be efficiently digested and metabolized by several species, including pigs, mink and salmonid fish species (Aas et al., 2006; Ahlstrøm et al., 2006; Hellwing et al., 2007). In Atlantic salmon, the uricolytic pathway seems to be well regulated to handle high dietary purine levels. However, the level of nucleic acids in BPM is relatively high compared with the contents of most other protein sources, excluding other single cell proteins. This may limit the possibility of using intact BPM for human consumption, because humans lack the enzyme uricase due to two nonsense mutations in the urate oxidase gene.

The present work aimed at studying the effects of growth substrate in BPM production and partial removal of nucleic acids on amino acid composition and digestibility in mink as a model animal species.

## MATERIAL AND METHODS

### *Bacterial protein meal*

Three types of bacterial protein meal (BPM) produced by aerobe bacteria fermentation with mainly *M. capsulatus* (Bath) were produced by Norferm Danmark A/S, Odense (Denmark): type BPMG was grown on natural gas in a pilot scale fermentor, type BPMM was grown on methanol in a laboratory scale fermentor, and type BPMM-NAR was grown on methanol and subsequently nucleic acid reduced using heat shock at 90°C and activation of endogenous nucleases (Larsen and Joergensen, 1996).

### *Animals and diets*

Digestibility experiments with mink (*Mustela vison*) were carried out at the Department of Animal and Aquacultural Sciences, The Norwegian University of Life Sciences, Ås. The animals were 7-month-old male mink of the standard genotype (initial body weight 2265±248 g), randomly allotted to three groups of six animals each. Norwegian protocols of ethical standards concerning experiments involving animals were followed.

Wet diets with approximately 30% of metabolizable energy (ME) from protein, and containing BPMG, BPMM or BPMM-NAR as the sole sources of crude protein and amino acids, were used (Table 1). The diets were made one day prior to start of the experiment. Individual daily rations of 130 g wet weight, corresponding to about 50 g DM and 1 MJ ME, were weighed into plastic cups, frozen at -20°C and stored at -20°C until start of thawing in a refrigerator about 20 h before feeding.

Table 1. Ingredient composition of diets fed to mink

| Ingredients | g kg$^{-1}$ as fed |
|---|---|
| Bacterial protein meal[1] | 194.2 |
| Maize starch (precooked) | 91.9 |
| Soyabean oil[2] | 87.2 |
| Cellulose powder | 15.7 |
| Vitamin and mineral premix[3] | 1.0 |
| Water[4] | 610.0 |

[1] grown on natural gas, methanol, or grown on methanol and nucleic acid reduced (Norferm Danmark A/S, Odense, Denmark); [2] Denofa AS, Fredrikstad, Norway; [3] Norsk Mineralnæring, Hønefoss, Norway. Ingredients per kg, IU: vit. A 2 000 000, vit. $D_3$ 200 000; mg: vit. E 50 000, vit. $B_1$ 15 000, vit. $B_2$ 3 000, vit. $B_6$ 3000, vit. $B_{12}$ 20, pantothenic acid 3000, niacin 5 000, biotin 30, folic acid 300, Fe (amino acid-chelated) 20 000, Zn oxide 7 500, Mn oxide 15 000 and Cu sulphate 1 250; [4] added to achieve the same total water content in all diets

The mink were housed individually in cages equipped for controlled feeding and quantitative faecal collection. The animals were fed the experimental diets during a 3-day preliminary period and a subsequent 4-day period for total collection of faeces. Feeding, collection of feed residues and collection of faeces were carried out once daily. The animals had free access to drinking water. Pooled faeces from each animal were freeze-dried, ground and sifted for removal of hair pending analysis.

*Chemical analysis*

Experimental diets and individually pooled faeces were freeze-dried and ground through a 0.5 mm screen. Dry matter (DM), crude protein (CP) (Kjeldahl N × 6.25) and ash were analysed according to standard procedures (AOAC, 1990). Crude fat content was determined by acid hydrolysis and subsequent petroleum ether extraction. Starch was determined according to McCleary et al. (1984). Amino acids, except tryptophan, were determined according to Method 994.12 of AOAC (1990) and tryptophan was determined according to Method 988.15 of AOAC (1990). Carbohydrate (CHO) content was calculated by subtracting protein (N × 6.25), fat and ash from the dry matter content. Nucleic acids (DNA and RNA) in the bacterial protein meals were determined by the diphenylamine method and the orcinol method, respectively (Herbert et al., 1971).

*Calculations and statistics*

Apparent nutrient digestibility was determined as ((a-b)/a) × 100, where a is nutrient intake and b is amount of nutrient in faeces. The true digestibility of protein and amino acids was calculated using previously estimated correction values for endogenous excretion adjusted according to DM intake, obtained by feeding graded levels of protein and linear regression equations. One-way analysis of variance was used to determine effects of diets on digestibility, using each mink as the experimental unit. The results are presented as least square means (LSMEANS) for each diet, and standard deviation (SD) as a measure of the variance. Data from one animal fed the BPMM-NAR diet were omitted due to low feed intake. Duncan's multiple range test was used to test differences in digestibility between diets. The significance level was set to $P<0.05$.

## RESULTS AND DISCUSSION

*Proximate composition and amino acid profile.* The contents of crude protein, fat, ash, DNA and RNA in BPMG (Table 2) agreed well with previously published data (Skrede et al., 1998). The BPMM contained similar levels of crude protein, fat, DNA and RNA compared with BPMG, whereas the starch content was lower and the ash content higher. The method used for reduction of nucleic acid contents to produce BPMM-NAR appeared to reduce ash levels in addition to reducing the DNA and RNA to about 1/3 of the levels in BPMM. The increase in crude protein content associated with nucleic acid reduction may be associated

Table 2. Proximate composition and amino acid contents of bacterial protein meal grown on natural gas (BPMG) or methanol (BPMM), or grown on methanol and nucleic acid reduced (BPMM-NAR)

| Treatment | Diet | | |
|---|---|---|---|
| | BPMG | BPMM | BPMM-NAR |
| *Proximate composition* | | | |
| DM, g $kg^{-1}$ | 941 | 940 | 947 |
| *g $kg^{-1}$ DM* | | | |
| crude protein | 737 | 748 | 779 |
| fat | 83 | 87 | 112 |
| starch | 4.3 | 2.4 | 2.2 |
| ash | 7.8 | 9.9 | 3.9 |
| CHO | 10.3 | 6.6 | 7.0 |
| DNA | 6.9 | 7.4 | 2.5 |
| RNA | 2.0 | 2.3 | 0.8 |
| *Essential amino acids, g 16 $g^{-1}$ N* | | | |
| lysine | 5.2 | 5.3 | 5.5 |
| threonine | 4.2 | 3.9 | 4.5 |
| methionine | 2.2 | 2.2 | 2.5 |
| tryptophan | 1.7 | 1.8 | 2.2 |
| valine | 5.9 | 5.6 | 6.3 |
| isoleucine | 4.5 | 4.1 | 4.7 |
| leucine | 7.4 | 7.2 | 8.4 |
| phenylalanine | 4.2 | 4.1 | 4.8 |
| histidine | 2.2 | 2.2 | 2.5 |
| arginine | 6.3 | 5.9 | 6.7 |
| *Non-essential amino acids, g 16 $g^{-1}$ N* | | | |
| aspartic acid | 8.3 | 8.5 | 9.4 |
| serine | 3.5 | 3.6 | 4.1 |
| glutamic acid | 10.6 | 10.0 | 10.6 |
| proline | 4.1 | 4.4 | 4.5 |
| glycine | 4.8 | 4.6 | 5.1 |
| alanine | 7.4 | 7.0 | 7.8 |
| tyrosine | 3.7 | 3.7 | 4.2 |
| cysteine + cystine | 0.6 | 0.6 | 0.7 |

with loss of ash and carbohydrates and is in agreement with Larsen and Joergensen (1996).

The amino acid composition of BPMG, determined as g per 16 g N (percent of crude protein), agreed reasonably well with earlier analyses (Skrede et al., 1998), except for slightly lower values for lysine and methionine. BPMG and BPMM contained similar levels of all amino acids, thus the difference in growth medium appeared to have no major effect on amino acid composition. The BPMM-NAR, however, revealed generally higher amino acid contents than BPMG and BPMM. This reflected all amino acids and was an expected effect of the removal of nucleic acids, which contribute to the crude protein content when analysed as total nitrogen.

*Digestibility.* The apparent CP digestibility of BPMG (Table 3) was similar to values obtained by Skrede et al. (1998), but slightly lower than found by Schøyen et al. (2005) when using BPM from an industrial-scale production plant. The use of methanol as growth medium (BPMM) rather than natural gas (BPMG) resulted in higher apparent and true CP digestibility, whereas nucleic acid reduction had no effect on CP digestibility.

Table 3. Digestibility of main nutrients in mink fed diets containing bacterial protein meal grown on natural gas (BPMG) or methanol (BPMM) without or with subsequent nucleic acid reduction (BPMM-NAR)[1], %

| Treatment | Diet | | |
|---|---|---|---|
| | BPMG | BPMM | BPMM-NAR |
| *Apparent digestibility* | | | |
| crude protein | 78.0 ± 1.4[a] | 84.9 ± 1.6[b] | 84.9 ± 2.7[b] |
| fat | 96.4 ± 0.8 | 96.4 ± 0.6 | 97.0 ± 0.6 |
| starch | 95.7 ± 0.3 | 95.6 ± 0.4 | 97.2 ± 0.7 |
| *True digestibility*[2] | | | |
| crude protein | 82.9 ± 1.4[a] | 89.7 ± 1.6[b] | 89.5 ± 2.6[b] |

[1] means ± SD, n=6 for diet with BPMG and BPMM, and n=5 for diet with BPMM-NAR
[2] calculated using endogenous excretion estimates obtained by linear regression
[a,b] numbers with different superscript within a row are significantly different ($P<0.05$)

Digestibility of crude fat and starch was not affected by treatment (Table 3). In the present study, the investigated BPM sources contributed only small fractions of dietary fat and starch, as soyabean oil was the main fat source, and maize starch was the major starch source. Hence, effects of different bacterial protein sources on digestibility of fat and starch were not expected. However, it is interesting to note the tendency towards slightly higher starch digestibility ($P=0.061$) of the diet containing BPMM-NAR compared with the other diets.

The amino acid digestibilities of BPMG (Table 4) were generally in good agreement with those determined in previous experiments (Skrede et al., 1998).

Thus, the high digestibilities of lysine and arginine, and the low digestibilities of cysteine and tryptophan were confirmed. The digestibilities of isoleucine and tyrosine were lower in the present study than found by Skrede et al. (1998).

Table 4. True digestibility[1,2] of amino acids in mink fed bacterial protein meal grown on natural gas (BPMG) or methanol (BPMM) without or with subsequent nucleic acid reduction (BPMM-NAR), %

| Treatment | Diet | | |
|---|---|---|---|
| | BPMG | BPMM | BPMM-NAR |
| *Essential amino acids* | | | |
| lysine | 93.2 ± 1.2[a] | 95.7 ± 0.9[b] | 96.0 ± 1.3[b] |
| threonine | 85.6 ± 2.7[a] | 90.0 ± 2.8[b] | 90.4 ± 4.2[ab] |
| methionine | 84.6 ± 1.6[a] | 93.7 ± 0.9[b] | 93.9 ± 1.4[b] |
| tryptophan | 70.0 ± 2.0[a] | 89.5 ± 1.6[b] | 89.2 ± 4.0[b] |
| valine | 88.8 ± 1.0[a] | 94.5 ± 1.1[b] | 94.0 ± 1.5[b] |
| isoleucine | 81.9 ± 1.2[a] | 91.7 ± 1.4[b] | 89.8 ± 2.0[b] |
| leucine | 84.2 ± 1.5[a] | 92.9 ± 1.3[b] | 92.5 ± 1.7[b] |
| phenylalanine | 79.4 ± 1.5[a] | 90.5 ± 1.5[b] | 91.0 ± 1.9[b] |
| histidine | 88.2 ± 1.0[a] | 93.7 ± 1.2[b] | 94.7 ± 1.9[b] |
| arginine | 93.1 ± 0.9[a] | 96.2 ± 0.8[b] | 96.3 ± 1.1[b] |
| *Non-essential amino acids* | | | |
| aspartic acid | 86.4 ± 1.6[a] | 91.8 ± 1.3[b] | 92.1 ± 1.8[b] |
| serine | 83.3 ± 2.2[a] | 89.7 ± 2.1[b] | 90.8 ± 3.1[b] |
| glutamic acid | 89.5 ± 1.6[a] | 92.1 ± 1.2[b] | 92.2 ± 2.1[b] |
| proline | 86.0 ± 2.6[a] | 91.1 ± 2.3[b] | 90.3 ± 3.6[b] |
| glycine | 84.0 ± 1.8[a] | 91.2 ± 1.3[b] | 90.7 ± 2.1[b] |
| alanine | 87.1 ± 0.8[a] | 94.1 ± 0.9[b] | 91.7 ± 2.0[b] |
| tyrosine | 75.4 ± 2.7[a] | 83.9 ± 3.2[b] | 85.2 ± 4.0[b] |
| cysteine + cystine | 76.7 ± 7.3 | 86.6 ± 6.5 | 88.8 ± 8.0 |

[1] means ± SD, n=6 for diet with BPMG and BPMM, and n=5 for diet with BPMM-NAR
[2] calculated using endogenous excretion estimates obtained by linear regression
[a,b] numbers with different superscript within a row are significantly different ($P<0.05$)

In studies by Kaushik and Luquet (1980) bacterial protein grown on methanol could be fed at high levels without adverse effects on growth rate and feed conversion ratio in rainbow trout. The amino acid digestibilities of BPMM and BPMM-NAR in the present study were significantly higher than those of BPMG, except for the highly variable cysteine + cystine digestibility. The greatest difference was seen with tryptophan, with a true digestibility of about 70% for BPMG and 90% for BPMM and BPMM-NAR. Amino acids with high digestibility in BPMG, i.e. lysine and arginine, were least affected by replacing natural gas with methanol as growth medium.

The improvement in digestibility of CP and amino acids obtained by using methanol instead of natural gas as carbon and energy source was probably related to morphological characteristics of the bacteria caused by different growing

conditions, resulting in reduced proportions of membrane-bound protein. The main bacteria in BPM, *M. capsulatus,* contain a complex system of internal membranes when methane is used as carbon and energy source. The key enzyme in the methane metabolism is methane monooxygenase (MMO), which catalyses the oxidation of methane to methanol. *M. capsulatus* (Bath) possesses two forms of MMO; the membrane-associated or particulate (pMMO) and the soluble (sMMO), and their expression in the cell is dependent upon the copper-to-biomass ratio in the culture (Dalton, 2005). The "copper switch" induces a range of physiological changes, differences in gene expression and the formation of intracellular membranes.

To our knowledge no experiments have been performed to study the formation of intracellular membranes in *M. capsulatus* (Bath) when grown on methane vs methanol. However, Hyder et al. (1979) have shown that *Methylococcus capsulatus* (Texas), when grown on methane, accumulates intracellular membranes, as opposed to when grown on methanol, when virtually no internal membranes were present. This has also been demonstrated in another methanotrophic bacterium (*Methylobacterium organophilum*), where the intracellular membranes were present when cells were grown with methane, but not when grown with methanol or glucose as the sole source of carbon and energy (Patt and Hanson, 1978). Thus when methane-utilizing bacteria are grown on methanol these internal membranes are not present, or may be present to a lesser extent.

The amino acid digestibilities of an extract from autolysed BPM with reduced content of membranes have been shown to be higher than those of a crude autolysed BPM (Schøyen et al., 2005). This suggests that poor digestibility of membrane-bound amino acids contributed to the rather low digestibility of some amino acids in BPMG. Vesicle formation of membrane fractions and proteins embedded in the membranes may render amino acids unavailable for digestion (Schøyen et al., 2005). The peptidoglycans in the cell wall of bacteria may be resistant to proteases due to the presence of D-amino acids. This would explain why a lower content of cell membranes in BPMM than in BPMG seems to result in considerably higher amino acid digestibility.

The true amino acid digestibilities of BPMM-NAR were similar to those of corresponding amino acids in BPMM. Thus, the process developed by Larsen and Joergensen (1996) for partial removal of DNA and RNA had no influence on amino acid digestibility. Removal of nucleic acids from BPM grown on mainly *M. capsulatus* may, however, be unnecessary, and may even reduce nutritional value for several species, including pigs, mink and Atlantic salmon. The nucleic acids are converted *in vivo* by nucleases to nucleotides, nucleosides and nucleobases, and recent studies show efficient digestion and absorption of nucleic acids from BPM (Mydland et al., 2008). In pigs and mink, increased intake of nucleic acid nitrogen from BPM resulted in increased allantoin excretion (Hellwing et al.,

2007). However, increasing levels of BPM have been fed without significant effects on nitrogen retention in pigs (Hellwing et al., 2007) and mink (Hellwing et al., 2005, 2007; Ahlstrøm et al., 2006). Recent studies by Aas et al. (2006) showed higher nitrogen retention when bacterial protein meal partially replaced high-quality fish meal in diets for Atlantic salmon. The latter findings indicate that dietary nucleic acids may have a nitrogen-sparing effect, although nucleotides as well as non-essential amino acids are endogenously synthesized. Moreover, there is evidence that nucleotides have an important role as semiessential nutritional components (Sànchez-Pozo and Gil, 2002).

The average true amino acid digestibilities of BPMG, BPMM and BPMM-NAR were 85.9, 92.2 and 92.0%, respectively. These values were slightly higher than the corresponding true CP digestibilities, indicating that non-protein nitrogen was less efficiently digested than the amino acids. This difference was apparent also for BPMM-NAR, where the non-protein fraction accounted for a reduced proportion of total nitrogen. This may indicate that the nucleic acids were digestible to approximately the same extent as the amino acids, as shown by Mydland et al. (2008), whereas other non-protein constituents may have been less digestible.

## CONCLUSIONS

Use of natural gas, consisting of mainly methane, or methanol as carbon and energy sources in the production of bacterial protein meal resulted in similar proximate composition and amino acid profile. Digestibility of crude protein and amino acids was significantly higher with methanol than with natural gas in the fermentation medium, most likely due to differences in morphology of the *Methylococcus capsulatus* cells. Reduction in nucleic acid content by endogenous nucleases had minor effects on amino acid composition and no effect on amino acid digestibility.

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