First report of the Scientific Committee for Animal Nutrition on Question 69 by the Commission on the use of protein products of fermentation from natural gas obtained by culture of *Methylococcus capsulatus* (Bath), *Alcaligenes acidovorans, Bacillus brevis, Bacillus firmus*, the living cells of which have been killed

(Opinion expressed on 28 April 1995)

TERMS OF REFERENCE (May 1994):

The Scientific Committee for Animal Nutrition (SCAN) and the Scientific Committee for Food (SCF) are requested to give their opinion on the following questions:

1. Does the protein product of fermentation from natural gas obtained by culture of *Methylococcus capsulatus* (Bath), *Alcaligenes acidovorans, Bacillus brevis, Bacillus firmus,* the living cells of which have been killed, have a nutritional value for the animal because it provides nitrogen or protein?

2. Can the use in animal nutrition of the protein product of fermentation from natural gas obtained by culture of *Methylococcus capsulatus* (Bath), *Alcaligenes acidovorans, Bacillus brevis, Bacillus firmus,* the living cells of which have been killed, result in risks for humans (consumer or user) or the animal health, or be prejudicial to the environment?

3. Does the use of the protein product of fermentation from natural gas obtained by culture of *Methylococcus capsulatus* (Bath), *Alcaligenes acidovorans, Bacillus brevis, Bacillus firmus,* the living cells of which have been killed, harm the consumer by impearling the distinctive features of animal products?

4. Can the above-mentioned protein product be monitored in feedingstuffs?

BACKGROUND

The Council of the European Union, when adopting Directive 82/471/EEC[1] considered it essential, before including a new product in one of the groups listed in the annex of this directive, to establish that it has the required nutritional value and that, when used sensibly, it has no detrimental effect on human or animal health or on the environment; and does not harm the consumer by impairing the distinctive features of animal products. With a view to providing all necessary guarantees, the Community procedure adopted should in certain cases of amendment of the annex make provisions for the compulsory consultation of the Committees created by Commission Decisions 74/234/CEE[2] and 76/791/EEC[3].

In accordance with the provisions laid down in the Article 6, amendments to be made to the annex as a result of developments in scientific or technical knowledge shall be adopted by the Standing Committee of Animal Nutrition in accordance with the procedure laid down in Article 13.

According to this article the Commission representative shall submit to the Standing Committee a draft of the measures to be adopted, and the Standing Committee shall deliver

1 Concerning certain products used in animal nutrition (O.J. No. L213, 21.07.82, p. 8)
2 Instituting a Scientific Committee for Food (SCF). (O.J. No. L136, 20.05.74, p.1)
3 Instituting the Scientific Committee for Animal Nutrition (SCAN). (O.J. No. L279, 09.10.76, p.35)

their opinion on the draft within a time limit set by the chairman according to the urgency of the matter, and shall decide by majority votes. In order to ensure that the product concerned complies with the principles set out in Directive 82/471/EEC[1], a dossier prepared in accordance with the provisions of Council Directive 83/228/EEC[4] should be prepared and, if requested, be the subject of consultation of members of the above-mentioned Scientific Committees set up by the Commission. This consultation is made compulsory for bacteria and yeasts by Article which 6 establishes that in the case of the products referred to in sections 1.1 (bacteria) and 1.2 (yeasts) of the annex, the Commission shall consult the SCF and the SCAN.

A request has been made to register the protein product of fermentation from natural gas obtained by culture of *Methylococcus capsulatus* (Bath), *Alcaligenes acidovorans, Bacillus brevis, Bacillus firmus,* the living cells of which have been killed according to the conditions set out in the attached table. It should be noted that, a product of similar nature was examined previously by the SCAN, and the opinion of the Committee expressed on 23 September 1985[5] in a report established jointly by the Scientific Committee for Animal Nutrition and the Scientific Committee for Food on the use in animal nutrition of protein products obtained from bacteria of the *Methylococcaceae* family[6].

A first report on the submission for registration of the protein product of fermentation from natural gas was considered both by the SCAN and the SCF in May 1994. In this report the documentation submitted in March 1993 was reviewed and certain additional information was requested by both committees at that time before a final answer to question No. 69 could be given to the Commission. A first supplementary dossier was provided in November 1994 which answered some of the questions of the committees. At the same time the submission was changed by restricting the use of the protein product in the feed of young growing animals, e.g. piglets, calves and fish in the early stages of growth. No application for the use in chicken was made, although data for this species were included in the original submission. Even this first supplementary dossier left some of the questions previously put by the SCAN and the SCF inadequately answered. The points still at issue were again transmitted to the submitter and a further supplementary dossier was submitted in February 1995. All additional information now supplied is included in this final report.

4 On the fixing of guidelines for the assessment of certain products used in animal nutrition. (O.J. No. L126, 13.05.83, p. 13)

5 Fifth Series 1986; Report EUR 1041EN. Catalogue Nº CD-NK-86-003-EN-C. p.51

6 In particular from *Methylophilus* cultivated in methanol (Pruteen)

Proposal for inclusion into the annexes of Directive 82/471

1	2	3	4	5	6	7
Name of product group	Name of Product	Designation of nutritive principle or identity of the micro-organism	Nutrient substrate (specifications if any)	Composition characteristics of the product	Animal species	Special provisions
1.1. Bacteria 1.1.2Bacteria cultivated on natural gas	1.1.2.1 Protein product of fermentation from natural gas obtained by culture of: *Methylococcus capsulatus* (Bath) *Alcaligenes acidovorans*, *Bacillus brevis*, *Bacillus firmus*, - the cells of which have been killed	*Methylococcus capsulatus (Bath)* NCIMB strain 11132, *Alcaligenes acidovorans* NCIMB strain 12387, *Bacillus brevis* NCIMB strain 13288, *Bacillus firmus* NCIMB strain 13280	Natural gas, (91% methane, 5,1% ethane 1,9% propane 0,4% isobutane 0,5% n-butane 1,1% other minor components), ammonia, mineral salts	Nitrogen expressed as crude protein minimum 65%	- Pigs - Calves - Fish	Declaration to be made on the label or packaging of the product: - the name: "Protein product obtained by fermentation of natural gas" - nitrogen expressed as crude protein - crude ash - crude fat - moisture content - instructions for use - declaration of the words "avoid inhalation" - For each animal species: recommended and maximum inclusion level expressed as percentage of the total nitrogen content of the complete feedingstuffs Declaration to be made on the label or packaging of the com-

						pound feedingstuffs: -the name: "Protein product obtained by fermentation of natural gas" - amount of the product contained in the feedingstuff"

OPINION OF THE COMMITTEE

The protein product presently being considered is another example of an edible bioprotein produced by cultivation of bacteria of the Methylococcaceae family together with certain other bacteria, for which product data on performance in livestock feeding have become available to the Committees and for which appropriate dossiers were supplied. BioProtein* is obtained by growing *Methylococcus capsulatus* (Bath), strain NCIMB-11123, aerobically on natural gas composed of 91% methane, 5.1% ethane, 1.9% propane, 0.4% isobutane, 0.5% n-butane and 1.1% other minor components. The bacteria oxidise methane through intermediate steps ending with CO_2 and thereby form a biomass. Ammonia is used as nitrogen source and appropriate mineral salts are added to satisfy the culture requirements.

The oxidation products arising during the fermentation process can inhibit, however, the growth of *Methylococcus capsulatus*, and it is therefore necessary to include other bacteria in the production line, which are able to utilise these inhibitory oxidation products. These are *Alcaligenes acidovorans DB3* (strain NCIMB 12387), *Bacillus brevis* DB4 (strain NCIMB 13288) and *Bacillus firmus DB5* (strain NCIMB 13280). These 4 bacterial strains form an ecosystem which in part ensures a stable production performance and in part protects the fermenting culture against unwanted contamination with other bacteria by occupying all niches which might be available to intruding micro-organisms.

Nutritional value

The protein product is marketed as a free-flowing non-dusty agglomerate (particle diameter 100-300 ìm). The crude protein content (Nx6.25) of the product is approximately 70% on a dry weight basis and includes minor contributions of nitrogen compounds other than protein, notable nucleic acids. The true protein content is 66% of dry weight. It also contains 9% crude fat, 7% crude ash, 7% nucleic acid, and a maximum of 100mg/kg copper. The N-free fraction is composed of 4.5% glucose, 2.4% starch and variable amounts of unidentified cellwall polysaccharides, and about 7% crude ash. The amino acid pattern, typical of a single cell protein, consists of lysine 6.5, methionine 2.8, methionine-cysteine 3.4, threonine 4.7. tryptophan 2.2, arginine 6.2, tyrosine 3.4 g/&§g N and a number of others in smaller amounts.

The true digestibility of the protein is 78.5% and the biological value 84% as determined in the rat. The NPU (net protein utilisation) is about 66% of the biological value with PU (protein utilisation = NPU x protein content) being 44.7%.

Further information on the amino acid profile of the protein product and their digestibility has now been supplied. the protein product is nutritionally characterised by a high protein content and a well balanced amino acid profile compared with other protein sources used in animal feeds.

The content of essential and semi-essential amino acids has been compared with that of fish meal, meat and bone meal, meat meal, blood meal, soybean meal, soyprotein concentrate and rape seed meal. The levels of methionine and cystine, the S-containing amino acids, and the lysine and arginine are somewhat lower, while the levels of threonine, tryptophan, leucine, isoleucine and phenylalanine are higher than those found in fishmeal (Pedersen et al., 1994).

The digestibility of the amino acids has also been studied in mink, pigs and poultry (Skrede *et al*., 1994) and values for veal calves and salmon are supplied in the second supplementary dossier (February, 1995). The D-amino acids are most likely to derive from the peptidoglycan murein that is present in the bacterial wall. They do not present a nutritional problem for the target species.

Information has now been supplied on the composition of the lipid fraction of the protein product. New data were obtained by using gas chromatography combined with mass spectrometry and a full list of all fatty acids present including cyclopropanoic acid (cycC17:0 or 9,10 methylene hexadecanoic acid) is now available. The corrected cyclopropanoic acid content is now calculated to be about 1.6%. In a three months feeding study in pigs no accumulation of cyclopropanoic acids was demonstrable in the body fat. The content of n-3 fatty acids in fish fed the protein product shows only a small reduction in C 22:5 and C 22:6 fatty acids when the diet included 37% of the protein product, allowing the conclusion that the cyclopropanoic acid does not interfere significantly with the elongases and desaturases involved in the synthesis of the nutritionally important n-3 fatty acids in salmon fed the protein product.

Apart from the sensory evaluation of chicken meat which was supplied in the original submission sensory evaluation data have now been made available for salmon fed for 2 years four diets containing the protein product and also for the back fat of pigs fed the protein product for 100 days. In neither instance was a negative effect on the quality of the edible products from animals and fish fed the protein product noted.

The protein product has been tested for its efficacy and nutritive value only in trials conducted under laboratory conditions. No field trials with pigs and veal calves are available, and no trials at all with calves starting at birth weight The adverse effect of 12% inclusion for veal calves is probably the result of nutritional imbalance. The protein product is proposed to be used at 8% in the feed of calves as a replacement of some of the skimmed milk in milk feeds. An experiment was carried out over 10 days to study the acceptability and functionality of calf milk replacers containing 10%, 20% and 30% protein product.

The results showed no acceptability problems arising even with a dietary incorporation of 30% bioprotein. But these experiments were not carried out with calves at birth weight. The protein product was, however, found to be unstable in calf milk replacers and caused faeces to be stiffer and to have an aberrant grey green colour.

In a second experiment on the digestibility of including either 10% or 20% of the protein product in calf feed there was a significant reduction in apparent digestibility of the crude protein (72% against 92%) and a similar reduction in the digestibility of carbohydrates (38% against 98%) but no effect on the digestibility of fat (95%) compared to the control diet. Only at the 10% inclusion level were daily weight gains and feed conversion ratios comparable to the control diet. The digestibilities of the amino acids were well below (10%-20%) those of skim milk.

In a third combined balance and growth study in calves 4%, 8%, 12%, 18% and 24% of the protein product were used in combination with 44%, 38%, 32%, 26% and 14.6% skimmed milk powder.

The feeds were also corrected for lysine, methionine and threonine content. Inclusion levels above 12% reduced the digestibility of the test diets. Weight gain, nitrogen retention, metabolisable energy content (2.8 Mcal/kg bioprotein against 4.5 Mcal/kg casein) and feed conversion ratio were reduced.

The nutritive value of the protein product for pigs in terms of protein and energy value was studied in piglets and compared with that of fishmeal. In a second experiment the protein digestibility of the bioprotein was 72% and the net energy was 8.96 MJ/kg.

In a 28 day study, using 120 weaned piglets aged 28 days, either 4%, 8% or 12% of the protein product were included in the feed. Daily weight gain was highest when 4% bioprotein were included (1 kg bioprotein replaced 0.85 kg LT fishmeal + 0.15 kg cereals, extra lysine was added to meet Danish recommendations). Inclusion of 12% yielded a reduced feed conversion ratio. These results support the inclusion of 8% protein product in pig feed as replacement for fishmeal, a level causing no adverse effects on the performance and health of piglets. However no dose-response studies over longer periods are available and no large field trials exist with 8% inclusion levels in pig feeds in EU countries.

The nutritive value of bioprotein for Atlantic salmon was studied in several trials. Fish weighing 70 g were maintained in seawater and fed feed with inclusion levels of 6.5%, 13% and 26% of the protein product. Growth was good and showed no difference in daily weight gains and feed conversion ratio between the groups. The highest inclusion level produced a tendency for reduced growth but no explanation is given for this effect.

In another dose-response trial in fresh water using fish of average weight of 650 g, up to 36% replacement of fish protein by the protein product caused no significant depression in growth rate compared to controls. Feed conversion ratio was also unaffected and no pathological effects attributable to the bioprotein were noted. At higher inclusion levels weight gain and feed conversion ratio were impaired.

Significant differences in growth were observed with atlantic salmon, in sea water with 19,3% of the protein product inclusion against 36% inclusion in the diet for 14 months from first feeding. Optimal inclusion levels averages 20%. This means that 25% of dietary amino acids can be provided by the protein product.

In a trial on juvenile fish weighing 60 g the protein and amino acid digestibility decreased linearly with increasing inclusion levels of 5%, 9.9%, 19.3% and 37% of the protein product. Fat digestibility and metabolisable energy of the diet also decreased but starch digestibility increased. The palatability of the composite feed was as good as that of the conventional fishmeal diet.

In a further diet performance trial over 4 periods, each of 28 days, in juvenile salmon weighing 0.2 g similar growth was obtained with up to 20% protein replacement by the protein product. Inclusion of 37% reduced growth and caused higher though unexplained mortality during the starting feeding period. The digestibility of the protein product was calculated to be 79%, of the fat 78% and of the starch 68%. There is insufficient information about the metabolism of the non-protein nitrogen fraction to estimate the metabolisable energy or net energy.

In another test using salmon weighing between 50 and 64 g there was no difference from controls with regard to growth and digestibility of the protein product for the first month. After 3 months the group fed the protein product showed better growth and better digestibility of amino acids. No adverse effects were seen with inclusion of 19% after 6 months. Fin erosion was noted in all groups.

The inclusion level of 19-20% in salmon diets (this means an inclusion of 14% protein) from Bioprotein seems the optimum. Seawater fishes support 35% inclusion level without differences in growth rate and feed conversion rate.

On the basis of the foregoing information the Committees concluded that the protein product appears to have a good nutritional value and to be an adequate protein source for fish. In pigs and calves adverse results occurred when the inclusion in feeds exceeds certain values.

The role of the comparatively high nucleic acid content and the high ash content is unclear and with some species, such as chicken, a palatability problem is apparent. No quantitative information has been supplied on the presence of D-amino acids but their presence is not regarded as of nutritional importance. The available efficacy trials are few in number, are only carried out in experimental groups, and are of short duration. No field trials have been reported. There are no efficacy trials in calves at birth weight nor in piglets weaned before 28 days of age.

Evaluation of risks

Methylococcus capsulatus (Bath), the bacterial strain used for the production of the protein product, is the type species for the family of Methylococcaceae. It represents about 90% of the stock culture. It occurs naturally in aerobic environments where methane is available. Growth occurs at temperatures between 37°C and 52°C. It is not known to be pathogenic or toxicogenic. Because it grows only on methane and at high temperatures pathogenic effects cannot be expected.

Alcaligenes acidovorans DB3 belongs to the family of *Pseudomonadaceae* and represents about 8% of the stock culture. The description *Comamonas acidovorans DB3* does not appear in Bergey's Manual (1984). This bacterium also occurs naturally and it is not known to carry virulence plasmids. The non-pathogenicity of this bacterium is further supported by the results of experiments carried out on mice by i.v. injection of 10^4 - 10^9 viable cells/kg b.w. Weight gain was not influenced by this treatment. No treatment-related macroscopic pathological changes were observed.

Bacillus brevis DB4 belongs to the genus Bacillus, but the species used is heterogeneous. It represents about 1% of the stock culture. The strain used in the manufacture of the protein product also occurs naturally and does not carry any known virulence plasmids. The non-pathogenicity is supported by the results of experiments carried out on mice by t.v. injection of 10^4 - 10^9 viable cells/kg b.w. There were no adverse effects on weight gain. One mouse died with signs of shock following the i.v. injection. No treatment-related macroscopic pathological changes were observed. However, food poisoning has been performed with a suspension of washed bacteria and oral test for toxin production is needed.

Bacillus firmus DB5 belongs to the genus Bacillus and the species used is heterogeneous. The strain occurs naturally and does not carry any known virulence plasmids. It represents about 1% of the stock culture. the non-pathogenicity is supported by the results of experiments on mice using i.v. injection of 10^4 - 10^9 viable cells/kg b.w. There were no adverse effects on weight gain and no mortalities. No treatment-related macroscopic pathological changes were observed.

The protein product stock and production cultures are kept freeze-dried and are regularly tested for composition and for the presence of pathogenic contaminants. Pathogenic risks for man or animals can only be expected, if viable cells of the production strains escape the fermenter or the sterilization procedure of the final product is inadequate. Because the optimum temperatures of the bacterial strains used all exceed 37°C and of the need for special growth requirements as well as the experimental evidence of non-pathogenicity it is unlikely that these bacteria can act as human or animal pathogens.
Heat-stable toxic substances are not expected in the sterilised product because of the absence of adverse effects in the feeding trials with calves, piglets, chicken and salmon.

The protein product is a sterile product as concerns the bacteria used for production of the biomass. The commercial product is highly contaminated but the type and number of contaminating micro-organisms do not differ from those found in similar bioproteins.

All production strains are genetically stable and unlikely to mutate into antibiotic-producing or toxin-producing strains. The fermentation product is regularly checked for the presence of toxins and antibiotics. The protein product does not constitute a microbiological risk to animals or man. Accidental release into the environment will have no deleterious consequences as all organisms of the production culture are already present in natural habitats. Nevertheless, the *Bacillus brevis* strain used should be tested orally to exclude the possibility of toxin production.

No immunological monitoring of workers exposed to the protein product has been carried out. No data have been supplied on possible exposure to dust from the spray-dried biomass or the agglomerated product.

Effects in target species

A feeding study in calves, starting at weight 110 kg, extending over 7 weeks, with 4%-24% protein product in the diet produced no significant toxicological effects, although inclusion levels at and above 18% reduced weight gain and feed conversion ratio and caused difficulties with digestion. The latter showed itself as faeces of reduced stiff consistency and a reduced feed intake. Apparent faecal digestibility coefficients of dry matter, ash, organic matter, crude protein, crude fat, carbohydrates, and iron decreased with increasing inclusion percentage. Nitrogen utilisation decreased similarly. Utilisation of ingested iron increased with increasing inclusion of the bioprotein. No clinical parameters were reported. The NEL appeared to be the inclusion level of 12% in the diet.

A feeding study in piglets, aged 28 days and weighing 25 kg, extending over 4 weeks, with 4%-12% protein product in the diet showed no significant effects on health, appetite and daily weight gain. At the 12% inclusion level the feed conversion ratio was significantly smaller. No

clinical parameters were reported. The NEL appeared to be a replacement level of 8% fishmeal in the diet.

Some 5 feeding studies in salmon, extending over 14-20 weeks, with 5%-70% protein product in the diet showed good growth and no effect on the feed conversion ratio up to the 33% inclusion level. No signs of hepatotoxicity attributable to the protein product were noted. The palatability test indicated a preference for the diets containing the protein product but the digestibility of protein and fat were reduced with increasing incorporation levels.

From these data it can be concluded that the inclusion of the protein product in the feeds of the above 4 animal species carries no appreciable risks for animal health, when added at a rate of up to 12% in the diet of calves weighing 110 kg but not at that level as milk protein replacer in calves at birth weight, up to 8% in the diet of pigs weighing 25 kg but not at that level in suckling pigs, up to 3% in the diet of broiler chicken, and up to 33% in that of salmon.

The statement, that in none of the feeding trials did any health problems, related to a disturbance in the microbial flora of the gut, arise is not substantiated by any experimental evidence.

Effects on the quality of animal products

As the protein product actually used as additive contains only normal feed ingredients, e.g. proteins, fat, carbohydrates and minerals, no toxic or other adverse residues are assumed to be present in the edible animal products. Tests for the presence of possible antibiotics and known toxins are carried out routinely on the added protein product. The organoleptic properties of the meat obtained from animals fed the protein product have been tested and found to be satisfactory.

Effects in laboratory animals

The nutritional studies provide evidence that this protein product is metabolized in the same way as conventional proteins.. Hence rigorous toxicological, metabolic and residue studies appear not to be meaningful.

No available data show that the bacterial strains used in production do not elaborate toxins and are not pathogenic. The analysis of the chemical composition of protein product has shown that nitrosamines are not present at the detection limit of 0.3ìg/kg dry matter, and that methanol and polycyclic aromatic hydrocarbons are not present at the detection limit of 5 mg/kg dry matter. No significant toxicity was noted in the feeding studies in target animals.

A 4-week oral feeding study in rats was carried out using 5 groups of Wistar rats, each of 5 males and 5 females, fed a standard diet containing added 0%, 5%, 10% and 15% of the protein product. A positive control group received added 15% of a standard protein. Only a few incidental clinical changes were noted. Bodyweight gain of all groups was comparable to controls. There were no consistent differences between the groups regarding food consumption. Serum urea levels of males and females in the top test and positive control groups were increased. Serum creatinine levels in females of these groups were reduced. The relative kidney weights of the 10% and 15% (females only) test groups and the positive control group were increased. These changes were the expected consequences of high protein intake. Gross pathology and limited histopathology showed no treatment-related changes.

A 90-day oral feeding study in rats was carried out using 5 groups of Wistar rats, each of 10 males and 10 females, fed a standard diet with added 0%, 5%, 10% and 15% of the protein product, the positive control group receiving added 15% standard protein.

No adverse clinical symptoms related to treatment were noted. Bodyweight, food consumption, water consumption and food conversion ratios were comparable to controls. Haematology was unremarkable.

The two top test groups showed a small rise in serum alanineaminotransferase levels indicating slight hepatocellular dysfunction. Serum ornithine carbamyltransferase levels were unchanged and hepatic histology was normal. Serum urea levels were increased in the top test and positive control groups. Urinary excretion of N-acetyl-Â-D-glucosaminase was increased in the top test groups and in males of the 10% test and positive control groups without any associated renal histopathology. This slight leakage of renal tubular enzymes was probably due to the high protein load but an additional toxic effect of the protein product cannot be excluded. The increase in female relative kidney weights of the top dose were probably related to the higher bodyweight. Gross and histopathology showed no treatment-related findings.

A 90-day oral feeding study in minipigs was carried out in 5 groups, each of 4 male and 4 female minipigs, given a standard diet with added 0%, 5%, 10% and 15% of the protein product, the positive control group receiving a corresponding high-protein diet.

There were no adverse clinical signs and no toxicologically significant changes in bodyweight, food consumption, haematology, clinical chemistry, urinalysis and organ weights compared to controls. Gross pathology and histopathology showed no treatment-related changes.

No studies have been carried out in laboratory animals on multigeneration-reproduction, teratogenicity, chronic toxicity and carcinogenicity. However, no toxic effects were noted on the reproductive organs in the laboratory animals examined. The submitter has undertaken to carry out reproduction studies, if use of the protein product is to be extended to animals capable of reproduction.

The protein product was examined for genotoxicity in a salmonella reverse mutation test using strains TA98, TA100, TA1535 and TA1537 +/- S9 and dose levels varying fro 0.63mg to 10 mg/plate. No increase in revertants was found, thereby confirming absence of mutagenic activity in this system.

The protein product was also tested in a mouse micronucleus test at dose levels of 1.25 g, 2.5 g and 5 g/kg b.w. No significant increase in polychromatic erythrocytes with micronuclei was seen, thereby confirming absence of mutagenic potential in this test system.

The absence of significant toxicity including genotoxicity and the anticipated digestive breakdown of the protein product appear to indicate that it is toxicologically safe as protein source in animal feeds at the proposed levels. Further animal testing is not deemed necessary.

Protective measures against dust inhalation for the production workers and the users are recommended pending information to be provided as to the allergenic potential of this bioprotein.

Effects on man

Skin and eye irritation potential was examined in rabbits and showed that the protein product was non-irritant to the skin and eyes. The allergenic potential of the protein product has not been investigated.

Effects on the environment

The protein product contains no substrate residues or heavy metal contaminants except for a maximum copper content of 100 mg/kg, dry matter. It carried no viable production organisms because of the sterilisation step in the production of the final product. Any escape into the environment of the production organisms from the fermenter causes no hazard as these organisms all occur naturally, are non-pathogenic, and carry no virulence plasmids.

Monitoring in foodstuffs

The protein product can be determined in feedingstuffs qualitatively by indirect immunofluorescence using antibodies specific against *Methylococcus capsulatus* (Bath) and quantitatively by a spectrofluorimetric method with a sensitivity better than +/- 1%. Techniques to determine the proportions of each of the constituent organisms are not described in the dossier.

Conclusions

The protein product obtained from methylotrophic bacteria has been the subject of a basic dossier and two supplementary dossiers prepared in accordance with the guidelines for the assessment of certain products used in animal nutrition. This report is therefore limited to the assessment of this particular protein product, in the light of the information provided.

The product examined has an acceptable nutritional value as a source of protein for feeding to animals provided the inclusion levels in feeds do not exceed the values set out in the suggested conditions of use. It is not suitable for ewe milk replacers and further extensions should fill the remaining gaps in the general nutritional information.

On the base of the information provided by the Firm, the product examined carries no appreciable risks for livestock, if the levels of incorporation do not exceed 8% in the ration of pigs starting at weight 25 kg, does not exceed 8% in the feed of veal calves starting at weight 80 kg, does not exceed 19% in the feed of freshwater salmon and 33% in the feed of seawater salmon up to 3 years. There were no data to protein level of inclusion in the feed of piglets and non ruminant calves.

It poses no appreciable risk on presently available evidence for the health of workers involved in its production, distribution and use, if adequate precautions are taken to prevent exposure to dust. Because the allergenic potential of the bioprotein has not been investigated a warning should be included on the label, that the dust may cause sensitisation by inhalation and may give rise to respiratory allergic reactions in susceptible people.

It carries no microbiological risks because of its origin from non-pathogenic naturally occurring bacteria, known not to produce antibiotics or toxins and not being present as viable organisms in the final product because of its sterilisation.

It has no adverse toxicological or genotoxic effects although reproductive toxicology, teratogenicity, chronic toxicity and carcinogenicity have not been specifically investigated in laboratory animals, and is free from harmful contaminants arising from the culture medium or manufacturing process but can contain up to 100 mg/kg dry weight of copper, which would be diluted when mixed into composite feeding stuffs. Its use in animal feed does not result in appreciable risks for the environment.

It carries no appreciable risk for the consumer from the consumption of products obtained from animals fed with a diet containing this protein product. The characteristics and organoleptic properties of such animal products from chicken, pigs and salmon have been investigated and show no deleterious properties.

It can be monitored in feedstuffs, although no techniques are described to determine the proportions contributed by each of the constituent 4 bacteria to the biomass.

Suggested conditions of use

During the examination of the registration files it has been observed growth depression in some target animal species, and that, based upon the information provided by the firm, it exists a lack of data concerning the metabolisms of cyclopropanoics and other non identified fatty acids, that are present in the product and may be present in the animal product lipids.

Further to these observations, the Committee has judged that it will be prudent to limit the conditions of usage of this product as follows:

- Growing pigs from 25 kg up to a 100 kg live weight.

- The quantity of inclusion in complete feedingstuffs should no exceed
    - 8% for piglets starting at 25 kg
    - 8% for veal calves starting at 80 kg
    -19% for salmon fish in fresh water
    -33% for salmon fish in seawater

- The amount of protein provided by the product should be expressed as percentage of the total protein content of the compound feedingstuffs

These declarations are to be made on the label or packaging of the compound feedingstuffs, and should be introduced in the usage conditions requested by the company (See annexed table)

Future extension of use to other species or type of animals

If in the near future the firm asks for an extension of use to other species or type of animals providing edible products to the human consumer the following information should be provided.

For chickens for fattening, data concerning the availability of the individual aminoacids at different inclusion levels to be able to elucidate the cause and mechanisms of the fall in performances and sufficient results to assess the optimum inclusion level to exclude nutritional imbalances from an overdosage of the product in their diet. For pigs: more field trials are required

For all animals providing edible products to the consumer, analysis of the true content of cyclopropanoic acid in their lipids, instead of the expected content by calculation.

References

Dossiers on BioProtein*, Sections I-IV (1994) by Dansk Bioprotein A/S, Denmark
Pedersen A.-T., Skrede A., 011i J. & Eggebo L.M. (1994) Report submitted Oct. 15, 1994.
Skrede A., Herstad O., Sundstol F., Overland M. & Mroz, Z. (1994) Report submitted Oct. 15, 1994.
Supplement (VII) dossier by Danks Bioprotein A/S (1995).

Suggested conditions of use

1	2	3	4	5	6	7
Name of product group	Name of product	Designation of nutritive principle or identity of the micro-organism	Nutrient substrate Specifications if any	Composition characteristics of the product	Animal species	Special provisions
1.1. Bacteria 1.1.2. Bacteria cultivated on natural gas	1.1.2.1 Protein product of fermentation from natural gas obtained by culture of: *Methylococcus Capsulatus* (Bath) *Alcaligenes Acidovorans*, *Bacillus brevis,* *Bacillus*	*Methylococcus capsulatus* (Bath) NCIMB 11132, *Alcaligenes acidovorans* NCIMB 12387, *Bacillus brevis* NCIMB 13288, *Bacillus firmus* NCIMB 13280	Natural gas, 91% methane, 5,1% ethane 1,9% propane 0,4% isobutane 0,5% n-butane 1,1% other minor components), ammonia, mineral salts	Nitrogen expressed as crude protein minimum 65%	- Pigs from 25 kg - Calves from 80 - Salmon Fish.	Declaration to be made on the label or packaging of the product: - the name: "Protein product obtained by fer- - nitrogen expressed as crude protein - crude ash - crude fat - moisture content - instructions for use - declaration of the words "avoid inhalation" - The quantity of inclusion in complete - 8% for piglets starting at 25 kg - 8% for veal calves starrting at 80 kg - 19% for salmon fish in fresh water - 33% for salmon fish in sea water Declaration to be made on the label or packaging of the compound feedingstuffs:

	firmus, -the cells of which have been killed					- the name: "Protein product obtained by fer- - Amount of the product contained in the - Amount of protein provided by the product