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 METHYLOMONADACEAE FAMILY

Opinion expressed 25 September 1985

TERMS OF REFERENCE (October 1983)

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

1. Do the products obtained from bacteria of the family of Methylomonadaceae and, in particular, from Methylophilus methylotrophus cultivated on methanol (Pruteen) (*) have a nutritional value for animals because they provide nitrogen or protein ?

2. Can the use in animal nutrition of products obtained from these bacteria and, in particular, from Pruteen (*) result in risks for human (consumer or user) or animal health, or be prejudicial to the environment ?

BACKGROUND

In accordance with the provisions of Council Directive 82/471/EEC of 30 June 1982 concerning certain products used in animal nutrition (**), Member States may, until such time as a Community decision has been taken, maintain authorizations granted within their territories before the date of application of the Directive concerning in particular products not listed under the product group indicated in Section 1.1. (Bacteria) of the Annex to the Directive.

(*) Registered trade name.
(**) OJ No L 213, 21.07.1982, p. 8

In accordance with the established procedure, the Commission consults the Scientific Committee for Animal Nutrition and the Scientific Committee for Food before producing a draft of the Community measures to be adopted for the compounds concerned.

OPINION OF THE COMMITTEES

Introduction

The Committees draw the Commission's attention to the fact that the term "Methylomonadaceae" has been replaced recently by the term "Methylococcaceae" (cf. Bergey's Manual of Systematic Bacteriology, vol. 1, Williams and Wilkins, Baltimore/London 1984). This family of bacteria includes various groups of rods, vibrios and cocci having the common characteristic of being able to utilize methane as their sole source of carbon and energy in earobic conditions.

Several bacteria of the family can be cultivated to produce edible bioproteins. However, the only exhaustive data on performance in livestock feeding at present available to the Committees were those concerning Pruteen (*), a bioprotein produced by Imperial Chemical Industries PLC, the dossiers of which were supplied. Pruteen (*) is obtained by growing Methylophilus methylotrophus (synonyms : Pseudomonas methylotropha, Methylomonas methylotropha), strain NCIB 10.515, on methanol. (Talbot C.J. et al., 1980).

In the early manufacturing process Pruteen (*) was not treated with hydrogen peroxide. The present procedure, which makes use of the treatment of the biomass with hydrogen peroxide before final dessication, was initially introduced as a bleaching measure. This treatment results in a partial oxidation of methionine to methionine sulphoxide and methionine sulphone, and cysteine and cystine to cysteic acid without major changes in the nutritional value of Pruteen (*). Of these conversion products, only methionine sulphone and cysteic acid, which account for a small proportion, are not bioavailable.

The only biologically significant modification noted was that the peroxide treated product was better tolerated by the chicken. On the basis of their similarity data available on both products have been used in the global evaluation of the safety of presently manufactured material. This evaluation refers exclusively to the use of Pruteen (*) in animal feedingstuffs. The material specified in the submission is not intended for human consumption without further processing.

1. Nutritional value

Pruteen (*) is marketed in two forms : granules (particle diameter : 500 microns) and powder (particle diameter : 25 microns). Its average crude protein content (N x 6.25) is 72.0 + 2% for the granules and 70.3 + 2% for the powder. The product contains 12.5 g nitrogen/100 g dry matter, of which 19.3% is in the form of nucleic nitrogen (13.8 g nucleic acid/100 g dry matter). The nitrogen of the cell wall of the bacteria (muramic acid + diaminopimelic acid + glucosamine and ethanolamine) represents 2.7% of the total nitrogen. The true protein content is 54.7 g per 100 g dry matter.

The amino acid composition of the protein of the product is (g/16 g nitrogen) : lysine 5.7; threonine 4.7; methionine 1.9 and cystine 0.7. Methionine is the first limiting amino acid in the rat. The availibility of lysine, methionine and thryptophan, determined in chickens, is respectively 89, 90 and 97 % (Abbey et al. 1980).

The chemical score of this protein is 59 as against that of the egg, 44 for soya, 66 for skimmed-milk powder and 74 for good quality fishmeal. The biological value of this protein, determined in the rat, varies from 66.2 to 76.8%, depending on the experiment. The addition to the diet of 0.2% of methionine raises this value to 88.4%.

Since the average digestibility of this protein is 90 + 2% in the rat, pig, calf and poultry, the net protein utilisation (NPU) is at least 90% of the biological value. In the rat and pig, the NPU is 65-70%. This figure is very close to that of soya (71%) but lower than that of good quality fishmeal (80%).

The constitutive fat of Pruteen (*) is accounted for essentially by phospholipids. The content in total lipids after addition of soya oil in amounts varying according to the form of the product, is of 8.5 + 1.5% for the granules and 13.2 + 2.0% for the powder. The fatty acids are chiefly made up of palmitic and palmitoleic acids. Linear fatty acids with an uneven number of carbon atoms are present in small quantities. 9, 10-methylene hexadecanoic acid (C 17) is the only cyclopropane acid present. According to Steel et al. (1977), the level of this acid in granula Pruteen (*) is 0.25% of dry weight, i.e. 3.2% of the total fatty acid. No cyclopropene acid has been detected.

The mean digestibility of the fat is 93% in the pig, calf and poultry for levels of incorporation of 6.5-37.5 of the ration. Mean metabolizable energy content of the product fed to poultry is 14.38 kJ/kg (Bolton and Blair 1974).

Pruteen (*) has been tested for its efficacy in numerous trials (Vogt et al. 1975, Hinks 1977, Braude et al. 1977, Waterworth and Heath 1981, Lloyd 1983). 64 trials grouping 194.250 subjects were carried out on poultry; 33 trials grouping 4.782 subjects on pigs and piglets, 69 trials grouping 8.150 subjects on calves and lambs, and 16 trials grouping 109.000 subjects on fish. Performances equal and sometimes superior to those of fishmeal and soya at isonitrogenous levels were obtained in mammals and birds with rations containing 5-10% of the product and fish with rations containing 30%.

On the basis of the foregoing information, Pruteen (*) has a good nutritional value as a source of protein for feeding to pigs, calves, poultry and fish.

2. Evaluation of risks

2.1. Pathogenicity and hypersensitivity

The bacterial strain used for the production of Pruteen (*) can be differentiated from other methylotrophic strains of the family of Methylococcaceae by variations in the composition of its cell walls (phospholipids, hydroxylated fatty acids, etc.) and DNA, and by its sensitivity towards antibiotics. On the other hand, the strain presents similarities to other gram-negative organisms, particularly E. coli and Salmonella spp., in its cell-wall structure.

On account of the obligate methylotrophic properties of this strain, it is reasonable to assume that viable cells which may normally escape the fermenter are unlikely to present any pathogenic risks for man or animals. This view-point is supported by the results of experiments carried out on mice by i.p. injection of 10^{10}-10^{12} viable or dead cells. Animal mortalities induced by either viable or dead cells were very similar and both significantly lower than mortalities ob- served with known pathogenic bacteria such as E. coli, Salmonella spp. and Staphylycoccus aureus. Moreover, no microbial proliferation was observed in the peritoneal cavity and in the organs of mice treated with viable cells.

Immunological monitoring of workers exposed to Pruteen (*) during its production has not revealed any allergic reaction, although exposure to dust concentrations exceeding 10 mg/m^3 resulted in influenza type symptoms and conjunctivitis which were probably irritative in nature. These findings stress the importance of controlling dust formation during the production, distribution and use. This control has been achieved by adopting special precautions when granulating or grinding (e.g. the addition of soya oil and use of a room with forced ventilation).

From these data, it emerges that no significant risks exist for the health of workers involved in production, distribution and use of Pruteen (*) if adequate precautions to prevent exposure to dust are taken.

2.2. Biological and toxicological aspects

2.2.1. Effects in target species

Feeding studies in pigs extending over 12-20 weeks with untreated or hydrogen peroxide treated Pruteen (*) used levels ranging from 2.25-30% in the diet. Growth rates and feed intake were improved but blood uric acid levels increased temporary at the 6.5% level. At higher levels of incorporation, feed intake, growth, kidney weights, liver weight, and adrenal weights were reduced. A 6-litter three generation study showed no pregnancy or litter abnormalities. A teratology study using levels of feeding from 8-18% did not show adverse effects. The 98-day feeding study with the treated-product showed slightly increased kidney weights compared to controls at the 30% test level but no histopathological abnormalities associated with this finding.

Feeding tests in calves extending over 16 weeks with 15-25% untreated Pruteen (*) produced no significant toxicological effects, although weight gain and feed conversion were reduced. Further studies with hydrogen peroxide - treated Pruteen (*) showed essentially the same effects (Sedgman, Roy and Thomas, 1985; Sedgman et al., 1985).
Nine different feeding experiments were carried out on broilers, layers and breeding hens with untreated or hydrogen peroxide-treated Pruteen (*) administered at levels of 2.5 to 25%. Dose levels above 10% reduced growth and lowered haemoglobin. At the 25% level liver and spleen weights increased as did the activity of the serum transaminases.

The livers of female birds developed necrotic and granulomatous lesions with the untreated product. Of the avian species tested (chickens, turkeys, quails, ducks), only chickens developed these hepatic lesions.

In two 8-week studies in broilers and one study in layers, the hydrogen peroxide-treated product did not increase the incidence of hepatic lesions at the level of 25% in the diet. The reason for this effect of the treatment with hydrogen peroxide is not known. Egg production and hatchability were not affected.

Trout fed on Pruteen (*) at 10-30% in the diet showed no adverse effects.

From these data, it emerges that Pruteen (*) as it is manufactured at present carries no appreciable risks for animal health when added at a rate of up to 7% in the diet of pigs, 10% in the diet of calves and poultry, and 30% in that of fish.

2.2.2. Effects on the quality of animal products

The use of Pruteen (*) at the recommended levels of incorporation (cf. point 2.2.1.) and at higher levels over long periods (10-30% for 16-17 weeks in pigs, 20% for 20 weeks in calves, 30% for 20 weeks in trout, 7.5-30% for more than 50 weeks in chickens) hardly alters fatty acid composition in the tissues and products of target species. Under the proposed conditions of use, the presence of trace amounts of C 17-cyclopropane acid was detected by capillary gas chromatography in the adipose tissues of pigs (up to 0.10% of total fatty acids) and in the egg yolks (0.11% of total fatty acids). No trace of the acid was found in the adipose tissues of chicken, calves or trout (limit of detection : 0.02% of total fatty acids). In pigs, the small quantities of cyclypropane acid are eliminated slowly. In man, degradation by beta and omega oxidation is probable (Lindstedt et al. 1974).

A comparative study of the technological and organoleptic properties of meat and eggs obtained from animals fed with Pruteen (*) and from those reared on conventional protein sources showed no significant difference.

2.2.3. Effects in laboratory animals

Numerous nutritional studies provide evidence that this bioprotein is metabolized in the same way as conventional proteins. With regard to toxicity studies, traditional toxicological tests are difficult to carry out on proteins because of the nutritional imbalance induced at high levels of incorporation in the diet. For this reason, isonitrogenous control diets based on casein were frequently included in the studies.

Four 90-day studies in rats using doses from 3.7-30% of untreated Pruteen (*) and two 90-day studies using 15 and 30% of the hydrogen peroxide-treated product showed little in the way of toxic effects. Serum levels of urea and allantoin, and kidney weights were increased in most tests only at doses of 30%. The slight but consistent decrease in haemoglobin was still within the normal range. Corticomedullary nephrocalcinosis without alteration of the kidney function was noted in female rats at doses of 15 and 30% and in male rats at the dose of 30%. The peroxide-treated materials was of the same toxicity as the untreated one. Several multigeneration reproduction tests on the untreated material showed no significant effects on reproductive function or litter parameters nor were there any teratological effects at 15 and 30% dietary levels.

A two-year chronic toxicity/carcinogenicity study using 7.5, 15 and 30% of untreated Pruteen (*) in the diet showed the effects of high protein intake and ionic imbalance at the levels of 15 and 30%. No carcinogenic effect was noted.

Beagle dogs from 6 weeks to 1 year were fed on a diet containing untreated Pruteen (*) at 30% and 60% levels and on an isonitrogenous casein diet. With the different diets, enlarged kidneys with fatty infiltration were observed and interpreted as a result of the protein overload. No urinary calculi were found. In the males, there was no significant difference in incidence between the casein controls and test dogs; in the females, dogs fed 60% Pruteen (*) had heavier kidneys than those which had been fed the isonitrogenous casein diet. 3 dogs out of 8 fed on a 60 % Pruteen (*) based diet and 1 dog out of 8 fed on a 30 % diet developed a transient weakness of hind limbs. Prolonged treatment produced hygromas of elbows and hocks. No cause could be identified. Among the species studied, only the dogs were affected and these effects appeared to be specific. One single experiment was done in Dalmatian dogs; 50 % Pruteen (*) diet raised serum urate.

The product was also submitted to mutagenicity tests. A dominant lethal test of untreated Pruteen (*) in mice using 15 % and 30 % in the diet, but with only 5-day male exposure, showed no mutagenic potential. A S. typhimurium reversion test, including S 9 metabolic activation, on a neutralised alkaline solution of both peroxide-treated and untreated material and of an in vitro enzymatic digest, and a mouse micronucleus test with the treated material also showed negative results. A host-mediated assay on hydrogen peroxide-treated product in mice with S. typhimurium TA 1537, TA 98 and TA 100 was inconclusive.

No mutagenic, carcinogenic or teratogenic effects have been observed in the studies reviewed with the product as prepared at present. No other adverse effects have been noted in laboratory animals if fed up to 7.5 % in the diet, provided the nutritional balance is observed.

3. Conclusions

In the light of available information, most of which is presented in this report, the Committees express the following opinion :

3.1. Of the protein products obtained from methylotrophic bacteria intended to be used as a source of protein in feedingstuffs, only Pruteen (*) has so far been the subject of a dossier forwarded to the members of the Committees and prepared in accordance with the "Guidelines for the assessment of certain products used in animal nutrition" (a). This opinion of the Committees is therefore limited to the assessment of this product.

3.2. In reply to question 1 of the Commission, the product examined has a good nutritional value as a source of protein for feeding to pigs, calves, poultry and fish.

3.3. In reply to question 2 of the Commission the product examined

- carries no appreciable risks for livestock if the level of incorporation does not exceed 7 % in the ration of pigs, 10 % in the ration of calves and poultry, and 30 % in that of fish,

- poses no appreciable risks for the health of workers involved in its production, distribution and use if adequate precautions are taken to prevent exposure to dust,

(a) OJ n° L 126, 13.05.1983, p. 23

- carries no appreciable risks for the consumer from the consumption of products obtained from animals fed with a diet containing it. In addition, the characteristics and organoleptic properties of such products are not different from those of products obtained from animals reared on conventional protein sources,

- which originates from non-pathogenic bacteria, has no toxicological effects and is free from any residues of the culture medium or harmful contaminants. Its use in animal feed does not result in appreciable risks for the environment.

3.4. The Committees cannot give an opinion on other products obtained from bacteria of the family of Methylomonadaceae even if cultivated under the same conditions as Pruteen (*), without assurance as to the equivalence of the characteristics of such products.

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