Commission of the European Communities

agriculture

REPORTS OF THE SCIENTIFIC COMMITTEE FOR ANIMAL NUTRITION (Third series)

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Directorate-General Agriculture

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C O N T E N T S

FOREWORD

This publication, which constitutes the third series of reports by the Scientific Committee for Animal Nutrition[1], contains the Committee's opinions on the safety of certain products proposed as additives in feedingstuffs and also "Guidelines for the assessment of certain products to be used as a source of proteins in animal nutrition".

Since new uses of known products or new products with beneficial effects on the quality of feedingstuffs and on the health or growth of animals were involved, it was necessary to ensure that the proposed conditions for use fulfilled the requirements of Community legislation on feedingstuffs and carried no risks for animals and, indirectly, for human beings and the environment. The scientifically-based opinions expressed by the Committee dispelled any doubts in this connection.

The "Guidelines", laid down by the Committee at the Commission's request, are designed to gather together the necessary documentation for the assessment of non-traditional products abtained from culturing micro-organisms and likely to be authorized as new protein sources in animal nutrition.

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(1) The first and second series of reports have been published by the Office for Official Publications of the European Communities in Luxembourg, under catalogue numbers CB-28-79-277-EN-C and CD-NK-80-002-EN-C respectively.

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(1) Set up by Commission Decision 76/791/EEC, 24 September 1976 (OJ N° L 279, 9.10.1976, p. 35)
(2) Chairman
(3) Vice-Chairman
(4) Resigned from Committee on 4 June 1980
(5) Resigned from Committee on 1 April 1980
(6) Commission of the European Communities, Directorate-General for Agriculture.

REPORT OF THE SCIENTIFIC COMMITTEE FOR ANIMAL NUTRITION ON THE USE OF FUMARIC AND MALIC ACIDS IN FEEDINGSTUFFS

Opinion expressed 15 January 1980

TERMS OF REFERENCE (March 1979)

The Scientific Committee for Animal Nutrition is requested to give an opinion on the following questions :

1. Do fumaric and DL-malic acids added to feedingstuffs act as preservatives ?

2. Can they have harmful effects on the animal organism ?

3. Can they have adverse effects on the quality of animal products ?

4. Taking into account the answers to the abovementioned questions, should the use of these acids as preservatives in feedinstuffs be continued and, if so, should the conditions of use be restricted ?

BACKGROUND

According to the provisions of Council Directive 70/524/EEC, of 23 November 1970, on additives in feedingstuffs (1), as last amended by the twenty-sixth Commission Directive of 18 December 1978 (2), Member States are authorized to make use, by way of derogation until 31 December 1979, of fumaric acid without specific conditions of use and malic acid for ensilaged feedingstuffs.

It is planned to authorize these products as preservatives in feedingstuffs throughout the Community.

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(1) OJ No L 270, 14.12.1970, p. 1
(2) OJ No L 39, 14. 2.1979, p. 11

OPINION OF THE COMMITTEE

1. Fumaric and DL-malic acid are used as preservatives in certain feedingstuffs because of their antifungal and antibacterial properties. Their efficacy varies according to the conditions of use.

2. Fumaric and L-malic acid are natural components of plant and animal organisms which are consumed daily. They participate in many biochemical processes and are intermediate products of the Krebs cycle. D-malic acid and DL-malic acid (racemic mixture of the D- and L-isomers) do not occur naturally.

   These acids have a very low acute chronic toxicity. Long-term oral administration of fumaric acid produced no ill effects in rats at a concentration of 1.2 % in the diet, in guinea pigs at 1.0 % and in dogs at 5.0 % (Landwirtschaftliche Schriftenreihe 1979). Long-term oral administration of DL-malic acid was without adverse effects in rats at concentrations of 0.05 % and 0.5 % in the diet. No adverse biological effects have been observed in dogs at a dietary concentration of 5 % (National Technical Information Service 1975).

   The Joint FAO/WHO Expert Committee on Food Additives established an ADI of 6 mg/kg body weight for fumaric acid. The Codex Alimentarius Committee on Food Additives approved the use of this acid in jams and jellies, subject to a maximum level of 3 g/kg, and the use of DL-malic acid in certain fruit and vegetable preserves, jams, jellies and some fruit juices. The establishment of an ADI for DL-malic acid was not considered necessary (Joint FAO/WHO Food Standards Programme 1979).

3. Fumaric and DL-malic acid are normal intermediate metabolites in the animal organism and have no adverse effects on the quality of animal products.

4. In view of the above, the Committee is of the opinion that the use of fumaric and DL-malic acid as preservatives in feedingstuffs does not constitute any risk for animal or human health and does not have any unfavourable effects on the quality of animal products. It appears unnecessary to lay down any specific conditions for this particular use of the two acids.

NOTE

Although the opinion of the Committee has not been requested on this issue, its attention was drawn to the interest in these products as growth promoters. A symposium held at Gelsenkirchen in 1978 (Landwirtschaftliche Schriftenreihe 1979) concluded that the addition of certain concentrations of fumaric acid to the ration appreciably improves the growth rate of piglets after weaning, and of calves. It also improves the feed conversion rate of fattening pigs and broiler chicken. Scipioni et al. (1979) are of the opinion that fumaric and malic acid do not affect the growth of piglets up to the age of two months. However, appreciable weight gain manifests itself from the third month onwards. Other recent studies have demonstrated that the addition of fumaric acid to the feeding stuffs for beef cattle resulted in a 5 % improvement in growth.

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REPORT OF THE SCIENTIFIC COMMITTEE FOR ANIMAL NUTRITION ON THE USE OF HYDROCHLORIC AND SULPHURIC ACIDS IN FEEDINGSTUFFS

Opinion expressed 15 January 1980

TERMS OF REFERENCE (March 1979)

The Scientific Committee for Animal Nutrition is requested to give an opinion on the following questions :

1. Do hydrochloric and sulphuric acids, added at low levels to feedingstuffs, act as preservatives ?

2. Can they have harmful effects on the animal organism ?

3. Can they have adverse effects on the quality of animal products ?

4. Taking into account the answers to the abovementioned questions, should the use of these acids in feedingstuffs be continued, and if so, should the conditions of use be restricted ?

BACKGROUND

According to the provisions of Council Directive 70/524/EEC, of 23 November 1970, on additives in feedingstuffs (1), as last amended by the twenty-sixth Commission Directive of 18 December 1978 (2), Member States are authorized to make use, by way of derogation until 31 December 1979, of hydrochloric and sulphuric acids for ensilaged feedingstuffs.

Authorization to use these products as preservatives in feedingstuffs, without specific conditions of use, was requested by one Member State.

(1) OJ No L 270, 14.12.1970, p. 1
(2) OJ No L 39, 14.02.1979, p. 11

OPINION OF THE COMMITTEE

1. The use of mixtures of mineral acids, particularly technical grade hydrochloric and sulphuric acids, for the preservation of ensilaged fodders goes back to the period 1930-1935. This method, which is known to be effective, consists of acidifying the fodders with a quantity of 5 - 10 % of mineral acids, diluted so as to obtain a pH of 3-4.

   More recently, it has been reported that the addition of 1 - 3 % concentrated hydrochloric or sulphuric acid (mixed with phosphoric acid) to liquid molassed feedingstuffs supplemented by non-protein nitrogen compounds enhanced the effectiveness of these compounds in ruminants. It is likely that this beneficial effect results from a better distributed daily consumption of non-protein nitrogen compounds. It is known that the acidification of feedingstuffs leads to a less massive and more frequent feed consumption in ruminants and that this method of feeding favours the assimilation of non-protein nitrogen compounds.

2. The use of hydrochloric and sulphuric acids under the conditions of use recommended for the preservation of ensilaged fodders does not present any danger for ruminants. The cases of acidosis and demineralization observed resulted either from an excessive consumption of acidified fodder or from feeding which was deficient in alkaline salts.

   The addition of 1 - 3 % hydrochloric or sulphuric acid to liquid molassed feedingstuffs, supplemented by non-protein nitrogen compounds, does not appear likely to produce metabolic disturbances. The consumption of these feedingstuffs by ruminants is limited. In addition the presence of heavy metals or other impurities in the technical grades of these acids (cf. Tables I and II) does not pose any problem at the levels at which these acids are used in feedingstuffs. According to the provisions of Council Directive 74/63/EEC of 17 December 1973 on the fixing of maximum permitted levels for undesirable substances and products in feedingstuffs (1), the maximum levels of arsenic, lead, fluorine and mercury tolerated in feedingstuffs for adult bovine animals are 2, 5, 50 and 0.1 mg/kg compound feedingstuff, respectively.

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(1) OJ No L 38, 11.02.1974, p. 31

Table I

Average levels of impurities in concentrated hydrochloric acid of technical quality	
SO_3	50 ppm
Cl^- (free)	0 - 20 ppm
Fe	0 - 10 ppm
Pb	none
As	none
Cu	none
F	none
Dry residue	0.04 %

Table II

Levels of impurities in sulphuric acid (66°B) of technical quality	
Calcination residue	max. 0.01 %
SO_2	max. 0.0003 %
Ammonium nitrogen	max. 0.0002 %
Nitric nitrogen	max. 0.0003 %
Cl	max. 0.0005 %
As	max. 0.00001 %
Fe	max. 0.002 %
Organic Matter	max. 0.0005 %
Sb	max. 0.0002 %
Mn	max. 0.00002 %
Cu	max. 0.0002 %
Zn	max. 0.004 %
Se	max. 0.0015 %
Pt	none
Ni	max. 0.00005 %
Ag	none
Co	max. 0.00015 %
Dry residue	max. 0.008 %
Ba	0.1 mg/l
Cd	< 0.01 mg/l
Cr	0.08 mg/l
Hg	< 0.01 mg/l
Pb	0.18 mg/l
F	< 1 mg/l

3. The addition of hydrochloric or sulphuric acid to ensilaged fodders or liquid molassed feedingstuffs containing non-protein nitrogen compounds is without effect on the quality of the animal products.

4. The Committee is of the opinion that the use of hydrochloric and sulphuric acids is acceptable for the preservation of ensilaged fodders and also for improving the effectiveness of non-protein nitrogen compounds added to liquid molassed feedingstuffs intended for ruminants. The addition of these acids to other types of feedingstuffs is only acceptable if supported by the relevant documentary evidence.

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REPORT OF THE SCIENTIFIC COMMITTEE FOR ANIMAL NUTRITION ON THE USE OF POLYETHYLENE GLYCOL 6000 AND OF A POLYOXYPROPYLENE-POLYOXYETHYLENE POLYMER IN FEEDINGSTUFFS

Opinion delivered on 15 January 1980

TERMS OF REFERENCE (March 1979)

The Scientific Committee for Animal Nutrition is requested to give its opinion on the following questions :

Could the use of polyethylene glycol 6000 and of a polymer of polyoxypropylene-polyoxyethylene (M.W. 6800 - 9000) for coating vitamins intended for incorporation into feedingstuffs under the conditions proposed (cf. Background)

- be harmful for productive livestock and/or pets ?
- result in undesirable effects on the quality of animal products ?

BACKGROUND

The autorization of the use in the Community of the products concerned as additives in feedingstuffs (group of emulsifiers, stabilizers, thickeners and gelling agents) was requested by a Member State. The proposed conditions of use are the following :

- Feedingstuffs intended for all animal species
- Maximum content :
  polyethylene glycol 6000 : 300 mg/kg of complete feedingstuffs
  polyoxypropylene-polyoxyethylene polymer (M.W. 6800 - 9000) : 50 mg/kg complete feedingstuffs.

OPINION OF THE COMMITTEE

1. Polyethylene glycols of high molecular weight (4000 - 6000) are used in pharmacy in the preparation of ointments, suppositories and tablets and they are also used in the preparation of cosmetics. They are water-soluble substances which act essentially as dispersants or binding agents.
   They are described in many pharmacopoeias.

   Polyoxypropylene-polyoxyethylene polymers are non-ionic surfactants used in pharmaceutical preparations and cosmetics either as emulsifiers or to facilitate gastro-intestinal absorption of certain active substances.

Polyoxypropylene-polyoxyethylene polymer (M.W. 6800 - 9000) as an emulsifying agent, in combination with polyethylene glycol 6000 as a dispersant, has been found suitable for the preparation of premixes of liposoluble vitamins for incorporation into feedingstuffs, particularly milk powders and milk replacer feeds.

The biologicial and toxicological properties of these products have been thoroughly investigated. Polyethylene glycols with a molecular weight ranging from 300 - 9000 are biologically inert when administered orally or applied dermally. Their oral LD 50 ranges from 40 to over 50 g/kg body weight for rat and from 20 to over 50 g/kg body weight for guinea pig. Long-term administration of polyethylene glycols (M.W. 1500 and 4000) at levels of 2 % in the diet of dogs and 4 % in the diet of rats had no adverse effects. Polyethylene glycol 6000 is not absorbed from the digestive tract and is not affected by the action of intestinal microorganisms. It is recovered quantitatively unchanged from the faeces. For these reasons, it is also used as a "marker" in studies of the process of digestibility and gastrointestinal absorption of certain nutrient principles.

The biological and toxicological properties of polyoxypropylene-polyoxyethylene polymers vary with their molecular weight and the weight ratio between the chains of polyoxypropylene and polyoxyethylene. The polymer of molecular weight 6800 - 9000, having about 80 % by weight of polyoxyethylene chains and 20 % by weight of polyoxypropylene chains, has little toxicity for rats, mice, guinea pigs, dogs or rabbits. The oral LD 50 for these species is above 15 g/kg body weight. The administration of the product for six months at levels of 0.1 and 0.05 mg/kg body weight in the diet of dogs and for two years at the levels of 3 % and 5 % in the diet of rats produced no adverse biochemical, haematological or histopathological changes and dit not affect growth or rate of survival. A marked reduction of growth was observed in the rat only at a concentration of 7.5 % in the diet. Orally administered, the product is not metabolized and is recovered quantitatively unchanged from the faeces.

No sensitization or irritant effects were found on repeated dermal application to dog, rabbit, guinea pig and man.

2. Since orally administered polyethylene glycol 6000 and polyoxypropylene-polyoxyethylene polymer are neither absorbed from the digestive tract nor metabolized in the organism, they are unlikely to influence the quality of animal products.

3. In view of the data presented, the Committee is of the opinion that the use of polyethylene glycol 6000 and of polyoxypropylene-polyoxyethylene polymer (M.W. 6800 - 9000), under the conditions proposed for the preparation of vitamin premixes intended for incorporation into feedingstuffs, has no harmful effects on animal or human health, nor will it affect the quality of animal products.

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REPORT OF THE SCIENTIFIC COMMITTEE FOR ANIMAL NUTRITION ON THE USE OF ARPRINOCID IN FEEDINGSTUFFS FOR CHICKENS

Opinion expressed 11 December 1980

TERMS OF REFERENCE (March 1980)

The scientific Committee for Animal Nutrition is requested to give an opinion on the following questions :

1. Does the use of the coccidiostat arprinocid in feedingstuffs for chickens, under the proposed conditions of use (see Background), result in the presence of residues in animal products ? Could these residues be harmful to the consumer ?

2. Could the use of this additive affect the development of resistance in bacteria ?

3. Could the excreted products, derived from the additive, be prejudicial to the environment ? If so, what is the nature of the risks ?

4. In the light of the answers to the above question, are the proposed conditions of use acceptable ?

BACKGROUND

Arprinocid was the subject of a submission for inclusion in Annex II, Section B, of Council Directive 70/524/EEC of 23 November 1970, concerning additives in feedingstuffs (1), under the following proposed conditions for use :

Species of animal : chickens for fattening.

Minimum and maximum content in complete feedingstuffs : 60 ppm (mg/kg).

Other provisions : use prohibited at least two days before slaughter.

(1) OJ No L 270, 14.12.1970, p. 1

OPINION OF THE COMMITTEE

1. Studies of the metabolism of arprinocid (9-(2-chloro-6-fluorophenylmethyl) 9H-purin-6-amine) in chicken, using the ^{14}C-methylenelabelled compound, show that the compound is rapidly metabolized to the 1-N-oxide, the hypoxanthine, the 2-chloro-6-fluorobenzylalcohol and the 2-chloro-6-fluorobenzoic acid. Only about 0.25 % of administered radioactivity is excreted as $^{14}CO_2$, establishing the metabolic stability of the methylene bridge. Ninety four per cent of the administered radioactivity appears in the mixed excreta; less than 0.1 per cent is found in each of the major organs and in the carcass except for 0.18 % in the liver and 0.37 % in the intestine stripped of its content.

   In surgically altered chicken, permitting separate collection of urine and faeces, 50 % of the administered radioactivity appears in the urine. About one third of the urinary activity is unchanged arprinocid, one third is the 1-N-oxide, one eigth the benzylalcohol derivative and one thirtieth each the hypoxanthine and the benzoic acid derivative. There are considerable species differences in the quantitative but not the qualitative distribution of the urinary metabolites. Residues of arprinocid in tissues and organs appear to consist essentially of 50 % arprinocid, 10 % hypoxanthine and 0.6 % of 1-N-oxide, and the remainder is unidentified metabolites. Residues are found mainly in the liver and kidneys, very little appearing in muscle, skin and fat.

   Residues in tissues and organs of chicken, fed for various times up to several weeks on a ration containing 70 ppm arprinocid, were determined by reverse isotope dilution assays or gas chromatography with electron capture (sensitivity : 50 ppb, limit of detection : 20-30 ppb).

   Three days after withdrawal only about 0.3 ppm residues were detectable in the liver and 0.15 ppm in the kidneys, while none could be detected in other tissues. Five days after withdrawal the liver residues ranged from 0.17 - 0.22 ppm. These liver residues consisted of at least two pools, one representing 85 - 90 % of the residues and consisting essentially of unchanged arprinocid (depletion halflife 1.7 days). The other pool, representing various metabolites, included the 1-N-oxide (depletion halflife 3.5 days). The liver residues appeared to be associated with the macromolecular cellular constituents and were thus largely not bio-available (only 18 %). There were no bio-available residues in liver and kidneys after 5 days withdrawal.

   ^{14}C-labelled arprinocid is well absorbed in rats, 65 % of the radioactivity appearing in the urine, 30 % in the bile and 5 % in the faeces during the 72 hours after administration. When liver containing ^{14}C-labelled arprinocid residues (0.2 ppm after 5 days withdrawal) was fed to rats, most of the activity appeared in the faeces, the net absorption being less than 0.05 ppm.

Arprinocid was investigated in short- and long-term toxicological studies in laboratory animals. The compound possesses hepatic and renal toxicity at high doses as well as having effects on the epithelial and cardiovascular systems manifested by necrosis of ears and tails. It appears to be teratogenic in the rat and mouse but not in the rabbit. This activity is due most likely to the 1-N-oxide metabolite. There was no evidence of carcinogenicity in the two rodent species (rat and mouse) tested nor was there any mutagenic potential demonstrable both in in vivo and in vitro tests. The no-adverse-effect level in the most sensitive species (mouse) was 1 mg/kg body weight. Separate investigation of the 1-N-oxide metabolite revealed no additional toxic potential.

The low bio-availability of the residues in the liver of chicken following relay toxicity tests adds a further safety factor.

2. The absence of activity of arprinocid on various species of micro-organisms tested in vitro (ten bacterial and two fungal strains) indicates that the product has no antimicrobial properties.

3. Studies on the excretion of arprinocid in chicken showed that, under the proposed conditions of use, about 95 % of the amount ingested appears in the excreta within 24 hours after administration. The excreted material is composed of 50 % arprinocid, 4 % 1-N-oxide, about 16 % polar water-soluble metabolites and 7 % bound to faecal solids.

   Aqueous solutions of arprinocid are sensitive to photodegradation, some 70 % being photolysed in 7 days. Tests performed under aerobic and anaerobic conditions showed a slow degradation of arprinocid in undiluted chicken excreta. When excreta were incorporated into different soils, the residues were retained strongly in the soil and little leaching by water occured. The halflife of residues in soil was about 56 days, being slowly degraded to CO_2 and organic volatiles by aerobic processes.

   Arprinocid had low toxicity for algae and fish but was moderately toxic for daphnia. No phytotoxicity was observed in tests carried out in millet, rice, beans, cabbage, cucumber, tomato, carrot and corn crops. These observations indicate that contamination of the environment is unlikely.

4. The Committee is of the opinion, in the light of the available facts, that the use of arprinocid in feedingstuffs for chickens, at a level of 60 ppm (mg/kg), is acceptable provided a withdrawal period of at least 5 days before slaughter is imposed.

REFERENCES

Dossiers Merck and Co Inc.

REPORT OF THE SCIENTIFIC COMMITTEE FOR ANIMAL NUTRITION ON THE USE OF INACTIVATED CULTURES OF SELECTED ENTEROPATHOGENIC STRAINS OF E. COLI IN FEEDINGSTUFFS FOR PIGLETS

Opinion expressed 11 March 1981

TERMS OF REFERENCE (June 1980)

The Scientific Committee for Animal Nutrition is requested to give an opinion on the following questions :

1. For what reasons can preparations of inactivated cultures of selected enteropathogenic strains of E. coli not be considered as vaccines ? If they are not vaccines, should feedingstuffs supplemented with these preparations not be considered as medicated feedingstuffs particularly because of their immunological effects ?

2. What are the effects of Intagen(x) on piglets and sows when this preparation is incorporated in the ration according to the proposed conditions of use (see Background) ? Are these effects significant in healthy farm livestock with no colibacillary infection symptoms ? Has the administration of Intagen in feedingstuffs for pregnant sows a significant influence on the effects observed in piglets ?

3. Are Intagen preparations other than the one mentioned in the dossier submitted in support of the request for its admission as an additive in feedingstuffs for piglets and sows, used for animal production ? Is Intagen used for human or veterinary therapeutic purposes ?

4. Could the use of Intagen under the proposed conditions (see Background) favour the selection of intestinal bacteria resistant to therapeutic agents used for pigs ?

5. Is this use without risk for :
   - the consumer ,
   - the people involved in livestock rearing ?
   - the environment ?

6. If Intagen satisfies the requirements for admission as an additive in feedingstuffs, what should be the administration period of the supplemented feed for piglets and sows and the maximum levels of the active principles in complete feedingstuffs ?

(x) Registered trade name

BACKGROUND

Intagen was the subject of a submission in Annex II, section F, of Council Directive 70/524/EEC, of 23 November 1970, concerning additives in feedingstuffs (1), under the following conditions of use :

Species of animal : piglets up to 10 weeks, pregnant sows.

Dose in complete feedingstuffs: 0.15 % minimum of a preparation containing from 5×10^4 to 2×10^5 hemagglutination inhibition units (HIU) per kg.

OPINION OF THE COMMITTEE

1. Intagen is a heat-treated, formalinised bacterial preparation from seven specific serotypes of E. coli, all known porcine enteropathogens; the heat treatment not only inactivates the bacteria, it also liberates the polysaccharide antigens from the cell walls. The product is incorporated in a mixture of whey powder, wheat flour and citric acid and the final product termed Intagen premix is a creamy white, free-flowing powder containing from 5×10^4 to 2×10^5 HIU/kg. Intagen, incorporated in the feed acts by stimulating the cells on the surface of the intestinal villi to produce specific IgA antibodies to the seven serotypes of E. coli used in its preparation.

   It should be noted that the intestine is equipped with a specialised surface immunity to counter the harmful effects of pathogenic bacteria. This antibody system is different from other immune systems in the body in that it can only be activated by a direct application of the antigen onto the absorptive cells covering the villi. Injectable vaccines are very inefficient in stimulating the surface immune system. A feature of the secretory immune system is that it is not long lasting unlike normal blood borne immunity in which a single course of vaccination will provide protection for a considerable period of time. Antibody production (IgA) by the secretory system persists only for as long as the stimulating antigens are present. Intagen does not have a long-term effect and must be continually administered. It acts by increasing resistance against specific pathogenic E. coli infection and thus feed containing it cannot be considered as medicated for therapeutic use.

2. Numerous trials with healthy young piglets have shown that incorporation of Intagen in the feed for an extended period of time improved weight-gain and feed conversion efficiency. In herds where E. coli infection was severe in the control piglets, its presence in the feed greatly reduced piglet mortality, increased the number of piglets weaned/litter and reduced more than half the requirement for other medication.

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(1) OJ No L 270, 14.12.1970, p. 1

In sows, the continuous ingestion of Intagen with the feed from 6 weeks prefarrowing has a limited effect in increasing the immunological activity of the colostrum.

3. To achieve maximum potency of antigen activity in the colostrum of sows it is recommended that at 3 weeks before farrowing the sows, already receiving Intagen premix in their feed (see 2. above) are given a single parenteral infection of 'Intagen Injectable'. This product is a sterile preparation of the E. coli polysaccharides prepared from the heat-treated formalinised preparation referred to in 1. above. Its mode of action consists in stimulating blood borne immunity; the effect is long lasting and entirely different from that of the Intagen premix included in the feed. There is a synergistic effect between the oral and parenteral route in stimulating antigenic potency of the colostrum.

   Neither product is used for human or veterinary therapeutic purposes.

4. There is no evidence that Intagen used under the conditions proposed (see 6. below) favours the selection of intestinal bacteria resistant to therapeutic agents. Studies have shown that during Intagen administration the numbers of coliform and sulphite-reducing bacteria inhabiting the intestine are reduced while the numbers of enterococci and lactobacilli are increased. There is no evidence of increased numbers of any harmful bacteria present in the intestine after treatment has been stopped. Indeed, since the product is only specific against certain serotypes of E. coli, the intestinal flora is normal after treatment ceases. There is no adaptation of the strains of E. coli used in the preparation of Intagen to its continued use and the question of resistance developing in other E. coli strains present within the intestine does not exist by virtue of the known mode of action of the product.

5. The nature of Intagen excludes any possibility of harmful residues in animal products resulting from the feeding of it to piglets or sows. There is no risk for the consumer. Tests with guinea pigs show the absence of danger due to inhalation; the effects are much less than those caused by an egg albumin aerosol. There is no adverse effect on the environment resulting from its use, indeed the opposite may be true since its use reduces the environmental burden of toxic E. coli species.

6. The Committee considers that the conditions of use of the product should be the following :

   a) in the feed of young piglets up to 77 days of age or when the animals reach 25 kg liveweight; maximum level in complete feedingstuffs : 1 % of a preparation containing 5×10^4 to 2×10^5 HIU/kg.

   b) in the feed of sows for the period of 6 weeks prefarrowing to 3 weeks postfarrowing; maximum level in complete feedingstuffs : 0.15 % of a preparation containing 5×10^4 to 2×10^5 HIU/kg.

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Dossiers Unilever Ltd.

GUIDELINES FOR THE ASSESSMENT OF CERTAIN PRODUCTS TO BE USED AS A SOURCE OF PROTEINS IN ANIMAL NUTRITION

GENERAL ASPECTS

This document is intended as a guide for establishing dossiers on non-traditional products obtained from culturing microorganisms and proposed as a new source of proteins in animal nutrition. These dossiers should enable an assessment of such products based on the present state of knowledge and should ensure their compliance with the fundamental principles laid down for permitting their use, which are the subject of the provisions of Article 6(2) of the proposal for a Council Directive of 26 July 1977, concerning certain products used in animal nutrition[1].

All the studies outlined in this document may be required and, if necessary, additional information may be requested. As a general rule, all the information necessary to establish the identity of the microorganism and the composition of the culture medium, and also the manufacturing process, characteristics, presentation, conditions of use, methods of determination and nutritional properties of the product must be provided. The same applies to the information necessary to assess the tolerance of the product by the target species and the risks for man and the environment, which could result directly or indirectly from the use of the product. The toxicological studies required for this purpose will depend on the nature of the product, the animal species concerned and the metabolism of the product in laboratory animals.

The documentation to be provided should include detailed reports, presented in the order and with the numbering proposed in these guidelines and should be accompanied by a summary. The omission of any proposed studies should be justified. The publications quoted as references should be attached.

OBSERVATIONS

The term "product", as used in these guidelines, refers to any non-traditional proteinaceous product obtained from culturing microorganisms in the state in which it will be presented as feedingstuff or component of a feedingstuff.

Any modification in the manufacturing process or in the conditions of use of a product will require notification and, if necessary, additional documentation for a new assessment.

The guidelines will be updated as new scientific knowledge develops.

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(1) OJ No C 197, 18.08.1977, p. 3

PRESENTATION OF STUDIES

I. Microorganism, culture medium and manufacturing process, characteristics of product, presentation and conditions of use, methods of determination.

II. Studies on the nutritional properties of the product.

III. Studies on the biological consequences of the use of the product in animal nutrition.

IV. Other relevant studies.

SECTION I : MICROORGANISM, CULTURE MEDIUM AND MANUFACTURING PROCESS, CHARACTERISTICS OF PRODUCT, PRESENTATION AND CONDITIONS OF USE, METHODS OF DETERMINATION

1. MICROORGANISM

1.1. Classification, morphology, biological properties, any genetic manipulation.

1.2. Innocuity, possible survival outside the fermenter and environmental consequences.

1.3. Constancy and purity of strains cultivated. Methods used to check these criteria.

2. CULTURE MEDIUM AND MANUFACTURING PROCESS

2.1. Composition of substrate, added substances, etc.

2.2. Manufacturing and purification processes. Methods used to check the constancy of composition of the culture product and the detection of any chemical and biological contamination during production.

2.3. Technical processes of preparation for use.

3. CHARACTERISTICS OF PRODUCT

3.1. Physical and physico-chemical properties : macro- and micromorphology, particle size, density, specific weight, hygroscopicity, solubility, solution characteristics (pH, rheological characteristics), electrostatic properties, etc.

3.2. Chemical composition :

3.2.1. Content of moisture, crude protein, crude fat, crude fibre, ash, nitrogen-free extract. Limits of variation.

3.2.2. Content of total, ammonium, amide, nitrate and nitrite nitrogen, true protein. Qualitative and quantitative composition of total and free amino acids, nucleic acids (purine and pyrimidine bases).

3.2.3. Qualitative and quantitative composition of total lipids : fatty acids, non-saponifiable matter, lipid soluble pigments, etc.

3.2.4. Qualitative and quantitative composition of carbohydrates and related substances.

3.2.5. Qualitative and quantitative composition of inorganic components.

3.2.6. Qualitative and quantitative composition of vitamins.

3.2.7. Qualitative and quantitative compositon of the other constituents : additives, residues of substrate and solvents, contaminants (in particular, polycyclic aromatic hydrocarbons, nitrosamines), etc.

3.3. Contamination by microorganisms during the manufacturing process.

3.4. Behaviour and stability of the product, as such and when mixed with feedingstuffs in current use, during storage.

4. PRESENTATION AND CONDITIONS OF USE

4.1. Proposed names for marketing the product.

4.2. Proposed formulations for marketing the product.

4.3. Intended use of the product in animal nutrition. Proposed concentrations in complete and complementary feedingstuffs for the animal species concerned.

5. METHODS OF DETERMINATION

Qualitative and quantitative methods for determination of the product in complete and complementary feedingstuffs.

N.B. : Description of these methods should be accompanied by information as to specificity, sensitivity, limits of detection, margin of error, possible interferences by other substances. Samples of the product in its various proposed presentations should be available.

SECTION II : STUDIES ON THE NUTRITIONAL PROPERTIES OF THE PRODUCT

1. ASSESSMENT OF PROTEIN VALUE

1.1. Chemical and/or microbiological studies.

1.2. Studies on laboratory animals (preferably rats) : digestibility, protein efficiency ratio (PER), biological value of the product compared with reference proteins.

2. STUDIES ON TARGET SPECIES

The following studies should be performed on each target species in comparison with a control group receiving, under the same conditions of nutritional balance, a diet in current use containing equivalent amounts of protein nitrogen.

2.1. Protein and energy supplementation value of the product in the rations under the proposed conditions of use at various physiological stages of the animals (growing period, pregnancy, laying, etc.).

2.2. Influence of the product under the proposed conditions of use on growth rate, feed conversion rate, morbidity, mortality, etc.

2.3. Optimum levels of incorporation of the product in the rations.

2.4. Effect of the product under the proposed conditions of use on the composition and on nutritive, technological and organoleptic quality of meat, offal, eggs and milk.

3. EXPERIMENTAL CONDITIONS IN THE STUDIES ON TARGET SPECIES

Give a detailed description of the tests performed and provide the following data :

3.1. Species, breed, age and sex of the animals, identification procedure.

3.2. Number of test and control groups; number of animals in each group (the number should be large enough for statistical analysis using suitable statistical parameters).

3.3. Levels of incorporation of the product, qualtitative and quantitative composition of the ration and its analysis.

3.4. Location of each experiment, physiological state and animal health conditions, rearing conditions (these should reflect those used in practice in the Community).

3.5. Exact duration of testing and date of the analyses performed.

3.6. Adverse effects which occured during the experiment and time of their appearance.

SECTION III : STUDIES CONCERNING THE BIOLOGICAL CONSEQUENCES OF THE USE OF THE PRODUCT IN ANIMAL NUTRITION

The studies outlined in this section are intended to permit assessment of the safety in use of the product in the target species, and of the risks for man and the environment which could result directly or indirectly from this use. The toxicological studies required for this purpose will depend on the nature of the product, the animal species concerned and the metabolism of the product in laboratory animals.

1. STUDIES ON TARGET SPECIES

The following studies should be performed on each target species in comparison with a control group receiving, under the same conditions of nutritional balance, a diet in current use containing equivalent amounts of protein nitrogen.

1.1. Maximum incorporation rates of the product in the ration without producing any adverse effect.

1.2. Effects of ingestion of the product under the proposed conditions of use on microorganisms of the flora of the alimentary tract and on colonization of pathogens in the alimentary tract.

1.3. Investigation under the proposed conditions of use of possible residues of the product (substrate, culture medium, solvents, contaminants) in animal products (meat, milk, eggs, etc.).

1.4. Investigation under the proposed conditions of use of possible residues of the product (substrate, culture medium, solvents, contaminants) in excreta.

2. STUDIES ON LABORATORY ANIMALS

2.1. Metabolism

Fate of the product in the animal : absorption, elimination, etc.

2.2. Mutagenicity

Investigations of potential mutagenicity due to contaminants (in particular mycotoxins) or residues of the product (substrate, culture, medium, solvents), including *in vitro* screening tests using metabolic activation systems.

2.3. Toxicological studies

The following studies should be performed in comparison with control groups receiving, under the same conditions of nutritional balance, a diet in current use containing equivalent amounts of protein nitrogen. Toxic effects should be investigated to elucidate their cause and mechanisms and to ascertain that they do not result from nutritional imbalance or from an overdosage of the product in the diet.

2.3.1. Subchronic toxicity (at least 90 days)

In general, these studies should be carried out on two animal species, one of which being a rodent. The product should be administered in the daily ration in at least two levels of incorporation. If possible, these should be chosen so as to determine a no-effect level and a level showing some adverse effect. The experimental groups should contain an adequate number of animals of each sex. A control group should always be included. All relevant biological data should be recorded at appropriate intervals, particularly data on growth rate, feed consumption, haematology, urine analysis, biochemical parameters, mortality, organ weights, gross pathology and histopathology of major organs and tissues. The results should be presented in detail and, as far as possible, should include statistical assessment.

2.3.2. Chronic Toxicity

In general, chronic toxicity studies should be carried out on two animal species, one of which being a rodent. The product should be administered in the daily ration in at least two levels of incorporation. Experiments should extend for a minimum of two years in the rat or 80 weeks in mice. The experimental groups should contain an adequate number of animals of each sex. A control group should always be included. The experiment, if continued beyond the minimum period, should be terminated when survival in any but the group with the highest level of incorporation has fallen to 20 %.
The biological examinations mentioned under item 2.3.1. should be carried out preferably on a small satellite group of animals at appropriate intervals throughout the experiment and on the surviving animals at the end of the experiment. For assessing carcinogenicity, particular attention should be paid to the time of appearance, the histological types of any observed tumours and their incidence. Any effect on the incidence of tumours and/or the incidence or progress of diseases should be assessed by reference to control groups, as indicated in paragraph 2.3. The result should be presented in detail and, as far as possible, should include statistical assessment.

2.4. Other studies

Reproduction studies should extend over at least two filial generations and may be combined with embryotoxicity including teratogenicity studies. Particular attention should be paid to fertility, fecundity and observation on post-natal development of litters.

2.5. Experimental conditions in the studies on laboratory animals

Give detailed description of the tests performed and provide the following data :

2.5.1. Species, breed, strain and sex of animals.

2.5.2. Number of test and control groups, number of animals in each group (the number should be large enough for statistical analysis using suitable statistical parameters).

2.5.3. Levels of incorporation of the product, qualitative and quantitative composition of the ration and its analysis.

2.5.4. General rearing conditions throughout the period of testing.

2.5.5. Exact duration of testing and date of examinations performed.

2.5.6. Rate and timing of deaths for the various test groups.

2.5.7. Pathological incidents which occurred during the experiment and time of their appearance.

3. STUDIES CONCERNING THE ENVIRONMENT

Depending on the nature of possible residues of the product (substrate, culture medium, solvents, contaminants) in excreta of target species, data on the fate of these residues in manure, soil and water and also their effects on soil biology, plant growth and aquatic life may be required.

SECTION IV : OTHER RELEVANT STUDIES

Depending on the nature and the conditions of use of the product, data on allergic effects, on irritation of the skin and mucous membranes of the eye, respiratory or digestive tract may be required to assess possible risks in handling the product and to prevent them.

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