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 CANDIDA YEASTS

Opinion expressed 3 May 1984

TERMS OF REFERENCE (January 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 yeasts of the Candida variety and, in particular, from those cultivated on n-alkanes have a nutritional value for animals because they provide nitrogen or protein ?

2. Can the use in animal nutrition of products obtained from yeasts of the Candida variety and, in particular, from those cultivated on n-alkanes 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 (0), Member States may, until such time as a Community decision has been taken, maintain authorizations granted within their territories before notification of the Directive concerning on the one hand products obtained from yeasts of the Candida variety and cultivated on n-alkanes and on the other hand products listed in Section 1.2.1. of the Annex to the Directive (yeasts cultivated on substrates of animal or vegetable origin) meeting requirements different from those laid down therein.

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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

The Committees draw the attention of the Commission to the fact that yeasts of the Candida variety are not a homogeneous group of micro-organisms in relation to their nutritional value and their pathogenicity (difference in virulence) for man and animals.

The Committees have made some general comments on the nutritional and pathogenicity aspects of Candida yeasts. In the absence of other relevant documentation they have evaluated the safety of only two specific products derived from two strains of Candida on which information has been provided, namely Toprina (*) and Liquipron (*).

Toprina (*) and Liquipron (*) are trade-marks for dried whole yeasts obtained by growing Candida lipolytica strain 246 and Candida strain ATCC 20275 IS respectively on n-alkanes containing culture media. The term Candida tropicalis is used in the text. However, there is no complete agreement on the actual classification of the Candida strain used as the source material for Liquipron (*).

1. Nutritional aspects

Candida yeasts may be broken down into groups according to the various substrates on which they can be grown, as indicated in the table below.

(*) Registered trade name

n-Alkanes	Methanol	Effluents and cellodextrins	Whey
C. tropicalis C. lipolytica C. utilis (b) C. maltosa (11)	C. boidinii (1)	C. pelliculosa (13) C. wickerhamii (a)	C. fabrianii (10) C. frigida (11) C. krusei (20)

(a) for the production of methanol

(b) also grown on sulphite solution (12), molasses and other sugar by-products

Extensive literature on the nutritional value of their proteins is available for C. lipolytica and C. tropicalis strains cultivated on n-alkanes. The crude protein content (N x 6.25) of products obtained from these micro-organisms varies from 60% to more than 70% on the basis of dry matter. Their protein efficiency (ratio between animal weight gain and quantity of proteins ingested) has been determined in various animal species (2, 3, 4, 5, 6, 8, 9, 19). In the rat, this efficiency is around 2.2-2.5 when the ration has been supplemented with 0.3% DL-methionine.

Except for low methionine levels, the amino-acid composition of C. lipolytica and C. tropicalis proteins is close to that of the egg. Lysine and threonine, limiting factors of the usual pig and poultry rations, are supplied in large measures by proteins obtained from Candida strains. At levels of 15% in the ration, these products cover 88% of chickens' and more than 100% of piglets' lysine requirements.

Studies on rats have shown that, at incorporation rates higher than 15% in the ration, the protein efficiency of products from C. lipolytica drops as the concentration increases in the same way as that of casein and whole egg which has been freeze-dried and heated at 60°C (7).

Provided nutritional balance is maintained, products from C. lipolytica and C. tropicalis strains can be used at levels up to 15%

in the rations of pigs, calves, lambs, poultry, pets, with the exception of the Dalmatian dog whose ration should not contain more than 3-4% of these yeasts (18). On the other hand, fish can be given high levels of them in their ration (14, 15, 16, 17). On account of their content in nitrogen compounds with high biological value, these products have a nutritional value comparable to or even higher than that of other sources of proteins commonly used in animal feed.

2. Risk assessment

2.1. Pathogenicity and hypersensitivity

2.1.1. General considerations on Candida strains

Viable Candida yeasts are so-called opportunistic pathogens, i.e. they may induce in predisposed hosts (human or animal organism) various forms of mycoses. Depending on exposure conditions, specific virulence of the strain and host factors, these mycoses may range from mild superficial infections to deep organ invasions (with predilection for kidney and brain) and generalized septicaemia which, if untreated, rapidly causes death (1, 6, 7). The skin, oral cavity and urogenital tract are more commonly affected in human beings, whereas the digestive tract is usually involved in young livestock (7, 8). Sometimes, a mycoallergy develops in addition to the underlying mycoses (7, 9). Both an overt and a latent infection during pregnancy may have serious consequences if viable cells of an opportunistic Candida strain are transmitted to the newborn at delivery.

Over the past ten years infections caused by Candida species have increased noticeably in number, severity and spectrum of causative species (14). This increase is reasonably attributed to the increase in predisposing factors which are generally a prerequisite for an organism being affected by a

candidosis. A severe form of candidosis may also occur in a healthy host when a high number of viable cells of the most virulent strains is administered. In humans, the predisposing factors include several metabolic disorders (e.g. diabetes), underlying bacterial or viral infections, treatment with broad- spectrum antibiotics, lowered resistance due to exposure to immunosuppressants (cytostatics or corticosteroids), congenital myelodeficiency and malignant disease (8, 14).

Candida-induced mycoses very often occur in animals in connection with the use of chemotherapeutics which, in warm-blooded animals, favour the development of yeasts by suppressing the normal intestinal flora (6). The use of medicated feedingstuffs may also result in an increased excretion of viable Candida yeast cells (5). Recently Candida yeasts have been shown to induce diseases also in freshwater fish (4).

Not all species of Candida are endowed with pathogenic properties for human or animal organisms (1, 6, 12, 14, 17, 16); this only happens when the optimal growth of the yeast occurs at the host body temperature and the yeast is able to withstand the various defence mechanisms that the host puts into play (among which phagocytosis is of utmost importance). The formation of mycelia or pseudomycelia is widely held to account for increased resistance of Candida to phagocytosis and for virulence in general (14), but other factors may contribute to the invasive properties of the micro-organism, particularly enzymes (mainly proteases) and cell glycoproteins (endotoxin-like substances) (7, 10, 16, 3).

A review of the current literature, covering both experimental and clinical reports, clearly shows that the following eight species of Candida are true pathogens (in this context "pathogenic" means "capable of inducing harmful effects in a

compromised host" with the understanding that the virulence will depend on the particular strain) : C. albicans; C. tropicalis; C. stellatoidea; C. glabrata; C. parapsilosis; C. pseudotropicalis; C. guilliermondii; C. krusei. According to Odds (14), all the traditional Koch's postulates, which still remain the orthodox criteria for judging a species to be regarded as pathogenic, are satisfied for these eight Candida species.

Moreover, the following Candida species have been occasionally isolated from human or animal tissues: C. claussenii; C. intermedia; C. brumptii; C. lipolytica; C. solani; C. ravantii; C. pulcherrima; C. ingens; C. lambica; C. macedoniensis and C. norvegensis. For these Candida species a strong aetiological association with a disease has not been documented but is, however, suspected. Lastly, C. viswanathii has been shown recently to be highly pathogenic in the mouse (2). C. ingens has been reported as a cause of septicaemia in surgical patients (15) and strains of C. utilis have been described which are virulent for the mouse and possess very active cytoplasmic toxins (10).

It must be stressed here that the information reported above about the pathogenicity of Candida organisms is derived either from clinical evidence in men and/or animals or from experimental infections in laboratory animals. There is a great lack of knowledge about the consequences of animal or human exposure to a "high" number of viable yeast cells or to repeated exposure to these cells. There is no doubt, however, that the continuous inhalation and/or ingestion of huge amount of viable yeast will affect the host immune system and may give raise to serious hypersensitivity reactions considering the strongly antigenic nature of the Candida cell surface.

Based on the above evidence, it may be concluded that the use of some Candida yeast strains for manufacturing single cell

proteins as animal feeds may pose serious risks not only to (i) workers (if exposed to viable yeast cells in production plants or during preparation and/or administration of feeds) and (ii) population groups (if exposed to emissions of viable yeast cells from manufacturing plants), but also to (iii) consumers of animal products containing viable yeast cells, (iv) farm animals exposed to viable yeast cells through contamination of the environment. Therefore, particular strains of Candida yeasts should not be permitted for industrial use in SCP manufacturing unless sufficient data are available to show that the strain employed is non-pathogenic and/or does not elicit serious hypersensitivity reactions.

2.1.2. Specific considerations relating to Candida strains used for manufacturing Toprina (*) and Liquipron (*)

C. lipolytica, strain No 246, and Candida sp., strain No ATCC 20.275 is cultivated on n-alkanes for manufacturing Toprina (*) and Liquipron (*) have been tested for pathogenicity in several laboratory animal species. The results obtained showed that the two strains did not proliferate but exhibited a tendency to persist especially in the kidneys of infected animals. This behaviour was, however, not significantly different from that of non-pathogenic C. utilis strains chosen as reference organisms. No data are available on the effects of the ingestion of Toprina (*) and Liquipron (*) on micro-organisms of the flora of the alimentary tract and on the colonization of pathogens in the alimentary tract.

Consequently, to protect consumers of animal products, workers involved in the preparation and administration of SCP-containing feeds, farm animals fed SCPs and the environmental organisms that might be exposed through contamination of the environment from mycoses and mycoallergies, Toprina (*) and Liquipron (*) should not be

permitted as animal feeds unless, in addition to the qualification of non-pathogenicity referred to in the previous section, they are also proven to be free of viable cells by a sensitive recognised method, the limit of detection of which is defined.

2.2. Biological and toxicological aspects

The biological effects of incorporating Toprina(*) or Liquipron(*) in the ration have been examined in a number of target species. The lipids of these products and probably of other Candida products from yeasts grown on n-alkanes contain a much higher percentage of odd-carbon-atom-number fatty acids, mainly C_{17}, than the usual dietary lipids. Investigations of the effect of the presence of these fatty acids in Toprina (*) and Liquipron (*) showed a dose-dependant accumulation of these fatty acids in the lipid fraction of all tissues as well as in milk and eggs in target species. Accumulation was greatest in the adipose tissue and involved mainly C_{15} and C_{17} fatty acids. Young poultry accumulated these fatty acids faster than older birds, while eggs accumulated more than body tissues. Similar accumulation was detected in the adipose tissue of mice, rats and monkey. These fatty acids were also present in the lipid fraction of brain, heart, liver and blood platelets. Accumulation usually reached a plateau after about 2 months feeding indicating steady state kinetics (1, 5, 29, 25).

Residues of n-alkanes ranging from 0.1 to 0.4% were detected in several samples of Toprina (*) and Liquipron (*) (9, 30). Investigations carried out on many target species have shown that n-alkane residues were also present in the adipose tissue and muscle of animals fed yeasts grown on n-alkane substrates. Accumulation levels reached a plateau usually within two months of continuous feeding of these yeasts in the ration (1, 6, 18, 26).

Subchronic feeding studies in rats, using levels up to 30% Toprina (*) or 20% Liquipron (*) in the diet showed no adverse findings (8). These studies included two 90-day studies with Toprina (*) and an 11 months study with Liquipron (*).

Lifespan studies were also carried out with both products. Two rat studies extending over 104 weeks and a mouse study lasting over 78 weeks were performed with Toprina (*) given at levels up to 30% in the diet. Apart from minor organ weight changes in rats, confined either to one sex or not dose-related, no carcinogenic or other adverse effects were noted in either species. Growth was somewhat reduced in all mice on yeast diet (17, 20, 22, 27).

A lifespan study in rats extending over 30 months with dietary levels of Liquipron (*) up to 26% showed an increased incidence of lymphomas in females and less so in the males. The rise in tumour incidence in females appeared to be related to dose (13). However, in the absence of knowledge of the incidence of these tumours in historical controls and with no mutagenicity data available, it is not possible to interpret these findings. These may well be due to epigenetic mechanisms. A second study, extending over 17 months, at a dietary level of 20% produced kidney calcification as the only adverse finding (23). A 28 months study in mice at a dietary level of 30% produced calcification in the hearts and kidneys of males and the kidneys of females. The only adverse finding in a two year dog study with Liquipron (*) was a dose-related reduction in body weight gain. Monkeys fed 2% Liquipron (*) for 2 years showed no adverse effects apart from diarrhoea and soft stools (24).

Reproductive and teratological studies with up to 30% of Toprina (*) in rats showed no adverse effects due to treatment (9, 21, 28a). Similar studies in mice with up to 27% Liquipron (*) produced only slight growth depression at the

highest test level. In rats the same top dose showed some adverse effects on pre- and post-implantation as well as on litter parameters. Maternal behaviour towards the litter also showed a dose-related adverse change. Some delayed ossification was also noted. Embryotoxicity was also noted when 17% Liquipron (*) was administered to pregnant rabbits (14, 15, 12).

The only existing mutagenicity test is a dominant lethality assay with Toprina (*), which was negative (28b). Liquipron (*) has not been tested for mutagenicity. The relay toxicity studies on Toprina (*) and Liquipron (*) did not yield any meaningful results (11).

A series of special studies was carried out on pregnant rats using the lipid fraction extracted from Toprina (*) at a dietary level equivalent to 75% Toprina in the diet. Examination of the progeny revealed some disturbance in the early myelination pattern in the brain and delayed maturation in post natal development (7). Similar effects were also produced by feeding the corresponding odd-carbon-atom-number fatty acids. These fatty acids were found in the lipids of treated rats, the milk and the progeny, this being evidence of passage accross the placenta (2, 3). Tissue microsomes associated with cytochrome b_5 and cytochrome b_5 reductase induction also contained these fatty acids but the mitochondria were normal. In contrast rats treated with up to 16% Liquipron (*) for 3 months and longer showed no effects on hepatic microsomal enzymes, haematological parameters, motor activity, coordination and neurotransmission except for slight increases in acetycholine and 5-OH-indoleacetic acid, slight reductions in liver triglycerides and adrenal cholesterol (10, 4, 5).

3. Conclusions

In the light of these data the Committees did not consider it feasible to assess the risks for the consumer of the use of protein products obtained from Candida yeasts cultivated on n-alkanes. The interpretation of some of the experimental findings was rendered difficult by the lack of certain basic data. In particular, information is missing on :

(a) the mutagenic activity of the relevant fractions of both Toprina (*) and Liquipron (*) biomass in in vitro systems, with and without metabolic activation, and in in vivo systems,

(b) the dose levels of lipids, extracted from these yeast products, which on ingestion do not produce any neurobehavioural and/or neuropathological changes in the progeny of treated laboratory animals,

(c) the dose levels of lipids extracted from the milk and eggs of animals ingesting these yeast products in their feed, which do not result in neuropathological and neurobehavioural changes in the progeny of treated laboratory animals,

(d) and the classical toxicological effects of fatty acids containing linear chains of 15 or 17 carbon atoms.

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