EUROPEAN COMMISSION
HEALTH & CONSUMER PROTECTION DIRECTORATE-GENERAL

Directorate B - Scientific Health Opinions
Unit B3 - Management of scientific committees II

Report of the Scientific Committee on Animal Nutrition on the assessment under directive 87/153/EEC of the efficacy of micro-organisms used as feed additives

(expressed on 18 February 2000)

TERMS OF REFERENCE (January 1999)

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

(1) What is the most appropriate way to assess the efficacy of micro-organisms used as feed additives in animal nutrition in order to improve animal production.

(2) When the product contains several different strains of micro-organisms, what data are necessary to justify the presence of each active component of the additive?

BACKGROUND

As part of the assessment process, the amended guidelines (Council Directive 87/153/EEC) require companies to demonstrate the efficacy of microbial products in each target species in terms of animal production. According to Article 2 of Directive 70/524 EEC one definition of an additive is a product intended:

"to improve animal production, in particular by affecting the gastro-intestinal flora or the digestibility of feedingstuffs;....".

The legislation services of the Commission have indicated that "animal production" should be interpreted as "animal performance", expressed in terms of an improvement in the efficiency of nutrient utilisation, or animal growth, or in the quality and yield of animal products or improved animal welfare. Other beneficial effects, such reduced morbidity or mortality, can be used as further evidence of the practical value of the product, but can not replace the fundamental requirement for proof of a performance effect as so defined.

The guidelines also require that when a microbial feed additive contains two or more strains, then the presence of each and every active component of the mixture should be justified in terms of their contribution to the overall efficacy of the product.

1. INTRODUCTION

1.1. Micro-organisms, in common with other biological products used in animal nutrition, exert effects which are not always uniform, are difficult to predict and operate by mechanisms which often are poorly understood (Teller and Vanbelle, 1991; Thomke and Elwinger, 1998). Responses may be very different in animals of different type and age. Products intended to act in the functioning rumen have actions and effects very different from those of products intended for non-ruminants or the post-ruminal tract of ruminants. When effective, microbial products can benefit performance within the definition of the term used by the Commission services. However, microbial products also can affect parameters that apparently escape this definition including product quality and effects on the environment.

1.2. A product that has its primary effect on the gut flora may produce a range of direct and indirect effects including some that occur within the tissues of the host. These may include modulation of an immune response (Matsuzaki, 1998; Dune *et al*., 1999) or control of opportunistic pathogens (Tortuero *et al*., 1995). In young animals in particular, effects of this nature may be manifested in a reduced morbidity and mortality. This is of benefit both to the welfare of the animal and to the producer, since it improves the total productivity of the system. The value of extending the interpretation of the guidelines to encompass effects which benefit total production and the consequences of this for the formal assessment of efficacy are considered here.

1.3. Extending the definition beyond performance effects could infer that microbial products should be considered as veterinary medicines. Although microbial additives can and do have an impact on the health of animals, this is through a broad effect on the system and is not targeted to specific pathogens.

2. DEMONSTRATING THE EFFICACY OF MICROBIAL PRODUCTS

2.1. Non ruminants

2.1.1. For non-ruminants, the concept of *probiosis*, essentially the ability of an intact gastro-intestinal flora to resist the overgrowth of any component or foreign strain, is well recognised (Fuller, 1989). Microbial (probiotic) products designed to help restore a “normal” flora after illness or following deliberate insult are of proven value, particularly as an adjunct to antibiotic or chemotherapy in humans (McFarland *et al*., 1994; Corthier, 1997). However, their value in the digestive tract of apparently healthy animals to provide added resistance to the overgrowth and other deleterious effects of opportunistic pathogens has proved more difficult to demonstrate.

2.1.2. Evidence would suggest that those microbial products able to affect/stabilise the gut flora of target animals do so only when the natural flora is in some way disturbed. This may occur at any time amongst a few individuals within a herd or flock or, in the case of

poor husbandry, change of diet or other externally imposed stress, it may involve a majority. Since there is no rapid and objective measure of the microbial status of the gut at any given point in time, adding a microbial preparation to feed can be seen as providing an insurance policy against any detrimental effect on performance mediated through the intestinal flora. Thus, it is reasonable to expect a microbial product **not** to affect animal performance where there is no significant disturbance to the flora. When only part of herd or flock is affected, then the addition of a microbial product is more likely to result in greater consistency in performance (i.e. as reduced standard deviation on any measured performance parameter) rather than an any overall improvement. The present inability to define and recognise situations in which microbial products are likely to have maximum effect is probably the prime cause of the inconsistency in response associated with microbial products and the inherent difficulty of being able to demonstrate their efficacy.

2.1.3. A range of beneficial responses may be generated in the compromised animal or bird in the presence of an appropriate microbial product depending on the origin and severity of the initial disturbance. Early changes to the flora or to immune competence may be detectable only in experimental animals. In production situations, particularly with young animals, the first visible and more readily detectable responses may be reduced morbidity and/or mortality (Pollman, 1992; Huis in't Veld and Havenaar, 1993). Only as the host's response to a microbial challenge is lifted, are enough nutrients spared to allow repartitioning to occur. If the response to the additive is sufficiently great, then repartitioning may allow changes in performance to be detected.

2.1.4. Meta-analysis of the use of microbial products in pigs have shown that effects on performance averaged over many trials are small but positive for young, rapidly growing animals - in the region of a 4% improvement in liveweight gain or feed conversion efficiency (Pollman, 1992; Rosen, 1992). However, effects are virtually absent in older animals and adults (~1%) (Rosen, 1992). There is also considerable variation, with 70% of trials with newly weaned piglets showing no or negative effects. This is not surprising since, if a microbial product benefits only a few individuals within a well-managed herd, this is unlikely to be seen in terms of herd performance. A similar limited and age related response has been noted in all other non-ruminant target species in which microbial products have been used (Jin *et al*., 1997).

2.2. Ruminants

2.2.1. Relatively few microbial products have been developed designed to improve the performance of a functioning ruminant by modulating rumen function and the activities of its microflora. Current products claiming this effect are based either on strains of yeast (*Saccharomyces cerevisiae*) or on spent culture medium from the

growth of *Aspergillus* species. Despite the obvious differences in the nature of these products, both have been shown to induce similar changes in the rumen, which can benefit production. When effective, both product types result in an improvement in the extent of plant cell wall (fibre) degradation in the rumen. This is usually accompanied by an increase in numbers of cellulolytic organisms, a change in proportion of volatile fatty acids and an increase in microbial nitrogen leaving the rumen (Wallace and Newbold, 1992; Yoon and Stern, 1996). The most likely mechanism underlying these changes is, for the *Aspergillus* product, the action of extracellular enzymes remaining in the spent medium, and for the yeast, the removal from the rumen liquor of traces of oxygen toxic to rumen cellulolyic bacteria (Newbold, 1995). However, other mechanisms have been postulated, including reduction of lactate concentration (Williams *et al*., 1991) or the provision of specific growth factors or metabolic intermediates (Dawson 1993, Varel and Kreikmeier, 1993).

2.2.2. Results obtained with the microbial products for ruminants are also highly variable and often unpredictable. There are, however, indications that diet and the nutritional demands of the host affect production responses. Effects of yeast and the *Aspergillus* products in dairy cows appear greatest at early rather than mid or late lactation. Generally, the response of both dairy cows and beef animals is greatest on high concentrate rather than high roughage diets (Huber *et al*., 1985; Williams *et al*., 1991) although this is not a universal rule.

2.3. Conclusion

The limited performance response of all livestock species to microbial products indicated by meta-analysis and shown in the majority of individual cases makes it difficult to detect a statistically significant effect against a background of the animal to animal variation seen in most production trials. Good experimental design will help to offset, but not totally eradicate, this problem.

3. JUSTIFYING ALL ACTIVE COMPONENTS OF A MULTI-COMPONENT MIXTURE

3.1. There are good practical and theoretical reasons why the use of animal trials to demonstrate the need for each component strain is not always feasible. Assuming that the effects of the individual components are additive, then spreading an already limited response over two or more strains makes it increasing unlikely that a statistically significant effect could be obtained. If the effects are not evenly spread then detecting a response from the least active agent further compounds the problem. A more extreme case would be where there was an element of synergy and where individual strains have demonstrable effects only in combination and not in isolation. Strains may also be included in a mixture to take account of changes or differences in the condition of the digestive tract in the target species. One strain may be selected to be active under particular conditions and another under different

physiological or microbiological circumstances. This could not be demonstrated in a single trial. In practice, since the precise conditions for effectiveness remain obscure, it may not be possible to define any appropriate trial conditions.

3.2. *In vivo* or *in vitro* data causally related to a performance response could provide an acceptable alternative strategy to define the contribution of individual strains. Any beneficial effect of a microbial product should be most readily detected though some change in the gut flora. However, so little is known about the mechanism(s) of action of microbial products that it would, in most cases, be difficult to establish a consensus about what is an appropriate microbiological measure. General evidence relating to protection from a natural or deliberately imposed microbial challenge could be provided in terms of reduced morbidity or mortality. More immediate microbiological or immunological measures, for example, a reduction in numbers of coliforms in the presence of the additive, the production of bacteriocins by one or more component strains or some change in host immune status, have not been causally related to performance.

4. RECOMMENDATIONS

4.1. On the way to assess the efficacy of microbial feed additives

4.1.1. SCAN recognises the difficulty of recreating the circumstances when a microbial preparation might be most effective. Nonetheless, companies should be required to demonstrate that any trials presented in a dossier were appropriately designed and that the numbers of animals used were sufficient to be able to detect a stated minimum response in terms of the claimed effect at a given level of confidence. Consideration should be given to the use of a replicated block design or equivalent for performance trials.

4.1.2. SCAN recommends that serious consideration should be given to the acceptance of broader measures of efficacy which have demonstrable value to the end-user, despite any possible conflict with existing legislative boundaries. Claims for microbial products essentially can be considered to fall within three categories:

- improved performance of the target species (the current basis for the recognition of microbial products as feed additives);

- reduced morbidity or mortality which improves the welfare of the target species and can lead to cost-savings for the producer through less veterinary intervention, reduced labour costs or by enabling increased numbers of animals to reach slaughter weight.

- benefits to the consumer through improved product quality (e.g. reduced cholesterol in eggs, reduction in milk fat content, reduced contamination of poultry with human enteropathogens) or indirectly through the environment.

4.1.3. A proven benefit to animal health alone should be sufficient to justify claims of efficacy. As indicated in the introduction, in the view of SCAN microbial products are feed additives within the meaning of the legislation and not veterinary products. Although they can and do have an impact on the health of animals, this is through a broad effect on the system and is not targeted to specific pathogens. When microbial challenge trials are used as evidence of efficacy, they can be considered as primarily a mechanism to perturb and then demonstrate restoration of microbial balance and only indirectly signal an effect on specific pathogen numbers or activity.

4.1.4. Whatever the measure of efficacy adopted, SCAN considers that, a minimum of three studies demonstrating a statistically significant ($p<0.05$) improvement should be provided. In the case of cattle and horses a lower level of probability could be accepted ($p<0.10$). The effects should be demonstrated with the lowest application rate claimed. In addition, data relating to any other trials which failed to reach statistical significance also should be supplied. The three significant studies preferably should be done in different locations

4.1.5. As a principle, the duration of studies designed to demonstrate the effectiveness of a microbial additive in enhancing some aspect of *animal performance* should be related to the time at which farm output is valued in economic terms and any benefit to the producer demonstrated at this stage. (see annex)

For example, for meat production this may be at slaughter or, where a young animal is sold on for growing and fattening, at the age at which the animal is sold. The point in production where the microbial additive is introduced should be left to the manufacturer but should be reflected in the claimed conditions of use. In the view of SCAN, it is insufficient to demonstrate efficacy for only part of a production period, unless the magnitude of the benefit achieved at this time is sufficient to provide the producer with a significant gain when the value of the produce is finally realised.

For other types of claims, the same principle should apply. Thus for egg or milk quality, the duration does not have to be the whole laying/milking period since the produce is sold (valued) on a regular basis throughout the period. However, authorisation for use should not be based on extrapolation of data and should only be for the period for which evidence of effect is provided. Similarly, claims relating to morbidity or mortality during the perinatal period need only cover this period provided claims are restricted to reduced veterinary input or the improved welfare of the animal – the benefits being immediate. Other claims for improved survivability may be better supported by measurement of survival to market weight.

4.2. For the justification of the different components of multi-strain products

4.2.1. SCAN considers the present requirement to justify the presence of each strain in a final product containing multiple strains based on evidence from animal trials is neither practical nor theoretically justified. Accordingly, SCAN suggests that any data that directly or indirectly supports the presence of individual strains in a mixture should be accepted. However, SCAN does recommend that any data submitted as justification be strain specific and not simply based on the generality of scientific data available in the literature.

4.2.2. SCAN questions the value of requiring a justification for the presence of each strain present in a mixture. Claims made by a manufacturer are for the mixture as a whole and not for its component parts. Provided that, as at present, each component strain is examined for safety, it is difficult to see how the safety of the consumer, the health and welfare of livestock or the confidence of the end-user is further benefited by this information.

5. REFERENCES

Corthier, G. 1997. Antibiotic-associated diarrhoea: treatments by living organisms given by the oral route (probiotics). In: *Probiotics 2 Applications and Practical Aspects* (Fuller, R. ed.) pp. 40-64. Chapman and Hall, London.

Dawson, K.A. 1993. Current and future role of yeast culture in animal production: a review of research over the last six years. In: *Biotechnology in the Feed Industry* (Lyons, T.P. ed.) pp. 269-291. Alltech Technical Publications, Nicholsville, USA.

Dunne, C., Murphy, L., Flynn, S., O'Mahony, L., O'Halloran, S., Feeney, M., Morrissey, D., Thornton, G., Fitzgerald, G., Daly, C. Kiely, B., Quigley, E.M.M., O'Sullivan G.C., Shannon, F. and Collins J.K. 1999. Probiotics from myth to realty. Demonstration of functionality in animal models of disease and in human clinical trials. *Anton. Leeuw. Int. J.G.* **76**, 279-292.

Fuller, R. 1989. Probiotics in man and animals. *J. Appl. Bacteriol*. **66**, 365-378.

Huber, J.T., Higginbotham, G.E. and Ware, D. 1985. Influenceof feeding Vitaferm, containing an enzyme-producing culture from *Aspergillus oryzae*, on performance of lactating cows. *J. Dairy Sci.* **68** (Suppl. 1), 30.

Huis in't Veld, J.H.J., and Havenaar, R. 1993. Selection criteria for microorganisms for probiotic use. In: *FAIR No. 6 Prevention and Control of Potentially Pathogenic Microorganisms in Poultry and Poultry Meat Processing 12. Probiotics and Pathogenicity* (Jensen, J.F., Hinton, M.H. and Mulder, R.W.A.W. eds) pp. 11-19. COVP-DLO, Beekbergen.

Jin, L.Z., Ho, Y.W., Abdullah, N. and Jalaludin, S. 1997. Probiotics in poultry: modes of action. *Worlds Poultry Sci. J.* **53**, 351-368.

Newbold, C.J. 1995. Microbial feed additives for ruminants. In: *Biotechnology in Animal Feeds and Animal Feeding* (Wallace, R.J. and Chesson, A. eds.) pp. 259-278. VCH , Weinheim.

Matsuzaki, T. 1998. Immunomodulation by treatment with *Lactobacillus casei* strain Shirota. *Int. J. Food Microbiol.* **41**, 133-140.

McFarland, L.V., Surawicz, C.M., Greenburg, R.N. 1994. A randomised placebo-controlled trial of *Saccharomyces boulardii* in combination with standard antibiotics for *Clostridium difficile* disease. J. Am. Med. Assoc. 271, 1913-1918.

Teller, E. and Vanbelle, M. 1991. Probiotics: facts and fiction. *Med. Fac. Landbouww. Rijksuniv. Gent* **56**, 1591-1599.

Pollman, D.S. 1992. Probiotics in swine diets. In: *Proceedings of the International Round Table on Animal Feed Biotechnology*, Ottawa Canada.

Rosen, G.D. 1992. Antibacterials in disease control and performance enhancement. Part II. *Feed Compounder* **12**, 20-23

Tortuero, F., Rioperez, J., Fernandez, E. and Rodriguez, M.L. 1995. Response of piglets to oral administration of lactic acid bacteria. *J. Food Protec.* **58**, 1369-1374.

Varel, V.H. and Kreikemeier, K.K. 1993. Influence of feeding *Aspergillus oryzae* fermentation extract (Amaferm) on in situ fiber degradtion, ruminal parameters and bacteria in non-lactating cows fed alfalfa or bromegrass hay. *J. Dairy Sci*. **71**(Suppl. 1), 287.

Wallace,R.J. and Newbold, C.J. 1992. Probiotics for ruminants. In: *Probiotics: the scientific basis* (Fuller, R. ed.) pp. 317-353. Chapman and Hall, London.

Williams, P.E.V., Tait, C.A.G., Innes, G.M. and Newbold, C.J. 1991. Effects of the inclusion of yeast culture (*Saccharomyces cerevisiae* plus growth medium) in the diet of dairy cows on milk yield and forage degradation and fermentation patterns in the rumen of sheep and steers. *J. Anim. Sci*. **69**, 3016-3026.

Yoon, I.K. and Stern, M.D. Effects of *Saccharomyces cerevisiae* and *Aspergillus oryzae* culture on ruminal fermentation in dairy cows. *J. Dairy Sci*. **79**, 411-417.

Annex

Recommended duration of experiments (period to be reported)

Calves (only milk replacer)	Total feeding period until slaughter
Calves (for fattening)	A minimum of 6 weeks
Calves (for rearing)	A minimum of 6 weeks
Cattle for fattening	From 100 kg (or according to local custom) until slaughter
Dairy cows	A minimum of 100 days; if the first 100 days of milking period are concerned, then remaining lactation period
Dairy cows (reproduction)	2 cycles
Sheep for fattening	From weaning until slaughter (according to local custom)
Goat for fattening	From weaning until slaughter (according to local custom)
Piglets	Until weaning (creep feed)
or	From weaning to 25 kg (or according to local custom)
Pigs for fattening	Growing or fattening period until slaughter
Sows (reproduction)	2 cycles
Rabbits for fattening	From weaning until slaughter
Breeding does	At least 2 cycles
Chicken for fattening	Day 1 to a minimum of 35 days (until slaughter)
Laying hens	A minimum of 6 months; if the first 6 months of the laying period are concerned, then total laying period
Turkeys for fattening	From day 1 to a minimum of 12 weeks (until slaughter)
Horses for fattening	A minimum of 12 months until slaughter

If validated experimental models are available (e.g. bacterial challenge), this may allow shorter duration tests.

If feeds for particular feeding purposes are concerned, the experimental duration may be adjusted to the respective recommended feeding time.