In present day culture regimes, animals are subjected constantly to intense management practices which tell upon their ability to remain healthy as they are driven to achieve high production standards. The system of the body most likely to succumb to such stressful conditions is the intestinal tract which constitutes a doorway for pathogenic microorganisms. Traditionally, farmers have used electrolytes and antibiotics to combat diseases and infection, but pressure is mounting for much greater control over the use of antibiotics and farmers are thus forced to look for alternative methods for healthier and quicker growth and disease control.

Invasion from such microorganisms and the resulting disease can be circumvented by the administration of probiotics in order to reinforce the beneficial intestinal microflora. Though many theories have been proposed about the use of probiotics, still many doubts their existence with as regard to their safety and efficiency.

Disadvantages of using antibiotics as feed additives

Though antibiotics have been used as feed additives, the associated toxicity, allergy, residues in food and resistance obtained after long term administration of low doses makes their use worthy on second thought. Also the indiscriminate use of broad-spectrum antibiotics may alter the normal gut flora by suppressing its growth and cause an overgrowth of pathogenic bacteria.

Because of decreased production of lactic acid and other fatty acids by the normal gut microflora, the growth of yeasts, fungi, Enterobacteriaceae and other pathogens gains prominence. They ascend into the normally and sparsely colonised small intestine and change their characteristics through gene transfer processes, and a state of intestinal dysbiosis is created. The ensuing diarrhoea is unresponsive to antibiotic therapy. These undesirable side effects are not encountered in the use of probiotics.

Definition and development of probiotics

The word ‘probiotic’ is derived from the greek meaning ‘for life’ and originally referred to a phenomenon observed when two organisms were cultured together, in which substances produced by one organisms stimulated the growth of the other. These growth promoting substances were referred to as ‘probiotics’. The term was subsequently used to describe living preparations of microbial cells, that could
be administered to animals, including humans with the aim of promoting the health of the consumer. This latter concept is derived from the observations of Elie Metchnikoff (Box 1). In the case of farm animals, faster weight gain for the same amount of food consumed (growth promotion, feed efficiency) has been of primary importance. Fuller (1989) redefined probiotics as 'a live microbial feed supplement which beneficially affects the host animal by improving its intestinal microbial balance’. This revised definition stressed the need for a probiotic to be viable. The principle of a probiotic product containing viable bacteria is that these bacteria become associated with the epithelial cells and are then trapped within the mucous layer (glycoalyx) and can grow and multiply.

Box 1. Elie Metchnikoff (1845-1916) and the elixir of life

Elie Metchnikoff, Nobel Laureate (for his discovery of phagocytosis) of the Institut Pasteur, Paris was interested in the scientific basis of ageing. According to Metchnikoff, the large bowel harboured microorganisms that produced substances that were toxic to the vascular and nervous systems. The toxic substances, as a result of absorption into the bloodstream, contributed to the ageing process. Thus, intestinal microorganisms were the aetiological agents of 'auto-intoxication', because they produced ammonia, amines and indole as a result of protein hydrolysis (putrefaction) in the digestive tract. Metchnikoff believed that his 'elixir of life' would prevent the putrefaction of milk, would it not have the same effect in the digestive tract if appropriate microorganisms were used. The inhabitants of Eastern European countries, some of whom were apparently extremely long-lived, consumed fermented milk as a constant part of their diet. Thus, yogurts were introduced to Western Europe as health-related foods.

Source: Tannock G.W. (1977)

Composition of probiotic preparations

Metchnikoff and his colleagues worked with Bulgarian bacillus an organism closely related to lactobacillus, starter of yoghurt (L. delbureckii subsp. bulgaricus) and to this day lactobacilli have remained the most commonly used probiotic organisms. Currently available probiotic preparations contain L. delbureckii subsp. bulgaricus, L. brevis, L. cellobiosus, L. lactis and L. reuteri. Of the bifidobacteria currently being used as probiotics are Bifidobacterium adolescentis, B. animalis, B. bifidum, B. infantis, B. longum and B. thermophilus. The yoghurt starter S. salivarius subsp. thermophilus is still a common probiotic organism (Table 1). Many commercial probiotic products currently on the market abroad also contain lactacid producing organisms (Table 2).

Table 1. Examples of microorganisms used in probiotic products

<table>
<thead>
<tr>
<th>Products for human</th>
<th>Products for farm animals</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lactobacillus acidophilus</td>
<td>Bacillus subtilis</td>
</tr>
<tr>
<td>Lactobacillus casei</td>
<td>Bifidobacterium bifidum</td>
</tr>
<tr>
<td>Lactobacillus plantarum</td>
<td>Streptococcus thermophilus</td>
</tr>
<tr>
<td>Lactobacillus reuteri</td>
<td>Bifidobacterium breve</td>
</tr>
<tr>
<td>Lactobacillus johnsonii</td>
<td>L. plantarum</td>
</tr>
</tbody>
</table>

The move towards intestinal isolates resulted in the use of Enterococcus faecium. Other species of streptococci used as probiotics are S. lactis, S. cremoris, S. diacetilactis and S. intermedius. Probiotics also contain bacteria belonging to the genera Lactobacillus, Pediococcus, Propionibacterium and Bifidobacterium. Yeasts (Saccharomyces coxerviae and Candida utilis) and moulds (Aspergillus niger and A. oryzae) are used in animal products. Probiotics may contain one or several (upto nine) strains of microorganisms and may be presented to the animal in the form of powder (loose or in capsules), tablets, granules or pastes (Table 3). They may be administered by direct insertion into the mouth or by inclusion in the food or water. Moreover, to achieve best results it is better to select an organism that is a normal inhabitant of the gut. Bacterial genera commonly detected as components of the human intestinal microflora are listed in Box 2.
In spite of careful selection of strains, permanent establishment of the probiotics may not be achieved in the intestinal tract and multiple dosing is essential if the full probiotic effect is to be obtained.

Box 2. Bacterial genera that are commonly detected as components of the intestinal microflora of humans

**Bacteroides**
Gram-negative, non-spore-forming bacilli. Obligate anaerobes. Obligate anaerobes. Acetic and lactic acids are produced primarily, in the molar ratio 3:2. Glucose is degraded exclusively and characteristically by the fructose-6-phosphate 'shunt' metabolic pathway.

**Clostridium**
Gram-positive bacilli that form endospores. Obligate anaerobes.

**Eubacterium**

**Fusobacterium**

**Peptostreptococcus**

**Ruminococcus**
Gram-positive coccus. Obligate anaerobes. Amino acids and peptides are not fermented. Fermentation of carbohydrates produces acetic, succinic and lactic acids, ethanol, carbon dioxide and hydrogen.

**Lactobacillus**
Gram-positive bacilli, non-spore-forming. Grow best under anaerobic conditions. Lactic acid is a major product of glucose fermentation.

**Escherichia coli**
Gram-negative rods, facultatively anaerobic. Citrate not utilized. Carbohydrates fermented to lactic, acetic and formic acids. Part of formic acid is split by a complex by hydrogenase system to give equal amounts of carbon dioxide and hydrogen. Lactose is fermented by most strains, but fermentation can be delayed or absent. Motile by means of peritrichous flagella or nonmotile.

Possible modes of action of probiotics

Three possible mechanisms have been proposed for the mode of action of probiotic preparations. These are summarize below:

1. Suppression of viable count by:
   a) production of organic acids, hydrogen peroxide and CO₂
   b) production of antibacterial compounds
   c) competition for nutrients or nutrient depletion
   d) competition for adhesion sites

2. Alteration of microbial metabolism by:
   a) increased enzyme activity
   b) decreased enzyme activity

3. Stimulation of immunity by:
   a) increased antibody levels
   b) increased macrophage activity

Though there is some experimental data to support these modes of action, there have been reports of increases in number of eggs produced and also individual egg weights.

**Characteristics of an effective probiotic**

An effective probiotic is required to operate under a variety of different environmental conditions and to survive in many different forms and therefore should have the following characteristics.

1. It should be prepared as a viable product on an industrial scale.
2. It should remain stable and viable for long periods under storage and field conditions.
3. It should have the ability to survive (not necessarily grow) in the intestine.
4. It must produce a beneficial effect in the host animal.
5. It should be non-pathogenic with no toxin production.

Beneficial claims made for probiotic supplementation are numerous and include:

1. Improved growth rate of farm animals due to suppression of a sub clinical infection with a growth depressing microorganism.
2. Improved utilisation of food which may be achieved by increased efficiency of existing digestive processes or by promoting the digestion of previously undigestible substances.
3. Improved health which includes resistance to infectious diseases either by direct antagonism or by stimulation of immunity.
4. Increased egg production in poultry animals.
5. Improved milk production by dairy cattle.

There have been reports of increases in number of eggs produced and also individual egg weights.

**Bacterial interactions in the gut**

Microbial interactions represent the main force which contributes to the homeostasis of the bacterial flora in the gut. This flora forms an ecosystem with its host, comprising (a) biotic components e.g. indigenous and transient microbes, and gastrointestinal epithelial cells which delimit the biotope; (b) abiotic components of dietary origin, namely those that have not been digested during their course through the small intestine and (c) endogenous components coming from saliva, gastric, pancreatic, hepatic and intestinal secretions or excretions, including enzymes, hormones, mucus, bile salts, urea, immunoglobulins, peptides and several other unknown components. All these components interact and the result of such interactions is compatible with the healthy survival of the host. When gastro intestinal disorders arise the ecosystem becomes destabilized. This emphasizes the importance of maintaining microbial interactions in a way that maintain the stability of the ecosystem and the optimum health for the host.

**Metabolic interactions in the gut**

The most important way in which a probiotic organism can exert a beneficial effect on its host is by modifying the metabolic processes of the host. Preparations which may be a consequence of the effect of probiotic bacteria are unable to displace the microbes. In addition, there is some thought that probiotics may also help in preventing the development of antibiotic resistance.
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of studies were carried out by Tanasomwang and Muroga and bacteriovory is widespread among a number of infancy with very little work having been and mariculture in particular are still in the stages of animal nutrition, but their advent into aquaculture Application of such probiotic organisms has beneficial effects.

Application of probiotics in marine culture

Probiotics are widely used in human and animal nutrition, but their advent into aquaculture and mariculture in particular are still in the stages of infancy with very little work having been carried out on the effects of potential probiotic strains on marine finfishes and shellfishes. However, it is known that bacteria account for over 80% of the total bio surface in seawater and bacteriovorv is widespread among a number of marine larvae (Axam et al., 1984). A series of studies were carried out by Tanasomwang and Muroga (1990) on the intestinal microflora of larval and juvenile stages of important species such as red sea bream (Pagrus major), black sea bream (Acanthopagrus schlegeli), Japanese flounder (Paralichthys olivaceus), rockfish (Sebastes schlegeli), tigerpuffer (Takifugu rubripes) and red grouper (Epinephelus aokura) in relation to the microflora of ambient water and feeds. Newly hatched larve and low bacterial populations which increased in direct proportion to the size of the fish up to 10^6 CFU/fish after commencement of feeding with live feeds. Feeding with artificial feed and minced fish caused a decrease. Average bacterial counts were 10^3, 10^2-10^3 and 10^4 CFU/ml or gin water, live diets, minced fish and artificial feed respectively. The bacterial genera recovered from the intestine were Vibrio, Pseudomonas, Acinetobacter, Moraxella, Cytophaga and Alcaligenes.

Based on these observations studies on finfishes have undertaken feeding the animals with the probiotic both directly and indirectly. Indirect feeding being carried out with the help of live food organisms like artemia nauplii and rotifers reared on probiotics (Gite Soupe 1989, 1991). In oral feeding studies, carried out with rainbow trout fingerlings using seven strains of bacteria isolated from the gut, superior growth and feed conversion efficiency was observed in animals fed probiotic diets as compared to control (Sridharr and Austin 1998).

Garriques and Arevalo (1995) have reported on the beneficial effects of nutritional probiotics in developing shrimp of high immunity. Mohamed (1996) used live heterotrophic bacteria as 50% replacement to microalgae in the diet of P. monodon larvae and reported marked improvement in survival and growth rate. Sridharr and Chandrasekar (1996) in evaluating the effect of feeding five strains of bacterial biomass to larvae of P. indicus also revealed the beneficial effects of probiotics on growth and survival of shrimp larvae. Also, mantis shrimp, feed with wheat bran fermented with B.licheniformis and Beauveria sp. fungi, fed to postlarvae of P. indicus showed enhanced growth and survival. Probiotics may therefore provide growth and survival. Probiotics may therefore provide growth factors and inhibit the proliferation of pathogens by stimulating the nonspecific immune response. The studies of Garriques and Arevalo (1995) and Gatesoupe (1994) also agree with these observations and recommend the use of probiotics in increasing the resistance to disease of animals.

Sridhar and Austin (1998) studied the resistance to infection by A. salmonicida in fingerlings of rainbow trout and reported higher survival and resistance in animals fed with probiotic diets as compared to control.

Chandrika (1999) has recently found that Bacillusspp. can be successfully used as probiotics in feed preparations and also for the management of diseases in intensive aquaculture operations, to control the attack of microbial diseases thereby reducing high shrimp mortality.

Though limited in number the information generated from these studies does highlight the effect of probiotics in increasing the production efficiency and resistance to disease of aquaculture organisms. Further studies on the real effect and mode of action of various probiotic preparations for marine finfishes and shell fishes would definitely strengthen mariculture practices.

Future prospects

The use of probiotics in animal husbandry is an accepted practice and is on the increase. However, the interaction of a microbial feed supplement with the host animal and its gut microflora resulting in the expression of the probiotic effect is by its nature a very complex phenomenon. The factors affecting the probiotic response are:

1. Composition of the host animal gut flora
2. Dosing regime which may be continuous or as a single dose
3. Age and type of animal, since physiology and immune status of an animal change as the animal gets older
4. Quality assurance which is viability of the probiotic product and strain variation among species
5. Type of preparation: Because of the variety of different forms, one type may be more suitable than another for a particular animal
6. Production methods of the probiotic product may cause variations in response for the same organism

Therefore, the future development of probiotic products for mariculture purposes is dependent upon the availability of effective and reliable preparations which would give consistently positive results. With the development of such products, the mode of action of probiotic organisms would be understood and it would be possible to look for key biochemical features in the laboratory and select potential candidates for field trials. Genetic engineering allows us to have unlimited access to new strains and with detailed information on the mode of action of probiotics we may be able to introduce the probiotic effect into an organism which permanently colonizes the intestinal tract. It may also be possible to incorporate protective
antigens from pathogenic bacteria into harmless intestinal commensals such as lactic acid bacteria and capitalize on their ability to stimulate the immune system. Genetic engineering would also aid to increase resistance to acid so that probiotics would survive passage through the stomach. Resistance to heat would also be an advantage enabling producers to include probiotics in feed without risking subsequent damage by the heat generated during the pelleting process.

An important development in probiotic research would be the production of non-viable probiotics. Once the biochemical basis of probiotic activity is known, it would be possible to produce the effect by feeding the substance responsible for the activity produced by the viable supplement. The yield can be improved by genetic manipulation without attending the problem of environmental release of genetically altered viable microorganisms.

Improved quality assurance of probiotic preparations should be maintained and their viability sustained throughout the stated shelf-life of the product. The live nature of probiotics creates unique features and problems compared with antibiotics and other drugs. Future research and development may enable us to identify the biochemical feature responsible for the probiotic effect and give rise to a second generation of probiotics which are nonviable.

**CONCLUSION**

The balance between normal and potentially pathogenic bacteria is altered in the intestine of animals subjected to stress. The result is domination by the pathogens giving rise to deteriorative changes like diarrhoea and decreased production performance in the host. Probiotics - products containing strains of lactic acid - producing beneficial organisms - are biologic tools that promote digestive balance. Probiotics supplement intestinal microflora with beneficial bacteria and create conditions non-conducive to the growth of pathogens. Along with decreasing intestinal colonization by pathogens, probiotics improve production efficiency by increasing average daily weight gain, feed consumption and feed efficiency.

The use of probiotics such as food additives is preferred over the use of antibiotics, because they have no problems associated with antibiotics viz. toxicity, allergy, residues in food, bacterial resistance and indiscriminate suppression of intestinal microflora. However, probiotics should not be considered as alternatives to antibiotics in disease therapy, but as a complementary therapy for restoring balance to the intestinal flora.

The mechanism of action of probiotics which include depletion of nutrients, production of acids and antimicrobial substances, competition for adhesion receptors in the intestine and immunostimulation create an environment incompatible to the growth of pathogens.

The probiotic product selected should be capable of implanting itself in the gut and should inhibit pathogens and as far as possible it should be a normal inhabitant of the intestine capable of tolerating low pH levels and the effects of bile. The derivation of such efficaceous probiotics requires substantial research and development, especially at the level of fundamental science.

**REFERENCES**


