Probiotics: An Alternative to Antibiotics

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Few topics confound a nutritionist or veterinarian more than an ingredient over which he has no control and in many cases even less information. Probiotics fall squarely into this category. A good deal of the interest in probiotics with regard to livestock has been based on the possible banning in some countries and the *actual* banning in others of antibiotics, otherwise called growth promoters, as routine feed additives.

The term probiotic was first used by Parker (1974) to describe "organisms and substances which contribute to intestinal microbial balance." The term probiotic originated from two Greek works meaning "for life" and contrasted with the term antibiotic which means "against life."

The concept of probiotics has often been maligned, in many cases deservedly so, and compared, at times, to witchcraft or voodoo. The purpose of this article is to review the current use of probiotics and explain why in certain instances the concept has failed while in others it has worked magnificently.

Belief in the beneficial effects of probiotics comes from Metchnikoff's (1907) original contention that the longevity of Bulgarian peasants was related to their consumption of large amounts of milk fermented with organisms such as *Lactobacillus acidophilus.* Metchnikoff speculated that detrimental microbes in the intestinal tract excreted substances that were harmful to the host. Through ingestion of beneficial organisms, which he believed were contained in yogurt, one could improve the intestinal environment through what is now referred to as "microbial population manipulation." Through the constant infusion of "friendly" organisms in the diet colonization of the gastrointestinal tract by disease-causing (pathogenic), "unfriendly" organisms was prevented and thus health and life expectancy improved. Thus was born the concept of microbial inoculation based on the principle of "competitive exclusion."

The idea of lactic acid bacteria either excreting metabolites which are harmful to pathogens or of excluding them from colonization sites received one of its first scientific endorsements as early as 1924 in the work of Marriott & Davidson. These authors reported that in Children's Hospital in Washington young infants fed similar quantities of fresh cow's milk or cow's milk to which lactic acid was added had higher mean daily weight gains. The use of acidified milk replacers and starter feeds whether acidified with organic acids or with natural lactic acid bacteria is a direct descendant of this early Washington work.

Nurmi & Rantala (1973) demonstrated the protective effect of the gut flora in young chickens. By dosing newly hatched chicks with feces from adult chickens, colonization of the cecum by *Salmonella infantis* was restricted. The microorganism responsible was anaerobic but was not specifically indentified out of the 48 strains containing predominantly lactobacilli and streptococci (Rantalla 1974). Numerous reviews have shown that the inclusion of *Lactobacillus acidophilus* to the feed or drinking water causes changes in intestinal microflora to occur (Sandine, et al 1972, Sheck 1976).

Typical of this work was that of Gilliland (1979) who showed that feeding humans unfermented milk containing cells of *Lactobacillus acidophilus* caused significant increases in the numbers of lactobacilli in their feces. Conversely, Ellinger et al (1978) observed decreases in the number of coliform bacteria in the feces of calves fed whole milk containing *Lactobacillus acidophilus.*Similarly, Muralidhara et al (1977) found that *Lactobacillus acidophilus* inclusion in the diet of young piglets decreased the number of *E. coli* in the digestive tract. The beneficial effects from inclusion of *Lactobacillus acidophilus* as a supplement to poultry feeds has been reported (Francis et al, 1978; Fuller, 1977; and Tortuero, 1973). Thus so long as active bacteria are added to feed or water there would appear to be no question but that a shift in microbial population away from toxin-forming *E. coli* and towards benevolent lactic acid producers can be achieved. This fact is fundamental to the probiotic approach. In addition, certain health benefits have been attributed to lactobacillus including alleviation of abdominal and intestinal disorders, reduction in dental caries and even anti-tumor activity (Yakult, 1980; Tsuchiya, et al 1982).

With so much reported research, how and when would a probiotic best be used in animal diets? Healthy animals are generally characterized by having a well-functioning intestinal tract. This fact is fundamental in the efficient conversion of feed for growth or production. A most important characteristic of a well-functioning intestinal tract is *the balance* of its bacterial microflora. A healthy intestinal tract has a preponderance of lactic acid-producing bacteria such as lactobacilli and streptococci. This equilibrium within the intestinal tract is upset any time the animal is put under stress. At this time, the balance swings in favor of the pathogen *E. coli.* For example, Smith (1954) showed that calves with severe diarrhea had, when sacrificed, an elevated level of *E. coli* in the abomasum.

This was confirmed by Ingram (1962) with less than 100 *E. coli* per ml in the duodenum of a healthy calf compared to levels some *million times higher* in diarrhetic animals.

Diseased pigs show similar changes in the small intestine. In both cases it is the ratio of*E. coli* to lactobacillus which is crucial. The faster specific growth rate of *E. coli* will always enable that microbe to become dominant. Hence lactobacillus and streptococcus populations must be maintained at high levels. Smith (1971) showed that while lactobaccilli counts were similar in healthy and diseased pigs, large differences existed in the *E. coli* population of the upper regions of the digestive tract *(Table 1)*: In fact, there was a 10,000 fold increase in the *E. coli* population in the case of a diseased pig.

TABLE 1

Bacterial Content of the Alimentary Tract in Eight Pairs of Healthy and Diseased Piglets.

	Small Intestine				Large
	1*	3	5	7	Intestine
E. coli					
Diseased	7.3	8.5	9.4	9.5	9.6
Healthy	3.6	4.8	7.3	8.3	9.0
Lactobacillus					
Diseased	8.1	8.4	8.6	7.4	8.8
Healthy	7.9	8.0	8.0	8.3	9.0

All counts: Log of Viable Bacteria

(From Smith, 1971)

*The seven levels of the small intestine examined were equal distances apart. Level 1 was distal to the abomasum and level 7 was proximal to the large intestine.

The importance of early administration of lactic acid bacteria was confirmed when Barnes (personal communication) demonstrated that turkey poults were already heavily infected with *E. coli* when they left the hatchery. Poult mortality was drastically reduced when *E. coli* levels were reduced but increased when levels went unchecked. He recommended that lactobacillus be administered in the drinking water and misted in the hatchery to insure early colonization of the bacteria. In the young pig *E. coli* can be detected in the feces within 2 hours of birth while lactobacillus is not detected for at least 18 hours.

How and where can probiotics help?

The probiotic hypothesis is that if sufficient lactic acid-producing bacteria can be introduced into the intestinal tract at a time when the balance has swung in favor of *E. coli* (stress or disease) or when no lactic bacteria are present (birth or following antibiotic treatment) then digestive upsets can be minimized or overcome. An example of such a stress period is when an animal is subjected to shipping stress. In addition to the lack of access to feed and water en route, intake procedures, environmental changes or unfamiliar feed mean feed intake will be low for a time. It is during this adaption period that many of the manifestations of "shipping fever" occur. Resistance being low, undesirable bacteria are able to proliferate. Maintaining healthy intestinal flora is critical during such transitional periods.

Weaning of piglets also represents a time of major stress to an animal. Pollman (1986) concluded that while results of adding lactobacillus to starter diets of pigs were variable, since 1977 they have been positive *(Table 2)*. The significance of the later results are related to the recognition by nutritionists that it is not the quantity of material added to a feed but the actual number of bacteria added that is important.

TABLE 2

Summary of Research Conducted with Starter Pigs Fed Probiotics

Culture	No. Studies	No. Pigs	Item	% Improvement Over Control
Lactobacillus	4	960	Gain	8.4
Fermentation product			Feed/Gain	4.8
Mixed	7	1052	Gain	2.5
Lactobacillus			Feed/Gain	6.8
Pure	2	227	Gain	8.6
Lactobacillus				

(Adapted from Pollmann, 1986)

The value of adding a probiotic to growing/finishing pigs is somewhat questionable. With the animals apparently not under stress and gut microflora well established, the infusion of additional bacteria brings negligible results. This is shown in Pollman's summary *(Table 3).*

TABLE 3

Summary of Research Conducted with Growing and Finishing Pigs Fed Microbial Cultures

Culture	# Of Studies	# Pigs	Item	% Response Over Control
Mixed	5	568	Gain	0.7
lactobacillus			Feed/Gain	1.6
Streptococcus	3	825	Gain	-1.8
faecium			Feed/Gain	-0.7

(Adapted from Pollmann, 1986)

The importance of stress also played a role in Mordenti's (1986) work *(Table 4)* and confirmed the value of employing lactic acid bacteria in feeds for piglets prior to and during weaning. The use of peptides in conjunction with lactobacillus improved performance. The combined administration reduced mortality following diarrhea, cut in half the incidence of digestive tract disorders and improved animal growth significantly. Also, while peptides or lactic bacteria alone foster animal productivity, their combination resulted in a synergy of action of undoubted scientific and practical interest.

Mordenti (1986) concluded that the results of their research confirmed the validity of the use of lactic acid bacteria in the prevention of pathological disorders of the digestive tract in piglets.

The key common factors in successful use of probiotics have been the presence of viable or living bacteria in the probiotics and of stress in the animals. In many negative university or institute studies either one or both of these factors have been absent.

TABLE 4

Probiotics and Peptides Associated: Effects on Weight Gain, Diarrhea and Mortality Rate from Birth to Weaning

Treatments[2]	A Control	B Peptides	C Probiotics	D Peptides & Probiotics
Piglets (no.)	471	480	484	499
Avg. birth wt (kg)	1.22	1.21	1.21	1.22
Avg. daily gain (g)	186[a]	195[b]	207[c]	221[d]
Diarrhea rate (%)				
1st week	20.4[a]	16.4[ab]	9.1[b]	6.0[b]
2nd week	28.8	28.8	21.4	14.7
3rd week	11.7[ab]	23.5[a]	7.8[b]	5.8[bc]
Mortality				
Total	11.7[a]	12.7[a]	11.5[a]	8.4[b]
Diarrhea	6.7[ab]	7.3[a]	5.9[b]	4.2[c]
Other	5.0	5.4	5.6	5.2

[1] Means of two trials carried out for a period of 34 days in autumn and winter.

[2] At birth (1st day of life) with: CONTROL (.2 g dried whey): PEPTIDES (.2 g whey + .2 g proteolysate); PROBIOTICS (.2 g whey with 200 million cells of *Streptococcus faecium*); PEPTIDES + PROBIOTICS (.2 g whey + g proteolysate + 200 million cells of *Streptococcus faecium*). All substances were suspended in 2 ml water.

[abcd] Means not sharing a common superscript letter are significantly different (P is less than .05).

(Adapted from Mordenti, 1986)

Why have probiotics not worked so many times?

Many reports have shown no response to *Lactobacillus acidophilus* or *Streptococcus faecium* inclusion in the diet during stress periods where a response would have been expected. It has been suggested that the viability of the bacteria *if* indeed any were present was probably the reason. The response to *Lactobacillus acidophilus* is dependent upon having sufficient quantities of viable bacteria present in the diet which have the potential to successfully colonize tha animal's intestinal tract. Gilliland (1981) examined 15 commercially available probiotic supplements and found that only two of them contained more than one million viable lactobacilli per gram.

Both Lyons (1986) and Fallon (1986), when discussing factors affecting the potential of probiotics to colonize the gut, stressed the importance of a) attachment to the gut epithelium which can allow slow-growing organisms to colonize, and b) the ability to grow in the gut environment where the successful colonizer can utilize available substrate and resist antibacterial agents present in the environment. The mode of adhesion of bacteria to the intestinal wall has been reported by Lyons (1986) and is illustrated in *Figure 1).* Barrow et al (1980) assayed a selection of bacteria isolated from the pig gut for the ability to adhere to stomach squamous cells in an in vitro study found that the number of bacteria adhering per cell ranged from 0 for *E. coli* to 42 for *Streptococcus salivarius* (Table 5). Fuller (1977) contended that an organism could establish itself in the stomach either by attaching to the epithelium or by rapid growth. When a lactobacillus strain selected on the basis of its high adhesion index and good growth in vitro was fed to pigs there was a significant reduction in coliform count in the stomach and duodenum. However, many bacteria are host specific; so a probiotic should ideally contain a microorganism isolated from that type of animal.

TABLE 5

Correlation Between Growth in Diet, Adhesion to Epithelium and Colonization of the Stomach Lumen

	L. acidophilus	L. salivarius	S. faecium	E. coli
Growth in Diet	1.8	0.8	3.9	4.4
Adhesion to Stomach Wall	+	+	—	—
Presence in Stomach Lumen	+	+	+	+

Growth in $\log_{10}$ viable count after 24h at 37°C.

(Adapted from Barrow et al, 1980)

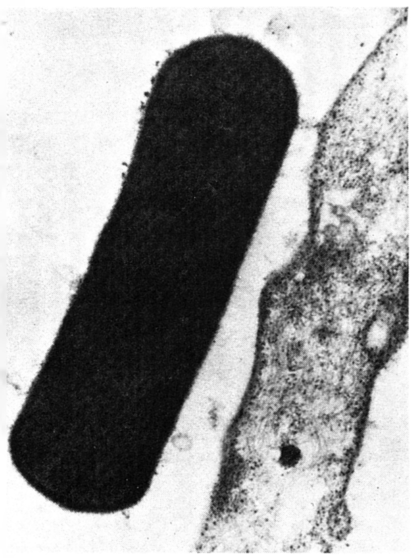
The effectiveness of probiotic bacteria are also dependent on their resistance to hydrochloric acid and to bile acids. It is well established that gastric acidity is an important barrier to gut colonization. It is essential therefore that the probiotic bacteria have the ability to survive acidic conditions in the stomach. Gilliland (1981) suggested that bile resistance was an important criterion in the selection of an effective probiotic and found that only one of 15 commercially available products examined contained more than one million bile resistant lactobacilli per gram. Many bacteria are known to metabolize bile salts and their growth to be stimulated by them.

For a probiotic, therefore, to be successful it must meet certain criteria. Alltech has the following specifications for their bacteria.

1. The bacteria must be capable of reaching and colonizing the intestinal tract (Figure 1). The bacteria spp is normally one isolated from the particular animal and is first grown up on a plate containing bile acids. At the end of the harvesting process the bacteria are screened again for bile resistance. Alltech quality control calls for 70% plus resistance.

FIGURE 1

Lactobacillus Adhering to Intestinal Tract



From Miller (1986)

2. The bacteria spp selected must be a rapid acid producer. Each bacteria has its own specific rate of acid production and only those capable of producing above a specific rate are acceptable. The importance of acid lies not only in its anti-*E. coli* effect but also because acidified conditions in the gastrointestinal tract or abomasum are more conducive to good nutrition. Recent work from Burnell et al (in press) confirmed this. In trials using a commerical product, Acid-Pak 4 Way, acidification increased weight gain in piglets on corn soya diets and improved feed efficiency in both corn and soya diets and diets containing milk products increased significantly (Table 6).

The pigs used in the study were weaned at 28 days. Earlier weaning when digestive enzyme levels were less developed might have given a greater response.

TABLE 6

Effect of Acid-Pak 4-Way on Performance of Weanling Pigs[a]

Acid %	0	1	0	1
Dried whey %	0	0	15	15
Daily gain (lb)	.643	.703	.717	.751
Daily feed (lb)	1.133	1.162	1.270	1.231
Feed/Gain	1.773	1.658	1.775	1.676

a Eight replicate pens of six pigs per pen; 16.3 to 35.9 lb; 28-day test.

From Burnell et al (in press)

3. The bacteria should be present in sufficient numbers to be significant. As both *E. coli* and lactobacillus are facultative anaerobes both can survive under similar conditions, but the specific growth rate of *E. coli* is so much faster that higher levels of the lactic acid bacteria must be present to ensure its survival.

4. The bacteria must be quickly activated and have a high specific growth rate.

The bacteria shoul have some anti-*E. coli* activity. The need for this last specification was confirmed by Jonsson and Olsson (1985) who reported that the production of antibacterial substances should be taken into consideration when selecting a lactobacillus product as a feed additive.

Recent Developments

The strains of bacteria used by Alltech are routinely screened against a range of pathogenic bacteria including *E. coli.* Typically these bacteria, having been grown, are separated from their growth medium and the medium itself is then monitored for anti-*E. coli* activity. The area of the zone of inhibition is related to the bacteria's ability to kill *E. coli* due to the production of some metobolite.

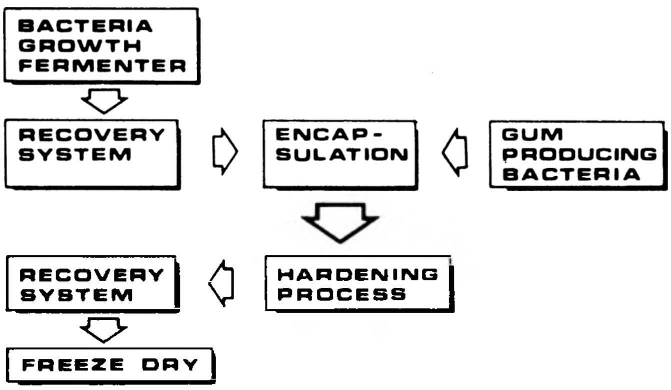
By altering bacterial growth conditions and by using gentle harvesting techniques (Figure 2), the level of this metabolite can be increased. The concentration of the metabolite is directly related to its *E. coli* and other pathogen-killing power.

A similar effect is obtained when using the purified antibiotic nisin, but as yet it has not been identified as a component of the medium.

Pollman (1986) also considered the practicality of handling a pure bacteria culture to be an area where nutritionists needed to be educated. Lyons (1986) showed that microencapsulation ensured liveability of the bacteria. In addition, however, both the growth conditions while propagating the lactobacillus and streptococcus, how they are harvested, and how they are protected prior to freeze drying is important. Recovery systems using centrifugation are known to severely weaken the cell walls and membranes making bacteria survival during freeze drying difficult. Using a patented microencapsulation process (Figure 2), Alltech coats each bacteria with a water soluble betaglucan which provides protection during drying and storage.

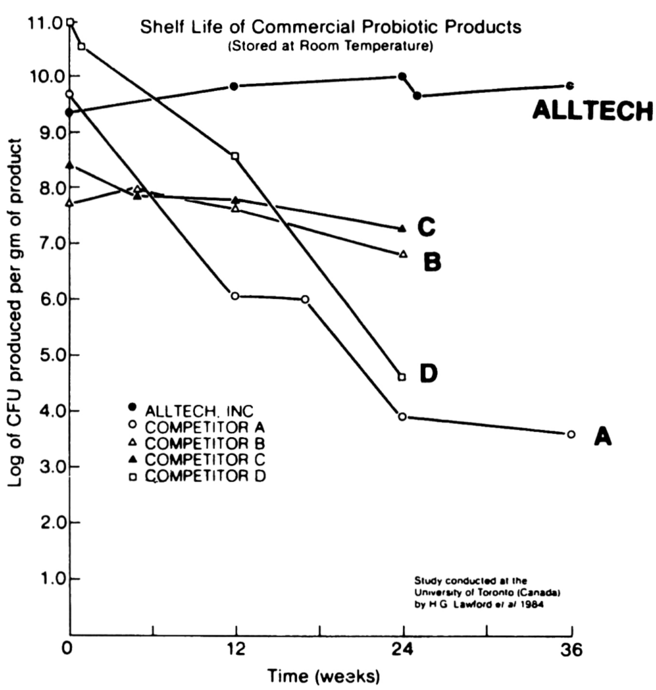
FIGURE 2

Alltech Encapsulation Process



Stability studies (Lawford 1985) at the University of Toronto recorded no loss in activity of this microencapsulated bacteria over a 36 month period at room temperature and reported it to be substantially greater than any other products analyzed (Figure 3).

FIGURE 3



The bottom line is having sufficient lactobacilli growing in the intestinal tract such that they can compete favorably with the *E. coli* population. Application to the feed should ideally be post-pelleting although some 40% of the microencapsulated bacteria could survive pelleting.

How does a probiotic work?

In general, Lyons (1986) contended that the phenomenon of lactic acid bacteria creating an environment which is unfavorable or even damaging for pathogenic bacteria is well known. However, its operational mechanism still remains to be exactly defined.

Among the theories put forward have been:

1. The production of lactic acid and the ensuing decrease of pH.
2. The production of hydrogen peroxide and its antibacterial action.
3. The production of natural antibiotic substances, particularly nisin, from streptococcus and acidophilin from lactobacillus.
4. An antienterotoxin activity principally against the enterotoxin of *E. coli.*
5. The adhesion to the cell walls of the intestinal tract, thus preventing colonization of the pathogen.

Lactic acid alone does not successfully inhibit *E. coli*, but activity of other metabolites produced by lactobacillus and streptococcus is improved by the lower pH.

Conclusions

While much research still remains to be done on probiotics, the eminent possibility of a ban on antibiotics makes their use in feed no longer a topic to be ignored. Too many solid field results are accumulating when a stable microencapsulated bacteria is used. In times of stress when the correct numbers of bacteria are added, positive results are assured.

Care must be taken however to insure the product being used is indeed a stable microorganism. Only then can valid conclusions be drawn.

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