The availability of large volumes of safe, quality food of animal origin at affordable prices remains the goal of modern food production and continues to be a challenge today. These food products should be produced from animals raised without excessive reliance on antibiotics to maintain animal health and growth, and with minimal environmental impact.

In the feed to food chain, where raw materials are being fed to farm animals resulting in finished food products, Total Nutrition plays an important role. In a nutshell, Total Nutrition encompasses the quality and safety of the feed, and the effects of nutrition on the optimum growth and the overall health of the animals. In the past, feed was merely considered as a source of nutrients such as carbohydrates, proteins, fats, vitamins and minerals. In practice, however, animal feed contains, in addition to the nutrients, other components such as antioxidants, enzymes, organic acids, biosurfactants and probiotics that exert a beneficial effect on the health and metabolism of the animal. Thus, in Total Nutrition feed has multiple properties encompassing nutritional, health and environmental functions.

For the effective digestion and absorption of nutrients from feed, the role and microflora of the gastrointestinal tract is critical. This organ is also the site where direct contact takes place with micro-organisms. This contact with both non-pathogenic and pathogenic micro-organisms from feed, water and the environment is continuous throughout the lifespan of the animals.

In healthy animals, the balance between the populations of beneficial micro-organisms and those with potential to cause infection is important to maintaining good gut and overall health of the animal, causing minimal stress to the animal. This management of animal health is important not just for the food animals but also for human health. The tripartite relationship influencing health status of host animal and disease avoidance is best illustrated as shown in Fig. 1.

In healthy animals, the intestinal microflora and the host immune system work together to provide resistance to enteric pathogens. Microbial colonisation of the animal has important health implications. A study that compared germ-free animals, or animals that do not require the residency of microbes to survive, to those that do concluded that the germ-free animals were much more susceptible to infection.

Thus, it is proposed that the healthy bacteria that inhabit the gastrointestinal tract may promote good health by negating the adverse activities of harmful commensals and invading microbes. Stressors such as heat, humidity, overcrowding and even antibiotics regime may kill beneficial bacteria causing imbalance in the microflora in the gut. The result may be decreased performance, increased mortality and morbidity. Indeed, the modulation of an unbalanced indigenous microflora forms the rationale of probiotic therapy.

**Nutrition-based health**

Probiotics are viable bacterial cell preparations or foods containing viable bacterial cultures or components of bacterial cells that have beneficial effects on the health of the host. By definition, a good probiotic strain should possess the following characteristics:

- Strain safety.
- Acid and bile stability.
- Ability to adhere to the epithelium.
- Selective maintenance of beneficial gut bacteria.
- Specificity against pathogens of interest.
- Speed of growth within the gut.
- Functional health characteristics.
- Suitability for use with other microbials.
- Stability to pelleting conditions.
- Undergone controlled evaluation to document health benefits in the target host.

There are many favourable effects associated with the use of beneficial micro-organisms in animal feed. Probiotics possess a combination of benefits that other alternatives cannot provide alone.

For example the production of organic acids, the competitive exclusion of enteric pathogens such as Escherichia coli, Campylobacter jejuni and Salmonella enteritidis, enhancing growth and viability of beneficial gut microflora, improved digestion and absorption of nutrients, production of antimicrobial substances and an enhanced immunity system of the host are all attributes that help probiotics to play a critical role in protecting the animal against enteric diseases.

The demands of modern society for large volumes of animal origin protein have led to intensive animal production where large numbers of animals are raised in relatively small areas.

This drive for high productivity has invariably exposed the animals to considerable amount of stress resulting in health maintenance problems and disease issues. This is

**Table 1. Influence of CloSTAT on weight gain, feed intake, FCR and mortality rate of broilers under experimentally induced necrotic enteritis.**

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Weight gain (g)</th>
<th>Feed intake (g)</th>
<th>FCR (g)</th>
<th>Mortality (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Negative control</td>
<td>869</td>
<td>1425</td>
<td>1.64</td>
<td>0.2</td>
</tr>
<tr>
<td>Negative challenged control</td>
<td>741</td>
<td>1378</td>
<td>1.86</td>
<td>35.9</td>
</tr>
<tr>
<td>Antibiotic challenged control</td>
<td>891</td>
<td>1470</td>
<td>1.65</td>
<td>17.2</td>
</tr>
<tr>
<td>CloSTAT challenged control</td>
<td>906</td>
<td>1513</td>
<td>1.67</td>
<td>9.4</td>
</tr>
</tbody>
</table>

Continued on page 12
Necrotic enteritis (NE) is an enterotoxæmic disease caused by the Gram positive, spore-forming anaerobe, *C. perfringens* that leads to the development of necrotic lesions in the gut wall resulting in mortality of poultry. It is a disease with complex epidemiology and pathogenesis. For over three decades, alpha toxin, a phospholipase C enzyme was thought to be the key virulence factor in the disease. However, recent research has implicated TpeL, a large toxin, and NeTB, a protein with similarity to pore forming toxins, as playing a role in the disease with NeTB almost always found in outbreak isolates from NE chickens. Although *Clostridium perfringens* is commonly found in the gastrointestinal tract of poultry, the occurrence of necrotic enteritis is, however, sporadic. Nevertheless, feed contaminated with *C. perfringens* has been implicated in outbreaks of necrotic enteritis in chickens. Studies have also shown that healthy chicken have a relatively low number of *C. perfringens* in its gastrointestinal tract, while the increase in the concentration of the bacteria has been correlated with the necrotic enteritis condition.

Antibiotics such as bacitracin and lincomycin are commonly used to treat poultry suffering from necrotic enteritis. However, due to the isolation of antibiotic-resistant strains of *C. perfringens* from chickens and turkeys, and a significant shift in consumer attitudes and legislation in the European Union and elsewhere towards raising food animals without drugs and medicines, this has meant that other non-antibiotic alternatives are now being sought. In this respect, the use of a probiotic in the feed to influence the gastrointestinal microflora in a manner beneficial to the health of the animal, thus, represents a major breakthrough in Total Nutrition based health.

**Active microbial strain**

In an attempt to develop an active microbial strain to inhibit *C. perfringens*, Kemin have formulated CloSTAT that contains a naturally occurring strain of *Bacillus subtilis* PB6 that was isolated from the intestinal tract of a healthy chicken. This patented proprietary strain, closely associated with the intestinal epithelium, was able to tolerate gastric and bile conditions.

The CloSTAT strain is able to form spores and this is a huge advantage in the strain’s survival during pellet formation, which is widely used for broiler feed production. CloSTAT is found to secrete a group of peptides that, while inactive towards beneficial gut bacteria such as *Lactobacillus* and *Bifidobacterium* spp., are inhibitory towards common enteric pathogens such as *Clostridium perfringens*, implicated in outbreaks of NE in broiler chickens and *Campylobacter* spp. in vitro well diffusion assays.

Using LC/MS and Q-TOF analyses, the active metabolites have been found to possess a cyclic structure comprising seven amino acids, collectively known as surfactins. The mode of killing action is due to the ability of the surfactin molecules to form pores on the cell walls of the bacteria using electron microscopy.

**Effect on birds with NE**

The bactericidal effect of CloSTAT against *C. perfringens* was clearly demonstrated in an in vivo 27-day broiler trial in which the condition of NE was experimentally induced. Birds were fed a corn-soya diet unsupplemented or supplemented with either bacitracin at 50g/t or CloSTAT at a dosage of 500g/t. All birds were orally inoculated with a mixed coccidial inoculum and, except for the posi-

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Weight gain (g/bird)</th>
<th>FCR (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Negative control</td>
<td>2111&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.91&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Antibiotic control</td>
<td>2100&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.89&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>CloSTAT</td>
<td>2208&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.81&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

*Table 2. Influence of CloSTAT on weight gain and FCR of broilers fed a corn-soy based diet (days 1 to 42).*
tive control group (non-medicated/unin-
fected birds), followed by a broth culture of
C. perfringens once daily for three days.
The experimentally induced necrotic enteri-
tis condition had a clear impact on the animal
performance affecting feed intake, weight
gain, FCR and especially mortality (Table 1).
In contrast, no significant difference was
observed between the antibiotic challenged
control group and the CloSTAT treated chal-
lenged group with respect to the infected
control group for weight gain and FCR even
when the birds were challenged. It is of inter-
est to note that birds receiving CloSTAT had
an even lower mortality rate than those
receiving the antibiotic.
When CloSTAT was added to broilers
raised under normal, unchallenged conditions
on a corn-soy diet supplemented with
CloSTAT they showed significantly better
weight gain and feed conversion ratio com-
pared to birds fed unsupplemented feed or
feed supplemented with a mixture of antibi-
otics as growth promoters (Table 2).
The same observations were also seen in
birds fed a wheat-based diet, where they reg-
istered a significantly better weight gain and
feed conversion ratio than birds not receiving
the B. subtilis PB6 strain (Table 3), suggesting
CloSTAT to be a suitable antibiotic growth
promoter or AGP replacement.

### Conclusion

The development of a complex stable gas-
троintestinal microflora is important for health
maintenance and disease avoidance.
Micro-organisms as feed additives can have
beneficial effects on animal health and perform-
ance. Establishing optimal conditions in the
gut during particularly stressful periods in the
growth of the animal is important to prevent
various enteric disorders such as necrotic
enteritis.
While the gastrointestinal health of animals
raised for food has relied very heavily on the
use of antibiotics and other drugs in the
recent past, the use of probiotics in feed rep-
resents a breakthrough in the Total Nutrition
based approach to animal health.
By maintaining a state of eubiosis in the gas-
trointestinal tract, active beneficial microbial
strains such as B. subtilis PB6 serve to
improve the growth rate and feed utilisation
of poultry.
Previous research has shown that when
cells of B. subtilis were fed to experimental
animals, the intestinal tract was recolonised
by beneficial micro-organisms such as
Lactobacilli spp.
Furthermore, the ingestion of Bacillus spp.

### Table 3. Influence of CloSTAT on weight gain, feed intake and FCR of broilers fed a
wheat-based diet (days 1 to 42).

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Weight gain (g/bird)</th>
<th>Feed intake (g/bird)</th>
<th>FCR (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Negative control</td>
<td>2612</td>
<td>4691</td>
<td>1.80</td>
</tr>
<tr>
<td>CloSTAT</td>
<td>2707</td>
<td>4640</td>
<td>1.71</td>
</tr>
</tbody>
</table>

B. subtilis PB6 is resistant to heat. The results
from animal trials show that ileal populations of
Clostridium spp. were significantly reduced in
broilers fed on CloSTAT.
In terms of growth performance, broilers in
the CloSTAT treatment group showed com-
parable or even better weight gain and/or
feed conversion rate over those in the nega-
tive control and antibiotic groups.
Furthermore, the spore forming property
of B. subtilis PB6 is a huge advantage in their
survival and application in pelleted feed.
Used in a Total Nutrition programme for
poultry, B. subtilis PB6 in particular, can help
to ensure a balanced intestinal microflora and
reduce losses from sub-clinical clostridial
infections and other enteric challenges.