Selected and specific probiot and Clostridium infections in

Food safety is a major issue in the poultry industry. Many efforts have been undertaken to reduce the level of salmonella's and clostridia in broilers. Egyptian trials showed that probiotics could support a program aimed at controlling these disease-causing microorganisms.

By Wiebe van der Sluis

Competitive exclusion and specific probiotics are natural control methods based on ensuring the bird has adequate gut micro flora to counter pathogenic bacteria colonization (e.g. clostridium, salmonella or pathogenic E.coli) in its digestive tract. In the early nineties Japanese researchers discovered that probiotics such as *Lactobacillus acidophilus* and *Streptococcus faecium* were able to reduce the severity of necrotic enteritis. Even before this finding various theories have been proposed to explain the mechanisms by which avian indigenous gut microorganisms protect their host against invading enteropathogens.

A barrier against salmonella

The bird's micro flora is potentially depleted for a period of time at hatching and following any medication with an anti-microbial. The gastrointestinal tract harbours a micro flora formed immediately after the bird is born and is an important barrier against colonization of potentially pathogenic microorganisms. The micro flora of the healthy bird consists of many microorganisms, of which *Lactobacilli, bifidobacteria* and *bacteroides* species are the most important groups. They constitute approximately 90% of the flora and also include *Enterobacteriaceaea, Enterococci* and *Clostridi*. Probiotics are able to inhibit the growth of potentially pathogenic microorganisms by lowering the pH through production of lactate, lactic acid and volatile fatty acids. The ceacum is the main site of *Salmonellae* colonization, and studies have shown that providing newly hatched chicks with intestinal microflora from adult chickens decreases the incidence of this colonization.

Clostridial infections

Four disease conditions are caused by clostridial infections. *C.colinum* is the cause of ulcerative enteritis; *C.perfringens* and *C.septicum* have been isolated from cases of necrotic enteritis and gangrenous dermatitis respectively. *C.botulinum* is the etiology of botulism. While *C.perfringens* is obligate anaerobic bacterium in the intestinal tract of chickens. The organisms are relatively innocuous unless cofactors occur such as dietary changes, severe stress, coccidiosis, or immunosuppressive infections. Toxins produced by clostridial organisms are responsible for many of these conditions. Alpha toxin produced by *C. perfringens* types A and C, and beta toxin produced by *C. perfringens* type C, are believed responsible for intestinal mucosal necrosis, the characteristic lesion of necrotic enteritis. Widespread use of antibiotics as therapeutic agents and growth promoters resulted in the development of resistant bacteria that made their subsequent use for therapy difficult and result in occurrence of antibiotic residues at the poultry products; the direction towards the use of competitive exclusion and probiotics are natural control methods based on ensuring the bird has an adequate gut micro flora to counter pathogenic bacterial colonization in the digestive tract. In poultry production several stresses result in depressing the dominant beneficial flora (change of feed, high pathogen pressure with resistant genes or high adhesion power, anti-microbials, density). A minimum level of positive lactic acid bacteria and its associated lactic acid produced is necessary to limit the proliferation of several pathogen germs.Fukata demonstrated in 1991 high

Table 1 - Intestinal colonization of C. perfringens in treated and untreated broilers with P. acidilactici (bacterial count / gm intestinal contents x 105)

Treatment	Clostridium perfringens infection				
	0 hr.	3 Ds PI	7 Ds PI	14 Ds PI	21 Ds PI
P. acidilactici	0	0	0.5*	1.5*	2.5*
Non-treated	0	1.25	2.75	12.5	25

*Significant difference (P <0.05)

Table 2 - Caecal colonization of C. perfringens in treated and untreated broilers with P. acidilactici (bacterial count / gm caecal contents x 105)

Treatment	Clostridium perfringens infection				
	0 hr.	3 Ds	7 Ds PI	14 Ds PI	21 Ds PI PI
P. acidilactici	0	0	0	0	0.5*
Non-treated	0	0	0.5	3.0	17.5

*Significant difference (P <0.05)

ics may reduce Salmonella broilers

Probiotics are able to inhibit the growth of potentially pathogenic microorganisms by lowering the pH through production of lactate, lactic acid and volatile fatty acids.

differences of Clostridium contamination between conventional broilers (without flora 9,28 log Clostridium perfringens /g digesta) and conventional broiler (<2 log /g digesta). Probiotics stimulate natural resistance of the organism by increasing the number of antibodies and the effectiveness of macrophages. The effect of probiotics on the organism is due to the better adhesion of the lactic acid bacteria to the intestinal epithelium in comparison with the pathogenic bacteria, and stopping the implementation of those bacteria over the mucus membranes of the intestine.

Trial material

In Egypt trials were conducted to better understand the process and possible effectiveness of *Pediococcus acidilactici* (Bactocell®) in reducing intestinal and ceacal colonization with *Salmonella typhimurium* and *Clostridium perfringens* type C in broilers. This probiotic, produced by Lallemand, France is the first to receive EU registration. It is classified as a lactic acid bacteria able to produce significant levels of lactic acid L+. The experiments, headed by Prof Dr MHH Awaad were carried out under controlled laboratory conditions in the Poultry Diseases Department of the Faculty of Veterinary Medicine at Cairo University. Five hundred and forty day-old meat type chickens (Hubbard) from a commercial hatchery were assigned into 9 equal groups (1-9) consisting of 60 birds each (3 replicate pens of 20 chicks each). All were kept in separate pens at a density of 12 birds / m^2 and fed a commercial balanced ration ad libitum. The chickens were vaccinated against Newcastle disease using Hitchner B1 vaccine at 5 day-old and La Sota vaccine at 18 days. The ration contained semduramicin at a concentration of 25 PPM as a coccidiostat. No antibiotics were added to the ration. The test groups received the probiotic at a 100 g/tonne feed level for the entire period of the experi-

Figure 1 - Weight gain of *P. acidilactici* treated and untreated broiler chickens

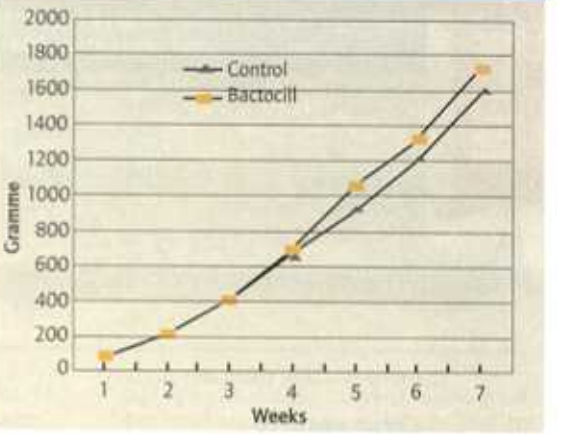

Figure 2 - The histopathological liver lesion score after E.coli infection in *P. acidilactici* treated and untreated groups

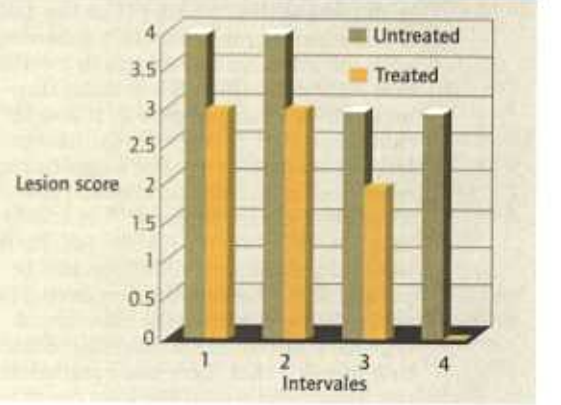

Figure 3 - Caecal colonization of *Salmonella typhymurium* in treated and untreated broiler chickens with *P. acidilactici*

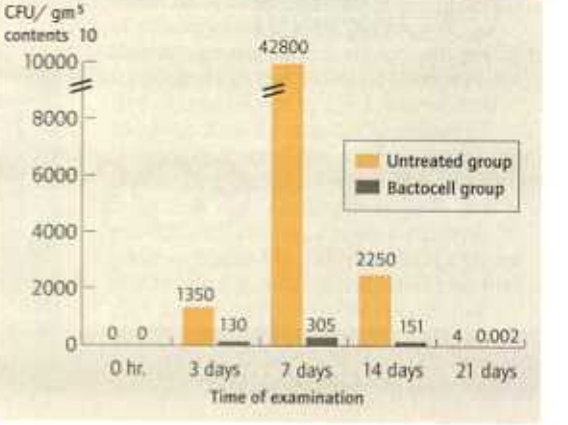

Table 3 - Intestinal colonization of *Lactobacilli* in treated and untreated broiler chickens with *P. acidilactici* (bacterial count / gm intestinal contents x 103)

Treatment	Infection										Non-infected controls				
	Salmonella typhimurium					Clostridium perfringens									
	0 hr.	3 Ds	7 Ds	14 Ds	21 Ds	0 hr	3 Ds	7 Ds	14 Ds	21 Ds	0hr	3 Ds	7 Ds	14 Ds	21 Ds
P. acidilactici	7	237*	27.5*	3250*	1	7	225*	32.5*	3250*	1.5	7	240*	210*	5250*	3
Non-treated +ve control	-	-	-	-	-	7	42.5	10	350	0.5	-	-	-	-	-
Non-treated +ve control	7	28.5	7.5	375	0.5	-	-	-	-	-	-	-	-	-	-
Blank	-	-	-	-	-	-	-	-	-	-	7	70	10	325	2

*Significant difference (P <0.05)

Table 4 - Ceacal colonization of *Lactobacilli* in treated and untreated broiler chickens with *P. acidilactici* (bacterial count / gm intestinal contents x 103)

Treatment	Infection										Non-infected controls				
	Salmonella typhimurium					Clostridium perfringens									
	0 hr.	3 Ds	7 Ds	14 Ds	21 Ds	0 hr	3 Ds	7 Ds	14 Ds	21 Ds	0hr	3 Ds	7 Ds	14 Ds	21 Ds
P. acidilactici	3.5	7.5	7	14	2.5	3.5	7	5.5	42.5	3	3.5	17.5*	27.5*	32.5*	12.5*
Non-treated +ve control	-	-	-	-	-	3.5	5.5	5	40	1.5	-	-	-	-	-
Non-treated +ve control	3.5	4	7.5	17.5	1	-	-	-	-	-	-	-	-	-	-
Blank	-	-	-	-	-	-	-	-	-	-	3.5	4	1.5	5	1.25

*Significant difference (P <0.05)

ment starting from day one.

Clear results

The daily weight gain and mean body weight of the birds that received the selected and specific probiotic was better than that of the untreated birds (*Figure 1*). The feed conversion of the treated group was with 1.85 better than the 2.025 of the control group. Mortality following an *E.coli* infection was 8% less in treated birds. They also showed less histopathological liver lesions (*Figure 2*). It also became clear that at the 95% significance level, *P. acidilactici* showed a significant reduction in intestinal and ceacal colonization of *C.perfringens* at 7, 14 and 21 days post infection (PI) (*Tables 1-2*). There was also a significant reduction in *S.typhimurium* cecal colonization during the entire 21 day period of the experiment (*Figure 3*). Intestinal *Lactobacilli* colonization revealed that there was a statistical significant increase in the total count in all treated groups as compared with the control groups at 3, 7 and 14 days PI (*Table 3*). Results of *lactobacilli* count in caecum revealed an increase only in the non-infected *P. acidilactici* treated group at 3, 7, 14 and 21 days as compared with its non- infected non-treated control group (*Table 4*).

Conclusion

Dr Awaad expressed that: "It would appear from these experiments that the use of *Pediococcus acidilactici* for broiler chickens can greatly assist in the control of *Salmonella typhimurium* and *Clostridium perfringens* colonization. It could be claimed that this product has a positive effect on diarrhea prevention, reducing mortality, stabilization and fast establishment of a balanced gut microflora and is an important concept for competitive exclusion. It will support the elimination of paratyphoid infections from broiler flocks and reduce the significance of animal food as a source of infection to man. Such a program will also be very important to reduce the incidence of paratyphoid and clostridium infection in poultry to a very low level". ■