SYNLAC Report

Study on *Lactobacillus acidophilus* LAP5 as a probiotic strain for poultry and livestock

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In farming process of livestock and poultry, maintenance of the balanced microflora in gastrointestinal tracts of animal is neglected. However, according to the earlier studies in Europe and America, the animal under stress conditions, like weaning, changing the type of feedstuff, molting, and transportation will reduce the gastrointestinal lactic acid bacteria (LAB) and increase *E. coli*. This change of microbiota not only lost 14% in weight gain, but also easily caused intangible losses in farming (Kenworthy and Crabb, 1963; Schulman, 1973).

When the animal is dying due to the infection or disease, it generally causes organ dysfunction. The main reason is that intestine barrier is damaged by infection, and thus pathogen or virus can easily translocate to the organs and cause function failure. At the same time, the immunity in the animal is decreasing since the immune response is abnormal in the intestine. Therefore, the healthy gut is the frontline of defense in the body of livestock and poultry. Probiotics, especially lactic acid bacteria (LAB), play an important role to maintain healthy intestine through the competition and exclusion of pathogens, modulation of immune system as well as the maintenance of intestinal barrier and functions.

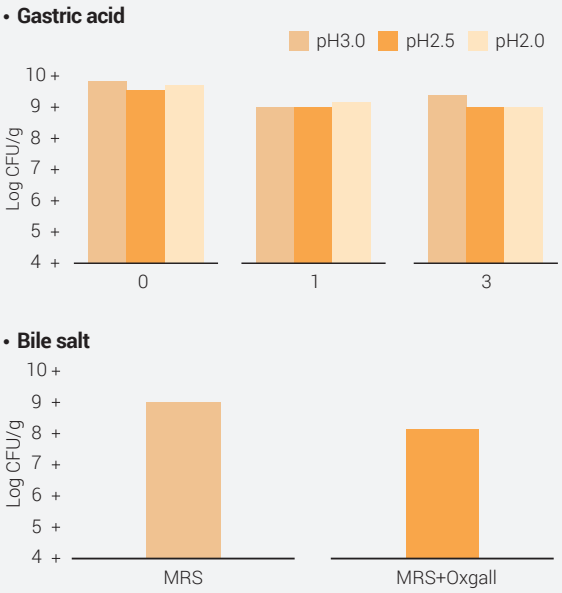
LAB are naturally inhabited microorganisms of human and animal gastrointestinal tracts, and was defined as a group of microorganisms that they are gram-positive, non-spore forming, and produce more than 50% lactic acid as an end-product when carbohydrates were metabolized. Many LAB are used for the prevention or alleviation of symptom of diseases. In 1908, Metchnikoff discovered the lactic acid-producing microorganisms can prevent diarrhea and intestinal disorders (Mercenier *et al.*, 2003), and he pointed out the GI microflora is closely related to human health. *Lactobacillus acidophilus* is the most widely used species as probiotic and has been studied broadly in the literatures. They could produce natural bacteriocins, like acidophillin, latolin, and acidolon, or produce hydrogen peroxide (H_2O_2) combining with lactic acid to inhibit most pathogens. Furthermore, they are the most important species to modulate innate or adaptive immune system (Konstantinov *et al.*, 2008; Liu *et al.*, 2010; Zhang *et al.*, 2008).

In the early 1900's, *Lactobacillus acidophilus* (*L. acidophilus*) as discovered in the GI tract of human, chickens, and pigs (Gilliland *et al.*, 1975). It is reported that supplementation of *L. acidophilus* contributes to building up a predominant good microbe in the GI tract and significantly improve diarrhea and immune system. In 1999, Kmet and Lucchini found that *L. acidophilus*, isolated from sow's intestine, reduced the incidence of diarrhea by the aggregation with diarrheagenic *E. coli* and the intestine peristalsis. This study showed that when pigs were infected with rotavirus, supplementing diet with *L. acidophilus* assisted in modulating immune factors like TGF-β in order to maintain balanced immunity, or raising the production of immunoglobulins from B cells which relives severe diarrhea caused by rotavirus (Azevedo *et al.*, 2012; Zhang *et al.*, 2008a,b). Some studies have reported that not all strains of the same species of the LAB have the same probiotic characteristics (Conway *et al.*, 1987; Lee and Salminen, 1995). That means probiotic is strain specific. To be a probiotic, the strain must to be further evaluated with its properties in the application. Taking the strain of *L. acidophilus* LAP5 (LAP5) originated from health piglet as example when it is applying in poultry.

Adherance to GI tract and tolerance to gastric acid / bile salt in Poultry and Livestock

The LAB serving as effective probiotics have two basic requirements, tolerace to gastric acid and bile salt in intestinal tracts, and colonization in the intestinal epithlium (Chou and Weimer, 1999; Hudault *et al.*, 1997; Bernet *et al.*, 1994; Chauviere *et al.*, 1992; Coconnier *et al.*, 2000).

Figure 1. LAP5 has great gastric acid and bile salt tolerance



Figur 2. The observation of the adhesive ability in intestinal epithelial cells

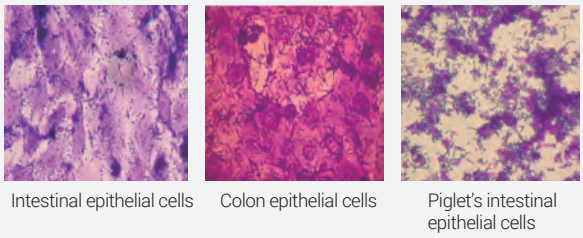


Figure 3. High adhesive ability of LAP5 to epithelial cells in chicken's gastrointestinal tracts

After consumption of LAB, there must to have enough amount of LAB to improve intestinal microflora for gut health. However, the biggest challenge for LAB is low pH of gastric acid and the hydrolysis of bile salts in the GI tract, which LAB would be easily destroyed and died in the digestive process. The strain of *L. acidophilus* LAP5 orginated from swine GI tract is able to survive the challenge of gastric acid and bile salt tolerance (Figure 1). In addition, LAP5 has good ability to adhere to intestinal epithelium of swine and poultry (Figure 2 and 3).

Inhibition of pathogens and modulation of immunity

The mechanisms for LAB to inhibit pathogens include the production of bacteriocins, competition and exclusion to the intestinal epithelium attaching site, or immunomodulation, etc. The patented LAP5 was documented to have all the capabilities mentioned above and is a very unique probiotic strain for livestock and poultry in the world. Specifically, it's reported that the LAP5 with excellent adhesive ability produces organic acids and antimicrobial substances against the growth of *Salmonella* and *E. coli.* The antimicrobial substances are insusceptible to temperature and digestive enzymes (Lin *et al.*, 2008; Tsai *et al.*, 2005), and moreover, it's found that the LAP5 showed a strong antagonistic effect against *Salmonella* invasion to liver and spleen cells through modulating immunity in the intestine, which would reduce the infection rate in animals (Table 1, Yu and Yu, 2006; Lin *et al.*, 2006).

Table 1. Effect of LAP5 strain on infection rate and invation of *Salmonella* to liver and spleen of broiler

- **Infection rate**

Item		Treatment			
		Control[1]	LAP5[1]	E29[2*]	LAP5+E29[1]
Day 7					
	Liver	0/5	0/5	5/5	2/5
	Spleen	0/5	0/5	4/5	2/5
Day 10					
	Liver	0/5	0/5	5/5	2/5
	Spleen	0/5	0/5	4/5	2/5

[1]n=5; [2]Day7, n=5; Day 10, N=6
*E29, *Salmonella typhimurium*

- **Invation of *Samonella***

Item		Treatment				
		Control	LAP5	E29*	LAP5+E29	SEM
		Log CFU/g				
Day 7						
	Liver	0	0	3.27±0.31	2.47±0.87	0.51
	Spleen	0	0	2.64±0.38	3.27±0.78	0.51
Day 10						
	Liver	0	0	2.26±0.32	1.87±0.2	0.29
	Spleen	0	0	3.62±0.62	N[a]	

[a]N, Log_{10} < 2.0
*E29, *Salmonella typhimurium*

(Yu and Yu, 2006)

Reduction of diarrhea

The piglets in age of 3 to 4 weeks have to be separated from the sow and get into the weaning stage. During this stage the piglets suffer from vigorous changes and stress, breast milk to solid feed, loss of immune protection from colostrum and the altering of farming environment, and the intestinal structure of piglets is easily destroyed by the changes. The results of destroyed structure may include the wearing of intestinal villi, abnormal renewal rate of intestinal cells, and unbalanced intestinal bacteria that causes diarrhea in piglet and the loss of farming cost. According to the statistical data of EU, the mortality is about 17% in weaning piglets, which leads to the biggest loss in the farming process. Therefore, it is very important to reduce diarrhea in piglets farming, especially in the critical developing period for the piglets after 14 days of weaning.

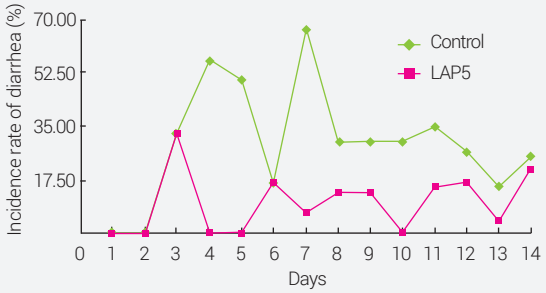
Feed of piglets formulated with LAP5 showed lower diarrhea episodes than the feed with antibiotics (Figure 4). Furthermore, in the field trail of chicken farming challenged with pathogen, LAP5 could help chicken to build the dominant microflora in GI tracts and reduce the infection by pathogens. The results also indicated that feed with LAP5 significantly increased the height of intestinal villi and the area of nutrient absorption, also promoted the growth of chicken and other health benefits (Yu and Yu, 2006; Chang and Yu, 2007). Altogether, LAP5 is effective on the prevention of diarrhea and the improvement of

Figure 4. LAP5 alleviates diarrhea in wearned piglets

Tested animal: 48 piglets wearned at 28 days of age

Control group: Feed with 0.114% antibiotics (STP-500) (Chlorteracycline 88 g/Kg, Sulfamethazine 88 g/Kg, Procaine Penicillin G 44 g/Kg)

LAP5 group: Feed with 0.1% LAP5 (10^9 CFU/Kg)



*Incidence of diarrhea in pigs for 14 days after wearning

animal health; in addition, it results no burdens to animals and the environment.

Lactobacillus acidophilus LAP5 is selected and developed for poultry and livestock

LAP5, developed jointly by National Chung Hsing University and Hung Kung University in Taiwan, is isolated from the GI tract of swine and is excellent in gastric acid/bile salt tolerance and adherence to intestinal cells. The advantages on animal performance have been approved, such as preventing the invasion from pathogens, reducing diarrhea, improving immunity, decreasing the mortality, and increasing the margins for farmer. All of results of field trials have been published in international journals.

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