

Application of Probiotics for the Production of Safe and High-quality Poultry Meat

Yong Ha Park¹, Farizal Hamidon², Chandraprasad Rajangan³, Kim Pong Soh⁴, Chee Yuen Gan⁵,
Theam Soon Lim⁶, Wan Nadiyah Wan Abdullah, and Min Tze Liong*

School of Industrial Technology, Universiti Sains Malaysia, 11800 Penang, Malaysia

¹*Department of Biotechnology, Yeungnam University and ProBionic Institute, Gyeongsan 38541, Korea*

²*Teroka Jaya Farm, Lot 204, 205 and 2, Mukim E. Titi Teras, Balik Pulau, 11000 Penang, Malaysia*

³*3M Malaysia Sdn Bhd, Level 8, Block F, Oasis Square No.2, Jalan PJU 1A/7A, Ara Damansara,
47301 Petaling Jaya, Selangor, Malaysia*

⁴*Premier Diagnostics Sdn Bhd, 7, Jalan USJ 19/2, 47620 UEP Subang Jaya, Selangor, Malaysia*

⁵*Analytical Biochemistry Research Centre, Universiti Sains Malaysia, 11800 Penang, Malaysia*

⁶*Institute For Research In Molecular Medicine, Universiti Sains Malaysia, 11800 Penang, Malaysia*

Abstract

Poultry industry has always been a dynamic and integral part of national economies in many countries. Economic losses incur especially in large-scale rearing facilities, often attributed to the deterioration of environmental conditions, poultry exposure to stressors and development of diseases. While antibiotics have been commonly used for prophylactic purposes and as growth stimulants, extensive documentation of antimicrobial resistance among pathogenic bacteria due to indiscriminate utilization of antibiotic in the industry has led to public and governmental outcries. Elimination of antibiotics from poultry production has thus encouraged intensive search for alternatives. In this review, we discuss the immense potential of probiotics to fill the gap as alternative growth promoters and evidences of beneficial effects of probiotic application in poultry production.

Keywords: Probiotic, Antibiotic, Broiler, Growth promoter, Gut health

Received August 8, 2016; Accepted September 27, 2016

Introduction

Increasing awareness on healthy foods have led to increasing interests on natural food products and nutraceuticals such as probiotics. Probiotics have been defined as 'living microorganisms which when administered in adequate amount confer a health benefits on the host' (FAO/WHO 2001). Probiotic microorganisms have shown much health beneficial effects via in-vivo trials, accompanied by much promising new potentials as developed by in-vitro experiments (Ewe *et al.*, 2010; Liong and Shah, 2005). In general, probiotics have been demonstrated to improve intestinal microbial balance, provide protection against gut pathogens and modulate immune system.

Trend for probiotics products was first observed to gain

momentum in Japan in late 1980 and soon spreaded into areas such as Asia Pacific, European Union and United States (Arora *et al.*, 2013). In present, given the greater understanding of the linkage between diet, nutrition and health, market for functional foods especially the probiotics, are rapidly expanding. The public has also been increasingly accepting alternative therapies which include probiotics, in replacing synthetic drugs.

The current probiotic market has reached \$33.19 billion (2015) and expected to reach \$46.55 billion by 2020, indicating a compound annual growth rate (CAGR) of 7.0%. The probiotic market was dominated by Asia Pacific, followed by Europe in 2014, attributed to the increasing demand for dietary supplements. The future probiotics market of Asia-Pacific is projected to be dominated by China, Japan and India (Markets and Markets, 2015).

Emergence of new probiotic products and needs has spurred increasing number of research groups exploring new probiotic strains and potential novel health function of probiotics (Fung *et al.*, 2011). While consumption of

*Corresponding author: Min Tze Liong, School of Industrial Technology, Universiti Sains Malaysia, 11800 Penang, Malaysia. Tel: +604-653-2114, Fax: +604-653 3675, E-mail: mintze.liong@usm.my

probiotics is previously known to restore and stabilize the gut microbial population, ongoing researches have suggested that probiotics have potentials in preventing cancer and ageing processes (Xu *et al.*, 2003), combating hypercholesterolemia (Choi *et al.*, 2014; Ooi *et al.*, 2010), and reducing risks and severity of myocardial infarction (Lam *et al.*, 2012) via various mechanisms of decreased in-vivo toxicity and normalized gut microbiota.

In addition to human consumption, probiotics are also increasingly applied to animals especially in poultry industries (Fig. 1), with the probiotic market for animals currently growing at a CAGR of 7.7% (Probiotics Ingredients Market, 2015). Probiotics are supplemented into animal feed such as that for ducks, broilers and chickens, cattles and in aquaculture for fishes and prawns. Genera of probiotic microorganisms commonly used for animals include *Bifidobacterium*, *Lactococcus*, *Lactobacillus*, *Bacillus*, *Streptococcus* and yeast such as *Candida*. Feeding of probiotics have been reported to have beneficial impacts on the commercial animals by enhancing weight gain, increasing feed conversion efficiency, increasing egg/milk production, lowering the incidence of disease as well as lowering mortality rates (Crittenden *et al.*, 2005).

Antibiotics as Growth Promoters for Poultry

The use of antibiotics in broiler production was first introduced in the 1940s to enhance growth and feed efficiency as well as reduce mortality (Castanon *et al.*, 2007). Consequently, addition of antibiotics as growth promoters in poultry feed became common practices without rigorous testing. The mechanisms of action in promoting growth induced by antibiotics is closely related to the reduction of pathogenic bacteria in the intestines (Dibner

et al., 2005) However, concentration of antibiotics used for growth promotion is often lower than the concentration used for therapy and prophylaxis, and the former concentration is commonly referred as “sub-therapeutic concentration”.

Antibiotics that are provided to healthy animals as growth promoters are usually at concentrations lower than 200 g per ton of feed for more than 14 d (Graham *et al.*, 2007) These sub-therapeutic doses of antibiotics often create a conducive condition to selection for resistance in bacteria. Association of the use of antibiotic growth promoters (AGPs) and the occurrence of resistant bacteria including *Campylobacter*, *Salmonella*, *Enterococcus* and *Escherichia coli* was well documented in previous studies. This close link has been most thoroughly studied on the connection between avoparcin and glycopeptide-resistant enterococci (Van Immerseel *et al.*, 2004).

It has been suggested that animal feed could serve as a reservoir for antibiotic-resistant bacteria that may rapidly spread across the food chain and eventually to the human. Propagation of antibiotic-resistant bacteria could spread to food products during slaughtering and processing. Past studies have shown that the presence of enterococci resistant to antibiotic in food products derived from animal fed with AGPs (McDonald *et al.*, 2001). Furthermore, the contaminations of antibiotic-resistant bacteria and active antibiotics have also been extensively documented to spread into the environment. An eight month period investigation on soil bacteria revealed that treatment of farmland with pig manure slurry elevated the level of resistant of the bacteria to tetracycline (Sengeløv *et al.*, 2003).

While the transmission of the antibiotic resistant bacteria to human could be attributed to the consumption of the food animals and products, a direct transmission of the bacteria could happen during the handling of the farm animals by the farm workers. High prevalence of vancomycin-resistant enterococci was reportedly to be detected in the fecal samples of healthy poultry workers at antibiotic-exposed farm (van den Bogaard *et al.*, 1997). Antibiotic resistant properties of the bacteria could also be acquired by other distantly related microorganisms via horizontal transfer of gene material, a process in which substantial amounts of DNA are inserted into and deleted from the chromosomes (Ochman *et al.*, 2000). As a result of the alarming risk of elevated resistance level of the bacteria towards antibiotics, the European Union (EU) has decided to ban the use of AGPs in poultry industry. This removal of antibiotics as growth promoters is soon implemented by countries such as the USA due to con-

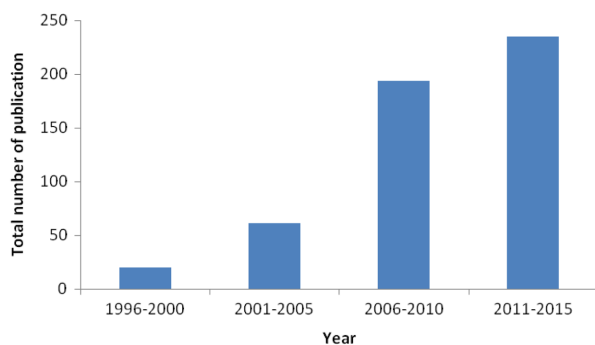


Fig. 1. Total number of publications (research article, review, conference paper, short survey and article in press) in Scopus (1996-2015), related to probiotics and poultry. Source from: www.scopus.com on 18 July 2015.

sumer pressure (Huyghebaert *et al.*, 2011).

Following the phasing out of use of AGPs, impact of the termination was assessed on the aspect of animal health and productivity. The discontinuation of AGPs has been reported to led to animal performance issues and increased incidence of animal diseases such as necrotic enteritis (Wierup, 2001). Enteric disease is one of the prime concern in poultry industry after the exclusion of AGPs due to reduction in productivity, increased mortality as well as associated food products contamination (Patterson and Burkholder, 2009). Other apparent syndromes reported as consequences of termination of AGPs use are bacterial overgrowth in small intestine, increased water content in faeces, malabsorption and poor condition of the gut (Huyghebaert *et al.*, 2011). Thus, with the ban on sub-therapeutic antibiotic usage, search for potential alternative to AGPs has gained momentum.

Application of Probiotics in Poultry Industry

Probiotic supplementation in farm industry dated back to the 1960s, although the details of selection for the poultry probiotics were often rarely provided (Santini *et al.*, 2010). According to Kabir (2009), in order to fit the criteria as functional probiotic for poultry production, the bacteria must possess the following desirable traits: the bacteria must be a gut inhabitant, it must be able to adhere to the intestinal epithelium and withstand harsh condition such as high acidity environment in stomach and tolerance to bile salts in the intestines, and competes against other gut microorganisms for colonization in the gastrointestinal (GI) tract, as well as able to exert beneficial effects in host and maintain high viability under normal storage condition and after industrial processes such as lyophilisation. Advancement in the research technology has currently enabled more promising selection of functional probiotics as many in-vitro assays have been made available for evaluation of competitiveness of the probiotics. Further selection of the most potential probiotic for poultry industry can be enhanced by monitoring the efficacy of administration via in-vivo study using live broilers.

Poultry animals are constantly subjected to various environmental stresses. Stressful experiences happen in broilers during their adaptation to post hatching period, transportation, processing at the hatchery and high stocking densities (Burkholder *et al.*, 2008) Also, before slaughtering, feed withdrawal are implemented on the birds in order to reduce intestinal volume, for the purpose of min-

imizing risk of carcass contamination following the rupture of intestinal tract during processing (May and Deaton, 1989). Fluctuation in temperature in seasonal environments has also been documented as additional stressors for poultry animals (Traub-Dargatz *et al.*, 2006). Exposure to stressors could eventually threaten the animals' health via weakening immune functions and predisposing the animals to enteric diseases induced by pathogenic microorganisms. Evidence of stress study on poultry animals has showed that fecal shedding of pathogens was markedly increased in the event of stress, which is one of the main factors that contribute to carcass contamination. This could subsequently pose threat to food safety and detrimental to human health. Thus, probiotic could prove to be a reliable strategy to control bacterial shedding and improve poultry animal welfare.

Benefits of Probiotics Application in Poultry Industry

Effects of probiotics against pathogens infection

Successful probiotics colonization is essential for immunoregulatory function and inhibition of pathogenic bacteria in the gastrointestinal tract. The inhibition of pathogen by probiotics is suggested to occur via competition for adherence site on the intestinal wall and nutrient as well as production of antimicrobial compounds (Patterson and Burkholder, 2003). Adherence of probiotics consists of *Lactobacillus acidophilus* and *Streptococcus faecium* to intestinal mucosa and subsequent colonization in the gut are able to reduce colonization and shedding of *Campylobacter jejuni* in the intestinal tract of chicken (Morishita *et al.*, 1997). Colonization of probiotic *Lactobacillus* strains have been demonstrated to have a preventive function against *Salmonella enterica* serovar Enteritidis infection in chicken (Van Coillie *et al.*, 2007). Approach using probiotics against pathogenic bacteria has been shown to be effective to reduce food-borne illnesses such as campylobacteriosis and salmonellosis in consumer in view of the absence of AGPs. Besides suppressing the colonization of *E. coli* as efficient as antibiotic bacitracin methylene disalicylate (BMD), application of probiotic using *L. sakei* Probio-65 was also observed to increase the population of lactic acid bacteria (Fig. 2). Lactic acid bacteria have been widely acknowledged for its importance in exerting inhibitory and antagonistic effects against pathogenic bacteria.

Numerous studies have been documented that the probiotics can exert antimicrobial effect against pathogenic

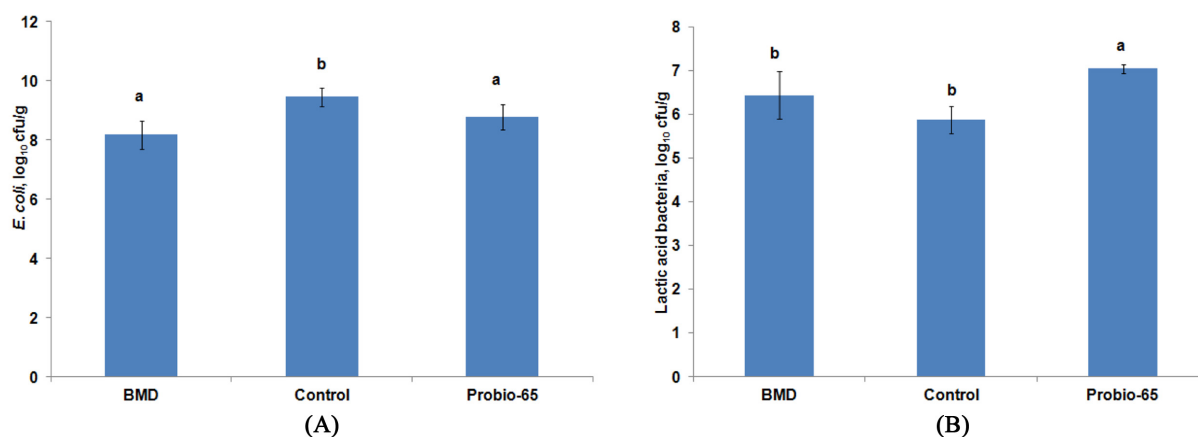


Fig. 2. Population of *E. coli* (Log 10 CFU/g) (A) and lactic acid bacteria (Log 10 CFU/g) (B) in the small intestinal contents of broiler chickens fed with antibiotic bacitracin methylene disalicylate (BMD; n=14), control basal diet (n=14; void of antibiotic or probiotic) and probiotic *Lactobacillus sakei* Probio-65 (n=18) at the end of the 42-day trial period. Error bars represented standard deviation of means. ^{a,b}Means with different lowercase superscript letters are significantly different ($p < 0.05$).

bacteria via production of metabolites such as short chain fatty acids (SCFAs) and bacteriocins. Increased concentration of butyric acid has been demonstrated to reduce *Salmonella* infection in poultry animals whereas elevated concentration of SCFAs as a result of probiotic *Bacillus subtilis* effectively reduced coliform counts while increased population of *Lactobacillus* in broiler chickens (Milián *et al.*, 2013; Van Immerseel *et al.*, 2004). On the other hand, bacteriocins which are ribosomally synthesized peptides or proteins with antimicrobial properties have been reported to show promising growth inhibition potential against intestinal pathogenic bacteria. Bacteriocins derived from *Lactobacillus salivarius* exhibit strong antagonistic activity against *Campylobacter jejuni* and Gram-positive bacteria (Pilasombut *et al.*, 2006). Similarly, bacteriocins produced by probiotic *Escherichia coli* strain have been shown to greatly reduce *Salmonella* contamination in poultry industry (Stern *et al.*, 2006).

Role of probiotics in stimulation of protective immune response have been proposed to be one of the main element that help suppress growth of potential gut pathogens in poultry animals (Panda *et al.*, 2007). Stable establishment of enteric microbiota by administration of probiotics is often associated with immunologically balanced intestinal inflammatory activity. It has been shown that failure in maintaining the balance of gut microbiota could initiate activation of the mucosal immune system and lead to chronic inflammatory response in the intestine (Haller *et al.*, 2000). Exaggerated synthesis of pro-inflammatory cytokines such as interleukin (IL)-12 and interferon (IFN)- γ in inflamed intestine has been demonstrated to have dev-

astating effect contributing to intestinal tissue damage (Pallone and Monteleone, 2001). Intestinal tissue damage accompanied by challenges imposed by opportunistic pathogens often lead to aberrant changes in gut microbiota. Gut inflammation and subsequent increased abundance of pathogenic bacteria decrease the animal performance and would eventually increase mortality rate of the broilers. However, this unfavorable condition could be reversed via probiotic supplementation in broiler diet. The administration of *L. sakei* Probio-65 reduced mortality of broilers as compared to chickens fed with antibiotic and chickens that were fed with feed void of antibiotics or probiotics (Fig. 3).

Effects of probiotics on immune responses

The dynamics of probiotics related to immune responses evaluated by Kabir *et al.* (2009) demonstrated that antibody production was elevated in broilers fed with probi-

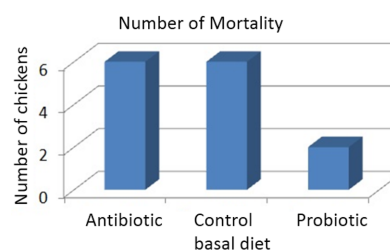


Fig. 3. Total number of death recorded in broiler chickens fed with antibiotic bacitracin methylene disalicylate (n=20), control basal diet (n=20; without antibiotic or probiotic) and probiotic *Lactobacillus sakei* Probio-65 (n=20) at the end of the 42-day trial period.

otics *Lactobacillus* compared to control chickens. The modulation of immune responses by probiotics is also apparently observed in broilers exposed to stress conditions. *Lactobacillus*-based probiotics administration was observed to ameliorate heat-stress related problems in broilers which are accompanied with improved antibody production as compared to controls (Zulkifli *et al.*, 2000). In addition, supplementation of probiotic *Lactobacillus* in broilers diet revealed that probiotic could enhance intestinal immunity against coccidiosis by altering population of intestinal intraepithelial lymphocyte (IEL) expressing surface markers CD3, CD4, CD8, and $\alpha\beta$ TCR (Dalloul *et al.*, 2003). Probiotics has also been suggested to augment Toll-like receptor (TLR) signaling in which TLR play a crucial role for activation of T-cells in the intestinal immune system. Recent study showed that probiotic product consists of *Lactobacillus fermentum* and *Saccharomyces cerevisiae* increased the level of mRNA expression of TLR-2 and 4 in the foregut of the chickens compared to those administered with control diet and antibiotic (Bai *et al.*, 2013). Furthermore, basal diet supplemented with probiotics mixture containing *Lactobacillus acidophilus*, *Lactobacillus casei*, *Enterotococcus faecium* and *Bifidobacterium thermophilus* elevated the concentration of IgG and IgM levels in turkeys and the enhancement of the immunoglobulins level have been proposed to contribute to more positive growth performance, production and resistance of the animals towards diseases (Cetin *et al.*, 2015).

Effects of probiotics on intestinal morphology

Numerous studies have been carried out to investigate the effects of probiotic administration on the histomorphology of the intestine. Dietary treatment with probiotic *Lactobacillus* sp. was reported to influence the villi

height and crypt depth in the small intestine of broilers (Bai *et al.*, 2013). The administration of *Lactobacillus sakei* Probio-65 increased villi height and crypt depth in jejunum of broilers as compared to chickens fed with antibiotic and chickens that were fed with feed void of antibiotics or probiotics (Fig. 4). Probiotics are proposed to increase the length of villi by activating cell mitosis and induce gut epithelial-cell proliferation (Samanya and Yamauchi, 2002). Increased of villi height by the probiotics is beneficial to the broilers as the increased surface area of the villi enhanced the absorption of nutrient (Caspary, 1992). On the other hand, deeper crypt depth promoted in the presence of probiotics allow higher turnover rate of villi tissue and replenish villi which may lost due to sloughing or inflammation in response to pathogen infection (Wegener, 2003). It has been suggested that alteration in villi length and crypt depth may lead to poor nutrient absorption, digestive enzymes secretion in the GI tract and eventually lower growth broilers performance (Xu *et al.*, 2003).

Pelicano *et al.* (2005) has described that villi in jejunum occur in zig-zag form, resembling wave pattern. It was suggested that the formation of villi in wave pattern enable better nutrient absorption than villi arranged in parallel or randomly positioned. Zigzag flux in the small intestine permits food to take a longer passage through the alimentary canal compared to the straight flux, and improve the contact between the nutrients and the absorption surface of the intestinal epithelium. Probiotic such as *Lactobacillus sakei* Probio-65 promoted waved-like arrangement of jejunum villi in broilers (Fig. 5), while this wave-like pattern was absent in the jejunum of broiler fed with antibiotic or were fed with feed void of antibiotics or probiotics. In addition, gut health of broilers was well-

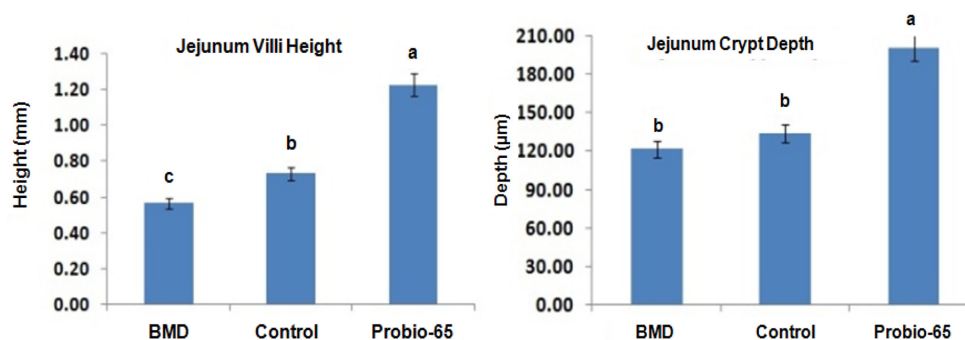


Fig. 4. Effects of administration of antibiotic bacitracin methylene disalicylate (BMD; n=14), control basal diet (n=14; without antibiotic or probiotic) and probiotic *Lactobacillus sakei* Probio-65 (n=18) for 42 days, on villi height and crypt depth in jejunum of broiler chickens. Error bars represented standard deviation of means. ^{a-c}Means with different lowercase superscript letters are significantly different ($p < 0.05$).

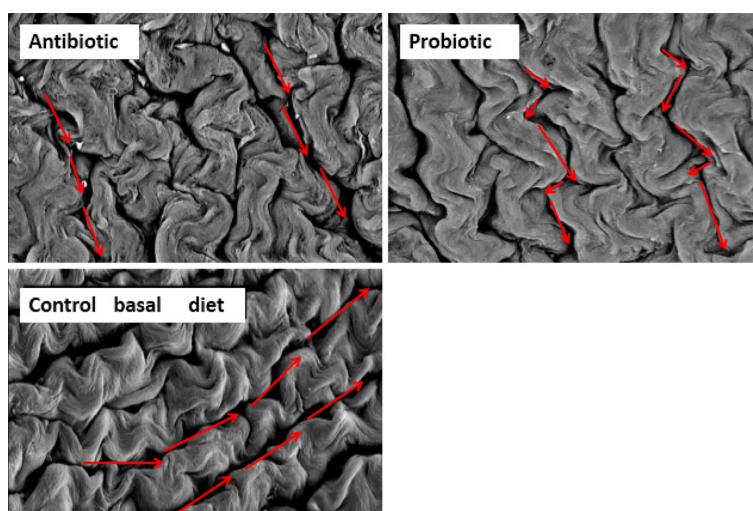


Fig. 5. Scanning electron microscopy of jejunal villi arrangement in broiler chickens administered with antibiotic bacitracin methylene disalicylate (BMD; n=14), control basal diet (n=14; void of antibiotic or probiotic) and probiotic *Lactobacillus sakei* Probio-65 (n=18) at the end of the 42-day trial period.

preserved as indicated by intact and densely packed microvilli upon administration with *L. sakei* Probio-65. On the other hand, the application of antibiotic led to less packed and scattered microvilli arrangement while chickens fed with feed void of antibiotics or probiotics showed normal arrangements of microvilli (Fig. 6). It has been suggested that reduced density of microvilli could compromise intestinal enterocyte integrity, leading to a reduced protective effect of the intestinal epithelium barrier (Merrifield *et al.*, 2009).

Probiotic administration could alleviate histological changes in the intestine induced by feed contaminant such as deoxynivalenol. Deoxynivalenol is one of the trichothecene mycotoxin commonly found in feedstuffs which involve in inhibition of protein synthesis that affects rapidly dividing cells such as those in GI tract (Leeson *et al.*, 1995). Study has evidenced that probiotic feed additive is able reverse the morphology of short and thin villi which are negatively impacted by the feed contaminant (Awad *et al.*, 2006).

Furthermore, promotion of gut health by probiotic bacteria further strengthen the potential of probiotics as emerging alternatives to antibiotics as growth promoters in poultry industry. Gut condition was well preserved in the presence of probiotic such as *Lactobacillus sakei* Probio-65, accompanied by healthy development of the intestines of as compared to control broilers that were not fed with probiotics. Unlike probiotics, antibiotic damaged jejunal villi tip with prevalent shedding at the end of the villi tips (red circle; Fig. 7). Injuries of the intestinal walls have

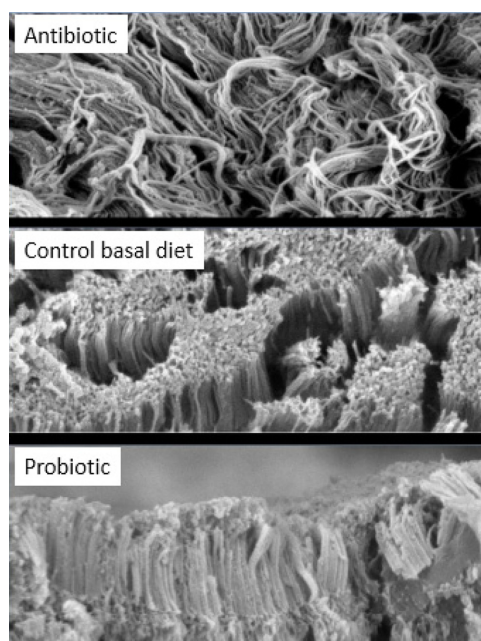


Fig. 6. Scanning electron microscopy of jejunal microvilli of broiler chickens fed with bacitracin methylene disalicylate (BMD; n=14), control basal diet (n=14; void of antibiotic and probiotic) and probiotic *Lactobacillus sakei* Probio-65 (n=18) at the end of the 42-day trial period. Magnification: 10,000X.

been much reported upon the administration of antibiotics, and are very often accompanied with thinning of the intestinal mucus layer and increased depletion of goblet cells (Wlodarska *et al.*, 2011).

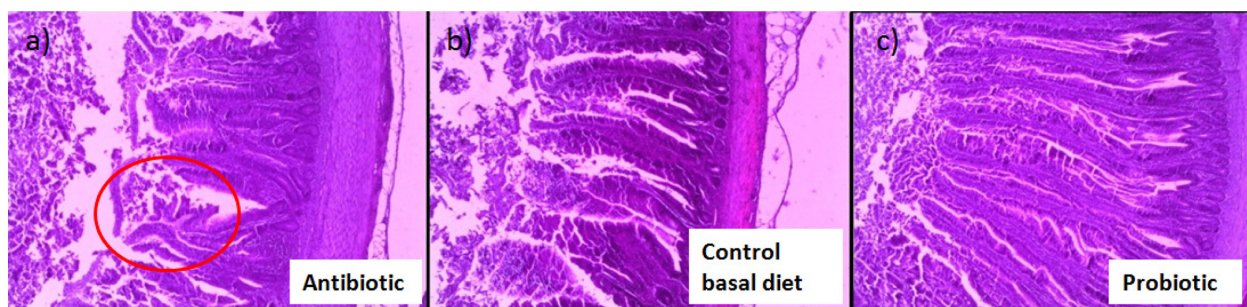


Fig. 7. Histological images of sections from jejunum tissue of broiler chickens fed with (a) antibiotic bacitracin methylene disalicylate (n=14), (b) control basal diet (n=14; without antibiotic and probiotic) and (c) probiotic *Lactobacillus sakei* Probio-65 (n=18) at the end of the 42-day trial period. Magnification 400X.

Effects of probiotics on growth performance

Effects of dietary supplementation of probiotics on growth performance of poultry animals have been extensively investigated. Most studies indicated that probiotics displayed great efficacy in promoting animal growth. Dietary inclusion of probiotics *Lactobacillus* has been reported to increase body weights and feed to gain ratio when compared to control broilers (Jin *et al.*, 1998). *Lactobacillus* inclusion in broilers nutrition also resulted in higher broiler productivity index which is measured based on daily weight gain, feed efficiency, and mortality (Timmerman *et al.*, 2006). While growth rates of the broilers are improved, the *Lactobacillus* administration reduced the mortality of the broilers which usually arised from pathogen infections. Moreover, probiotics supplementation to diet improved feed intake, feed efficiency, and carcass yield of broilers (Denli *et al.*, 2003).

Recent evaluation of effects of probiotic supplementation on digestive enzymes activity in broiler chickens revealed that the probiotic *Bacillus coagulans* NJ0516 promotes higher activity of protease and amylase compared with controls. This finding suggests that the higher activity of the enzymes may lead to better digestibility of protein and starch, which in turn explains better growth in broilers fed with probiotics rather than control basal diet (Wang and Gu, 2010). On the other hand, dietary supplementation of probiotic *Lactobacillus sporogenes* lowered serum level of total cholesterol, low-density lipoprotein (LDL) cholesterol, very low-density lipoprotein (VLDL) cholesterol and triglycerides (Panda *et al.*, 2006). Hypolipidaemic effect of probiotics on broilers was similarly reported by Kalavathy *et al.* (2003) where abdominal fat deposition was reduced by mixture of 12 probiotic *Lactobacillus* strains compared to control diet. The amount of subcutaneous fat beneath the skin of broiler chickens was

lower in chickens fed with diet added with probiotic *Lactobacillus sakei* Probio-65 and higher in chickens fed with antibiotic BMD (Fig. 8). Such marked differences have been reported to be attributed to the roles of antibiotics that promote adiposity via disruption on gut microbiota and energy balance (Liou and Turnbaugh, 2012).

Effects of probiotics on meat quality

There is widespread agreement that probiotics supplementation could improve meat quality of broilers. Intramuscular lipid content is involved in determining meat quality particularly nutrition, tenderness, odor, tastes and flavor characteristics. Endo and Nakano (1999) reported a greater tendency of higher ratio of unsaturated fatty acids to saturated fatty acids in pectoral and thigh meat of broilers fed with probiotics-supplemented diet containing *Ba*-

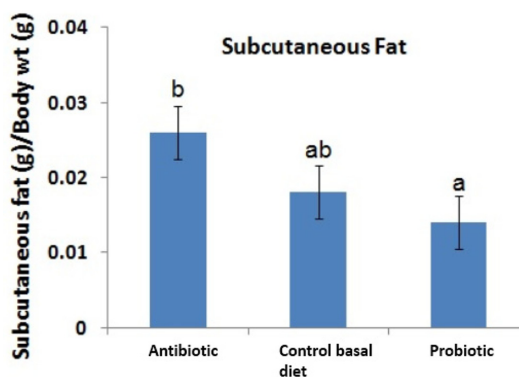


Fig. 8. Weight of subcutaneous fat of broiler chickens fed with antibiotic bacitracin methylene disalicylate (BMD; n=14), control basal diet (n=14; without antibiotic and probiotic) and probiotic *Lactobacillus sakei* Probio-65 (n=18) at the end of the 42-day trial period. Error bars represented standard deviation of means. ^{a,b}Means with different lowercase superscript letters are significantly different ($p < 0.05$).

cillus, *Lactobacillus*, *Streptococcus*, *Clostridium*, *Saccharomyces* and *Candida*. The results suggested that the fat in meat was converted into favorable fat in the presence of probiotics, which in turn contributed to smoother meat texture. Improved tenderness which was indicated by decreased shear force was reported by Yang *et al.* (2010) when probiotic *C. butyricum* was added in diet of broiler. The decreased in shear force observed in the study was positively correlated with increased muscular fat content. The same study also demonstrated that probiotic inclusion in broiler diet modulated fatty acid composition in breast meat of broiler by increasing omega-3 fatty acids concentration especially eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) while contents of omega-3 fatty acids in meat of control broilers remain relatively low.

Sensory assessment on chicken meatballs conducted by Mahajan *et al.* (2000) revealed that overall organoleptic scores in terms of appearance, texture, juiciness and overall acceptability were higher in probiotic *Lactobacillus* fed broilers than counterparts fed with traditional basal diet. Evaluation of effects of probiotic on skin color of broiler elucidated that probiotic *Lactobacillus salivarius* could increase xanthophyll accumulation in tissue, thus improving the visual appearance of meat products (Zhu *et al.*, 2009). Meat in broilers fed with probiotics *Lactobacillus acidophilus*, *Lactobacillus casei*, *Bifidobacterium bifidum*, *Aspergillus oryzae*, *Streptococcus faecium* and *Torulopsis* sp displayed higher content of moisture, protein and ash compared to the control (Khaksefidi and Rahimi, 2005). The results indicated that chicken fed with probiotics has better retention of minerals especially phosphorus, calcium and nitrogen as well as protein efficiency ratio. Higher protein efficiency ratio may subsequently help promote meat yield as observed by Hossain *et al.* (2012) where addition of probiotics increased breast meat absolute and relative weight. Besides, carcass quality of broilers was also reported to be improved by probiotics with lesser occurrence of *Salmonella* contamination.

Conclusions

Biotechnology plays an essential role in the development of effective poultry feed. Feed composition is crucial to promote growth and maintain health of broilers. A great amount of research has provided strong emphases that maximizing feed utilization could determine the functionality of feed. A well-balanced diet sufficient in nutrient and energy is also of significant importance to maintain gut in healthy state. In view of this, the concept of

probiotics as feed additives has garnered much attention and support. Significant work and studies have increasingly demonstrated that probiotics provide means to a balanced gut microbiota in poultry, maintaining health status in broilers, preserving gut condition and improving immune system as well as enhancing nutrient absorption, which are all crucial and needed to promote growth of broilers.

Acknowledgements

This work was supported Teroka Jaya Farm (Penang, Malaysia), 3M Malaysia Sdn Bhd (Selangor, Malaysia), and Premier Diagnostics Sdn Bhd (Selangor, Malaysia). The authors would like to thank Chai Soo Teng, Choong Yi Lee, Maya Emira bt Mad Saad, Mohammad Syafiq b Abdullah, Muhammad Aizat b Mat Saad, Nor Shaqira bt Azlan, Nur Diana bt Abdul Jalil, Nur'ain bt Arzmi, Nur'Ain bt Zulkefli, Nurul Saffa bt Zainal Abidin, Ooi Bin Ying, Bee Yen Shan, Tan Hwee Fend, Tang Bu Xian, Teoh Leong Sin, Tunku Syed Iskandar Al-Qadri b tunku Syed Azhar, Yasmin Syafikah bt Razali, Yu Tiam Meng, Zayani bt Mukhtar and Zhameir Shafiq b Mohd Ilias for their roles in executing the project on broilers, Mr Mohd Zaini Baharuddin (Kilang Memproses Makanan Ternakan, Kota Kuala Muda, Kedah) for pelletizing the feed, and Dr. Kiran Thakur and Loh Yung Sheng for reading of this work.

References

1. Arora, M., Sharma, S., and Baldi, A. (2013) Comparative insight of regulatory guidelines for probiotics in USA, India and Malaysia: A critical review. *Int. J. Biotechnol. Wellness Ind.* **2**, 51-64.
2. Awad, W. A., Böhm, J., Razzazi-Fazeli, E., Ghareeb, K., and Zentek, J. (2006) Effect of addition of a probiotic microorganism to broiler diets contaminated with deoxynivalenol on performance and histological alterations of intestinal villi of broiler chickens. *Poultry Sci.* **85**, 974-979.
3. Awad, W. A., Ghareeb, K., Abdel-Raheem, S., and Böhm, J. (2009) Effects of dietary inclusion of probiotic and synbiotic on growth performance, organ weights, and intestinal histomorphology of broiler chickens. *Poultry Sci.* **88**, 49-56.
4. Bai, S. P., Wu, A. M., Ding, X. M., Lei, Y., Bai, J., Zhang, K. Y., and Chio, J. S. (2013) Effects of probiotic-supplemented diets on growth performance and intestinal immune characteristics of broiler chickens. *Poultry Sci.* **92**, 663-670.
5. Burkholder, K. M., Thompson, K. L., Einstein, M. E., Applegate, T. J., and Patterson, J. A. (2008) Influence of stressors on normal intestinal microbiota, intestinal morphology, and susceptibility to *Salmonella enteritidis* colonization in broilers. *Poultry Sci.* **87**, 1734-1741.
6. Caspary, W. F. (1992) Physiology and pathophysiology of

- intestinal absorption. *The Am. J. Clin. Nutr.* **55**, 299-308.
7. Castanon, J. I. R. (2007) History of the use of antibiotic as growth promoters in European poultry feeds. *Poultry Sci.* **86**, 2466-2471.
 8. Cetin, N., Güçlü, B. K., and Cetin, E. (2005) The effects of probiotic and mannanoligosaccharide on some haematological and immunological parameters in turkeys. *J. Vet. Med. A.* **52**, 263-267.
 9. Choi, S. B., Lew, L. C., Yeo, S. K., Nair Parvathy, S., and Liong, M. T. (2014) Probiotics and the BSH-related cholesterol lowering mechanism: a Jekyll and Hyde scenario. *Cr. Cr. Rev. Biotechn.* **35**, 392-401.
 10. Crittenden, R., Bird, A. R., Gopal, P., Henriksson, A., Lee, Y. K., and Playne, M. J. (2005) Probiotic research in Australia, New Zealand and the Asia-Pacific region. *Curr. Pharm. Des.* **11**, 37-53.
 11. Dalloul, R. A., Lillehoj, H. S., Shellem, T. A., and Doerr, J. A. (2003) Enhanced mucosal immunity against *Eimeria acervulina* in broilers fed a *Lactobacillus*-based probiotic. *Poultry Sci.* **82**, 62-66.
 12. Denli, M., Okan, F., and Celik, K. (2003) Effect of dietary probiotic, organic acid and antibiotic supplementation to diets on broiler performance and carcass yield. *Pak. J. Nutr.* **2**, 89-91.
 13. Dibner, J. J. and Richards, J. D. (2005) Antibiotic growth promoters in agriculture: History and mode of action. *Poultry Sci.* **84**, 634-643.
 14. Endo, T. and Nakano, M. (1999) Influence of a probiotic on productivity, meat components, lipid metabolism, caecal flora and metabolites, and raising environment in broiler production. *日本畜産学会報*, **70**, 207-218.
 15. Ewe, J. A., Wan-Abdullah, W. N., and Liong, M. T. (2010) Viability and growth characteristics of *Lactobacillus* in soy-milk supplemented with B-vitamins. *Int. J. Food Sci. Nutr.* **61**, 87-107.
 16. FAO/WHO. (2001) Report of a joint FAO/WHO expert consultation on evaluation of health and nutritional properties of probiotics in food including powder milk with live lactic acid bacteria. Córdoba, Argentina (October 1-4, 2001).
 17. Fung, W. Y., Yuen, K. H., and Liong, M. T. (2011). Agrowaste-based nanofibers as a probiotic encapsulant: Fabrication and characterization. *J. Agri. Food Chem.* **59**, 8140-8147.
 18. Graham, J. P., Boland, J. J., and Silbergeld, E. (2007) Growth promoting antibiotics in food animal production: An economic analysis. *Public Health Rep.* **122**, 79.
 19. Haller, D., Bode, C., Hammes, W. P., Pfeifer, A. M. A., Schiffrin, E. J., and Blum, S. (2000) Non-pathogenic bacteria elicit a differential cytokine response by intestinal epithelial cell/leucocyte co-cultures. *Gut.* **47**, 79-87.
 20. Hossain, M. E., Kim, G. M., Lee, S. K., and Yang, C. J. (2012) Growth performance, meat yield, oxidative stability, and fatty acid composition of meat from broilers fed diets supplemented with a medicinal plant and probiotics. *Asian Australas. J. Anim. Sci.* **25**, 1159.
 21. Huyghebaert, G., Ducatelle, R., and Van Immerseel, F. (2011) An update on alternatives to antimicrobial growth promoters for broilers. *Vet. J.* **187**, 182-188.
 22. Jin, L. Z., Ho, Y. W., Abdullah, N., and Jalaludin, S. (1998) Growth performance, intestinal microbial populations, and serum cholesterol of broilers fed diets containing *Lactobacillus* cultures. *Poultry Sci.* **77**, 1259-1265.
 23. Kabir, S. M. (2009) The role of probiotics in the poultry industry. *Int. J. Mol. Sci.* **10**, 3531-3546.
 24. Kalavathy, R., Abdullah, N., Jalaludin, S., and Ho, Y. W. (2003) Effects of *Lactobacillus* cultures on growth performance, abdominal fat deposition, serum lipids and weight of organs of broiler chickens. *Brit. Poultry Sci.* **44**, 139-144.
 25. Khaksefidi, A. and Rahimi, S. (2005) Effect of probiotic inclusion in the diet of broiler chickens on performance, feed efficiency and carcass quality. *Asian Australas J. Anim. Sci.* **18**, 1153.
 26. Lam, V., Su, J., Koprowski, S., Hsu, A., Tweddell, J. S., Raffie, P., Gross, G. J., Salzman, N. H., and Baker, J. E. (2012) Intestinal microbiota determine severity of myocardial infarction in rats. *The FASEB J.* **26**, 1727-1735.
 27. Leeson, S., Diaz, G., Gonzalo, J., and Summers, J. D. (1995) Poultry metabolic disorders and mycotoxins. (No. 04; SF995, L4.).
 28. Liong, M. T. and Shah, N. P. (2005) Bile salt deconjugation and BSH activity of five bifidobacterial strains and their cholesterol co-precipitating properties. *Food Res. Int.* **38**, 135-142.
 29. Liou, A. P. and Turnbaugh, P. J. (2012) Antibiotic exposure promotes fat gain. *Cell Metab.* **16**, 408-410.
 30. Mahajan, P., Sahoo, J., and Panda, P. C. (2000) Effect of probiotic (Lacto-Sacc) feeding, packaging methods and seasons on the microbial and organoleptic qualities of chicken meat balls during refrigerated storage. *J. Food Sci. Tech. Mys.* **37**, 67-71.
 31. May, J. D. and Deaton, J. W. (1989) Digestive tract clearance of broilers cooped or deprived of water. *Poultry Sci.* **68**, 627-630.
 32. McDonald, L. C., Rossiter, S., Mackinson, C., Wang, Y. Y., Johnson, S., Sullivan, M., Sokolow, R., DeBess, E., Gillbert, L., Benson, J. A., Hill, B., and Angulo, F. J. (2001) Quinupristin-dalfopristin-resistant *Enterococcus faecium* on chicken and in human stool specimens. *N. Engl. J.* **345**, 1155-1160.
 33. Merrifield, D. L., Dimitroglou, A., Bradley, G., Baker, R. T. M., and Davies, S. J. (2009) Soybean meal alters autochthonous microbial populations, microvilli morphology and compromises intestinal enterocyte integrity of rainbow trout, *Oncorhynchus mykiss* (Walbaum). *J. Fish Dis.* **32**, 755-766.
 34. Milián, G., Rondón, A. J., Pérez, M., Bocourt, R., Rodríguez, Z., Raniilla, M. J., Rodríguez, M., and Carro, M. D. (2013) Evaluation of *Bacillus subtilis* biopreparations as growth promoters in chickens. *Cuban J. Agr. Sci.* **47**.
 35. Morishita, T. Y., Aye, P. P., Harr, B. S., Cobb, C. W., and Clifford, J. R. (1997) Evaluation of an avian-specific probiotic to reduce the colonization and shedding of *Campylobacter jejuni* in broilers. *Avian Dis.* **41**, 850-855.
 36. Ochman, H., Lawrence, J. G., and Groisman, E. A. (2000) Lateral gene transfer and the nature of bacterial innovation. *Nature* **405**, 299-304.
 37. Ooi, L. G., Bhat, R., Rosma, A., and Liong, M. T. (2010) A synbiotic containing *Lactobacillus acidophilus* CHO-220 and inulin improves irregularity of red blood cells. *J. Dairy Sci.* **93**, 4353-4544.

38. Pallone, F. and Monteleone, G. (2001) Mechanisms of tissue damage in inflammatory bowel disease. *Curr. Opin. Gastroen.* **17**, 307-312.
39. Panda, A. K., Rao, S. V. R., Raju, M. V., and Sharma, S. R. (2006) Dietary supplementation of *Lactobacillus sporogenes* on performance and serum biochemico-lipid profile of broiler chickens. *Poultry Sci.* **43**, 235-240.
40. Panda, A. K., Reddy, M. R., Rao, S. R., Raju, M. V. L. N., and Praharaj, N. K. (2000) Growth, carcass characteristics, immunocompetence and response to *Escherichia coli* of broilers fed diets with various levels of probiotic. *Arch. Geflügelkd.* **64**, 152-156.
41. Patterson, J. A. and Burkholder, K. M. (2003) Application of prebiotics and probiotics in poultry production. *Poultry Sci.* **82**, 627-631.
42. Pelicano, E. R. L., Souza, P. A., Souza, H. B. A., Figueiredo, D. F., Boiago, M. M., Carvalho, S. R., and Bordon, V. F. (2005) Intestinal mucosa development in broiler chickens fed natural growth promoters. *Rev. Bras. Cienc. Avic.* **7**, 221-229.
43. Pilasombut, K., Sakpuaram, T., Wajjwalku, W., Nitisingpraser, S., Swetwiwathana, A., Zendo, T., Fujita, K., Nakayama, J., and Sonomoto, K. (2006) Purification and amino acid sequence of a bacteriocins produced by *Lactobacillus salivarius* K7 isolated from chicken intestine. *Songklanakarin J. Sci. Technol.* **28**, 121-131.
44. Probiotic Ingredients Market by Function (Regular, Preventative, Therapy), Application (Food & Beverage, Dietary Supplements, & Animal Feed), End Use (Human & Animal Probiotics), Ingredient (Bacteria & Yeast), and by Region - Global Trends & Forecast to 2020. Publishing Date: July 2015. Report Code: FB 2269. [accessed on November 9, 2015]. Available from <http://www.marketsandmarkets.com/Market-Reports/probiotic-market-advanced-technologies-and-global-market-69.html>
45. Samanya, M. and Yamauchi, K. E. (2002) Histological alterations of intestinal villi in chickens fed dried Bacillus subtilis var. natto. *Comp. Biochem. Phys. A.* **133**, 95-104.
46. Santini, C., Baffoni, L., Gaggia, F., Granata, M., Gasbarri, R., Di Gioia, D., and Biavati, B. (2010) Characterization of probiotic strains: An application as feed additives in poultry against *Campylobacter jejuni*. *Int. J. Food Microbiol.* **141**, 98-108.
47. Sengeløv, G., Agersø, Y., Halling-Sørensen, B., Baloda, S. B., Andersen, J. S., and Jensen, L. B. (2003) Bacterial antibiotic resistance levels in Danish farmland as a result of treatment with pig manure slurry. *Environ. Int.* **28**, 587-595.
48. Stern, N. J., Svetoch, E. A., Eruslanov, B. V., Pereygin, V. V., Mitsevich, E. V., Mitsevich, I. P., Pokhilenko, V. D., Levchuk, V. P., Svetoch, O. E., and Seal, B. S. (2006) Isolation of a *Lactobacillus salivarius* strain and purification of its bacteriocin, which is inhibitory to *Campylobacter jejuni* in the chicken gastrointestinal system. *Antimicrob. Agents Chemother.* **50**, 3111-3116.
49. Timmerman, H. M., Veldman, A., Van den Elsen, E., Rombouts, F. M., and Beynen, A. C. (2006) Mortality and growth performance of broilers given drinking water supplemented with chicken-specific probiotics. *Poultry Sci.* **85**, 1383-1388.
50. Traub-Dargatz, J. L., Ladely, S. R., Dargatz, D. A., and Fedorka-Cray, P. J. (2006) Impact of heat stress on the fecal shedding patterns of *Salmonella enterica* Typhimurium DT104 and *Salmonella enterica* Infantis by 5-week-old male broilers. *Foodborne Pathog. Dis.* **3**, 178-183.
51. Van Coillie, E., Goris, J., Cleenwerck, I., Grijspeerdt, K., Botteldoorn, N., Van Immerseel, F., De Buck, J., Vancanneyt, M., Swings, J., Herman, L., and Heyndrickx, M. (2007) Identification of lactobacilli isolated from the cloaca and vagina of laying hens and characterization for potential use as probiotics to control *Salmonella enteritidis*. *J. Appl. Microbiol.* **102**, 1095-1106.
52. Van den Bogaard, A. E., Jensen, L. B., and Stobberingh, E. E. (1997) Vancomycin-resistant enterococci in turkeys and farmers. *New Engl. J. Med.* **337**, 1558-1559.
53. Van Immerseel, F., Fievez, V., De Buck, J., Pasmans, F., Martel, A., Haesebrouck, F., and Ducatelle, R. (2004) Microencapsulated short-chain fatty acids in feed modify colonization and invasion early after infection with *Salmonella enteritidis* in young chickens. *Poultry Sci.* **83**, 69-74.
54. Vyas, U. and Ranganathan, N. (2012) Probiotics, prebiotics, and synbiotics: gut and beyond. *Gastroenterol. Res. Pract.* **2012**, Article ID 872716.
55. Wang, Y. and Gu, Q. (2010) Effect of probiotic on growth performance and digestive enzyme activity of Arbor Acres broilers. *Res. Vet. Sci.* **89**, 163-167.
56. Wegener, H. C. (2003) Antibiotics in animal feed and their role in resistance development. *Curr. Opin. Microbiol.* **6**, 439-445.
57. Wierup, M. (2001) The Swedish experience of the 1986 year ban of antimicrobial growth promoters, with special reference to animal health, disease prevention, productivity, and usage of antimicrobials. *Microb Drug Resist.* **7**, 183-190.
58. Wlodarska, M., Willing, B., Keeney, K. M., Menendez, A., Bergstrom, K. S., Gill, N., Russell, S. L., Vallance, B. A., and Finlay, B. B. (2011) Antibiotic treatment alters the colonic mucus layer and predisposes the host to exacerbated *Citrobacter rodentium*-induced colitis. *Infect. Immun.* **79**, 1536-1545.
59. Xu, Z. R., Hu, C. H., Xia, M. S., Zhan, X. A., and Wang, M. Q. (2003) Effects of dietary fructooligosaccharide on digestive enzyme activities, intestinal microflora and morphology of male broilers. *Poultry Sci.* **82**, 1030-1036.
60. Yang, X., Zhang, B., Guo, Y., Jiao, P., and Long, F. (2010) Effects of dietary lipids and *Clostridium butyricum* on fat deposition and meat quality of broiler chickens. *Poultry Sci.* **89**, 254-260.
61. Yason, C. V., Summers, B. A., and Schat, K. A. (1987) Pathogenesis of rotavirus infection in various age groups of chickens and turkeys: Pathology. *Am. J. Vet. Res.* **48**, 927-938.
62. Zhu, N. H., Zhang, R. J., Wu, H., and Zhang, B. (2009) Effects of *Lactobacillus* cultures on growth performance, xanthophyll deposition, and color of the meat and skin of broilers. *J. Appl. Poultry Res.* **18**, 570-578.
63. Zulkifli, I., Abdullah, N., Azrin, N. M., and Ho, Y. W. (2000) Growth performance and immune response of two commercial broiler strains fed diets containing *Lactobacillus* cultures and oxytetracycline under heat stress conditions. *Brit. Poultry Sci.* **41**, 593-597.