Animal Feed
The Way Forward

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Prof. Dr. Loh Teck Chwen

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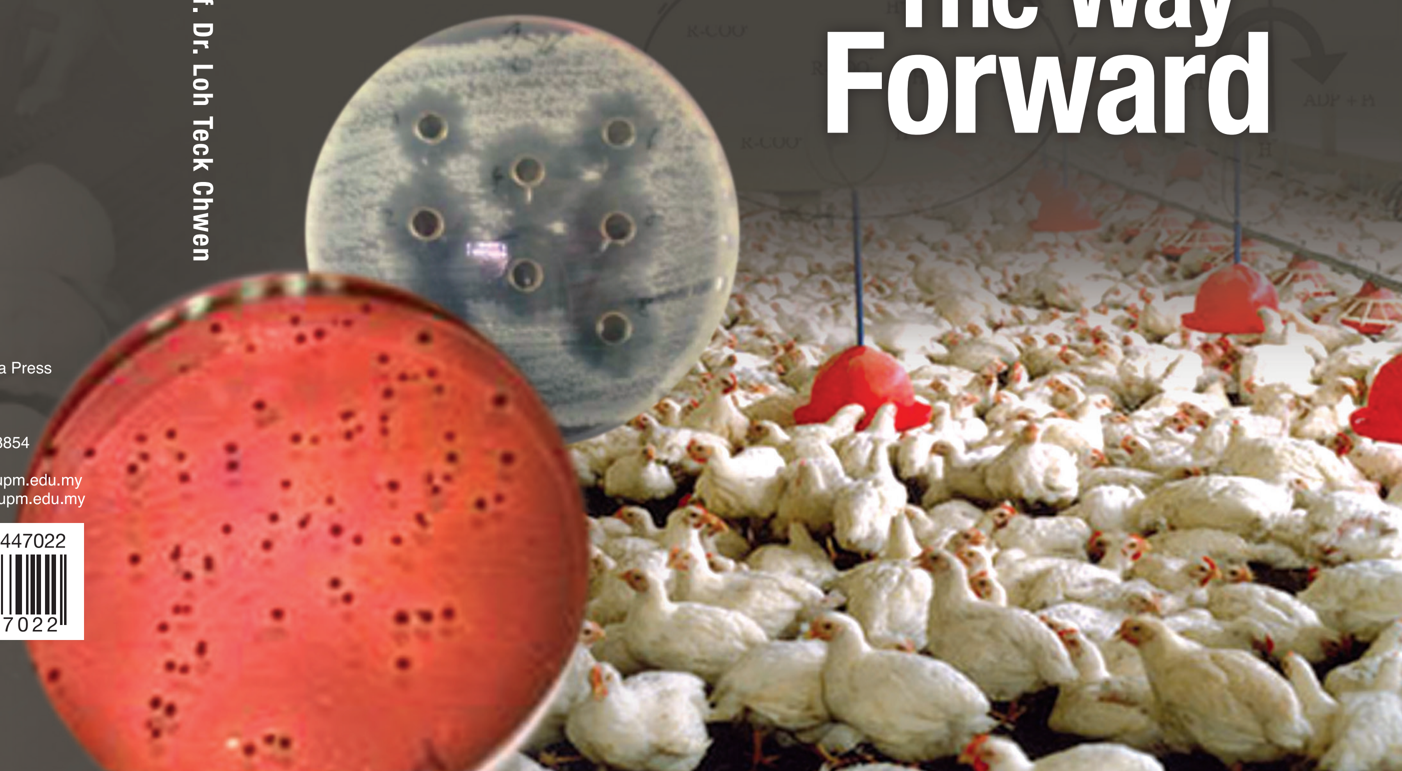

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The Way Forward

PROFESSOR DR. LOH TECK CHWEN

Animal Feed
The Way Forward

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Loh Teck Chwen

ABSTRACT

The poultry and livestock industry is a globalised industry and poultry is one of the fastest growing sectors in Malaysia. Malaysians are one of the highest poultry meat consuming populations in the world with per-capita consumption of about 40 kg/person/year and the overall consumption is expected to increase over the years. Even though our self-sufficiency level (SSL) is high for poultry and swine production, there is a need to meet the increasing meat demands, not just in the local but also in the international market. Animal feedstuffs and feed additives, which are largely imported, are getting costlier by the day resulting in a higher cost of production which is a major setback for our local animal production industry. This has prompted animal scientists to seek alternatives to formulate a more cost effective feed that is able to meet the minimum nutrient requirements of animals without compromising the quality of the output. Focus has been given to postbiotic metabolites produced from lactic acid bacteria (LAB) and other additives such as prebiotic, probiotic, organic acids and phytogenic compounds due to various factors such as food safety concerns. Locally available agricultural waste which is produced abundantly by agriculture sector, such as palm kernel cake, is important in our research, as feedstuff for animal feeding. The mechanisms of postbiotic metabolite actions and their importance are discussed herein. Today's lecture covers the need for constant research in this area and to explore newer approaches for animal production. On the application aspect, this lecture will provide substantial information on future prospects of environmental friendly feed additives and precision in feed formulation.

ABSTRAK

Industri ayam dan ternakan adalah industri global dan merupakan sektor yang berkembang paling pesat sekali di Malaysia. Rakyat Malaysia adalah antara populasi yang makan daging ayam tertinggi di seluruh dunia dengan penggunaan per kapita sekitar 40 kg/orang/tahun, dan penggunaan keseluruhan dijangka meningkat pada tahun-tahun akan datang. Walaupun tahap sara diri (SSL) kita adalah tinggi dalam pengeluaran ayam dan babi, adalah keperluan kita untuk memenuhi permintaan daging yang semakin meningkat bukan sahaja pada tahap tempatan, malah di pasaran antarabangsa. Bahan makanan haiwan dan bahan aditif makanan yang kebanyakkannya diimport telah menjadi semakin mahal yang boleh menyebabkan kos pengeluaran yang tinggi dan ini merupakan satu kekangan utama dalam industri pengeluaran haiwan. Ini telah mendorong para saintis haiwan untuk mencari alternatif bagi merumuskan satu pemakanan kos efektif yang boleh memenuhi keperluan nutrien minimum bagi haiwan tanpa menjejaskan kualiti pengeluaran. Fokus telah diberi kepada penggunaan metabolik postbiotik, yang dihasilkan oleh bakteria asid laktik (LAB) dan juga aditif yang lain seperti prebiotik, probiotik, asid organik dan bahan fitogenik bagi mengurangkan kos pengimportan. Penggunaan sisa pertanian tempatan yang sedia ada yang dikeluarkan oleh sektor pertanian tempatan (contoh: hampas isirong palma) juga merupakan penyelidikan yang penting untuk kami gunakan dalam pemakanan haiwan ternakan. Mekanisma-mekanisma tindakan metabolik postbiotik serta kepentingannya juga dibincangkan dalam buku ini. Kuliah hari ini merangkumi keperluan penyelidikan berterusan dan penerokaan pendekatan baharu dalam pengeluaran haiwan. Dalam aspek aplikasi pula, kuliah ini akan memberi maklumat yang penting bagi prospek masa depan penggunaan bahan aditif yang lebih mesra alam dan formulasi pemakanan yang jitu.

INTRODUCTION

The poultry and livestock industry is the most dynamic and ever expanding segment of the global market. Population growth, urbanisation and income growth are the factors that serve as the driving force behind the development of the world livestock sector that is growing at an unprecedented rate.

Due to the rising world population as well as the meat consuming population, the global demand for poultry and livestock meat and related products is on an upward swing. The world population, now surpassing seven billion, is forecasted to reach 9.3 billion by the year 2050. This will necessitate more intensified meat production in the global meat industry to keep up with growing demand to provide food and livelihood for billions of people. Worldwide consumption of pork, beef and other livestock is projected to double by 2020, although this prediction may be dampened by the recent economic downturn. It is projected that annual meat production will increase from 218 million tonnes in 1997-1999 to 376 million tonnes by 2030, worldwide.

At the same time income growth has made various types of meat more affordable for many throughout the globe. Developing countries now register higher meat consumption due to economic development, changing lifestyles and eating habits, in par with industrialised countries. Furthermore, urbanisation stimulates improvements in infrastructure, including cold chains, making trade of perishable goods more viable. A more diversified diet can be observed among city dwellers compared to the rural communities, while the spread of the food service industry, including fast food chains, has led to more regular consumption of meat.

Pork is the most highly consumed meat globally, followed by poultry and beef, at a ratio of 4-3-2, while demand for poultry is growing at the most rapid rate. Malaysians show high preference for poultry meat followed by pork and beef (Figure 1). This is because poultry production is cheaper resulting in more affordable market price, while additionally there are fewer religious or cultural limitations to eating chicken.

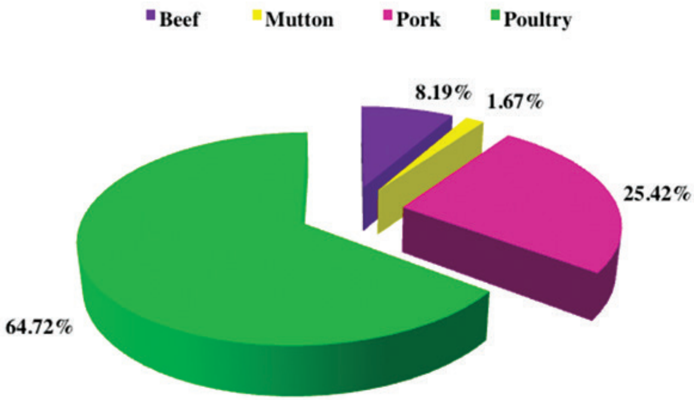


Figure 1 Percentage of different types of meat consumed by the Malaysian Population in the year 2014

Source: Agrofood Statistics, 2014

Poultry is the main source of protein for our country's population whereby the per capita consumption is among the highest in the world, at over 40kg/person/year, and overall consumption is expected to increase in the coming years. According to the reports of FAO (2014), when the Malaysian population was 28.4 million in the year 2010, our poultry meat consumption was a whopping 38.3kg/person/year; while the Asian and world poultry meat consumption was only 8.8 kg/person/year and 13.6 kg/person/year, respectively. Now that our population has reached 30.7 million in 2015, and is postulated to reach 33.0 million by the year 2020, we can expect an increase in the demand for poultry meat over the next few years (Table 1).

Table 1 Malaysian, Asian and world population (current data and projected) and poultry meat consumption

	Human population (million)				**Poultry Meat Consumption (kg/person/year)**			
	2000	2010	2015	2020	2000	2007	2010	2014
Malaysia	23.4	28.3	31.1	32.9	31.5	37.0	38.2	46.6
Asia	3,717.4	4,165.4	4,384.9	4,581.5	6.6	8.1	9.2	n.a
World	6,127.7	6,916.2	7,324.8	7,716.7	11.0	13.1	14.1	n.a

Source: FAO, 2014

In Malaysia, the production of broiler meat was 1,440,000 MT and 1,460,000MT for the years of 2014 and 2015, respectively, with annual growth rates of 1.69% and 1.39%. Commercially bred broilers account for 67% and layer hen for 25% of our total poultry production. Malaysia is largely self-sufficient in poultry meat production and its output is expanding slowly. Our beef production is much lower compared to poultry meat, whereby the production of beef and veal meat was only 56,000 MT in 2014 and 58,000 MT in 2015, with a growth rate of 3.70% and 3.57%, respectively.

As at the year 2014, our self-sufficiency levels (SSL) for pork (93.87%) and poultry (104.87%) were high, but a fairly depressing picture was seen for the production of beef (24.84%) and mutton (13.10%), despite various livestock development plans implemented over the years.

Data on our local production, per capita consumption and self-sufficiency levels for beef, mutton, swine, poultry (Table 2) and livestock products (Table 3) from the Department of Veterinary Services, Malaysia, show that we are still not self-sufficient in certain sectors, leading to dependency on outside sources for our local meat demands.

Table 2 Production and per capita consumption of poultry and livestock in Malaysia from 2009-2015

Commodity	2009	2010	2011	2012	2013	2014	2015
Cattle/Buffalo							
Beef							
Production *(Metric Tonnes)*	42, 178	46, 510	48, 835	51, 277	51, 738	52, 202	50,493
Per Capita Consumption *(kg/person/year)*	5.35	5.45	5.76	6.15	6.74	6.94	7.05
Self Sufficiency Level *(%)*	28.26	30.12	29.17	28.26	25.67	24.84	23.50
Goat/ Sheep							
Mutton							
Production *(Metric Tonnes)*	2, 161.9	2, 386.5	3, 091.5	4, 806.2	4, 321.4	4, 575.1	4,367.3
Per Capita Consumption *(kg/person/year)*	0.69	0.69	0.69	0.83	0.96	1.15	1.25
Self Sufficiency Level *(%)*	11.20	12.13	11.73	19.71	15.02	13.10	11.46
Swine							
Pork							
Production *(Metric Tonnes)*	206, 026	234, 000	214, 308	218, 471	217, 422	215, 675	215,760
Per Capita Consumption *(kg/person/year)*	19.00	21.65	21.01	19.13	18.74	18.79	18.70
Self Sufficiency Level *(%)*	97.50	95.36	94.57	95.60	95.35	95.66	94.62

Table 2 Production and per capita consumption of poultry and livestock in Malaysia from 2009-2015 *(cont'd.)*

Commodity	2009	2010	2011	2012	2013	2014	2015
Chicken/ Ducks							
Poultry							
Production *('000 Metric Tonnes)*	1, 202.00	1, 295.60	1, 289.90	1, 374.37	1, 458.09	1, 495.53	1,613.92
Per Capita Consumption *(kg/person/year)*	41.11	43.32	43.58	44.40	46.49	47.12	50.67
Self Sufficiency Level *(%)*	104.72	105.55	105.36	104.88	104.85	104.87	104.48

Source: DVS, 2016

Table 3 Production and Per Capita Consumption of poultry and livestock products in Malaysia from 2009-2015

Commodity	2009	2010	2011	2012	2013	2014	2015
Chicken/ Duck Eggs							
Production							
(million eggs)	9, 270	9, 826	10, 358	10, 736.7	11, 399.3	12,127.1	12,917.5
('000 Metric Tonnes)	556	590	621.48	644.2	683.96	727.62	775.05
Per Capita Consumption *(egg/person/year)*	283	303	309	308	319	352	373
Self Sufficiency Level *(%)*	117.53	114.63	115.35	118.17	119.35	113.79	113.55
Milk							
Production *(million litres)*	62.30	67.00	70.87	72.41	73.99	75.27	76.04
Per Capita Consumption *(kg/person/year)*	25.41	27.85	18.52	35.45	32.38	35.17	35.68
Self Sufficiency Level *(%)*	8.79	8.49	7.22	6.92	7.64	7.07	6.99

Source: DVS, 2016

Animal Feed Ingredients as the Main Cost of Production

Our beef and mutton production is inadequate to meet even our domestic demands, making us dependent on imported meat from other countries. When we look deeper into the factors that are contributing to the inefficiency of our production, it is observed that many farms are still under the management of small farmers who are not as well equipped as larger commercial farms with proper infrastructure and established facilities. Furthermore, the related high cost of production is a major barrier for any plans to completely terminate dependency.

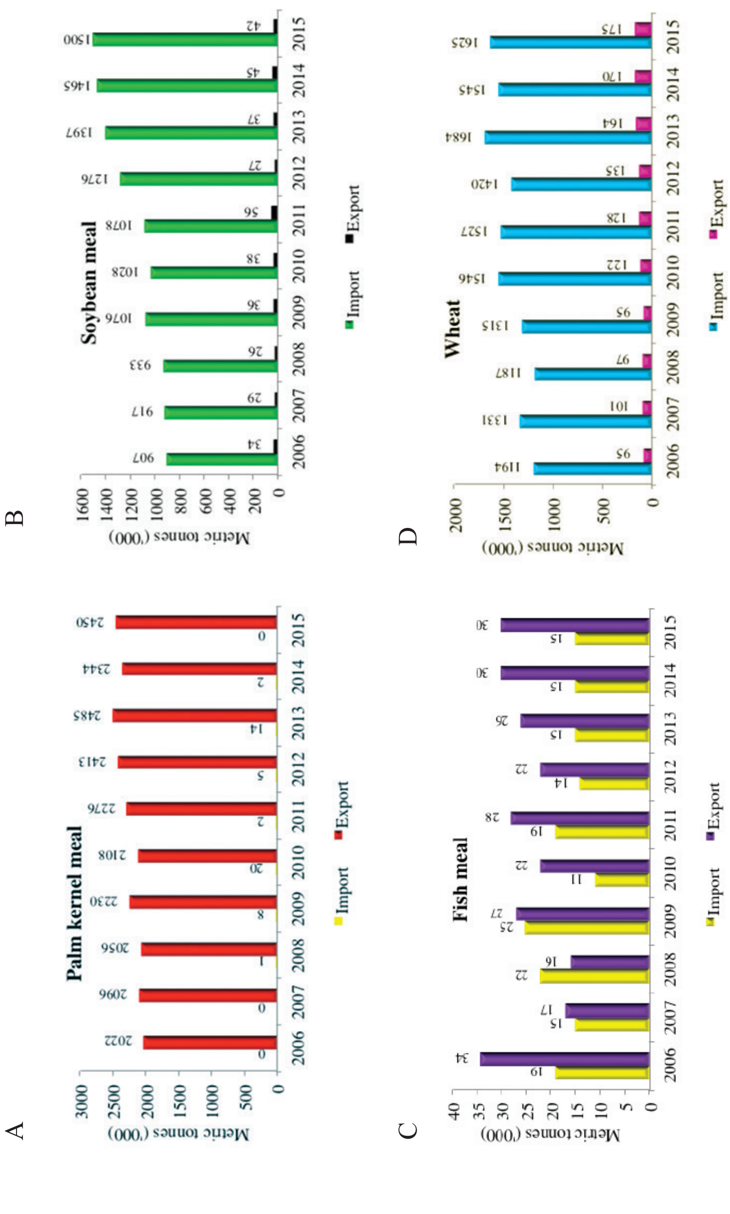
Feed for the consumption of animals constitutes a major proportion of the cost of production. The feed ingredients are costly as the predominantly used raw ingredients are not produced locally and are mostly imported. Feed ingredients imported to fulfil the demands of our poultry and livestock industry includes maize, wheat, meat and bone meal, skim milk powder, whole milk powder, dried whey powder, groundnut cake, soybean meal, dicalcium phosphate, monocalcium phosphate, salt, sesame cake, corn gluten meal, tapioca and a number of micro ingredients (Loh, 2002g).

International trade of raw materials is the key to the global feed industry whereby such feed are formulated and milled locally. A nutritive diet is required to ensure optimal growth of the animals and their meat quality. Animal feed and a related balanced diet is essentially required for supply of appropriate energy for efficient amino acid and protein use by the animals. Furthermore, proper energy to protein ratio is necessary for optimised protein and energy utilisation. Protein requirements vary by species, age of the animals as well as their growth stage.

Protein sources in animal feed could be from various sources, such as plant origin, legumes, oil meal crops and animal origin.

Wheat, soybean and maize meal are those of plant origin whereby the latter two have high crude protein content and balanced amino acid composition, making them ideal to be used in high performance monogastric diets. Oil meal crops such as palm kernel cake (PKC) which are the by-products of oil palm production are also used in animal feed. Since Malaysia is one of the leading palm oil producers, it produces a large amount of what is referred to as agriculture 'waste products'. Local availability and adaptation to growing conditions is an added advantage for the use of oil meal crops in animal feed. Efficient utilisation of these agriculture waste products as animal feed sources is a smart move aimed to transform waste to wealth. However, the disadvantage is that oil meal crops have lower nutritive value due to their high fibre content. Measures to increase nutritive value with pre-treatment of oil meal crops prior to feeding have demonstrated promising effects (Alshelmani *et al.*, 2013, Alshelmani *et al.,* 2014, 2016a & b). Protein sources of animal origin, such as meat, bone- and fish- meal, provide good nutritional sources of calcium and phosphorus in animal diets.

The import and export of palm kernel, soybean meal, fish meal, wheat and corn (Figure 2) highlight that our self-sufficiency level is high only for palm kernel meal and fish meal while we still remain highly dependent on importation for other feed ingredients.


A
Palm kernel meal
Metric tonnes ('000)
0 500 1000 1500 2000 2500 3000
2006 2007 2008 2009 2010 2011 2012 2013 2014 2015
Import
Export
0 2022
0 2096
1 2056
8 2230
20 2108
2 2276
5 2413
14 2485
2 2344
0 2450
B
Soybean meal
Metric tonnes ('000)
0 200 400 600 800 1000 1200 1400 1600
2006 2007 2008 2009 2010 2011 2012 2013 2014 2015
Import
Export
907 34
917 29
933 26
1076 36
1028 38
1078 56
1276 27
1397 37
1465 45
1500 42
C
Fish meal
Metric tonnes ('000)
0 5 10 15 20 25 30 35 40
2006 2007 2008 2009 2010 2011 2012 2013 2014 2015
Import
Export
19 34
15 17
22 16
25 27
11 22
19 28
14 22
15 26
15 30
15 30
D
Wheat
Metric tonnes ('000)
0 500 1000 1500 2000
2006 2007 2008 2009 2010 2011 2012 2013 2014 2015
Import
Export
1194 95
1331 101
1187 97
1315 95
1546 122
1527 128
1420 135
1684 164
1545 170
1625 175

E

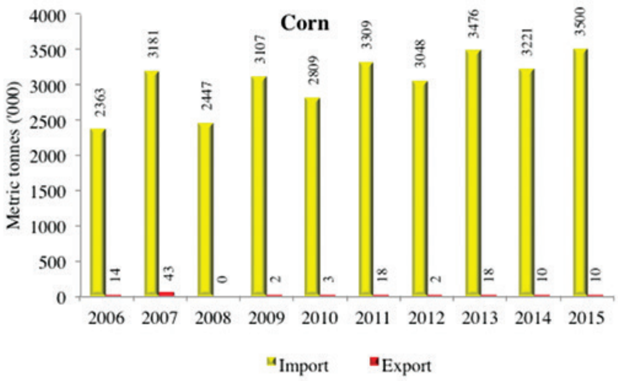


Figure 2 Import and export of: (A) Palm kernel meal; (B) Soybean meal; (C) Fish meal; (D) Wheat; and (E) Corn (2006-2015)

Source: USDA, 2016

Even though we export certain feedstuffs to other countries, the amount is far too low to compensate for the amount we spend on the importation of other feedstuffs, leading to a deficit in the Balance of Trade (Figure 3). This is the prime cause leading to the increase in the cost of production.

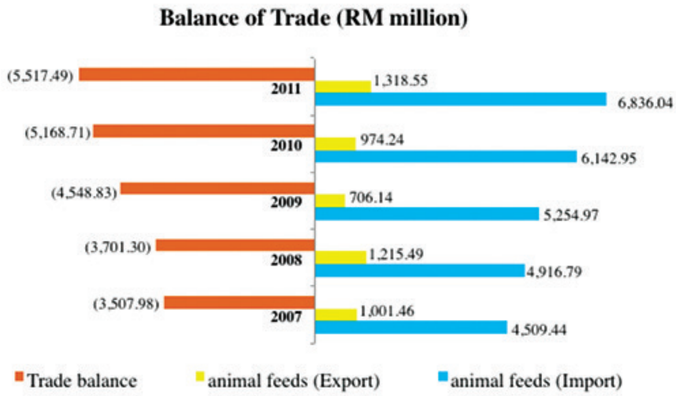


Figure 3 Balance of Trade for the importation and exportation of animal feed

The Use of Feed Additives in Feed Formulation

The cost of production is also affected by the feed additives that are widely used in the diets of the animals for various benefits. The addition of these feed additives increase the nutritive content of the diet thereby improving feed efficiency and the growth of the animals. The various types of feed additives commonly used in the industry and their functions are as follow:

Table 4 Examples of feed additives and their functions

Feed additives	Roles/ Functions
Antibiotics/ drugs	Disease prevention/ medication
Coccidiostats	Control parasites
Xanthophyll	Makes egg yolks yellow
Hormones	Increases growth
Tranquilizers	Calms nerves of cattle, turkey
Antioxidants	Prevents feed from getting rancid
Pellet Binders	Keeps feed in pellet form
Flavouring agents	Makes feed taste better

Source: DVS, 2008

Antibiotics as Growth Promoters

Supplementation of antibiotics in the feed of breeding animals as additives is a common practise in the poultry and livestock industries. Antibiotics serve as growth promoters (AGP), whereby it enhances the growth rate and production performance of the breeding animals when ingested. The use of antibiotics as AGP, even at low doses, result in weight gain of healthy animals that are fed nutritionally complete feed (Jukes, 1977). Thus, antibiotics are widely used to maximise output and quality production. The history of using AGP dates back 60 years following researches that observed enhanced growth in poultry (Moore *et al.,* 1946) and in swine (Jukes *et al.,*1950). A few examples of antibiotics commonly used as feed additives include bacitracin, manganese, neomycin, soframycin, tetracyclines and penicillin (Castanon, 2007).

The action of the antibiotics is focused on the gut of the animals where it stabilises the intestinal microflora which leads to improved general performance. It is important to maintain stabilised intestinal microflora as these animals are vulnerable to potentially pathogenic microorganisms, mainly, *Escherichia coli, Salmonella* spp*., Clostridium perfringens* and *Campylobacter sputum*. The action of these pathogenic bacteria results in increased incidence of disease and depressed growth performance of breeding animals. The consequences are also evident further down the food chain when illness caused by these food-borne pathogens are reported resulting from contaminated raw or undercooked poultry and red meats being consumed by humans or pets. *Campylobacter*, *Salmonella* and *E. coli* are often present in fresh meat and poultry (Todd, 1997).When consumed by humans, these food-borne pathogens cause food poisoning, bloody diarrhoea, haemolytic uremic syndrome, crampy stomach pain, high fever and, in serious cases, can be fatal.

Despite its usefulness, concerns about the usage of AGP arose following the discovery of the development of antimicrobial resistance in a few pathogenic bacterial species such as, *Salmonella*, *E. coli* and LAB, and transference of the antibiotic resistance genes from the animal to human microbiota (Greko, 2001). The prevalence of antibiotic resistance in LAB strains in Malaysia was recently documented following tests on faeces samples of broiler chickens collected from a number of wet markets in the Klang valley. *Lactococcus lactis* subs *lactis, Lactobacillus paracasei*, *Lactobacillus brevis, Lactobacillus curvatus, Lactobacillus plantarum, Leuconostoc lactis mesenteroids subsp mesenteroides/ dectranium* and *Pediococcus pentosaceus* were the most common LAB that were isolated from the faeces samples. It was further reported that most of the isolated LAB were resistant to a wide range of antibiotics such as nalidixic acid, bacitracin, gentamycin, ciprofloxacin, sulphamethoxazole, kanamycin, tetracycline, streptomycin and vancomysin (Shazali *et al.,* 2014).

In another report, the prevalence of antimicrobial resistance towards *E. coli* infection in diarrhoeic piglets was studied in 10 farms in Penang, Perak and Selangor. The *E. coli* was highly resistant to oxytetracylcine (100%), nalidixic acid (96.8%), trimethoprim-sulfadimthoxine (95.1%), chloramphenicol (91.9%), enrofloxacin (90.3%), ampicillin (85.5%), kanamycin (74.2%) and neomycin (71.3%) (Loh *et al.,* 2006). Further, study on the occurrence of antimicrobial resistance of *Salmonella* spp. in finishing pigs was carried out at 12 pig farms in Seberang Perai, Malaysia. Nine out of the 12 farms sampled, which constitutes 75.0% of our total sampling, tested positive for *Salmonella typhimurium* and most of the isolates showed a relatively high level of antimicrobial resistance (Choe *et al.,* 2011).

Serious consequences surface when microorganisms become resistant to treatments that humans have relied on for years. Future treatments can be rendered unsuccessful and it becomes increasingly challenging to combat the threat to not only human, but to animals and plant health. In addition, AGP has residual effects on tissues long after withdrawal. Furthermore, there are also reports on the use of AGP leading to genotoxicity and allergies.

This issue has been under scrutiny for many years, resulting in the imposition of a ban on the usage of AGP as a precautionary measure. In 1986, Sweden became the first nation to eliminate the use of AGP. Denmark banned the use of avoparcin in 1995, followed by the Commission of the European Union which banned the use of antibiotics as feed additives in all European Union member states beginning from 1st January 2006. The use of many other AGPs was ceased following this and many other countries including the United States began surveillance on the use of AGP in animal feed and decided to phase it out and ultimately ban the use of a few antibiotics. On the global level the World Health Organisation (WHO), Food and Agriculture Organisation of the United Nations (FAO) and the World Organisation of Animal Health (OIE) have agreed on the implementation of the WHO global principles for the containment of antimicrobial resistance in animals intended for food (World Health Organisation, 2004). Antibiotics can, however, be used for veterinary purposes, by prescription only.

Increasing restrictions on the use of AGP have prompted a search for alternatives. Potential alternatives should preferably share similar mechanisms to AGP and carry out their role mainly by ensuring that balanced intestinal microbiota is maintained and at the same time serve as antagonists to pathogenic bacteria. It should therefore be able to exhibit optimised animal performance and increased nutrient availability. Although to date there are no equivalent replacements for AGP, potential alternatives, such as organic acids, methane inhibitors, dietary fibres, probiotics (Abdulla *et al.*, 2017a), prebiotics, postbiotics, herbs, spices or botanicals (Rosen, 1996) as well as phytogenic compounds such as essential oils, have been widely explored for over a decade. Strategies also include the best possible combinations of these pronutrients.

POSTBIOTICS: A POTENTIAL ALTERNATIVE TO ANTIBIOTICS

Probiotics, prebiotics, synbiotics and postbiotics have recently gained preference to be used as in-feed additives as they have demonstrated desired effects on the host.

- Probiotics are viable microorganisms, sufficient amounts of which reach the intestine in an active state and thus exert positive health effects. Probiotics usually possess criteria as:
    i. non-pathogenic;
    ii. able to survive during processing and storage of the feed;
    iii. resistant to bile and acidic environment; and
    iv. producers of inhibitory compounds (organic acids and antibacterial activities).
- Prebiotics on the other hand are indigestible carbohydrates that leave a desired effect on the host by selective growth stimulation or activation of one or more beneficial bacteria in the large intestine and thus act as a food for probiotics. When probiotics and prebiotics are combined, they are termed as synbiotics.
- Postbiotics are non-viable bacterial products or metabolic by-products from probiotic microorganisms that have a biological activity in the host. The postbiotics produced by LAB species possess myriad beneficial probiotic effects on the growth of animals, particularly gut health, when used as additives in animal diet (Loh et al., 2010a, Choe et al., 2012, Thu et al., 2011b)

We have explored extensively the use of postbiotics as in-feed additives, mainly produced by the species *Lactobacillus plantarum.* Being a member of the lactic acid bacteria, *L. plantarum* is widely used in the industry and has been granted ‘Generally Recognised as Safe' (GRAS) status. Metabolites produced by LAB have gained wide attention for inclusion in the feed of poultry and livestock as growth promoters and as a potential substitute for in-feed antibiotics (Thanh *et al.,* 2009, Loh *et al.,* 2010a, Thu *et al.,* 2010, Choe *et al.,* 2012). The use of metabolites is preferred over the use of live microorganisms for this purpose due to their advantages in terms of storage, transportation and handling. As a matter of fact, even probiotics such as LAB have acquired resistance to antimicrobials commonly used in human and animal health sciences (Shalini and Ramashwar, 2005; Shazali *et al.*, 2014), especially those encoded by plasmids which can be transferred between organisms.

We first embarked on the study of postbiotic metabolites by carrying out a series of *in-vitro* studies in which we selected different strains (RG11, RG14, RI11, UL4 and RS5) of *L. plantarum* previously isolated from Malaysian fermented food (Foo *et al.,* 2003b, Moghadam *et al.,* 2010). The molecular characterisation of *plw* and *pln*EF structural genes loci of bacteriocin genes harboured in *L. plantarum* I-UL4 indicate that it is a novel multiple bacteriocin producer (Tai *et al.*, 2015). *L. plantarum* exhibited high inhibitory activity against a few pathogenic bacteria (indicator organisms) and was thus considered a suitable candidate to be further tested in the feeding trials (*in vivo* study). Furthermore, higher bacteriocin inhibitory activity was observed when glucose or yeast extract was optimised as carbon and nitrogen sources, respectively. (Ooi *et al.,* 2015).

Metabolites produced from *L. plantarum* display broad antagonistic activities (Thu *et al.*, 2011a, Choe *et al.,* 2013, Kareem *et al.,* 2014, Thanh *et al.*, 2010, Lim *et al.*, 2005, Foo *et al.*, 2005) showing its capability to inhibit pathogens from various species. The postbiotic metabolite inhibits the growth of Gram-positive (e.g.: *Bacillus cereus, Staphylococcus aureus, Listeria monocytogens,* *Streptococcus pneumonia, Enterococcus faecalis, Enterococcus faecium* and *Pediococcus acidilactici)* and Gram-negative (e.g.: *Escherichia coli* and *Salmonella typhimurium)* bacteria (Savadago *et al.*, 2004). Upon discovering its profound benefit on growth performance from the animal studies, we then progressed to researching the use of combinations of metabolites from a few strains of *L. plantarum*. The postbiotic combinations showed improved growth performance and were in fact, more effective than using postbiotic metabolites of a single strain in broiler (Loh *et al.,* 2010a) and pig (Thu *et al.,* 2011b) feed.

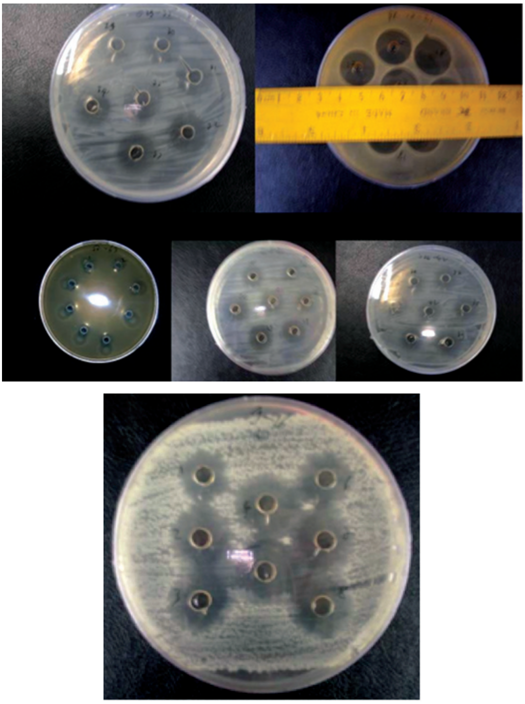


Figure 4 Formation of clear and distinct zones surrounding the well indicates the positive bacteriocin-inhibitory activity of LAB

Postbiotics Provide a Balanced Gut Microflora

Ideally, the intestine allows harmonious habitation of commensal bacteria and at the same time protects the host from various pathogens and toxins. Bacterial pathogens cause inflammation whereby uncontrolled inflammation can lead to tissue injury and necrosis. Therefore, the growth and habitation of commensal bacteria is important in order to maintain intestinal homeostasis. At birth, the new-born's intestine is sterile and almost absent of microbes. It then undergoes transitions and begins to acquire a diverse set of commensal bacteria. The gastrointestinal microflora consist of as many as five hundred bacterial species that outnumber the host cells by 10:1. At this point, supplementation of beneficial bacteria via feed seems to be an effective option as the population of these commensal bacteria could thus be increased. Bacteria in the gut benefits the host by playing vital roles in digestion, absorption and storage of nutrients, intestinal homeostasis, protection against injury as well as, the development and control of epithelial immune response and function. Probiotic bacteria protect against intestinal inflammation and injury by restoring and supplying the essential commensal strains. LAB are the most common bacteria used as probiotics. The population of LAB in the gut further increases via competitive exclusion of pathogens as LAB persevere in the competition for attachment sites and nutrients with different species within the niche. At this point, supplementation with prebiotics complements the probiotics by ensuring its continued survival whereby prebiotics provide the required resources to promote the growth of commensal bacteria.

The high population of beneficial bacteria exhibits bactericidal properties by synthesising agents such as bacteriocins and organic acids. The use of postbiotic metabolites in feed is also able to bring about similar effects as they mainly consist of different levels of organic acids and bacteriocins (Foo *et al.,* 2005, Thanh *et al.,* 2009, Thu *et al.,* 2011a, Moghadam *et al.*, 2010). Bacteriocins are a group of ribosomal synthesised antimicrobial peptide produced by various genera of bacteria which can kill or inhibit bacterial strains closely-related or non-related to the producing bacteria. The bacteriocins will however not harm to the producer bacteria since they possess specific immunity proteins. Bacteriocins are one of the bio weapons against microorganisms due to their specific characteristics of large differences in structure and function, natural resources and being stable to heat. In addition, bacteriocins are not toxic to eukaryotic cells; are inactivated by proteases during the digestion process; and are active against food borne pathogens and food spoilage bacteria (Chen and Hoover, 2003). These properties enable bacteriocins to have an advantage in food preservation and storage. Their benefits are that they are safe and natural agents; their use reduces the need for chemical preservatives; and they can decrease the extension of thermal treatments (Nath *et al.*, 2014).

The antimicrobial action of bacteriocins is due to its high affinity targets and low-affinity membrane interactions (Figure 5). It binds with high affinity to the lipid molecules of the bacterial membrane via a hydrophobic carrier for peptidoglycan monomer and form pores that increases the membrane permeability. The bacteriocin-lipid interaction compromises the incorporation of precursor units, and hence blocking the biosynthesis of the bacterial cell wall. The pore formation causes a loss of membrane integrity which induces a passive efflux of small intracellular metabolites through the lipid bilayer. Following the loss of ions (potassium, phosphate), amino acids and ATP, the proton-motive force is reduced or dissipated leading to cell death (Hsu *et al.*, 2002).

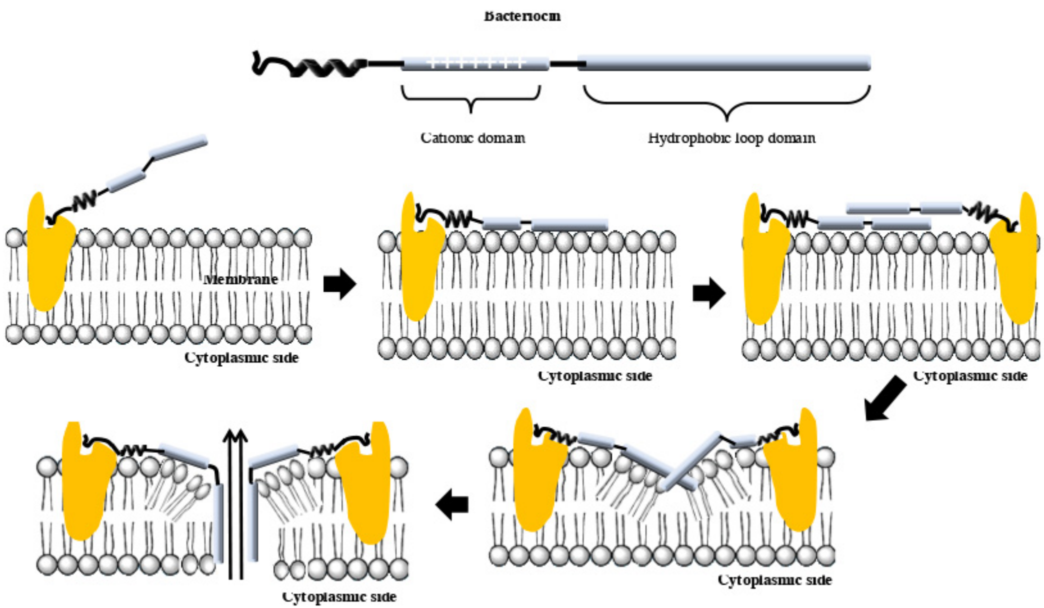


Figure 5 The hydrophobic face of the peptide (shaded dark) and hydrophilic face (shaded light). Positively charged amino acids of bacteriocins interact with negatively charged phospholipids of the bacterial membrane to form a pore. Pore formation disintegrates the membrane resulting in the loss of small intracellular metabolites eventually causing cell death. Adapted from Ennahar *et al.* (2000) with slight modifications.

Apart from bacteriocins, postbiotic metabolites also contain organic acids which are both bacteriostatic and bactericidal. Short chain acids such as lactic acid and acetic acid are associated with potent antimicrobial activity. The undissociated organic acids are lipophilic, enabling them to cross the cell membrane of microbial cells via carrier-mediated transport mechanism. Once inside the cell, the higher cytosolic pH causes the acids to dissociate, releasing hydrogen ions, which consequently reduces the intracellular pH (Figure 6). The low pH inhibits the synthesis of vital microbial enzymes and depresses the enzyme activities, thus affect the microbial metabolism. In order to redress the balance, the cell is forced to use the energy to expel protons out across the membrane via the H^+-ATPase pump to restore the cytoplasmic pH to normal. This is sufficient to kill the cell over a period of exposure to organic acids. Furthermore, expelling protons leads to accumulation of acid anions in the cell, which inhibits intracellular metabolic reactions, such as the synthesis of macromolecules and the disruption of internal membranes (Lambert and Stratford, 1998). LAB are less sensitive to difference across the cell membrane and thus remain unaffected.

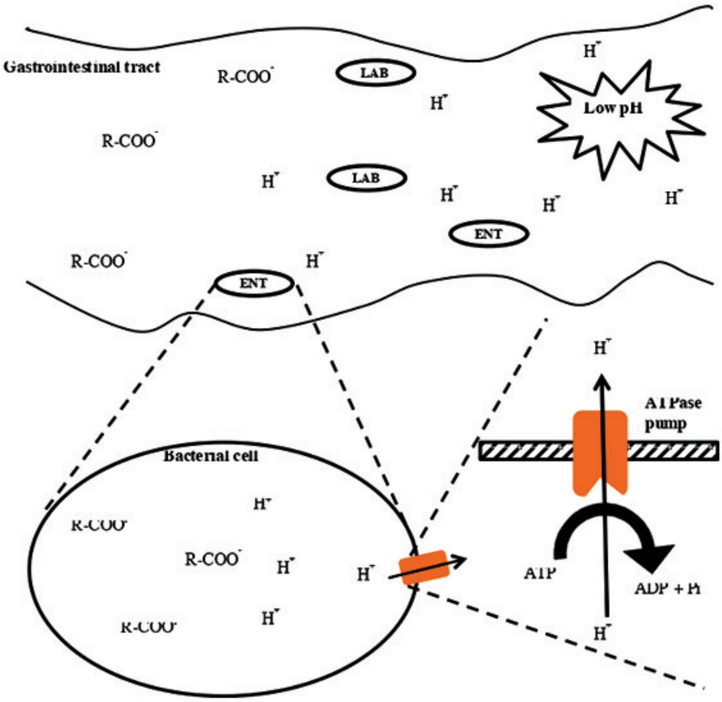


Figure 6 Lipophilic organic acids cross the cell membrane of microbial cells and dissociate within the cell, releasing H^+ and consequently reducing intercellular pH. In the effort to restore cytoplasmic pH to normal, cells are forced to use energy to expel protons out across the membrane via H^+- ATPase pump. This largely affects microbial metabolism leading to cell death.

The acidic condition in the stomach initiates pepsin activity that is necessary for protein digestion. Metabolites produced by existing bacteria in the gut were able to reduce pH further via fermentation process (Foo *et al.*, 2005). The low pH condition seems favourable for the growth of the existing intestinal LAB population and inhibits the viability of the intestinal *Enterobacteriaceae* (ENT) population. In bringing about this microbial balance, postbiotics are implicated in 3 ways:

1. Provide a healthier gut environment when the pathogenic bacterial load in the gastrointestinal microflora is reduced by its bacteriostatic and bactericidal effect. The animal's health and performance are negatively impacted as the bacteria compete with the host for nutrients, secrete toxic compounds and induce an ongoing immune/inflammatory response in the gastro intestinal tract. While reducing the amount of toxins produced by the bacteria in the intestine, the nutrients are fairly preserved against bacterial destruction.
2. Modulation of the immune response. Following the reduction in the opportunistic pathogens in the gut, various endemic subclinical infections are prevented, thus reducing the metabolic costs of the (innate) immune system.
3. Production of volatile fatty acids (VFA). LAB and other gut microbiota ferment various substrates like lactose, biogenic amines and allergenic compounds into short chain fatty acids and other organic acids and gases. High VFA concentration creates an unfavourable environment for many unwanted bacteria such as *Salmonella* and *E.coli.*

We hypothesised that these favourable characteristics could be achieved by the action of postbiotic metabolites. From our studies on incorporating postbiotic metabolites produced by *L. plantarum* in the feed of animals, we observed the successful reduction of pH, increased LAB population, reduced ENT population and increased VFA in layer hens (Loh *et al.*, 2014, Choe *et al.,* 2012, Loh *et al.,* 2007a), post weaning piglets (Loh *et al.*, 2013a, Thu *et al.*, 2011b), broilers (Rosyidah *et al.*, 2011, Loh *et al.*, 2013b, Thanh *et al.,* 2009) and rats (Loh *et al.,* 2009a, Foo *et al.*, 2003a, Foo *et al.,* 2003b, Foo *et al.*, 2003c, Loh *et al.*, 2008c). These parameters could be evaluated by analysing the faeces (Figure 7) or digesta obtained from the animals. The pH values, LAB count and ENT count in the faeces of hen (Table 5) broilers (Table 6) and piglets (Table 7) showed that feeding animals with a combination of metabolites can elicit a desired outcome.

Table 5 Faecal pH, lactic acid bacteria and *Enterobacteriaceae* counts in hens following treatments supplemented with different postbiotic metabolite combinations

	Treatments			
Weeks	**Control**	**COM246**	**COM345**	**COM456**
pH				
24	7.04 ± 0.03	7.05 ± 0.04	7.01 ± 0.05	7.03 ± 0.03
26	6.98 ± 0.04	7.02 ± 0.03	6.99 ± 0.03	7.01 ± 0.04
28	7.02 ± 0.04^{a}	6.98 ± 0.04^{a}	7.00 ± 0.02^{a}	6.82 ± 0.05^{b}
30	6.98 ± 0.05^{a}	6.86 ± 0.04^{b}	6.87 ± 0.05^{b}	6.78 ± 0.03^{c}
32	6.94 ± 0.06^{a}	6.65 ± 0.05^{b}	6.54 ± 0.04^{c}	6.52 ± 0.05^{c}
Lactic acid bacteria counts (log CFU/g)				
24	6.45 ± 0.03	6.48 ± 0.06	6.42 ± 0.06	6.41 ± 0.07
26	6.39 ± 0.11	6.32 ± 0.07	6.31 ± 0.12	6.34 ± 0.08
28	6.48 ± 0.12	6.57 ± 0.10	6.53 ± 0.11	6.60 ± 0.19
30	6.56 ± 0.18^{b}	6.61 ± 0.16^{b}	6.68 ± 0.14^{b}	6.92 ± 0.11^{a}
32	6.62 ± 0.16^{c}	6.91 ± 0.18^{b}	6.68 ± 0.13^{c}	7.28 ± 0.10^{a}
34	6.45 ± 0.21^{c}	6.88 ± 0.18^{b}	6.90 ± 0.10^{b}	7.32 ± 0.12^{a}

Table 5 Faecal pH, lactic acid bacteria and *Enterobacteriaceae* counts in hens following treatments supplemented with different postbiotic metabolite combinations *(cont'd.)*

	Treatments			
Weeks	**Control**	**COM246**	**COM345**	**COM456**
Enterobacteriaceae counts (log CFU/g)				
24	7.43 ± 0.08	7.47 ± 0.06	7.51 ± 0.05	7.56 ± 0.09
26	7.50 ± 0.05	7.52 ± 0.06	7.51 ± 0.06	7.46 ± 0.07
28	7.38 ± 0.10	7.41 ± 0.18	7.32 ± 0.14	7.25 ± 0.12
30	7.16 ± 0.08[a]	6.89 ± 0.09[b]	6.78 ± 0.10[b]	6.83 ± 0.07[b]
32	6.92 ± 0.16[a]	6.33 ± 0.11[c]	6.55 ± 0.08[b]	6.31 ± 0.12[c]

Means in the same row not sharing a common superscript are significantly different (P<0.05).
SEM: Data are means of 25 cages of 2 hen per cage per treatment.
COM246 is basal diet supplemented with 0.6% metabolites of TL1, RI11, and RG11.
COM345 is basal diet supplemented with 0.6% metabolites of RS5, RI11, and RG14.
COM456 is basal diet supplemented with 0.6% metabolites of RI11, RG14, and RG11.

Source: Loh *et al.,* 2014

Table 6 Faecal LAB and ENT count and VFA of broilers at week 6 of treatments supplemented with different dosages of metabolites from COM3456

Parameters	Dietary treatments †						
	-ve control	+ve control	0.1% Com 3456	0.2% Com 3456	0.3% Com 3456	0.4% Com 3456	0.5% Com 3456
LAB and ENT count, log CFU/g							
LAB	6.04 ± 0.11^{b}	6.80 ± 0.15^{a}	6.74 ± 0.12^{a}	6.91 ± 0.14^{a}	6.65 ± 0.15^{a}	6.72 ± 0.11^{a}	6.43 ± 0.10^{a}
ENT	4.88 ± 0.10^{a}	4.81 ± 0.18^{a}	4.28 ± 0.12^{b}	3.94 ± 0.19^{b}	4.10 ± 0.11^{b}	4.32 ± 0.14^{b}	3.94 ± 0.11^{b}
VFA, mM							
Acetic acid	50.39 ± 1.96^{c}	59.56 ± 4.51^{bc}	62.77 ± 7.51^{bc}	62.33 ± 4.87^{bc}	69.88 ± 5.74^{ba}	81.27 ± 5.51^{a}	64.21 ± 3.77^{bc}
Propionic	1.96 ± 0.43^{a}	2.09 ± 0.76^{a}	3.33 ± 1.13^{a}	1.80 ± 0.51^{a}	1.83 ± 0.28^{a}	2.01 ± 0.21^{a}	1.44 ± 0.15^{a}
Butyric	2.22 ± 1.12^{ba}	1.96 ± 0.90^{ba}	2.53 ± 0.86^{a}	1.13 ± 0.43^{ba}	0.64 ± 0.07^{b}	0.86 ± 0.08^{b}	0.68 ± 0.05^{b}
Others	0.55 ± 0.10^{a}	0.46 ± 0.12^{a}	0.83 ± 0.25^{a}	0.73 ± 0.14^{a}	0.46 ± 0.08^{a}	0.54 ± 0.07^{a}	0.65 ± 0.12^{a}
Total	52.29 ± 2.08^{c}	62.71 ± 5.68^{bc}	68.32 ± 8.79^{bac}	65.68 ± 5.71^{bac}	72.74 ± 6.04^{ba}	84.23 ± 5.51^{a}	66.63 ± 3.77^{bac}

a-cmeans SEM with different superscript are significantly different ($P<0.05$). †Diets supplemented with different dosages (0.1-0.5%, w/w) of metabolite powder of COM3456 (a combination of 4 strains RS5, RI11, RG11 and RG14). Each treatment consisted of 6 replicates.

Source: Loh *et al.,* 2010a

Table 7 The pH value, LAB and ENT population (log_{10} CFU/g) in piglets subjected to different dietary treatments

Items	Negative control	Positive control	Met 1	Met 3	Met 5	SEM	*P*-value
	Dietary treatments						
Digesta							
pH	6.25^{a}	6.22^{a}	6.23^{a}	6.06^{b}	6.06^{b}	0.05	0.02
LAB	6.40^{bc}	6.25^{c}	6.64^{b}	6.98^{a}	7.11^{a}	0.11	0.00
ENT	4.59^{a}	4.52^{a}	4.62^{a}	4.62^{a}	4.56^{a}	0.03	0.26
Ratio LAB: ENT	1.39^{c}	1.38^{c}	1.44^{bc}	1.51^{ab}	1.56^{a}	0.02	0.00
Faeces							
pH	6.54^{a}	6.54^{a}	6.50^{a}	6.24^{b}	6.23^{b}	0.08	0.02
LAB	6.51^{c}	6.12^{d}	6.92^{b}	7.29^{a}	7.32^{a}	0.10	0.00
ENT	5.34^{a}	5.05^{ab}	5.24^{a}	4.77^{bc}	4.57^{c}	0.11	0.00
Ratio LAB: ENT	1.22^{c}	1.22^{c}	1.32^{b}	1.53^{a}	1.60^{a}	0.02	0.00

Note: The results were presented as mean values ± SEM. Means expressed with different superscripts letters within the same row were significantly different at $P<0.05$.

Met 1 is a treatment with 0.1% metabolite combination; Met 3 is a treatment with 0.3% metabolite combination; Met 5 is a treatment with 0.5% metabolite combination.

Source: Loh *et al.,* 2013a

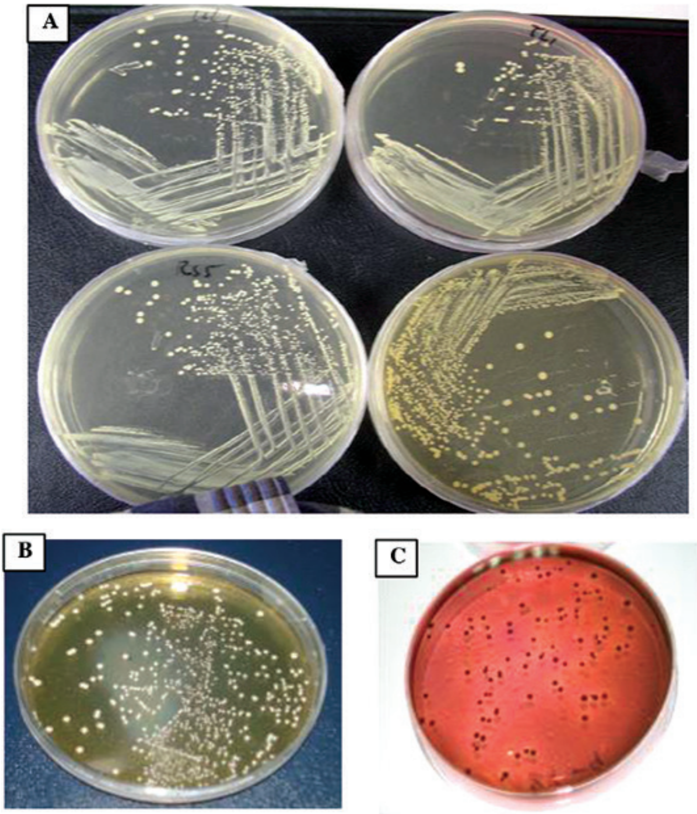


Figure 7 (A) Agar streak plates of bacteria from faecal samples to isolate bacterial colonies. (B) Colonies of *Lactobacillus* formed on MRS-agar (DE Man, ROGOSA and SHARPE). (C) Colony formation of ENT on EMB-Agar (Eosine-Methylene-blue lactose sucrose agar)

A balanced gut microflora is essential as it mediates various vital morphological and physiological processes within the animal's (innate) body. Intestinal bacteria are associated with mucosal cell turnover, vascularity, muscle wall thickness, motility, baseline cytokine production, digestive enzyme activity and cell-mediated immunity (Macdonald and Monteleone, 2005). In addition, intestinal microflora make important metabolic contributions to vitamin K, folate and short-chain fatty acids (SCFA), such as butyrate, a major energy source for enterocytes, and also mediate the breakdown of dietary carcinogens (Hooper *et al.,* 2002). Postbiotic metabolites were able to shift the gut microflora toward a more beneficial balance resulting in better growth performance and reduced severity of diarrhoea incidence in post weaning piglets (Thu *et al.,* 2011b, Loh *et al.,* 2013a) and increased growth in broilers (Loh *et al.,* 2010a, Rosyidah *et al.*, 2011, Thanh *et al.*, 2009). Conversely, growth rate was not improved in rats (Loh *et al.,* 2009a, Foo *et al.,* 2003a, Foo *et al.,* 2003b) as reduced water and feed intake was observed due to the undesirable taste of these metabolites. Tables 8 and 9 show the growth parameters of broilers and piglets fed with metabolites.

Table 8 Growth performance of broilers at week 6 of treatments supplemented with different dosages of metabolites from COM3456

Parameters	**Dietary Treatments †**						
	-ve control	**+ve control**	**0.1% COM3456**	**0.2% COM3456**	**0.3% COM3456**	**0.4% COM3456**	**0.5% COM3456**
Body weight, kg	2.23 ± 0.04^{d}	2.45 ± 0.04^{ba}	2.38 ± 0.03^{bc}	2.40 ± 0.03^{bac}	2.37 ± 0.03^{bc}	2.49 ± 0.03^{a}	2.32 ± 0.03^{c}
Weight gain, kg	2.19 ± 0.04^{d}	2.41 ± 0.04^{ba}	2.34 ± 0.03^{bc}	2.36 ± 0.03^{bac}	2.33 ± 0.03^{bc}	2.44 ± 0.03^{a}	2.28 ± 0.02^{c}
Average daily gain, kg	52.12 ± 0.98^{d}	57.29 ± 0.85^{ba}	55.65 ± 0.70^{bc}	56.08 ± 0.74^{bac}	55.52 ± 0.71^{bc}	58.21 ± 0.66^{a}	54.38 ± 0.59^{c}
Feed intake, kg	3.80 ± 0.08^{a}	3.92 ± 0.08^{a}	3.98 ± 0.08^{a}	3.96 ± 0.06^{a}	3.94 ± 0.06^{a}	4.00 ± 0.09^{a}	3.84 ± 0.03^{a}
Feed conversion ratio	1.73 ± 0.04^{a}	1.66 ± 0.02^{a}	1.73 ± 0.02^{a}	1.70 ± 0.02^{ba}	1.72 ± 0.02^{a}	1.63 ± 0.04^{b}	1.70 ± 0.02^{ba}

$^{a-c}$means ± SEM with different superscripts are significantly different ($P<0.05$). †Diets supplemented with different dosages (0.1% - 0.5%, w/w) of metabolite powder of COM3456 (a combination of 4 strains RS5, RI 11, RG 11, and RG 14). Each treatment consisted of 6 replicates.

Source: Loh *et al.*, 2010a

Table 9 Growth performance, diarrhoea score and nutrient digestibility of piglets subjected to different dietary treatments

	Dietary treatments						
Items	**Negative control**	**Positive control**	**Met 1**	**Met 3**	**Met 5**	**SEM.**	***P*-value**
Growth							
Initial BW, kg	6.63^{a}	6.49^{a}	6.64^{a}	6.54^{a}	6.34^{a}	0.26	0.92
Final BW, kg	15.43^{b}	16.75^{ab}	15.85^{ab}	16.83^{ab}	17.39^{a}	0.59	0.15
ADG, g/day	251.8^{c}	293.2^{ab}	263.2^{bc}	293.9^{ab}	315.7^{a}	13.51	0.01
DFI, g/pig/day	465.4^{c}	474.5^{bc}	476.0^{bc}	485^{ab}	4.93^{a}	5.48	0.02
FCR	1.87^{ab}	1.62^{b}	2.02^{a}	1.74^{ab}	1.59^{b}	0.11	0.07
Diarrhoea score							
Days 0 to 17	0.40^{a}	0.09^{b}	0.25^{ab}	0.16^{ab}	0.13^{b}	0.07	0.12
Nutrient digestibility							
Protein digestibility, %	65.48^{ab}	64.41^{b}	65.13^{ab}	66.32^{ab}	68.06^{a}	0.94	0.08
Energy digestibility, %	68.76^{a}	68.33^{a}	68.10^{a}	68.95^{a}	70.12^{a}	0.65	0.24
AME of diets, kcal/kg	2761.3^{a}	2751.2^{a}	2724.0^{a}	2745.4^{a}	2841.5^{a}	38.89	0.27

Note: The results were presented as mean values ± SEM. Means expressed with different superscript letters within the same row were significantly different at $P < 0.05$. Met 1 is a treatment with 0.1% metabolite combination; Met 3 is a treatment with 0.3% metabolite combination; Met 5 is a treatment with 0.5% metabolite combination. BW, body weight; DFI, dietary feed intake; FCR, feed conversion ratio; AME, apparent metabolisable energy.

Source: Loh *et al.*, 2013a

POSTBIOTICS ALTER GUT MORPHOLOGY

Alteration in the gut morphology has been reported in animals fed with antibiotics. The related parameters were thus evaluated in the animals fed with postbiotic metabolites. The underpinning mechanism involves an inflammatory process. Pathogens in the normal microflora in the intestinal epithelium may contribute to the changes of the permeability of the villi surface. This may lead to invasion of pathogens, modifying metabolism and absorption of nutrients, resulting in chronic inflammation in the intestinal epithelium. Intestines have been described as an organ which is in a state of constant controlled inflammation. Inflammation in the intestine normally results in the accumulation of inflammatory cells in the mucosa, leading to a thicker intestinal wall and decreased villus height. Reduced absorptive functions occur in short villi with the reduction in the villi surface area. Additionally, reduction of enzyme activities, such as mucosal lactase and sucrase, lactase and alkaline phosphatase, alkaline phosphatase and dissaccharidase and total lactase phlorizin hydrolase and mucosal protein concentration, were observed in these short villi. This condition is entirely reversed when AGP was fed to animals, whereby AGP lowers the level of inflammation and reduces influx and accumulation of inflammatory cells resulting in thinner lining of the small intestine and increased nutrient absorption. Furthermore, intestinal crypt is invaginations of the epithelium around the villi and is lined by epithelial cells which secrete enzymes. The base of the crypts is constantly dividing to maintain the structure of the villi. An increase in the crypt depth would therefore produce more developed villi. Similarly, antimicrobial activity of metabolites which inhibits the growth of many gut pathogens was able to demonstrate increased villi height and crypt depth in layer hen (Choe *et al.,* 2012), broilers (Thanh *et al.*, 2009, Loh *et al.,* 2010a) (Table 10) and postweaning piglets (Thu *et al.,* 2011b).

Table 10 Villi height and crypt depth in broilers at week 6 of treatment supplemented with different dosages of metabolites from COM3456

Parameters	Dietary treatments †						
	-ve control	+ve control	0.1% COM3456	0.2% COM3456	0.3% COM3456	0.4% COM3456	0.5% COM3456
Villi height, μm							
Duodenal	1683 ± 19^{a}	1756 ± 26^{b}	1752 ± 16^{b}	1865 ± 22^{c}	1871 ± 15^{c}	1984 ± 26^{d}	1765 ± 18^{b}
Jejunal	1157 ± 9^{a}	1303 ± 13^{c}	1373 ± 21^{d}	1390 ± 19^{b}	1272 ± 19^{bc}	1532 ± 24^{e}	1224 ± 20^{b}
Ileal	719 ± 12^{a}	788 ± 13^{b}	913 ± 14^{d}	778 ± 9^{d}	826 ± 12^{c}	832 ± 12^{c}	849 ± 12^{c}
Crypt depth, μm							
Duodenal	275 ± 10^{b}	306 ± 8^{c}	247 ± 8^{a}	296 ± 7^{bc}	289 ± 7^{bc}	237 ± 4^{a}	237 ± 7^{a}
Jejunal	219 ± 7^{a}	276 ± 9^{c}	276 ± 7^{c}	235 ± 7^{a}	218 ± 5^{a}	226 ± 3^{a}	255 ± 4^{b}
Ileal	134 ± 4^{bc}	115 ± 3^{a}	185 ± 3^{f}	165 ± 5^{e}	137 ± 3^{c}	124 ± 3^{ab}	151 ± 2^{d}

$^{a\text{-}f}$means ± SEM with different superscripts are significantly different ($P<0.05$). †Diets supplemented with different dosages (0.1-0.5%, w/w) of metabolite powder of COM3456 (a combination of 4 strains RS5, RI11, RG11 and G14). Each treatment consisted of 20 replicates.

Source: Choe *et al.*, 2012

Postbiotics Reduce Cholesterol Levels

The use of postbiotic metabolites has also proved to be beneficial when addressing issues of high level of cholesterol in meat and egg yolk. Increased activity of LAB in the gut system did exhibit a profound effect in reducing cholesterol levels. Loh *et al.* (2003) puts forward a few mechanisms by which LAB might be involved in reducing cholesterol levels. LAB:

1. Inhibit the enzymes that synthesise cholesterol, thus reducing cholesterol production.
2. Enhance the activity of bile salts hydrolase, whereby this enzyme catalyses the deconjugation of the bile salts to liberate free primary bile acids. Deconjugated bile salts are less soluble and less efficiently reabsorbed into the intestinal lumen than conjugated bile salts, therefore, increased amounts of free bile acids which will be excreted in the faeces (Figure 8).
3. Interfere with recycling and enhance the excretion of bile salts which signals the host to utilise more cholesterol from the pool within the body to synthesise new bile salts.
4. Inhibit absorption into the body by binding with the cholesterol and utilising them for the synthesis of bacterial cell walls.

Similar mechanisms were proposed by a group of researchers from Korea (Lee *et al.,* 2009) in year 2009.

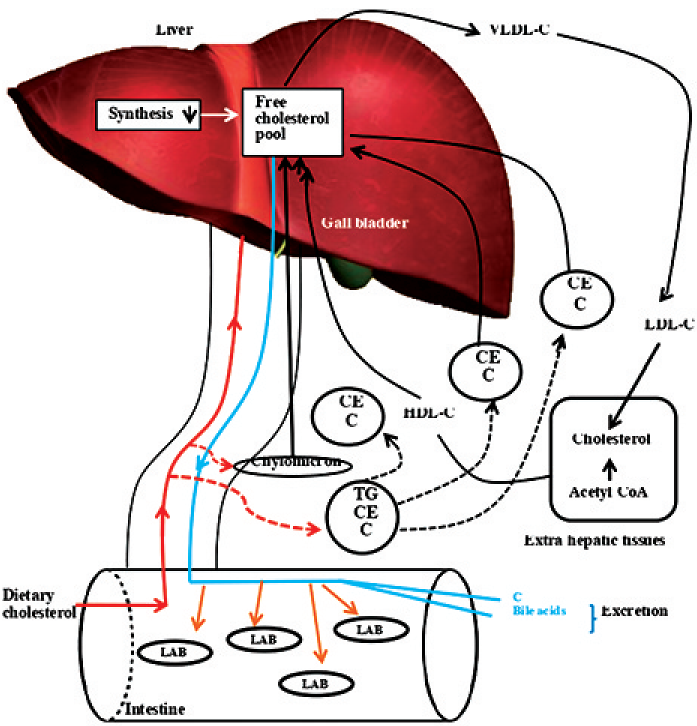


Figure 8 Transport of cholesterol within the host body. TG, triacylglycerol; C, free cholesterol; CE, cholesteryl ester; VLDL, very low density lipoprotein; LDL, low density lipoprotein; HDL, high density lipoprotein. LAB bacteria in the GI tract use the free cholesterol for the synthesis of bacterial cell walls. This interferes with the recycling of cholesterol. This increases bile salt excretion, resulting in the synthesis of new bile salts by using more cholesterol from the cholesterol pool.

Feeding trials involving the use of postbiotic metabolites in the feed showed successfully lowered cholesterol levels in the animals. Reduced plasma cholesterol was observed in layer hens (Loh *et al.,* 2014, Choe *et al.,* 2012), post weaning piglets (Thu *et al.,* 2010), broilers (Loh *et al.,* 2013b) and rats fed with metabolites of *L. plantarum* I-UL4 and spray-dried metabolite of *Lactococcus lactis* RW18 (Loh *et al.*, 2009a, Foo *et al.,* 2003b, Loh *et al.,* 2008d), but not in rats offered *Lactococcus lactis* RW18 in drinking water (Foo *et al.,* 2003a). Apart from lowered breast meat cholesterol levels, reduced cholesterol esters concentration and VLDL particles as well as increased bile salt conjugating ability of LAB was observed in broilers (Loh *et al.,* 2013b).

In efforts to increase the quantity and quality of egg yield, it was found that supplementation with postbiotic metabolites was able to successfully increase the number of hen/day egg production (Loh *et al.,* 2014, Choe *et al.*, 2012). At the same time, a significantly lower level of yolk cholesterol was observed in the eggs of layer hens fed with postbiotic metabolites (Loh *et al.,* 2014, Choe *et al.,* 2012) (Table 11). The cholesterol reduction is related to the depletion in cholesterol synthesis by the liver. In laying hens, the liver is the major site for cholesterol synthesis. Laying hens usually synthesise more cholesterol than their body require. The synthesised cholesterol is then secreted into the blood stream, carried by the very low density lipoprotein particles across the ovarian membrane and subsequently deposited in the developing yolks through the oocyte vitellogenesis receptor. Thus, for the laying hen, a major excretion pathway of cholesterol seems to be excretion via the egg yolk (Figure 9). A reduction in cholesterol synthesis in the liver results in lower levels of cholesterol being present in the blood circulation making it less available to be deposited into the egg yolk. The production of eggs with lower yolk cholesterol is much preferred by consumers as it is a healthier option for general health, particularly for those affected by cardiovascular diseases.

Table 11 Egg yolk and plasma cholesterol following treatments supplemented with various concentrations of metabolites from COM456 at 31 weeks of age

	Dietary treatments[1]							
	Control	**0.3% COM456**	**0.6% COM456**	**0.9% COM456**	**1.2% COM456**	**SEM**	**Linear**	**Quad**
Yolk weight (g)	16.33	16.32	16.31	16.30	16.34	0.05	NS	NS
Yolk cholesterol (mg/100g)	12.73[a]	12.17[b]	11.36[c]	11.96[b]	12.13[b]	0.17	*	*
Yolk cholesterol (mg/yolk)	209.62[a]	199.71[b]	187.31[d]	195.74[c]	199.43[b]	2.28	*	*
Plasma (mg/dl)	186.28[a]	168.12[b]	152.62[c]	128.56[d]	125.42[d]	1.52	*	NS

[1]COM456 is a combination of 3 strains, RI11, RG14 and RG11.

[2a-d]Means in the same row not sharing a common superscript are significantly different ($P<0.05$).

[3]SEM: standard error of means (pooled).

[4]Linear or quadratic response estimated using orthogonal polynomial contrasts (NS: non-significant; *$P<0.05$).

[5]Data are means of 50 cages of 2 hens per cage.

Source: Choe *et al.*, 2012

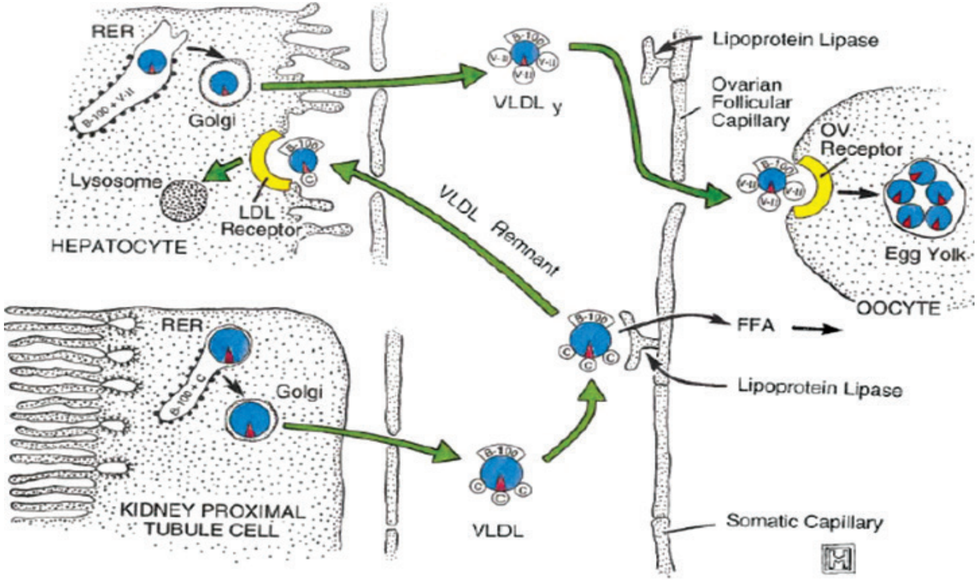


Figure 9 Mechanism of the availability and excretion of cholesterol in the egg yolk. VLDL, very low density lipoprotein; LDL, low density lipoprotein; RER, rough endoplasmic reticulum; OV receptors, oocyte vitellogenesis receptors

Source: Walzem *et al.,* 1999

Prebiotics are nondigestible food ingredients that induce the growth or activity of microorganisms. Prebiotics possess indirect antimicrobial effects due to the production of fermentation products such as bacteriocins and short chain fatty acids. Prebiotics also encourage the production of organic acids by microflora in the gut and thus increases acidification of the gut contents. This creates a gut microenvironment that inhibits the proliferation of pathogenic bacteria. Most importantly, prebiotics act as fermentation energy sources (Wang *et al.*, 2010) for particular members of the microbiota, enhancing their numbers, as well as the postbiotic effects.

The use of inulin, a prebiotic, was however not able to provide a desired outcome when it was included in the feed of pigs. No significant effects on live weight gain feed conversion ratio (FCR) and P_2 backfat thickness of pigs were observed (Loh *et al.*, 2010b). Thus, a combination of prebiotics and postbiotics was tested in an *in vitro* study where we were able to demonstrate more effective inhibition of various pathogens due to the synergistic effect of postbiotics and inulin. When this combination was fed to broiler chickens, and its carcass, meat and bone quality subsequently evaluated. Nevertheless, it was observed that the postbiotics and inulin combination had a beneficial effect on the meat quality, body weight, feed efficiency, mucosa architecture, liver insulin like growth factor 1 (IGF1) and growth hormone receptor (GHR) mRNA expressions, as compared to commercially used antibiotics (Kareem *et al.*, 2015, Kareem *et al.*, 2016a). In addition, postbiotics RG14 supplementation 0.15% + 1.0% inulin was found to be the best combination in diets of broiler chickens as the growth performance and population of beneficial bacteria (LAB) was improved while the populations of ENT was reduced. Furthermore, an increase in the acetic acid concentration was observed which could be associated with the alterations in the ileal cytokine expression (Kareem *et al.,* 2016b).

Other benefits of postbiotic metabolites have also been documented. The proteinaceous component of postbiotic metabolites has the potential to induce different levels of human mammary gland adenocarcinoma (MCF-7) cancer cells' death. This could lead to the establishment of postbiotic metabolites as a human health supplement and as a cancer preventive agent (Tan *et al.*, 2015).

The Future of the Animal Feed Industry with Postbiotics

Our studies have shed some light on various properties of postbiotics which shift the physiological state towards a more desirable one, without causing stress or altogether altering the normal physiology of the animals. The use of postbiotics is definitely a safer option as it does not involve live organisms, totally eliminating all possibilities of development of antibiotic resistance or other related issues. Further, in line with the effort to discover a more natural option, this alternative is also suitable for our local climate and conditions, which has been proven by our numerous and extensive studies (Figures 10, 11 and 12). Its profound benefits and various properties allow us to firmly believe that postbiotics would be the best substitute to antibiotics. Further, more optimisation and research in this area may in fact yield better outputs than that with the use of AGP. Steps towards adapting or incorporating this option into our local farm regimens should therefore be encouraged and given serious consideration by the relevant parties.

Figure 10 Broilers reared in a closed house system (Top)
Layers bred in two tier battery cages (Below)

Figure 11 Feeding activity of layer hens in two tier battery cages

Figure 12 Pigs feeding in their respective pens (Top)
Sow and suckling piglets (Below)

ALTERNATIVE FEED ADDITIVES

Diets offered to animals in the basal form might not be able to provide all the essential nutrients and requirements of the animals which is vital for their continuous survival. This may lead to the animals experiencing nutrient deficiency. Furthermore, retarded growth or other diseases caused by incomplete diets lead to high mortality rates which causes economic wastage. Many approaches, either the supplementation of a specific mineral (Loh *et al.*, 2001b, Loh *et al.,* 2002d) or trace elements, have been studied to meet at least the minimum nutrient requirements of the animals.

Medium-Chain Triacylglycerol As Feed Additive and Energy Source

High mortality rate of pre-weaning piglets is a major problem faced in the swine industry and has always represented significant economic wastage. Weaning is a stressful and abrupt period. This is when the piglets are separated from the sow and they lose maternal protection, their major source of nutrition and external defences. The rate of mortality at this stage can range from 10 to 14% and usually occurs during the first seven days post-partum. The root of this problem is attributed to various factors which include nutrition, thermoregulation, behavioural factors, genetic (Loh *et al.*, 2002f), immunological factors and stockmanship (Loh, 2003).

Weaned piglets might show no live weight change or even a slight weight loss in the week after weaning (Loh *et al.,* 1999) due to a temporary reduction in voluntary feed intake and poor energy and nitrogen digestibility. As the growth performance of piglets in the first week affects greatly its future growth performance (Loh *et al.*, 1998), post-weaning mortality could be reduced by maximising the growth performance of nursing animals during the pre-weaning period. Pre-weaning growth depends solely on the milk produced by the dams as the colostrum and milk fats are primarily utilised for the deposition of body fat in the new born mammals. Importantly, milk composition could be altered by the diet to an extent. In our studies using rats, we successfully verified that fat supplementation in a dam's diet during late pregnancy could improve the pre-weaning survival of the offsprings by improving the fat content (Loh *et al.*, 2002a, Loh *et al.,* 2002b).

Very low density lipoprotein (VLDL) plays a pivotal role in supplying triacylglycerol for milk production. Milk fats are derived from *de novo* synthesis within the mammary gland from lipids of dietary origin or lipids mobilised from adipose tissue. High levels of triacylglycerol could cause hypertriacylglycerolaemia in late-pregnant sows. High fat content in pigs can liberate into fatty acids and be incorporated into very low density lipoproteins (VLDL) by the liver which is then secreted into the blood stream. The supplementation of different types of fats might also influence the concentration of plasma triacylglycerol and the size of the VLDL (Loh *et al.,* 2003c). Since fats deposited in the adipose tissue are derived from plasma protein, increased VLDL in plasma might have a relationship with the backfat thickness deposition in growing pigs (Loh *et al.*, 1997, Loh *et al.,* 2001a). The characterisation of plasma VLDL of commercial broiler and crossbred village chickens was studied by Tan *et al.* (2005) and the relationship of VLDL with abdominal fat deposition in chickens was demonstrated by Loh *et al.* (2011b).

Several inconsistencies have been reported on the effects of fat inclusion in the diet of sows on their energy reserves. We thus attempted an experiment which involved offering fat in the form of medium-chain triacylglycerol (MCT) to pre-weaning piglets as a means of improving survivability. MCT has a few advantages over fats. MCTs are passively diffused, rapidly absorbed and transported to the liver. Additionally, MCTs have lesser tendency to be stored as body fat and do not require bile for emulsification and digestion. Further, most MCTs are metabolized in the liver and the energy provided might be used as alternative fuel sources by the muscles and brain under starvation condition. The outcome of our experiment showed better growth performance of piglets treated with MCT (Table 12). Weight gain was more pronounced when the piglets were fed with MCT together with the milk from the mother and they also showed improved gut morphology (i.e. higher duodenal, jejunal and ileal villus height) (Loh *et al.*, 2013c).

Table 12 Body weight and weight gain of piglets at day 1, 6 and 8 after farrowing

Body weight and weight gain (kg)	**Treatments**		
	Control	**MCT + milk**	**MCT + fasting**
BW1	1.72 ± 0.04	1.63 ± 0.05	1.51 ± 0.20
BW6	2.42 ± 0.05^{a}	2.59 ± 0.06^{b}	2.27 ± 0.06^{a}
BW8	2.71 ± 0.06^{a}	2.96 ± 0.07^{b}	2.63 ± 0.07^{a}
WG-1	0.69 ± 0.04^{a}	0.97 ± 0.04^{b}	0.75 ± 0.08^{a}
WG-8	0.99 ± 0.05^{a}	1.33 ± 0.05^{b}	1.11 ± 0.12^{ab}

n=150. The results are presented as mean ± standard error of mean (SEM). $^{a-b}$ Values within each row with different superscripts were significantly different (P<0.05).

Source: Loh *et al.,* 2013c

Fermented Feed as Feed Additive

In Malaysia, fermented products were initially included in the feed of farm animals to improve performance while at the same time it has been employed as a viable alternative to in-feed antibiotics. The pleasant flavour, aroma and texture of fermented products are an added advantage and are generally appreciated when included in the diet. Fermented products are rich in LAB, low in ENT and pH, and the presence of essential PUFA, such as linoleic, linolenic, arachidonic EPA, DPA and DHA, is highly consistent and reproducible (Law *et al.,* 2006).

The weaning stage of the piglets is a very critical stage as it is abrupt and stressful as they are subjected to a combination of stress factors that increase their susceptibility to diseases. Post weaning diarrhoea is a common problem encountered by piglets, whereby the affected herd may have mortality rates greater than 25% and a morbidity rate greater than 80%, which results in great economic loss (Svenden *et al.*, 1974). Loh *et al.* (2003a) have also documented that fermented feed has a positive effect in terms of increasing the feed intake of post-weaning piglets and consequently the growth rate of those post weaning piglets were significantly increased. In our study, we offered a fermented fruit mixture comprising locally available fruits, such as lime 16% and sugar cane juice 32%, which was crushed and mixed thoroughly with 52% rice bran and combination cultures of LAB. The mixture was fermented for 7 days at 70 - 80°C before 10% and 20% of the mixture was offered together with basal diets. Interestingly, inclusions of 10% of fermented fruits were able to elicit weight gain in the animals (Table 13). Inclusions of fermented fruits of up to 20% in the diet however affected the palatability of the diets where lower feed intake was observed and poor growth rate was recorded in animals allocated in that group, possibly due to the odour of the feed caused by low pH. This feed formulation was also able to provide a balanced gut microflora as we observed a reduced pH and ENT population as well as an increase in the LAB population in the faeces' of piglets (Loh *et al.,* 2003a) and rats (Foo *et al.,* 2003d, Loh *et al.*, 2003b).

The use of fermented products as a dietary manipulation (comprising 9% lime, 1% molasses, 53.5% rice bran, 35% *Rastrelliger kanagurta* fish, 1% vinegar and 0.5% starter culture on *L. plantarum* isolated from 'tempeh') in laying hens diet was studied by Loh *et al.* (2007a). The inclusion of fermented products of up to 6% contributed to heavier egg weight and shifted the microflora composition of the layer's gastrointestinal tract towards a beneficial balance. Laying hens however did not show a desired effect in terms of growth performance and laying performance (egg production) with this dietary manipulation. Further, egg yolk cholesterol and plasma cholesterol concentrations were substantially reduced (Loh *et al.,* 2009b). This was probably because the fish included in the diet contained high levels of n-3 fatty acids, which decreases circulating triacylglycerides and therefore limits the availability of lipids for yolk formation. This assumption was further supported when the level of total n-3 and DHA in the egg yolk was found to be higher in hens fed with diets of 9% fermented fish. Hence, this dietary manipulation with fermented fish has high potential to be used in efforts to produce eggs with enhanced n-3 fatty acid content. Lowered cholesterol levels in the plasma were also demonstrated in rats fed with fermented products (Foo *et al.*, 2003d, Loh *et al.*, 2003b).

Phytogenic Compounds

Due to antimicrobial properties exhibited by herbs, spices, essential oils and extracts or mixtures of natural substances from plant, these have been extensively used in poultry rearing, and are therefore potential replacements for antibiotic growth promoters in the diet (Zulkifli *et al.,* 2012, Loh *et al.*, 2002e, Loh *et al.,* 2008a). It has been claimed that the performance of animals consuming these compounds have improved via the stimulation of amylase and protease enzyme secretions (Patel and Srinivasan, 1996). Feed ingredients from plants are rich in phytate phosphorus, but the availability of P is low mainly due to the phytic acid present in the feedstuffs. So, phytate has to be hydrolysed to inorganic compounds containing P within the digestive tract. Since poultry is unable to secrete sufficient amounts of endogenous phytase in the gastrointestinal tract, addition of inorganic phosphorus such as dicalcium phosphate in the diet is desirable to meet the requirement of P for growth. This will however increase the feed cost directly. In addition, unabsorbed phosphorus is excreted, creating environmental pollution. We observed that the combination of phytogenic substances (with matrix values) with microbial phytase had a synergistic effect on enhancing growth performance and apparent digestibility of broilers (Loh *et al.*, 2008a).

Table 13 Effect of fermented feed on the growth performance of piglets

Treatments	AF	Ab	10% FF	20% FF
Initial body weight (kg)	6.03 ± 0.75^{a}	6.60 ± 0.23^{a}	6.10 ± 0.46^{a}	6.15 ± 0.45^{a}
Final body weight (kg)	13.80 ± 1.17^{ab}	17.23 ± 2.43^{a}	15.57 ± 0.29^{a}	10.53 ± 0.87^{b}
Average daily gain (kg/d)	0.22 ± 14.8^{ab}	0.30 ± 31.9^{a}	0.28 ± 11.9^{a}	0.15 ± 18.4^{b}
Total feed intake (kg)	16.91 ± 1.16^{ab}	20.96 ± 1.56^{a}	17.86 ± 1.22^{a}	11.95 ± 1.86^{b}
Feed conversion ratio	2.17 ± 0.13^{ab}	2.04 ± 0.40^{ab}	1.78 ± 0.03^{b}	2.62 ± 0.04^{b}

The results are presented as mean values ± SEM. a, b within each row, means with different alphabets are significantly different ($P<0.05$).
AF, basal diet, antibiotic free
Ab, basal diet, with antibiotic
10% FF, basal diet + 100g per kg fermented fruits
20% FF, basal diet + 200g per kg fermented fruits

Source: Loh *et al.,* 2003a

Other Feed Additives

The protein source is the most expensive component in broiler diets as the price of common protein sources like soybean meal is on the rise worldwide. Furthermore, the ingestion of proteins increases the nitrogen emission by the animals. Consequently, dietary manipulations in the form of low crude protein diets have been attempted. Such low crude protein diets should however be supplemented with adequate essential amino acids as they are not synthesisable by the body. Supplementing synthetic amino acids also offers advantages in obtaining a positive outcome in many instances. Synthetic amino acids improve the hen/day egg production and shows desired effects such as an increase in the small intestine villus height and promotes beneficial effects on the faecal microflora in layer hens (Tenesa *et al.*, 2016).

Animals that are fed methionine deficit diets or low crude protein diets, have insufficient methionine causing deterioration in body weight and overall growth performance, mostly during the starter period. Methionine and arginine are two important amino acids that are involved in cell division, protein synthesis and tissue growth. Methionine is a methyl donor involved in polyamine biosynthesis, immune response and blood lipid levels. We therefore experimented with supplementing putrescine, a polyamine that promotes anabolic processes like the synthesis of DNA, RNA and protein as well as, increases amino acids intake in the methionine deficit diets of broilers. No distinct benefits in the growth performance of broilers were observed when putrescine was added to their diets. However, putrescine supplementation resulted in increased antibody levels when the broilers were fed methionine deficient diets (Hashemi *et al.,* 2014a) and improved energy efficiency ratio of the birds, highlighting the advantages of using putrescine in low crude protein diets (Hashemi *et al.,* 2014b).

Dietary putrescine has a positive effect on small intestine villus height and crypt depth, particularly at a younger age (Hashemi et al., 2014c).

Organic acids, either as individual acids or blends of acids, are included in animal feed due to the antimicrobial activity whereby intolerant species such as *E.coli,* Salmonella and Campylobacter could be diminished. Likewise, acidifiers and organic acids exhibit improved protein and energy digestibility by reducing microbial competition with the host for nutrients and endogenous nitrogen losses. These acids also help in the secretion of immune mediators, reduce the production of ammonia and at the same time, eliminate microbial metabolites that are growth-depressing. The inclusion of acidifiers and organic acids result in the same mechanism as discussed in detail earlier whereby they are able to provide a balanced gut microflora, providing optimum conditions for the benefit of animal growth. We have demonstrated the outcomes of using acidifiers, organic acids or the combination of these with other substances in the feed of pigs (Loh *et al.,* 2008b, Loh *et al.*, 2010a, Loh *et al.,* 2002e) and broilers (Rosyidah *et al.,* 2011, Loh *et al.*, 2007b).

Animal Diets

While the global consumption of poultry products, such as meat and eggs, is on the rise so is the demand for poultry's main feedstuffs. Meanwhile, the prices of the feedstuffs, especially resources that are high in protein and energy, such as soybean meal and yellow corn, respectively, are fluctuating. This contributes to increased production costs leading to a high market price for poultry and poultry products. As a result, there is a push to find alternatives for soybean meal and yellow corn as feed for monogastric animals such as poultry and swine.

Meanwhile, agro-industrial by-products that are considered as agro-waste are produced in abundance. It has been a priority for many developing countries, principally the agriculture-based countries, to fully utilise these agro-wastes for more beneficial purposes. Due to the fact that these agro-waste by-products still contain a certain amount of nutrients they may be able to cater for the requirements of farm animals. Among the commonly used agro-waste by-products are wheat bran, rice bran, cotton seed meal, copra meal and palm kernel cake. Malaysia, being one of the top producers of palm (*Elaeis guineensis*) oil, generates important by-products, such as palm kernel meal (PKM), palm kernel cake (PKC) and palm kernel expeller (PKE), all of which differ in content depending on the method used for extraction of oil from the kernel. For example, PKE contains as much as 15-17% of protein but a rather poor amino acid profile (deficient in lysine, methionine and tryptophan). Conversely, there are some limitations in using these agrowaste by-products in animal feed due to the presence of high fibre content. PKE contains up to 58-78% nonstarch polysaccharides (NSP), such as xylan and mannan, from the total fibre content and some other anti-nutritional factors. High level of crude fibre gives it a coarse texture and gritty appearance and the composition makes it a rather moderate quality feed ingredient for ruminants but unsuitable for monogastric animals.

Enzymes are not very effective in breaking down the NSP to monomeric sugars within the gastro-intestinal tract of poultry suggesting that pre-treating PKE with enzymes before feeding would be a better option. This has resulted in recent interest in the use of exogenous enzymes or cellulolytic and hemicellulolytic enzymes to degrade the by-products prior to feeding. Due to its complex chemical structure, the fibre of PKE requires a combination of enzymes, including mannosidases, galactosidases, glucosidases and xylanases, to release the full potential of fermentable sugars to be of use for monogastric animals.

The combination of cellulolytic and hemicellulolytic bacteria in solid state fermentation leads to the production of different types of enzymes at the same time, whereby synergistic effects of the cellulolytic and hemicellulolytic enzymes can break down different types of β-glucosidic lingkages in NSPs. Further, bacteria belonging to the genus *Bacillus* have the ability to adhere to the substrate particles and produce filamentous cells in order to penetrate and degrade the substrate effectively. Cellulolytic and hemicellulolytic bacteria are capable of degrading the cellulose, hemicellulose, xylans and mannan molecules. The use of bacteria, specifically, *Paenibacillus curdlanolyticus* and *Paenibacillus polymyxa,* have shown higher capacity in degrading PKC effectively (Alshelmani *et al.,* 2013, Alshelmani *et al.,* 2014) and thus improve the nutritive value of PKC (Alshelmani *et al.*, 2014) through solid state fermentation.

When PKC fermented by *P. polymyxa* ATCC 842 was tested in feeding trials, an inclusion of up to 15% showed no adverse effects on nutrient digestibility, growth performance and meat quality and no apparent difference in the gut morphology. At the same time the feeding and gut microflora of the birds were shown to be significantly improved (Alshelmani *et al.,* 2016a, Alshelmani *et al.*, 2016b).

The ability of fungus in degrading these complex structures has also been extensively studied. Fungi were found to have higher enzyme activities than bacteria. However, the treatment of these agro waste products with fungus is less preferred as the secondary products from fungi such as mycotoxins would depress the growth of the animals, rendering it rather unsuitable to be used. Since this effect is generally absent when the degrading process is carried out using bacteria, bacteria is preferred over fungus.

In our study, when we treated PKE with exogenous enzymes, there was an increase of about 200 folds in the total reducing sugar (glucose, mannose, galactose and xylose) (Saenphoom *et al.,* 2011). Furthermore, the enzymes treated PKE had higher cellulose and mannanase activities indicating an increase in metabolisable energy (Saenphoom *et al.,* 2011, Saenphoom *et al.,* 2013). This effect was however not reflected in the growth performance of broiler chickens fed with the treated PKE. It has however been shown that PKE can be included by up to 5% in the grower diet and 20% in the finisher diet without any significant negative effect on the feed conversion ratio in broilers (Saenphoom *et al.,* 2013), as replacement for other more costly feedstuff. In addition to increasing metabolisable energy, the exogenous enzyme treatment also enhances the vital nutrients (protein, fat and nitrogen free extract).

Our study on rats indicated that the inclusion of PKC in their diets by up to 25% had no adverse effects on their growth performance. However, rats fed with PKC diets showed a different blood lipid profile, whereby they had bigger size of VLDL with low phospholipid content but with similar number of VLDL in the plasma. Plasma triacylglycerol was reduced due to a decrease in both low density lipoprotein and high density lipoprotein. This is due to the high fibre content in PKC (Loh *et al.,* 2002c).

Vegetable oils such as palm oil, soybean oil and linseed oil are also used as supplements in broiler diets to increase productivity and energy concentration. Furthermore, fats used in diets increase palatability, improve feed texture and reduce the dustiness of broiler feed. Abdulla *et al.* (2015) and Abdulla *et al.* (2016a) demonstrated that the supplementation of palm oil, soybean oil and linseed oil increased the proportion of oleic, linoleic and α-linolenic acids, respectively, in broiler breast muscles. This study concluded that palm oil has positive effects on the meat firmness quality and growth performance of broilers compared to other vegetable oils that are rich in linoleic and α-linolenic acids. However, soybean oil was found to be more effective in increasing the body weight of the birds compared to linseed oil (Abdulla *et al.,* 2017b). The fatty acid compositions of palm oil, soybean oil and linseed oil were different, but the apparent metabolisable energy of these oils was similar (Abdulla *et al.*, 2016b). Furthermore, it was suggested that blending oils is an attractive way to increase the apparent metabolisable energy of oil that is rich in saturated fatty acids for poultry, by adding oil rich in unsaturated fatty acids (Abdulla *et al.,* 2016b). The advantages of combinations of oils on fatty acid composition, fat deposition, lipogenic gene expression and performance of broiler feed diets supplemented with different sources of oil have also been demonstrated (Khatun *et al.,* 2017).

Soy lecithin, a by-product from the processing of soybean oil, contains various phospholipids such as phosphatidylinositol, phosphatidylethanolamine and phosphatidylcholine. Phospholipids are essential components of cell membranes found in living cells and functions in the regulation of lipid metabolism and therefore provide energy. Another by-product of soybean oil is an omega-6-polyunsaturated fatty acid that contains a high level of linoleic acid. Lecithin is an important source of choline in broiler diets and has been reported to improve broiler productivity (Huang *et al.,* 2007). An inclusion of up to 2% of soy lecithin improved egg weight of aged layer hens but not egg production, feed conversion efficiency, as well as, egg quality and meat texture (Akit *et al.*, 2016).

A feed production trial was conducted to study the effect of synthetic emulsifier and natural biosurfactant on the processing and quality of pelletised broiler feed. A corn-soy based broiler diet was formulated with fixed ratio 2:1 of oil-to-water with two types of emulsifiers, namely glyceryl polyethylene glycol ricinoleate synthetic emulsifier and lysophosphatidylcholine natural biosurfactant. The treatment diets were manufactured by a commercial feed mill where electricity cost and meal temperature were measured during the process of milling. Composite samples of the pelletised feed were collected from different process points and tested for physical properties, chemical stability and biostability. Even though the outcomes showed that the use of different types of emulsifiers did not improve electricity consumption, the diets supplemented with emulsifiers showed improved pellet quality. The higher ($p<0.05$) intact form of crumble and pellet in the starter and grower feeds were attributed to the better gelatinisation of starch as a result of greater meal moisture, higher conditioner temperature and decreased frictional heat. Furthermore, no deteriorating effect was observed in hydrolytic rancidity (AV), oxidation rancidity (PV), mold count, moisture content and water activity. However, there were no apparent differences in the use of either the synthetic emulsifier or natural biosurfactant (Cheah et al., 2017a). Following the production trial, a feeding trial of diets supplemented with synthetic emulsifier and natural biosurfactant was attempted in broiler chickens. The emulsifiers were able to enhance the dietary fat utilization efficiency and improve bird performance only in the starter phase but were insignificant after 14 days of age. The effect of the emulsifier was however not observed in low metabolisable energy diets (Cheah et al., 2017b).

With feedstuffs getting costlier by the day and increased interest in finding other options to be used as feed stuff, considerable attention has been focused on the potential role of intensive earthworm culture and vermicompost (Loh *et al.,* 2005) as a source of animal feed, primarily proteins. Due to the earthworm's high protein content, which is approximately 58-71% of its dry weight, and high essential amino acid, it is a potential candidate for use in commercial feedstuffs, especially for poultry. The study by Loh *et al.* (2009c) showed that earthworm meal of between 10-15% in the diet of broilers could be a partial replacement for soybean and fish meal. Earthworm meal showed up to 63% digestibility of crude protein and also increased the LAB count, but no effect on the ENT count or faecal pH in broilers has been reported.

SUMMARY

Our researches on the use of postbiotic metabolites as in-feed additives have proven its myriad benefits. Furthermore, our studies were not only extensive (comprising various species) but consistent and reproducible results were also obtained. We have also able to shed some light on the mechanisms of action of postbiotic metabolites. Even though the advantages of postbiotic metabolites have been reported in various parts of the world, it is believed that our studies, which were carried out under the local climate and conditions, may resemble better the possible outcomes when this feeding regimen is adopted in the local animal farming industry.

Our future plan is to give an attempt to optimise the feed-formulation. We thus hope to be able to derive ideal and precise formulations incorporating postbiotic metabolites for poultry and swine feed and to further explore this option for ruminants. It is hoped that this feeding regimen will gain support from our local farmers and authorities so that action could be initiated for commercialisation in not just our local market but on a larger scale, internationally.

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BIOGRAPHY

Loh Teck Chwen was born on 16th January, 1968, in Gadek, Alor Gajah, Melaka. He obtained his Bachelor's degree in Doctor of Veterinary Medicine in 1993 from Universiti Pertanian Malaysia, now Universiti Putra Malaysia, before pursuing his PhD. in Wye College, University of London, United Kingdom. His academic journey began when he was first appointed as a tutor from 1993-1997, and he subsequently assumed the position of lecturer in the Faculty of Veterinary Medicine and Animal Science in 1998. He then continued his service as a lecturer in the Faculty of Agriculture. Throughout the duration of 14 years (1997-2011), he moved up the ranks from Lecturer to Full-Professor and is currently serving as the Head of the Animal Science Department, Faculty of Agriculture (since 2011). Through his position, he has established close relationships with private industries and increased the number of academic awards from industries to acknowledge the achievements of his students.

As a lecturer, he undertakes undergraduate courses for students from Bachelor of Agriculture (Animal Science), Doctor of Veterinary Medicine and Diploma in Animal Health and Production programmes. He also plays a role as academic advisor for undergraduate students and has been a co-ordinator and examiner of students' final year project seminars. Furthermore, he is actively involved in revising, planning, proposing and designing the undergraduate and post-graduate curricula. He was also awarded the Innovation and Commercialisation award during the *Majlis Gemilang Academia Putra* in year 2010.

In addition to teaching, Prof. Loh has supervised undergraduate students for their final year projects and also local and foreign post-graduate students, whereby to date he is the principal supervisor for 9 PhD and 16 Master students, and has co-supervised 21 PhD and

Master students. He also had the privilege to be theses examiner for 12 students from several local and international universities, including University of Melbourne, Australia and Anamalai University, India.

In terms of research, Prof. Loh has an impressive publication track record whereby he has authored and co-authored more than 100 journal papers. As a research leader, he has presented research papers at international, national and regional conference meetings. He has also been invited as speaker to a number of national and international seminars and congress. His proficiency and competence are well recognised as he has been entrusted with various research grants, such as the Fundamental Research Grant Scheme and Long-Term Research Grant Scheme from the Ministry of Higher Education, Putra grant from UPM, Technofund from Ministry of Agriculture and Agro-Based Industry of Malaysia, e-Science fund from the Ministry of Science, Technology and Innovation and many other grants from the private sector. The total funding he has received to date for research projects exceeds five million ringgit, from both local grants and international donors. Over the last decade, his research focus has been principally in the field of poultry and livestock animal feed and production and he also aims to venture into researches involving ruminants.

Prof. Loh has widely contributed to the field of animal production from the department level up to the national level. He is a permanent member of the National Animal Feed Standards and Specification, and has been a member of the National Advisor Council of Agriculture Training (Majlis Penasihat Latihan Kebangsaan), Ministry of Agriculture and Agro-Based Industry Malaysia since 2010. Prof Loh also holds the position of Vice-President (since 2012) of the Malaysian Society of Animal Production. It is worth mentioning that he was the Organizing Chairman of the 1st ASEAN

Regional Conference on Animal Production, and 35^{th} (2014) Annual Conference of the Malaysian Society of Animal Production.

Since 1999, he has participated in various professional bodies involving with Research Policies Drafting as well as in auditing and training. Furthermore, he has also been a technical panel of grant evaluation for the Ministry of Science, Technology and Innovation (eScience and Technofund) since 2012 and the Ministry of Education, Malaysia, since 2013. Early 2017, he was invited by the Research Council of United Kingdom to be a panel member for research proposals from prestigious universities of UK. He is also a fellow member of the Federation of Livestock Farmers Association Malaysia in which he serves as the vice-chairman of its technical committee. He has also been appointed as Associate-Editor-In-Chief of the Journal of Revista Brasileira de Zootecnia, Brazil and Section-Editor of the Media Peternakan, Indonesia, and a regular reviewer for several other international journals. Among his numerous accomplishments is his success in bidding for his team to host the Asian-Australasian Animal Production Congress in Malaysia in year 2018.

Despite his remarkable achievements in his area of expertise, he aspires to expand his horizons in terms of networking and forming collaborations with various partners at the international level. He is enthusiastic about bringing our local animal production to a whole new level and at the same time is passionate about producing more Malaysian researchers who are competent in the international arena. He is happily married with Professor Dr. Foo Hooi Ling, who is also a lecturer in the Faculty of Biotechnology and Biomolecular Sciences, UPM, and has three lovely daughters, Loh Xiao Tian, Loh Xiao Ting and Loh Xiao Xuan.

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