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BROILER INDUSTRY IN PENINSULAR MALAYSIA

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ABSTRACT

The poultry sector is an integral part of livestock industry in Peninsular Malaysia. The tremendous growth of the sector has been largely propelled by the private sector. It has evolved into a progressive, organized and developed industry with an annual production of eggs and meat valued between RM1.78 billion to RM6.03 billion annually. The significant contribution of the poultry sector in supplying the protein needs of the population is reflected by the higher percentage share of the sector to the total livestock value. The special status of chicken is due to its general acceptance among the multi ethnic population since there are no religious taboos attached to it, unlike beef, mutton and pork, which are unacceptable to certain sections of the population. Besides, chicken is the cheapest source of animal protein. The broiler industry basically consisted of backyard farming operations prior to the 1950's. The growth of the industry gained momentum in the 1960's and 1970's being aided by several factors including a liberal import policy on high quality breeds from Australia, Canada, USA and Europe, the adoption of modernization and technological innovations in farming system, private sector involvement in the feed milling industry, the highly efficient integrated production system, competent veterinary services and regulation and enforcement being in placed. Production of broiler chicken has continued to increase since 1990 from 348,500 tones of broiler meat produced in 1990, it increased steadily to over 1.2 million tones in 2011. The domestic consumption of broiler meat has also increased steadily from 296,240 tones in 1990 to 918,000 tones in 2011. The popularity of chicken in the diet of the local population is reflected by the high percapita consumption of poultry meat. The percapita consumption of poultry meat exceeded 37 kg per annum in 2012. The main driving forces behind the growth in percapita consumption are increases in incomes, strong demand for animal proteins, the increasing urbanization of the population and the relatively cheaper price of chicken as compared to other meat and fish. The total broiler population in Peninsular Malaysia was 118.5 million birds in 2012, with Johor being the largest producing state, followed by Perak, Terengganu and Negeri Sembilan. The structure of the broiler industry consists of breeding farms (Grand Parent Stock (GPS) and Parent Stock (PS), hatcheries as well as broiler farms producing chickens for meat. The GPS are wholly imported to produce PS, which are in turn used in producing eggs for hatching. Day old chicks (DOC) are produced by hatcheries either owned by the breeder farms, which usually are having facilities, or by independent hatcheries, which only perform the function of hatching. These DOCs are supplied to broiler farms to produce table birds. The other important segment of the industry comprises the feed mills, feed ingredient suppliers, the pharmaceutical firms, the equipment firms, poultry slaughterhouses and the food

processing firms. At present, there are four broiler GPS companies which import GPS to produce PS DOC for their own farms, and for sale to other breeders. These GPS companies, which are integrators, supplied about 96% of parent stock day-old chicks requirements in 2012, with the shortfall being imported from Australia and UK. In 2012, there are only 23 breeding companies with 76 farms in operation in Peninsular Malaysia. Of these, 8 companies were integrators and 15 were non-integrators. In 2012, integrators accounted for 73% of the total production of broiler DOC compared to 49% in 2000. The ten largest producers comprising seven integrators and three non-integrators contributed about 76.5% of the total DOC production. The challenges faced by the industry includes increasing cost of production, free World Trade, fierce market competition, consumer buying power and choice, traceability, disease outbreaks, competition of land use with other industries and development and environment pollution as well as public nuisance.

The production of safe and wholesome high quality poultry and poultry products will drive the future direction of the poultry industry. In this respect, priority will be given to establishing more innovative production and processing facilities, enhancing quality assurance, expanding the practice of product specification, identification and traceability and conducting more risk management.

FOOD SAFETY FOR POULTRY MEAT AND EGGS AS A COMPONENT OF FOOD SECURITY IN EMERGING ECONOMIES

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ABSTRACT

The World Food Summit celebrated in Rome in 1996 declared that Food Security exists when all people, at all times have physical and economic access to sufficient, safe and nutritious food to meet their dietary needs and food preferences for an active and healthy life. Food safety is the assurance that food will not cause harm to the consumer and is therefore an inherent component of food security. If food security is to be achieved, efforts towards the sustainable production of enough and affordable food must be accompanied by the implementation of strategies to mitigate food safety risks. In developing countries, the main food security concerns are the production of sufficient food and the need to keep food prices affordable. In these settings, livestock and poultry diseases are a concern as they compromise the production of enough animal-derived food (meat, milk, eggs) and the availability of draught power and manure for crop production. Food safety in this environment is managed mainly by the consumer and is facilitated by short, stable and transparent food chains that consumers understand well. As countries experience the transition towards emerging economies the market for food products evolves and food safety becomes a barrier for international trade access. Emerging economies face the need to design and implement food safety systems to adhere to international trade agreements and food safety rules. The shift from a focus on production to a more integral approach to food security with explicit consideration of food safety issues is well exemplified by China, where in recent years a number of systems to monitor food safety have been implemented culminating with the establishment, in March 2013, of a new China Food and Drug Administration.

Poultry and egg production can be used as a case that illustrates some of the challenges faced by emerging economies to address the food safety/food security challenge. In recent years, the world's total poultry meat consumption has grown from 66 million tonnes in 2000 to an expected 106 million tonnes in 2013, around 40% in Asia. Global egg production is expected to reach 65 million tonnes in 2013, from around 51 million tonnes in 2000. Growing demand for poultry products has been accompanied by the expansion of large-scale integrated systems which continue to increase their share of the total poultry production in countries such as Malaysia or Thailand. These new systems are part of global and complex food supply chains bringing on new food safety vulnerabilities that have to be managed within complex logistic networks and considering local and international trade regulations. The scale of food safety management in these complex food supply chains is illustrated by the recent announcement of a $10M investment by a major international corporation to improve efficiency and food safety of a poultry

processing facility in Thailand. The establishment of systems to appropriately manage food safety in expanding and increasingly complex poultry and egg production systems is a major concern for emerging economies, but in addressing the food safety challenge, emerging economies must also ensure that such new systems do not make food unaffordable to the poor; in other words, the food safety component of food security should be addressed without compromising the affordability component. The coexistence of modern, large-scale, integrated systems and small-scale production poses additional challenges to emerging economies. Hazard analysis and critical control points systems for large scale production may need adaptation before they can be used to manage food safety in small-scale production and informal poultry chains; experiences in countries such as Indonesia show that a combination of infrastructural changes and behaviour change intervention through participatory work may be particularly effective at promoting strategies to mitigate food safety risks in the small-scale chain.

Food safety is an inherent component of food security. The transition from developing to emerging economy is associated with a gradual shift in emphasis from the “food production” to the “food safety” component of food security. In emerging economies, the expansion of complex large-scale poultry systems and the need to ensure access to international markets require these countries to establish new systems to mitigate food safety risks. As in developed countries, the management of safety should be implemented in the food chain as a whole, from animal feeding to the consumer, with HACCP as the fundamental food safety management tool and risk analysis as the commonly accepted framework to support decisions on what constitutes a level of practical, achievable reduction of risk. Increasingly, the dimension of “food acceptance” will need to be considered also in emerging economies, whereby animal derived food products must not only be affordable and safe, but also produced in a way that is acceptable for the consumer on the basis of cultural, environmental and animal welfare considerations.

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HOW TO FEED BROILER FOR MAXIMUM PROFITABILITY

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ABSTRACT

Maximum profitability of broiler raisings is related with the broiler performance however maximum performance of broiler is not necessary to be the cheapest cost of production. It is possible to maximize performance to meet a breeder standard but it may require a huge investment or higher cost of feed. Maximum profit is achieved by the lowest cost to produce 1 kg live-weight or 1 kg of broiler meat; therefore it is critical to optimize all conditions (genetic, housing, environment, health/biosecurity and feed/feeding system) in order to reduce cost. Broiler production is not considered traditional farming any more, it is an animal industry to produce meat for human consumption therefore each country or company have to be competitive in order to survive in the business. Competitive broiler production is determined by several factors including lower feed and labor costs, good business climate, vertical integration/coordination, economies of scale and access to technology. In term of cost production, feed contribute around 60 percent of total cost but when day old chick production cost is counted from parent stock farm, additional feed cost should be added at another 6%, therefore feed and feeding system is very critical to be optimized based on advance technologies currently available. Several techniques can be adopted to reduce cost of feed and feeding, include nutrition, formulation, feed production and feeding system at farm level. With modern breed currently developed, precise nutrition (amino acids requirements, ratio and stage of growth) becomes important to meet bird requirements in every stage of growth. In regard to feed formulation, the nutritionist should focus on three most expensive nutrients including energy, protein (amino acids) and available phosphorus in order to reduce cost; however the meeting nutrients requirement for maximum growth may be different with nutrient content to produce lowest cost of formulation. A breeding company (Cobb) recommended 3 different recommendations for feeding broiler for different purposes. Formulation should also consider proper selection of feed ingredients and accurate measurement or estimation of nutrient contents. Many feed additives have been developed and marketed by different companies around the world, however the use of feed additives (enzymes, probiotics, functional, herbals etc.) should be critically examined if an additive provides benefit to reduce cost of feed and feeding and withdrawal feed can be developed to reduce cost of feed by removing additives not necessary for production. In term of feed production, feed particle size and pellet quality would be critical for broiler performance, optimum particle size for different feed ingredient can be different and improving pellet quality should be easily achieved by process adjustment modern feedmill. When a feed has been produced properly, it is critical that all balanced nutrients found in ration should be delivered in such a way so that the bird can consume, ingest and absorb efficiently to produce meat. Stress factors from environmental conditions and disease should be minimized in order

bird to achieve better performance. Measurement of performance should be conducted for each farm and the results can be bench-marked against other farms in the company or other countries in the world. Finally continued adoptingnew and advance information and technologies would be critical to reduce cost of production and compete with rest of the world.

CHALLENGES IN CONTROLLING VIRAL DISEASES OF POULTRY

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ABSTRACT

As in many countries including Malaysia, poultry industry has become a major contributor to the country's economy primarily due to the rapid revolutionary of the industry and advances made in poultry management, nutrition, genetics and better diagnosis and control of diseases. As the poultry industry is expanding, much is needed to be done to improve the efficiency of production, which directly depends on the control and prevention of diseases. Infectious diseases are one of the major causes of economic losses in poultry industries. In many instances, no specific signs are associated with a particular disease. Besides clinical signs and findings from post mortem examinations, diagnostic tools based on serological and molecular detection are used to confirm the causative agents. However, the identification of causative agents and the detection of specific antibody responses in relation to a clinical problem are complicated due to the concurrent infections and improper use of vaccines. Currently, the poultry industry is threatened by more virulent viruses of endemic diseases or by exotic and emerging diseases that can cause major economic losses to this sector. The emergence and re-emergence of avian influenza virus (AIV), particularly the highly pathogenic avian influenza (HPAI) H5N1, the presence of endemic low pathogenic avian influenza (LPAI) H9N2 in poultry flock and recently the detection of novel H7H9 posed significant threat to the poultry industry and public health sector. Catastrophic diseases such as HPAI H5N1 is not easy to overlook, the real challenge is to confront H7N9, H9N2 and other LPAI which act in concert with other factors such as management, environment, nutrition and concurrent infections which form continuous threat to the entire poultry production system. The repeated outbreaks of diseases caused by variant strains of infectious bronchitis virus (IBV), velogenic Newcastle disease viruses (NDV) and more virulent viruses of infectious bursal disease virus (IBDV), infectious laryngotracheitis virus (ILTV) and Marek's disease virus (MDV) in well managed poultry flocks have prompted the need to evaluate the underlying factors contributing to the failure of vaccination to provide complete protection against clinical infections and transmission of disease. Vaccination failure can be classified as primary vaccination failure where the chickens do not develop adequate antibody titer and succumbed to a field disease outbreak. However, in secondary vaccination failure, the chickens develop adequate immune response but then immunity wanes over time. In the field, vaccination failure is complex involving various factors associated with the vaccine strains and vaccination program, the virulence of field pathogens and the host immune competence. In many situations, immunosuppressive agents primarily MDV, IBDV, and chicken anemia virus play an important role in increasing the susceptibility of chickens to opportunistic infections and/or suppressing effective vaccine induced responses.There is no doubt diagnostic tools, vaccines and vaccination equipment

have improved over the years. However, despite these advancements, conventional laboratory diagnosis using serological tests and conventional live and killed vaccines are used extensively in health and disease management of poultry. As the poultry industry become more intensive, accurate, economical and practical laboratory diagnostic tools are important for effective control of disease outbreaks. The advancements in the use of molecular detection method using real-time PCR approach, highly automated instruments for antibody detection and development of rapid on site antigen capture assays for virus antigen detection may have significant impact in the field of disease prevention and control. In the area of vaccinology, most of the advances in the development of the so called recombinant vaccines against poultry disease are based on the development of recombinant protein or synthetic peptide vaccines, recombinant vector vaccines using selected virus or bacteria as carriers and DNA plasmids as genetic vaccines. However, very few recombinant vaccines are available commercially. The majority of these newer vaccines are live recombinant viral vectors based on fowlpox virus and herpesvirus designed to deliver specific gene(s) to stimulate the host' immune system. Recently, a few new live recombinant viral vector vaccines based on avian adenovirus and reverse genetic NDV and AIV are making their way in several countries. However, the use of vaccines against field viruses also contribute to the emergence of variant or more virulent viruses that are able to escape existing vaccine induced immunity as what have been reported for several RNA viruses namely AIV, IBV and IBDV. Similar phenomenon was also reported for DNA viruses such as MDV and ILT, where the use of different live attenuated ILT vaccine strains contribute to the emergence of new pathogenic ILT strain affecting poultry. It is clear that progress has been made in the control and prevention of viral diseases of poultry. However, a comprehensive approach is needed for disease control requiring consideration of the interactions between management, nutrition, poultry genetics and immune functions against infectious disease. It is envisioned that the progress in functional characterization of the chicken genome, avian transgenic technology and further improvement of poultry vaccines and therapeutics will further increase the global competitiveness of poultry industry.

STRATEGIES TOWARD HPAI-FREE ASEAN BY 2020

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INTRODUCTION

Since the Highly Pathogenic Avian Influenza (HPAI) was officially declared in early 2004, seven of ASEAN member's states have experienced the outbreaks. The countries are Cambodia, Indonesia, Lao PDR, Malaysia (Peninsular), Myanmar, Thailand and Vietnam. The other three countries, namely, Brunei Darussalam, Philippines and Singapore fortunately remained free. The impact on poultry industry in ASEAN was substantial due to poultry mortality and culling destruction as well as the market being scared of the zoonotic effect and possibly pandemic consequence. The World Bank estimated the losses by mid-2005 to be in excess of US$10 billion. Each affected country responded to the outbreaks individually or with assistance of international technical agencies and other donor countries. Accordingly, ASEAN also responded by formation of the ASEAN HPAI Taskforce which was mandated by the Twenty-Sixth Meeting of the ASEAN Ministers on Agriculture and Forestry (AMAF), which was held on 7th October, 2004 in Yangon. The main objectives of the initiative were to coordinate cooperation for the prevention, control and eradication of HPAI in the region. This paper discussed the ASEAN responses and strategies in achieving an HPAI-Free ASEAN by 2020.

RISKS OF OCCURRENCE AND STATUS OF HPAI IN ASEAN COUNTRIES

The ways the HPAI outbreaks spread in ASEAN countries reflected that the disease is truly a trans-boundary animal disease. The disease agent is capable of crossing country border, both land and sea. In ASEAN countries, the disease spread progressively from "source country" to ASEAN Member's states that are sharing land border and subsequently to other ASEAN member countries. The disease is also capable of spreading from "source country" to other country separated by sea. Epidemiological data on HPAI H5N1 virus clades supported these phenomena. As such, countries like Lao PDR, Myanmar and Vietnam which have land borders with endemic HPAI H5N1 country have a highrisk level of getting infection or recurrence of HPAI outbreaks. The risks are associated with movement of poultry including day old ducks and chicks and other poultry farming inputs. Initial outbreaks in Myanmar were hypothesized to be associated with migratory birds as well as importation of fertile eggs from neighboring countries. Intra-ASEAN poultry movement and poultry associated activities also has been stipulated to be the other mode of the disease spread in Cambodia, Thailand and Malaysia. In contrast, the situation in Indonesia is unique, although there was no conclusive evidence on risks associated with introduction of the disease in 2003, the author believed biological products movement from "source country" to Indonesia was perhaps the reason for occurrence of the first outbreak. Since earlier episode of HPAI outbreaks until now, Member's State HPAI situation have progressed. Based on the HPAI situation in

Member's States the ASEAN might be divided into four categories, namely, Category 1: Brunei, the Philippines and Singapore countries still free of HPAI, Category 2: Thailand and Malaysia countries successfully eradicated the disease, Category 3: Cambodia, Lao PDR and Myanmar country experiencing sporadic HPAI outbreaks and Category 4: Indonesia and Vietnam HPAI H5N1 virus is persistent. Strategies to prevent, control and eradicate HPAI in ASEAN are formulated based on these categories.

STRATEGIES TOWARD HPAI-FREE ASEAN BY 2020

In 2010 ASEAN Member's State in collaboration with International Agencies developed the Roadmap for an HPAI-Free ASEAN by 2020. The roadmap focuses on three main broad strategies for prevention, control and eradication for the region. Firstly, is to maintain existing freedom status (HPAI-Free Member's State) through vigilant border controls and minimising risks of introduction. Secondly, is to strengthen early detection capabilities and respond quickly and effectively once the disease agent is detected, for those countries with sporadic outbreaks. Thirdly, and the most challenging task, is to gradually control and eradicate the infection through progressive zoning based on risk management for the most significant transmission pathways, for Member's States where the disease agent is persistent in their duck and chicken population. In order to achieve that, seven key areas need to be in-placed. Such areas are as the following: (1) Strong Veterinary Services - animal health and legislation, veterinary epidemiology (capacity to detect, report, monitor, investigate and respond to disease threats and outbreaks), diagnosis (confirm and carry out virus characterization), responding to HPAI outbreaks, and farm certification and accreditation. (2) Progressive zoning - achieving disease-free status in progressive manner, expanding the free-status, capacity in mitigating risks of cross-border incursions and applying the compartmentalization approach. (3) Vaccines and vaccination - capacity for pre- and post-vaccination surveillance, ensuring vaccines quality, availability of vaccines, development of sound vaccination, engagement of relevant stakeholders, particularly the private sector in implementing vaccination program including exit strategies, and preparedness to implement an effective vaccination strategy. (4) Containment of infections and outbreaks (stamping-out or culling) - stamping out policies, capacity in eliminating the virus from infected environment, mechanisms to encourage reporting, cooperation of farmers and producers in implementing culling policies. (5) Surveillance - capacity to detect and respond appropriately to the presence of H5N1 virus infection, recognising risk areas and products, conducting cost effective surveillance system and launch appropriate and timely response at both the national and sub-national levels and integrating surveillance systems between animal and human health. (6) Sustainable market chain policies and interventions in reducing risks of spreading and contamination to poultry and human populations - capacity in better understanding of the market chain and the risks of disease transmission along the market chain, implementing strategies and structural changes to manage risks in the production and market chains, and conducting socio-economic assessment. (7) Bio-security as a long-term preventive measure to keep the HPAI virus out of the farms/flocks - capacity of designing and implementing effective bio-security measures. The plan and activities in line with the roadmap strategies will be

implemented at national level and closely linked with the FAO/OIE Global Framework for the control of transboundary animal diseases. A strategic schedule that includes key milestones achievement will be monitored to ensure the attainable of HPAI freedom in ASEAN by 2020.

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FOOD SECURITY AND POLICY RESPONSES WITH SPECIAL REFERENCE TO THE POULTRY INDUSTRY

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ABSTRACT

The right to food is the basic tenet of the food security policy of any nation. The guiding framework is based on the World Food Summit 1996 interpretation which stated that: food security as existing when all people, at all times, have physical and economic access to sufficient, safe and nutritious food that meets their dietary needs and food preferences for an active and healthy life (FAO, 2003). Most developing economies had translated their food security drive by a number of policy measures to ensure availability, accessibility, and utilisation. The food security agenda in the twenty-first century faces a totally new set of challenges. Domestically, the competition for resources (land, labour and capital) continues to intensify as urbanisation and industrialisation grow rapidly. Limited investment in food and agriculture have made this sector lagged on all fronts; productivity, efficiency and development. The effect of climate change is showing, aggravated further by unsustainable practices such as overuse of chemical fertilisers, and poor water management. The international market also poses the bigger challenge to developing countries' food security in particular "extreme volatility". The course of the global food system is no longer in the main determined by the resolution of demand and supply fundamentals. External shocks are emerging from a complexity of sources and are having a profound influence in causing vulnerability in food systems. The detrimental impact of volatility is further magnified by structural problems such as: poor infrastructure, poor supply response, inefficient market, and susceptibility to climactic disturbances.

Malaysia is self-sufficient in some of the food commodities such as poultry meat (128%), eggs (115%), pork (102%), and fisheries (102%). However, she is not self-sufficient in commodities such as rice (71%), fruits (66%), vegetables (41%), beef (29%), mutton (11%) and dairy milk (5%) (MOA, 2010). The food trade deficit grows from year to year, from RM1 billion (US$0.33 billion) in 1990 to RM12 billion (US$ 4 billion) in 2011 (DOS, 2011). Like any other developing countries, Malaysia has enjoyed the benefits of cheaper food imports. This dependence however has its price as it disincentivised the country to seek ways and means to improve productivity and efficiency. Although the country is self-sufficient in poultry meat, there are still pertinent issues with regards to production sustainability, accessibility and utilisation, particularly food safety. The production is mainly based on imported feed. The share of food price to the overall consumer price index is 30.3% which indicates little margin for changes in food prices. Efforts to improve food safety, which is a market failure, must be evaluated in terms of their impact on additional costs and returns to producers, risk reduction, economic gains for the domestic

industry, and positive spillovers for food safety in the domestic food system. To the extent that the externality costs are borne by society, it is unlikely that the supply and demand functions will fully embody the economic consequences of the consumption of the food. In the National Agro-food Policy (2011–2020), the overall objective with respect to the poultry industry is to improve productivity and competitiveness to ensure food security for the nation and increase exports and sufficient supply to consumers at reasonable prices. The initiatives include new technology adoption, R&D in feed production, and improve surveillance for disease free poultry production, in particular adoption of good agricultural practices. On top of these, the industry requires better market information system, effective risk management system, and social safety nets.

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RECENT ADVANCES IN METHIONINE NUTRITION FOR POULTRY

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ABSTRACT

Methionine, which has a nonpolar thioether group in its side chain, is a nutritionally essential sulfur-containing amino acid that is present in plant in only low levels, diminishing their value as a source of good-quality dietary protein. Methionine is classified as a first limiting amino acid in commercial poultry feed manufactured primarily from plant-derived ingredients. It plays several important roles as an essential amino acid not only in protein synthesis but also in cellular metabolic methyl donation, co-enzyme S-adenosylmethionine formation, and provision of precursors for metabolic pathways, control of several key metabolite levels for biochemical processes, and polyamine synthesis, as well as a sulfur donor. Absorption and transportation in the intestines of different sources of methionine differ because multiple systems are involved. Methionine supplementation in a low-protein diet alleviates the negative effects of heat stress. Methionine also improves immune response through direct effects (protein synthesis and breakdown) and indirect effects (through its derivatives). The supplementation of methionine in feed improves the amino acid balance and consequently promotes growth performance by enhancing feed efficiency, increasing protein synthesis, and decreasing fat synthesis. As various factors influence methionine requirements, recommended levels for commercial strains vary and should be considered to achieve optimum performance.

Methionine is a basic metabolite in plant cells. Apart from its role as a protein constituent and its central importance in the initiation of mRNA translation, methionine indirectly regulates a variety of cellular processes as the precursor of SAM, which is the primary biological methyl donor. Livestock and poultry cannot synthesize some of the amino acids used as the building blocks of their proteins and must obtain these so-called essential amino acids from their diet, methionine is one of these essential amino acids. Methionine, one of two sulfur-containing amino acids, has a nonpolar thioether group in its side chain. It donates its methyl group to any of a number of possible acceptors through S-adenosylmethionine (SAM), and three of its four remaining carbon atoms are converted to the propionate of propionyl-CoA, a precursor of succinyl-CoA. Methionine and cysteine are particularly significant among amino acids because they play numerous roles in protein metabolism. They may be considered to be the principal sulfur-containing amino acids among all amino acids that are incorporated into proteins, although homocysteine and taurine also play important physiological roles.

Methionine and cysteine are unique among amino acids in that they contain sulfur. The other amino acids are comprised only of carbon, hydrogen, oxygen, and nitrogen atoms. Although both sulfur and oxygen belong to the same group (Group 6) of the

periodic table and, therefore, are capable of forming similar covalent bonds, methionine and cysteine analogs, in which the sulfur atom is replaced by oxygen, do not serve the same functions. One of the critical differences between oxygen and sulfur is sulfur's lower electronegativity. Indeed, oxygen is the second-most electronegative element in the periodic table. This accounts for the presence of sulfur in methionine; replacement of the sulfur with oxygen would result in a much less hydrophobic amino acid. Cysteine readily forms disulfide bonds because of the ease with which it dissociates to form a thiolate anion. On the other hand, serine, which differs from cysteine only in its substitution of oxygen for sulfur, does not readily make dioxide bonds. This difference results from the fact that thiols are much stronger acids than are alcohols, so that the alcohol group in serine does not dissociate at physiological pH levels. Substitution of oxygen for sulfur in SAM would produce so powerful a methylating agent that it would promiscuously methylate cellular nucleophiles without the need for an enzyme (Brosnan and Brosnan, 2006).

Diets with an amino acid imbalance or methionine deficiency normally increase heat production (Sekiz *et al*., 1975) and induce more negative effects from heat stress when environmental temperatures are high (Bunchasak and Silapasorn, 2005). Balancing the amino acid composition in the diet with methionine supplementation improves production performance through pathways of polyamine metabolism (Gonzalez-Esquerra and Leeson, 2006), while glutathionine (derived from methionine) may reduce damage from oxidative stress. Sulfur amino acids are one of the most abundantly used amino acids for synthesis of immune function-related molecules. Their effects can be divided into two routes: the first is a sufficient metabolic supply of SAA from the diet and tissue protein breakdown that supports the synthesis of myriad proteins and peptides involved in the normal functioning of the immune system, and the second involves the three major products of SAA (glutathione, homocysteine, and taurine), which influence inflammatory aspects of immune response (Grimble, 2006).As various factors influence methionine requirements, recommended levels for commercial strains vary and should be considered to achieve optimum performance.

Broiler nutrition recommendations according to the nutrition guideline of strains

Strain	Stage	Nutrition recommendation (%)				Relative to lysine	
		TSAA		Methionine		TSAA	Methionine
		Total	Digest	Total	Digest		
Ross 308[1]	Starter	1.07	0.94	0.51	0.47	74	38
	Grower	0.95	0.84	0.45	0.42	76	38
	Finisher	0.60	0.76	0.41	0.38	78	39
Cobb[2]	Starter	0.98	0.86	0.56	0.50	74	38
	Grower	0.96	0.84	0.53	0.48	75	40
	Finisher1	0.88	0.77	0.48	0.43	78	41
	Finisher2	0.80	0.7	0.44	0.40	78	41
ArborAcres[3]	Starter	0.97	0.86	0.53	0.46	71	39
	Grower	0.85	0.75	0.46	0.41	72	39
	Finisher1	0.78	0.69	0.42	0.37	73	39
	Finisher2	0.77	0.68	0.42	0.37	76	41
NRC (1994)[4]	Starter	0.90	—	0.50	—	82	46
	Grower	0.72	—	0.38	—	72	38
	Finisher	0.60	—	0.32	—	71	38

[1] Starter (0–10 days), grower (11–24 days) and finisher (25-slaughter).
[2] Starter (0–10 days), grower (11–22 days), finisher1 (23–42 days) and finisher2 (42-slaughter).
[3] Starter (0–14 days), grower (15–28 days), finisher1 (29–36 days) and finisher2 (37-slaughter).
[4] Starter (0–21 days), grower (22–42 days) and finisher (43–56 days).

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BENEFITS OF ADDING SODIUM BUTYRATE, A SODIUM SALT OF THE SHORT CHAIN FATTY ACID BUTYRIC ACID, IN THE FEED OF BROILERS AND OTHER FARM ANIMALS

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ABSTRACT

There is more and more evidence that butyric acid possesses interesting characteristics that make it "not just an acid". In addition the well documented anti bacterial effect, sodium butyrate is known to stimulate the production of pancreatic secretions, including enzymes. It will also stimulate hormones, such as insulin, which in turn stimulates epithelium development. Sodium butyrate also improves the absorption of electrolytes and reduces the incidence of diarrhoea, providing the ileal and the hindgut mucosa with a preferred energy source. Recently, more and more data suggests positive impacts on the immune defence of the animal. Physiological effects of sodium butyrate have been demonstrated in several species and can be summarized as follows: control of the intestinal barrier, pathogen reduction, increase of mucin synthesis, regulation of the immune response, and positive effects on the intestinal epithelium, such as supplying energy to the colonocytes and enterocytes, and enhancing the intestinal cells proliferation, differentiation and maturation. The better development of the intestinal epithelium results in an increased intestinal surface, ensuring a better digestion. However, not all butyrates are equal. Using a specific production process to protect sodium butyrate, it is possible to ensure a gradual release along the digestive tract. This will maximize the efficacy of this important additive. Therefore, it is important to know the advantages and drawbacks of the different technologies that can be used to protect the sodium butyrate.

Keywords: Sodium butyrate, animal performance, antibacterial effect, epithelium development, regulation of the immune response

INTRODUCTION

The use of organic acids in poultry nutrition is quite well accepted. Besides the reduction of pH that limits the development of pathogens and helps in the digestion of proteins at crop level, some acids also have the ability to enter the gram-negative bacteria and disrupt their metabolism. One of the organic acids, butyric acid, is a carboxylic acid with the chemical formula CH3CH2CH2-COOH. Butyric acid is a natural product of the bacterial fermentation of the carbohydrates in the large intestine of monogastrics, or in the rumen of ruminants. However, butyric acid is volatile and corrosive, so when thinking in a practical way, we must use a salt, sodium butyrate, which is appropriate for use in pelleted feed, as it is stable during processing. Once sodium butyrate reaches the stomach of the bird (proventriculus and gizzard), it will quickly release the sodium and, due to the low pH, butyrate will be rapidly converted to the undissociated form, also termed butyric acid. This form is the one responsible for the antimicrobial activity, as butyric is strongly lipophilic and can diffuse across the membranes of bacteria (especially gram negative). As we go further down the digestive tract, pH increases and the proportion of butyric acid decreases while the proportion of butyrate increases. This is where we observe the trophic effect of butyrate (and butyric) on the intestinal epithelium, because these 2 forms can be absorbed by the enterocytes thanks to different transporters.

TROPHIC EFFECT OF SODIUM BUTYRATE

Sodium butyrate is a preferred source of energy for the enterocytes. This will result in better development of the intestinal villi, and also in stronger gut lining, as it was demonstrated in many species such as poultry, swine, but also calves or aquatic species. Different publications show an increase in epithelial regeneration of the intestinal microvilli, together with an enlargement, producing an increase of the intestinal absorption area when using sodium butyrate in feed. Mallo *et al*. (2011) demonstrated that the digestible energy of the diet and the digestibility of the protein, as well as the lengths and widths of the intestinal villi, can be significantly improved by the addition of sodium butyrate.

ANTIBACTERIAL EFFECT OF SODIUM BUTYRATE

It has been demonstrated that butyric acid can inhibit the growth of bacteria of the group of Enterobacteriaceae (*Salmonella*, *Escherichia coli* etc). This is because its undissociated form can freely diffuse across the bacterial membrane. Once inside the cytoplasm of the bacteria, the acid dissociates, thus releasing free hydrogen ion and reducing the pH, which causes internal cell damage. Sodium butyrate is particularly effective against *Salmonella* (Fernández-Rubio *et al*., 2009), and one very unique aspect of butyric acid is its ability to negatively affect the virulence of this pathogenic bacteria through modification of their genetic expression.

SODIUM BUTYRATE AND IMMUNE DEFENCE

Research in various animal species indicates that adding sodium butyrate to the diet results in better resistance to a challenge, which can be explained by two mechanisms: anti-inflammatory effect, and reinforcement of the intestinal defence barrier. More specifically in poultry, recent studies reveal some additional features. For instance, a recent work by Sunkara *et al*. (2011), demonstrated that sodium butyrate is capable of inducing host defence peptides (HDPs) and enhancing disease resistance in chickens, suggesting that dietary supplementation of butyrate has potential for further development as a convenient antibiotic-alternative strategy to enhance host innate immunity and disease resistance.

THE IMPORTANCE OF A PROPER PROTECTION

Recent results confirm that sodium butyrate partially protected with vegetable fats ensures efficacy all along the gastrointestinal tract as well as higher level of active substances when compared to the coated products, requiring a lower dose per kg of feed to achieve the same level of sodium butyrate.

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CONTROL OF MYCOPLASMA CAN MINIMIZE THE EFFECT OF ALL RESPIRATORY INFECTIONS AND GREATLY REDUCE ANTIBIOTIC DEPENDENCE

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ABSTRACT

The potentiating effect of MG and MS infections on the impact of respiratory viruses (NDV, APMV and AIV) and bacterial respiratory diseases (Coryza, EPEC, ORT and Fowl Cholera) is well recognized. Effective control of mycoplasma infections offers chicken and egg producers great advantages in decreasing the need for antibiotics and the impact of these infections. Although antibiotics are (initially) effective at controlling mycoplasma and bacterial respiratory diseases in poultry the development of resistance means that this is not sustainable even in the medium term. The best long term strategies for mycoplasma control are live vaccines that are safe and prevent vertical transmission. It is important to have criteria for success for mycoplasma control programme and realize that vaccination must take into account antibiotic interventions and other interactions. These criteria will include biological and economic parameters.

Keywords: Avian mycoplasma, antibiotic reduction, potentiation of respiratory infections

INTRODUCTION

Synergistic effects of mycoplasma infections with other "simple/uncomplicated" infections has been long recognised but perhaps forgotten. The chronic nature of mycoplasma infections in chickens and their propensity to make infections with NDV, IBV, AIV (especially H9) and APMV virus have been demonstrated in laboratories, indeed it is very hard in the laboratory to produce respiratory disease with *Mycoplasma synoviae* without adding respiratory viruses or vaccines. Bacterial infections of the respiratory tract in chickens have also been potentiated by mycoplasma infections. Indeed in the field often many potential pathogens are present and can be identified but there role in respiratory disease is difficult to ascertain. For example in broilers in Germany Cheesy broilers (airsacculitis in the slaughter house) have been considered to be ORT for a long time but the potentiating effect of MS has recently been considered. The observation that MS may be triggering *E. coli* peritonitis at the beginning of lay (Raviv *et al.* 2007) in the USA and Europe may be due to MS infection being acquired at the time of transfer to a multiage layer site. It is easier to see if the effect of LaSota NDV in broilers in the field. This vaccine cannot be used in broilers after one day of age if those broilers are mycoplasma positive without the use of antibiotics to dampen down post vaccinal reactions. Commonly the use of LaSota at 10 days will need antibiotic administration at 18 days in mycoplasma positive chicks. This has seen lentogenic/apathogenic strains of NDV (VG/GA, V4, Ulster etc) used in broilers in some areas. Obviously here the antibiotics are not affecting the viral infection. Similarly coryza and fowl cholera is more chronic in mycoplasma infected birds (usually older than broilers). This effect is seen with MG and/or MS. Antibiotics can certainly help control bacterial diseases and maybe reduce infections but the emergence of resistance strains can limit the long term usefulness of this strategy. Contamination of poultry products with

antibiotic residues and antibiotic resistance determinants (genes, plasmids etc) are also public health issues.

MATERIALS AND METHODS

MG and MS field strain freedom has been successfully achieved by biosecurity in many areas including UK, USA, NZ. Some places have only effectively controlled MG including Israel, Iran, Brazil, Germany, France, the Netherlands and individual integrators. Indeed the MS status of many places is hard to tell because of antibiotic usage in lay. In some areas they have controlled MG by ts-11 vaccination of breeders (best if they are MG free as DOC) in Australia, South Africa, Lebanon (see Barbour *et al.* 2000) and China. MG and MS control by combined vaccination has been used in Australia, Philippines and Indonesia. This later strategy is particularly attractive as both mycoplasma infections have the same control strategy (antibiotics for MS may be incompatible with live vaccination for MG). F strain vaccination has not been used for breeders in the USA although ts-11 has been used for MG control in the face of MG epidemics. Where F strain has been used overseas vertical transmission and residual pathogenicity in vaccinated birds and their progeny is sometimes seen (where antibiotics are not used).

RESULTS AND DISCUSSION

In Australia the vaccines ts-11 (MG) and MSH (MS) have been used extensively in layers and breeders in Australia for the last twenty years and now most chickens (layers, breeders or broilers never have antibiotic in their whole lives. Concurrently, we were able to largely effectively control Coryza with a vaccine although this is no longer available and coryza has not re-emerged as a problem. Fowl cholera is still a problem on some sites especially those with earthen floors (layers and breeders) and free range layers. Some other problems are emerging in free range layers including AIV (mostly H7) and erysipelas. Mycoplasma free broilers do not need routine antibiotics at 18-22 days; horizontal infection is not a big problem during the short life of a broiler. As people decrease antibiotic usage sometimes some previously unidentified problems can emerge. Most commonly *Brachyspira* infection (Avian intestinal spirochaetosis) may emerge as a problem – repeated egg production drops that respond to antibiotics including pencillins, diarrhoea (collapsing manure cones and caramel stained eggs), no increase in mortality. In fact until a country reports *Brachyspira* in chickens it seems they have not seriously tried to decrease antibiotic usage (acidification of water may control). Antibiotics may also be controlling Salmonella infection including SG (not present in Australia), SE (not present in Australia), other invasive and non invasive Salmonella (present in Australia - control at egg and breeders with vaccination) and *E. coli*.

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THE LATEST TECHNOLOGY IN CONTROLLING NEWCASTLE DISEASE

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ABSTRACT

Newcastle disease virus (NDV) causes illness in many avian species and typically manifests in respiratory and gastrointestinal or nervous system (or both) symptoms. The most severe form of Newcastle disease (ND) can result in mortality rates exceeding 90% in susceptible chicken flocks. Different strategies have been used to control ND, but vaccination is by far the most popular approach. Numerous conventional live and inactivated ND vaccines have been used for many decades in routine vaccination protocols in the poultry industry. Nevertheless, this condition still causes many problems around the world, especially due to interference of maternally derived antibody (MDA) and problems with vaccine application in the field. ND vaccines can also cause post-vaccination reaction and interference with infectious bronchitis vaccines. Such drawbacks with conventional vaccines led to the development of live viral vector vaccines, also known as recombinant vaccines. A particular success story has been the development of a recombinant vaccine using as a vector the turkey herpesvirus (HVT) to express the Newcastle disease virus fusion protein. The HVT possess a large DNA where foreign gens, such as F gen from NDV, can be safely inserted into non-essential region of the HVT genome along with an appropriate promoter, without disturbing the infectivity. The recombinant HVT ND (rHVT ND) has demonstrated strong protective efficacy to different NDV genotypes in many chicken producers around the world. This construction has also significantly reduced viral shedding into the environment as well as inducing long lasting protective immune in long living birds. Data will be presented and discussed showing recent results of rHVT ND obtained from broilers and layers flocks from major poultry producer countries.

Keywords: Newcastle Disease virus, recombinant HVT vaccines

POULTRY SLAUGHTERING IN PERSPECTIVE TO HALAL SLAUGHTER

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ABSTRACT

Halal Poultry Slaughtering is often considered differently from Poultry Slaughtering Guidelines due to religious implication. An overview on the current practices of poultry slaughtering and poultry meat inspection process indicate more similarities and can provide better understanding of the basic requirement process involved in Halal Poultry Slaughtering. Understanding issues pertaining to Halal Slaughter such as stunning, mechanical slaughter, animal welfare and slaughtering process will further indicate its rationale and relevant to the industry.

FACTORS AFFECTING POULTRY PRODUCTION WITH THE MEDIATING EFFECT ON POULTRY WELFARE

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ABSTRACT

In poultry welfare and production, quality and quality control require good management throughout the entire production chain to follow performance upgrading and therefore increase profitability, and secondly the achievement of some products appropriate to the standards. People involved in poultry production must realize that the main purpose of the entire production system is customer requirements satisfaction. Improving quality is possible when all the stages of production of poultry meats are incorporated in the quality control methods and those methods are simple and practical. The welfare of poultry is a key concern to veterinarians and poultry producers in developed as well as developing countries of the world. In developing countries where poultry welfare may not currently be a primary concern at present, it is likely to become more important in the future as the global trend of increasing awareness of animal welfare and production develops further. The purpose of the study was to investigate the factors which increase the poultry production with the mediating effect of poultry welfare in developing countries like Malaysia. Quality management is a major factor which impact on poultry welfare which leads to increase in production. In quality management, some factors are very important which indirectly effect on production with mediating effect of poultry welfare such as disease, nutrition, genetics, availability of drugs and vaccines, climate, environmental management and stockman ship, consumer and customer requirements and perceptions (retailers) as well as food safety. There is a need to feed a rapidly growing world population with projected global population of 10 billion by 2030. This paper summarizes what we consider to be the important animal welfare and good production factors in poultry and to discuss some of the challenges to be faced by the poultry veterinarian and the poultry producer in this respect in the 21st century.

Keywords: quality management, quality control, animal welfare, poultry welfare, poultry production

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FOOD SECURITY – SOLVING THE GREATEST ISSUE OF OUR TIME

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ABSTRACT

"We have enough solutions to create a food secure world. Do we have enough courage, enough leadership and enough urgency to deliver?" - Jeff Simmons, Elanco. A food secure world is one in which everyone can afford and access an adequate quantity and quality of food. On October 31st, 2011, the world's population shot past the 7-billion mark on its way to reaching 9 billion or more by the year 2050. We will live our lives between the 7 and the 9. But the steepest part of the growth curve is happening between now and 2020. Beyond 2030, growth rates taper. Population growth is not infinite. In fact, population is already declining in countries like Japan, Germany and Russia. Populations in Europe and China will begin declining within the next decade. Beyond population growth, 3 billion people will enter the middle class during these years. In fact, more people will join the middle class between now and 2020 than any other time in history. While "middle class" means different things in different places, to put it simply, billions of people will live a better life and that's all starting now. Regardless of the specific income figure, one thing is consistent: as income growth one of the first things most people do with more income is to improve their diets by eating more meat, milk and eggs. In fact, the United Nations Food and Agriculture Organization (FAO) predict a 60% increase in demand for meat, milk and eggs by 2050. Increasing demand will also mean increasing prices. FAO and the Organization for Economic Co-operation and Development (OECD) expect in the next decade beef, pork and sheep meat prices to climb 11%, 17% and 4%, respectively. According to the World Wildlife Fund, the Earth takes 1.5 years to regenerate the renewable resources we use in a single year. On August 20th, 2013, we crossed the line where annual resource consumption exceeded the planet's ability to replenish. In eight months we exhausted the natural resources that should last all year – and every year that date is moving up by a few days. On this course, by 2030 we'll require double the planet's resources to meet our needs. We have to produce more, and do it with less. We need to be careful when we make insular decisions that use more resources. For example, in a meta-analysis of 66 studies, researchers from McGill University in Canada and the University of Minnesota found that organic methods produce 25% less food than conventional farming on the same land area.

CALIBRIN®Z REDUCES GUT INFLAMMATION AND IMPROVES GROWTH PERFORMANCE IN NECROTIC ENTERITIS-AFFECTED YOUNG BROILERS

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ABSTRACT

Necrotic enteritis (NE) is considered among the most important Clostridial poultry diseases globally with estimated annual economic loss of over $2 billion largely due to medication and impaired growth performance. Due to increasing restrictions on the agricultural use of antibiotics in the US, novel strategy is needed to reduce the NE-associated gut damage. In this paper, we describe an effective dietary strategy to mitigate *C. perfringens*-induced gut inflammation and to improve growth performance using thermally processed clay, Calibrin-Z (CAZ). One hundred, 1d-old Ross male chickens were randomly allotted into 5 treatment (TRT) groups and challenged with 10,000 *Eimeria maxima oocysts* orally at d-14 followed by 10^9 CFU of a virulent field strain of *C. perfringens* at d-18 to induce NE. The 5 TRT groups were a non-infected control, a NE-infected control, and NE-infected groups fed diets containing 0.25% or 0.5% of CAZ or 22 mg/kg virginiamycin (VM). Body weights, intestinal gut lesions, serum toxin levels, serum antibody levels, and lymphocyte transcripts encoding inflammatory cytokines were measured in the intestine and spleen as immunological parameters of protective immunity. Birds fed diets with 0.25% or 0.5% CAZ or VM showed significantly improved body weight gain which was not different from that of non-infected birds when compared to the NE-infected control birds. Birds fed 0.5% CAZ showed the greatest numerical improvement in body weight gain, with 15.2% improvement compared to the NE control group. Intestinal lesion scores were reduced in TRT groups fed 0.25 or 0.5% CAZ or VM as compared to the untreated NE-infected control, with no difference between the CAZ- and VM-fed groups. Serum antibodies to *C. perfringens* α-toxin and NetB toxin increased in all NE-infected groups as compared to the non-infected control. Birds fed 0.5% CAZ showed significantly lower ($P<0.05$) serum α-toxin levels post CP infection compared with the non-supplemented NE-infected control. Normalized transcripts for IL-8, IL1β, and iNOS, showed variations between treatments. In general, birds fed 0.25% or 0.5% CAZ showed lower mRNA for TNFSF15 in the intestine, and splenic inflammatory markers, IL-8, LITAF, iNOS and TNFSF15 transcripts were down-regulated as compared to the NE-infected control. These results suggest that the Calibrin-Z effectively reduced the ill effects of NE-induced gut damage thus enhancing host resistance to NE. These protective effects were equivalent or better than those of the antibiotics used in the study. Although the mechanism of clay-mediated protection from NE needs to be investigated, these results are indicative of beneficial effects of thermally processed clay (Calibrin-Z) as an alternative strategy to reduce NE-induced production loss.

Keywords: Necrotic enteritis, broiler, cytokines, immunity, clay

PREVENTION STRATEGY OF VIRAL DISEASES IN POULTRY USING 1-DEOXYNOJIRIMYCIN

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ABSTRACT

Several virus diseases in the global livestock industry is become a problem. 1-Deoxynojirimycin (DNJ) is a kind of alkaloids that inhibit the growth of virus in animal. Typically, mulberry tree contains many kinds of alkaloids including 1-deoxynojirimycin. 1-Deoxynojirimycin has the strongest antiviral activity than other alkaloids. In order to infect animal cells, most animal viruses require specific glycoprotein. 1-Deoxynojirimycin inhibites viral glycoprotein synthesis in the animal cells that make to abnormal virus particles. So 1-deoxynojirimycin can inhibit to several virus such as Influenza, Newcastle, Pneumo, Marek, Infectious bronchitis, Infectiouslaryngotracheitis virus in poultry and Circo, PRRSV, PED in swine. Biotopia is developed mass production of the 1-deoxynojirimycin by fermentation technique using bacteria as a *Bacillus subtilis* MORI-91 and commercialized the feed supplement product. 1-Deoxynojirimycin can prevent viral diseases in livestock industry.

GROWTH PERFORMANCE OF BROILER CHICKENS FED LOW-PROTEIN DIETS WITH CONSTANT CONCENTRATION OF LIMITING AMINO ACIDS UNDER A CLOSED-HOUSE ENVIRONMENT

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ABSTRACT

A study was conducted to determine the effect of feeding low-protein diets with constant concentration of lysine, methionine, threonine and trypthopan on the growth performance of broiler chickens raised in a closed-house environment. Feeding broilers low-protein diets reduced body weight (day 35) but not feed intake and feed conversion ratios (day 1-35).

Keywords: low protein diets, growth performance, feed conversion ratio

INTRODUCTION

Crude protein (CP) is the costliest ingredient. The crude protein (CP) concentration in the diet is usually carried out by stipulating its minimum requirement for broiler (Sklan and Plavnik, 2002). One approach to determine the appropriate CP level in diet is by reducing the CP concentration while supplementing fixed amount of either essential or non-essential amino acids (Pesti, 2009). The objective of this study was to determine the effect of feeding low-protein diets with constant concentration of lysine, methionine, threonine and trypthopan on the growth performance of broiler chickens raised in a closed-house environment.

MATERIALS AND METHODS

A total of 168 day-old, male broiler chickens were assigned to 42 cages in groups of six in an environmentally-controlled house. From day 21 onwards, temperature was maintained at 23±1°C and relative humidity was 65-73%. Equal number of chicks were fed isocaloric diets with 21 (control), 19.5, 18 or 16.5% CP from day 1-20 and 19 (control), 17.5, 16 or 14.5% CP from day 21-35 (Table 1). Body weight and feed intake were recorded and feed conversion ratios (FCR) and average daily gain (ADG) were calculated. All data were subjected to ANOVA and Duncan's multiple range tests were used to separate means.

RESULTS AND DISCUSSION

On day 35, broilers fed 21-19% CP and 19.5-17.5% CP had similar body weights and average daily gain (Table 2). Feeding 18-16% CP and 16.5-14.5% CP diets resulted in lower body weight and ADG when compared to controls. Kamran *et al.,* (2008) reported that supplementing crystalline AA to reduce the dietary protein level by 3% supported optimal growth and feed efficiency but higher reduction was detrimental to broiler chickens. We noted that feed intake and FCR were not affected by CP level. In conclusion, under a closed-house environment, although lower CP diets reduced body weight, FCR was not affected.

Table 1. Composition of starter and finisher diets (%)

Ingredients	Diet							
	Starter				Finisher			
	CP21%	CP19.5%	CP18%	CP16.5%	CP19%	CP17.5%	CP16%	CP14.5%
Corn	59.0	64.0	66.0	69.0	62.52	68.48	73.00	76.46
Soybean	34.0	29.6	25.5	21.15	26.99	22.13	18.20	13.65
Palm oil	3.0	2.0	2.4	2.3	4.92	3.65	2.76	2.56
Monocalcium phosphate	1.7	1.75	1.8	1.8	1.69	1.79	1.80	1.80
Limestone	1.2	1.25	1.24	1.25	1.49	1.26	1.60	1.50
Salt (NaCl)	0.5	0.5	0.5	0.5	0.5	0.50	0.50	0.50
Sand	0	0.12	1.58	2.8	1.01	1.027	0.818	1.98
Vitamin premix	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05
Mineral premix	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25
Lysine	0.17	0.3	0.38	0.48	0.34	0.48	0.55	0.66
Methionine	0.13	0.15	0.18	0.2	0.17	0.193	0.21	0.23
Threonine	0	0.03	0.1	0.18	0.07	0.151	0.21	0.29
Tryptophan	0	0	0.02	0.04	0	0.035	0.052	0.08
Calculated values								
ME (kcal/kg)	2950.70	2948.08	2952.45	2948.75	3050.54	3050.38	3050.08	3050.13
Protein	20.96	19.56	18.02	16.49	19.02	17.50	16.18	14.55
Lysine	1.20	1.23	1.21	1.20	1.20	1.22	1.20	1.20
Methionine	0.46	0.46	0.47	0.46	0.46	0.46	0.46	0.46
Threonine	0.84	0.80	0.79	0.80	0.79	0.79	0.79	0.79
Tryptophan	0.24	0.21	0.21	0.20	0.20	0.20	0.20	0.20
Analysed values								
Crude Protein	21.30	20.89	19.00	17.70	19.10	17.96	16.17	14.70
Lysine	1.041	1.019	1.015	0.912	1.012	0.972	1.020	1.011
Methionine	0.473	0.487	0.478	0.519	0.482	0.471	0.461	0.491
Threonine	0.710	0.696	0.608	0.646	0.681	0.719	0.695	0.701
Tryptophan	0.184	0.171	0.191	0.176	0.191	0.186	0.188	0.179

Table 2. Mean (±SEM) body weights, feed intake, feed conversion ratios and average daily gains in broilers

Diets	Body Weight (g) (day 35)	Feed Intake (g/bird) (day 1-35)	Feed Conversion Ratio (day 1-35)	Average Daily Gain (g/bird) (day 1-35)
21-19%CP	1886^{a}±66	3358^{a}±109	1.79^{a}±0.06	54^{a}±2
19.5-17.5%CP	1827^{ab}±30	3353^{a}±78	1.84^{a}±0.04	52^{ab}±1
18-16%CP	1707^{b}±32	3201^{a}±73	1.88^{a}±0.04	49^{b}±1
16.5-14.5%CP	1738^{b}±38	3100^{a}±126	1.78^{a}±0.05	50^{b}±1

$^{a, b}$ Means within a column with no common superscripts differ at P≤0.05.

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GROWTH PERFORMANCE OF HEAT-STRESSED BROILER CHICKENS FED EXTRUDED CANOLA MEAL

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ABSTRACT

A study was conducted to determine the effect of dietary inclusion 0, 10, 20 and 30% of extruded canola meal (ECM) on performance of boiler chickens under heat stress. From day 29-35, birds were exposed to either 36±1°C or 23±1°C. Feeding heat-stressed birds with 30% ECM did not depress growth performance but not those under unheated condition. In conclusion, 30% ECM can be included in the diets of heat-stressed broiler chickens without any adverse effect on performance.

Keywords: heat stress, broiler, growth performance, extruded canola meal

INTRODUCTION

Canola meal does not only have high protein content (40%) but also has a well-balanced amino acid composition (Newkirk and Classen, 2002). However, the high glucosinolate and fibre content limit the use of canola meal in chicken diets (Bell, 1993). One of the possible approaches to diminish the antinutritional factor and enhance the bioavailability of nutrients in canola meal is through extrusion process. Extrusion process may increase the accessibility of proteins to enzymatic breakdown (Bhattacharya and Hanna, 1988). The objective of the present study was to evaluate the effect of feeding diets with various levels of extruded canola meal (ECM) on growth performance of broiler chickens under heat stress condition.

MATERIALS AND METHODS

A total of 240 day-old male broiler chickens were housed in two environmentally controlled rooms. On day 1, chicks were fed isocaloric and isonitrogenous diets with 0, 10, 20 or 30% of ECM (Table 1). From day 29-35, equal number of birds from each dietary group was subjected to either 36±1°C or 23±1°C throughout. Relative humidity varied from 60-75%. Weight gain and feed intake during the heat treatment were recorded and feed conversion ratios were calculated. All data were subjected to ANOVA and Duncan's multiple range tests were used to separate means.

RESULTS AND DISCUSSION

There were significant diet x temperature interactions for weight gain and FCR (day 28-35) (Table 2). Irrespective of diet, the heat treatment resulted in poorer weight gain and FCR. Among the heat-challenged birds, ECM can be included in the diets up to 30% without any adverse effect on weight gain and FCR. However, under unheated condition, feeding diets with more than 10% ECM was detrimental to both weight gain and FCR. Under heat stress condition, broiler chickens may not able to express their full genetic potential because of environmental constraint. Hence, it is possible to feed 30% ECM diets without any adverse effect on broiler chickens. The

poor performance showed by birds fed more than 10% ECM could be attributed to high glucosinolate content, which may impair thyroid functions (Mushtaq *et al.*, 2007). In conclusion, under chronic heat stress condition, broiler chickens can be fed 30% ECM diets without any detrimental effect on growth performance.

Table 1. Ingredient and nutrient composition of experimental finisher diets

	Levels of extruded canola meal			
Item (%)	0%	10%	20%	30%
corn	57.43	55.89	53.57	51.25
Soybean meal	28.2	18.82	10.99	3.16
Extruded-canola meal	0.00	10.00	20.00	30.00
Corn gluten	5.00	6.00	6.00	6.00
Palm oil	5.22	5.23	5.51	5.79
Dicalicium phosphate	1.33	1.25	1.15	1.05
Limestone	1.32	1.28	1.24	1.20
Premix	1.00	1.00	1.00	1.00
Salt	0.25	0.25	0.25	0.25
DL-Meth	0.04	0.02	0.01	0
Choline Chloride	0.08	0.08	0.08	0.08
Sodium Bicarbonate	0.05	0.05	0.05	0.05
Calculated composition				
ME ,Kcal/ kg	3199	3200	3200	3200
CP%	20.0	20.0	20.0	20.0

Table 2. Mean (±SEM) weight gains (WG) and feed conversion ratios (FCR) of broiler chickens where the diet x temperature interactions were significant

Diet	**WG (day 29-35)**		**FCR (day 29-35)**	
CM %	L	H	L	H
0.00	608.50±24.73^{ax}	385.53±21.93^{y}	1.80±0.03^{by}	2.5±0.0.13^{x}
10	584.33±32.24^{abx}	359.43±37.83^{y}	1.87±0.04^{by}	2.55±0.18^{x}
20	523.00±18.43^{bx}	404.13±28.2^{y}	2.06±0.04^{ay}	2.43±0.15^{x}
30	521.00±16.22^{bx}	423.63±30.76^{y}	2.0±0.02^{a}	2.3±0.16

$^{a, b}$ Means within a column with no common superscripts differ at p≤0.05.

$^{x, y}$ Means within with a row no common superscripts differ at p≤0.05.

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EFFECT OF YEAST (*Saccharomyces cerevisiae*) AS FEED ADDITIVE ON THE PERFORMANCE OF BROILER CHICKENS

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ABSTRACT

The effect of yeast (*Sachharomyces cerevisiae)* as feed additive on the performance of broiler chickens was determined with 450 7-day-old mixed sexed Hubbard broiler chicks. Starter and finisher broiler diets were supplemented with five graded levels (0.0, 0.5, 1.0, 1.5 and 2.0 g per kg) of yeast in a completely randomised design (CRD) with 90 birds per treatment and 30 per replicate. Parameters measured were broiler daily weight gain, final live weight, feed and protein intakes, feed conversion ratio and protein efficiency ratio. Results showed that diets supplemented with yeast significantly ($P<0.05$) improved all the performance indices measured compared to the control (0.0g yeast) diet. Diet of 1.0 g yeast gave a final live weight of 2297.33 g while 2.0 g yeast diet gave a feed conversion ratio of 2.03. The conclusion was that yeast could be a good broiler feed additive and that broiler diets could be supplemented with up to 2.0 g yeast per kg.

Keywords: Broiler chickens, feed additive, performance, yeast

INTRODUCTION

Studies have been conducted using yeast in the diet of layers (Anyawale *et al.,* 2006), broilers (Azizollah *et al*., 2009) but not with broilers in the humid tropics of Nigeria. Ordinarily, poultry lack the enzymes (cellulases, hemi-cellulases and xylanases) to digest high fibre diets (Oyedeji *et al*., 2010). *Sacharomyces cerevisiae* has unidentified growth factors (Paryard and Mahmoudi, 2008). Yeast could improve broiler performance through improvement in energy and protein utilization, and may increase the ability of broilers to degrade fibrous materials in feeds. The objective of this study was to determine the effect of yeast as feed additive on the performance of broiler chickens.

MATERIALS AND METHODS

The study was conducted at the Research and Teaching Poultry Farm of Michael Okpara University of Agriculture, Umudike with 450 Hubbard 7-day-old broiler chicks. The chicks were brooded and reared in a deep litter (wood shavings) house. Water and feed were provided *ad libitum* during the rearing periods. The chicks were vaccinated against Newcastle disease at day 1 and 14, and Gumboro disease at day 9 and 21. The experimental maize-soybean starter diet had 22.04% CP and 14.45 ME (MJ/kg) and finisher diet 20.56% CP and 14.67 ME (MJ/kg). Graded levels (0.0, 0.5, 1.0, 1.5 and 2.0 g per kg) of yeast were used to supplement the diets in a completely randomized design (CRD). Each treatment was replicated three times, 90 birds per treatment and 30 broilers per replicate. Birds were weighed at 7^{th} day and subsequently on a weekly basis. Final live weight minus initial live weight gave weight gain; feed offered minus quantity not consumed gave feed intake. A 20 kg top loading weighing scale (Goat Brand^{R}) was used to weigh feed and birds at 7.00-8.00 am local time. Data collected were subjected to analysis of variance (ANOVA)

for a CRD (Steel *et al.*, 1997). The differences among treatment means were separated using Duncan's Multiple Range Test.

RESULTS AND DISCUSSION

Diets supplemented with yeast significantly (P<0.05) improved daily weight gain, final live weight, daily feed and protein intakes, feed conversion ratio and protein efficiency ratio (Table 1). Broilers fed control (0.0 g yeast) diet consistently had poor performance compared to those fed yeast diets. There were no significant (P>0.05) differences among the broilers fed yeast diets in all indices measured except daily feed intake. The improved performance of broilers fed graded levels of yeast in feed could be attributed to the presence of glucans and fructo-oligossaccharides in yeast (Rutz *et al.*, 2006). It has also been reported that broiler diets supplemented with yeast improved feed intake, weight gain, body weight and feed conversion ratio (Paryard and Mahmoudi, 2008).

Table 1. Performance of broilers fed diets supplemented with graded levels of yeast (*Saccharomyces cerevisiae*)

Parameters	0.0g	0.5g	1.0g	1.5g	2.0g	SEM
Initial live weight (g)	165.33	166.00	165.80	166.10	166.30	5.17
Final live weight (g)	1957.66^{b}	2184.67^{a}	2297.33^{a}	2174.33^{a}	2270.33^{a}	23.47
Daily weight gain (g)	36.58^{c}	41.20^{ab}	43.50^{a}	40.98^{ab}	42.94^{a}	0.81
Daily feed intake (g)	83.93^{c}	88.45^{ab}	92.87^{a}	90.76^{a}	86.98^{b}	1.22
Feed conversion ratio	2.29^{a}	2.15^{b}	2.13^{b}	2.21^{ab}	2.03^{b}	0.09
Daily protein intake (g)	17.44^{c}	18.38^{ab}	19.27^{a}	18.90^{a}	18.07^{b}	0.07
Protein efficiency ratio	2.10^{b}	2.24^{ab}	2.26^{a}	2.17^{ab}	2.38^{a}	0.09
Mortality (%)	5.56	4.44	3.33	4.44	3.33	0.02

a,b,c: Means within the same row with different superscripts are significantly (P<0.05) different.

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EFFECTS OF FEEDING FERMENTED SAGO PITH MEAL ON THE GROWTH PERFORMANCE OF BROILER CHICKENS

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ABSTRACT

Rhizopus sp. was used to improve the nutritive value of sago pith meal by solid state fermentation (SSF). The optimized conditions for SSF were moisture 50ml/50 g substrate, pH 6.0, 30°C and 1% ammonium sulphate for 44h incubation. The crude protein increased but the crude fibre decreased slightly. Feeding broiler chickens with diet containing fermented SPM (Diet C) increased the average weight gain to 1.33±0.143 kg but group fed on diet containing untreated SPM (Diet B) had lower weight gain 1.27±0.162 kg. The FCR of Diet C group was higher than FCR of Diet A group; 2.15±0.243 and 1.99±0.316, respectively. The organs to live weight ratios of liver, bursa, heart, spleen and gizzard were not significantly different and the hepatic histopathology did not show any abnormalities. Fungal fermentation enhanced the nutritive value of SPM and broilers fed diet containing 25% of fermented SPM during the grower-finisher period showed better feed efficiency when compared to broilers fed untreated SPM.

Keywords: Sago pith meal, *Rhizopus sp.,* solid state fermentation, broilers

INTRODUCTION

Local feedstuffs such as palm kernel cake, rice bran, cassava meal and sago products have been identified as alternative feeds to partially substitute feeds like corn and soybean for poultry. However, their low nutritive values limit their inclusion in poultry diet. Solid state fermentation (SSF) by microbial cultures was proposed as a strategy for enhancing the nutritive value of feeds (Mitchell and Lonsane, 1992). Sago pith meal (SPM) has been used in poultry feed formulations in the South East Asian countries. This study was undertaken to evaluate SSF by fungal culture as a mean of enhancing the nutritive value of SPM and to investigate the effects of feeding diets containing fermented SPM during the grower-finisher period on the growth performance of broiler chickens.

MATERIALS AND METHODS

Solid state fermentation: Nutrient composition of dry sago pith meal (SPM) was determined according to standard procedures. *Rhizopus* sp. isolated from tempeh was used for SSF. The fermentation was optimized in flask system using autoclaved substrate through a series of fermentation parameters. The optimized condition was used in the scaling up and mass production in laboratory fermenter.

Feeding trials: Diets containing untreated and fermented SPM were fed during the grower-finisher period to 180 1-day-old Cobb broilers. Three diets were: A - control with 61% corn meal; B- diet containing 36% + 25% untreated SPM and C-diet containing 36% + 25% fermented SPM. At day 22, the chicks were assigned randomly into 3 groups with 6 replicates. Weight gains and total feed intake were measured

weekly and at day 42 they were sacrificed for organs weights, liver histopathology and serum biochemical parameters.

RESULTS AND DISCUSSION

The optimized conditions for SSF were moisture 50ml/50 g substrate, pH 6.0, 30°C and 1% ammonium sulphate for 44h incubation. The crude protein increased but the crude fibre decreased slightly (Table 1). When compared to corn meal, the protein content in fermented SPM was lower and this may limit the amount of inclusion in poultry diet. Feeding broiler chickens with diet containing fermented SPM (Diet C) increased the average weight gain to 1.33±0.143 kg but group fed on diet containing untreated SPM (Diet B) had lower weight gain 1.27±0.162 kg (Table 2). The FCR of Diet C group was higher than FCR of Diet A group; 2.15±0.243 and 1.99±0.316, respectively. The organ to live weight ratios of liver, bursa, heart, spleen and gizzard were not significantly different and the hepatic histopathology did not show any abnormalities.

Table 1. Nutrient composition of treated and untreated sago pith meal

Nutrient composition	**Dry matter**	**As % of dry matter**			
		Crude protein	Crude fibre	Ash	Gross energy (cal/kg)
SPM (untreated)	88.2	1.6	6.0	5.3	3608
SPM (fermented)	89.1	3.4	5.5	6.3	3495
Corn meal*	89.0	8.5	2.2	4.0	3350

*Corn meal as comparison (NRC, 1994)

Table 2. Body weight and feed conversion ratio (FCR) of chicken fed with broiler finisher diet containing 25% SPM

Parameters	**Diet A (Control)**	**Diet B (+ 25% Untreated SPM)**	**Diet C (+ 25% Fermented SPM)**
Initial weight at day 22 (kg)	0.56 ± 0.083	0.55 ± 0.066	0.53 ± 0.066
Final weight at day 42 (kg)	2.01 ± 0.205	1.82 ± 0.183	1.86 ± 0.177
Weight gain	1.45 ± 0.192 ^{a}	1.27 ± 0.162 ^{c}	1.33 ± 0.143 ^{b,c}
Feed intake/bird (kg/bird)	2.83 ± 0.086 ^{b}	2.89 ± 0.129 ^{a}	2.83 ± 0.075 ^{b}
FCR	1.99 ± 0.316 ^{c}	2.32 ± 0.357 ^{a}	2.15 ± 0.243 ^{b}

^{a,b,c}: Values are means ± S.D. Means with different supercripts within a row are significantly different ($P<0.05$)

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EFFECT OF PROTEASE ON APPARENT CRUDE PROTEIN AND METABOLISABLE ENERGY DIGESTIBILITIES IN BROILER CHICKENS FED LOW PROTEIN DIET

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ABSTRACT

A study was conducted to determine the effects of two types of commercial protease on apparent crude protein and metabolisable energy digestibilities of 27 day-old boiler chickens. Apparent crude protein (ACP) and metabolisable energy (AME) were reduced in low protein diet (CP16). Protease B significantly increased ACP and AME digestibility.

Keywords: commercial protease, crude protein, metabolisabe energy

INTRODUCTION

The quality of a protein source depends on the presence of essential amino acids content and availability (Ravindran *et al*., 2005) and protein antinutritional factors (Frikha *et al*., 2012). Adding protease in feed may help to hydrolyze the peptide bonds thus improve the availability and digestibility of protein in feed (Angel *et al*., 2011; Liu *et al*., 2013). The objective of the present study was to qualify and quantify the efficiency of two commercial proteases on apparent crude protein (ACP) and apparent metabolisable energy (AME) in normal (Cobb standard) and low protein diet.

MATERIALS AND METHODS

A total of ninety-six, 21-day-old male broiler chicks were equally assigned to two levels of dietary proteins; 19% and 16%. Both diets were iso-caloric and meet the Cobb requirement for digestible lysine, digestible metionine, digestible threonine and digestible tryptophan (Table 1). Each diet was added with protease A (200 ppm) or protease B (500 ppm). A diet without protease was considered as a control group. Ileal digesta were collected after 5 days of feeding (Soleimani *et al.,* 2010). Titanium was used as a marker. All data were analyzed using GLM of SAS (SAS Institute, 2002).

RESULTS AND DISCUSSION

Lower dietary crude protein decreased ACP and AME (Table 2). Protease B significantly improved the ACP (both diets) and AME (19% crude protein diet). Protease may help to hydrolyze the undigested protein present in the GIT (Angel *et al*., 2011). Hence, addition of protease B may allow the use of lower crude protein diet in broiler chickens.

Table 1. Composition of experimental diets

		19% crude protein			16% crude protein			
Ingredient	Basal diet	No enzyme	Protease A	Protease B	Basal Diet	No enzyme	Protease A	Protease B
Corn	61.45	60.010	60.010	60.010	66.84	65.970	65.970	65.970
Dehulled Soybean Meal	29.62	29.080	29.080	29.080	16.63	16.960	16.960	16.960
Canola Meal	0.250	1.220	1.220	1.220	5.00	4.810	4.810	4.810
Palm Kernel Ccake	0.120	0.210	0.210	0.210	2.93	2.850	2.850	2.850
Palm Oil	5.500	5.940	5.940	5.940	5.15	5.460	5.460	5.460
Limestone	1.130	1.110	1.110	1.110	1.12	1.120	1.120	1.120
Sodium Chloride	0.340	0.340	0.340	0.340	0.34	0.340	0.340	0.340
MDCP	1.280	1.280	1.280	1.280	1.29	1.290	1.290	1.290
Premix	0.125	0.125	0.125	0.125	0.125	0.125	0.125	0.125
Lysine HCL	0.030	0.030	0.030	0.030	0.27	0.270	0.270	0.270
DL-Methionine	0.120	0.120	0.120	0.120	0.15	0.150	0.150	0.150
Threonine	0.020	0.020	0.020	0.020	0.14	0.140	0.140	0.140
Antioxidant	0.015	0.015	0.015	0.015	0.015	0.015	0.015	0.015
Titanium Oxide	-	0.500	0.500	0.500	-	0.500	0.500	0.500
Protease	-	-	0.020	0.050	-	-	0.020	0.050
Calculated values								
ME (kcal/kg)	3180	3180	3180	3180	3180	3180	3180	3180
Crude protein, %	19.0	19.0	19.0	19.0	16.0	16.0	16.0	16.0
Lysine	1.06	1.05	1.05	1.05	1.04	1.06	1.06	1.06
Methionine	0.42	0.42	0.42	0.42	0.42	0.41	0.41	0.41
Threonine	0.75	0.75	0.75	0.75	0.73	0.73	0.73	0.73
Dig. Lysine	0.95	0.95	0.95	0.95	0.95	0.95	0.95	0.95
Dig. Methionine	0.39	0.39	0.39	0.39	0.39	0.39	0.39	0.39
Dig. Threonine	0.65	0.65	0.65	0.65	0.65	0.65	0.65	0.65
Analysed values (DM basis)								
	-	19.29	20.14	19.45	-	16.22	17.14	16.38

*The inclusion levels of Protease A and Protease B were based on recommended dosage by the supplier as top-up basis, 200 ppm and 500 ppm respectively.

Table 2. Effects of protease and dietary crude protein on apparent crude protein and metabolisable energy in broiler chickens

	Apparent Crude Protein Digestibility		**Apparent Metabolizable Energy Digestibility**	
Diet (%)	CP19	CP16	CP19	CP16
Control	$91.49^{a,y} \pm 0.50$	$87.89^{b,y} \pm 0.83$	$86.99^{y} \pm 0.46$	84.96 ± 1.06
Protease A	$91.01^{a,y} \pm 0.60$	$88.75^{a,xy} \pm 1.05$	$86.46^{y} \pm 0.44$	84.9 ± 1.20
Protease B	$92.91^{a,x} \pm 0.38$	$90.31^{b,x} \pm 0.41$	$89.50^{x} \pm 0.43$	86.9 ± 1.13

^{a,,b} Means within with a row no common superscripts differ at P≤0.05.

$^{x,\,y}$ Means within a column with no common superscripts differ at P≤0.05.

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EFFECT OF SUPPLEMENTING LOW–PROTEIN DIETS WITH ESSENTIAL AMINO ACIDS ON GROWTH PERFORMANCE OF BROILER CHICKENS UNDER THE HOT AND HUMID TROPICAL CONDITIONS

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ABSTRACT

A three weeks trial was conducted to determine the effect of lowering crude protein (CP) with optimal amino acid (AA) profile on performance of broilers under tropical hot and humid environment. Five isocaloric (3023 ME/kg) experimental diets in which the CP was gradually reduced by 1.5% from 22.2% to 16.2% were formulated to meet or exceed NRC (NRC, 1994) recommendations from 1-21d except for the protein and metabolizable energy. Body weight (BW) and weight gain (WG) were significantly (P≤ 0.001) decreased, feed intake (FI) significantly (P≤0.05) decreased, whereas, feed conversion ratio (FCR) was significantly (P≤ 0.0003) increased when CP reduced.

Keywords: crude protein level, amino acid, broilers performance, tropical conditions

INTRODUCTION

The price of crude protein (CP) ingredients like soybean meal is on the rise worldwide. Therefore, it is important economically to formulate diets to meet the exact amino acid (AA) requirements for optimum performance under the hot, humid tropical conditions. Besides reducing feed costs, the ability to lower CP may result in decreased nitrogen excretion (Bregendahl *et al.*, 2002). However, it is still unclear to what extent the AA supplementation can replace the CP without adversely affecting broiler performance in such environment. Therefore, the objective of current study was to determine the effect of amino acid-fortified diets with lower CP on growth performance of broilers under hot and humid tropical condition during starter period.

MATERIALS AND METHODS

A trial was conducted in a naturally ventilated, open sided house from 1–21days. A total of 375 day-old male broiler chicks (Cobb x Cobb) were obtained from a commercial hatchery. On day 1, the chicks were randomly assigned to five dietary groups. Each group had five replicates with 15 chicks per pen. Five isocaloric (3023 ME/kg) experimental diets in which the dietary protein was gradually reduced by 1.5% from 22.2% to 16.2% were formulated to meet or exceed NRC 1994 recommendations from 1-21 days except for the CP and metabolizable energy (Table 1).

Table 1. Nutritional composition of the experimental diets

Ingredient	Level of crude protein (%)				
	16.2	17.7	19.2	20.7	22.2
ME (kcal/kg)	3023	3023	3023	3023	3023
Crude protein (%)	16.2	17.7	19.2	20.7	22.2
Calcium (%)	0.94	0.94	0.94	0.94	0.94
Available phosphorus (%)	0.45	0.45	0.45	0.45	0.45

Table 2. Effect of dietary protein level on performance of broilers from 1–21 d[1]

Item	Level of crude protein (%)				
	16.2	17.7	19.2	20.7	22.2
BW (g per bird)	632 ± 2[c]	747 ± 4[b]	805 ± 29[a]	816 ± 7[a]	830 ± 18[a]
WG (g per bird)	594 ± 2[c]	707 ± 5[b]	762 ± 29[a]	778 ± 7[a]	792 ± 19[a]
FI (g per bird)	953 ± 14[b]	1047 ± 7[a]	1043 ± 46[a]	1084 ± 16[a]	1078 ± 30[a]
FCR (feed/ gain)	1.60 ± 0.03[a]	1.48 ± 0.02[b]	1.37 ± 0.07[c]	1.39 ± 0.01[bc]	1.36 ± 0.01[c]

[1]Data are means of 5 replications of 15 birds per pen.

[a - c] Means within rows followed by different superscript letters are significantly different (P≤0.05).

RESULTS AND DISCUSSION

Birds fed 19.2% CP diet had similar body weight and FCR as those fed 22.2% CP (Table 2). Feeding 16.2% and 17.7% CP diets resulted in a significantly lower body weight and weight gain (Table 2). Chicks fed 16.2% CP diet showed the poorest FCR when compared to other groups. The poor growth performance of broilers fed excessive low crude protein level could be due to several factors. Among these, the change in dietary potassium or dietary electrolyte balance (Waldroup, 2000), decreased feed intake (Aftab *et al.,* 2006), the lack of sufficient nitrogen amount for non-essential AA synthesis (Waldroup, 2007) and too low Gly + Ser concentration (Dean *et al.,* 2006) in birds fed 16.2% CP. In conclusion, dietary CP can be reduced up to 19.2% without any adverse effect on growth performance of chicks from day 1-21.

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PHYSICAL PROPERTIES OF PALM KERNEL CAKE

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ABSTRACT

A systematic sieving method (500 g sample; 50 Hz, 20 min) was used to obtain mean sizes of 6.38, 3.72, 2.40, 1.50, 0.75, 0.40, 0.15mm of palm kernel cake (PKC). The highest mass percentage was 20.9% for 0.15 mm particle size. The lowest mass percentage of 7.6% was observed for 2.40 mm particle size. The mean particle size of PKC was 1.79±0.09 mm. The swelling capacity and water retention capacity of different particle sizes of PKC ranged from 2.13 to 3.81 ml/g dry matter and 1.98 to 2.66 g/g dry matter of sample, respectively. The highest protein content was observed in 0.40 mm particle size while the lowest was in 1.50 mm. The ash, crude fat and crude fiber contents were significantly ($P<0.05$) different among particle sizes of PKC.

Keywords: particle size, water holding capacity, water retention, proximate analyses

INTRODUCTION

Palm kernel cake (PKC), has been well studied for chemical properties, but minimal studies on the hydration properties such as water retention and swelling capacities ofdifferent particle sizes. The PKC contains 78% hemicellulose and 12% cellulose. Hydration and chemical properties are important information for the incorporation of PKC in broiler diet. Therefore, the main objective of this study was to characterize PKC in terms of particle size and to determine how different sizes affect the hydration capacity and chemical composition.

MATERIALS AND METHODS

Palm kernel cake samples were obtained from an oil palm factory in Klang, Selangor. Particle size distribution of PKC was determined using the method by (Saw *et al*., 2012) using the following nominal apertures; 8.00, 4.75, 2.80, 2.00, 1.00, 0.50 and 0.30 mm. The mass percentage was obtained by dividing the weight of each particle size by the total weight, while t he mass mean diameter was calculated for each particle size by multiplying the mean size and mass percentage. The values were added and divided by 100. The swelling capacity and water retention capacity were determined according to the method described by Robertson *et al*. (2000). The proximate analyses of crude fat, fiber, protein and ash were according to standard AOAC methods (AOAC, 2005).

RESULTS AND DISCUSSION

The highest mass percentage was shown by particle size 0.15 mm (20.9±0.87%), followed by 1.5 mm (15.0±0.14%). Particle size 2.40 mm had the lowest mass percentage of 7.6±0.20%. The variation of the oil extraction process led to the production of various particle sizes. The highest swelling capacity was shown by 1.50 mm particle size, followed by 3.72 mm. The difference in swelling capacity could be due to the presence of water soluble materials (Robertson *et al.,* 2000). The highest water retention capacity was shown by particle size 0.40 mm, followed by 0.75 mm.

Crude protein content was significantly (P<0.05) higher in particle size 0.40 mm. The ash content was higher in particle sizes of 0.40 mm and 0.15 mm. The crude fat of particle size less than 3.72 mm was almost twice than larger particle sizes. High crude fiber content was observed in particle size of 3.72, 1.50 and 2.40 mm, probably due to the presence of higher amount of shells. Particle sizes showed significant effects on swelling capacity as well as water retention capacity. Significant differences in crude protein, ash, crude fat and crude fiber contents were observed among different particle sizes of PKC.

Table 1. Hydration properties of different particle sizes of PKC

Particle size (mm)	Swelling capacity (ml/g PKC)	Water retention capacity (g/g PKC)
>8.00	2.93 ± 0.00^{c}	2.15 ± 0.15^{b}
6.38	2.91 ± 0.01^{c}	2.13 ± 0.00^{b}
3.72	3.73 ± 0.09^{a}	1.98 ± 0.02^{cb}
2.40	2.75 ± 0.04^{c}	1.96 ± 0.08^{cb}
1.50	3.81 ± 0.03^{a}	1.81 ± 0.07^{cb}
0.75	3.53 ± 0.01^{ab}	2.66 ± 0.03^{a}
0.40	2.94 ± 0.09^{c}	2.71 ± 0.05^{a}
0.15	3.33 ± 0.25^{b}	2.16 ± 0.18^{b}
Whole PKC	2.13 ± 0.01^{d}	2.53 ± 0.00^{a}

Different superscripts indicate significant difference (P<0.05).

Table 2. Chemical composition of different particle sizes of PKC

Particle size (mm)	Proximate composition (%)			
	Crude fat	Crude fiber	Crude protein	Ash
>8.00	5.0 ± 0.83^{c}	23.2 ± 1.26^{c}	17.4 ± 0.32^{abc}	4.4 ± 0.04^{b}
6.38	4.4 ± 0.40^{c}	24.6 ± 1.19^{abc}	17.0 ± 0.39^{bc}	4.5 ± 0.04^{b}
3.72	8.8 ± 0.20^{ab}	27.7 ± 1.10^{a}	16.7 ± 0.36^{dc}	4.2 ± 0.02^{c}
2.40	9.7 ± 0.31^{a}	26.0 ± 1.83^{abc}	16.6 ± 0.38^{cd}	4.3 ± 0.03^{c}
1.50	9.0 ± 0.18^{ab}	27.5 ± 0.43^{ab}	16.2 ± 0.20^{d}	4.4 ± 0.13^{bc}
0.75	9.5 ± 0.24^{ab}	24.3 ± 0.19^{bc}	17.6 ± 0.33^{ab}	4.5 ± 0.09^{b}
0.40	8.5 ± 0.26^{b}	23.4 ± 1.06^{c}	17.9 ± 0.00^{a}	4.7 ± 0.04^{a}
0.15	9.8 ± 0.34^{a}	23.9 ± 1.21^{c}	17.7 ± 0.21^{ab}	4.9 ± 0.01^{a}
Whole PKC	5.4 ± 0.01^{c}	24.1 ± 1.22^{c}	16.8 ± 0.03^{cd}	4.4 ± 0.06^{bc}

Different superscripts indicate significant difference (P<0.05).

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COMPARATIVE GROSS MORPHOMETRY EVALUATIONS OF THE GASTROINTESTINAL TRACT OF EDIBLE BIRD'S-NEST SWIFTLET *(AERODRAMUS FUCIPHAGUS)* AND HOUSE SWIFT (*APUS NIPALENSIS)*

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ABSTRACT

The comparative gross morphometrical evaluations of the gastrointestinal tract of two species of aerial insectivorous bird from Apodidae family; Edible Bird's-Nest Swiftlet (*Aerodramus fuciphagus*) and House Swift (*Apus nipalensis*) were conducted. The results revealed that the actual weight and length as well as the relative weight and length of the esophagus were significantly higher (P≤0.05) in *Apus nipalensis* as compared to the *Aerodramus fuciphagus*. The actual weight and length of *Apus nipalensis* were higher but relatively significantly lower *(*P≤0.05*)* compare to *Aerodramus fuciphagus*. Similarly, the actual weight and length of the duodenum of *Apus nipalensis* was higher than *Aerodramus fuciphagus* but the relative weight and length found to be higher *(*P≤0.05*).* The values of remaining intestinal segments showed a similar pattern with the duodenum.

Keywords: Gastrointestinal tract, Apodidae, morphometrical evaluation

INTRODUCTION

Edible Bird's-Nest Swiftlet *(Aerodramus fuciphagus)* or EBN Swiflet and House Swift (*Apus nipalensis*) are both insectivorous birds belong to Apodidae family that forages on insects as their feed (Chantler, 1999; Birdlife Int., 2013). The gross morphology of the avian gastrointestinal tract (GIT) had been studied for centuries to understand the relationship between avian and the diet consumed which had been demonstrated to have related to their diet (McLelland, 1989). This morphometrical evaluation of the GIT had been used as the indicator to understand the anatomical as well as the development of the tract itself (Duritis and Mugurevics, 2011). Thus, the objective of the study was to determine the differences between them by using the morphometric evaluation.

MATERIALS AND METHODS

A total of 6 birds of *Apus nipalensis* and 7 birds of EBN Swiftlet were used in this study. The birds were euthanised with barbiturates at 60mg/kg (AVMA, 2007). Whole GIT was removed from the abdomen. The length of GIT was measured using the standard stainless steel ruler. The GIT was cut and divided into several sections according to Waugh *et al*. (2007). The weight and length of each section were measured and recorded. The results were tabulated based on the actual weight and length, and the ratio to body weight and GIT length according to Duritis and Mugurevics (2011), analyzed and significant level was set at P≤0.05.

RESULTS AND DISCUSSION

The length of the esophagus in EBN Swiflet was 3.50±0.14 cm which is longer in House Swift with 4.70±0.24 cm. The weight of the esophagus was also higher in

House Swift (0.29±0.04 g) as compared to EBN Swiftlet (0.08±0.01 g). The relative weight and length of the esophagus was also significantly higher (P≤0.05) in House Swift as compared to EBN Swiftlet. The weight of EBN Swiftlet stomach was 0.40±0.02 g, while *Apus nipalensis* was 1.13±0.05 g. The length of stomach in EBN Swiftlet was 2.43±0.13 cm which was not significantly different from House Swift (2.45±0.03 cm). The relative weight of the stomach in EBN Swiftlet was 4.90±0.17 g which was significantly higher (P≤0.05) to the relative weight of the stomach in *Apus nipalensis* (4.28±0.20 g). The relative stomach length was also significantly higher (P≤0.05) in EBN Swiftlet as compared to House Swift. The weight of duodenum in *Aerodramus fuciphagus* was 0.12±0.01 g and 0.23±0.01 g for House Swift. The actual weight of duodenum in House Swift was higher but relatively lower (P≤0.05) than the EBN Swiftlet. The length of duodenum showed no significant differences between two species with 2.97±0.15 cm in EBN Swiftlet and 2.98±0.19 cm in House Swift respectively. The remaining intestinal weight of *Apus nipalensis* showed higher values than the EBN Swiftlet but relatively lower (P≤0.05) than the House Swift. The length of remaining intestines of EBN Swiftlet was 7.17±0.19 cm while in House Swift which significantly higher the lengths were 7.83±0.15 cm and 9.75±1.75 cm in male and female, respectively. The relative remaining intestinal length was significantly higher (P≤0.05). Only male birds can be analyzed with Mean±SE due to inadequate number of females. The higher relative value of the gastrointestinal tract in *Aerodramus fuciphagus* may correlate to the functional properties of the tract itself or the body requirements.

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EFFICACY OF A *BACILLUS SUBTILIS* PROBIOTIC ON GROWTH PERFORMANCE AND INTESTINAL MORPHOLOGY OF BROILER CHICKENS

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ABSTRACT

Effects of feeding probiotic (*Bacillus subtilis;* BS) or antibiotic growth promoter (AGP) alone or in combination on growth performance and intestinal morphology of broilers was studied. A total of 480 day-old male Cobb-400 broilers were grouped into 4 treatments and fed different diets: **(1)** corn-soybean meal based control (C), **(2)** C+AGP (100 ppm of oxytetracyclin and neomycin), **(3)** C+BS (500 g/T) and **(4)** C+BS+AGP. At day 42, feeding BS or AGP alone numerically improved body weight gain (BWG) of broilers compared with control whereas the combination of BS+AGP resulted in higher BWG indicating a synergistic effect. Feed conversion ratio improved (P≤0.05) when broilers were fed BS, or BS+AGP diets compared with C diet. Significant increase in duodenal villus height was observed when broilers were fed BS or AGP alone compared with those fed C diet. Feeding BS or BS+AGP diet increased (P≤0.05) the VH of jejnum and ileum compared with C diet, whereas VH was reduced in ileum of broilers fed AGP diet.These results indicate that BS can be a viable alternative or synergistic partner to AGP.

Keywords: Probiotic, AGP, growth, intestinal morphology, broilers

INTRODUCTION

Probiotics are live microbial feed supplements which positively impact the host by improving its intestinal microbial balance. They are considered to be one of most promising alternatives to antibiotic growth promoters (AGPs) to promote gut health. Among the probiotics, *Bacillus*-based probiotics seem to be most suitable candidates for in-feed applications because of their spore forming ability. These spores are tolerant to heat, harsh pH conditions, pressure, coccidiostats and antibiotics. Furthermore, *Bacillus* germinates in the gut and grows as vegetative cells, and can exert several benefits to poultry including reduction of pathogens, improved intestinal health and growth performance. A study was conducted to evaluate the effects of feeding a *Bacillus subtilis*-based probiotic alone or in combination with AGP on growth performance and intestinal morphology of broilers.

MATERIALS AND METHODS

A total of 480 Cobb-400 male birds were randomly allocated to 4 dietary treatments with 6 replicate pens of 20 birds each. The four dietary treatments were: (1) Corn-soybean meal based control (C), (2) C+AGP (100 ppm neomycin and oxytetracyclin), (3) C+*Bacillus subtilis* probiotic (BS; 500 gm/T of feed), and (4) C+AGP+BS. The starter and finisher diets were offered in mash form from 0–21 and 22–42 days of age, respectively. The diets were formulated to meet the requirements of all nutrients for commercial broiler chickens under Malaysian rearing conditions. Birds were maintained in a deep litter open-sided poultry house and feed and water were provided *ad libitum*. Body weight (BW) and feed intake (FI) were recorded weekly and body weight gain (BWG) and feed conversion ratio (FCR) were calculated. At the end of experiment, a total of 20 birds/treatment weighing close to the average body

weight of the respective pen was selected and were used to study villus height (VH) of duodenum, jejunum and ileum. Data was analyzed using a randomized complete block design following GLM procedures of SAS (1998) with each pen being used as the experimental unit. The means of the treatments were compared by Duncan's multiple range test (Duncan, 1955). Treatment differences were considered significant when P≤0.05.

RESULTS AND DISCUSSION

Feeding BS or AGP alone numerically improved BWG of broilers compared with C while the combination of BS + AGP resulted in higher BWG indicating a synergistic effect. Feed intake was significantly lower in broilers fed BS alone compared with other groups. While FCR significantly improved when broilers were fed BS or BS+AGP diets compared with C (Table 1). Significant increase in duodenal VH was observed when broilers were fed BS or AGP alone compared with C. However synergistic effect was observed in broilers fed a combination of BS and AGP (Table 1). Feeding BS or BS+AGP diet significantly increased the VH of jejunum and ileum compared with C diet whereas VH was reduced in ileum of broilers fed AGP diet.

Table 1: Growth performance and intestinal morphology of broilers at 42 days

	Control (C)	C+AGP	C+BS	C+AGP+BS	SEM
Growth					
BWG (kg)	2.09	2.13	2.15	2.18	0.01
FI (kg)	3.65^{a}	3.66^{a}	3.55^{b}	3.60^{a}	0.05
FCR (kg/kg)	1.75^{a}	1.73^{ab}	1.66^{b}	1.65^{b}	0.02
Intestinal morphology (VH, µm)					
Duodenum	1682.47^{c}	1756.38^{b}	1752.00^{b}	1871.62^{a}	9.7
Jejunum	1183.38^{c}	1200.43^{c}	1407.74^{a}	1310.50^{b}	9
Ileum	738.83^{b}	676.96^{c}	872.35^{a}	843.80^{a}	7

$^{a-c}$Means in the same row with common superscript are not significantly different (P > 0.05).

This study indicated that BS can positively influence all segments of small intestinal alone or in combination with AGP. Therefore, improvement in intestinal morphology is one of the mechanisms for the observed improvement in growth performance of broiler chickens when fed BS. In conclusion, BS is a viable alternative or synergistic partner to AGP as evidenced by improved growth performance and small intestinal morphology.

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MORPHOLOGICAL EVALUATION OF SUBMANDIBULAR SALIVARY GLANDS OF WHITE (*AERODRAMUS FUCIPHAGUS*) AND BLACK (*AERODRAMUS MAXIMUS*) EDIBLE BIRD'S NEST SWIFTLET

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ABSTRACT

The *Aerodramus fuciphagus (A. fuciphagus)* and *Aerodramus maximus (A.maximus)* are known to use their saliva as the nest building material during breeding season. The histochemical characterizations of Edible Bird's Nest swiftlet *Aerodramus fuciphagus (A. fuciphagus)* and *Aerodramus maximus (A.maximus)* submandibular salivary glands were studied and evaluated in 5 male and 5 female birds. The birds were humanely euthanised using 15mg/kg sodium phenobarbital. The histochemical staining used in this study were Alcian Blue-PAS, PAS, Aldehyde fuchsin-Alcian Blue and Alcian Blue at pH 1 and pH 2.5. This study demonstrated that the submandibular salivary glands were weakly sulphated mucins producing glands and acidic carboxylated mucins which are mass produced to build the nest as well as lubricating of the food ingested.

Keywords: EBN swiftlets, submandibular salivary gland, histochemical

INTRODUCTION

The *Aerodramus fuciphagus (A. fuciphagus)* and *Aerodramus maximus (A.maximus)* are insectivorous birds contained within the swift family and known to use their saliva as the nest building material during breeding season. In Malaysia, *A. fuciphagus* were commercially ranched in modified bird's houses in commercial business town area and also rural farming area. The *A. maximus* were only found in the natural caves of Sabah and Sarawak (Koon, 2000). In Sabah, they can be found in the Gomantong and Madai Caves. The knowledge and information on the structure, composition and localizations of mucins of salivary glands in *A. fuciphagus* and *A. maximus* has never been reported. Thus, this study aimed to define the morphology of the *A. fuciphagus* and *A. maximus* salivary glands and to evaluate their histochemistry components.

MATERIALS AND METHODS

Five (5) male and female birds of each *Aerodramus fuciphagus (A. fuciphagus)* and *Aerodramus maximus (A.maximus)* were trapped using net in PTH Tersat, Terengganu and Gomantong Caves, Sabah respectively. They were humanely euthanised using 15 mg/kg sodium Phenobarbital (Vetoquinol®) intramuscularly. The tissues were processed and routinely stained using Haematoxylin and Eosin (H&E) stain. Histochemical staining were done for Alcian Blue pH 1 and Alcian Blue pH 2.5 to differentiate and identify the different acid mucins, Alcian Blue-PAS (for acid mucins and neutral mucins), PAS (for α Amylase) and Aldehyde fuchsin-Alcian Blue (to differentiate the sulphated and carboxylated mucins). The histological

appearance like color intensity and distribution was determined and described for each gland.

RESULTS AND DISCUSSIONS

The histological evaluations of the submandibular salivary gland of both sexes of the *A. fuciphagus* and *A. maximus* showed no morphological difference. The glands consisted of tall columnar cells with flattened nuclei which can be found at the basement membrane. The glands were divided into numerous lobules and each lobules contained numerous secretory units and separated by the supporting tissue septa. The lobules were supplied by the numerous blood vessels, nerves and large secreatory duct. The hyperthrophy and hypothrophy of the tall columnar cell were seen in the enlarged submandibular glands in both sexes of both species. The nuclei of the mucous cells were characteristically condensed and flattened against the basement membrane and the cytoplasm. Numerous mucigen granules can be seen at the supranuclear region due to poor staining of the mucous acini but was not clearly seen in the hyperthrophy condition of the salivary glands.

The submandibular glands were satisfactorily alcianophilic reaction in Alcian blue pH 2.5 as compared to pH 1. This showed that the submandibular glands were also weakly sulphated mucins producing glands. Staining using Aldehyde Fuschin–Alcian Blue Staining showed a showed that the gland was stained blue mostly at the apical region which indicated that the sublingual salivary gland produced carboxylated mucins. A combined Alcian blue and PAS staining showed slightly different stain intensity in submandibular glands of *A. fuciphagus* which was lightly stained compared to *A. maximus* but both tissues were stained blue indicating that they were acidic mucins producing gland. The PAS-positive reaction revealed the presence of sialidase-labile sialomucins in this gland. The secretion of salivary mucins containing sialomucins with terminal sialicacid residues linked to b-galactose-N-acetylgalactosamine or a N-acetylgalactosamine often located in distinct secretory elements which was similar to those reported for lingual glands in the chicken (Gargiulo *et al.*, 1991), kingfisher, parrot, sparrow, pigeon (Nalavade and Varute, 1977) and quail (Menghi *et al.*, 1993) which may contribute to the acidity of the saliva produced.

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CHITOSAN COATED ALGINATE-XANTHAN GUM-β-CYCLODEXTRIN BASED MICROCAPSULES ENHANCED pH RESISTANCE AND HEAT TOLERANCE OF *LACTOBACILLUS* SP. LAB12 *IN VITRO*

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ABSTRACT

Probiotics have become increasingly recognised for their importance in poultry production. Given their lack of detrimental effects, probiotics has emerged as good alternative for antibiotics. This has resulted in their extensive use in improvement of poultry's performance. The susceptibility of probiotics to gastric acid and heat however, remain as major challenges for effective delivery and pelleting process of poultry feed. The present study aimed to enhance the pH resistance and thermo tolerance of a potential probiotic by incorporating *Lactobacillus plantarum* LAB12 into alginate (Alg)-xanthan gum (XG)-β-cyclodextrin (β-CD) based microcapsules coated with chitosan (Ch). The LAB12-loaded microcapsules were characterised in term of their physicochemical properties. Cell viability was assessed by subjecting LAB12-loaded microcapsules under conditions of low pH and high temperature. The results indicated that the microcapsules were fairly large (1.3 mm), achieving an encapsulation efficiency of 96%. When compared to free cells, the microencapsulated LAB12 showed significantly ($P<0.05$) higher viability when exposed to pH 1.8 (+51%) and direct heat at 75^{o}C (+48%) and 90^{o}C (+47%). The outcomes strongly implied the possible use of LAB12 incorporated into Alg-XG-β-CD-Ch as stable supplement in poultry feed.

Keywords: probiotic lactobacilli, alginate, xanthan gum, β-cyclodextrin, chitosan

INTRODUCTION

Malaysia has one of the highest per capita consumption rates for chicken in the world. In order to limit the controversial use of antibiotic growth promoter (AGP), alternative feed supplement based on probiotic preparation have been comprehensively studied to improve performance in poultry production. Probiotics or lactic acid bacteria (LAB) are live microorganisms which can confer health benefits for the host when administered in sufficient amount. The stability of probiotics in feed, however, remains a major challenge as they are susceptible to gastric acid in their host and high temperature during pelleting process. The present study aimed to address these issues by enhancing pH resistance and thermo tolerance of probiotics through microencapsulation. In this regard, a Malaysian potential probiotic, *Lactobacillus* sp. LAB12, was incorporated into microcapsule with a unique blend of alginate (Alg), xanthan gum (XG), β-cyclodextrin (β-CD) and chitosan (Ch). Alg, a liner polysaccharide, has been widely used as encapsulation agent for probiotics formulation given its mild gelling condition. Its low stability in acidic environment however, limits its use as an effective microcapsule for delivery of probiotics to intestine (Cook *et al.*, 2011). XG, another natural polymer, elicits excellent protection for probiotics against acidic condition. On the other hand, the oligosaccharide β-CD, is known for its water solubility and ability to form inclusion complex with hydrophobic guest molecule. β-CD containing core compounds are not

only highly heat stable, but also highly resistant to chemical degradation. Enteric coating with Ch promised protection against acid, heat and storage condition with ability to release at the targeted site (Cook *et al.*, 2011). By combining the individual strengths of Alg, XG, β-CD and Ch, the viability of probiotics under harsh conditions could be enhanced.

MATERIALS AND METHODS

Microcapsules were prepared by Extrusion and Freeze Drying Techniques. The encapsulation efficiency (EE) of LAB12 was evaluated using the Spread Plate Technique**.** The resultant microcapsules were then analyzed for their physicochemical properties using the environmental scanning electron microscopy (ESEM), particle size analyzer, Fourier Transform Infrared (FTIR) spectrometer and Differential Scanning Calorimeter (DSC). For cell viability at low pH and high temperature, the microcapsules were exposed to pH 1.8 for 120 min and direct heat of 75 ^{o}C and 90^{o}C for 30 sec and 5 sec, respectively.

RESULTS AND DISCUSSION

Physicochemical characteristic of Alg-XG-β-CD-Ch

The bead size of the microcapsules was fairly large (1.3 mm), providing good protection to LAB12. Despite high degree of shrinkage and irregularity, the microcapsules showed good surface smoothness. The reduced melting temperature of the microcapsules indicated good miscibility of the polymers. This was confirmed by chemical interaction between polymers observed in FTIR spectra analysis.

Viability of microencapsulated LAB12 at low pH and high temperature

The microencapsulated LAB12 (EE=96%) showed significantly ($P<0.05$) improved viability at pH 1.8. When compared to free cells, Alg-XG-β-CD-Ch microcapsules enhanced viability of bacteria by 51% after 120 min of treatment. On the other hand, exposure of microencapsulated LAB12 to direct heat at 75^{o}C (30 sec) and 90^{o}C (5 sec) improved viability by 48% and 47% respectively, as opposed to free cells.

CONCLUSION

Alg-XG-β-CD-Ch microcapsules minimized viability loss of LAB12 in harsh conditions. This implies the possible use of LAB12 loaded Alg-XG-β-CD-Ch microcapsules as stable supplement in poultry feed.

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EFFECT OF DIETARY SUPPLEMENTATION OF L-GLUTAMINE AND L-GLUTAMIC ON ACUTE PHASE PROTEIN AND HEAT SHOCK PROTEIN 70 RESPONSES TO HEAT STRESS IN BROILER CHICKENS

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ABSTRACT

Broiler chickens were fed 0.5% AminoGut (AG) (contained a mixture of L-Glutamine and L-Glutamic) for various durations and subjected to either 34°C (4 hours daily from day 21-42) or 24°C. Heat treatment and AG supplementation increased heat shock protein (HSP) 70 expression in the liver and duodenum on day 42. Serum concentration of ceruloplasmin was neither affected by heat nor AG supplementation. It was concluded that AG supplementation may improve heat tolerance in broiler chickens through elicitation of HSP70 reaction.

Keywords: Heat stress, heat shock protein, acute phase protein, aminoGut

INTRODUCTION

Glutamine (Gln), a non- essential but conditional essential amino acid may enhance cellular fuel cellular survival against a variety of stressful stimuli through heat shock protein (HSP70) expression (Singleton and Wischmeyer, 2007). Gln attenuates the release of pro inflammatory cytokine, and protects cells against ischemia or reperfusion injury. Acute phase proteins (APP) are a group of blood proteins that change in concentration in farm animals subjected to external and internal challenges such as infection, inflammation or stress (Murata *et al*., 2004). The aim of this study was to investigate the effect of AminoGut (AG), a commercial dietary supplement (Ajinomoto, Chuo-ku, Tokyo, Japan) containing a mixture of L-glutamine and L-glutamic on APP (ceruloplasmin) and HSP 70 reactions in heat-stressed broiler chickens.

MATERIALS AND METHODS

A total of 360 day-old male broiler chicks (raised in temperature-controlled rooms) were assigned to: (i) basal diet (control), (ii) basal diet + 0.5% AG from day 1-21, (iii) basal diet + 0.5% AG from day 1-42, (4) basal diet + 0.5% AG from day 21-42. From day 21 to 42, birds from each diet were subjected to 34°C for 4 hours daily or 23°C throughout. On day 42, blood samples (8 birds from each diet-temperature subgroup) were collected for serum ceruloplasmin concentration (Martinez *et al*., 2006). Following blood sampling, birds were killed and their livers and duodenums were removed for determination of HSP70 (SDS-PAGE and immunoblot analysis). All data were subjected to ANOVA and Duncan's multiple range tests were used to separate means.

RESULTS AND DISCUSSION

Heat treatment significantly increased HSP70 expression in both liver and duodenum (Table 1). The higher HSP70 expression could be attributed to its cellular protective role under stressful condition. Irrespective of duration, dietary supplementation of AG augmented HSP70 expression (Table 2). The protective effects of Gln-induced HSP70 expression are associated with a significant enhancement of β-O- linked N-acetyl glucosamine (O-GlcNAc) modification which subsequently increased the level of endo-nuclear HSF-1 expression (Gong and Jing, 2011). Both heat and AG supplementation had negligible effect on serum levels of ceruloplasmin. In summary, dietary AG supplementation could be beneficial in enhancing heat tolerance in broiler chickens.

Table 1. Effect of heat challenge on HSP70 density and serum ceruloplasmin concentration in broiler chickens on day 42

Treatment	Parameters		
	Deodenum (HSP70)	Liver (HSP70)	Ceruloplasmin (mg/ml)
Heated	9.64 ± 0.35^{a}	4.43 ± 0.16^{a}	8.42±0.46
Unheated	5.76 ± 0.38^{b}	3.59 ± 0.14^{b}	7.15±0.86

$^{a-b}$ Means ± SEM within a column with no common superscripts differ at P<0.05.

Table 2. Effect of diet on HSP 70 density and serum ceruloplasmin concentration in broiler chickens on day 42

Treatment	Parameters		
	Deodenum (HSP70)	Liver(HSP70)	Ceruloplasmin (APP)
control	5.57 ± 0.73^{b}	3.32 ± 0.17^{b}	8.04±0.87
AG: 1-3wks	8.21 ± 0.72^{a}	4.43 ± 0.30^{a}	6.97±0.75
AG: 3-6 wks	8.18 ± 0.61^{a}	4.02 ± 0.16^{a}	7.04±1.24
AG: 1-6 wks	8.09 ± 0.82^{a}	4.41 ± 0.23^{a}	9.22±0.86

$^{a-b}$ Means ± SEM within a column with no common superscripts differ at P<0.05.

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PROXIMATE AND AMINO ACID VALUES OF SOYBEAN MEAL FROM EXPORTING COUNTRIES

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ABSTRACT

The large variation in the world environmental conditions in which soybeans are grown combined with the differences in varieties and agricultural practices may result in soybeans and soybean meal (SBM) with varying chemical composition. Therefore, this study was carried out to compare the proximate and amino acid values of SBM collected from five different countries (Argentina, Brazil, China, India and USA). The variation was found in crude protein, ash, fat, crude fiber and certain amino acid values of SBM. Brazilian SBM was high while USA and Argentina SBMs were low in protein content. The ash and fiber contents were higher for the samples from India while fat content was higher in samples from Brazil and Argentina. Thr, Ser, Glu, Ile, Phe and Arg contents of SBM from Brazil were higher than that of other four countries. Asp, Val, His, Trp, and Pro contents of SBM were similar in all five countries. In conclusion, variations were found for proximate content and certain amino acid values of SBM collected in different countries. The variations in certain amino acid contents were independent of either other amino acids or of crude protein.

Keywords: Soybean meal, proximate analysis, amino acid, nutrition

INTRODUCTION

Soybean (*Glycine max*) is one of the most valuable oil seed crops. It serves as a feed for poultry, livestock and aquaculture, source of protein for the human diet and biofuel feedstock. On an average, crushing soybeans yield 79% soy meal and 18% soy oil. Soybean meal (SBM) is the most common feed ingredient used as a protein source in diet for non-ruminant species because of its relatively high concentration of protein (44% to 49%), excellent amino acid profile and dependable supply (Wang *et al*. 2011). Currently, Argentina is the leading exporter of SBM followed by Brazil, USA and India (USDA, 2013). Malaysia is one of the top 10 importers of SBM (USDA, 2013), using mainly for broiler and swine feed industry. Amino acid profile of SBM is excellent for most types of poultry, and when combined with corn or sorghum, methionine is usually the only limiting amino acid (Leeson and Summers, 2005). The large variation in the world environmental conditions in which soybeans are grown combined with the differences in varieties and agricultural practices, meal processing conditions such as de-hulling, moisture content, temperature and drying time create soybean and SBM with varying quality (Thakur and Hurburgh, 2007). The objective of this study was to compare the chemical composition (proximate and amino acid contents) of SBM originated from different countries.

MATERIALS AND METHODS

Soybean meal samples, collected at random from importers were from Argentina (31), Brazil (7), China (8), India (8) and USA (25). Samples were ground and stored at 4°C, pending proximate and amino acid content analyses. Moisture, crude protein, ash, fat and crude fiber contents were analyzed using Official Methods of Analysis (AOAC, 2007). Acid hydrolysis, performic acid oxidation and alkaline hydrolysis were

carried out to determine amino acid content (AOAC, 2007). The data mean of the different countries were evaluated using Tukey's test.

RESULTS AND DISCUSSION

The proximate and amino acid values of the SBM samples by country of origin are presented in Table 1. The variation was found in crude protein, ash, fat, crude fiber and certain amino acid values. Crude protein content was the highest from Brazil and the lowest from USA and Argentina. The ash and crude fibre contents were high in samples from India, while fat content was high in samples from Brazil and Argentina. Thr, Ser, Glu, Ile, Phe and Arg values from Brazil were higher than that from other countries.

Table 1. Nutrient composition (mean ± SEM, 88% dry matter basis) of soybean meal from different countries

Content (%)	Argentina	Brazil	China	India	USA
Crude Protein (CP)	45.65 ± 0.15^{a}	48.64 ± 0.25^{c}	46.79 ± 0.511^{b}	45.89 ± 0.25^{ab}	45.64 ± 0.14^{a}
Ash	5.89 ± 0.03^{b}	5.80 ± 0.05^{ab}	5.54 ± 0.04^{a}	6.73 ± 0.20^{c}	5.96 ± 0.05^{b}
Fat	1.65 ± 0.07^{ab}	2.16 ± 0.09^{b}	1.46 ± 0.22^{a}	1.11 ± 0.12^{a}	1.41 ± 0.12^{a}
Crude Fiber (CF)	3.52 ± 0.04^{b}	3.59 ± 0.07^{b}	4.93 ± 0.25^{c}	6.20 ± 0.07^{d}	3.08 ± 0.06^{a}
Aspartic Acid (Asp)	5.04 ± 0.02	5.43 ± 0.06	5.22 ± 0.09	5.22 ± 0.06	5.14 ± 0.12
Threonine (Thr)	1.72 ± 0.01^{a}	1.83 ± 0.03^{b}	1.75 ± 0.03^{a}	1.70 ± 0.02^{a}	1.70 ± 0.01^{a}
Serine (Ser)	2.53 ± 0.02^{a}	2.78 ± 0.03^{b}	2.61 ± 0.06^{a}	2.57 ± 0.04^{a}	2.54 ± 0.02^{a}
Glutamic Acid (Glu)	8.17 ± 0.05^{a}	8.89 ± 0.10^{c}	8.48 ± 0.13^{ab}	8.55 ± 0.10^{b}	8.22 ± 0.05^{a}
Glycine (Gly)	1.88 ± 0.01^{ab}	2.00 ± 0.04^{c}	1.94 ± 0.03^{bc}	1.92 ± 0.02^{ab}	1.84 ± 0.01^{a}
Alanine (Ala)	1.91 ± 0.01^{a}	2.02 ± 0.02^{b}	1.96 ± 0.04^{ab}	1.93 ± 0.04^{a}	1.88 ± 0.01^{a}
Cystine (Cys)	0.62 ± 0.01^{ab}	0.64 ± 0.01^{b}	0.55 ± 0.08^{a}	0.57 ± 0.02^{ab}	0.62 ± 0.01^{ab}
Valine (Val)	2.46 ± 0.05	2.59 ± 0.18	2.51 ± 0.12	2.56 ± 0.14	2.38 ± 0.04
Methionine (Met)	0.62 ± 0.01^{ab}	0.65 ± 0.01^{b}	0.54 ± 0.08^{a}	0.58 ± 0.02^{ab}	0.63 ± 0.01^{b}
Isoleucine (Ile)	1.82 ± 0.01^{a}	1.97 ± 0.03^{b}	1.87 ± 0.03^{a}	1.86 ± 0.03^{a}	1.81 ± 0.01^{a}
Leucine (Leu)	3.71 ± 0.03^{a}	3.94 ± 0.05^{b}	3.83 ± 0.08^{ab}	3.80 ± 0.07^{ab}	1.81 ± 0.01^{a}
Tyrosine (Tyr)	1.32 ± 0.02^{a}	1.48 ± 0.04^{b}	1.40 ± 0.04^{ab}	1.40 ± 0.03^{ab}	1.32 ± 0.01^{a}
Phenylalanine (Phe)	2.22 ± 0.01^{ab}	2.41 ± 0.03^{c}	2.30 ± 0.04^{b}	2.30 ± 0.03^{b}	2.19 ± 0.01^{a}
Lysine (Lys)	2.86 ± 0.01^{a}	2.98 ± 0.04^{b}	2.92 ± 0.04^{ab}	2.88 ± 0.05^{ab}	2.85 ± 0.01^{a}
Histidine (His)	0.98 ± 0.01	1.03 ± 0.03	0.99 ± 0.03	1.01 ± 0.04	0.97 ± 0.01
Arginine (Arg)	3.30 ± 0.01^{a}	3.53 ± 0.04^{c}	3.38 ± 0.06^{ab}	3.42 ± 0.06^{b}	3.29 ± 0.01^{a}
Tryptophan (Trp)	0.56 ± 0.01	0.56 ± 0.02	0.57 ± 0.02	0.56 ± 0.02	0.57 ± 0.01
Proline (Pro)	2.02 ± 0.02	2.14 ± 0.04	2.12 ± 0.11	2.09 ± 0.05	2.02 ± 0.02

$^{a-c}$ Means within a row without a letter in common differ (P<0.001); SEM: standard error of mean

Asp, Val, His, Trp, and Pro contents were similar between countries. Methionine content from Brazil and USA was the highest, and China, the lowest. Lysine from Brazil was the highest and from Argentina and USA was the lowest. The amino acids that showed variation were independent of either other amino acids or of the protein content. This variation in SBM composition might be due to differences in genetics, location, fertility, agronomic conditions, pre- and post harvest processing variables, as well as storage condition and period of storage. It has been reported that CP content (Thakur and Hurburgh, 2007) and amino acid profile (Frikha *et al.,* 2012) of SBM depend on their origin. In conclusion, differences were found for

proximate content and certain amino acid values of SBM collected from different countries.

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EFFECTS OF COMMERCIAL OLIGOSACCHARIDES ON GROWTH OF SELECTED BENEFICIAL AND NON-BENEFICIAL GUT BACTERIA OF BROILERS

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ABSTRACT

Prebiotics, which are digestive-resistant low molecular weight oligosaccharides, have been known to able to selectively promote the growth of beneficial bacteria. It is one of the substances that is currently being studied as feed supplement to improve the growth performance of chickens. Several commonly known oligosaccharides that are well documented as prebiotics are such as inulin (INU), isomaltooligosaccharides (IMO), xylooligosaccharides (XOS), nigerooligosaccharides (NOS) and gentioologosaccharides (GOS). Although some of these oligosaccharides have been reported to enhance the growth of beneficial bacteria, the effects on non-beneficial bacteria is limited. Therefore, in the present study, the effects of XOS, IMO, GTO, INU and FOS on the growth of six strains of *Lactobacillus* and three strains of non-beneficial bacteria (*Escherichia coli*, *Salmonella typhimurium* and *Salmonella enterica*) were investigated. It was observed that only IMO supported the growth of all the tested *Lactobacillus* spp. Other prebiotics only supported selected strains. Interestingly, the three non-beneficial bacteria were also able to grow in all the tested prebiotics, except inulin. This may indicate that inulin is a more superior prebiotic in comparison to the other tested candidates.

Keywords: Prebiotic, broilers, beneficial bacteria, non-beneficial bacteria

INTRODUCTION

Prebiotics are low molecular weight carbohydrates in the range of 3-10 degree of polymerization (DP). Its special chemical structure allows fermentation by only a limited number of bacteria in the gastrointestinal system. Previous studies showed that ingestion of prebiotic confers a lot of benefits not only in human but also in animals such as broilers. Incorporation of prebiotics in feeds have been reported to increase body weight of chickens, altered gastrointestinal microbial community by enhancing beneficial bacteria population, aiding minerals and calcium absorption in host and also reducing the cholesterol level in serum (Kim *et al*., 2011; Abrams *et al*., 2005; Pereira and Gibson, 2002). Studies on effects of prebiotics on non-beneficial bacteria, which are also present in the gut flora, are however limited. Thus, in the present study, the effects of five selected commercial prebiotics (xylooligosaccharide [XOS], somaltoligosaccharide [IMO], gentioligosaccharide [GTO], fructooligosaccharide [FOS], inulin [INU]) on a series of beneficial and non-beneficial bacteria were conducted.

MATERIALS AND METHODS

Beneficial bacteria such as *L. reuteri* C1 (C1), *L. salivarius* I24 (I24), *L. brevis* I218 (I218), *L. brevis* ATCC 14869 (LB), *L. galinarium* ATCC 33199 (LG), *L. reuteri* ATCC 23272 (LR) and non-beneficial bacteria e.g. *S.enterica* NCTC 4444 (SE), *S. thyphimurium* NCTC 13348(ST), *E. coli* ATCC 25922 (EC) were cultured in modified de Man-Rogosa-Sharpe (MRS) broth (Merck) and modified Nutrient broth (NB) (Merck), respectively. In these modified media, the carbon source of the basal media (MRS

and NB) was removed and replaced with respective filter sterilized prebiotics (XOS, IMO, GTO, INU and FOS) which were dissolved in water (1 g/ml). The final concentration of the prebiotic in the media was 1% (w/v). Glucose (1% [w/v]) was used as the positive control. The modified MRS and NB were inoculated with 1% (v/v) 18-h-old bacteria and incubated under recommended condition at 37°C for 24 h. Both modified basal medium supplemented with 1% (v/v) prebiotic or glucose without inoculum were used as negative control. At the end of the incubation period, the turbidity of the culture was determined at OD 620 nm. All experiments were carried out in triplicates.

RESULTS AND DISCUSSION

Among the prebiotics, only IMO was able to support the growth of all tested strains of *Lactobacillus*, although the growth varied in different prebiotics (Figure 1). The highest growth was observed in LB. GTO was able to support the growth of all *Lactobacillus* strains, except I24. XOS and inulin were able to support three and four strains of *Lactobacillus*, respectively. Only one strain of *Lactobacillus* (LG) was able to grow in FOS. All the non-beneficial bacteria (EC, ST and SE) were able to grow in all the prebiotics tested, except inulin. Good growth was observed for EC when IMO and XOS were used. These results may indicate that inulin is a more superior prebiotic in comparison to the other tested oligosaccharides. Nevertheless, the growth of non-beneficial bacteria in the oligosaccharides may also be due to impurities in the commercial products. DNS assay showed that among the tested prebiotics, inulin contained the least reducing sugars (data not shown).

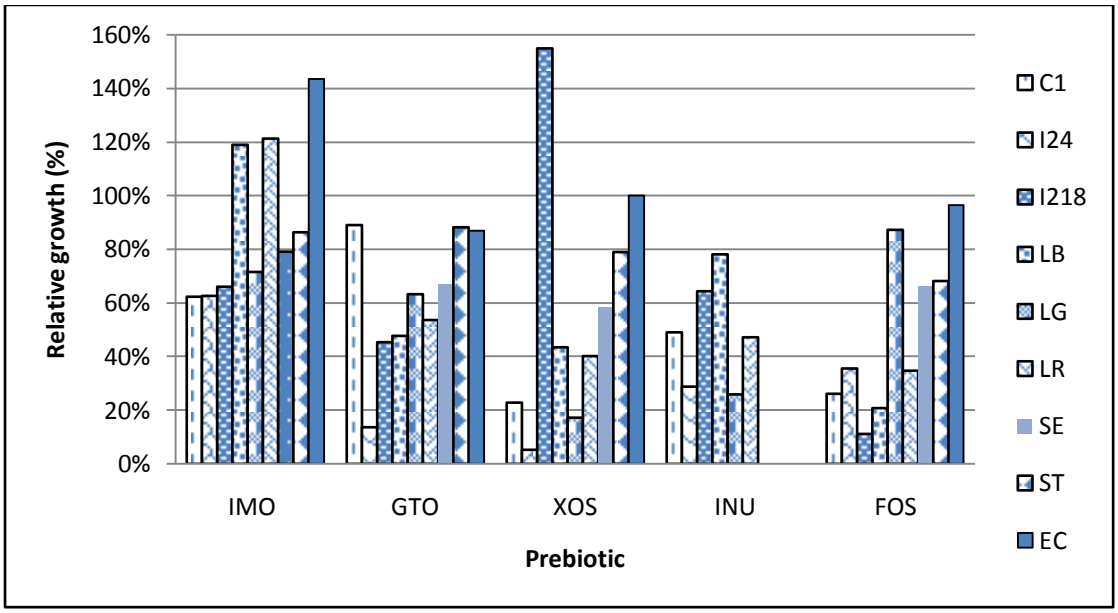

Figure 1. Relative growth of bacteria strains in media supplemented with commercial prebiotic

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EFFECTS OF PROBIOTIC, PREBIOTIC AND SYNBIOTIC ON GROWTH PERFORMANCE, CAECAL BACTERIAL POPULATIONS AND SERUM LIPID CONCENTRATIONS OF BROILERS

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ABSTRACT

A total of 360 one-day-old male broiler chicks were randomly assigned to four dietary treatments: basal diet (control) (T1), basal diet + 0.1% commercial probiotic (consisted of 11 probiotic *Lactobacillus* strains) (T2), basal diet + 1.0% prebiotic isomaltooligosaccharides (IMO) (T3), basal diet + 0.1% commercial probiotic + 1.0% prebiotic IMO (synbiotic) (T4). Body weights of chickens were measured at 1, 21 and 42 days of age. Feed intake and feed conversion was determined. At days 21 and 42, 18 chickens/treatment were euthanised and blood samples were collected for serum lipid analysis. Caecal contents were also collected from 6 euthanised chickens/treatment group and enumerated for lactobacilli, bifidobacteria, *E. coli* and total aerobes. The results showed that broiler chickens fed T2, T3 or T4 diet had significantly ($P<0.05$) higher body weight gain and better feed efficiency than those fed control diet (T1) at 1 to 42 days of age. The supplementation of probiotic, prebiotic and synbiotic also significantly ($P<0.05$) increased the caecal populations of lactobacilli and bifidobacteria and decreased the caecal *E.coli* and total aerobe populations. In addition, supplementation of T2, T3 or T4 diet significantly ($P<0.05$) lowered the serum total cholesterol, LDL cholesterol and triglycerides concentrations of broiler chickens at 42 days of age. The results indicated that prebiotic IMO (1.0%) and its symbiotic were effective in improving the performance of broiler chickens and in conferring other health benefits on the chickens.

Keywords: probiotic, prebiotic, synbiotic, broiler performance, caecal microflora

INTRODUCTION

Lactobacilli are the predominant lactic acid bacteria used as probiotics for animals, particularly poultry. Prebiotics are substances that act as microbial modulators and are defined as "nondigestible food ingredients that beneficially affect the host by selectively stimulating the growth and/or activity of one or a limited number of bacteria in the colon and thus improve host health" (Gibson and Roberfroid, 1995). Oligosaccharides are nondigestible carbohydrates with prebiotic potential, stimulating the growth of friendly bacteria (probiotic) present in the colon. The synbiotic concept arises from the combination of probiotic and prebiotic. Synbiotic has synergistic effects promoting growth of existing strains of beneficial bacteria in the colon as well as improving the survival and growth of newly added probiotic strains. In the present study, the effects of a prebiotic oligosaccharide (isomaltooligosaccharides)(IMO), used singly or in combination with a commercial *Lactobacillus* probiotic (forming a synbiotic), were investigated in broiler chickens. The parameters measured were growth performance, caecal bacterial populations and serum lipid concentration.

MATERIALS AND METHODS

A total of 360 one-day-old male broiler chicks were randomly assigned to four dietary treatments. The treatments were: basal diet (control)(T1), basal diet + 0.1% commercial probiotic (consisted of 11 probiotic *Lactobacillus* strains) (T2), basal diet + 1.0% prebiotic IMO (T3), basal diet + 0.1% commercial probiotic + 1.0% prebiotic IMO (synbiotic) (T4). Body weights of chickens were measured at 1, 21 and 42 days. Feed intake was recorded daily on per cage basis, and feed conversion ratio (FCR) was determined. At days 21 and 42, 18 chickens/treatment were euthanised and blood sample from each chicken was collected and analyzed for total cholesterol, HDL cholesterol, LDL cholesterol and triglycerides. Caecal contents were also collected from 6 euthanised chickens/treatment group and enumerated for lactobacilli, bifidobacteria, *E. coli* and total aerobes.

RESULTS AND DISCUSSION

The results showed that broilers fed T2, T3 or T4 diet (i.e. probiotic, prebiotic or synbiotic diet, respectively) had significantly ($P<0.05$) higher body weight gain ($P<0.05$) than those fed control diet (T1) from 22 to 42 days and 1 to 42 days. From 1 to 21 days, the feed intake was significantly lower ($P<0.05$) in broilers fed T2, T3 or T4 diet than in those fed control diet (T1). However, the feed intakes of birds from 22 to 42 days, and 1 to 42 days were not significantly different among the treatments ($P>0.05$). The FCR of broilers fed control diet (T1) was higher ($P<0.05$) than those fed other treatments and there were no significant differences between diets T2, T3 and T4 from 1 to 21 days, 22 to 42 days and throughout the whole experimental period (1 to 42 days). The serum total cholesterol levels of chickens fed T2, T3 and T4 diets were significantly lower ($P<0.05$) than those fed control (T1) diet at 21 and 42 days of age. At 42 days of age, there were no significant differences in HDL cholesterol levels of chickens among all the treatment groups, but LDL cholesterol levels were significantly ($P<0.05$) lower in T2, T3 and T4 supplemented chickens when compared to control (T1) chickens. At 21 and 42 days of age, chickens supplemented with T2, T3 or T4 diet showed significantly ($P<0.05$) lower triglyceride levels than control (T1) chickens. At 21 days of age, T2, T3 and T4 diets significantly ($P<0.05$) increased caecal populations of lactobacilli and bifidobacteria, but decreased caecal *E. coli* populations in chickens. At 42 days of age, caecal lactobacilli and bifidobacteria populations significantly ($P<0.05$) increased with T2, T3 and T4 diets, while caecal *E. coli* populations decreased with T3 and T4 diets and caecal total aerobe population decreased with T2 and T4 diets. In conclusion, the results of the study showed that prebiotic IMO (1.0%), supplemented singly or in combination as a synbiotic, was effective in improving the performance of chickens, increasing the beneficial caecal bacterial populations of lactobacilli and bifidobacteria, decreasing the non-beneficial caecal *E.coli* and total aerobe populations, and in lowering the serum total cholesterol, LDL cholesterol and triglycerides concentrations.

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IMPROVEMENT OF INDIGENOUS CHICKEN PRODUCTION IN HOT AND HUMID ENVIRONMENT OF BANGLADESH

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ABSTRACT

The study was undertaken to assort improved, heat tolerant and economically viable genotype of chicken in the hot humid environment of Bangladesh. Sixty nine (69) offspring of Naked Neck and Fifty five (55) crossbred were produced through crossbreeding between indigenous Aseel chicken (cock) and Naked Neck (NN) female for the study. The average body weight of crossbred flock was higher (331.63±3.95 g) and (1216.71±61.44 g) than the control flock (236.27±12.89 g) and (738.61±71.84 g) in two months (2-m) and five months (5-m) of age, respectively. Average live weight (g) of male in crossbred flock was higher 370±4.86 and 1300±68.58 at 2m- and 5-m compared to control flock, respectively. Mortality and predator loss was lower (6.23±3.03% & 4.87±3.82%) and (3.64% & 4.00%) up to 2-m and from 2-m to 5-m, respectively in the crossbred flock. It was found that crossbred under semi scavenging system of management in the hot and humid environment of Bangladesh.

Keywords: Native chicken, crossbred, environment, body weight, mortality

INTRODUCTION

Poultry play a very important role for income generation and poverty alleviation. Indigenous chickens are genetically growth depressed but there is heavy weight Aseel chicken in the less hot area which can contribute through breeding with Naked Neck chicken potential for hot dominant gene. The genetic distances between the indigenous and Aseel was relatively large which almost corresponded to the differences between the breeds. Thus, an attempt was undertaken to improve body weight of indigenous Naked Neck chicken by crossbreeding with Aseel to suit in the hot and humid environment of Bangladesh.

MATERIALS AND METHODS

The study was conducted in the hot humid area (Sapahar, Naogaon) of Bangladesh. Based on previous experience households (HH) were selected and trained. Hens were distributed among five (5) households for each of breeding flock containing ten hens and a cock for production of F_{-1} control (NN male x NN female) and crossbred (Aseel male x NN female). Hens were fed with balanced diet and eggs were collected for natural hatching. Chicks were provided with 50% feed of their daily requirement up to 5-m and vaccinated against Newcastle and Pox disease. Data were collected on body weight (at 2-m and 5-m), mortality and predator loss (from records of dead birds) by their age, sex and genotypes, respectively and statistically analyzed with SPSS using one way ANOVA and T-test.

RESULTS AND DISCUSSION

Results are shown in Table 1, respectively. Average body weight of F_{-1} crossbred flocks at 2-m of age was 331.63±3.95 g and control flock was 236.27±12.89 g ($P<0.05$), respectively and during 5-m of age 1216.71±61.44 g and control flock was

738.61±71.84 g with significant difference (P<0.05). Naked Neck and Full Feathered male and female progeny were produced. In crossbred flock average body weights of Full Feathered male was higher (376.80±14.29 g) than Naked Neckmale 365 g at 2-m of age. However, at 5-m average weight of Full Feather female was higher (1600.00±68.58 g) than the Naked Neck female (1033.00±42.34 g). In control flock highest weight was in case of Naked Neck male (267.65±23.60 g) than Full Feathered male (260.60±31.12 g), Naked Neck female 209.35±22.83 g and Full Feathered female 207.50±17.79 g, respectively in 2-m. In the control flock body weight of Naked Neck male was higher (835.00±89.61 g) than Full Feather male (618.46±35.50 g), Naked Neck female 805.80±127.02 g was higher than Full Feather female (695.20±93.99 g), respectively. However, the trend of the weight gain of the crossbred flock was higher than the control flock (P<0.05) which is in agreement with the result of Zaman *et al*. (2004). There was an interaction effect between the sex and breed on the growth rate at 2-m and 5-m of age (P<0.05).

Table 1. Mortality and body weight of chicken at 2 months and 5 months of age

	Predator Loss (%)		Mortality (%)		Body weight (g)	
Types of Chicken	2 months	5 months	2 months	5 months	2 months	5 months
Crossbred	3.64	4.00				
Naked Neck male			9.17	-	365	865
Naked Neck female			8.46	4.17	258	1033
Full Feathered male			3.06	6.25	376	1368
Full Feathered female			4.23	9.09	326	1600
Average			6.23	4.87	311	1261
Control flock	13.04	4.92				
Naked Neck male			17.25	10.76	267	835
Naked Neck female			3.83	12.20	209	805
Full Feathered male			9.20	9.38	260	618
Full Feathered female			-	8.71	207	695
Average	8.34	4.46	7.57	10.26	236	738

Lower mortality at the age of 2-m and 2-m to 5-m were calculated in crossbred flock (6.23±3.04% & 4.88±3.82%, respectively). Hoque *et al*. (2003) reported 8.33% mortality of NN. F_{-1} of crossbred was found more adaptive in protecting and escaping from the predator in the conventional management.

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RESPONSE TO DIETARY SUPPLEMENTATION OF L-GLUTAMINE AND L-GLUTAMATE IN BROILER CHICKENS RAISED AT DIFFERENT STOCKING DENSITIES UNDER THE HOT, HUMID TROPICAL CONDITIONS

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ABSTRACT

This research was conducted to evaluate the effect of AminoGut™ (a mixture of Gln and Glu) 0.5% for first 3 weeks and 6 weeks on performance of broiler chickens stocked at 10 birds/m^2 and 15 birds/m^2. Supplementing 0.5% AminoGut™ did not affect growth performance and serum acute phase protein (APP) level. The high stocking density impaired growth performance and increased serum APP concentration as compared to the low stocking density.

Keywords: growth performance, broiler chicken

INTRODUCTION

Glutamine (Gln) is a neutral free amino acid and can be synthesised from glutamate (Glu) primarily in the skeletal muscle. Gln has been shown to improve growth performance and humoral immune response in poultry (Dai *et al*., 2009). Work in cattle (Arthington *et al*., 2003) suggested that stress may induce acute phase protein (APP) response. The objective of the present study was to evaluate the effect of supplementing diets with AminoGut™ (Ajinomoto, Chuo-ku, Tokyo, Japan) (a mixture of Gln and Glu) on growth performance and serum APP concentration in broiler chickens raised at different stocking densities under the hot, humid tropical conditions.

MATERIALS AND METHODS

A total of 928 one day-old male chickens were stocked at 0.100 m^2/bird (LD) or 0.067 m^2/bird (HD) and fed (i) diet + AminoGut™ at 0.5 % (AG) for the first 3 weeks (AG1-21), (ii) diet + AG for 6 weeks (AG1-42), (iii) diet + virginiamycin 0.02 % for 6 weeks (VM) and (iv) unsupplemented diet (control). Body weight and feed intake were recorded weekly. Blood samples were collected on day 42 to determine the level of ceruloplasmin (CER), ovotransferin (OVT), α-1 acid glycoprotein (AGP) in broilers chickens. Serum AGP concentration was determined by radial immunodiffusion using a commercial kit. Serum CP concentration was measured according to procedure of Martinez *et al.* (2006). Radial immunodiffusion methods were used to measure OVT level according to the modified method of Mancini *et al*. (1965).

RESULTS AND DISCUSSION

Birds fed control and AG supplemented diets had similar weight gains and FCR throughout the period of study (Table 1). Similarly, Gln supplementation at 0.5% did not enhance growth performance of broilers. From 3-6 weeks, the VM birds had greater weight gains than those provided AG and better FCR than the other groups. From 3-6 weeks, HD was detrimental to both weight gain and FCR when compared

to the LD group. While diet had no significant effect on APP, HD significantly elevated CER, OVT and AGP on day 42 compared to those of LD (Table 2). These finding suggested that APP is a potential stress biomarker in poultry. In conclusion, AG supplementation had negligible effect on growth performance. Stress attributed to HD elevated serum levels of APP in broiler chickens.

Table 1. Mean (±SEM) weight gains and feed conversion ratios (FCR) in broilers by diet and stocking density

	Weight gain (g)		FCR	
Diet	**1-3 wks**	**3-6 wks**	**1-3 wks**	**3-6 wks**
Basal	811± 8 [a]	1045± 46 [ab]	1.47 ± 0.01[a]	2.27± 0.02[a]
AG1-21	812± 7 [a]	996± 43 [b]	1.49 ± 0.02 [a]	2.35 ± 0.02[a]
AG1-42	801± 4 [a]	1002± 56 [b]	1.46 ± 0.01[a]	2.27 ± 0.03[a]
Virginiamycin	832± 13 [a]	1090± 49 [a]	1.46 ± 0.01[a]	2.09 ± 0.03[b]
Density				
Low density	812± 6 [a]	1152± 21 [a]	1.49 ± 0.01[a]	2.12± 0.02[b]
High density	816± 7 [a]	915± 11 [b]	1.45 ± 0.01[a]	2.42 ± 0.03[a]

[a,b] Means within a column with no common superscripts differ at P<0.05.

Table 2. Mean (±SEM) serum levels of ceruloplasmin (CER), α-1 acid glycoprotein (AGP), ovotransferin (OVT) in broilers by diet and stocking density.

Diet	**CER, µg/ml**	**AGP, ng/ml**	**OVT, mg/ml**
Basal	11.11± 1.72 [a]	874± 83 [a]	1.44± 0.15 [b]
AG1-21	7.41± 0.71 [a]	955± 71 [a]	2.51± 0.33 [a]
AG1-42	9.86± 1.61 [a]	1034± 136 [a]	2.17± 0.32 [ab]
Virginiamycin	9.75± 1.31 [a]	792± 94 [a]	1.63± 0.34 [ab]
Low density	7.52± 0.64[b]	711± 51 [b]	1.4± 0.16 [a]
High density	11.34± 1.13 [a]	1081± 61 [a]	2.5± 0.25 [b]

[a,b] Means within a column with no common superscripts differ at P<0.05.

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DEVELOPED AND UNDEVELOPED SUBMANDIBULAR SALIVARY GLANDS OF EBN SWIFTLET (*AERODRAMUS FUCIPHAGUS)*

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ABSTRACT

One of the major salivary gland producing saliva in swiftlet species is the submandibular gland. The developed glands are clearly seen under the lower mandible of the swiftlet. Since it is the largest visible salivary gland, it is considered as the major source of saliva production towards the building of the nest. This study was conducted to find out whether all flying swiftlets are making nest for their offspring. The findings revealed that based on the development of the swiftlets salivary glands, not all the flying swiftlets are able to build nest. Some of them were still juvenile where the glands were undeveloped and some were mature and active birds where the glands were well developed.

Keywords: Swiftlets, *Aerodramusfuciphagus,* edible bird nest (EBN), saliva, salivary gland

INTRODUCTION

Swiftlets are birds that are greatly found in caves and specialized build house commonly presence in South East Asia. These birds are classified into four types of genera which are *Aerodramus*, *Hydrochous*, *Schoutedenapus* and *Collocalia.* Currently, researchers more focusing on the bird itself since it may greatly expand the economy based on the nest that being produced. The *Aerodramus fuciphagus* (*A.fuciphagus*) is one of the genus *Aerodramus* that able to build nest with high nutrients content. The nests or also known as edible bird nest (EBN) were consumed since centuries ago and it is believed that EBN serves as therapeutic herbal medicine (Lin *et al*., 2009). The EBN is made up from the saliva of the swiftlets (Ma and Liu, 2012).

MATERIALS AND METHODS

A total of eight swiftlets (*A. fuciphagus*) were captured from Sri Iskandar, Perak, Malaysia coordinated at N 04°20.824' E 100°52.826'. The birds were captured by mist net (FAO, 2007).The birds were called using swiftlet song. Prior to post mortem, the birds were euthanised with an overdose of barbiturate at 60mg/kg via brachial vein (AVMA, 2007). Post mortem was conducted immediately after euthanasia and the morphology of submandibular gland was examined both macroscopically using standard histological examination (H&E staining). The study protocol was approved by UPM Animal Ethic committee.

RESULTS AND DISCUSSIONS

Although morphologically the size of the swiftlets has no difference (almost uniform), the size of the submandibular salivary glands was not related to the size of the body.The well develop submandibular glands were large and lobulated, and clearly seen under the lower mandible (Fig. 1A). Histological examination showed the gland architecture with the secretion can be seen clearly at the middle of the gland. This showed that the glands are at actively producing saliva. The undeveloped

submandibular glands were small and less lobulated (Fig. 2B). Histological examination showed the gland architecture with less gland cells and very much less secretion at the middle of the gland. The undeveloped submandibular glands may be due to the birds were still juvenile or the birds were in the inactive season where there was no nesting activity.

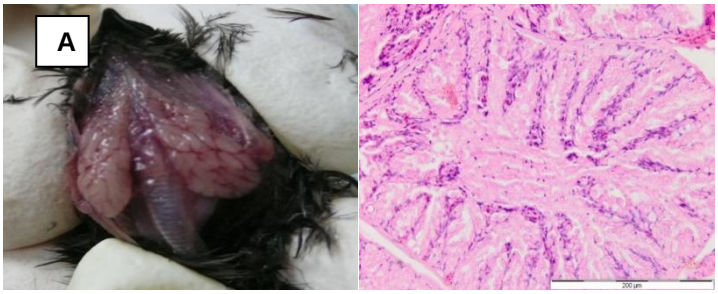

Figure 1A. Photographs showing well-developed submandibular glands, large and lobulated, histologically well-developed submandibular gland.

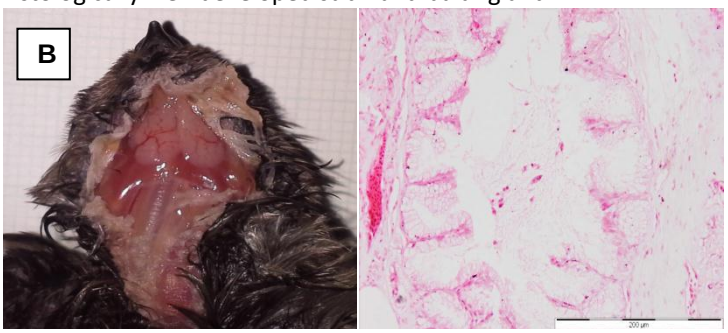

Figure 2B. Undeveloped submandibular glands, small and less lobulated and histologically undeveloped submandibular gland with less gland cells and very much less secretion materials at the middle.

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DETERMINING THE BIOLOGICAL EFFECTS OF FUNCTIONAL AMINO ACID SUPPLEMENTATIONIN LOW CRUDE PROTEIN DIET ON GROWTH PERFORMANCE OF BROILER CHICKEN

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ABSTRACT

This experiment was conducted to determine the biological effect of functional amino acid supplementation in low crude protein diet on growth performance of broiler chicken. A total of 288 male Cobb 500 chicks were allocated into 8 treatment groups and were fed with essential amino acids supplementation in low crude protein diet. Diets contain 21% crude protein until 18% crude protein and supplemented with amino acid (L- lysine, DL-methionine and L-threonine). This experiment was conducted for 6 weeks and the feed intake, body weight, feed conversion ratio were measured weekly. There was no significant different ($P>0.05$) in feed intake among the treatment groups. The reducing level of crude protein from 21% until 18% supplemented with amino acid (T2-T8) was significantly ($P<0.05$) higher in body weight (WG), average daily gain (ADG) and total weight gain (TWG) as compared with T1. Overall result shows 19% crude protein contained optimum level of amino acidshad better BW, ADG, FI and lower FCR. Thus, with this reduction of crude protein it will reduce the production costs concurrently maintain the broiler performance.

Keywords: Low crude protein, amino acid

INTRODUCTION

In poultry industry, farmers have suffered tremendous increased of the cost due to fluctuation of feed price in recent years (Baker, 1997; Kidd *et al.*, 2001; Lepleaideur, 2004) resulting in increased market price of animal products. Protein is one of important nutrients in feedstuff that needs to be met for the basic nutrient requirement of animals. This nutrient involves substantial part of the cost of feeding (Mtimuni, 1995). Protein is known for its sources of amino acid and ideal protein concept contains all of amino acid in exact amount and proportion in order to maintain and fulfill the chicken requirement and reduce the feed cost (Baker *et al.,* 1994). Therefore, this experiment was conducted to determine the biological effect of functional amino acid supplementation in low crude protein diet on growth performance of broiler chicken.

MATERIALS AND METHODS

A total of 288 male Cobb^{500} broiler chicken (live weight: 44.78±3.70 g) were used in current experiment with 8 treatments (6 replicates and 6 chicks per replicates). The chickens were offered with starter (21% to 18% crude protein) and finisher diets (18% to 15% crude protein) supplemented with three commercial amino acids (L-Lysine, DL-Methionine and L-Threonine). The dietary treatments were T1: negative control (starter: 21% CP without supplemented commercial amino acid; finisher: 18% CP without supplemented amino acid); T2: positive control (starter: 21% CP with amino acid supplementation; finisher: 18% CP with amino acid supplementation); T3:

(starter: 20.5% CP with amino acid supplementation; finisher: 17.5% CP with amino acid supplementation); T4: (starter: 20% CP with amino acid supplementation; finisher: 17% CP with amino acid supplementation); T5: (starter: 19.5% CP with amino acid supplementation; finisher: 16.5% CP with amino acid supplementation); T6: (starter: 19% CP with amino acid supplementation; finisher: 16%CP with amino acid supplementation); T7: (starter:18.5 % CP with amino acid supplementation; finisher: 15.5%CP with amino acid supplementation); and T8: (starter:18% CP with amino acid supplementation; finisher: 15% CP with amino acid supplementation). The amino acids in starter and finisher diets from different treatment groups were adjusted to similar level. This experiment was conducted for 6 weeks and the feed intake were measured weekly by deduction of the balance of feed from the quantity originally supplied to the chicks. The body weights were measured individually.

RESULTS AND DISCUSSION

The reducing level of crude protein from 21% until 18% supplemented with amino acid (T2-T8) was significantly ($P<0.05$) higher inbody weight (WG), average daily gain (ADG) and total weight gain (TWG) as compared with T1.The T1 birds had lower average daily gain (ADG) as compared with those birds fed with dietary supplemented with amino acids and this result was consistent with the findings (Kamran *et al.,* 2004). T6 had higher ($P<0.05$) WG compared to other treatment groups. Feed conversion ratio (FCR) for T1 was significantly higher ($P<0.05$) as compared with treatment that supplemented with amino acids. This result contradicted with previous study (Hill, 1989) that low CP supplemented with essential amino acids gave slightly increased in FCR. In previous study by Kamran *et al.* (2004), 20% crude protein with essential amino acid had better FCR. However, in current study T6 with 19% CP had better BW, ADG, FI and lower FCR. In conclusion, T6 (19% CP starter diet and 16% CP finisher diet with amino acid supplementation) had better BW, ADG, FI and lower FCR in the dietary of low crude protein supplemented with amino acid that able to fulfill the optimal requirement of broiler chicken performance and at the same time reduce the cost of animal feed.

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EFFECT OF EFFECTIVE MICROBES (EM) SUPPLEMENTATION ON VILLAGE CHICKEN BROILER PERFORMANCE, COCCIDIOSIS BURDEN AND INTERNAL ORGAN CHANGES

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ABSTRACT

A study was conducted to evaluate the effects of supplementation of effective microbes (EM) on body weight gain, coccidiosis burden and organ morphological changes of village chicken broilers. A total of 40 broilers at the age of 28 d were kept at the same managerial, environmental and hygienic conditions except different dietary treatments: A (EM supplemented starting 4 weeks old), B (EM supplemented since 1-d-old), C (negative control) and D (positive control). For EM treatment birds, both groups received 5% of EM Bokashi mixed with chicken pellets and EM EMAS added into non-chlorinated drinking water (1:1000) daily. Results of 5 weeks experiment showed that birds fed with EM had higher average weight gains than the control groups ($P>0.05$). Compared to control, EM treatment significantly decreased coccidiosis burden (oocyst count) from week 3 onwards ($P<0.05$). Enlarged dark-mahogany spleen and excessive fat covering gizzard was observed in positive control birds. From necropsies descriptive observation, no hemorrhagic intestinal gross lesion was observed in both EM treated groups where birds in Group B demonstrated the largest cecum and the smallest small intestine size. The results of this study suggest that EM shows effect on weight gain, coccidiosis burden, gizzard fat layer and internal organs of village chicken broilers.

Keywords: Effective microbes, broiler performance, coccidiosis burden, organ morphology

INTRODUCTION

Many literatures suggest that the microbial preparations have beneficial effects in poultry production (e.g. improvements in growth rate and feed efficiency, prevention of intestinal infections by pathogenic microorganisms and improved nitrogen utilization) (Safalaoh, 2006). Early feeding with microbial preparations or probiotics influenced healthy intestinal development and secondary lymphoid organs (Yegani, 2008). This study was carried out to evaluate the effectiveness of EM supplementation on body weight gain, coccidiosis burden and organ morphological changes of village chicken broiler.

MATERIALS AND METHODS

This study was conducted with 40 (28 d of age) male village chicken broilers obtained from a commercial hatchery. They were weighed individually (upon arrival and at weekly intervals during the treatment) and assigned at random to four experimental treatments as follows: A (basal diet supplemented with both types of EM (supplied from Department of Veterinary Services, Kuala Langat, Selangor) starting 4 weeks

old); B (basal diet supplemented with both types of EM since 1-d-old); C (negative control, basal diet without any feed additive); D (positive control, basal diet with anticoccidial drug). Body weight gain and oocyst count was performed weekly. After 5 weeks of treatment, three birds in each group were slaughtered randomly where spleen, gizzard, ceca and small intestines were removed for descriptive observation on organ morphological changes and intestinal gross lesion. Statistical software (SPSS ver. 17) was used to analyse the data.

RESULTS AND DISCUSSION

Birds fed diet supplemented with EM had higher body weight gain ($P>0.05$) than the control groups. The results were in contrary with previous studies (Safalaoh, 2006; Wondmeneh *et al*., 2011) that have shown significant increased in body weight gain as it was conducted in larger population and longer period of treatment. Oocyst count was significantly dropped in both EM treated groups ($P<0.05$) from week 3 onwards. In necropsies descriptive observation, the secondary lymphoid organ in positive control birds, demonstrated enlarged dark-mahogany spleen which possibly related to high dose of drug used by direct effect on splenic cells or as a side effect of disturbances in other organs, such as the liver, or systems, such as the haemato-immunological system (Petroianu, 2007). Excessive fat that covering the gizzard was also observed in positive control birds compared to both EM treated groups. No clear mechanisms have been reported responsible for the reduction of lipid synthesis by effective microorganisms. It might be due to increase in beneficial bacteria such as *Lactobacillus* in the effective microbes that decrease the activity of acetyl-CoA carboxylase, which is the rate-limiting enzyme in fatty acids synthesis (Jerome, 1970). In the present study, gross intestinal lesion marked by pin-point haemorrhages at the upper and lower tract of intestine of birds in Group D due to heavy infestation was observed. No hemorrhagic lesion was detected in negative control group as the infestation was lower than the positive control group from the early period of the study and also in EM treated groups. Descriptive internal organ changes analysis showed larger cecum (large intestine) and smaller small intestine of birds in Group B compared to other treatment groups. This study suggests that EM shows effect on growth performance, coccidiosis elimination and changes of internal organs in village chicken broilers.

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EFFECT OF PREBIOTIC, PROBIOTIC AND SYNBIOTIC ON PERFORMANCE OF LAYERS AND CHOLESTEROL CONTENT OF EGG YOLK

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ABSTRACT

This study evaluated the effects of prebiotic, probiotic and synbiotic on the performance of laying hens and the cholesterol contents of their egg yolks. One hundred and sixty Hisex Brown hens, aged 20 weeks old, were randomly assigned to four dietary treatments: basal diet (control), basal diet + prebiotic, basal diet + probiotic, and basal diet + prebiotic + probiotic (synbiotic). The results showed that there were no significant differences ($P>0.05$) among the treatments in the feed intake, feed efficiency, egg mass, egg production and egg weight of 20-27 weeks old layers. However, at 28-35 weeks old, these parameters improved significantly ($P<0.05$) in the prebiotic-, probiotic- and synbiotic-fed hens compared to the control hens. The egg yolk cholesterol content of the prebiotic-, probiotic- and synbiotic-fed hens were significantly ($P<0.05$) lower than that of the control hens at 27 weeks of age, but there were no significant differences ($P>0.05$) among treatments at 35 weeks of age. This study showed that prebiotic, probiotic or synbiotic improved the performance of layers from 28-35 weeks of age and reduced egg yolk cholesterol at 27 weeks of age.

Keywords: prebiotic, probiotic, synbiotic, layer performance, egg yolk cholesterol

INTRODUCTION

The rampant utilization of antibiotic growth promoters for animal production has led to the development of antibiotic-resistant bacteria and antibiotic residues in animal products. Probiotics and prebiotics, or their combination (synbiotics) has been considered as potential alternatives. This study was initiated to assess the effects of a prebiotic, a probiotic and a combination of the two as a synbiotic on the performance of layers and the cholesterol content of egg yolk.

MATERIALS AND METHODS

A total of 160 Hisex Brown hens, 20 weeks old, were used in this study. They were placed in an open layer house with individual cages. The layers were randomly assigned to one of the following four dietary treatments: (1) basal diet (control), (2) basal diet + 1% commercial probiotic (PrimaLac), (3) basal diet + 1% isomatooligosaccharide (IMO) (prebiotic diet), and (4) basal diet + 1% IMO + 1% PrimaLac (synbiotic diet). Four replicates, each with 10 hens, were used for each treatment. The experimental period was 16 weeks (20-35 weeks of age). Feed intake was determined weekly, eggs were collected once daily and weighed individually. The egg production (%) was calculated and feed efficiency (feed intake: egg mass) was determined on a weekly basis. At 27 and 35 weeks of age, egg yolks were analysed for total cholesterol using the direct saponification method (Fletouris *et al.,* 1998). The cholesterol content of egg yolk was measured using gas chromatography

with 5a-cholestane as the internal standard. All data were analysed using SPSS software (SPSS Inc., 1999).

RESULTS AND DISCUSSION

From 20-27 weeks of age, the feed intake, feed efficiency, egg mass, egg production and egg weight of laying hens fed the four dietary treatments were not significantly different ($P>0.05$). These results are similar to those of Zarei *et al.* (2011) in which supplementation of dietary inclusion of prebiotics, probiotics or synbiotic to hens has no significant effect on their feed intake, feed efficiency, egg mass and egg production. However, from 28-35 weeks of age, hens fed with prebiotic, probiotic or synbiotic had significantly ($P<0.05$) better feed intake (106.21-107.10 g/hen/day), feed efficiency (1.96-2.01), egg mass (53.21-54.34 g), egg production (90.94-92.64%) and egg weight (58.15-58.57 g) than those fed control diet (feed intake, 102.04 g/hen/day; feed efficiency, 2.19; egg mass, 46.70 g; egg production, 82.14% and egg weight, 56.71 g). To date, there is a lack of study on the effect of prebiotic and synbiotic on laying hens, but studies on probiotics (Kalavathy *et al.*, 2005) showed that the feed efficiency, egg production and egg mass of hens were improved from 28-35 weeks of age. Supplementation of prebiotic, probiotic or synbiotic to laying hens significantly ($P<0.05$) lowered the egg yolk cholesterol content (11.60-11.65 mg/g) when compared to the control (12.71 mg/g) at 27 weeks of age. However, at 35 weeks old, there were no significant differences ($P>0.05$) among the treatment groups in the egg yolk cholesterol content. In conclusion, supplementation of prebiotic, probiotic or synbiotic improved the performance of laying hens from 28-35 weeks of age and reduced the cholesterol content of egg yolk at 27 weeks of age.

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PROXIMATE AND AMINO ACID CONTENTS OF CORN FROM DIFFERENT COUNTRIES

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ABSTRACT

The large variation in the world environmental conditions in which corn is grown combined with the differences in varieties and agricultural practices may result in corn with varying chemical composition. Therefore, local corn samples were collected in several countries and analyzed for proximate and amino acid composition.Significant differences were found for chemical composition of corn originated from seven different countries. Indian and Indonesian corn were high while Brazilian corn was low in protein content. Fat content was high for the corn samples from Brazil and Indonesia and low for USA corn. Cys, Val and Lys of the corn samples were similar from different countries of origin, while other amino acids (Asp, Thr, Ser, etc) showed differences. In conclusion, variation was found for proximate content and certain amino acid values of corn collected in different countries. This variation in certain amino acid contents was independent of either other amino acids or of crude protein.

Keywords: corn, proximate analysis, amino acid, nutrition

INTRODUCTION

Domestic corn production in Malaysia is insignificant. The dominant supplier for Malaysia is Argentina with over 40 percent market share, followed by India and Brazil (USDA, 2012). The feed industry usually uses corn that is equivalent of USA grade 2. This gradation is based on various attributes such as test weight per bushel, percentages of heat-damaged or broken kernels, and foreign material (USDA, 2004). Many of the factors that comprise USA corn grades for potential nutrient availability are based on physical attributes only, such as weight or appearance (USDA, 2004). Therefore, chemical variables may be better predictors of nutritional value compared with more generalised designations such as bushel weight, which has been shown to be a poor estimator of feeding value (Leeson *et al*., 1993; Dale, 1994). The chemical composition of corn may be inconsistent and can vary by genetics, location, soil type, fertility, rainfall and other environment factors. The objective of this study was to determine the proximate and amino acid contents of corn originated from different countries.

MATERIALS AND METHODS

Commercial feed grade local corn samples were collected in Argentina (3), Brazil (3), India (6), Indonesia (8), Philippines (5), Thailand (10) and USA (96) and stored in HDPE bottles. Samples were ground and stored at 4°C for pending proximate and amino acid content analyses. Moisture (930.15), protein (988.05), ash (942.05), fat (920.39) and fiber (962.09) contents were analyzed (AOAC, 2007). Acid hydrolysis (994.12), performic acid oxidation (994.12) and alkaline hydrolysis (988.15) were carried out to determine amino acid content (AOAC, 2007). The significance of the differences between countries means were evaluated using Tukey's test.

RESULTS AND DISCUSSION

The composition of the corn samples by country of origin is presented in Table 1. Indian and Indonesian corn were high while Brazilian corn was low in crude protein (CP) content. The CP of corn from Philippines was lower than that from India but was not different from Argentina, Indonesia, Thailand and USA. The CP content of corn samples in this study were within previously reported range of 7.1-9.4% (Cowieson, 2005). The variation in nutritive value of corn protein might be due to cultivar, type of grain, growing conditions, grain drying temperature or due to other factors (Lasek, *et al*., 2012). Fat content was high for the corn samples from Brazil and Indonesia and low for USA corn. Fat content of all corn samples were within range as previously reported which are 3.4 to 5.2%, (Lasek, *et al*., 2012). Ash and fiber contents for each country varied independently, with lower levels found in Brazilian and USA corn, respectively.

Table 1. Nutrient composition (mean ± SEM, 88% dry matter basis) of corn from different countries

Content (%)	Argentina	Brazil	India	Indonesia	Philippines	Thailand	USA
Crude Protein	7.53 ± 0.18^{abc}	7.13 ± 0.15^{a}	8.57 ± 0.24^{d}	7.94 ± 0.15^{cd}	7.82 ± 0.07^{bc}	7.75 ± 0.09^{abc}	7.24 ± 0.04^{ab}
Fat	3.87 ± 0.47^{bc}	4.43 ± 0.15^{c}	3.32 ± 0.21^{ab}	4.06 ± 0.08^{c}	3.82 ± 0.13^{bc}	3.91 ± 0.07^{bc}	3.19 ± 0.03^{a}
Ash	1.13 ± 0.03^{b}	0.97 ± 0.03^{a}	1.15 ± 0.02^{b}	1.10 ± 0.02^{b}	1.08 ± 0.05^{ab}	1.14 ± 0.03^{b}	1.08 ± 0.01^{ab}
Crude Fiber	2.00 ± 0.06^{ab}	1.93 ± 0.07^{ab}	2.17 ± 0.15^{b}	2.03 ± 0.08^{ab}	2.08 ± 0.07^{b}	2.08 ± 0.04^{b}	1.72 ± 0.02^{a}
Aspartic Acid (Asp)	0.50 ± 0.01^{ab}	0.46 ± 0.02^{a}	0.53 ± 0.01^{b}	0.50 ± 0.10^{ab}	0.49 ± 0.01^{ab}	0.47 ± 0.01^{a}	0.48 ± 0.01^{ab}
Threonine (Thr)	0.28 ± 0.01^{ab}	0.25 ± 0.02^{a}	0.30 ± 0.01^{b}	0.27 ± 0.01^{ab}	0.27 ± 0.01^{ab}	0.27 ± 0.01^{ab}	0.25 ± 0.01^{a}
Serine (Ser)	0.40 ± 0.02^{bc}	0.33 ± 0.02^{a}	0.43 ± 0.01^{c}	0.40 ± 0.01^{bc}	0.38 ± 0.01^{abc}	0.38 ± 0.01^{abc}	0.35 ± 0.01^{ab}
Glutamic Acid (Glu)	1.49 ± 0.02^{bcd}	1.30 ± 0.04^{a}	1.60 ± 0.06^{d}	1.45 ± 0.03^{abcd}	1.54 ± 0.03^{cd}	1.39 ± 0.02^{abc}	1.34 ± 0.01^{ab}
Glycine (Gly)	0.30 ± 0.01^{b}	0.29 ± 0.02^{ab}	0.31 ± 0.01^{b}	0.30 ± 0.00^{b}	0.30 ± 0.01^{b}	0.29 ± 0.01^{ab}	0.26 ± 0.01^{a}
Alanine (Ala)	0.61 ± 0.01^{ab}	0.55 ± 0.03^{a}	0.66 ± 0.02^{b}	0.63 ± 0.01^{b}	0.63 ± 0.02^{b}	0.59 ± 0.01^{ab}	0.54 ± 0.01^{a}
Cystine (Cys)	0.17 ± 0.00	0.16 ± 0.01	0.18 ± 0.01	0.17 ± 0.01	0.18 ± 0.01	0.16 ± 0.01	0.18 ± 0.01
Valine (Val)	0.39 ± 0.05	0.50 ± 0.02	0.41 ± 0.02	0.52 ± 0.02	0.50 ± 0.01	0.52 ± 0.01	0.44 ± 0.01
Methionine (Met)	0.14 ± 0.01^{a}	0.15 ± 0.01^{ab}	0.18 ± 0.01^{b}	0.17 ± 0.01^{ab}	0.16 ± 0.01^{ab}	0.16 ± 0.01^{ab}	0.17 ± 0.01^{ab}
Isoleucine (Ile)	0.23 ± 0.01^{a}	0.21 ± 0.01^{a}	0.26 ± 0.01^{b}	0.24 ± 0.01^{ab}	0.23 ± 0.01^{a}	0.22 ± 0.01^{a}	0.22 ± 0.01^{a}
Leucine (Leu)	1.04 ± 0.04^{b}	0.96 ± 0.04^{ab}	1.17 ± 0.06^{c}	1.10 ± 0.03^{bc}	1.09 ± 0.04^{bc}	1.03 ± 0.01^{b}	0.88 ± 0.01^{a}
Tyrosine (Tyr)	0.29 ± 0.03^{bc}	0.26 ± 0.01^{ab}	0.25 ± 0.01^{a}	0.33 ± 0.01^{c}	0.37 ± 0.01^{d}	0.31 ± 0.01^{bc}	0.28 ± 0.01^{abc}
Phenylalanine (Phe)	0.38 ± 0.01^{b}	0.33 ± 0.01^{a}	0.45 ± 0.02^{c}	0.36 ± 0.01^{ab}	0.36 ± 0.01^{ab}	0.32 ± 0.01^{a}	0.34 ± 0.01^{ab}
Lysine (Lys)	0.24 ± 0.01	0.23 ± 0.01	0.25 ± 0.01	0.25 ± 0.01	0.24 ± 0.01	0.24 ± 0.01	0.23 ± 0.01
Histidine (His)	0.19 ± 0.01^{a}	0.18 ± 0.01^{a}	0.24 ± 0.01^{b}	0.19 ± 0.01^{a}	0.19 ± 0.01^{a}	0.18 ± 0.01^{a}	0.17 ± 0.01^{a}
Arginine (Arg)	0.36 ± 0.01^{ab}	0.33 ± 0.01^{a}	0.39 ± 0.01^{b}	0.38 ± 0.01^{b}	0.38 ± 0.01^{b}	0.36 ± 0.01^{ab}	0.34 ± 0.01^{a}
Tryptophan (Trp)	0.06 ± 0.01^{c}	0.04 ± 0.01^{a}	0.05 ± 0.01^{bc}	0.05 ± 0.01^{bc}	0.05 ± 0.01^{ab}	0.05 ± 0.01^{bc}	0.05 ± 0.00^{bc}
Proline (Pro)	0.65 ± 0.01^{abc}	0.63 ± 0.05^{ab}	0.78 ± 0.04^{d}	0.71 ± 0.05^{abc}	0.67 ± 0.04^{abc}	0.71 ± 0.04^{bc}	0.56 ± 0.01^{a}

$^{a-d}$ Means within a row without a letter in common differ (P<0.001); SEM: standard error of mean.

Cys, Val and Lys contents of the corn samples from different origin were similar. Met content for corn samples from India and Argentina was higher and lower, respectively. Trp content of corn from Argentina was high while low in Brazilian corn. Indian corn was high in Thr while Brazilian and USA corn showed lower values. Thr content in corn from Argentina, Indonesia, Philippines and Thailand were similar. Other amino acids (Asp, Ser, Glu, Gly, Ala, Ile, Leu, Tyr, Phe, His, Arg and Pro) showed difference among countries. The amino acids showed variation independent of either other amino acids or protein content. The variation in corn chemical composition might be due to differences in genetics, location, fertility, agronomic conditions, pre- and post harvest processing variables, storage condition and period of storage. In conclusion, variation was found for proximate content and certain amino acid values of corn collected from different countries.

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DETERMINING BIOLOGICAL EFFECTS OF FUNCTIONAL AMINO ACID SUPPLEMENTATION IN LOW CRUDE PROTEIN DIET ON GROWTH PERFORMANCE OF LAYER CHICKEN

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ABSTRACT

This investigation sought to determine the biological effects of functional amino acids on the growth performance of layer chickens. A total of 144 Hisex Brown hens were allocated into 4 groups that were each fed low crude protein diet from 16 to 32 weeks of age. Diets contained 17.5% CP (control group), 17.5% CP, 17% CP and 16.5% CP and were supplemented with limiting AA (methionine, lysine and threonine). Weekly feed intake, egg weight, hen-day egg production and feed conversion ratio (FCR) were collected. There was no significant difference ($P>0.05$) among the treatment groups on overall feed intake, egg weight and egg mass. Significant higher ($P<0.05$) overall hen-day egg production was observed in all treatment groups compared with the control. Overall, 17.5% CP diets resulted in better egg production than other treatments.

Keywords: crude protein, amino acid, laying hen, growth, production

INTRODUCTION

The use of low-protein, amino acid-supplemented diets for various classes of poultry has been the subject of numerous investigation. According to Amaefule *et al*. (2000), lysine, methionine, methionine plus cystine and tryptophan are the major amino acids that are limiting in the practical feeds for laying hens. Inadequate knowledge about the essential amino acids requirements of laying hens may have been the reason for the inferior performance of hens (Keshavarz *et al*., 2004). Thus, the main objective of this study was to determine the effect of diet supplemented with essential amino acids on growth performance of layer.

MATERIALS AND METHOD

Feeding trial

A total of 144 16-week old layer hens of Hisex brown were housed in two-deck battery-type cage block equipped with nipple drinkers and feed trough. Each treatment consisted of six replications and six birds per replicate. Feed was supplied according to Hisex Brown Management Guide and water was provided daily *ad libitum*. The birds were randomly assigned to four dietary treatments; 17.5% CP (negative control), 17.5% CP, 17% CP and 16% CP (addition of commercial methionine, lysine and threonine).

Sampling and laboratory analysis

Feed intake, egg weight, hen-day egg production and feed conversion ratio (FCR) were calculated. Data were analysed by one way analysis of variance (ANOVA) using the General Linear Model (GLM) procedure by Statistical Analysis System (SAS, 1998). Duncan's Multiple Range Test System was used to compare the significant difference between the treatments at $P<0.05$.

RESULTS AND DISCUSSION

There was no significant difference ($P>0.05$) among the treatment groups on overall feed intake, egg weight and egg mass. Decreasing protein level in diets usually does not depress feed intake (Chaiyapoom *et al*., 2005). Significant higher ($P<0.05$) overall hen-day egg production was observed in all treatment groups supplemented with amino acids combination compared with the control. Egg production was improved significantly by increasing dietary protein levels and addition of 0.1% methionine and 0.12% lysine to 16% CP diet (Zeweil *et al*., 2011). Low protein diet (17.5% CP) supplemented with functional amino acids resulted in significantly improved egg production. Therefore, implementing reduced crude protein with amino acid balanced diets for laying hen would maintain layer performance concurrently reduce the production cost.

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HEAVY METALS IN MARINE MUSSELS AND MANGROVE SNAILS: ARE THE MOLLUSCS MEALS SAFE AS POULTRY FEEDS TOO?

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ABSTRACT

The concentrations of Cd, Cu, Pb and Zn were determined in the total soft tissues of snails *Nerita lineata* and mussels *Perna viridis* collected from Peninsular Malaysia. The metal levels were compared with established permissible limits or guidelines for human consumption to predict the possible toxicological consequences if those molluscs were to be used as poultry feeds. It is found that Cd, Cu and Zn were well below the guidelines while Pb levels in the snail populations exceeded the guidelines. However, it is assumed that the toxicological effects on the animals would be lesser due to two main reasons: i) poultry feeds are usually composed of a multiple composition and the molluscs feeds made up of < 50%; ii) the tolerance of molluscs to the toxins and metals could be better when compared to human. Thus, the molluscs meal can be potentially used as poultry feeds similarly to the fish meal.

Keywords: Molluscs meals, heavy metals, poultry feeds

INTRODUCTION

Poultry has higher requirements for sulphur-containing amino acids (especially methionine and cysteine) than any other food-producing animals due to the plumage protein. According to Jonsson and Tauson (2011), the first limiting nutrients in typical laying hens' diets are the sulphur-containing amino acids, particularly methionine. As supplementation of synthetic methionine is not allowed in organic production, it is crucial to have alternative high-quality protein feed ingredients. In commercial broiler production, fish meal is generally used as a high quality protein source for its rich amino acids content. Therefore, the use of marine molluscs as poultry feed could be the answer. The objective of this study was to compare heavy metals level in the snails (*Nerita lineata*) and green-lipped mussels (*Perna viridis*) from Peninsular Malaysia with established permissible limits and guidelines for human consumption, and to predict the possible toxicological consequences if those molluscs were to be used as poultry feeds.

MATERIALS AND METHODS

Snails and mussels were collected from 11 and 8 sampling sites, respectively in Peninsular Malaysia. Methods of analysis were adapted from Yap *et al*. (1999), while analytical procedures were checked with Certified Reference Material (CRM) for dogfish liver (DOLT-3, National Research Council Canada). The present dry weight basis was converted into wet weight basis for *N. lineata* (Cheng, 2008) and *P. viridis* (Yap *et al.*, 1999).

RESULTS AND DISCUSSION

The mean metal concentrations for *Nerita* snails' (µg/g wet weight) ranges are 1.32-5.37 for Cu, 21.37-27.18 for Zn, 0.21-10.50 for Pb, and 0.14-1.30 for Cd. For *Perna* mussels, the concentrations (µg/g wet weight) ranges are 0.48-3.07 for Cu, 8.67-20.36 for Zn, 0.27-1.50 for Pb, and 0.07-0.31 for Cd. For Cd, only snail population from Lukut exceeded some the maximum permissible limits (1.0 µg/g wet weight)(FAO, 1983; MRC, 1985; USFDA, 2001). The Pb levels in all the snails' population except for Kg. Pasir Puteh had exceeded all guidelines while only mussels from Kg. Pasir Puteh were close to the Pb guideline and others were well below. In comparison to all the established guidelines for human consumption, the levels of Cu and Zn in the snails and mussels were below all the guidelines. Nevertheless, Cd and Pb can potentially cause kidney damage at any concentrations (Abou-Arab *et al*., 1996). Cu and Zn are essential minerals and required cofactors for enzymatic reactions for all molluscs (Hambidge, 2000). Although the comparisons of present metal levels were done with the guidelines for human consumption (not poultry), it is assumed that the toxicological effects on the animals would be lesser due to: i) poultry feeds are usually composed of a multiple composition and the molluscs feed made up of <50%, ii) the tolerance of molluscs to the toxins and metals could be better when compared to human. The use of snails and mussels as poultry feeds has been widely reported in the literature (Diomande *et al*., 2008; Sogbesan *et al*., 2012). Kacem *et al*. (2009) reported the evidence of the presence of okadaic acid in *M. galloprovincialis* in the Bizerte Lagoon despite the fact that mussels are potential high protein source in poultry feeds. Jönsson and Holm (2010) found that mussel meal functions as a novel protein source for laying hens and those toxic mussels at this level may be included in the feed without negative effects on parameters evaluated in this study. Waldenstedt and Jönsson (2006) reported that poultry could tolerate moderate levels of toxins without detrimental effects on health and with no residues in meat and eggs. Thus, molluscs meal can be used as potential poultry feeds similarly to the fish meal.

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IMPACT OF REPLACING VITAMIN E WITH GRAPE POLYPHENOL ON THE PERFORMANCE OF BROILERS

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ABSTRACT

Grape Polyphenols is a good antioxidant and could be used as an alternative and cheaper source. It has also been reported that it works synergistically with Vitamin E. Four iso-nitrogenous and iso-caloric starter and finisher diets was formulated and gradually replaced with vitamin E. These four treatments were fed to four different groups from days 1-21 (starter) and days 22-42 (finisher). Data regarding performance was collected weekly. Result showed non-significant impact on growth performance. In conclusion, grape polyphenols can be used in place of vitamin E without any untoward effects on performance of broilers.

Keywords: vitamin E, grape polyphenol, broilers

INTRODUCTION

Grape (Vitisvinifera) is one of the world's largest fruit crop (FAO, 2007) with approximate annual production of 61 million metric tons (Dorri *et al*., 2012). The grape by-products collected during destemming grape crushing and pressing (Dorri *et al*., 2012) or collected after juice extraction and wine industry processing are used either as animal feed or ethanol production (Viveros *et al.,* 2010). Several researchers have reported the importance of by products from wine processing as plant materials particularly rich in a wide range of polyphenols (Dorri et al., 2012). These residues could be used to extract the polyphenols. It was reported that the polyphenols extracted from grapes not only possess more antioxidant properties but also excellent property of thermo-stability and work synergistically with vitamin E (Gladine *et al*., 2007). At present, many evidences exist which supports the antimicrobial activity of polyphenols by changing the gut physiology and modifying morphology of intestine (Lopez-olive *et al*., 2006). Very limited data available in literature in relation to the effects of replacing vitamin E with GPP, thus, the present study has been planned to investigate the replacement effects of vitamin E with GPP on immunity, antioxidant status, tissue pathological change and growth performance of broilers.

MATERIAL AND METHOD

Two hundred eighty day old chicks (Hubbard) were randomly divided into four groups having seventy chicks in each. Each group was further portioned into seven replicates with ten chicks per replicate. Each replicate was placed in separate pens, all birds was reared under identical management system. Vaccines were done according to local schedule of Poultry research institute (PRI) Rawalpindi. Four isonitrogenous (22.5% CP) and isocaloric (ME 3200 Kcal/Kg) starter and finisher diets was formulated and fortified with 0+100 (C), 25+75 (LGP), 50+50 (MGP) and 75+25 mg (HGP) GPP and vitamin E per kg of diet, respectively. Feed intake, body weight

gain and feed conversion ratio of each treatment was recorded on weekly basis and it was divided into three phases' starter (1-21 day), finisher (22-35 day) and starter cum finisher phase (1-35 day). Data collected from the trial was analyzed by analysis of variance (ANOVA) techniques in a Completely Randomized Design (Steel *et al.*, 1996). The software used for statistics analysis was SPSS 17.0 (SPSS Inc., USA).

RESULTS AND DISCUSSION

The feed intake, body weight and feed conversion ratio showed linearly non-significant ($P>0.05$) effect among all treatment groups in starter, finisher and starter cum finisher phase. A significant ($P<0.05$) quadratic response was observed in weight gain in starter phase. The findings of present study have shown that replacement of vitamin E with Grape polyphenol did not affect growth performance in starter or finisher phases. Similar results have been reported by (Dorri *et al.,* 2012) who fed different levels of grape pomace (GP) to broilers and concluded that inclusion of GP have non-significant impact on growth. Several researchers (Brenes *et al.,* 2010; Viveros *et al.,* 2011) have reported similar no-significant impact of grape extracted polyphenols on growth performance. In conclusion, present study has confirmed previous studies that polyphenols extracted from grape by products also did not alter growth performance of broilers.

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EFFECT OF FEEDING FERMENTED PALM KERNEL CAKE ON PERFORMANCE OF BROILER CHICKENS

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ABSTRACT

A total of 245 Cobb 500 one-day old chicks were raised in open house system at the poultry unit, Universiti Putra Malaysia. The birds were randomly distributed on 7 dietary treatments with 5 replicates per treatment. The chicks were fed 0, 5, 10, 15% palm kernel cake (PKC), and 5, 10, 15% fermented palm kernel cake (FPKC). The birds were fed *ad libitum*, and the trial lasted for 42 days. The objective of the experiment was to determine the effect of feeding FPKC on broiler performance. The results obtained showed that body weight (BW) and body weight gain (BWG) were significantly ($P<0.05$) decreased for broilers fed diet with 10% and 15% PKC compared to those groups fed diet with 0% PKC, whereas feed intake (FI) and feed conversion ratio (FCR) were not significantly ($P>0.05$ different. No significant effect in BW and BWG for broilers fed 5% PKC or 5, 10, 15% FPKC. The birds fed 10 and 15% FPKC produced significantly ($P<0.05$) higher BW and BWG compared to those groups fed 10 and 15% PKC, respectively. The study showed that FPKC can be included up to 15% to broiler diets without any adverse effect.

Keywords: FPKC, PKC, broiler performance

INTRODUCTION

It is well known that Malaysia is one of the largest worldwide producers of palm oil. Consequently, it has abundant amounts of PKC which consider as agro-industrial waste after the process of oil extraction from palm fruits. The challenge of using the PKC to be included to poultry diets is the presence of high levels of crude fibers (CF), coarse texture, gritty appearance (McDonald *et al.*, 1995; O'Mara *et al.*, 1999; Sundu and Dingle, 2002) and non- starch poly saccharide (NSP) such as mannan, xylan and cellulose (Sundu and Dingle, 2002; Alimon, 2004; Francech and Brufau, 2004). Therefore, it was reported that feeding broiler more than 12.5% (Boateng *et al.*, 2008) or 15% PKC (Soltan, 2009) without enzyme would be led to decrease their performance.

MATERIALS AND METHODS

A total of 245 Cobb 500 one-day old chicks were obtained from local hatchery in Malaysia. The chicks were wing-banded, individually weighed and randomly distributed into 7 groups with 5 replicates per treatment and 7 birds per cage. The broilers were fed with diets containing 0, 5, 10, 15% PKC and 5, 10, 15% FPKC *ad libitum*, and the trial lasted for 42 days. BW was individually recorded each week, and FI was recorded each week per cage. BWG and FCR were calculated. The data were analysed by using General Linear Model procedure of the statistical analysis system (SAS, 2003). Tukey's test was used to compare the means of treatments at probability 0.05 ($P<0.05$).

RESULTS AND DISCUSSION

Table 1 shows the effect of feeding FPKC on broiler growth performance at 42 days. The birds fed with 10 or 15% FPKC were not significantly (P>0.05) affected, whereas the BW and BWG of broiler fed with the same level of PKC was significantly (P<0.05) decreased. No significant effect (P>0.05) was observed in FI and FCR among dietary treatments. The reduction in BW and BWG in birds fed with 10 or 15% PKC may be due to the presence of high levels of NSP as well as the CF, whereas the improvement being observed in broiler fed with 10 or 15% FPKC could be referred to the reduction of NSP and CF as a result of previous fermentation. This finding was in agreement with (Iluyemi *et al.*, 2006; Dairo and Fasuyi, 2008) when they referred to the improvement of FPKC quality as a result of the fermentation. In conclusion, the FPKC can be substituted for yellow corn in broiler chickens' diets up to 15%.

Table1. Effect of graded levels of PKC and FPKC in broiler chickens performance

	PKC level				FPKC level			
	0%	5%	10%	15%	5%	10%	15%	SEM*
BW (g)	2107.33^{a}	1995.59^{abc}	1881.75^{bc}	1876.13^{c}	2106.14^{a}	2102.10^{a}	2017.93^{ab}	31.88
BWG (g)	2067.55^{ab}	1936.66^{bcd}	1860.82^{cd}	1836.87^{d}	2076.62^{a}	2062.40^{ab}	1977.50^{abc}	32.14
FI (g)	3589.59	3447.70	3459.97	3483.58	3593.22	3527.38	3499.35	65.90
FCR (g:g)	1.75376	1.81756	1.76871	1.71823	1.62722	1.71690	1.70823	0.08

*Mean±SEM^{a-d} means with different superscripts in the same raw are differ significantly (P<0.05)

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INFECTIOUS BURSAL DISEASE VACCINE DELIVERY VIA TOPICAL APPLICATION IN 18-DAY-OLD SPECIFIC PATHOGEN FREE EMBRYONATED CHICKEN EGGS

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ABSTRACT

The purpose of this study was to investigate the effect of vaccination against infectious bursal disease (IBD) via topical application of IBD vaccine (MyVAC UPM93) either with or without liposomes as vaccine carrier in 18-day-old specific pathogen free (SPF) embryonated chicken eggs. The study demonstrated that the IBD vaccine group alone could induce high and protective level of IBD antibody titre (2545±1884 ELISA unit) in the chicks at 14 days of age. In contrast, IBD antibody titre was not detected in the liposomes, combination of liposomes with IBD vaccine and control groups of chickens. It appears that further study is needed to improve the liposome as a vaccine carrier to accelerate vaccine delivery to the target organ and enhance antibody titre. It was concluded that topical application of IBD vaccine (MyVAC UPM93) in 18-day-old SPF embryonated chicken eggs can be an effective vaccination route against IBD.

Keywords: Infectious bursal disease, vaccine delivery, topical application route, liposome

INTRODUCTION

Developments of nanoparticles as carrier-based vaccines have received a lot of attention due to their potential to provide effective immunisation. Nanoparticles are solid particles with the size around 10 to 1000 nm (Kreauter, 1996). Liposomes, being composed from phospholipids and can function adroitly complements the natural lining of nearly every cell. This therefore creates a natural bond and/or affinity for the liposomes to deliver their drugs or vaccines to the cells in the live body (Rosenblum and Chen, 1995). Infectious bursal disease (IBD) is one of the major viral diseases for the poultry industry worldwide. IBD causes death to susceptible chickens as well as immunosuppression which lead to a variety of secondary infections and high mortality. IBD can be controlled and prevented by proper vaccination and biosecurity. Vaccination as early as 18-day-old embryonated eggs known as *in ovo* vaccination has been practiced, although puncturing the eggshell and membrane dramatically increased embryonic mortality, regardless of whether any material is injected into the egg (William, 2005). Alternatively, topical application onto eggshell could provide better solution and could reduce mortality. A few studies have been done in relation to the topical application of drugs onto the crocodile eggs (Muller *et al*., 2007) that have similar characteristic with chicken eggs (Astheimer *et al*., 1989). Thus, the objective of this study was to determine the effect of vaccination against IBD via topical application of IBD vaccine (MyVAC UPM93) either with or without liposomes as vaccine carrier in 18-day-old specific pathogen free (SPF) embryonated chicken eggs.

MATERIALS AND METHODS

Specific-pathogen free (SPF) embryonated chicken eggs and IBD vaccine (MyVACUPM93) were obtained from Malaysian Vaccines and Pharmaceuticals (MVP) Sdn. Bhd. Positive liposome kit (Product No: L4395) was obtained from Sigma Malaysia. Sterile double distilled water (1.0 mL) was added into the vial that contained dry powder of liposomes at room temperature. Then, by using vortex, the hydrated liposomes were shaken for 60 seconds. A sample was taken for determination of size and zeta potential. After that, by using sterile pipette, IBDV were combined with hydrated liposomes. The vial was stored at 4-6˚C. Twenty four, 18-day-old SPF embryonated chicken eggs were marked with pencil and the surfaces of the eggshells were cleaned by using 70% ethanol. Then, the eggs were divided into 4 groups namely the control, liposome, combination of IBDV and liposome and IBDV. Samples (0.1 mL) were sprayed onto eggshells accordingly. After half an hour, eggs were incubated at 37°C until all the eggs hatched. Serum was collected from all groups of chickens at 14 days of age for detection of IBD antibody titre using the ELISA technique.

RESULTS AND DISCUSSION

The mean size of pure liposome, IBDV and combination of liposome and IBDV were 1441$\pm$313 nm, 1827$\pm$182 nm and 2842$\pm$168 nm, respectively. These averages of sizes were quite far from the expected values and contradicted with the early hypothesis. This could be due to the homogenization procedure which was not performed before the samples were sprayed onto the eggshells in the study. The average of zeta potential of empty liposome, IBDV and combination of liposome and IBDV were +192 mV, -18.0 mV and -12.1 mV, respectively. In order to maintain stability and pH of liposomes, phosphate buffered saline (PBS) was found to be the better choice to dilute the liposomes rather than using distilled water. Although the size IBDV, liposomes and combination of both were more than 1000 nm, they were still under the range diameter size of pores in the eggshells of the chickens which varied from 110 to 4140 nm (Tan *et. al*., 1992). Hence, there are possibilities of the vaccine and the carriers to be able to pass through the eggshells of the chickens. Neither clinical signs nor gross and histological lesions of the bursa of Fabricius were observed in all groups of chickens throughout the study. It is interesting to note that the IBD vaccine group alone could induce high and protective level of IBD antibody titre (2545 $\pm$ 1884 ELISA unit) in the chicks at 14 days of age. In contrast, IBD antibody titre was not detected in the liposome, combination of liposome with IBD vaccine and control groups of chickens. It appears that further study is needed to improve the liposome as a vaccine carrier to accelerate vaccine delivery to the target organ to enhance antibody titre. It was concluded that topical application of IBD vaccine (MyVAC UPM93) on 18-day-old SPF embryonated chicken eggs can be an effective vaccination route against IBD.

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ISOLATION OF *ESCHERICHIA COLI* FROM VARIOUS ORGANS OF BROILER CHICKENS WITH COMPLICATED CHRONIC RESPIRATORY DISEASE

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ABSTRACT

Escherichia coli was isolated from broiler chickens in 17 farms in the state of Johor (6 farms), Melaka (6 farms) and Perak (5 farms), Malaysia with clinical signs and lesions of complicated chronic respiratory disease (CCRD). The affected live chickens which showed clinical signs of distended abdomen, respiratory distress, weak, stunted and ruffled feather, whilst the dead chicken with distended abdomen were selected in the study. Upon necropsy, pericarditis, air sacculitis, perihepatitis, ascites, splenitis and peritonitis were the common lesions recorded. Samples of heart, liver, spleen, air sac and peritoneal swab were collected for *E. coli* isolation and identification. *E. coli* was isolated from 40% (2/5), 67% (4/6) and 60% (3/5) farms in Johor, Melaka and Perak, respectively. Heart (26%) and spleen (26%) were the common samples positive for *E. coli* followed by the liver (22%), air sacs (17%) and peritoneal swabs (9%).

Keywords: *Escherichia coli*, avian pathogenic *E. coli*, colibacillosis, broiler chickens, complicated chronic respiratory disease

INTRODUCTION

The term avian pathogenic *E. coli* (APEC) is used for *E. coli* isolated from avian colibacillosis. The agent is well known as the primary cause of morbidity, mortality and condemnation of carcasses in the poultry worldwide. The bacterium is responsible for 20% to 40% mortality in broiler chickens depending to the severity of the disease. Colibacillosis commonly occurs in 3 to 12 weeks old broiler chickens and characterised by extraintestinal disease. Complicated chronic respiratory disease (CCRD) is the most common form of colibacillosis that is frequently followed by septicaemia (Ewers *et al*., 2004). Clinical signs of CCRD varied from unapparent to total unresponsiveness just prior to death. The primary lesions are airsacculitis, perihepatitis, pericariditis, ascites and peritonitis (Barnes *et al*., 2008; Blanco *et al*., 1998). APEC strains possess virulence factors that enable the organisms to live extraintestinal life and each strain has a number of virulence factors with several combinations of genes (Circella *et al*., 2012). Currently, there is little known of the specific virulence genes and combination of genes associated with strains causing the disease. Vaccination is the only method of effective prevention. Therefore, this study was conducted to isolate *E. coli* from broiler chickens with CCRD and to identify the infected organs. The molecular characteristics and pathogenicity of the isolates will be further determined for future development of vaccine against the disease.

MATERIALS AND METHODS

Seventeen commercial broiler farms in the state of Johor (6 farms), Melaka (6 farms) and Perak (5 farms) with history of complicated chronic respiratory disease (CCRD) were selected in this study. Five live chickens with clinical signs of CCRD such as distended abdomen, respiratory distress, weak, stunted and ruffled feathers and five dead chickens with distended abdomen were selected from each farm. Post mortem was conducted and the gross lesions were recorded. Samples of heart, liver, spleen, air sac and peritoneal swabs were collected for bacterial isolation and identification. All samples were cultured on Mac Conkey agar at 37˚C for 24 hours. Gram staining was performed to select gram negative and small rods bacteria. Isolates were then subcultured onto blood agar and incubated at 37˚C for 24 hours to harvest the pure culture. Standard biochemical tests for *E.coli*; oxidase urea, triple sugar iron (TSI), sulfide-indole-motility (SIM) and citrate were used to identified the isolates.

RESULTS AND DISCUSSION

The study showed that *E. coli* was isolated from 40% (2/5), 67% (4/6) and 60% (3/5) farms in state of Johor, Melaka and Perak, respectively in chickens with CCRD or gross lesions of pericarditis, air sacculitis, perihepatitis, ascites, splenitis or peritonitis. These may associate with the issues of antibiotic resistance and effectiveness of antibiotic therapy in the control and prevention of the disease. Antibiotic sensitivity test needs to be conducted for the isolates. Samples of heart (26%) and spleen (26%) were the common samples positive for *E. coli* followed by the liver (22%), air sacs (17%) and the peritoneal swabs (9%). The isolation of *E.coli* from multiple organs of chicken with CCRD is an indicative for invasiveness of extraintestinal *E.coli*. Pathogenesis of APEC started with initial step of infection such as by colonization of *E.coli* at the respiratory tract, followed by crossing and penetration into the mucosa of air sacs, and then multiplication in the blood stream and internal organ such as liver, heart and spleen (Levine *et al*., 1983). Production of deleterious effect from cells and tissues leading to lesions developed followed with clinical signs. However, the knowledge of specific and roles of each combination of invasive strain virulence genes in the pathogenesis is poorly understood. The molecular characteristics and pathogenicity of the isolates in the present study will be further determined for future development of vaccine against the disease.

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ADAPTATION AND ATTENUATION OF FOWL ADENOVIRUS IN MAMMALIAN CELL LINE

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ABSTRACT

Highly pathogenic Fowl Adenoviruses (FAdVs) have been recognised as the primary pathogen of inclusion body hepatitis (IBH) in many poultry producing areas and thus, there is a need to develop vaccine for the control and prevention of the disease. It was the objective of the study to determine the adaptation and attenuation of FAdV (UPM1137) isolated from chickens with IBH in mammalian cell line (Vero cells). Confluent monolayer of Vero cells were inoculated with 0.1mL FAdV inoculum and monitored daily until day 7 post inoculation (pi) for cytopathic effect (CPE). CPE was recorded on day 6 pi on the first (P1) and subsequent passages up to passage 5 (P5). It was further confirmed that the CPE belong to FAdV by polymerase chain reaction (PCR) and sequence analysis. The virus successfully adapted and attenuated up to P5 with presence of amino acids changes. Phylogenetic trees revealed both samples from original homogenate embryo liver (P0) and P1 infected Vero cells (P1) were classified under FAdV group E strain (serotype 8). However, the FAdV at P5 could not be assigned to any clusters within groups as it might be due to a new strain of FAdV.

Keywords: Fowl Adenovirus (FAdVs), Vero cells, adaptation, cytopathic effect (CPE), polymerase chain reaction (PCR)

INTRODUCTION

Highly pathogenic fowl adenoviruses (FAdVs) have been recognised as the primary pathogen of inclusion body hepatitis (IBH) commonly caused by serotype 8 and 9. FAdVs are usually isolated and grown in embryonated eggs and in primary cell cultures such as chicken embryo livercells and chicken kidneycells producing typical cytopathic effect (CPE) of clumping and balooning of the cells. There are only few studies on propagation of the virus in Vero cells sincethe virus appears to replicate better in homologous than in heterologous cells culture system (Aghakhan and Pattison, 1974). However the use of a continuous cell line such as Vero cells has several advantages over the use of primary cell culture, as it can be made available easily and continuously. It also provides fast growth of confluent monolayer cells which takes only about 24 hours for Vero cells and is capable to produce CPE as early as first passage after inoculated with avian viruses (Barta *et al.,* 1984). It was the objective of the study to determine the adaptation and attenuation of FAdV isolated from chickens with IBH in Vero cells.

MATERIALS AND METHODS

Samples of liver, gizzard and proventriculus were collected from 25 to 27-week-old commercial layer chickens during outbreak of IBH with total mortality of 3% within a week and decrease in egg production. Upon necropsy, haemorrhagic hepatitis and severe haemorrages and ulceration of the gizzard were noted. The samples were further inoculated into 9-day-old embryonated specific pathogen free (SPF) eggs. Samples of liver were collected from the embryo and processed for the preparation

of the virus inoculum (UPM1137). The FAdV inoculum (0.1 mL) was inoculated onto monolayer Vero cells and incubated for 30 minutes at 37°C for adsorption of virus into cells. A maintenance medium, DMEM with 1% FBS was then provided into the flask and kept under controlled atmosphere with 5% CO_2 and 85-90% humidity. Cultures were observed daily for 7 days post inoculation (pi) for CPE formation. For inoculation into next passage, the virus was harvested at the time of optimum CPE by freezing and thawing three times at -20°C (Barta *et al.,* 1984). The supernatant were collected for inoculum in the subsequent passages and the method was repeated for the following passages. Samples from the virus inoculum (P0) and supernatant from first passage (P1) and fifth passage (P5) in Vero cells were extracted and amplified using published primers known as H1/H2 and H3/H4 (Raue and Hess, 1998). The PCR product was visualized in a 1% agarose gel with ethidium bromide, purified and sequenced. The sequences were analysed and phylogenetic tree was constructed using ClustalW Multiple Alignment software programmes.

RESULTS AND DISCUSSION

The study showed that UPM1137 FAdV isolate was successfully adapted and attenuated in Vero cells. CPE was recorded on day 6 pi at the first and subsequent passages. The major type of CPE produced was rounding of the cells. Samples of homogenate liver embryo (P0) and viral supernatant from P1 and P5 was positive for FAdV with their expected size 1219 bp (H1/H2 primer) and 1319 bp (H3/H4 primer). Nucleotide sequence changes were recorded in P5 with presence of substitutions, additions and deletions in various positions after comparing with P0 and P1. Substitutional of amino acids indicates presence of mutations of the virus gene after subsequent passage in the Vero cells recorded in P5. The hexon is a major antigenic protein of the adenovirus and consists of conserved regions (pedestal P1 and P2), which are located more inside the virion and the variable loops (L1 to L2), which protrude from the surface. These loops contain the type-specific neutralizing epitopes (Toogood *et al*., 1992). Phylogenetic trees which were constructed using 27 field isolates from the Genebank revealed both samples from P0 and P1 were classified under Group E strain (serotype 8). However, P5 sample could not be assigned to any clusters within groups and as it might be a new strain of FAdV gene. It was concluded that the FAdV isolate (UPM1137) had been successfully adapted and attenuated following five passages in Vero cells.

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ADAPTATION OF INFECTIOUS BRONCHITIS VIRUS IN MAMMALIAN CELL LINE

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ABSTRACT

Infectious bronchitis virus (IBV) is the causative agent of chicken infectious bronchitis (IB), an acute, highly contagious viral respiratory, urinary and reproductive tracts disease. Two different IBV inoculums (UPM1212) preparation was used in the study following inoculation of the virus into African green monkey kidney cell line (Vero cells) up to three consecutive passages. The study showed that the inoculums prepared directly from kidney of IBV infected chicken showed cytophatic effect (CPE) starting from the second (P2) to third passages (P3). The CPE was characterised by formation of syncytium, giant cell, dendritic shaped cell and finally plaque formation. The polymerase chain reaction (PCR) results confirmed the presence of IBV in the Vero cells at P2 and P3. In contrast, no CPE was observed in Vero cells inoculated with inoculums prepared from allantoic fluid following inoculation of the sample into specific pathogen free (SPF) embryonated chicken eggs. The PCR results were negative for IBV.

Keywords: Adaptation, cytophatic effects (CPE), infectious bronchitis virus, Vero cells

INTRODUCTION

Infectious bronchitis virus (IBV), a prototype of the coronaviridae family in new order Nidoviridales causes an acute and highly contagious disease of the respiratory, urinary and reproductive tracts of chickens (Collisson *et al*., 1992). IBV infection causes severe economic losses to the poultry industry worldwide. Animal cell cultures have been used for cultivation of viruses since the 1950's because it is an excellent host for virus growth. It was reported that some IBV strains were successfully propagated in BHK-21 cells (Otsuki, 1979) and adapted in mammalian cell line (Vero cells) by serial passages and produce cytopathic effects (CPE) (Cunningham *et al*., 1972). The use of Vero cell line has several advantages over the primary cell culture of avian origin. Vero cells are easy to handle and maintain and free from vertically transmitted extraneous virus of avian origin (Hassan *et al*., 1996). Vero cells are widely used for production of high quality vaccines for human and animals. Thus, the objective of this study was to determine the adaptation of recent isolate of IBV (UPM1212) in Vero cell line for the future development of vaccine against the disease.

MATERIALS AND METHODS

Recent IBV isolate (UPM1212) was obtained from broiler chickens during an outbreak of infectious bronchitis (IB) in a farm. Two types of virus inoculums were prepared prior to inoculation of the virus in Vero cells: inoculums prepared directly from infected kidney of chicken and inoculums prepared from allantoic fluid following inoculation of the sample into specific pathogen free (SPF) embryonated chicken eggs. The Vero cells were cultured in 25cm^2 flasks with double minimum essential medium (DMEM, Gibco) and supplements contained 5% fetal bovine serum (FBS, Gibco), sodium bicarbonate and antibiotic penicillin streptomycin (Penstrep,

Gibco) and incubated at 37°C. Cultured cells were observed carefully under inverted microscope until the formation of semi confluent monolayer was formed. At this stage, the growth medium was discarded and cells were infected with 0.25mL of IBV inoculums. After an hour of adsorption at 37°C incubation, the maintenance medium with 1% FBS were added and the monolayer cells were kept at 37°C. Cultured cells were observed daily for cellular morphologic changes and presence of cytopathic effect (CPE) up to 7 days post inoculation (pi). The virus was harvested as the culture was subjected to three cycles of alternate freezing and thawing. The supernatant was harvested and centrifuged at 600xg for 15 minutes and stored at -70°C. Harvested passage 1 (P1) virus was infected again onto Vero cells using the same media and techniques. The virus harvested from the second passage was designated as passage 2 (P2): similarly for the subsequent passage three (P3). All RNA extractions were carried out with TRIzol® Reagent (Invitrogen, USA) according to the manufacturer's instructions. The RT-PCR was conducted as described by Zarirah *et.al.* (2009). The PCR products were analyzed on a 1% agarose gel containing 1.5 µl of Midori Green DNA stain (Nippon Genetics Europe GmbH, Germany).

RESULTS AND DISCUSSION

The study showed that there were no alteration detected in mock-infected monolayers on passage one (P1) for both inoculums. At the second (P2) and third passages (P3), it was observed that formation of CPE spread quickly from the original virus infected cells to the surrounding cells. The CPE was characterised by formation of syncytium, giant cell, dendritic shaped cell and finally formation of plaque. PCR results confirmed the presence of IBV (470 bp) in the Vero cells at P2 and P3. In contrast, CPE was not observed in Vero cells inoculated with inoculums prepared from allantoic fluid following inoculation of the sample into SPF embryonated chicken eggs. The PCR results were also negative for IBV. IBV replication in Vero cells resulted in typical CPE; rounding up and fusion of infected cells to form multinucleated giant syncytia, detachment of infected cells from culture dish and eventually cell lysis and death. It was previously reported that the Beaudette strain of IBV grown in chicken embryos adapted to the serially propagated Vero cell line for 65 passages and produce CPE (Fang *et al.*, 2005). The multiplication of IBV in chicken embryo differed from strain to strain; some IBV strains produced a CPE while other strains did not grow so well (Lominiczi, 1974). The present study demonstrated that IBV (UPM1212) was able to adapt and propagate in mammalian cell line (Vero cells). Thus, there is a need to study the molecular characteristics, pathogenicity and titre of the virus, and perhaps further passages for future development of tissue culture based IB vaccine.

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DEVELOPMENT OF *IN SITU* PCR TECHNIQUE FOR DETECTION OF VERY VIRULENT INFECTIOUS BURSAL DISEASE VIRUS STRAIN

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ABSTRACT

A very virulent infectious bursal disease virus (vvIBDV) strain (UPM0081) was inoculated orally in 4-week-old specific pathogen free (SPF) chickens. Control was included and these chickens were not inoculated with the virus. The chickens in both groups were sacrificed at various intervals and samples of bursa of Fabricius, caecal tonsils, liver, spleen and kidney were fixed in 10% buffered formalin, processed and embedded in wax. A digoxigenin-labeled probe was designed according to the sequence of VP2 gene (vvIBDV). By application of *in situ* PCR technique, the probes were evaluated as a marker of the gene and to subsequently detect the presence of the virus. The technique was carried out on tissues of organs collected at 24, 48, 72, 96 and 120 hours post-inoculation (pi). The virus was detected in all samples at all times of sampling. The virus was not detected in all samples from the control group. It was concluded that this study has successfully developed an *in situ* PCR technique for detection of vvIBDV by formation of specific probes complementary to VP2 gene.

Keywords: *in situ* PCR, infectious bursal disease (IBD), very virulent IBD virus, tissues, detection probes

INTRODUCTION

Infectious bursal disease (IBD) is a drastically contagious disease that affects young chickens leading to high mortality and economic losses. Nevertheless, the disease is overtaken by lots of articles; an ambiguity environs its pathogenesis. For instance, the time duration within which the virus reaches target organ is still not known (Zhang *et al.,* 2010). It is indeed vital to develop a sensitive and rapid technique in the detection of the virus *in situ* in the cells or tissues. The objective of this study was to develop *in situ* PCR technique for detection of very virulent infectious bursal disease virus (vvIBDV) by formation of specific probes complementary to VP2 gene.

MATERIALS AND METHODS

Twenty one, 4-week-old specific pathogen free (SPF) chickens were divided into two groups: 15 and 6 chickens for groups A and B, respectively. Chickens in group A were orally inoculated with 0.1 mL of vvIBDV ($10^{7.5}$ EID_{50}/mL) UPM0081 strain while group B acted as the control group. Feed and water were given *ad libitum*. Three chickens each from group A were sacrificed at 24, 48, 72, 96 and 120 hours post inoculation (pi). The chickens in the control group were sacrificed at 0 and 120 hours of the trial. On necropsy, samples of bursa of Fabricius, caecal tonsils, liver, spleen and kidney were fixed in 10% buffered formalin, processed and embedded in wax. The protocol of one step RT-PCR was conducted as described by Kataria *et al*. (2001). Specific probes labeled with digoxigenin for detection of vvIBDV were designed. They were 5-ACCGTCCTCAGCTTACCCAC-3 (nt 1-10) (Genbank Accession No. GQ131540). Samples were hybridized after incubation in a buffer for 16 hours at 42°C (Morel and Raccurt, 2002). Digoxigenine-labeled probes were detected by Dig Nucleic Acid detection Kit

(Roche) for colour detection with NBT/BCIP (nitroblue tetrazolium/5-bromo-4-chloro-3-indolyl phosphate) (Morel and Raccurt, 2002).

RESULTS AND DISCUSSION

The study showed that IBDV was detected in the bursa of Fabricius, caecal tonsils, liver, spleen and kidney at 24, 48, 72, 96 and 120 hours pi. The virus was not detected from all samples in the control group at 0 and 120 hours of the trial. It was concluded that the study has successfully developed an *in situ* PCR technique for detection of vvIBDV by formation of specific probes complementary to VP2 gene. This probe is useful for the future study on the pathogenesis, tissue tropism and differentiation of IBDV strains.

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DEVELOPMENT OF *IN SITU* PCR TECHNIQUE FOR DETECTION OF VELOGENIC NEWCASTLE DISEASE VIRUS STRAIN

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ABSTRACT

A velogenic Newcastle disease virus (vNDV) strain (AF2240) was inoculated intranasally in 3-week-old specific pathogen free (SPF) chickens. Control was included and these chickens were not inoculated with the virus. The chickens were sacrificed at various intervals and samples of brain, caecal tonsils, liver, spleen, and trachea were fixed in 10% buffered formalin, processed and embedded in wax. A digoxigenin-labeled probe was designed according to the sequence of fusion gene (vNDV). By application of *in situ* PCR technique, the probe was evaluated as a marker of the gene and to subsequently detect the presence of the virus. The technique was carried out on tissues of chickens collected at 3, 6, and 7 days post inoculation (pi). The virus was detected in all of the tissues at days 3, 6 and 7 pi. The virus was not detected in the control group. It was concluded that this study has successfully developed an *in situ* PCR technique for detection of vNDV by formation of specific probes complementary to the fusion gene.

Keywords*: in situ* PCR, Newcastle disease (ND), velogenic ND virus, tissues, detection probe

INTRODUCTION

In situ PCR technique combines PCR power to amplify and *in situ* hybridization ability to localize target sequences. For *in situ* PCR to be of great success, it is necessary for further optimization of PCR cycling, fixation step, tissue processing, amplification of reagents and detection procedure to be done (Morel and Raccurt, 2002). *In situ* PCR could provide a technique to detect minute quantities of DNA and RNA in undamaged cells and tissues (Morel and Raccurt, 2002; Motalleb, 2009) and may allow detection of agent entry into the host as early as possible in understanding the pathogenesis of a disease (Zhang *et al*. 2010). It was the objective of this study to develop an *in situ* PCR technique for detection of velogenic Newcastle disease virus (vNDV) with the use of specific probe complementary to fusion gene.

MATERIALS AND METHODS

Six, 3-week-old specific pathogen free (SPF) chickens were orally inoculated with vNDV (10^5 EID_{50} /0.1 mL) AF2240 strain, whilst another six chickens remained un-inoculated and acted as the control group. Three chickens from the control group were sacrificed at days 0 and 7 of the trial for sampling. Three and two chickens from the vNDV inoculated groups were sacrificed at day 3 and 7, respectively for sampling, while samples were also collected from one dead chicken at day 6 pi. On necropsy, samples of brain, caecal tonsils, liver, spleen and trachea were fixed in 10% buffered formalin, processed and embedded in wax. The protocol of one step RT-PCR was conducted as described by Kataria *et al*. (2001). Specific probe labeled with digoxigenin for detection of velogenic NDV was designed. Its sequence was VC22, 5'-AAGGAGGCAGAAACGCTTTATA-3'; (Li and Zhang, 2004). Samples were hybridized after incubation in a hybridization buffer for 16 hours at 42°C (Morel and Raccurt,

2002). Digoxigenine-labeled probes were detected by Dig Nucleic Acid detection Kit (Roche) for colour detection with NBT/BCIP (nitroblue tetrazolium/5-bromo-4-chloro-3-indolyl phosphate) (Zhang *et al*. 2002; Morel and Raccurt, 2002).

RESULTS AND DISCUSSION

The study showed that vNDV was successfully detected in the brain, caecal tonsils, liver, spleen and trachea at 3, 6 and 7 days pi. The virus was not detected from all samples in the control group at 0 and 7 days of the trial. The study has successfully developed an *in situ* PCR technique for detection of vNDV by formation of specific probes complementary to fusion gene. This probe is useful for the future study on the pathogenesis, tissue tropism and differentiation of NDV strains.

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COMPLETE CODING SEQUENCE AND PHYLOGENETIC ANALYSES OF VERY VIRULENT INFECTIOUS BURSAL DISEASE VIRUS ISOLATE OF MALAYSIA

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ABSTRACT

A complete nucleotide (nt) coding sequence of infectious bursal disease virus (IBDV) isolate of Malaysia (UPM04190) was determined. In segment A, 3260 nt was identified, consisting of 2 overlapping open reading frame, predicted to be the VP5 of 465 nt with 154 amino acids and the polyprotein of 3039 nt with 1012 amino acids. In segment B, 2825 nt was obtained and consisted one open reading frame with 2640 nt predicted to be the 879 amino acids of VP1. Comparison of the deduced amino acids revealed 8 unique amino acids which were conserved only in UPM04190. Of all these amino acids, 3 were found in VP1, 3 were in VP2, and 2 were in VP5. The overall branching pattern of the phylogenetic trees revealed a similar pattern. Taken together, nt sequence and phylogenetic analyses revealed and suggested that our local isolate UPM04190 share a high homology with the very virulent strains. Analysis of the IBDV nt and amino acid sequence are best targeted at both genomes.

Keywords: Infectious bursal disease virus, sequence analysis, phylogeny, nucleotide, segment A and B

INTRODUCTION

Infectious bursal disease (IBD) is an acute, highly contagious viral infection of young chickens caused by the IBD virus (IBDV). IBDV consists of 2 segments, designated as A and B. Segment A encodes 2 overlapping proteins which are the VP5 and a polyprotein that give rise to VP2, 4, and 3. Segment B encodes only one protein, VP1. Although amino acids within the variable region of VP2 represent the molecular basis for antigenic variation, definite hot spot that determines pathogenicity has not been identified (van den Berg, 2000). It is believed that both segments are required for the expression of IBDV and contribute to the replication in the bursa of Fabricius. Nucleotide changes throughout the genome probably contribute to the multigenic nature of virulence. It has been demonstrated that VP2 is not the sole determinant of the pathogenic phenotype of IBDV and VP1 may play an important role in the virulence (Boot *et al.*, 2005). Besides, with the emergence of reassortant IBDV (Jackwood *et. al.*, 2011) the characterization is best targeted at both genome segments. It was the objective of the study to determine a complete coding sequence and characteristics of IBDV isolate of Malaysia.

MATERIALS AND METHODS

The IBDV sample homogenates (UPM04190) were propagated on the 9-day-old specific pathogen free (SPF) embryonated chicken eggs. On day 4 post inoculation, the chorioallantoic membrane (CAM) tissue from the embryo was collected and total RNA was isolated using Trizol® (Invitrogen, USA). The full-length PCR fragments of segments A and B were generated using the primers complementary to the 3'-terminus of the plus sense strain of IBDV, of either Segment A or B. The amplified PCR products were then purified and cloned into vectors. The positive clones were

sent for nucleotide sequencing (Macrogen, Ltd. Korea). The sequences were analyzed by BLAST and assembled to generate complete sequences in BioEdit Sequence Alignment Editor. Open reading frame and amino acids were predicted. Nucleotide and deduced amino acid sequences were aligned with 20 previously published full-length IBDV sequences using the ClustalW and these sequences were used to construct phylogenetic trees using Neighbor-Joining method with 1000 bootstrap replicates. Both analyses were conducted in MEGA version 4 programmes.

RESULTS AND DISCUSSION

Upon analysis, 3260 nt of segment A was identified, with 2 overlapping open reading frame, predicted to be the VP5 of 465 nt with 154 amino acids and the polyprotein of 3039 nt with 1012 amino acids. In segment B, 2825 nt was obtained and consist of one open reading frame with 2640 nt predicted to be the 879 amino acids of VP1.Nucleotide BLAST for the 2 segments showed that these sequences have 98% similarity with the very virulent (vvIBDV). Amino acids BLAST on the deduced VP5, polyprotein, and VP1 also showed 99% similarity with vvIBDV. The deduced amino acids sequence of VP1, VP5 and polyprotein contained all the unique and common vvIBDV amino acids reported previously. However, comparison of the local isolate with the selected full-length sequences revealed 3 unique amino acids residues at VP1, not observed in other strains. They were D240G, E677K, and L693H. Besides, 3 unique substitutions of VP2 were all at the hypervariable region, they were the D212N, Q249E, and I264M. In VP5, it has a unique substitution at position 133, from L-I. Another unique substitution at the position 150 from a stop codon to arginine had led to terminal extension of VP5. The overall branching pattern of the phylogenetic trees from the nt coding sequence of VP1, VP5, polyprotein, VP2, VP4 and VP3 revealed a similar pattern. Two major clades were observed. The serotype II IBDV formed a distinct lineage. The vvIBDV formed a monophyletic group that resides within the lineage of serotype I IBDV. Our local isolate falls within this group. The classical, attenuated and the variant strains make up a separate group in the serotype I IBDV lineage. Taken together, nucleotide sequence and phylogenetic analyses has revealed and suggested that our local isolate UPM04190 share a high homology with the vvIBDV strains. It is clearer now that no definite molecular structure in IBDV has been identified to be responsible for its virulency as nt and amino acids changes in both segments affect the degree of virulence. Therefore, analysis of the IBDV nt and amino acid sequence are best targeted at both genomes.

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INFECTION WITH VERY VIRULENT INFECTIOUS BURSAL DISEASE VIRUS CAUSES INFILTRATION OF T CELLS INTO BURSA OF FABRICIUS

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ABSTRACT

Infectious bursal disease (IBD) is an immunosuppression disease, causing high mortality rate in young chickens and instigated by Infectious Bursal Disease Virus (IBDV). Numerous studies have shown that IBDV can infect B cells and induce apoptosis. However, the effect of the virus on T cell is not clear. This finding provides evidence that IBDV induce infiltration of CD4 and CD8 T cells into bursa during acute phase of the infection.

Keywords: IBDV, CD4, CD8, T cells, chicken

INTRODUCTION

Infectious bursal disease virus (IBDV) is the causative agent of Gumboro's disease, a highly infectious and immunosuppressive disease affecting young chickens. The virus targets primarily IgM^+ B cells and replicates mostly in the bursa of Fabricius (Sharma *et al.*, 2000). Although many well described studies on IBDV's interaction with B cells have been done, not much is known about its interaction with T cells. T cells are important to produce full protection against virus infection. A study done by Rautenschlein *et al*., (2002) showed that T cells are needed to achieve complete immunity protection against IBDV. This study addresses the effects of IBDV infection on bursal T cells.

MATERIALS & METHODS

Chickens were inoculated with very virulent strain of IBDV strain UPM0081 through conjunctiva and bursal were harvested from chickens at days 0 (control), 2, 3, 4 and 5. The organ was processed into single cells suspension and isolation of lymphocytes was carried out. The purified lymphocytes then were stained with antibodies. Results were analyzed using flow cytometry.

RESULTS & DISCUSSION

The results suggested that IBDV induced infiltration of CD4 T and CD8 T cells into bursa. Currently, we are investigating the mechanism of IBDV induced apoptosis in T cells.

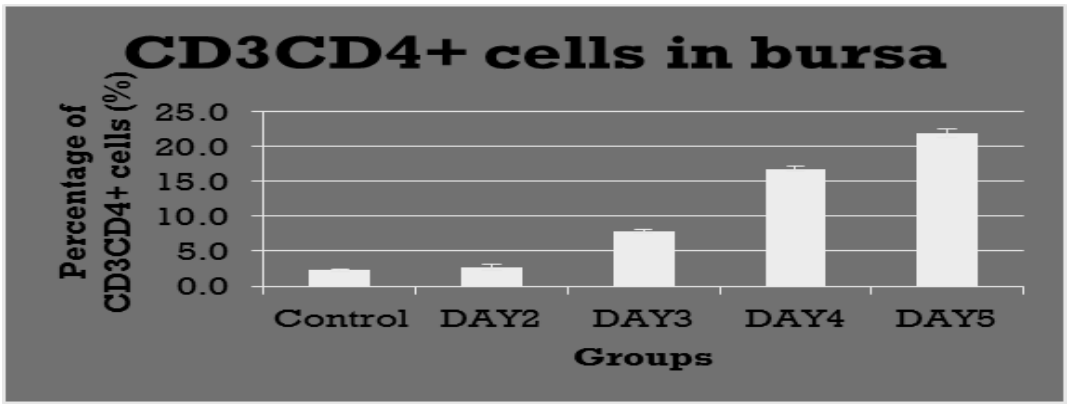

Figure 1. Bursa's lymphocytes cells were stained with Mouse Anti-Chicken CD3-FITC and CD4-RPE antibody and analyzed using flow cytometry. Immunophenotyping results showed increase of CD4 T cells infiltration into bursa from 2.270±0.16% at day 0 to 21.844±0.61% at day 5.

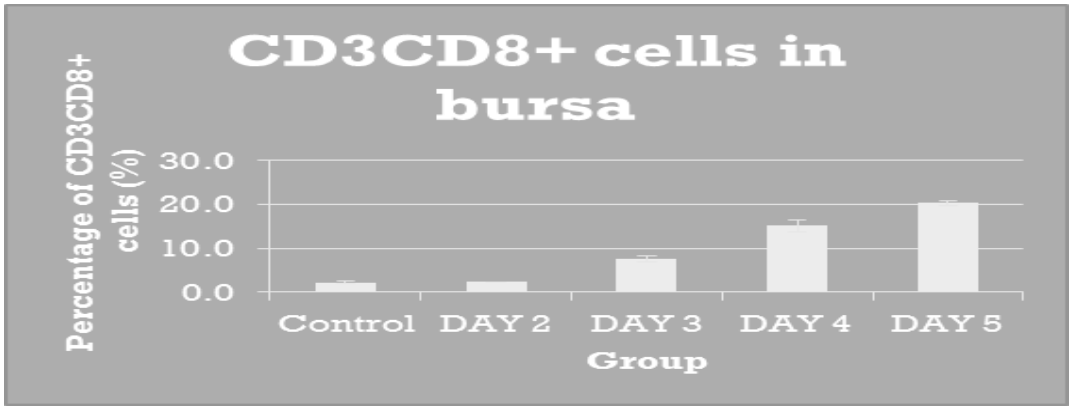

Figure 2. Bursa's lymphocytes cells were stained with Mouse Anti-Chicken CD3-FITC and CD8-APC antibody and analyzed using flow cytometry. Immunophenotyping results showed increase of CD8 T cells infiltration into bursa from 2.133±0.25% at day 0 to 31.321±0.47% at day 5.

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THE THREAT OF PERSISTENCE ANTIMICROBIAL RESISTANCE ON GLOBAL FOOD ANIMAL PRODUCTION: AN ESSENTIAL ROLE OF POULTRY FARMERS AND VETERINARIANS TO AVOID THE INEVITABLE

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ABSTRACT

Antimicrobial agents are essential tool in treatment, control and prevention of infectious diseases that have devastating effects on animal health and welfare, sustainable livestock production in addition to the control of animal infections that could be passed on to humans (Marshall *et al.,* 2011). According to the US National Research Council and Institute of Medicine states, "The benefit to human health in the proper use of antibiotics in food animals is related to the ability for these drugs to combat infectious bacteria that can be transferred to humans by either direct contact with the sick animal, consumption of food contaminated with pathogens from animals, or proliferation into the environment" (FDA, 2012). However imprudent use of these agents can lead to "the ability of microorganisms to survive or even grow in the presence of a given concentration of an antimicrobial agent, that is usually sufficient to inhibit or kill microorganism of the same species" and this phenomenon is termed as antimicrobial resistance (Anonymous, 2003), thus rendering the agents incapacitated to perform the above mention essential functions and hence may be a problem of global magnitude.

Food security is said to exist when all people, at all times, have physical, social and economic access to sufficient, safe and nutritious food which meets their dietary needs and food preferences for an active and healthy life (World Food Summit, 1996). Despite the understanding that access to sufficient nutritionally adequate food is a basic human right and also a mutual need that is shared by every human being, yet it was estimated in 2011-2013, over 12% of the global population are unable to meet their dietary energy requirement (suffering from chronic hunger) and the vast majority of this population are from developing countries (FAO, 2013).

Poultry industries are the most flexible and fastest growing of all livestock sectors. They represent the largest global domesticated animal division and contribute significantly to global economic growth and food security (FAOSAT, 2013). Studies have indicated that major losses in poultry production can lead to malnutrition, hunger, poverty, and economic losses depending on community settings or conditions (Conan *et al*., 2012). The United Nations (UN) estimates that by 2030, the global population will be eight billion, whom are mainly average income earners, and meat consumption per person per year will increase by 26%, chicken meat in particular (FAO, 2010). The International Food Policy Research Institute has also forecasts, that about 40% of the total global animal protein will be contributed by poultry industries by 2015 (IFPRI, 2000).

However in trying to achieve the global demand of poultry protein, farmer tends to misuse antibiotics, especially for the purpose of prophylaxis and growth promoting effect, hence promoting the global challenge of antimicrobial resistance. Organisms that acquired antimicrobial resistance tend to cause infectious diseases that are characterized by high cost of medication, resistance to multiple classes of antibiotics, prolong duration of infection, increase cost of management, decrease animal production and persistence or dissemination of the infectious agent among humans, animals and natural environment. These factors will jeopardize the tremendous contribution that is continuously given by poultry industries through the provision of easy access to safety, quality and nutrition food of animal origin, poverty alleviation and global economic growth (OIE, 2003).Today, multidrug resistance (MDR) that is, non-susceptibility to at least one agent in three or more antimicrobial categories, is showing an increasing trend at an alarming rate. It would be unthinkable to have a pandrug-resistant (PDR) bacteria, being resistant to all agents in all antimicrobial categories,

causing a serious infectious disease as this would spill a disaster for animal and public health (Magiorakos *et al*., 2012).

Hence in order to avoid the threatening effect of antimicrobial resistance on global economy and food security, poultry farmers have special role to play, through adherence to the judicious use of antibiotics, using them only when prescribe by the professional, use in accordance with prescription provision such as treatment regime, dosage, dosage intervals, duration of treatment, withdrawal period and amount of drug to be delivered, depending on dosage and population size; farmers need to cooperate with their veterinarians in the establishment of appropriate preventive strategies such as routine heath monitoring, vaccination, fumigation and routine medication. Poultry farmers need to establish effective biosecurity measures, by ensuring isolation of sick animals and proper disposition of infected carcasses, establishment of sound and sustainable waste management disposal system; they should also have appropriate storage condition for the maintenance of antimicrobials, and finally provide maximum support to the relevant authorities' in-charge of monitoring and surveillance of antimicrobial resistance (OIE, 2003; FDA, 2012).

Keywords: Food security, poultry production, antimicrobial resistance, poultry farmers

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IN VITRO EVALUATION OF PKE PREBIOTIC ON *LACTOBACILLUS SP.*

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ABSTRACT

Palm kernel expeller had been viewed as a by-product with high fiber content, mainly in the form of mannan, with cellulose and xylan making up the remaining portion. Partial hydrolysis of these hemicelluloses will produce short chain oligosaccharides, representing a good source of prebiotic oligosaccharides. The aim of this study was to increase the content of PKE oligosaccharides using heat and enzymes and evaluates its efficacy in supporting growth of *Lactobacillus sp. in vitro*. Results showed that PKE oligosaccharides can support growth of three different *Lactobacillus sp*. ($P<0.05$), and highest growth was observed in steam + enzyme treatment. This study showed the potential of PKE as a source of prebiotic in supporting growth of *Lactobacillus sp*.

Keywords: *Lactobacillus*, oligosaccharides, Palm kernel expeller, prebiotics

INTRODUCTION

The use of enzyme to improve nutritive value of PKE for incorporation in poultry ration has been extensively documented, however, with inconsistent results in term of growth performance and feed utilization. There have also been reports of beneficial effect on the use of PKE for enhancement of beneficial bacteria in gut of broilers (Fernandez *et al.*, 2002), suggesting the potential use of PKE as prebiotic source. This beneficial effect could be attributed to the oligosaccharides content in PKE, which might be increased through enzymatic fermentation. The aim of this study was to evaluate the effect of enzyme treatment on availability of PKE oligosaccharides to support growth of *Lactobacillus sp*.

MATERIALS AND METHODS

PKE extracts; raw PKE extract (PKE), enzyme-treated PKE extract (PKE_{ENZ}), and steam + enzyme-treated PKE extract ($SPKE_{ENZ}$) was prepared separately by shaking the PKE in distilled water at 4°C for 24 h, centrifuged at 10,000 rpm for 10 min, and filtered through Whatman No. 1 filter paper. PKE_{ENZ} and $SPKE_{ENZ}$ were PKE fermented with enzyme produced by *Aspergillus terreus* (60% moisture, 1% enzyme, 55°C) for 24 h prior to extraction. PKE filtrate was lyophilized by freeze drying to obtain solid PKE extract.

The PKE-extracts obtained through the different pre-treatment methods were assessed for their prebiotic ability to support growth of three *Lactobacillus* strains (*L. gallinarum* I 26, *L. salivarius* I 24, and *L. brevis* I 218). Each PKE-extract (30 g/L) was prepared in de-ionized water and sterilized by filtering each solution through 0.2 µm filters. PKE-extract, basal MRS broth with glucose (Control) and basal MRS broth without glucose (Control-Blank) was then inoculated with an overnight inoculum (18 h old) and incubated anaerobically at 37°C for 24 h. After incubation, each culture was vortex for 30 s, and absorbance was read at 620 nm.

RESULTS AND DISCUSSION

Mannan is the major non-starch polysaccharides (NSP) component of PKE, with cellulose and xylans making up the remainder portion. These hemicellulose polysaccharides are mainly polymers of monosaccharides such as D-mannose, D-galactose, D-xylose, D-glucose and L-arabinose (Alang *et al.*, 1988). Thus, it seems possible that the hydrolysis of PKE hemicellulose would produce a mixture of oligosaccharides with mannanoligosaccharides (MOS) being the major product. MOS have been widely reported to support growth of beneficial bacteria including *Lactobacillus*. However, the growth of bacteria differed even between different strains of the same species (Kneifel *et al.*, 2000). In agreement to the above report, results of this study showed that growth of all *Lactobacillus* species on MRS (Control) varied significantly ($P<0.05$) with *L. sallivarius* I 24 exhibited the highest growth potential followed by *L. gallinarum* I 16 and *L. brevis* I 218 (Table 1). Results of this study also show that all the three strains could grow on PKE-extract but their growth varied significantly among species and the different PKE-extracts ($P<0.05$). In general, growth of bacteria in the enzyme-treated PKE-extracts (particularly that of $SPKE_{ENZ}$) was higher than that in the untreated PKE, because of the higher mono- and oligo-saccharides content in the enzyme-treated PKE (data not included).

Table 1. The growth of *Lactobacillus sp*. on PKE-extract after overnight incubation

Strain	Growth (OD_{620})			
	Substrate			
	MRS	PKE	PKE_{ENZ}	$SPKE_{ENZ}$
L. salivarius I 24	1.254 ± 0.026^{Aa}	0.332 ± 0.029^{Ab}	0.383 ± 0.023^{Ac}	0.463 ± 0.070^{Ad}
L. gallinarum I 16	1.125 ± 0.016^{Ba}	0.242 ± 0.023^{Bb}	0.314 ± 0.038^{Bc}	0.334 ± 0.005^{Bc}
L. brevis I 218	1.004 ± 0.055^{Ca}	0.226 ± 0.005^{Bb}	0.417 ± 0.005^{Cc}	0.613 ± 0.026^{Cd}

[1] Results are mean value for 3 replicates ± Standard deviation.
[2] $^{a-d}$ means in the same row with different lowercase letter are significant different ($P<0.05$).
[3] $^{A-C}$ means in the same column with different uppercase letter are significantly different ($P<0.05$).

ACKNOWLEDGEMENT

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DETECTION OF *MYCOPLASMA GALLISEPTICUM* BY REAL TIME PCR USING *gapA* GENE

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ABSTRACT

Mycoplasma gallisepticum (MG) is responsible for chronic respiratory disease which leads to a lot of economic losses to the poultry industry. Therefore, it is very important to detect this organism early and efficiently. MG can be diagnosed by three ways: isolation identification, serological method and molecular detection method. In this study, a SYBR green real time PCR assay by using gapA gene was developed for the detection of MG. This assay was powerful both from the aspect of specificity and sensitivity as it amplified only MG at early cycle when run together with 20 other avian Mycoplasma species and it can detect a minimum of 26 pg/µl of DNA template.

Keywords: *Mycoplasma gallisepticum*, PCR, SYBR green real time PCR, *gapA* gene

INTRODUCTION

Mycoplasma gallisepticum, is the most economically important pathogenic avian Mycoplasma has a worldwide distribution. *Mycoplasma gallisepticum* economically affects the poultry industry through decreased egg production (Carpenter *et al.*, 1981), increased mortality (McLaren *et al.*, 1996) and reduced feed efficiency (Ley *et al.*, 1997). MG can be diagnosed by three way including isolation identification, detection of specific antibodies and detection of its DNA (Bradbury, 2001). However, molecular detection method especially real time PCR is more specific and sensitive when compared to other methods. Therefore, the objectives of this study were to develop a SYBR Green real time PCR assay for the detection of *Mycoplasma gallisepticum* and to detect the presence of MG in commercial and village chicken flock of Malaysia using the developed real time PCR.

MATERIALS AND METHODS

Samples were collected from choanal cleft of broiler, layer and village chicken by using sterile cotton swabs from different states in Peninsular Malaysia. Conventional salt-based method was used to extract genomic DNA with some modifications. The SYBR Green I real time PCR amplification was carried out using the CFX96 Real Time PCR System (Bio-Rad, USA). After completion of the amplification the efficiency and specificity was determined.

RESULTS AND DISCUSSION

For the detection of MG, real time PCR method using gapA 5F+6R primer set of gapA gene developed in this study was really beneficial on the aspect of efficiency, detection limit, specificity and rapidity. The R^2 value was 0.997, E=94.1% and the detection limit was 260 ng/µl to 26 pg/µl, which was the clear indication of high

efficiency and sensitivity about this protocol. Moreover, a total of 20 other avian Mycoplasma species were tested by this protocol and only MGS6 (reference strain of MG) showed early amplification with 26.05 Ct value. This was also a quick detection method of MG as it gave result within an hour in comparison with the conventional PCR where other study showed that detection of MG by conventional PCR using gapA 5F+6R primer set of gapA gene took 3 hrs and also need to do post PCR processing (Zahraa *et al.*, 2011). The results of screening by real time PCR indicated high prevalence rate of MG in Malaysia although the farmer carried out vaccination program in commercial poultry farm. Although these farms had vaccination and treatment history, the high presence of MG may be due to the horizontal transmission; through infected birds, eggs, wild birds, vehicles or fomites (Jordan, 1985).

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DETERMINATION OF AVIAN INFLUENZA VIRUS H5N2 INFECTION IN CHICKEN INNATE IMMUNE CELLS

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ABSTRACT

Infection with low pathogenic avian influenza virus in birds and mammals is not severe and usually give a mild clinical signs comparing to the highly pathogenic avian influenza virus. Once the virus enter the immune system of an organism, the first defense mechanism which consist of the antigen presenting cells and NK cells will react on the virus by activating the innate and adaptive immunity system. N2 gene from H5N2 strain A/duck/Malaysia/8443/04 was detected using SYBR Green real-time PCR from the infected bone marrow dendritic cell, macrophage and also natural killer cells. Comparing the increment of viral load among 6h, 12h and 24h, there were no significant difference between the viral load detected in bone marrow dendritic cells and natural killer cells. Meanwhile, for macrophage the viral load decreased gradually over time.

Keywords: viral load, H5N2, dendritic cells, macrophage, natural killer cells

INTRODUCTION

Avian influenza virus (AIV) is a type A influenza virus that affects the respiratory, digestive and/or nervous systems of many species of domestic and wild birds. Cellular components of the avian innate immune system are very similar to the mammalian immune system which comprise of macrophages, dendritic cells (DC) and natural killer (NK) cell (Gobel *et al.,* 2001; Wu *et al.,* 2010). In addition, antigen-presenting cells (APC) such as DC and macrophage are critical in processing and presenting pathogen antigens and activating adaptive immunity (Wu *et al.,* 2010). Virus infections generally up-regulate MHC antigens and co-stimulatory molecules on APC and induce cytokines and chemokine expressions (Xing *et al.,* 2008). On the other hand, NK cells are a key frontline defense against a number of pathogens such as parasites, intracellular bacteria, and viruses (Achdout *et al.,* 2010). The susceptibility of low pathogenic AIV on DC, macrophage and NK cells were compared in this study via detection of viral load in these cells using SYBR green I based real-time PCR.

METHODS AND MATERIALS

Bone marrow were obtained from femurs and tibia and cultured with or without cytokines (GM-CSF and IL-4) to obtain DC and macrophage (Wu *et al.,* 2010). On the other hand, IEL NK cell were isolated from duodenum and purified using 28-4 MAb (gift from Gobel, T.W) (Gobel *et al.,* 2001). The enriched DC, macrophage and NK cells were infected separately with 100 $TCID_{50}$ H5N for 2 hours. After a washing step, the cells were harvested at 6h, 12h and 24h post-infection and subjected to RNA extraction using RNeasy Plus Mini Kit (Qiagen, USA) according to manufacturer's instructions. The first strand of cDNA was performed with Superscript III Reverse Transcriptase Kit (Invitrogen, UK) followed by the amplification of N2 gene H5N2

A/duck/Malaysia/8443/04 isolate with Sensifast SYBR No-ROX Kit (Bioline, UK) as described by the manufacturer's protocols. The standard curve was generated by carrying out a serial of 10-fold dilution on AIV H5N2 A/duck/Malaysia/8443/04 isolate.

RESULTS AND DISCUSSIONS

The viral load in different AIV H5N2 infected cells harvested at different time points (Table 1). In general, the virus was detected in all cell types as early as 6h post-infection. Both infected DC and NK cells showed no significant different in viral load detection from 6h to 24h post-infection suggesting that no or only limited progeny was produced. However, the amount of viral load found in infected macrophage was gradually decreased from 6h to 24h post-infection.

Table 1. Determination of viral load in dendritic cells, macrophage and natural killer cells upon infection of AIV H5N2 A/duck/Malaysia/8443/04 isolate at different time points

Cells	Hours Post-Infection	Viral RNA Copy Number (Log_{10})
Dendritic cells	0	Not detected
	6	15.43±0.06
	12	15.41±0.07
	24	15.06±0.02
Macrophage	0	Not detected
	6	15.20±0.07
	12	14.72±0.09
	24	13.12±0.09
Natural killer cells	0	Not detected
	6	12.40±0.16
	12	12.33±0.02
	24	11.45±0.02

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CONSTRUCTION OF A PLASMID ENCODING CHICKEN MITOCHONDRIAL ANTIVIRAL SIGNALING (MAVS) GENE

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ABSTRACT

The significance of the role of mitochondria incontending viral infection is gaining worldwide interest. Its ability to elicit molecular signals to other immune components enables new perspective in the development of novel antiviral agents. This study explored the possibility of isolating the mitochondrial antiviral signaling (MAVS) gene from chicken tissue and cloning it into cloning vector. Prior to cloning, RNA extraction was carried out from chicken samples and converted to cDNA. Polymerase chain reaction (PCR) amplified product was ligated to the vector pJET1.2. The resulting plasmid construct pJET1.2/CARDIF was then transformed into competent cells and later sequenced and analysed by restriction enzyme analysis. BLAST analysis of the construct yields 99% similarity with *Gallus gallus* CARDIF mRNA gene.

Keywords: Mitochondrial antiviral signaling gene, antiviral response, type I interferon

INTRODUCTION

A considerable amount of literature has been published on the significance of mitochondria in innate immunity. Recent studies have also showed that the mammalian innate immunity is activated via pathogen-associated molecular pattern (PAMP) proteins such as Toll-like receptors (TLR) and retinoic acid-inducible gene I (RIG-I)-like receptors (RLRs) that interact with mitochondrial antiviral signaling (MAVS) gene (Koshiba *et al*., 2011). MAVS is also known as CARDIF, IPS-1 or VISA (Liniger *et al.*, 2012). Characterization of MAVS during viral infection will provide new leads in the development of novel antiviral molecules against viral infection in animals. The role of MAVS in immunity has been vastly documented as an important type I interferon (IFN) inducer in mammalians (Seth *et al.*, 2005; Scott, 2010) and also in fish (Biacchesi, 2009). It is postulated that MAVS genes helps in enhancing chicken innate immunity by modulatinginflammation, the production of cytokines and it also helps in regulating apoptosis of viral-infected cells. Therefore, this study aimed at the isolation of the gene and construction of a plasmid encoding the MAVS gene.

MATERIALS AND METHODS

Chicken spleen RNA was extracted using RNeasyPlus Mini Kit by Qiagen and converted to cDNA using RevertAid H Minus First Strand cDNA Synthesis Kit by Fermentas. The oligonucleotides ch-CARDIF-F and ch-CARDIF-R (Liniger *et al.*, 2012) was employed to amplify MAVS gene from chicken spleen cDNA using*i*-StarMAX II PCR master mix from Intron Biotechnology.The generated PCR product was gel electrophoresed and gel purified using MEGA-spin Agarose Gel DNA Extraction Kit (Intron Biotechnology, Korea). The purified PCR product was processed to produce blunt ends for the cloning process. The polished PCR product was ligated to pJET1.2/blunt Cloning Vector and later transformed into competent cells *E.coli*

TOP10 strain. The recombinant plasmids were extracted using DNA-spin Plasmid DNA Purification Kit by Intron Biotechnology. Some of the purified plasmid was digested with the restriction enzyme *Bgl*II and later gel electrophoresed and purified.

RESULTS AND DISCUSSION

The MAVS gene was successfully amplified via PCR with a product size of 1926 bp. The resulting plasmid construct following ligation of the gene to the vector pJET1.2 was labeled as pJET1.2/CARDIF. Duplicate samples electrophoresed on agarose gel indicated undigested and digested samples (Figure 1) with the lowest bands at lanes 2 and 4 are the genes of interest. Sequence analysis of the cloned PCR product illustrates 99% similarity with the CARDIF mRNA gene of *G. gallus* (HQ845772.1). Characterisation by restriction enzyme analysis demonstrates the insertion of the MAVS gene into the vector. The cloned gene can be transferred to an expression vector for more in-depth study of its function in immunity against viral infection.

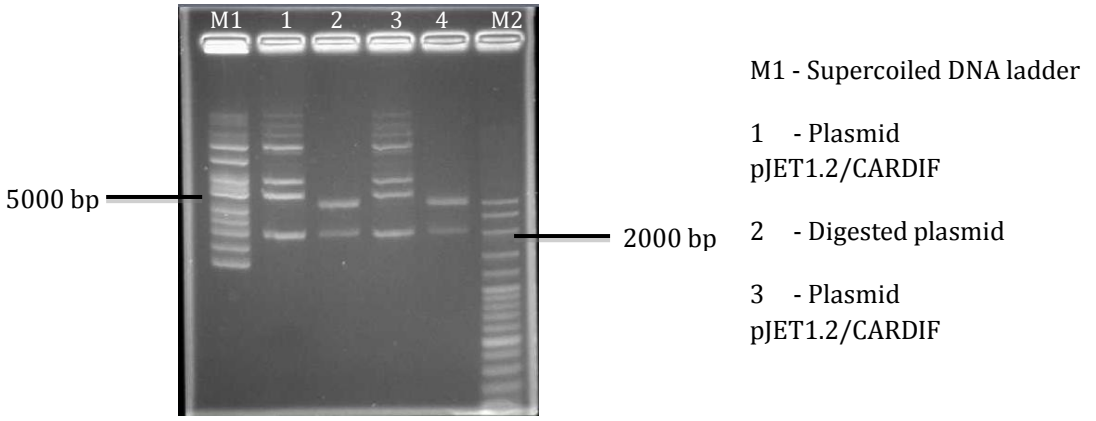

Figure 1

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PHYLOGENETIC ANALYSIS OF HAEMAGLUTININ GENE OF AVIAN INFLUENZA VIRUS ISOLATE VRI1803/04

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ABSTRACT

An isolate of avian influenza virus (AIV) designated as VRI1803/04 obtained from Veterinary Research Institute, Ipoh, Perak was used for identification and characterisation using the conventional one step reverse transcription polymerase chain reaction (RT-PCR). Total RNA of the virus was obtained from allantoic fluid of specific-pathogen-free (SPF) embryonated chicken eggs extracted using conventional method. The complete sequence of H3 gene (1701 bp) of VRI1803/04 was determined. The H gene has 89% homology to H3 gene from A/duck/Ukraine/1/63 (H3N8).

Keywords: Avian influenza virus, Nucleotide Sequencing, allantoic fluid, polymerase chain reaction, reverse transcription

INTRODUCTION

Highly pathogenic avian influenza virus (HPAI) is a highly contagious viral disease causes severe disease in domestic poultry including chickens and turkeys. Avian influenza viral genome consists of 8 segments (ranging from 890 to 2341 nucleotides) encode for 8 different genes, haemagglutinin (H) gene, neuraminidase (N) gene, matrix (M) gene, nonstructural (NS) gene, nucleoprotein (NP) gene, and three polymerase (PA, PB1 and PB2) genes (Hoffmann *et al*., 2001). The surfaces proteins include hemagglutinin (H) and neuraminidase (N) play important role for the production of protective immune response (Collins *et al.*, 2002). In addition, characterization of AIV isolates is based on the H and N proteins. Presently 17 H and 10 N subtypes have been detected. Hence, there are 170 possible subtype combinations of AIV.

MATERIALS AND METHODS

An isolate of AIV labelled as VRI1803/04 was kindly obtained from Veterinary Research Institute in Ipoh, Perak, Malaysia. The virus was propagated in 11 day-old specific-pathogen-free (SPF) embryonated chicken eggs by allantoic sac route. The eggs were incubated at 37^{o}C for 5 days. The viral RNA was extracted from allantoic fluid using TRI Reagent® LS (Molecular Research Centre, INC) as described by the manufacturer. A universal primer set of the full-length amplification of all influenza virus H gene was used (Hoffman *et al.,* 2002). The RT-PCR was performed using a one-step Access RT-PCR system (Promega, USA). The purified DNA encoded for H regions was then sent to First Base for DNA sequencing (ABI PRISM 377 DNA Sequencer, Applied Biosystem). The obtained sequences were compared to selected H gene sequences from GenBank by the BLAST (Basic Local Alignment Search Tool) search program of National Center for Biotechnology Information (http://www.ncbi.nlm.nih.gov/).

RESULTS AND DISCUSSION

Conventional RT-PCR resulted in the amplification of a product of 1700 bp. The nucleotide sequence covering approximately 1.7 kbp (1701 bp) of VRI1803/04 isolate was identified. Sequence analysis and comparison showed that the H gene of VRI1803/04 has 89% homology to H3 gene from A/duck/Ukraine/1/63 (H3N8) under accession no FJ802401. Figure 1 shows the phylogenetic tree of H gene from VRI1803/04 isolate clustered with A/duck/Ukraine/1/63 (H3N8).

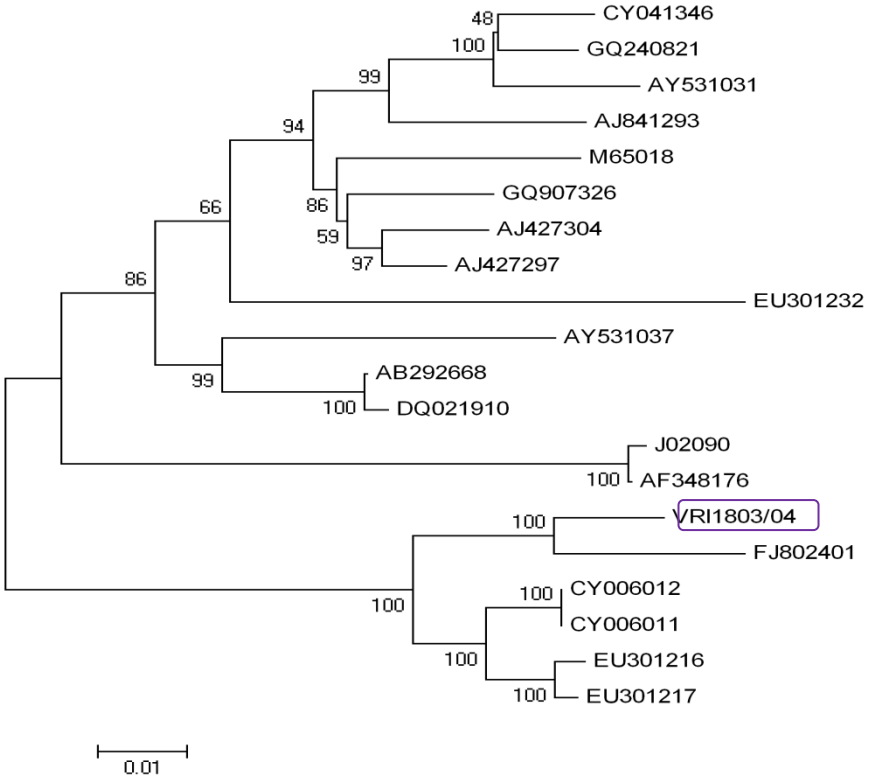

Figure 1. Phylogenetic tree was generated by Neighbor-Joining method based on 1701 bp nucleotide sequences of hemaglutinin gene region of VRI 1803/04 AIV isolate.

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RECOVERY OF A MALAYSIAN RECOMBINANT NEWCASTLE DISEASE VIRUS STRAIN

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ABSTRACT

Newcastle disease virus strain AF2240 is a viscerotropic velogenic strain that is used as a vaccine challenge virus in Malaysia. The identification of the full length genome will be a crucial platform for further studies of this isolate. In this study, we fully sequenced the genome of a derivative of this strain named AF2240-I. The 15,192 nt long genome contains a 55-nt leader sequence at the 3' whereas the trailer region consists of 114-nt at the 5'. The intergenic sequences between the NP-P, P-M, M-F, F-HN and HN-L genes comprise 1, 1, 1, 31, and 47 nt respectively. The acknowledged cleavage site of fusion protein showed amino acid sequence of 112-R-R-Q-K-R-F-117 which corresponds to those of virulent NDV strains. Phylogenetic analysis of the whole virus genome showed that the strain AF2240-I belongs to genotype VIII and is more closely related to velogenic strains QH1, QH4, Fontana, Largo and Italien than to other strains of NDV. Differences were noticed in the HN and M gene between AF2240 and its derivative AF2240-I. This is the first report of a complete genome sequence of an NDV strain isolated in Malaysia.

Keywords: Newcastle disease virus, genome analysis

INTRODUCTION

Newcastle disease virus (NDV) or avian paramyxovirus 1 remains a constant major threat to commercial poultry production (Alexander 1998). The strain AF2240-I is a derivative of the strain AF2240; a viscerotropic velogenic local strain that is used as a vaccine challenge virus in Malaysia. The genome of NDV contains a non-segmented single-stranded negative-sense RNA in the order of 3'-5'. The RNA holds 6 genes and is of approximately 15.2 kb. The genome is flanked by a leader and a trailer sequence at its 3' and 5' ends, respectively. Meanwhile, each gene contains an untranslated region (UTR) which is known to play a role in viral genome transcription, translation and virulence effect (Krishnamurthy and Samal 1998, de Leeuw and Peeters 1999). In this study, we first fully sequenced the genome of AF2240-I which is a platform towards the use of reverse genetics technology to rescue the virus.

MATERIALS AND METHODS

NDV strain AF2240 was from virus stock available at Biotech 2.0, Faculty of Biotech and Biomolecular Sciences, Universiti Putra Malaysia. It was grown in 9-day-old embryonated SPF chicken eggs and purified. Viral genomic RNA was isolated with Trizol reagent (Invitrogen, USA). The cDNA was made with RevertAid Premium first strand cDNA synthesis kit (Thermo, USA) using gene specific primers. All PCR products were amplified using Platinum *Pfx* polymerase (Invitrogen, USA) followed by gel purification with QIAquick Gel Extraction Kit (Qiagen, USA) and cloned into pJET 1.2/blunt cloning vector using CloneJET PCR Cloning Kit (Thermo, USA). Several independent clones were sequenced from both directions to verify the plasmid insertion. The leader and trailer sequences respectively at the 3'- and 5' -terminal ends of the viral RNA were determined by rapid amplification of cDNA ends (RACE). All alignment and phylogenetic analysis were performed with MEGA5 software

(Molecular Evolutionary Genetics Analysis, version 5.0) by neighbour-joining method at 1000 bootstrap values.

RESULTS AND DISCUSSION

The genome of AF2240-I is 15,192 nt in length, which follows the rule of six for a competent viral replication. Full length sequence analysis showed that the strain AF2240-I belongs to genotype VIII. The amplification of HN gene of the strain given (initially presumed AF2240) has indicated the presence of Arg 403 residue in the HN gene which was reported to be absent in the HN gene of strain AF2240. A frameshift was also observed between the amplified M gene of AF2240-I and the published sequence of AF2240. The 3'-leader and 5'-trailer regions of AF2240-I consist of 55 nt and 114 nt respectively. The first three IGSs of AF2240-I, the NP-P, P-M and M-F gene junctions each had only 1 nt. The remaining two IGSs, F-HN and HN-L contain 31 and 47 nt. Whole genome comparison was performed on AF2240-I and 63 other NDV strains. It was shown that strains QH1, QH4, Fontana, Largo and Italien shared the highest identity of nt (>90%) with strain AF2240-I, in contrast to the strains of Class I which showed the least nucleotide identity. QH1 and QH4 are of genotype VIII whilst Fontana, Largo and Italien belong to genotypes VI, V and IV respectively. Previous studies based on the F gene have shown that strain AF2240 belongs to genotype VIII (Yu *et al*., 2001). A phylogenetic tree of the F gene and full length of AF2240-I show similar result and suggest a phylogenetic clustering with strains QH1 and QH4 (genotype VIII). Availability of the complete genome sequence of strain AF2240-I for the first time in Malaysia is crucial in engineering its full length virus for various applications. The most profound application of genetically engineered virus is for vaccine development and as a therapeutic agent. In addition, we observed that the HN protein comprises 582 amino acids and that this length has not been noticed in other NDV strains worldwide.

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MOLECULAR CHARACTERISATION AND PATHOTYPING OF RECENTLY ISOLATED NEWCASTLE DISEASE VIRUS ISOLATES BASED ON F PROTEIN'S CLEAVAGE SITE

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ABSTRACT

Intensive vaccine programs have been implemented in many countries including Malaysia, but Newcastle disease virus (NDV) outbreaks have occurred, even in well-vaccinated farms. Hence, the present study was aimed to characterize five NDV isolates obtained from NDV vaccinated broiler farms in 2011 based on sequence and phylogenetic analysis of partial fusion gene. All the isolated NDV strains showed that they are categorized as velogenic NDV based on the presence of multi-basic protease cleavage sites, ^{112}RRRKRF^{117}. In addition, phylogenetic analysis showed that the isolates can classified under the genotype VII, subgenotype VIId.

Keywords: Newcastle Disease Virus (NDV), Genotyping, Phylogenetic Analysis

INTRODUCTION

Newcastle disease (ND), a highly contagious disease of birds, is one of the major causes of economic losses in the poultry industry. Genome of NDV is composed of six genes which are corresponding to six structural proteins: nucleoprotein (NP), phosphoprotein (P), matrix (M), fusion (F), hemagglutinin-neuraminidase (HN), and the RNA polymerase (L) (Alexander 2008). The HN and F are two glycoproteins which play important role in pathogenesis and inducing virus neutralising antibodies which represent the primary protective component induced by ND vaccines (Seal *et al.,* 2002). This study describes the molecular characterization of NDV isolated from vaccinated broiler farms that had experienced high morbidity and mortality.

MATERIALS AND METHODS

Tissue samples suspected of ND outbreaks were received from various ND vaccinated broiler farms in Penang, Perak and Johor. The samples were homogenized using sterile pestle and mortar in 1x PBS solution. Homogenized samples were centrifuged and supernatant were transferred to new sterile tubes. The samples were freeze thaw for 3 times. RNA from each homogenized sample was extracted using TRIzol® RNA Isolation Reagents (Invitrogen, USA). Reverse Transcriptase-PCR were performed using Promega Access Quick™ RT-PCR System to detect the cleavage site of fusion glycoprotein of NDV (Berhanu *et al.,* 2010). Specific band with 535 bp PCR products was purified using QIAquick PCR Purification Kit (QIAGEN, Germany) before sending for sequencing. Percent nucleotide identity and sequence editing were carried out using BioEdit software package version 7.1.3.0. Nucleotide analysis, prediction of amino acid sequences, and alignments were performed by MEGA version 5.2.

RESULTS AND DISCUSSION

The amino acid sequence of the protease cleavage site, ^{112}RRRKRF^{117}, reveals that all of the isolates, maintained multiple basic amino acids motifs within the penta-amino acid sequence of the F0 cleavage signal between residues 113 and 116 and phenylalanine (F) on the residue 117 of the N-terminus. Based on the World Organization for Animal Health (OIE), NDV with an ICPI equal to or greater than 0.7 and/or possesses multiple basic amino acids at the C-terminus of the fusion protein cleavage site is classified as velogenic strain (OIE, 2009). In addition, ICPI and MDT studies showed that one of the isolate, IBS002 which has been used as challenge virus for NDV vaccine efficacy study has an ICPI of 1.76 and MDT of 51.2 confirming the virus is of velogenic pathotype (unpublished results). Phylogenetic tree generated based on the variable portion of the F gene between nt 47 - 435 of the 5 new isolates and other 35 local and foreign NDV isolates worldwide separated into 10 potential clusters corresponding to the different genotypes of NDV. All 5 isolates were belonged to previously established subgenotypeVIId (Figure 1). Considering the topology of the phylogenetic tree, these Malaysian isolates might have a common origin including the two isolates in 2010 in Malaysia.

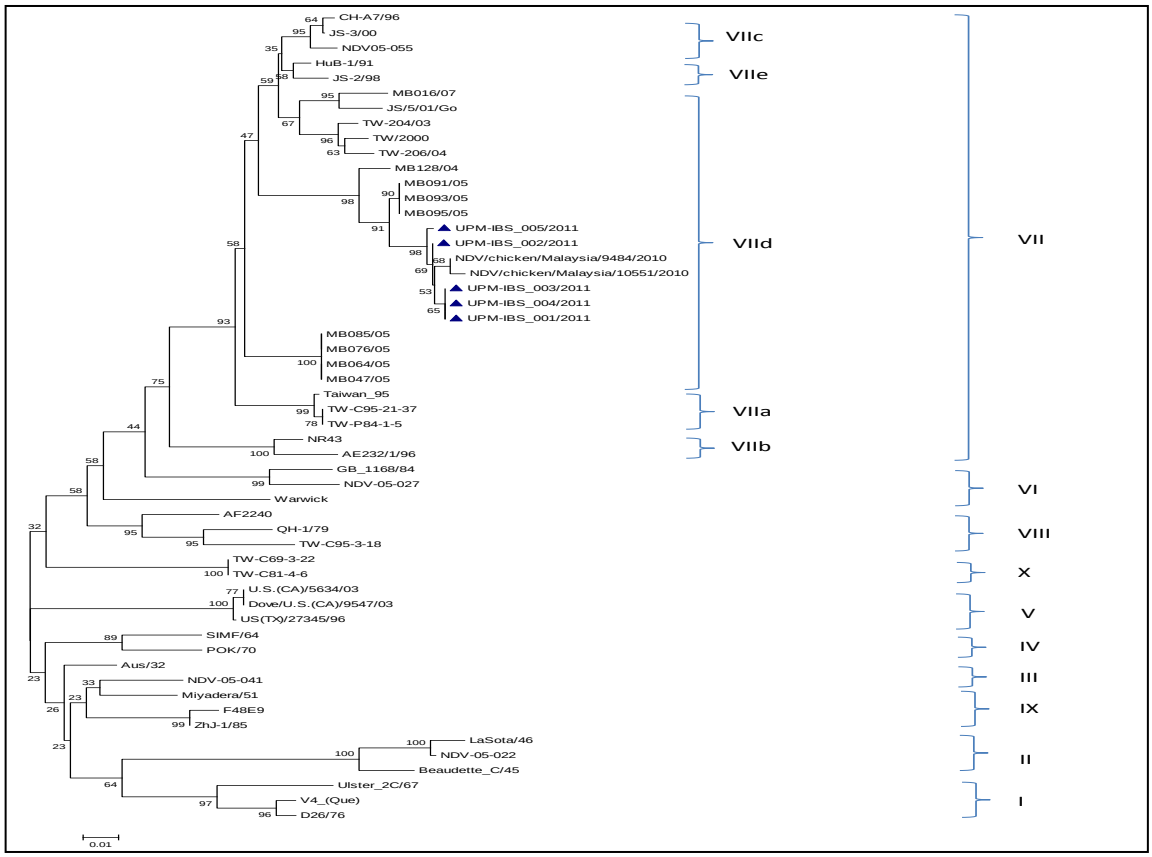

Figure 1. Phylogenetic relationship among 54 NDV isolates based on F gene nucleotide sequences between position 47 and 435. The phylogenetic tree was constructed using neighbour-joining method on MEGA 5. The characterised isolates are indicated by the blue triangles.

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RESPONSES OF ENRICHED CHICKEN B LYMPHOCYTES POPULATION TOWARDS INFECTION OF DIFFERENT GENOTYPES OF VELOGENIC NEWCASTLE DISEASE VIRUS

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ABSTRACT

Newcastle disease (ND) is a major disease in poultry industry that caused high loss and mortality. Fundamental study on the virus-host interactions is needed to address the issue of repeated outbreaks of NDV in Asia. In this study, the effect of inflammatory stress on the viability toward the development of humoral immunity was investigated in chicken IgM+ B lymphocytes when infected with different genotypes of velogenic NDV. When infected with NDV genotype VII UPM/IBS/002/2011 and genotype VIII AF2240, reduction of IgM+ B lymphocytes population and infiltration of macrophage was observed in the chicken bursa of Fabricius. The increment of macrophage population subsequently resulted in the elevation of nitric oxide (NO) content in the infected chickens with AF2240 causing higher increment of NO compared to UPM/IBS/002/2011. In brief, both genotypes of NDV strains caused different immune response in chicken enriched B lymphocytes upon virus infection.

Keywords: Newcastle disease, B lymphocytes, macrophage, chicken

INTRODUCTION

Newcastle disease virus (NDV) was reported to infect avian species and resulted in respiratory, neurological or gastrointestinal disease depending on the different strains of NDV (Alexander, 1998). In this study, two different genotypes of velogenic NDV isolates from Universiti Putra Malaysia were used namely NDV genotype VII and NDV genotype VIII strain AF2240. Although pathogenesis of different NDV pathotypes has been studied, no study was carried out to evaluate and compare the effect of different velogenic NDV strains on enriched B lymphocytes population in chicken. The prime objective of this study was to evaluate the *in vivo* immunoregulation of chicken enriched B lymphocytes following infection with UPM/IBS/002/2011 (Genotype VII) and AF2240 (Genotype VIII). The lymphocytes population changes in chicken bursa of Fabricius was also immunophenotyped to investigate the possible immune response of chicken B lymphocytes after being infected with NDV.

MATERIALS AND METHODS

NDV Genotype VIII AF2240 and Genotype VII UPM/IBS/002/2011 were obtained from Laboratory of Vaccine and Immunotherapeutics, Institute of Bioscience (IBS). The virus were inoculated and propagated in 9-day-old embryonated chicken eggs. Immunophenotyping of chicken bursa of Fabricius was carried out using FITC-labeled CD3, PE-labeled CD4, IgM and KUL1 and PerCP-labeled CD8 antibodies (Southern Biotech, USA). Nitric oxide level was measured using Griess Reagent (Invitrogen, USA). IgM+ B lymphocytes were enriched from total cells using LS column and microbeads (Miltenyi Biotec, Germany). Apoptosis study was carried out using AO/PI and AnnexinV FitC kit (BD Biosciences, USA).

RESULTS AND DISCUSSION

In this study, viability of chicken B lymphocytes population, which is a crucial element in the humoral immunity, was assessed and correlated with the viral load and inflammation in spleen and bursa infected with different genotype of velogenic NDV. When infected with NDV genotype VII UPM/IBS/002/2011 and genotype VIII AF2240, reduction of IgM+ B lymphocytes population and infiltration of macrophage was observed in the chicken bursa of Fabricius (Figure 1). The pattern of increasing macrophage population is reported to be similar in NDV infected chicken spleen (Rasoli *et al.,* 2013).

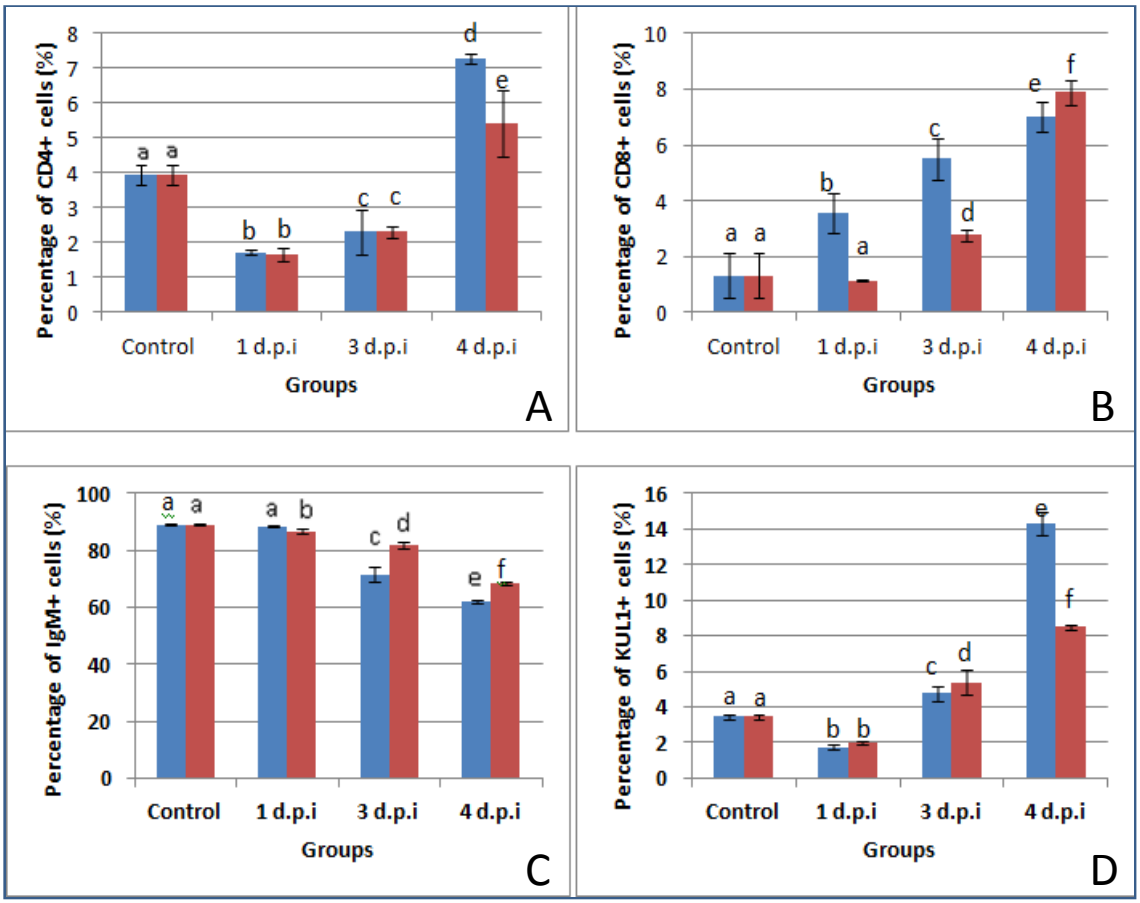

Figure 1. The percentage of CD4+ (a), CD8+ (b), IgM+ (c) lymphocytes and KUL1+ (d) in the bursa of 3-week-old chickens infected with AF2240 (blue) and UPM/IBS/002/2011 (red) strains of NDV at 1, 3 and 4 days post infection. Groups labelled with different superscript are significantly different (P≤0.05).

Nitric oxide can be synthesized from activated macrophages (Thomas and Martha, 1995). The increment of macrophage population subsequently resulted in the elevation of nitric oxide (NO) content in the infected chickens with AF2240 causing higher increment of NO compared to UPM/IBS/002/2011. This result suggest that different genotype of NDV caused a different response in B lymphocytes towards the infection.

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IN OVO VACCINATION AGAINST NEWCASTLE DISEASE VIRUS USING A DNA VACCINE CO-EXPRESSING THE F AND HN GENES AND DEXTRAN-SPERMIN AS A VEHICLE FOR DNA DELIVERY

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ABSTRACT

Newcastle disease virus (NDV) is recognized as a fatal disease virus affecting domestic and wild avian species. Different types of vaccines are under testing to ensure that there is efficient control. In the present study, a DNA vaccine, namely pIRES-HN/F, alone or in mixture with Dextran-spermine, as a vehicle, was applied for *in ovo* vaccination to NDV. The antibody titer in the chickens immunised by the pDNA was higher compared to the chickens immunised with pDNA-Dextran complex. The produced antibodies failed to control the acute NDV infection after challenge and the severity of infection in the most of the dead chickens showed variation. Immunisation with the pDNA/Dextran-spermine nanoparticles induced low level of antibody titer compared with the pDNA alone. Therefore, it was concluded that Dextran-spermine was not effective to facilitate the entrance of pDNA into the cell by *in ovo* vaccination.

Keywords: Newcastle disease virus, DNA vaccine, *in ovo* vaccination, Dextran-spermine

INTRODUCTION

DNA vaccine is an approach to achieve specific immune system activation (Takami *et al.* 2012). The advantages of DNA vaccines have made it much attractive for the poultry industry. In DNA vaccine delivery, intradermal or intramuscular injections are the most commonly used approaches in adult animals whilst there are few studies are involving embryonic administration (Johnston *et al.* 1997; Oshop *et al,.* 2003; Moura *et al.,* 2007). Avian embryo is competent immunologically after the 17th day of embryonic stages (Sharma and Witter 1983; Sharma 1986; Oshop *et al.,* 2003). Therefore, it was hypothesized that DNA vaccine could be administered into the amniotic sac of avian embryos to induce immunity at this stage. The efficiency of DNA vaccines delivery can be improved by using cationic lipids and/or polymers. Cationic polysaccharides are biodegradable and non-toxic which can be changed simply to develop physicochemical properties (Berscht *et al.,* 1995). Dextran-spermine (D-SPM) polycations have found to be active in transfecting a wide range of cell lines in vitro. Therefore, the aim of this study was to use cationic polysaccharide -Dextran-spermine- for *in ovo* delivery of the DNA vaccine against NDV.

MATERIALS AND METHODS

The pIRES vector (Clontech, USA) was used to construct a DNA plasmid encoding the F and HN proteins of NDV AF2240 strain. The amplified fragments of the F and HN genes were cloned into the vector to construct pEIRSHN/F DNA plasmid. The construct was purified using plasmid purification kit (QIAGEN, Germany). The pDNA/Dextran-spermine complex was prepared by mixing the DNA plasmid and

Dextran-Spermine in aqueous solution and gently mixed at 37°C for 30 min. The reliability of covering pDNA by dextran-spermine was tested on 1% agarose gel. Size and zeta potential of nanoparticles were determind using a laser particle size analyzer (Malvern, Zeta Worcestershire, UK). Eighteen day-old embryonated SPF eggs were inoculated with either pIRES/HN/F, pIRES/HN/F-D-SPM complex or the empty plasmid. After hatching, serum antibody titer was determined using indirect ELISA Kit (IDEXX, USA) and hemagglutination inhibition (HI) test was conducted as previously described (Allan and Gough 1974). Four weeks after vaccination, the chickens were challenged with intranasal administration of 10^5 egg infection dose (EID_{50}) of virulent NDV. The chickens were monitored daily for 10 days and the numbers of dead chickens were recorded.

RESULTS AND DISCUSSION

The HI test carried out on serum samples with 4 HA units of the purified NDV AF2240 strain, did not show significant difference between pDNA-Dextran-spearmint group (3.5±0.51) and pDNA-injected group (3.43±1.22). All the chickens in all the positive and vaccinated groups died of NDV disease. Some immunised chickens died with less severe clinical signs. The results showed that pDNA/Dextran-spermine complex can induce low level of antibody titer compared to the pDNA alone. Immunisation with pIRES-HN/F induced significant level of antibody titer even though it was not strong enough to confer protection from the virus challenge. Low antibody titer induced in the chickens immunised with the pDNA-nanoparticle can be related to the disability of Dextran-spermine in introducing pDNA to the embryo cell. This is the first study on applying Dextran-spearmint for the delivery of pDNA *in ovo*. Generally, finding safe and efficient nano delivery of pDNA into chicken embryo cells is still a dominant undertaking in today's biotechnological study.

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TRANSCRIPTOMIC ANALYSIS ON SUSCEPTIBILITY OF DIFFERENT INBRED CHICKEN LINES TOWARDS VERY VIRULENT INFECTIOUS BURSAL DISEASE VIRUS INFECTION

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ABSTRACT

Previous studies have identified differential expression of immune-mediated genes related to inflammatory response in chickens with different genetic susceptibility to IBDV infections. However, the mechanisms of genetic resistance against IBD are not known. RNA sequencing through NGS technologies provide an excellent platform to study differentially expressed genes of known or unknown functions to better define mechanisms of host resistance. Therefore, this study aimed at investigating susceptibility of different inbred chicken lines toward very virulent IBDV through transcriptomic analysis. This analysis allows for quantification and comparison of gene expression. Bioinformatics analysis of this data will allow function annotation of differentially expressed genes, indicating possible roles in the response to infection. Gene of interest, virus load detection, indel, SNP and CNV between different lines and breeds will be validated using qPCR. This study is expected to provide information that able to decipher the genetic resistance of chickens towards IBDV infection.

Keywords: infectious bursal disease, chicken, RNA, genes, vaccines

INTRODUCTION

Infectious bursal disease virus (IBDV) is the causative agent of Gumboro disease, a highly contagious and acute viral disease affecting all major poultry producing areas. The disease is controlled using live and inactivated vaccines. However, due to the virus induced immunosuppression and the presence of very virulent (vv) IBDV, vaccination failures are commonly reported. In order to develop an improved vaccine, fundamental study on IBDV and chicken immune system is crucial in elucidating the mechanism and immunes responses develop by the chicken upon IBDV infection (Ruby *et al.,* 2006). For this reason, next-generation sequencing (NGS) technology, current widely used platform to study host-pathogen interaction has add clear advantages as it allow researcher to quantify differentially expressed genes without prior knowledge of gene sequence (Wang *et al.,* 2009), thus enabling functional genomic analysis of host resistance and immune responses towards a particular infectious agent.

MATERIALS AND METHODS

Bursal tissues from 6 different inbred chicken lines; vvIBDVinfected and control bursal tissues were collected at 3d p.i. Total RNA were isolated from bursa according to the manufacturer's protocol (Qiagen). RNA analysis were done using Bioanalyzer by measuring RNA quality (28S:18S ratio>1.8) and integrity (RIN>8.0). PolyA RNA was purified from 5-10 ug of high-quality total RNA with Sera-Mag oligo (dT) beads (Illumina, USA) according to manufacturer's protocol. Upon polyA RNA selection, the RNA was fragmented and undergoes cDNA synthesis. The second-strand cDNA synthesis was treated with dUTP to conserve the original RNA sequence as part of

strand-specific sequencing through paired-end sequencing method. The cDNA were sequence in Illumina HiSeq 2000. Sequence analysis was performed using *Gallus_gallus 4.0* as reference genome whilst, unmapped sequence reads were analysed using Velvet *de novo* assembly approach.

RESULTS AND DISCUSSION

The chicken cDNA libraries were successfully sequenced and generated more than 50 million reads for each of the chicken lines with library size between 154±23.8 and 181±15.8 nt. Approximately, 52 million clean reads were generated with 96% of them with read length of 70-79 nt. Nearly 80% of the annotated chicken genes, which is around 14,300 genes were successfully detected from the reference genome with a cut-off value of FPKM≥1E-5. Differentially expressed genes were defined as genes that were measured to be at least 4-fold difference in expression level between infected and normal chickens ($\log_2$ fold-change<-2 or $\log_2$ fold-change>2) with q-value<0.05. The resulting differentially expressed genes were further analysed for gene enrichment and pathway enrichment analysis using DAVID. Several pathways that have identified to be associated with differentially expressed genes are cytokine-cytokine receptor interaction, JAK-STAT signaling pathway and MAPK signaling pathway. Apart from performing reference genome mapping, *de novo* assembly was also carried out from the unmapped sequence which later were functionally annotated via several protein databases. De novo assembly identified around 10,828 transcripts based on the NR and Swiss-Prot database. Further analysis is currently underway in identifying the roles of some of these genes in modulating IBDV infection and host resistance.

Table 1. Summary of significantly differentially expressed genes following reference assembly

	Line1	Line2	Line3	Line4	Line5	Line6
Up-regulated	2,004	1,317	1,950	1,283	954	1,475
Down-regulated	968	878	805	1,010	751	916
Unique (Infected)	114	22	54	11	3	62
Unique (Control)*	20	27	10	4	1	20
Total	3,119	2,245	2,828	2,309	1,709	2,474

* Genes that were not expressed in infected samples and expression did not pass the detection threshold level.

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INVOLVEMENT OF SPLENIC DENDRITIC CELLS DURING INFECTIOUS BURSAL DISEASE VIRUS INFECTION IN CHICKENS

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ABSTRACT

Infectious bursal disease virus (IBDV) is attracted to lymphoid cells and especially those of B-lymphocyte origins. Recent studies have shown that IBDV also can infect macrophage. However, the involvement of dendritic cells (DC) in IBDV infection is not clear. The objective of this study was to examine the effect IBDV vaccine and very virulent (vv) strains on avian derived splenic DC. Enriched DCs were cultured from spleen of infected SPF chickens with vaccine and vvIBDV strains of IBDV. The infected DCs were analysed for morphology study, immunophenotyping, proliferation activity, and immunofluorescent antibody test (IFAT). Both IBDV strains inhibit DC proliferation. However, the inhibition is higher in vvIBDV strain group. However, the expressions of IBDV antigens from both strains were detected in splenic DC.

Keywords: Dendritic Cells, Infectious Bursal Disease Virus, Vaccine, vvIBDV

INTRODUCTION

Infectious bursal disease is caused by IBDV, a highly contagious immunosuppressive agent of young chickens. The virus can be divided into three different strains; classical, variant and very virulent (Saif, 1991). IBDV mainly infects Bursa of fabricus where it infects and destroys actively dividing IgM-bearing B cell (Hirai *et al.,* 1981) and macrophages (Khatri *et al.,* 2005). The importance of DC in infection and immune responses of chickens is poorly studied.

MATERIALS AND METHODS

Generation of chicken splenic DC and infection with IBDV

Spleens were isolated from 3 weeks SPF chickens following infection with 0.5 MOI of vaccine (D78) and vv strains of IBDV (UPM0081). The DCs were purified by excluding B cells and macrophage cells population and cultured in the presence of GM-CSF and IL-4 (Zhiguang and Pete, 2011).

Splenic DC Morphology

Light microscopy and scanning electron microscope (SEM) were used to assess the morphology of the splenic DC.

Immunophenotyping

Two-colour flow cytometry was used to determine the phenotype of the enriched population of DC by staining using monoclonal antibodies CD86-APC and MHC II-PerCp.

IBDV detection on DC

IBDV VP2 antigen was identified using IFAT. In IFAT, the cells were stained using mouse monoclonal (IBDV9) to IBDV (Abcam, UK) conjugated with FITC fluorescent dyes (Innova Bioscience).

RESULTS AND DISCUSSIONS

Morphologically, immature DC and mature DC are distinctive as mature DC has dendrites characteristics (Figure 1). Proliferation study using BrdU after infection with vaccine and vvIBDV strains shows that DC proliferation activity is inhibited by IBDV infection (data not shown). Based on IFAT, IBDV antigen was detected in the infected DC (Figure 2). Hence, the expressions of IBDV VP2 antigen of vaccine and vv strains were detected in splenic DC.

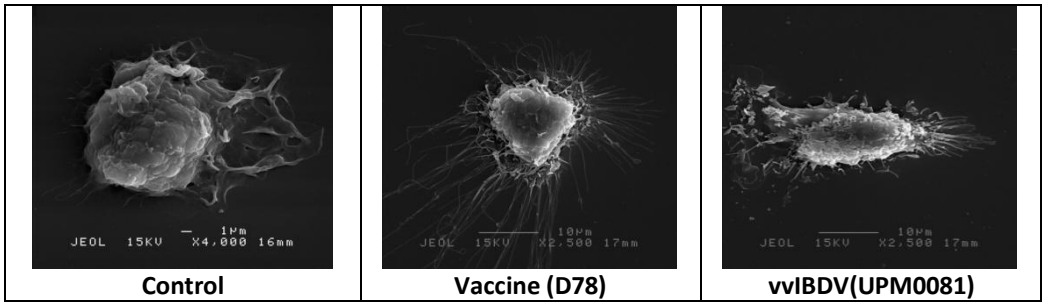
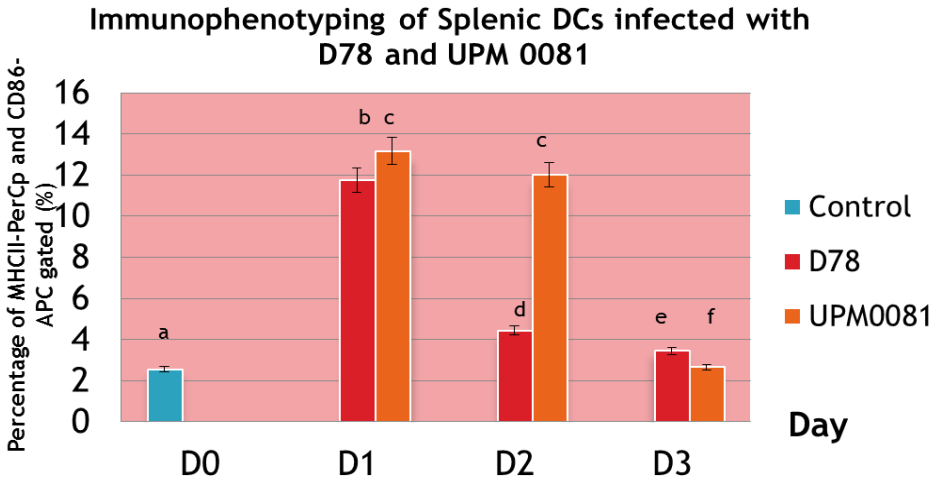


Figure 1. Scanning electron microscopic analysis of infected splenic DC.



*abcdef Values with different superscripts differ significantly at p<0.05

Figure 2. Immunophenotyping of Infected Sp-DC.

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WILD BIRDS AS A PROBABLE SOURCE OF COLONIZATION OF *CAMPYLOBACTER* IN POULTRY

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ABSTRACT

The study was carried out to detect the presence of *Campylobacter* in wild birds caught around chicken and duck farms and also in the chickens and ducks in the farms. Of the 70 birds belonging to six species, 18.6% were positive for *Campylobacter* and of the 101 chickens and 104 ducks sampled, 40% were *Campylobacter*-positive. *Campylobacter jejuni* were frequently isolated from birds, chickens and ducks. The wild birds may play a role in the spread of campylobacters to chickens in the farms.

Keywords: wild birds, *Campylobacter*, poultry

INTRODUCTION

Campylobacter and *Salmonella* have been frequently reported in poultry in the farms and isolated from carcasses in processing plants and retail outlets. These bacteria are of public health concern because upon ingestion of inadequately cooked contaminated meat and contact by handlers with poor hand hygiene could result in gastroenteritis. A number of factors are reported as sources of these bacteria in farm animals, among which include the presence of wild birds around the farms. The aim of this study was to detect the occurrence of *Campylobacter* in wild birds near poultry farms and in poultry in the farms.

MATERIALS AND METHODS

Birds were humanely trapped using mist net which was set up near to chicken houses and duck areas in the farms. Each bird was photographed, marked (to avoid from being resembled) and after the cloacal swab was taken, the bird was immediately released. Cloacal swabs were also taken from the chickens and ducks in the farms. The swabs were transported under chilled condition. Each swab was directly streaked on *Campylobacter* blood free agar and incubated under microaerophilic condition for 48h. The presumptive *Campylobacter* colonies were subjected to Gram stain for cellular morphology (Gram negative, curved rods) and wet mount for motility test; those suspected as *Campylobacter* were subcultured for identification using biochemical tests, which included urease, hippurate hydrolysis and indoxyl acetate hydrolysis tests. The isolates were then confirmed and speciated using mPCR assay.

RESULTS

A total of 70 birds belonging to six species were trapped and sampled near six poultry farms. The most common species caught was plain backed sparrows and Rock pigeon. Eighteen point six percent (18.6%) of these birds were found positive for *Campylobacter* and of these, 61.5% were identified as *C. jejuni*. From the 101

chickens and 104 ducks sampled in the farms, 40% were *Campylobacter*-positive and 80-100% were *C. jejuni.*

DISCUSSION

A number of studies reported that birds are often healthy carriers of several zoonotic viruses, bacteria, fungi and protozoa and given their ability to fly freely and some over a great distances such as migratory birds, these birds can spread these pathogens in the environment including grazing pastures, park areas, surface water and also to animals in the farms (Abulreesh *et al.,* 2007). A previous study found 18.1% of the 127 birds caught (which was later released) were positive *Campylobacter* (Saleha *et al.,* 2001). Craven et al. (2000) in a study in Georgia, United States found the wild birds caught near chicken houses carried *Salmonella* spp., *C. jejuni* and *Clostridium perfringens* and suggested that upon gaining entry into poultry houses have the potential to transmit these pathogens to poultry. Waldenstrom et al. (2003) isolated *Helicobacter canadensis* which was described as an emerging human pathogen from wild geese. An interesting study on salmonellosis in wild birds in pigs in farms by Andres et al. (2012) reported that the odds of detecting *Salmonella*-positive birds captured in a pig farm was more than 16 times higher than those from areas far (>2 km) from pig farms; this is partly because the high concentrations of insects would lead to *Salmonella* readily available for insectivorous birds as the study found *Salmonella* infection to be negatively related to mostly granivorous birds. Also, such a scenario could possibly occur with campylobacters in poultry farms because insects tend to be attracted to the feed and litter in the houses. Thus in this study, the birds could play a role in the spread of campylobacters to poultry in the farms through their droppings and/or they could have picked up the bacteria from the poultry litter and contaminated houses. Larger samples and locations and genotypic relatedness among isolates need to be studied.

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CYTOKINES AND IMMUNE RELATED GENE EXPRESSION ALTERATION IN CHICKEN HD11 CELL LINE FOLLOWING VERY VIRULENT INFECTIOUS BURSAL DISEASE VIRUS INFECTION

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ABSTRACT

Studies have showed that infectious bursal disease virus (IBDV) can infect macrophage and stimulate proinflammatory cytokines. To date, only a few cytokines were evaluated from IBDV infected macrophages. Using multiplex GeXP assay, we were able to quantify up to 27 cytokines and immune related gene expressions from vvIBDV infected chickens HD11 macrophage cell line.

Keywords: Infectious bursal disease virus (IBDV), PCR, GeXP, Cytokines

INTRODUCTION

Very virulent infectious bursal disease virus (vvIBDV) induced infectious bursal disease (IBD), is an important viral disease affecting poultry farms worldwide (Van Den Berg, 2000). IgM bearing B-cells found in gut-associated lymphoid organ and bursa of Fabricius are the major targets of IBDV (Withers *et al.,* 2005). *In vitro* study also showed that macrophage is susceptible to IBDV infection (Khatri and Sharma, 2006). However, the details of cytokines changes in IBDV infected macrophage are still unclear. This study evaluated multiple cytokines expression following *in vitro* infection of chicken macrophage cell line with vvIBDV.

MATERIALS AND METHODS

Chicken monocytes/macrophage cell line, HD11 (Beug *et al.*, 1979) was infected with vvIBDV (UPM0081) at MOI of 0.1 for 3 hours at 37°C. After incubation, the cells were washed, and further incubated with new media for 3, 21 and 45 hours. Then, total RNA was extracted using RNeasy® Plus mini kit (Qiagen, Germany). GenomeLab eXpress Profiler was used to detect 27 sets of immune related genes and 1 house-keeping gene (Rasoli *et al.,* 2013).

RESULTS AND DISCUSSION

GeXP data illustrates an up-regulation of proinflammatory cytokines such as IL-1β and iNOS in IBDV infected HD11 cells as early as 6 hours post-infection (Table 1). All the analysed chemokines (CCL4, CXCLi1 and CXCLi2); Th1-like cytokines (IL-12α and IL-18); TLR-3; MHCI and iNOS were also up-regulated. On the other hand, both IL-10 and MHCII expressions were down-regulated following vvIBDV infection. Other cytokine and immune-related genes were not significantly changed.

Table 1. Relative fold changes in gene expression of HD11 cells infected with 0.1 MOI of UPM0081 at 6, 24 and 48 hours post-infection

Gene	6 hpi	24 hpi	48 hpi
Chemokines			
CCL4	2.47±1.03	3.89±0.85	2.69±0.78
CXCLi1	3.39±0.45	1.99±0.35	1.38±0.47
CXCLi2	2.24±0.41	6.44±0.75*	3.84±0.40
Th1			
IL-12α	2.10±0.39*	3.43±0.31	1.08±0.15
IL-18	1.75±0.06*	2.46±0.23*	1.11±0.24
Proinflammatory			
IL-1β	1.48±0.43*	5.53±0.37	1.17±0.48*
iNOS	3.67±0.53*	3.52±1.16*	5.57±0.61
Toll-like receptors			
TLR-2-1	0.79±0.01	0.95±0.04	0.83±0.17
TLR-3	1.20±0.12*	2.00±0.90*	3.22±0.14*
TLR-4	1.36±0.30	0.92±0.14	1.27±0.14
TLR-5	1.20±0.34	0.91±0.30	1.00±0.02
TLR-7	0.79±0.03	1.00±0.27	0.99±0.14
IL-10 family			
IL-10	0.41±0.23	0.50±0.33	0.86±0.16
Others			
MHCI	0.83±0.01	0.53±0.12	2.59±1.18
MHCII	0.43±0.08	0.53±0.12	0.57±0.30
TGF-β3	0.52±0.07	0.64±0.09	0.59±0.18
IL-15	0.89±0.21	1.02±0.55	0.98±0.44
IL-16	0.85±0.20	0.92±0.05	1.14±0.26

Note: Significant differences ($P<0.05$) in the expression level are indicated with an asterisk.

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A COMPARISON BETWEEN BIO-MOS AND PALM KERNEL EXPELLER AS MANNAN-OLIGOSACHARIDS SOURCES ON THE ILEAL MICROBIAL POPULATION IN BROILER CHICKENS

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ABSTRACT

A total of 60 1-d-old male broiler chicks (Cobb 500) in a finisher period (29-42d) were allotted to 3 dietary treatments namely a control diet, a diet with 20 g/kg Bio-Mos, and a diet containing 200 g/kg of an enzymatically-treated PKE. There were no differences in the ileal population of *Lactobacillus* and *Enterococcus* or *Enterobacteriaceae* among the experimental groups. But, birds fed diets containing PKE or Bio-oOs had a lower population of *Escherichia coli* than the control group. The results showed that PKE potentially has comparable prebiotic features as commercial Bio-Mos for poultry.

Keywords: palm kernel expeller, Bio-Mos, ileum bacteria, broilers

INTRODUCTION

Palm kernel expeller (PKE) contains β-mannan which enhances the chicken immune system as does mannan originated from yeast cell wall (Allen *et al*., 1997). It seems that acid hydrolysis or physical digestion of β-mannan in the gizzard, produces simple form carbohydrates, including manno-oligosaccharide or mannose. The present study aimed to compare the effects of Bio-Mos (Alltech, USA), as mannanoligosaccharide based derivative of the outer cell wall of *Saccharomyces cerevisiae* and an enzymatically-treated PKE on the growth performance, ileal microbial populations, and nutrients digestibility in broiler chickens.

MATERIALS AND METHODS

A total of 60 1-d-old male broiler chicks (Cobb 500) were allocated to 12 pens (5 chicks each) to receive 3 dietary treatments with 4 replicates for each treatment. For starter and grower periods (1 to 28 d), the chicks were fed on a standard diet. During finisher period (29 to 42 d) the birds were fed one of the 3 isonitrogenous and isocaloric diets including a control diet, a diet with 20 g/kg Bio-Mos, and a diet containing 200 g/kg of an enzymatically-treated PKE. The birds were slaughtered at the age of 42 d and ileal digesta were collected from 12 birds per treatment. Within each treatment, 10 grams of the digesta from 3 birds were pooled and used for a total of 4 replicates per treatment for DNA extraction. Real-time PCR was performed with the BioRad CFX96 Touch (BioRad, Hercules, CA, USA) using optical grade plates. Real-time PCR data for each bacteria quantity were obtained as follows: plasmid DNA of *Methanobrevibacter ruminantium*, obtained from cloning process using the TOPO TA cloning Kit (Invitrogen, USA), was used to prepare the standard for total bacteria. To prepare the standards for other groups of bacteria, DNA extracts from pure cultures of *Lactobacillus brevis*, *Escherichia coli*, *Enterococcus faecium,* and *Enterobacter cloacae* were used. To calculate the amount of DNA in digesta samples, calibration standards constructed by amplifying known amounts of target DNA and

were used to convert the Ct values into quantities of DNA. The final data were calculated as a ratio of absolute quantities of each bacterial group to total *Eubacteria*.

RESULTS AND DISCUSSION

The bacterial populations of ileal digesta as a ratio of the total bacteria population are shown in Table 1. There were no differences in the ileal population of *Lactobacillus* and *Enterococcus* or *Enterobacteriaceae* among the experimental groups. However, the birds fed PKE or Bio-Mos containing diets had a lower population of *Escherichia coli* than the control group ($P<0.05$).

Table 1. Effect of the palm kernel expeller and Bio-Mos supplement on the bacterial populations of ileal digesta in broiler chickens

	Ileal bacteria population (as the log of bacterial cell number per ml of the liquid phase of digesta)			
	Lactobacillus	E*scherichia coli*	E*nterococcus* genus	Enterobacteriacea e family
PKE containing diet	2.78×10^{-4}	$2.38\times 10^{-8\ b}$	3.46×10^{-4}	3.36×10^{-5}
Bio-Mos containing diet	1.65×10^{-4}	$8.28\times 10^{-8\ b}$	2.92×10^{-4}	3.19×10^{-5}
Control diet	2.36×10^{-4}	$4.62\times 10^{-7\ a}$	3.34×10^{-4}	6.63×10^{-5}
SEM	1.8×10^{-5}	4.67×10^{-8}	2.01×10^{-5}	9.96×10^{-6}

[a,b]: means with different superscripts within same column differ significantly ($P\leq0.05$).

Mannan oligosaccharides have been reported to have receptor sites for the fimbriae of *E. coli* which results in an elimination of these particular bacteria as the digesta flows out (Spring *et al*., 2000). Because the birds were housed in raised wire-floor cages in the present study, it is acknowledged that performance could be different if birds were grown on less hygienic litter- floor pens. Further work is needed to elucidate the prebiotic properties of PKE.

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EFFECTS OF ENZYME TREATMENT AND SHELL CONTENT OF PALM KERNEL EXPELLER ON PERFORMANCE AND NUTRIENT DIGESTIBILITY IN BROILERS

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ABSTRACT

One hundred day-old male broiler chicks were allocated to 20 pens. At 29-42 d, the birds were fed one of the five experimental finisher diets: palm kernel expeller (PKE) free diet (control) and four experimental diets containing 200 g/kg of normal PKE, low shell PKE, enzymatic treated PKE or low shell-enzymatic treated PKE. Enzyme treatment increased mannose content drastically followed by glucose and xylose. Enzyme treatment also increased the mannan-oligosaccharides content in PKE with mannobiose made up the major component. Surprisingly, the average daily weight gain and feed conversion ratio of the birds fed low shell PKE diet was inferior than those in the control and other PKE treatment groups. Digestibility of dry matter, ash and crude protein in diets containing PKE were lower than the control diet. The lower growth rate in chicks fed low shell PKE containing diet could be because of the increased β-mannan concentration in this product when the shell was removed.

Keywords: palm kernel expeller, performance, digestibility, broilers

INTRODUCTION

Palm kernel expeller (PKE) contains up to 30% β-mannan which is a linear polysaccharide composed of repeating β-1-4 mannose with 1-6 galactose and glucose units (Jaafar and Jarvis, 1992). β-mannans are highly viscous components and this property categorizes them as anti-nutritional factors. Exogenous enzymes degrade β-mannan to mannan-oligosacharides with prebiotic properties (Ferket, 2004). This study was designed to investigate the effects of the enzyme treatment and shell content of PKE on performance and nutrients digestibility in broiler chickens.

MATERIALS AND METHODS

Palm kernel expeller was obtained from a palm kernel oil extraction mill from Selangor, Malaysia. Part of the normal or enzyme treated PKEs were sieved to remove the shell and the remaining portion was used as it is. Reducing sugars and the main mannan-oligosaccharides in PKE samples were determined using HPLC. One hundred day-old male broiler chicks (Cobb-500) were allocated to 20 pens. For starter and grower periods (1-28 d) chicks were fed commercial diets and during the finisher period (29-42 d) were offered PKE free diet or diets containing 200 g/kg normal PKE, low shell PKE, enzymatic treated PKE or low shell -enzymatic treated PKE. At 29 and 42 days of age, birds were weighed and feed consumption was recorded for feed conversion computation. Celite (*Celite* Corp., Santa Barbara, CA) was added at 15 g/kg to all finisher diets as an acid insoluble ash (AIA) marker for digestibility assay.

RESULTS AND DISCUSSION

Enzyme treatment increased mannose content of PKE drastically (Figure 1) and also the mannan-oligosaccharides content in PKE, with mannobiose made up the major component followed by mannoteriose and mannotetrose (Figure 2).

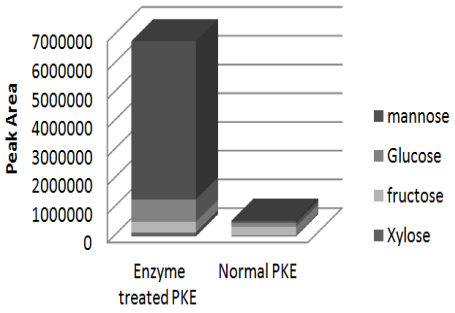

Figure 1. Effects of enzyme treatment on content of reducing sugars in PKE samples

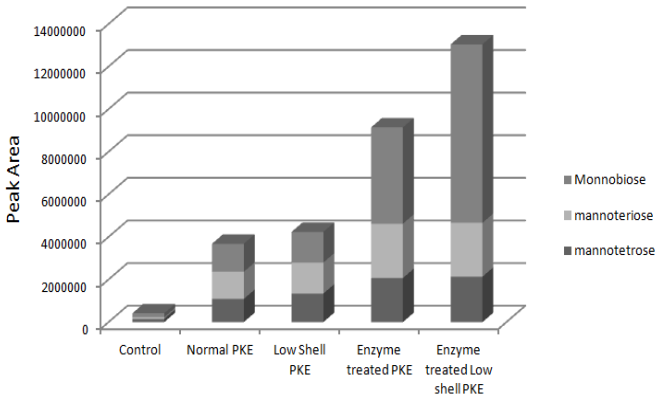

Figure 2. Mannan-oligosaccharides content of the experimental diets

Feed intake was not affected by inclusion of PKE in the diet. Surprisingly, the average daily weight gain and feed conversion ratio of the birds fed low shell PKE diet were poorer than those in the control and other PKE treatment groups ($P<0.05$). Digestibility of dry matter, ash and crude protein in diets containing PKE were lower than the control diet ($P<0.05$)

Table 1. Effects of different PKE sources on the broiler chickens performance

	Avg Daily weight gain (g/bird)	Avg Daily feed intake (g/bird)	Feed conversion ratio
Control	104.4[a]	210.7	2.02[b]
Normal PKE	99.1[a]	204.2	2.06[b]
Low shell PKE	77.6[b]	207.6	2.69[a]
Enzyme treated PKE	96.2[a]	214.5	2.23[b]
Enzyme treated Low shell PKE	95.5[a]	207.2	2.17[b]

[a,b]: means with different superscripts within the same column differed ($P<0.05$)

The dietary supplementation of 20% PKE in the feed regardless of its shell content or enzyme treatment decreased nutrient digestibility. The lower performance of the birds fed low shell PKE could be attributed to its higher β-mannan concentration after removal of the shell particles. However, enzymatic degradation of β-mannan of PKE alleviated these adverse effects.

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PROSPECTS FOR ORGANIC POULTRY IN PAKISTAN – AN OVERVIEW

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ABSTRACT

The objective of this study was to provide an assessment of the potential for organic poultry farming in Pakistan and, in particular, to identify the possible technical, financial and market constraints on the development of organic chicken egg and table meat production enterprises. In the last few years, poultry industry has transformed from just back yard poultry to highly intensive commercial farming, but the issues of food safety and quality remains unaddressed. Hence, organic poultry farming has become as an approach to address these issues. Organic poultry husbandry practices focus on natural living conditions with outdoor access, preventative health management with a prohibition of antibiotics or other drugs (although vaccines can be used), and 100% organic feed. Moreover it also will be a cheaper means of food in developing countries like Pakistan which have very less resources like power.

Keywords: Organic poultry farming, Backyard, Food safety

TOWARDS A MORE SUSTAINABLE POULTRY SECTOR IN MALAYSIA: A CASE FOR SHORT FOOD SUPPLY CHAINS

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ABSTRACT

Despite Malaysia's current self sufficiency of 125% in poultry meat and eggs the industry is facing challenges such as the soaring global prices of imported feed ingredients, consumers concerns over the safety of poultry products, health and environmental issues, and animal welfare concerns. The Malaysian poultry industry must re-orient itself to address these pressing issues and to ensure that the industry is sustainable and continue to contribute to the national food security. This paper presents a case for short food supply chains (SFSCs) as a viable solution to the conventional long food supply chains and elaborates that SFFCs can address the health, environmental and animal welfare issues of poultry industry.

Keywords: Malaysian poultry industry, short food supply chain, animal welfare, health and environmental issues

INTRODUCTION

Despite Malaysia's current self sufficiency of 125% in poultry meat and eggs, the industry is facing challenges such as the soaring global prices of imported feed ingredients, consumers concerns over the safety of poultry products, health and environmental issues, and animal welfare concerns.

It cannot be denied that industrial agriculture has led to the production of enormous amounts of food, using minimal labour and significantly lowering operation costs. However, many of the true costs of the industrial meat production industry are part of a flow on effect; meaning the price tag does not reflect the cost of the effects on the public's health, rural communities, animal welfare and the environment. The poultry industry, for example, formulates feeds and breeding techniques often used to abet animals reach their market weight, precociously. Inputs may include hormones, antibiotics and agricultural chemicals in the feed – all of which carry their own risks to consumer health (RESET, 2013). This factory style of meat production is the standard system in countries like the United States, which have seen a handful of corporations, such as Tyson and Cargill, gain monopoly power over the meat sector due to the consolidation of operations. As formal chains become more prevalent, they compete strongly with semi-regulated local chains, which eventually collapse. The transition has happened fastest for poultry products and this trend has resulted in the loss of livelihoods for small scale commercial producers and traders almost everywhere it has occurred; many studies have categorised the Malaysian poultry industry as oligopolistic (Zainalabidin, 2007). To rectify the aforementioned shortcomings, the way forward is to; promote *Microenterprise* in *Short Supply Chain* using *Appropriate Technology,* working towards *Right Livelihoods,* incorporating *Human Scale Development and* by giving due rights to the *Invisibles (*Baluch, 2013*)*.

Economic Impacts

One of the most commonly reported economic benefits associated with local food supply chain or short food supply chains is that of increased income for the producer; they are able to add a price premium when selling through SFSCs (Pearson *et al.*, 2011). Benefits can be found in rural development and economic regeneration. There is evidence that local farming systems and short chains do have a higher multiplier effect on local economies than long chains, with impacts also on maintaining local employment, particularly in rural areas. SFSC enable synergies with other regional economic activities and often contribute to an increase in job satisfaction and organisational capacity within rural communities, greater consumer trust in food systems, and reductions in food miles or waste; Transportation is the largest end-use contributor toward global warming in the United States and many other developed countries (Wakeland *et al*., 2012).

Social Impacts

Much research stresses that building relationships of trust is a central component and an important benefit of LFS/SFSCs. A study of Polish Farmers' Markets (Sinnreich, 2007) found that the building of relationships between consumer and producer is 'essential' and provides a 'unique experience'. DeLind (2011) discusses the market in terms of 'community'; as place-building and improving of relationships around neighbourhood-based, food-related activities (Abatekassa and Peterson, 2011). Lawson *et al*. (2008) found that the continued reference to community dimensions in relation to New Zealand farmers' markets can only arise because "farmers are willing to come together and recognise the potential benefits that emerge from cooperative activity."The local food movement also reflects an increasing interest by consumers in supporting local farmers, and in better understanding the origin of their food (Pirog, 2009). In more marginal areas, these benefits can help counter the abandonment of agriculture, out-migration and 'greying' populations (Roep and Wiskerke, 2006). SFSCs can be seen as new sources of value added which can be retained locally and can act as a catalyst for rural economic regeneration and dynamism (Du Puis and Goodman, 2005).

Environmental Impacts

Claims for environmental benefits in the referenced sources include: reduction in 'food miles' and carbon footprint for local food, positive impacts on agro-biodiversity and reduction in the use of agrochemicals for organic farms. Other environmental impacts of intensive farming which have not been covered extensively in the literature on SFSCs are: loss of biodiversity, destruction of habitats, pollution of soil and water from pesticide and fertiliser use, eutrophication, soil erosion and degradation, and deforestation (IAASTD, 2008; McMichael, 2008; Wiskerke, 2009). The importance of ethical values and the higher uptake of environmentally sound practices are *de facto* elements in favour of a positive impact of SFSCs in the EU.

CONCLUSION

SFSCs can be thought of as examples of 'local food movements' which are often driven and supported primarily by urban residents. They emphasize co-operation rather than competition and seek to sustain as many producer livelihoods as possible

rather than reduce the number of producers to those that can most profitably exist. In both traditional and neo-traditional types of SFSC, social values are central and people are motivated to participate not only because they will receive produce in return but also they want to support the initiative and its values. Supply chains needn't be as long with too many middle men trying to muscle in; too many stages in the supply chain with each one trying to make more/cut corners. Long supply chains provide opportunities for the unscrupulous. They always will whatever the rules may be on traceability (Agrichat UK, 2013).

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DETECTION OF BONE MORPHOGENETIC PROTEIN 2 WITHIN AVIAN GENOME USING PCR-BASED METHOD

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ABSTRACT

Bone Morhogenetic Protein 2 was first discovered by Urist (1965) when he implanted the demineralized bone in rat muscle pouch which result in bone formation at the implantation site. The presence of BMP 2 protein was further confirmed by Sampath and Reddi (1981) when they discovered the removal of BMP 2 protein caused the matrix failed to induce new bone formation. On the other hand, new bone formation was observed with the presence of BMP 2. Ever since then, the effects of BMP 2 has been actively study and lots of clinical trial have been done using recombinant BMP 2 protein produce from mammalian host but not from avian. Thus, the aim of this study is to detect the presence of BMP 2 gene within the avian genome. In order to amplify the BMP2 gene from avian genome, a set of primer was design based on the *Gallus gallus* nucleotides sequnces from NCBI (Accession: NM_204358.1). The two primers designed for this study were; forward primer (BMP2F) 5'-ATGGTTGCCGCCACCCGCTC-3' and reverse primer (BMP2R) 5'-TCAGCGGCACCCGCAGCCCT-3'. DNA from few types of avian species were extracted from the respectively species feathers followed Richard and Jayaraj (2010) protocol and further subjected to PCR screening. The annealing temperature for this particular set of primer was set at 71.9°C. The PCR results were then send for sequencing. As shown in Figure 1.0, the PCR results in 1% agarose gel (w/v), the expected PCR products size was 1179 bps but the PCR product only shown about 950 bps instead of 1179 bps. However the sequencing results of these PCR products shown up to 94% similarity, when blast against the BMP 2 gene from NCBI. Despite the PCR products size were lesser than the expected size but it should be able to function in avian system as clinical report from Sample *et al.* (2008) had proven that recombinant human BMP 2 in calcium phosphate matrix as a bone graft substitute can be used for treatment of a comminuted, open humeral fracture in a whooping crane. Whereby, the *Homo sapiens* BMP 2 gene sequence (Accession: NM_001200.2) only shown 79% of similarity when blast against the *Gallus gallus* BMP 2 gene sequence.

Keywords: BMP 2, recombinant BMP 2

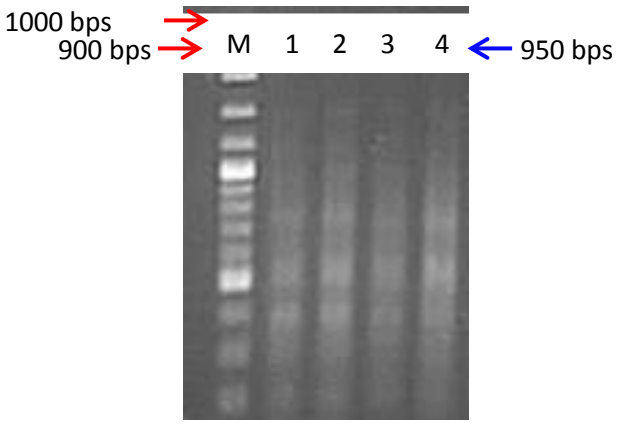

Figure 1:PCR results various Avian species.
M = 100 bps marker; Lane 1= Female Broiler DNA; Lane 2= Male Broiler DNA; Lane 3= Pigeon DNA, Lane 4= Macaw DNA.

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PHYSIOLOGICAL ASSESMENT OF HUNGER IN FEMALE BROILER BREEDERS

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ABSTRACT

The aim of current experiment was to investigate several physiological indicators of stress (corticosterone, heat shock protein, acute phase proteins and serotonin) in broiler breeders subjected to feed restriction. The birds were subjected to five different levels of feed restriction and ad libitum feeding as control group from 28 to 42 days of age. There was a linear increase in serum levels of corticostrone with severity of feed restriction. Only severe levels of feed restriction significantly increased heat shock protein 70 expression in the brain and plasma serotonin concentration. Feed restriction had negligible effect on serum levels of alpha-1 acid glycoprotein, ceruloplasmin and ovotranferrin.

Keywords*:* Broiler breeder, feed restriction, serotonin, heat shock protein 70, acute phase protein

INTRODUCTION

To restrict growth rate, broiler breeders are subjected to severe feed restriction that may compromise their welfare. Reliable indicators of stress are thus required to assess the effect of feed restriction on welfare of broiler breeders. Although plasma corticosterone (CORT) is a common physiological indicator of stress, the reliability is inconsistent (Savory *et al*., 1993). Hence, other more reliable measures of physiological stress in avian species are needed. The objective of this study was to determine the responses of acute phase proteins [ceruloplasmin (CP), ovotransferrin (OVT) and alpha-1 acid glycoprotein (AGP)], heat shock protein (HSP) 70 and plasma serotonin concentration (SERO) to various levels of feed restriction in female broiler breeders.

MATERIALS AND METHODS

Two-hundred day-old female broiler breeder chicks were reared at cages in an environmentally controlled room. From day 28 to 42, birds were assigned to one of 5 feeding regimens; (1) ad libitum feeding (AL); (2) 75% feed restriction (75AL); (3) 60% feed restriction (60AL); (4) 45% feed restriction (45AL) and (5) 30% feed restriction (30AL). On day 42, blood samples were collected from 8 birds per treatment for measurement of CP, AGP, OVT, CORT and SERO. The birds were killed and the brain samples were removed for determination of HSP70 expression. CORT and SERO were determined using commercial ELISA kits. Serum AGP concentration was determined by radial immunodiffusion using a commercial kit. Serum CP concentration was measured according to procedure of Martinez et al (2007). Radial immunodiffusion methods were used to measure OVT level according to the modified method of Mancini *et al.* (1965). The expression of HSP70 was determined by SDS – PAGE and immunoblot analysis. All data were subjected to ANOVA and Duncan's multiple range test was used to separate means.

RESULTS AND DISCUSSION

Results are presented in Table. Irrespective of age, there was a linear increase in CORT with severity of feed restriction. Feed restriction significantly increased HSP70 response as compared to AL. The mean HSP70 densities of 30AL, 45AL, and 60AL chickens, which did not differ, were significantly higher than those of 75AL. Similar findings have been noted in broiler chickens (Zulkifli *et al*., 2002). When living organisms are exposed to both thermal and non-thermal stressors, the synthesis of most proteins is retarded but HSP are rapidly synthesised (Etches *et al*., 1995). Serotonin, a neurotransmitter, is most persistently linked to stress-induced pathophysiological consequence (Dohms and Metz., 1991). In the present study, only 45AL and 30AL birds had significantly higher SERO than the AL group.

Table 1. The pooled effect of feeding regimen and age on mean relative levels of CORT, HSP70, SERO, AGP, OVT and CP in female broiler breeder

	CORT	HSP 70	SERO	AGP	OVT	CP
Treatment [1]	(ng/ml)		(ng/ml)	(µg/ml)	(µg/ml)	(mg/ml)
AL	0.81±0.12^{c}	0.54±0.09^{b}	9.48±0.28^{c}	288.60±36.61	245.00±47.93	5.95±0.41
75AL	0.94±0.06^{c}	0.61±0.06^{b}	9.95±0.25^{c}	281.33±34.72	214.41±18.22	5.46±0.69
60AL	1.60±0.08^{b}	1.07±0.09^{a}	10.00±0.27^{c}	289.26±36.24	195.83±17.32	4.99±0.49
45AL	1.92±0.11^{b}	1.21±0.08^{a}	14.19±0.53^{b}	328.25±28.06	195.25±12.77	6.90±0.43
30AL	2.95±0.31a	1.14±0.11^{a}	15.91±0.38^{a}	234.81±18.84	210.79±21.12	5.14±0.46

^{a,bc} Means within a column with no common letters differ at $P < 0.05$.

[1]AL = fed ad libitum; 75AL, 60, 45 and 30= 75%, 60%, 45% and 30% of fed ad libitum.

All levels of feed restriction had negligible effects on AGP, CP and OVT. Acute phase proteins (APP) are a group of blood proteins that change in concentration in animals subjected to external or internal challenges, such as infection, inflammation, surgical trauma or stress (Gonzàlez *et al*., 2008). The present findings suggested that although feed restriction was stressful (as indicated by CORT and HSP70), the treatments did not elicit APP reaction in broiler breeders. In conclusion, CORT is a reliable measurement of physiological stress response to feed restriction in broiler breeders when compared to HSP70 and SERO. Feed restriction did not evoke any alteration in the serum concentration of AGP, CP and OVT.

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MORPHOLOGY COMPARISON OF SWIFTLET SPECIES FROM DIFFERENT HABITAT IN MALAYSIA

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ABSTRACT

In Malaysia, nest of *Aerodramus fuciphagus* (white-nest swiftlet) and *Aerodramus maximus* (black-nest swiftlet) are harvested for commercial purposes as one of most valuable animal product. Swiftlet taxonomy has been controversial due to numerous undefined parameters. Morphological differences between swiftlet species from different habitats remain unclear. This study found that *A. fuciphagus* from natural habitat are generally larger in size compared to man-made habitat and *A. maximus* are larger compared to *A. fuciphagus*. We postulated the different in body size is due to dietary behavior of the swiftlets.

Keywords: Swiftlet, Morphology, Habitat, Species

INTRODUCTION

Swiftlets are insectivorous bird origin from South East Asia. Species that produce nests and harvested commercially in Malaysia are *Aerodramus fuciphagus* and *Aerodramus maximus* (Supaluck, 2002). Swiftlets have been controversy in terms of taxonomy due to the lack of distinguishing morphology characteristics (Chantler *et al*. 2000). The genera of swiftlets had been shuffled several times based on morphological characteristics, ability to echolocate, nesting area and molecular evolution (Thomassen, 2005). In Malaysia, swiftlet colonise in both natural cave and artificial man-made structures with control microenvironments to resemble a cave (Koon and Cranbrook 2002). Although the contents of the bird nest were well studied, however the morphology comparison of swiftlets species from different habitats still remain unclear and require further investigation.

MATERIALS AND METHODS

A total of 20 *A. fuciphagus*, 4 *A. maximus* and 8 little swift (*Apus affinis*) as out group, were captured using mist net from Perak (N 04^{o}20.824' E 100^{o}52.826'), Terengganu (N 05^{o}01.966' E 103^{o}01.260') and Sabah (N 5^{o}31.46.5 E 118^{o}4'.29.6). Sample measurements: weight, wing chord, wingspan, bill length, tarsus length, body length, tail length, gender and species were measured and recorded. The data were analysed based on Multivariate Analysis of Variance (MANOVA) using SPSS version 21.

RESULTS AND DISCUSSION

The species of the swiftlet were confirmed based on sequencing of the cytochrome b gene (data not shown). Our study shows that *A. fuciphagus* from natural cave are generally larger in size but not significant and *A. maximus* is significantly larger

compared to *A. fuciphagus* with 95% confidence level (Figure 1 and 2 and Table 1). We postulated the different in size is due to dietary behavior of the swiftlets.

Studies have showed that dietary behaviour of *A. fuciphagus*, Glossy Swiftlet (*Collocalia esculenta*) and Mossy-nest Swiftlet (*A. salangana*) are similar in food diversity but differ in pray average body length; while *A. maximus* have distinct diet pattern and consume larger pray (Lourie and Tompkins, 2000). In addition, cave swiftlet often fly close to or even under the forest canopy (Medway 1962b), where large prey may be more common at lower altitudes; swiftlet from man-made houses normally forage at higher altitude at rural area where the average size of pray available are significantly smaller. Besides dietary preference, morphological differences may also related to genetic profiles of the birds which require future studies.

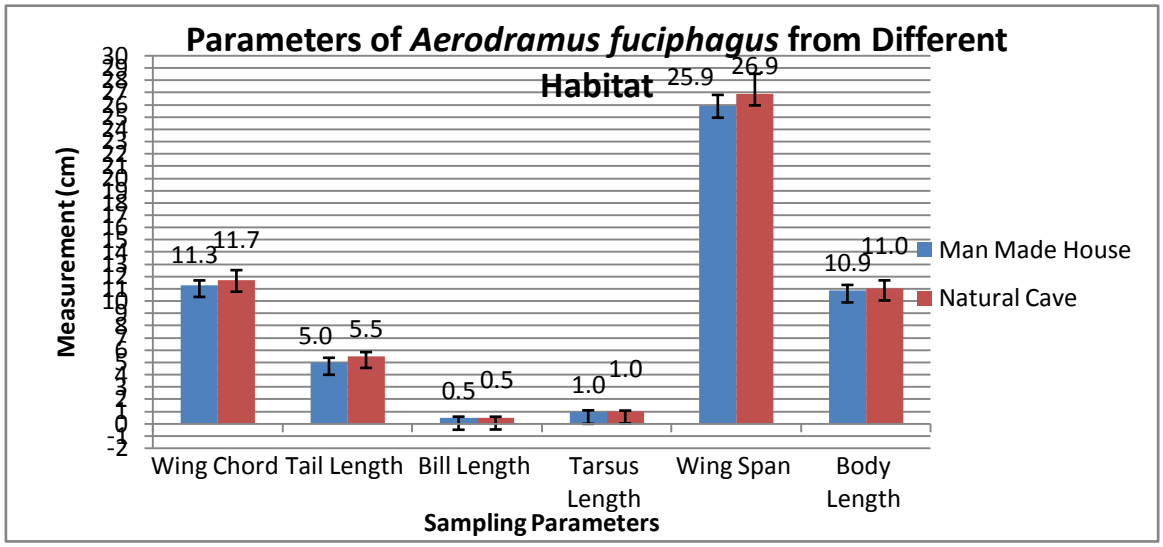

Figure 1.Morphology comparison of *Aerodramus fuciphagus* from different habitat.

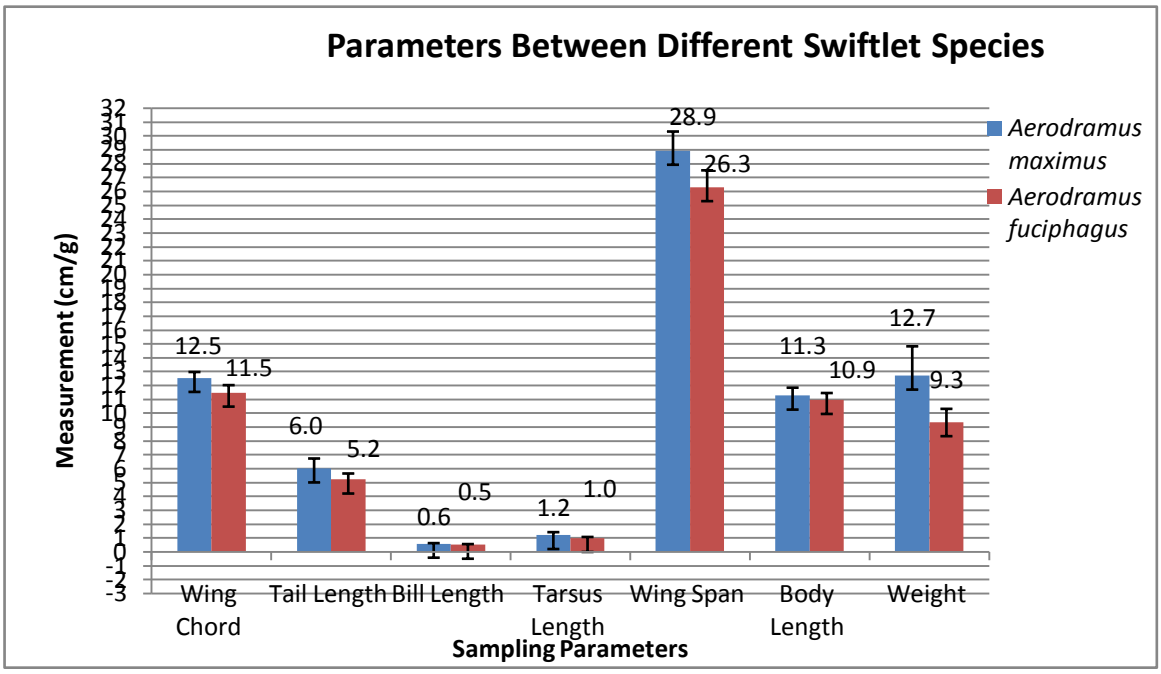

Figure 2.Morphology comparisons of swiftlet species.

Table 1. Descriptive statistic analysis

Tests of Between-Subjects Effects

Source	Dependent Variable	Type III Sum of Squares	df	Mean Square	F	Sig.
Species	weight	27.130	1	27.130	12.092	.005
	wing cord	2.829	1	2.829	6.697	.024
	wing span	11.774	1	11.774	6.702	.024
	tarsus	.114	1	.114	7.619	.017
	bill	.012	1	.012	8.779	.012
	tail	.014	1	.014	.024	.881
	body length	.293	1	.293	.789	.392
Habitat	weight	6.059	1	6.059	8.619	.010
	wing cord	1.213	1	1.213	4.464	.052
	wing span	4.441	1	4.441	2.876	.111
	tarsus	2.414	1	2.414	.624	.442
	bill	.005	1	.005	1.629	.221
	tail	19.135	1	19.135	144.266	.000
	body length	.104	1	.104	.383	.545

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