
UNIVERSITI PUTRA MALAYSIA

# PATHOGENICITY AND IMMUNOGENICITY OF LIVE ATTENUATED AND INACTIVATED FOWL ADENOVIRUS IN COMMERCIAL BROILER CHICKENS

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# PATHOGENICITY AND IMMUNOGENICITY OF LIVE ATTENUATED AND INACTIVATED FOWL ADENOVIRUS IN COMMERCIAL BROILER CHICKENS

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It is hereby certified that I have read this project paper entitled "Pathogenicity and Immunogenicity of Live Attenuated and Inactivated Fowl Adenovirus in Commercial Broiler Chickens" by Chong Zheng Zhe and in my opinion it is satisfactory in terms of scope, quality and presentation as partial fulfilment of the requirement for the course VPD 4999 – Project.

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## ABBREVIATIONS

| | |
|---|---|
| FAdV | Fowl Adenovirus |
| AAV | Avian Adenovirus |
| ℃ | Degree Celcius |
| HE | Hematoxilin and Eosin |
| IBH | Inclusion Body Hepatitis |
| IBD | Infectious Bursal Disease |
| CAV | Chicken Anemia Virus |

## ABSTRAK

Abstrak daripada kertas projek yang dikemukakan kepada Fakulti Perubatan Veterinar untuk memenuhi sebahagian daripada keperluan kursus VPD 4999 – Projek.

**PATHOGENISITI DAN IMMUNOGENISITI ADENOVIRUS AVIAN YANG HIDUP DILEMAHKAN DAN MATI DALAM AYAM PEDAGING KOMERSIAL**

**Oleh**

**Chong Zheng Zhe**

**2017**

**Penyelia: Profesor Dr. Mohd Hair Bejo**

Adenovirus avian (FAdV) ialah sejenis virus DNA yang merupakan pathogen utam inkulsi badan hepatitis (IBH) dalam ayam. Wabak IBH melanda seluruh dunia dan dilaporkan dalam beberapa negeri di Malaysia Program vaksinasi sangat penting untuk mengawal wabak penyakit IBH. Tetapi, ia masih tiada pengajian tentang pengembangan vaksin bagi jangkitan FAdV. Objektif kajian ini adalah mengkaji pathogenisiti dan immunogenisit FAdV (UPM1137) yang hidup dilemahkan dan mati dalam ayam pedaging komersial. Lima puluh empat anak ayam Cobb 500 berumur sahari dikategorikan kepada empat kumpulan, dinamakan kumpulan A, B, C dan D (kawalan). Makanan dan air disediakan secara *ad-libitum.* Ayam di kumpulan A, B dan C disuntik dengan 0.2mL FAdV mati dengan titer $10^{6.5}$ $TCID_{50}$/0.2mL, 0.1mL FAdV hidup dilemahkan dengan titer $10^{5.2}$ $TCID_{50}$/0.1mL, kombinasi 0.2mL FAdV mati dan 0.1mL FAdV hidup dilemahkan pada hari pertama (12 ayam dalam satu kumpulan) dan pada

umur 14 hari (6 ayam dalam satu kumpulan). Berat ayam ditimbang dan darah diambil sebelum nekropsi pada umur 14 dan 28 hari, kecuali persampelan juga dibuat pada hari pertama dalam kumpulan D. Semasa nekropsi, lesi mata kasar dan berat hati direkodkan, dan hati diambil untuk kajian histopathology. Kajian ini menunjukan tiada tanda klinikal atau lesi matakasar direkodkan dalam semua ayam sepanjang kajian. Berat badan ayam pada umur 14 dan 28 hari tidak berbeza secara ketara ($p>0.05$) dalam semua kumpulan. Nisbah berat hati kepada berat badan daripada kumpulan C lebih tinggi secara ketara ($p<0.05$) berbanding dengan kumpulan A dan D pada umur 28 hari. Tiada lesi matakasar dan histologi dilihat pada hari kesemua ayam sepanjang kajian. Titer antibodi FAdV kumpulan D ialah 938±1596 pada hari pertama dan tidak dapat dikesan pada umur 14 dan 28 hari. Titer antibodi FAdV kumpulan C lebih tinggi secara ketara ($p<0.05$) berbanding dengan kumpulan B dan D pada umur 28 hari. Kesimpulanya, FAdV yang hidup dilemahkan dan mati yang digunakan mempunyai pathogenisiti yang rendah tetapi dapat merangsang produksi antibodi dalam ayam. Kombinasi FAdV yang hidup dilemahkan dan mati mempunyai immunogenisiti yang tinggi berbanding dengan FAdV yang hidup dilemahkan.

*Kata kunci:* Adenovirus avian (FAdV), ayam pedaging komersial, hidup dilemahkan, mati, pathogenisiti, immunogenisiti.

ABSTRACT

An abstract of the project paper presented to the Faculty of Veterinary Medicine in partial fulfilment of the course VPD 4901-Project.

**PATHOGENICITY AND IMMUNOGENICITY OF LIVE ATTENUATED AND INACTIVATED FOWL ADENOVIRUS IN COMMERCIAL BROILER CHICKENS**

**By**

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**2017**

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Fowl adenovirus (FAdV) is a non-enveloped DNA virus which is the primary pathogen of inclusion body hepatitis (IBH) in chickens. IBH outbreak is worldwide distributed and being reported in different states of Malaysia. To control the outbreak in poultry industry, vaccination programmme is crucial. However, there is lack of research regarding development of local vaccine against FAdV infections. The objective of this study was to determine pathogenicity and immunogenicity of live attenuated and inactivated FAdV (UPM 1137) in commercial broiler chickens. Fifty four, 1-day-old Cobb 500 broiler chicks were divided into four groups, namely groups A, B, C and D (control). Feed and water were provided *ad-libitum*. The chicks in groups A, B and C

were inoculated with inactivated FAdV (0.2mL) with virus titre of $10^{6.5}$ $TCID_{50}$/0.2mL, live attenuated FAdV (0.1mL) with virus titre of $10^{5.2}$ $TCID_{50}$/0.1mL, combination of the inactivated (0.2mL) and live attenuated (0.1mL) FAdV, respectively on day 1 (12 chicks of each group) and day 14 of age (6 chicks of each group). Body weight and blood samples were collected prior to necropsy on days 14 and 28 of age, except sampling was also conducted on day 1 of age in the control group. On necropsy, the gross lesions and liver weight were recorded, and samples of liver were collected for histological examination. The study showed that neither clinical signs nor gross lesions were recorded throughout the trial. The body weight of chicken on days 14 and 28 were not significantly different ($p>0.05$) among all the groups. The liver to body weight ratio of group C was significantly higher ($p<0.05$) than groups A and D on day 28. There was no gross and histopathological lesions seen in the liver of all the chicken throughout the trial. The FAdV antibody titre in group D (Control) was 938±1596 on day 1 of age and was not detected on day 14 and day 28 of age. However, the FAdV antibody was induced at high titre in all the inoculated groups at day 14 and day 28 of age. The FAdV antibody titre of group C was significantly ($p<0.05$) higher than groups B and D on day 28. In conclusion, live attenuated and inactivated FAdV used in the study have a low pathogenicity but able to induce production of antibody among the chickens. The combination of live attenuated and inactivated FAdV has higher immunogenicity compared to sole usage of live attenuated FAdV.

*Keywords:* Fowl Adenovirus (FAdV), commercial broiler chicken, live attenuated, inactivated, pathogenicity, immunogenicity.

# INTRODUCTION

Fowl adenovirus (FAdV) belongs to family *Adenoviridae*, genus *Aviadenovirus*. It is grouped into five different species (A to E) and further subdivided into 12 serotypes from chickens, turkeys, and other species that share a common antigen (Mase *et al.*, 2009). Adenovirus infections are ubiquitous in commercial farmed chickens, and probably in all avian species (McFerran and Smyth, 2000). It has been isolated from both sick and healthy birds. FAdV is associated with outbreaks of inclusion body hepatitis (IBH), hydropericardium syndrome, respiratory disease, necrotizing pancreatitis or gizzard erosions (Ono *et al.*, 2003).

There were IBH outbreaks in commercial broiler chickens from different states in Malaysia in 2004, 2008 and 2011 (Juliana *et al.*, 2014). Norina *et al.* (2016) also reported IBH cases on broiler chickens flocks happening in Malacca and Johore in December 2015. Recently, five FAdV isolates of Malaysia involved in IBH outbreaks have been successfully propagated in SPF embryonated chicken eggs and characterized as FAdV-8b (Juliana *et al.*, 2014). According to Mettifogo *et al.* (2014), FAdV is the causal agent of IBH/ hydropericardium syndrome outbreak. It is usually seen in meat producing chickens between the ages of 3-7 weeks. It is associated with sudden onset mortality which peaks within 3-4 days and stop by days 5-6. It has low morbidity and a mortality ranges between 5-10%, but can reach to 30% (McFerran and Smyth, 2000). Affected chickens show lethargy, huddling, ruffled feathers and inappetance (Norina *et al.*, 2016).

Upon post mortem, in naturally infected chickens, the liver was pale, swollen and presence of multiple petechiae haemorrhages (Saifuddin and Wilks, 1992). Besides,

multiple hemorrhages may be also seen in the thymus, breast muscle and intestinal tract. In some cases, severe mucosal erosion in gizzards can be seen (Ono *et al.*, 2003). According to Norina *et al.* (2006), on histological examination of the liver, there are presence of numerous eosinophilic and basophilic intranuclear inclusion bodies in the hepatocytes.

The spread of FAdV can occur through horizontal and vertical transmission. The bird-to-bird transmission of virus in a flock occurs horizontally by the oral-fecal route and further spread take place by mechanical means and by contamination with infected feces (Hafez, 2010). Vertical transmission occurs through the embryonated eggs. According to Girshick *et al.* (1980), stress periods can reactivate the viral shedding. The infection in breeder may indicate vertical transmission and the continuous production of infected progenies (Pereira *et al.*, 2014).

## 1.1 Hypotheses

The first null hypothesis was live attenuated FAdV UPM 1137 is non-pathogenic and non-immunogenic, whereas the alternative hypothesis was it is low pathogenic and high immunogenic. The second null hypothesis was inactivated FAdV UPM 1137 is non-pathogenic and non-immunogenic, whereas the alternative hypothesis was it is low pathogenic and high immunogenic. The last null hypothesis was combination of live attenuated and inactivated FAdV does not induce higher immune response compared to single inoculum, whereas for the alternative hypothesis, the combination of live attenuated and inactivated FAdV is able to induce higher immune response compared to single inoculum.

## 1.2 Objectives

The objective of this study was to determine the pathogenicity (clinical signs, gross and histological lesion) and immunogenicity (FAdV antibody induced by chickens) of live attenuated and inactivated FAdV in commercial broiler chickens.

# LITERATURE REVIEW

## 2.1 Background

The family *Adenoviridae* is divided into 2 genera, the *Mastadenoviruses* infecting mammals and the *Aviadenoviruses* infecting birds (Russell *et al.*, 1995). Adenovirus infection are ubiquitous in commercial farmed birds, probably in all avian species (McFerran and Smyth., 2000). Chickens can be infected by fowl adenovirus (FAdV), belonging to the genus *Aviadenovirus,* egg drop syndrome (EDS) virus, belonging to the genus *Atadenovirus* and turket hemorrhagic enteritis (HE) virus, belonging to the genus *Siadenovirus* (Zhao *et al.*, 2015).

FAdV is further classified into five species (A to E) and 12 serotypes (Benko *et al.*, 2000). According to McFerran (1981), FAdV can be isolated from both healthy and sick birds due to the presence of maternal antibodies and low virulence of some strains. It is associated with outbreaks of inclusion body hepatits (IBH), hydropericardium syndrome, respiratory disease, necrotizing pancreatitis or gizzard erosion (Ono *et al.*, 2003).

There were IBH outbreaks in commercial broiler chickens from different states in Malaysia in 2004, 2008 and 2011 (Juliana *et al.*, 2014). It is usually seen in meat producing chickens between ages of 3-7 weeks (Hair-Bejo, 2005). It is associated with sudden onset mortality which peaks within 3-4 days and stop by days 5-6. It has low morbidity and a mortality ranges between 5-10%, but can reach to 30% (McFerran and Snyth, 2000).

## 2.2 Aetiology

### 2.2.1 Classification

FAdV has been grouped into 5 species (FAdV-A to FAdV-E) with 12 serotypes (FAdV-1 to 8a and -8b to 11) based on restriction enzyme digest pattern and serum cross neutralization test (Hess, 2000).

### 2.2.2 Morphology

Adenovirus are non-enveloped icosahedral of 70-90 nm in diameter (Andrewes *et al.,*1978). The nucleic acid is linear, double-stranded deoxyribonucleic acid (McFerran and Smyth, 2000). The capsid consists of 252 capsomers with 240 hexons (non-vertex) located on sides and edges of the particle and 12 apical pentons (vertex) provided with one or two thin fibres (Ackermann and Berthiaume, 1995). According to Harrach 2001), for genus *Aviadenovirus*, there are two fibres projecting from each vertex, 21-29 nm in length except FAdV-C (17-20 nm) and FAdV-A (different lengths: 11 and 47 nm).

## 2.3 Transmission

Adenovirus can spread through vertical transmission. According to McCraken and Adair (1993), vertical transmission is reported as a very efficient way to spread from parent birds to progenies. Adenovirus is transmitted through the embryonated eggs. There is report that establishment of latent infection with FAdV in chickens (Grgic *et al.*, 2006). Stress periods, such as the onset of egg production can reactivate the viral shedding (Girshick *et al.*, 1980).

Horizontal transmission is also important in spreading the virus. It could happen through feces, contaminated egg trays, crates and trucks (Hafez, 2010). Adenovirus could be transmitted in all excretions, but the highest titers are found in the feces (Adair and Fitzgerald, 2008). It takes place mainly by direct fecal contact but was also achieved by aerial contact over short distances, with infection spreading at a very slow rate for several weeks (Cook, 1974). In broilers, peak excretion occurred between 4 and 6 weeks of age (McFerran, 1981). This could be due to the decrease of maternally derived antibodies.

### 2.4 Clinical Signs

Inclusion body hepatitis (IBH) is usually seen in meat-producing chickens aged 3-7 weeks (Smyth and McNulty, 2008). It is associated with sudden onset mortality which peaks within three to four days post infection and stop by day five to six post infection. It has low morbidity and a mortality ranges between five to ten percent, but can reach to thirty percent. Affected chickens show clinical signs of depression, poor feed conversion, poor weight gain, ruffled feathers, and die or recover within two days (Smyth and McNulty, 2008 ; McFerran *et al.*, 2000).

### 2.5 Pathology

#### 2.5.1 Gross Lesions

The gross lesions upon necropsy include mild to moderate enlargement of liver with pale, friable and fatty change appearance, and areas of haemorrhages and congestion as well as pale and slightly enlarged kidney (Hair-Bejo, 2005). In cases of hydropericardium syndrome, there is an accumulation of clear straw-coloured fluid in the pericardium sac (Adair and Fitzgerald, 2008).

### 2.5.2 Histological Lesions

Histologically, necrotizing hepatitis and pancreatitis numerous eosinophils intranuclear inclusions and infrequently basophilic inclusions, are found in the hepatocytes (McFerran *et al.*, 2000). Besides, there is presence of mixed infiltration of lymphocytes and heterophils throughout the liver parenchyma (Saifuddin and Wilks,1992). Ono *et al.* (2003) states that a histologically degenerative koilin layer, necrotic mucosa, intranuclear inclusion bodies in the glandular epithelial cells, inflammatory cell infiltrations in the lamina propria, submucosa, and a muscle layer are seen in gizzard.

### 2.6 Pathogenesis

The virus is transmitted via horizontally through feces or vertically through embryonated eggs (McFerran and Smyth, 2000). The virus may enter the host body by inhalation or direct ingestion of contaminated feed. According to Smyth and McNulty (2008), using natural route of exposure, initial growth of fowl adenoviruses occur mainly in the epithelium of both the large and small intestine. Virus can be detected in liver, kidney, respiratory tract, bursa of Fabricius, spleen, bone marrow due to the occurrence of viremia. *Aviadenovirus* is excreted in the feces for about three weeks.

Girgic *et al.* (2006) also states that there is establishment of potential lifelong carrier in the cases of latent infection. During a stressful condition, there will be reactivation of latent virus, in which causing a recall of serum antibody to the common group antigen. So, virus may be transmitted through eggs if the hens are laying. According Smyth and McNulty (2008), FAdV is commonly isolated from hens around the time of peak egg production.

## 2.7 Diagnosis

The diagnosis of poultry disease is generally based upon case history, clinical signs and post mortem examination (Hafez, 2010). The laboratory diagnosis can be classified into direct and indirect method. Histological investigation can be done by staining of suspected cells or tissues with heamtoxylin and eosin to demonstrate typical intranuclear inclusion (Gallina *et al.*, 1973). For isolation and identification of viruses, the specimen of choice are feces, pharynx, kidney and affected organs, such as liver, in cases of IBH (Adair and Fitzgerald, 2008). According to McFerran and Smyth(2000), chick embryo liver or chick kidney cells are the best cell culture media. But, embryonated eggs are insensitive for primary isolation of most aviadenoviruses (Adair and Fitzgerald, 2008).

Rapid examination can be done by using electron microscope, as the virus morphology is typical. Besides, the most rapid method of confirming the presence of adenovirus is indirect immunofluorescence (Adair and Fitzgerald, 2008). Virus neutralization tests have been widely applied to identify the serotype of virus. However, Hafez (2010) states that it is labour intensive and expensive. Molecular technique, such as PCR is now widely used. PCR is applied to detect subgroup 1 adenoviruses and can be used to allocate isolates to species and to serotypes (Adair and Fitzgerald, 2008).

For serology, indirect immunofluorescent test is much more sensitive and rapid and is inexpensive (Adair *et al.*, 1980). To detect group-specific antibodies, another inexpensive but sensitive method is the enzyme-linked immunosorbent assay (ELISA). It can be used as a preliminary test before confirmation (Hafez, 2010).

## 2.8 Control and Prevention

Proper biosecurity and disinfection programmes are important keys in controlling and preventing the infection in chickens. The proper management, cleaning and disinfection of premises and equipment, restricted entry of visitors and vaccination crews in poultry houses play a significant role in prevention of disease (Hafez, 2010). However, according to Adair and Fitzgerald (2008), adenoviruses are resistant to inactivation, so the eradication of adenovirus from commercial flocks is questionable. Herdt *et al.* (2013) states that specific control of FAdV through vaccination is available. There has been a report on emulsion vaccine in which was highly effective against the hydropericardium syndrome in both experimental trials and field trials (Roy *et al.*, 1999). By vaccinating day-old birds with apathogenic FAdV-1 strain (CELO), absence of any clinical signs or pathological changes in gizzard was demonstrated (Grafl *et al.*, 2014). According to Junnu *et al.* (2015), humoral immunity induced by the inactivated FAdV serotype 2 vaccines could be a tool of IBH control in both breeders and their progenies. But, the dominant serotype responsible for IBH outbreaks in Malaysia is FAdV-8b (Juliana *et al.*, 2014).

# MATERIALS AND METHODS

## 3.1 Experimental Chicken

Fifty four day old Cobb 500 commercial broiler chickens (DOC) were reared in wire-floored isolated houses. They were given food and water *ad-libitum.* Chicks were allocated and separated randomly into four groups namely the groups A, B, C and D, in which there were 12 DOC each for groups A, B and C and 18 DOC in group D. The chicks in group A were inoculated with 0.2mL inactivated FAdV subcutaneously with virus titre of $10^{6.5}TCID_{50}$/ 0.2mL on day old. Six of them were sacrificed on day 14 and the remaining six chicken were given a booster on day 14. The chicks in group B were inoculated with 0.1mL live attenuated FAdV subcutaneously with virus titre of $10^{6.2}TCID_{50}$/mL on day old. Six of them were sacrificed on day 14 and the remaining six chicken were given a booster on day 14. The chicks in group C were inoculated with 0.2mL inactivated and 0.1mL live attenuated FAdV subcutaneously on day old. Six of them were sacrificed on day 14 and the remaining six chicken were given a booster on day 14. Six DOC from group D were sacrificed on the first day. Six of them were sacrificed on day 14. The chickens were monitored for any clinical abnormality at least twice daily. On day 28, all chicken were sacrificed with cervical dislocation. The body weight was recorded and serum sample was collected from each chicken prior to sacrifice. The gross lesions of the carcasses were recorded and the liver were fixed in 10% buffered formalin for histopathological examination (Appendix, 1).

### 3.2 Avian Adenovirus Isolates

The avian adenovirus used in the study was UPM 1137. It was originated from a field outbreak on 25 to 27 weeks old layer chickens. The clinical signs include reduced feed consumption, drop egg production, and increased thin and broken egg shelled formation in the farms. Post mortem was done and shown the lesion of gizzard erosion on the keratin layer, flabby proventriculus with friable and pale liver. Starting from 23 weeks to 27 weeks of age, there was a total mortality of 2.04%. Histology was done on the liver, gizzard and proventriculus sent. It revealed presence of intranuclear inclusion bodies in all samples. PCR was done and revealed positive for FAdV infection.

### 3.3 Gross Pathology

On necropsy, the gross pathological changes of the birds were recorded. The liver was weighed and collected for histopathological study. The serum samples were analysed for FAdV antibody titers using enzyme linked immune sorbent assay (ELISA) technique.

### 3.4 Histopathology

The liver, both in the control and FAdV groups were fixed in 10% buffered formalin for at least 24 hours, the tissue were trimmed and subsequently dehydrated in series of alcohol, cleared with xylene and embedded in paraffin wax using an automatic tissue processor (Leica). Tissues were sectioned at about 4 micrometer using microtome and mounted on glass slides, followed by dewaxing and later stained with Hematoxylin and Eosin (HE). The tissues were then carefully examined under microscope using x4, x10, x40 and x100 objectives for histological changes (Toro *et al.*, 2002).

### 3.5 Enzyme Linked Immune Sorbent Assay

The enzyme linked immune sorbent assay (ELISA) test was carried out according to the methods described by BioChek, London, UK. Each test sample was diluted to 1:100 in sample diluent reagent. FAdV Gp 1 coated plate was removed from sealed bag and the location of samples were recorded on template. Negative control (100µL) was added into wells A1 and B1. Negative control (100µL) was added into wells C1 and D1. Diluted samples (100µL) was added into the appropriate wells. The plate was covered with lid and incubated at room temperature for 30 minutes. The contents of wells were aspirated and washed for 4 times with wash buffer (350µl per well). The plate was inverted and tapped firmly on absorbent paper until no moisture was visible. Conjugate reagent (100µL) was added into the appropriate wells. The plate was covered with lid and incubated at room temperature for another 30 minutes. The wash procedure was repeated. Substrate reagent (100µL) was added into appropriate wells. The plate was covered with lid and incubated at room temperature for 15 minutes. Stop solution (100µL) was added to appropriate wells to stop reaction. The microtitre plate reader was blanked on air and the absorbance of controls and the samples were recorded by reading at 405nm.

### 3.6 Statistical Analysis

The data for body weight, liver weight, liver to body weight ratio and FAdV antibody titre of chickens were analysed with one-way analysis of variance (ANOVA) using SPSS version 22.0. Statement of statistical significance are based on $p<0.05$.

# RESULTS

## 4.1 Clinical Signs

All the chickens were feeding and drinking well. No abnormal clinical signs of FAdV and no mortality was observed in all groups of chicken throughout the trial.

## 4.2 Body Weight

The body weight of the day old chicks was 0.05±0.01 kg. After 28 days of trial, the body weight of the chicken in groups A, B, C and D were 1.64±0.10 kg, 1.64±0.06 kg, 1.63±0.07 kg and 1.86±0.03 kg, respectively. The body weight of chicken on day 14 and day 28 were not significantly different ($p>0.05$) among all the groups (Figure, 1; Appendix, 2).

## 4.3 Liver Weight

The liver weight of the day old chicks was 2.10±0.13 g. On day 14 of trial, the liver weight of the chicken in groups A, B, C and D were 14.68±0.77 g, 15.80±0.71 g, 15.30±0.81 g and 16.21±0.95 g, respectively, showing non-significant difference ($p>0.05$) between the groups. On day 28 of trial, the liver weight of chicken in groups A, B, C and D were 32.33±1.41 g, 36.17±1.20 g, 40.67±1.91 g and 38.67±1.33 g, respectively (Figure, 2). The liver of group A were significantly ($p<0.05$) lower than Group C and D (Appendix, 3).

## 4.4 Liver to Body Weight Ratio ($X10^{-2}$)

The liver to body weight ratio for day old chick was 4.09±0.15. On day 14 of trial, the liver to body weight ratio of the chicken in group A, B, C and D were 3.12±0.07, 3.30±0.12, 3.36±0.08 and 3.46±0.09, respectively, showing non-significant difference

($p>0.05$) between groups. On day 28 of trial, the liver to body weight ratio of the chicken in groups A, B, C and D were 1.99±0.07, 2.21±0.07, 2.53±0.22 and 2.08±0.77, respectively (Figure, 3), in which the ratio of Group C was significantly higher ($p<0.05$) than of Group A (Appendix, 4).

**4.5 Gross Lesion**

The day old chicks livers were yellowish. The livers in days 14 and 28 of ages were in dark red, and no enlargement was seen. No gross lesion was observed in all the liver samples from groups A, B, C and D on day 14 and 28 of ages (Figure, 4; Figure, 5).

**4.6 Histopathology**

No histopathological lesion was seen in all the liver samples from groups A, B, C and D on days 14 and 28 of ages (Figure, 6; Figure, 7). There was no intranuclear inclusion bodies present in the hepatocytes.

**4.7 Enzyme Linked Immunosorbent Assay**

The FAdV antibody titre of the day old chicks was 938±651 and was not detected on days 14 and 28 of age. On day 14 of trial, the antibody titre of the chicken in groups A, B and C were 3797±980, 1777±600 and 3447±2141, respectively, showing non-significant difference ($P>0.05$) between groups. On day 28 of trial, the FAdV antibody titre of the chicken in groups A, B and C were 4302±2234, 1104±264 and 6312±2232, respectively (Figure, 8), in which the FAdV antibody titer of Group C were significantly higher ($p<0.05$) than groups B and D (Appendix, 5).

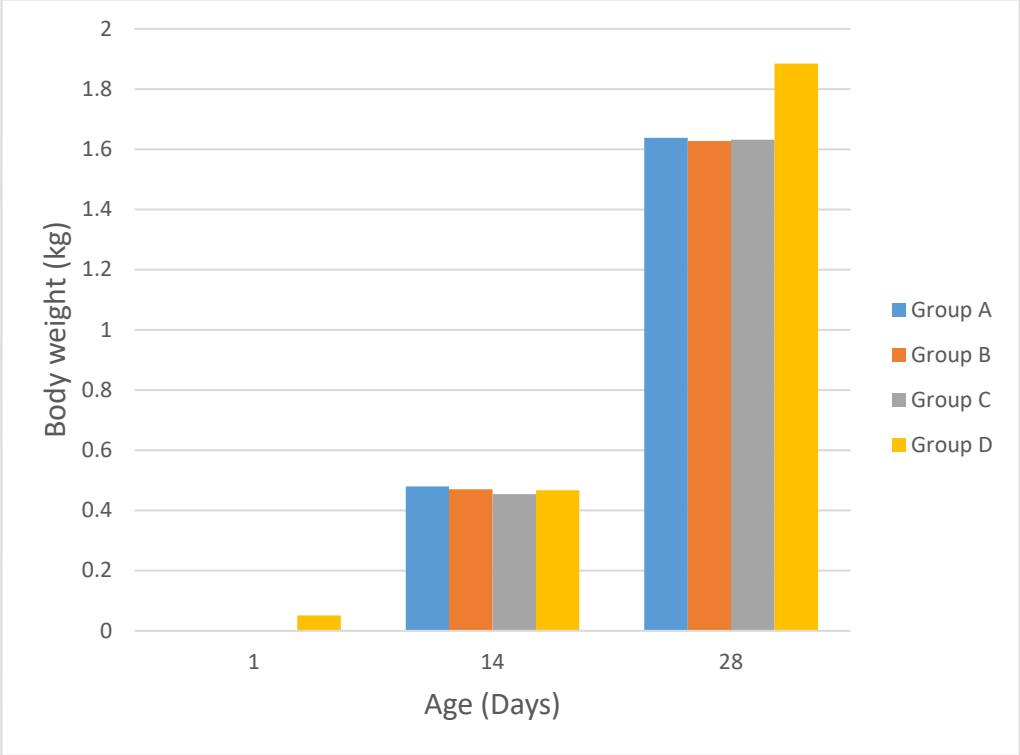


**Figure 1:** Body weight of chickens throughout the trial.

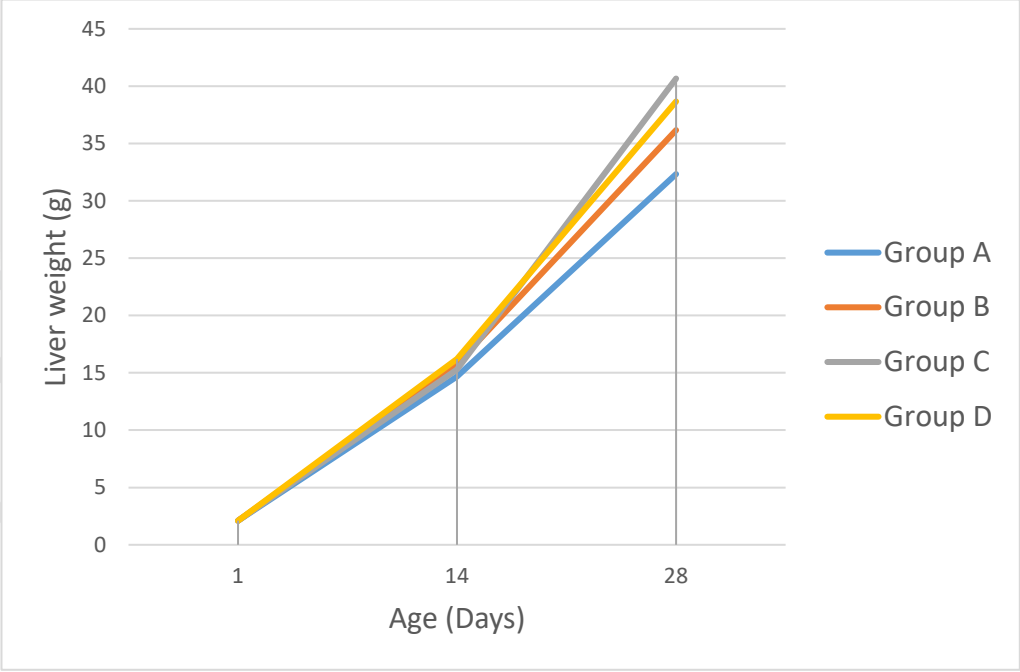


**Figure 2:** Liver weight of chickens throughout the trial.

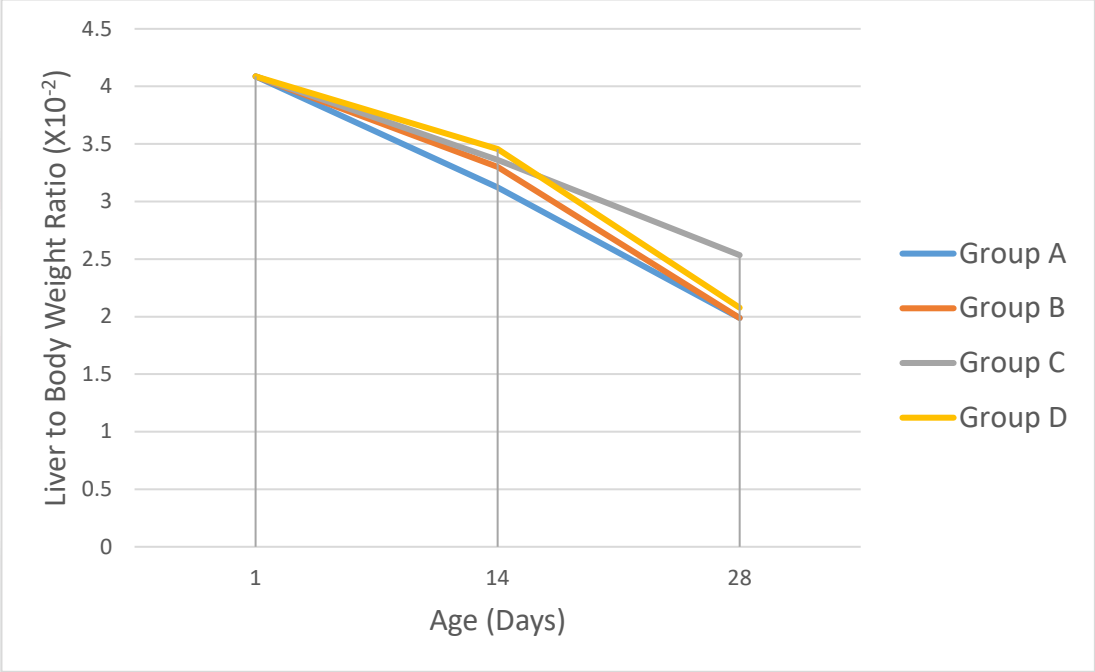


**Figure 3:** Liver to body weight ratio of chickens throughout the trial.

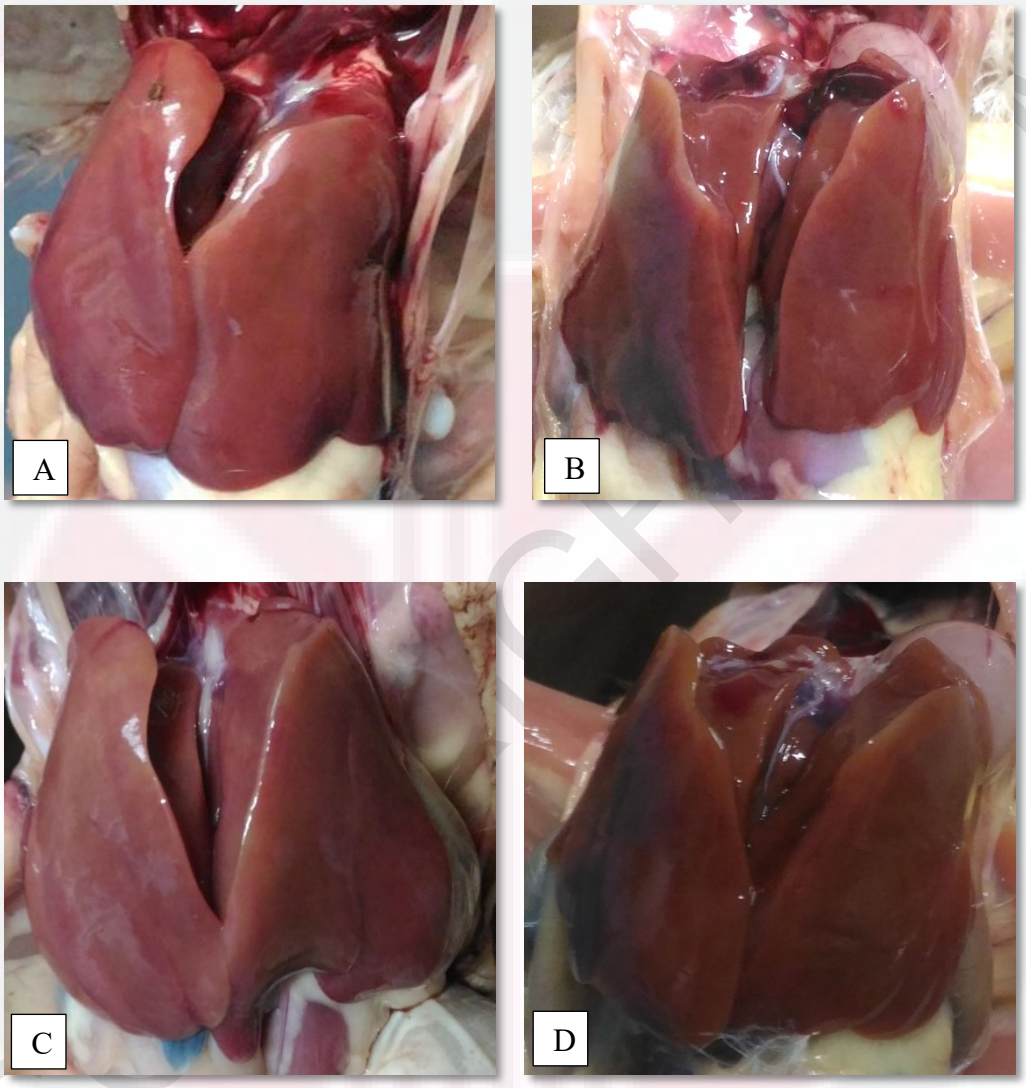


**Figure 4:** Normal livers seen in chickens from groups A, B, C and D on day 14 of age. No gross lesion was observed.

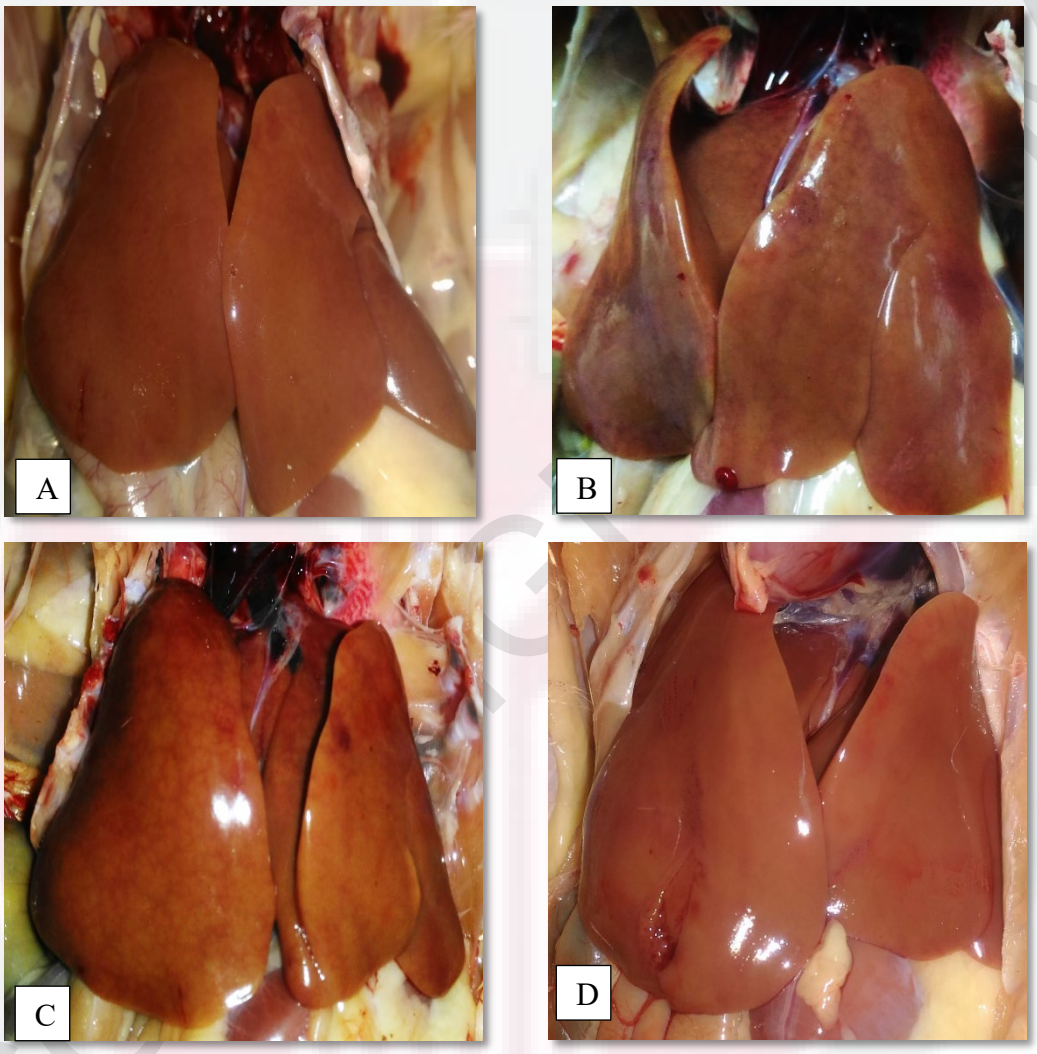


**Figure 5:** Normal livers of chickens from groups A, B, C and D on day 28 of age. No gross lesion was observed.

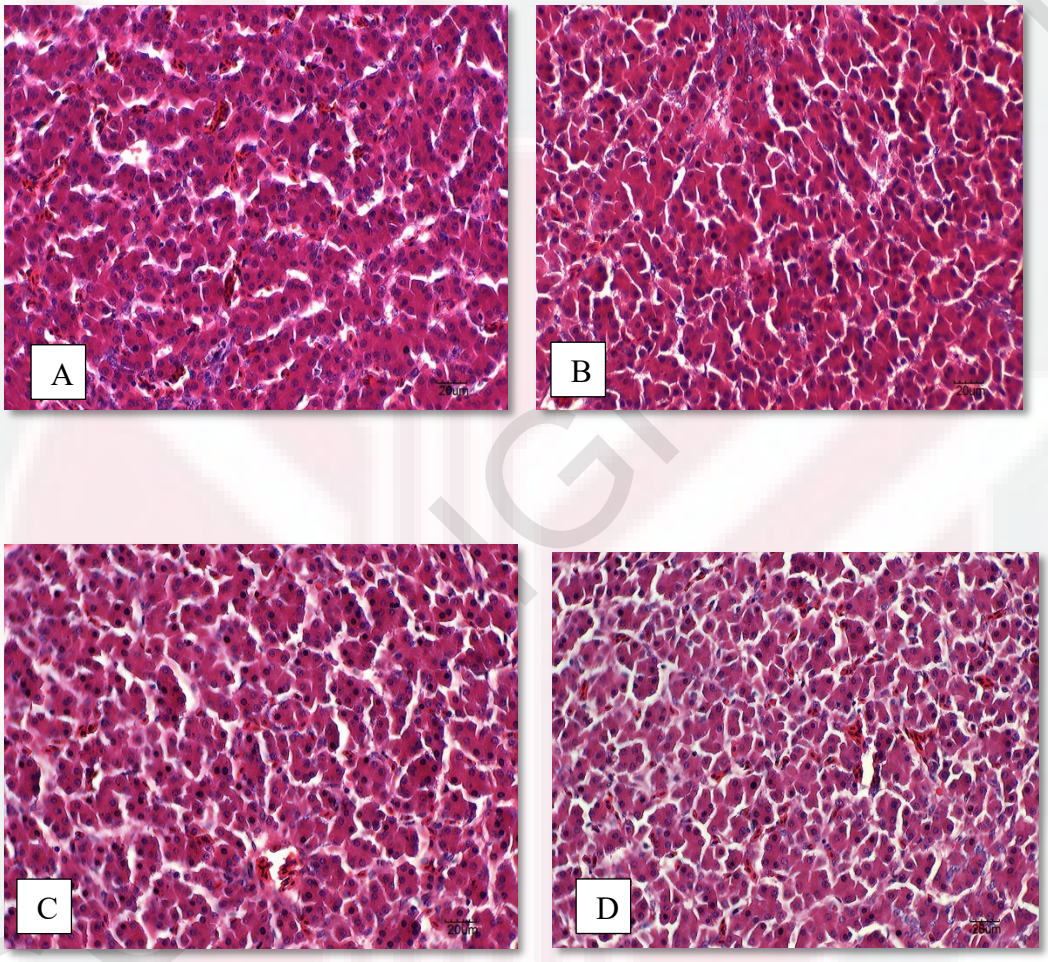


**Figure 6:** Livers of chickens from groups A, B, C and D on day 14 of age. No histopathological lesion was observed. HE, 40X. Bar= 20µm.

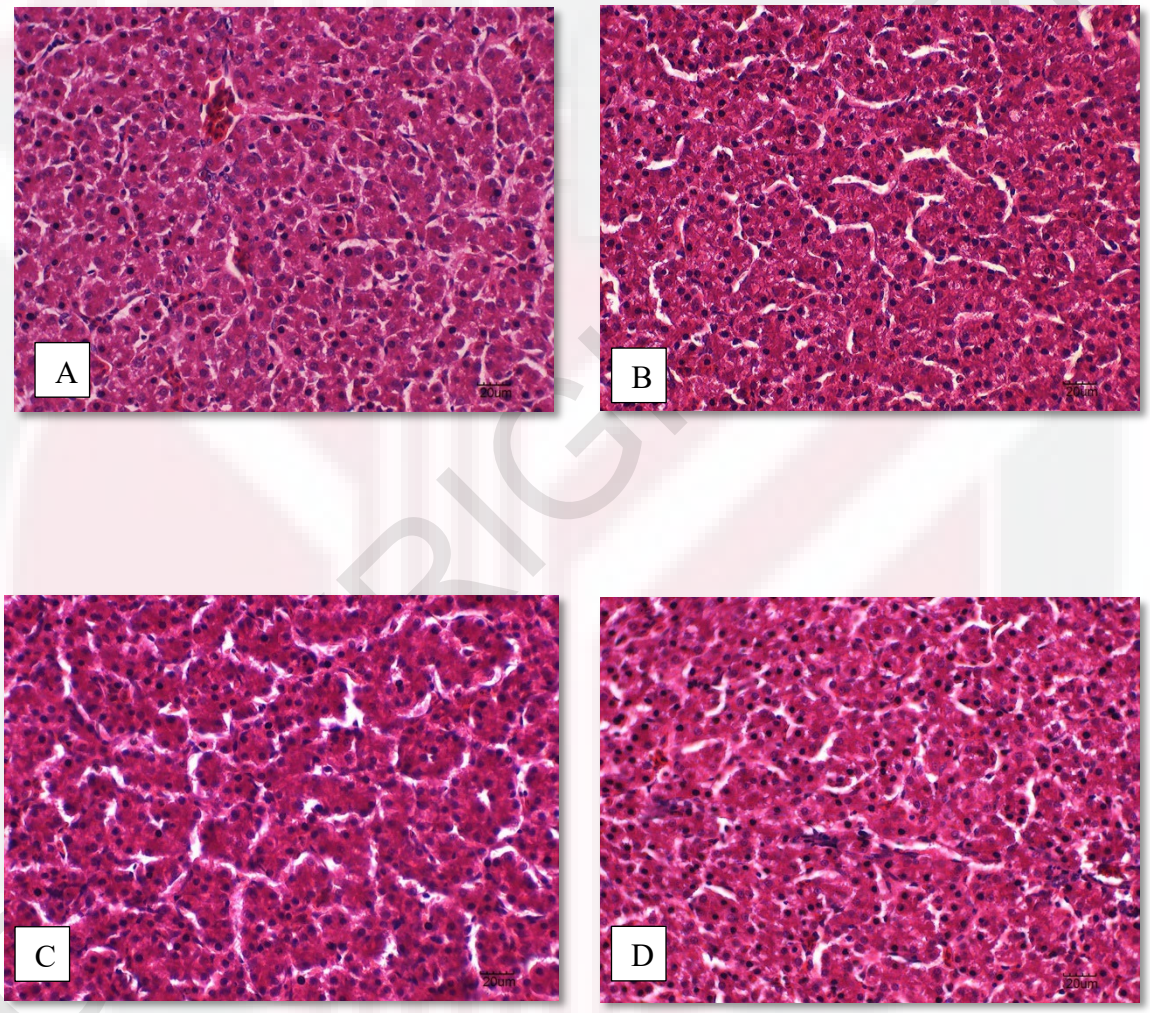


**Figure 7:** Livers of chickens from groups A, B, C and D on day 28 of age. No histopathological lesion was observed. HE, 40X. Bar= 20µm.

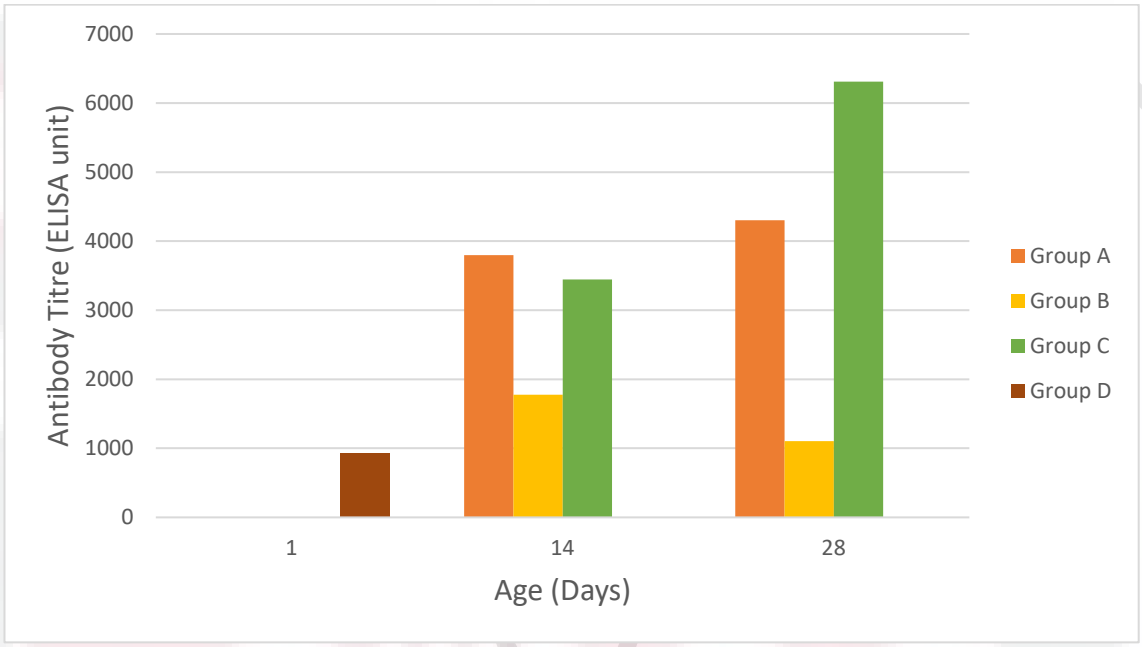


**Figure 8:** FAdV antibody titer of chickens throughout the trial.

## DISCUSSION

In this study, there was no clinical signs of IBH shown and no mortality occurred in any commercial broiler chickens throughout the trial. It did not reproduce the clinical signs of lethargy, huddling, ruffled feather and inappetance as reported in typical inclusion body hepatitis outbreak in Malaysia (Norina *et al.*, 2016). Besides, there was absence of gross and histological lesion such as swollen, pale liver with hemorrhages and multifocal necrosis as shown in typical IBH cases. Basophilic or eosinophilinc intranuclear inclusion bodies were found frequently in the hepatocytes in a field outbreak of IBH in chickens (Almenesh *et al.*, 2012). The basophilic intranuclear inclusion body consist of adenovirus particles, whereas the eosinophilic inclusions contain only fibrillar granular material and filaments (Weissenbock and Fuchs, 1995). So, it indicates that both inactivated and live attenuated FAdV (UPM 1137) is in low pathogenic to commercial broiler chickens.

In this study, there was also no significant difference ($p>0.05$) between groups in terms of the body weight on days 14 and 28 of all the commercial broiler chickens in different groups. According to Junnu *et al.* (2015), low mean body weights may be caused by the disease intervention resulting in decreased feed intake which was not happening in this trial. Thus, it shows that the inoculation of vaccine did not affect the growth performance of the broiler chickens, in which the body weight of commercial broiler chicken reached to more than 1.6 kg at 28 days of age. Based on the broiler performance objectives and guidelines from Cobb Vantress (2015), female and male Cobb 500 should be weighing at 1.4kg and 1.6kg on 28 days of age.

Moreover, the liver to body weight ratio of Group C was significantly higher ($p<0.05$) than the Group A on day 28. This could be due to the virus replication took place in the liver organs. According to Junnu *et al.* (2015), virus in live vaccines do replicate in the liver. Besides, Ekanayake (2009) states that the presence of intranuclear inclusion body in liver was a constant observation of typical IBH cases, and indicates liver as FAdV replication sites.

Based on the results of immunogenicity studies, the mean FAdV antibody titre of day old chicks was 938±1596, which indicates high possibility of the infection in the breeder flock, thus passing maternal antibody to the progenies. However, the level of maternal antibody in control group wane and not detected at days 14 and 28 of age. This was in line with Adair and Fitzgerald (2008) that the level of maternal antibody in broiler chickes wane at around 3 weeks of age. Furthermore, the presence of maternal antibody in an unvaccinated flock of broiler chicken indicates that there may be occurrence of field challenge in Malaysia poultry farms. This is in line with Alvarado *et al.*(2007) that higher maternal antibody levels in commercial broiler might be due to constant exposure of broiler breeders to adenovirus strains under typical field conditions, with the development of immunity and transfer of high levels of maternal antibodies to their broiler progeny. Alvarado also states that a previous infection in the grandparent flocks lead to the development and transmission of neutralizing antibodies to their progenies. Thus, development of local vaccine is in need for the industry.

Furthermore, combination of inactivated and live attenuated FAdV vaccine produced a significant higher ($p<0.05$) FAdV antibody titer than solely usage of live attenuated FAdV vaccine. It is similar with a study on Newcastle disease (ND) reported by

Bennejean *et al.* (1978), in which the live vaccine produces appreciable lower and clearly less stable titres than the combination of live and inactivated vaccine of the inactivated vaccine alone. By combining both forms of vaccines, the best immune response can be induced. It is because the fast response by live attenuated FAdV elicited the primary immune response. At the same time, the slow release of inactivated FAdV antigen acted as the booster. This also agreed with Turblin (2009) that simultaneous administration with a live vaccine and killed vaccine produces early, strong and lasting immunity. Not only that, based on study from Foitse *et al.* (1998), antibody response induced by the combined killed-in-oil vaccines administered concurrently with live virus was better than that induced in all other group. He reported that concurrent administration of oil emulsion and live Newcastle disease (ND) vaccine induce the best antibody response. It should be a progressive stimulation of an active immunity while the passive acquired immunity declines (Bennejean *et al.*, 1978). Wanasawaeng *et al.* (2009) also reported that all chickens receving combined vaccines from live and inactivated ND vaccine revealed higher HI antibody titers than chickens that received single live vaccine.

## CONCLUSIONS

It was concluded that live attenuated and inactivated FAdV (UPM 1137) has a low pathogenicity but able to induce production of FAdV antibody in commercial broiler chickens. The combination of live attenuated and inactivated FAdV has higher immunogenicity compared to sole usage of live attenuated FAdV.

## RECOMMENDATIONS

Sample size of the study can be increased in future studies. Moreover, efficacy of live attenuated and inactivated FAdV UPM1137 should be tested by challenge trial with pathogenic field FAdV isolate. Lastly, other routes of administration of live attenuated FAdV should be considered.

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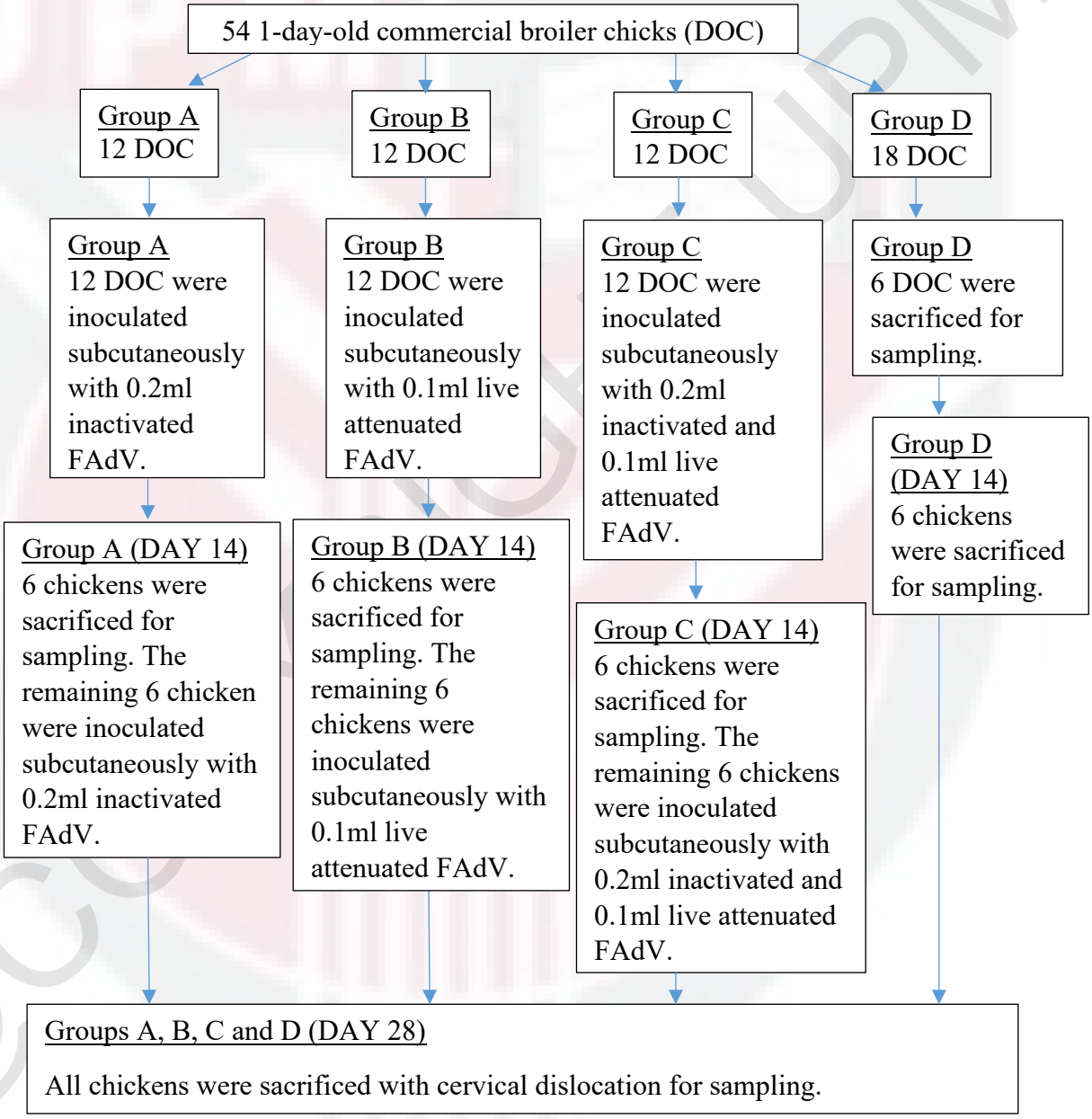
## APPENDIX 1

**Experimental design for pathogenicity and immunogenicity study of live attenuated and inactivated FAdV UPM1137**




Sampling; the body weight and blood samples (each 1ml) were collected prior to necropsy. On the necropsy, the gross lesion and liver weight were recorded. Sample of liver were fixed in 10% buffered formalin for histological examination.

## APPENDIX 2

**Body weight of chickens throughout the trial.**

| Age (days) | Body Weight (kg) | | | |
|---|---|---|---|---|
| | Group A | Group B | Group C | Group D |
| 1 | 0.05±0.01 [a] | | | |
| 14 | 0.48±0.02[b] | 0.47±0.02[b] | 0.45±0.02[b] | 0.47±0.02[b] |
| 28 | 1.64±0.10[c] | 1.64±0.06[c] | 1.63±0.07[c] | 1.86±0.03[c] |

Each value is the mean ± standard deviation of 6 chickens from each group.

[a-b-c] means within row with no common superscripts differs at $p<0.05$.

## APPENDIX 3

**Liver weight of chickens throughout the trial.**

| Age (days) | Liver Weight (g) | | | |
|---|---|---|---|---|
| | Group A | Group B | Group C | Group D |
| 1 | $2.10\pm0.13^{a}$ | | | |
| 14 | $14.68\pm0.77^{b}$ | $15.80\pm0.71^{b}$ | $15.30\pm0.81^{b}$ | $16.21\pm0.95^{b}$ |
| 28 | $32.33\pm1.41^{c}$ | $36.17\pm1.20^{c,d}$ | $40.67\pm1.91^{d}$ | $38.67\pm1.33^{d}$ |

Each value is the mean ± standard deviation of 6 chickens from each group.

$^{a\text{-}b\text{-}c\text{-}d}$ means within row with no common superscripts differs at $p<0.05$.

## APPENDIX 4

**Liver to body weight ratio of chickens throughout the trial.**

| Age (days) | Liver to Body weight ratio ($10^{-2}$) | | | |
|---|---|---|---|---|
| | Group A | Group B | Group C | Group D |
| 1 | $4.087 \pm 0.153^{a}$ | | | |
| 14 | $3.12 \pm 0.07^{b}$ | $3.30 \pm 0.12^{b}$ | $3.36 \pm 0.08^{b}$ | $3.46 \pm 0.09^{b}$ |
| 28 | $1.99 \pm 0.07^{c}$ | $2.21 \pm 0.07^{c,d}$ | $2.53 \pm 0.22^{c,d}$ | $2.08 \pm 0.77^{c,d}$ |

Each value is the mean ± standard deviation of 6 chickens from each group.

$^{a\text{-}b\text{-}c\text{-}d}$ means within row with no common superscripts differs at $p<0.05$.

## APPENDIX 5

**FAdV antibody titer of chickens throughout the trial.**

| Age (days) | FAdV Antibody Titer | | | |
|---|---|---|---|---|
| | Group A | Group B | Group C | Group D |
| 1 | 938±651[a] | | | |
| 14 | 3797±980[b] | 1777±600[b] | 3447±2141[b] | Not detected |
| 28 | 4302±2234[c] | 1104±264[c,d] | 6312±2332[d] | Not detected |

Each value is the mean ± standard deviation of 6 chickens from each group.

[a-b-c-d] means within row with no common superscripts differs at $p<0.05$.