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Pathogenicity and Immunogenicity of Live Attenuated and Inactivated Fowl Adenovirus in Commercial Broiler Chickens

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Abstract: Fowl Adenovirus (FAdV) is a non-enveloped DNA virus which is the primary pathogen of Inclusion Body Hepatitis (IBH) in chickens. IBH outbreaks were reported worldwide and was first reported in Malaysia in 2005 due to FAdV strain of serotype 8b infection. It was objective of the study to determine pathogenicity and immunogenicity of live attenuated and/or inactivated FAdV strain of serotype 8b (UPM1137) of Malaysian isolate in commercial broiler chickens. The 54, 1-day-old Cobb 500 broiler chicks were divided into four groups, namely groups A-D. Feed and water were provided ad-libitum. The chicks in groups A-C were inoculated with inactivated FAdV (0.2 mL) with virus titer of $10^{6.5}$ $TCID_{50}$/0.2 mL, live attenuated FAdV (0.1 mL) with virus titer of $10^{5.2}$ $TCID_{50}$/0.1 mL and the combination of the inactivated (0.2 mL) and live attenuated (0.1 mL) FAdV, respectively at day old and day 14 post-inoculation (pi). Body weight and blood samples were collected prior to necropsy at days 14 and 28 pi, except sampling was also conducted at day 0 pi in the group D (control). 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 and histological lesions were recorded in all group of chickens throughout the trial. The body weight of chickens at days 14 and 28 pi 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 at day 28 pi. The FAdV antibody titer in group D (control) was 938±1596 on day old and was not detected at days 14 and 28 pi. However, the FAdV antibody was induced at high titer in all the inoculated groups at days 14 and 28 pi. The FAdV antibody titer of group C was significantly ($p<0.05$) higher than groups A and B at day 28 pi. It was concluded that the live attenuated and inactivated FAdV are safe and able to induce FAdV antibody titer in broiler chickens with moderate level of maternally derived antibody at day old of age. The combination of live attenuated and inactivated FAdV was able to induce higher antibody titer when compared to sole use of live attenuated or inactivated FAdV. It has high potential to be used as vaccination strategy against IBH outbreaks.

Key words: Fowl Adenovirus (FAdV), commercial broiler chicken, live attenuated, inactivated, pathogenicity, immunogenicity

INTRODUCTION

Fowl Adenoviruses (FAdVs) are ubiquitous non-enveloped DNA virus and comprised of 12 serotypes emerged from five molecular groups species designated as letter A-E (Benko *et al*., 2005). FAdV infection was reported worldwide and is a major threat to poultry industry with serious economic losses (Saifuddin *et al*., 1992; Zadrvec *et al*., 2011). FAdV was identified as primary agent of IBH with immunosuppressive effect in the affected chickens (Hussain *et al*., 2012; Saifuddin and Wilks, 1992). The infection can cause high mortality up to 30% and poor production in chickens (McFerran and Smyth, 2000).

In recent years, most of the virulent strains of FAdV isolated during IBH outbreaks in broiler and layer chickens were characterized as FAdV strains of either serotypes 4, 8b, 9 or 11 (Maartens *et al*., 2014; Morshed *et al*., 2017; Juliana *et al*., 2014; Kajan *et al*., 2013; Ahmad, 2015). Currently, only FAdV group E species of serotype 8b is the cause of IBH and gizzard erosion outbreaks in chickens in Malaysia (Juliana *et al*., 2014; Sohaimi *et al*., 2018). Effective prevention and control of the disease can be achieved with proper biosecurity and vaccination programme, particularly with the nature of virus which is highly resistant in environment (Junnu *et al*., 2015).

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Development of live attenuated FAdV vaccine has been major focus of attempt by many researchers due to its low pathogenicity in chickens and elicits an excellent immune response with high protection rate (Mansoor *et al*., 2011). Continuous passage of the virus in chicken embryos or cell cultures resulting virus attenuation with diminish virulence gene (Sohaimi *et al*., 2018; Mansoor *et al*., 2011; Ali *et al*., 2015). On the other hand, inactivated FAdV vaccine was extensively used for rapid control of IBH outbreaks in commercial premises (Junnu *et al*., 2015; Kim *et al*., 2014; Kumar *et al*., 1997). Inactivated vaccine either in the form of autogenous or chick-embryo propagated vaccine was reported to be used in breeder and broiler chickens in the prevention and control against IBH outbreaks (Alvarado *et al*., 2007; Cowen, 1992). It was objective of the study to determine the pathogenicity and immunogenicity of live attenuated and/or inactivated FAdV isolate of serotype 8b (UPM1137) of Malaysian isolate in commercial broiler chickens.

MATERIALS AND METHODS

Virus: FadV isolate namely, UPM1137 was obtained from an outbreak of IBH and gizzard erosion in 25-27 weeks old commercial layer chickens. Mortality was recorded at 2% with clinical signs of reduced feed consumption and drop in eggs production. Upon necropsied, liver was pale and friable with erosion in koilin layer of gizzard. The isolate was positive for FAdV and characterized as serotype 8b under group E species (Sohaimi *et al*., 2018). Virus inoculum from liver sample was prepared based on methods described previously (Alemnesh *et al*., 2012). Live attenuated FAdV was obtained from virus isolate following 15th consecutive passage in primary Chicken Embryo Liver (CEL) cells with virus titer of $10^{6.2}$ $TCID_{50}$/mL. For inactivated FAdV isolate, the virus isolate from CEL cells at 5th passage was inactivated with Binary Ethylenimine (BEI) at concentration 0.002 M/L, according to, previous protocol (Habib *et al*., 2006). The inactivated inoculum was combined with aluminium potassium sulfate as adjuvant and virus titer was adjusted to $10^{6.5}$ $TCID_{50}$/ 0.2 mL.

Experimental design: The 54 day-old Cobb 500 commercial broiler chicks 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-D in which there were 12 chicks each for groups A-C and 18 chicks in group D. The chicks in group A were inoculated with 0.2 mL inactivated FAdV Subcutaneously (SQ) with virus titer of $10^{6.5}$ $TCID_{50}$/0.2 mL at day old. All chicks in group B were inoculated with 0.1 mL live attenuated FAdV via. SQ route with virus titer of $10^{6.2}$ $TCID_{50}$/mL at day old. The chicks in group C were inoculated with 0.2 mL inactivated and 0.1 mL live attenuated FAdV via. SQ route at day old. Six chickens in each group were sacrificed with cervical dislocation at day 14 post-inoculation (pi) and the remaining six chicken were given a booster at day 14 pi. All chicks in group D remained uninoculated and acted as the Control group. Six chicks from group D were sacrificed at days 0 and 14 pi. At day 28 pi, all chickens were sacrificed. The chickens were monitored for any clinical abnormality at least twice daily. The body weight was recorded and serum sample was collected from each chicken prior to sacrifice. On necropsy, the gross lesions were recorded and the liver was weighed prior to fix in 10% buffered formalin for histopathological examination. The serum samples were analysed for FAdV antibody titers using Enzyme Linked Immune Sorbent Assay (ELISA) technique. The study was conducted under approval of Institutional Animal Care and Use Committee (IACUC), Universiti Putra Malaysia with AUP No: FYP-2016/FPV.045.

Statistical analysis: The data for body weight, liver weight, liver to body weight ratio and FAdV antibody titer of chickens were statistically analyzed with one-way Analysis of Variance (ANOVA) using SPSS Version 22 with significance value $p<0.05$.

RESULTS AND DISCUSSION

Clinical signs: No abnormal clinical signs were recorded in all group of chickens throughout the trial.

Body weight: The body weight of chicks was 0.05±0.01 kg at day old and was continuously increased to 1.64±0.10, 1.64±0.06, 1.63±0.07 and 1.86±0.03 kg at day 28 pi in A-D, respectively. They were no significantly different ($p>0.05$) among all group of chickens at days 14 and 28 pi (Fig. 1).

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

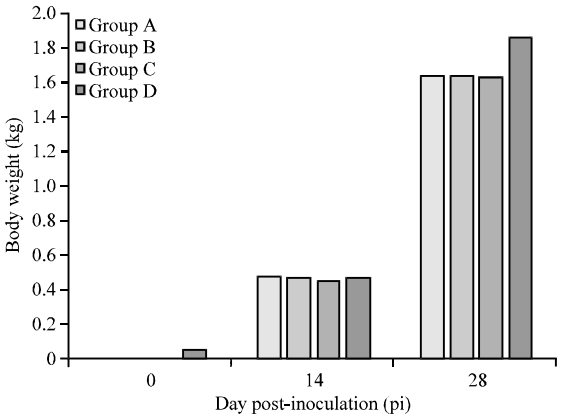

Fig. 1: Body weight of chickens following inoculation with live attenuated and/or inactivated FAdV UPM 1137 from days 0-28 pi in groups A (Inactivated FAdV), B (Live attenuated FAdV), C (Combination of live attenuated and inactivated FAdV) and D (Control) throughout the trial. There are no significant finding in body weight between groups ($p>0.05$)

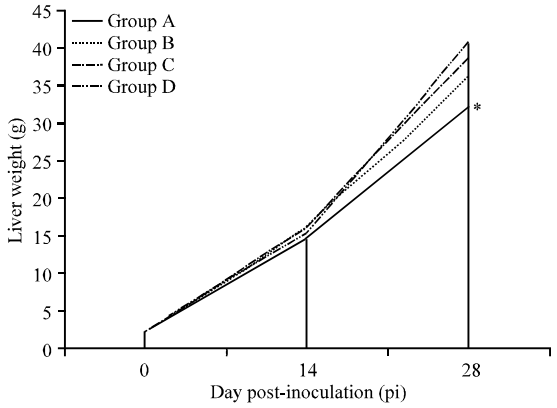

Fig. 2: Liver weight of chickens following inoculation with live attenuated and/or inactivated FAdV UPM 1137 from days 0-28 pi in groups A (Inactivated FAdV), B (Live attenuated FAdV), C (Combination of live attenuated and inactivated FAdV) and D (Control) throughout the trial. The liver of group A were significantly ($p<0.05$)*lower than groups C and D

Liver to body weight ratio ($X10^{-2}$): The liver to body weight ratio for day old chick was 4.09±0.15. At day 14 pi, the liver to body weight ratio of the chicken in groups A-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. At day 28 pi, the liver

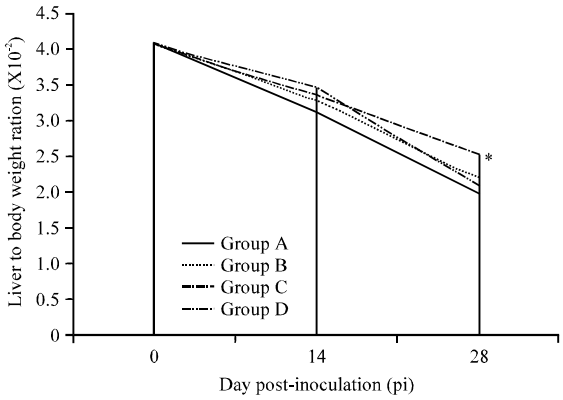

Fig. 3: Liver to body weight ratio of chickens following inoculation with live attenuated and/or inactivated FAdV UPM1137 from days 0-28 pi in groups A (Inactivated FAdV), B (Live attenuated FAdV), C (Combination of live attenuated and inactivated FAdV) and D (Control) throughout the trial. The ratio of group C was significantly higher ($p<0.05$)*than of group A

to body weight ratio of the chicken in groups A-D were 1.99±0.07, 2.21±0.07, 2.53±0.22 and 2.08±0.77, respectively in which the ratio of group A was significantly lower ($p<0.05$) than of group C (Fig. 3).

Gross and histopathological lesions: Grossly, liver was normal in all groups at days 0, 14 and 28 pi (Fig. 4 and 5). There were no abnormal finding in liver under histopathological examination (Fig. 6 and 7).

FAdV antibody titer (ELISA unit): The FAdV antibody titer of day old chicks was 938±651 and it was not detected at days 14 and 28 pi in the control group. In other groups, the antibody titer were 3797±980, 1777±600 and 3447±2141 in groups A-C, respectively, at day 14 pi without significant difference ($p>0.05$) between groups. At day 28 pi, the FAdV antibody titer of chickens in groups A-C were 4302±2234, 1104±264 and 6312±2232, respectively in which the antibody titer of Group C were significantly higher ($p<0.05$) than groups A and B (Fig. 8).

The study demonstrated that FAdV strain of serotype 8b (UMP1137) of Malaysian isolate either as the attenuated or inactivated virus was safe and immunogenic in broiler chickens with moderate level (938±1596 ELISA unit) of Maternally Derived Antibody (MDA) at day old of age. This MDA was not detected at days 14 and 28 of age and could suggest that FAdV vaccination is require in broiler chickens reared in farms with high challenge of FAdV infection (Adair and Fitzgerald, 2008). It should be

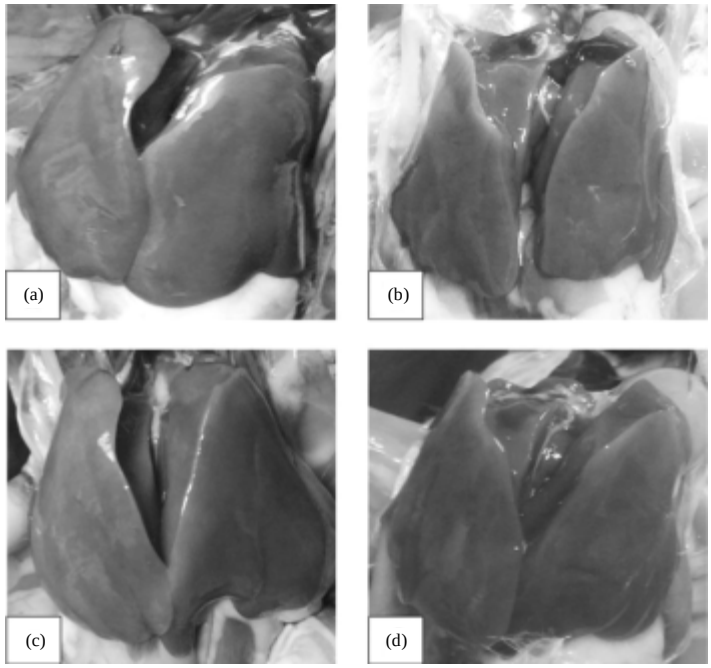

Fig. 4: Normal livers in chickens following inoculation with different FAdV inoculums in groups: a) Inactivated FAdV; b) Live attenuated FAdV; c) Combination of live attenuated and inactivated FAdV and d) Control at days 14 pi indicated, respectively. No gross lesion was observed

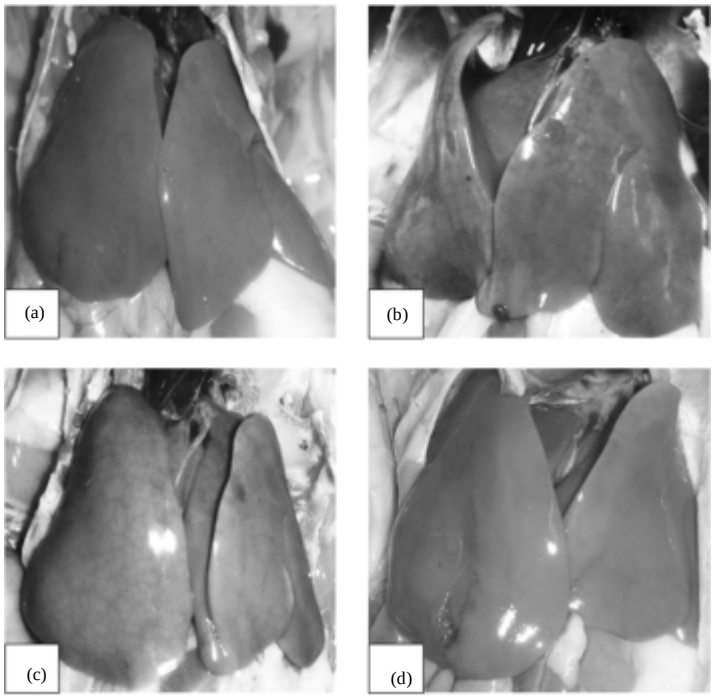

Fig. 5: Normal livers in chickens following inoculation with different FAdV inoculums in groups: a) Inactivated FAdV; b) Live attenuated FAdV; c) Combination of live attenuated and inactivated FAdV and d) Control at days 28 pi indicated, respectively. No gross lesion was observed

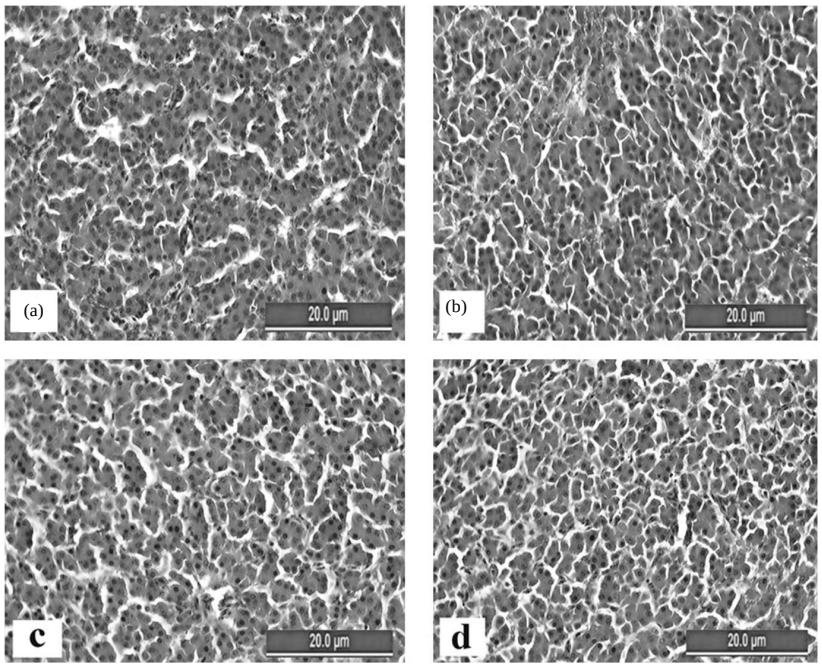

Fig. 6: Normal histological finding of liver following inoculation with different FAdV inoculums in groups: a) Inactivated FAdV; b) Live attenuated FAdV; c) Combination of live attenuated and inactivated FAdV and d) Control at day 14 pi indicated, respectively. HE, 40X. Bar = 20 µm

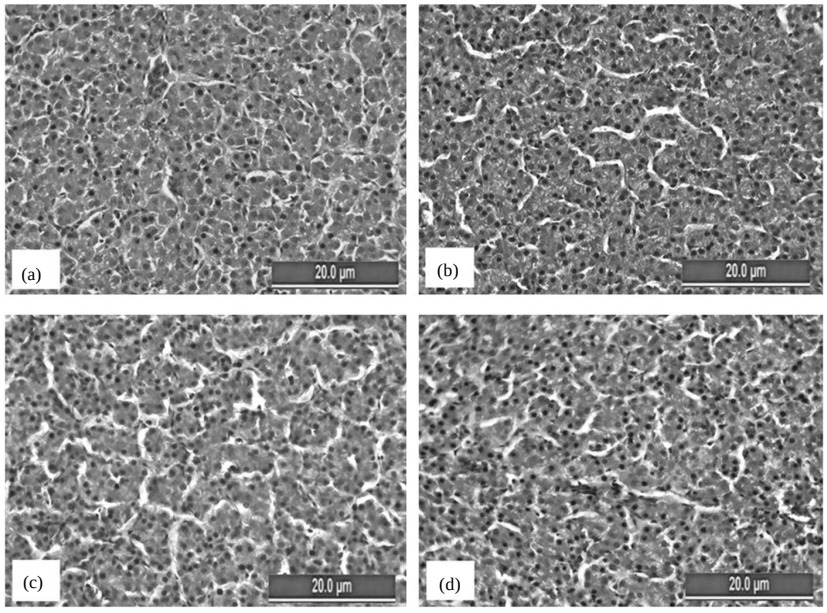

Fig. 7: Normal histological finding of liver following inoculation with different FAdV inoculums in groups: a) Inactivated FAdV; b) Live attenuated FAdV; c) Combination of live attenuated and inactivated FAdV and d) Control) at days 28 pi indicated, respectively. HE, 40X. Bar = 20 µm

a progressive stimulation of an active immunity while the passive acquired immunity declines (Bennejean *et al*., 1978).

The combination of inactivated and live attenuated FAdV could produce a significantly higher ($p<0.05$) FAdV antibody titer than solely use of live attenuated or

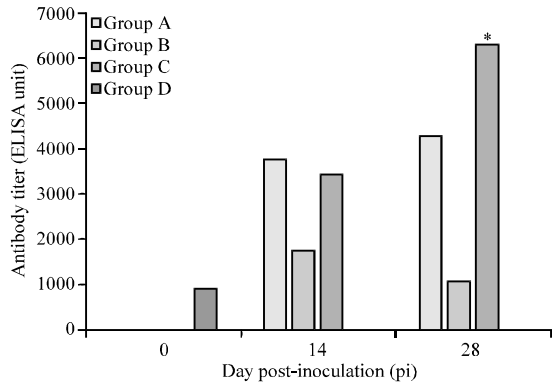

Fig. 8: Fowl Adenovirus (FAdV) antibody response in broiler chickens following inoculation with live attenuated and/or inactivated FAdV UPM1137 between groups: a) Inactivated FAdV; b) Live Attenuated FAdV; c) Combination of Inactivated and Live Attenuated FAdV and d) Control from day 0-21 pi. The antibody titer was significantly higher in group C ($p<0.05$)* compared to that in groups A and B

inactivated FAdV. It was previously reported that the combination of live and inactivated Newcastle Disease (ND) vaccine could induce high and stable antibody titer than the used of either live or inactivated vaccine alone (Bennejean *et al*., 1978; Wanasawaeng *et al*., 2009). It was suggested that the fast response by live attenuated FAdV elicited the primary immune response. At the same time, the slow release of inactivated FAdV antigen enhanced by adjuvant acted as the booster. Simultaneous administration with a live vaccine and inactivated vaccine produces early, strong and lasting immunity (Turblin, 2009). The ND antibody response induced by the combined killed-in-oil vaccines administered concurrently with live virus was better than that induced in all other single group (Folitse *et al*., 1998). However, it is important to take note that the total virus titer that the chickens received in the combination group in the present study was higher than the live attenuated or inactivated group. The study also showed that the live attenuated FAdV in Chicken Embryo Liver (CEL) cells was safe and could induce FAdV antibody in broiler chicken with MDA. Neither, clinical signs nor the gross lesions were recorded in the inoculated chickens. Chickens infected with FAdV will show clinical signs of lethargy, ruffled feather and inappetance and lesions of swollen and pale liver with hemorrhages and multifocal necrosis as well as basophilic or eosinophilinc intranuclear inclusion bodies in the hepatocytes (Hair-Bejo, 2005; Norina *et al*., 2016; 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). The low pathogenicity of live attenuated FAdV in the present study is likely due to diminish of virulence gene after consecutive passages of the virus in CEL cells (Sohaimi *et al*., 2018). The live attenuated and inactivated FAdV in the present study did not interfere the growth performance of the broiler chickens, in which the body weight reached more than 1.6 kg at day 28 pi (Cobb-Vantress, 2015).

CONCLUSION

It was concluded that the live attenuated and inactivated FAdV strain of serotype 8b of Malaysian isolate (UPM1137) are safe and able to induced FAdV antibody titer in the broiler chickens with moderate level of MDA and did not interfere the performance of the chickens at 28 days of age. The combination of live attenuated and inactivated FAdV could induce high level of FAdV when compared to the live attenuated or inactivated virus alone. It has high potential to be used as vaccination strategy against IBH outbreak.

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