How to Cite:
Mahdi, A. A., & Kaab, H. T. (2022). Evaluation of AGP, SSA concentration with the relationship of antibody titer in broiler chicken vaccinated with IBD virus. *International Journal of Health Sciences*, *6*(S5), 11594–11602. https://doi.org/10.53730/ijhs.v6nS5.11997

Evaluation of AGP, SSA concentration with the relationship of antibody titer in broiler chicken vaccinated with IBD virus

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***Abstract*---**Gumboro or Infectious bursal disease (IBD) is one of the most important diseases inpoultry industry. IBD causes economic losses as a result to mortality and reduction in performance and medicine expenses(de S. B. Fróes *et al.*, 2018). IBD is Severe acute disease of 3-6-week-old birds is associated with high mortality, but a less acute or subclinical disease is common in 0-3-week-old birds (Sali, 2019). IBD virus causes lymphoid depletion of the bursa and if this occurs in the first 2 weeks of life, significant depression of the humoral antibody response may result. The current study aims to measurement of antibody titer and acute phase proteins (AGP and SSA) in broiler chickens subjected to the Gumboro vaccine to explore the intensity of the vaccine strain and the response of both the early innate immune response and late humoral immune responses (antibody titers) raised against the vaccine. A handed Ross 308 chicks were divided into three groups, control, intermediate and intermediate plus vaccine. At 10 days old, the chicks were vaccinated and then blood samples collected at 12, 24 hours, and 10 and 15 days. The results shown, both vaccines were induced immune responses and raised antibody titer with significant increment at $P<0.05$ as expected. AGP was the significantly increase post vaccination ($P<0.05$) at 12 and 24 hours and it had a strong correlation to the antibodies raised at 15 days post vaccination ($P<0.01$). Whereas, the increment of the SAA response was not significant.

***Keywords*---**Acute phase protein, AGP, SAA, Antibody, Chicken, Infectious bursal disease, Vaccination.

Introduction

Infectious Bursal Disease (IBD), or Gumboro, is a highly contagious disease of poultry, characterized by severe immunosuppression (Van Den Berg, 2000). The birds surviving IBD suffer from poor feed conversion rate, poor growth, decreased egg production and quality, and reduced efficiency of vaccines. The economic losses due to IBD are not limited to the direct effect of the diseases, but also from decreasing the overall immune status of the flock leading it to be vulnerable to other diseases characterized by a high mortality rate such as Newcastle disease and Infectious Bronchitis (Kegne and Chanie, 2014). The immune suppression is a consequent to damage of Bursa of Fabricius, the target of IBDV, led to severe suppression of humoral and cellular immunity in the early stages of the bird's life (Rautenschlein et al., 2002).

The current study aims to measurement of antibody titer and two types of acute phase proteins (AGP and SAA) in broiler chickens subjected to the Gumboro vaccine to explore the intensity of the vaccine strain and the response of both the early innate immune response and late humoral immune responses (antibody titers) raised against the vaccine (Alhasnawi and Aljanaby, 2022). This investigation could link the early innate responses to the late antibody titers that may be used as an early indicator for the immune solidity responses of the vaccine.

Materials and methods

Housing and vaccination of birds

A nearby commercial hatchery provided 180 chicks for the current study population, one-day old Ross 308 Broiler chicks were employed in this experiment.According to the Ross 308 farming guide management, all of the chicks were kept and fed a standard commercial diet. Chicks were divided into three groups, 60 chicks in each group. The first group is control, the second group is intermediate Gumboro vaccine and last one is intermediate plus Gumboro vaccine. Chicks were vaccinated at 10 days old, by oral route with 1ml by disposable syringe after the vaccine is dissolved in water. Blood samples collected at 12 and 24 hours, 10-, 15- and 20-days post vaccination. After collection, the blood samples were placed in a centrifuge for 3000 cyclesin 15 minutes to separate the blood components and collect plasma that wassubjected to analysis. Collected plasma was allocated into 100 µl in the Eppendorf tube to store the plasma to analysis by ELISA kit for (AGP, SAA and antibody).

Results

The antibody titer was raised against the Gumboro vaccine post-vaccination.The two types of vaccines are used to raise antibodies against the IBDV to protect against the Gumboro disease Figure-1 demonstrates the level of the antibody titers post vaccination in both vaccinated groups comparison to the control group. Show in Figure-1 the elevation in both types of vaccines was a significant difference compare to the control group. However, the intermediate plus was higher than the intermediate vaccine.

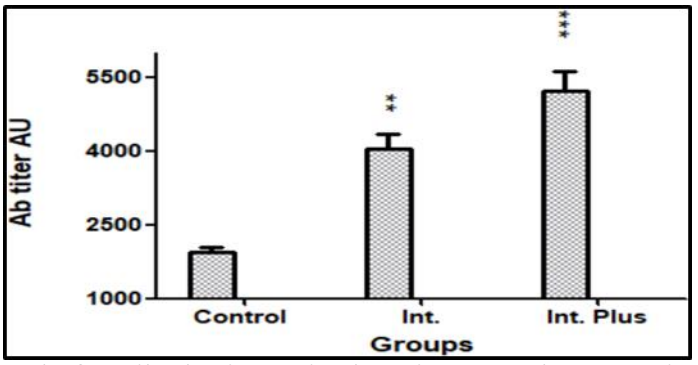

Figure 1: the level of antibody titer raised against Gumboro vaccine 10 days post vaccination in both groups intermediate and intermediate plus with significant difference compare to the control group ($P<0.05$).

Acute phase protein responses

The intermediate and intermediate plus Gumboro vaccine. The results revealed in Figure-2 shows a significant elevation of the AGP to hit the peak at 24 hours post-vaccination. The results showed the AGP level of the intermediate plus vaccine was higher than that of the intermediate and the differences were significantly important compare to the level of the control group. The response of the AGP at different sample times against the vaccine intermediate and intermediate plus, the increment was significantly higher in intermediate plus at 24 hours at $P<0.01$ compare to the control group. Figure-2 illustrates the correlation between the acute phase protein's early response with the late antibody titer raised against the Gumboro vaccine.

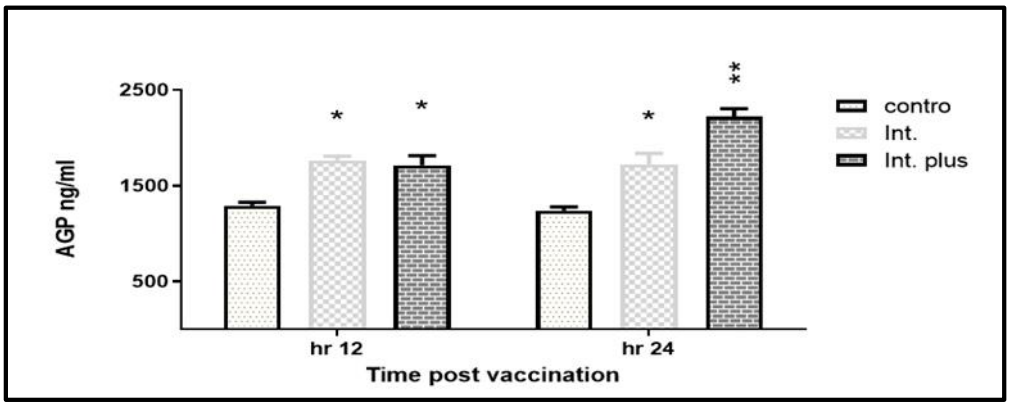

Figure 2: AGP response to the Gumboro vaccine, the level of the AGP in both groups of the vaccines was significantly difference ($P<0.05$) at 12 and 24 hours post vaccination in both treatment groups compare to the control group

The correlation between the acute phase protein's early responses with the late antibody titer raised against the Gumboro vaccine. The correlation of the AGP response at the first-day post-vaccination of both groups with the Ab titer raised against the vaccine after 10 days post-vaccination the results showed a positive significant correlation ($P < 0.05$) of the AGP and the antibody titer. The response

of the early increment of the AGP to the vaccine correlated to the late antibody titer. The correlation was significantly positive at a level of P< 0.05with r= 0.65.

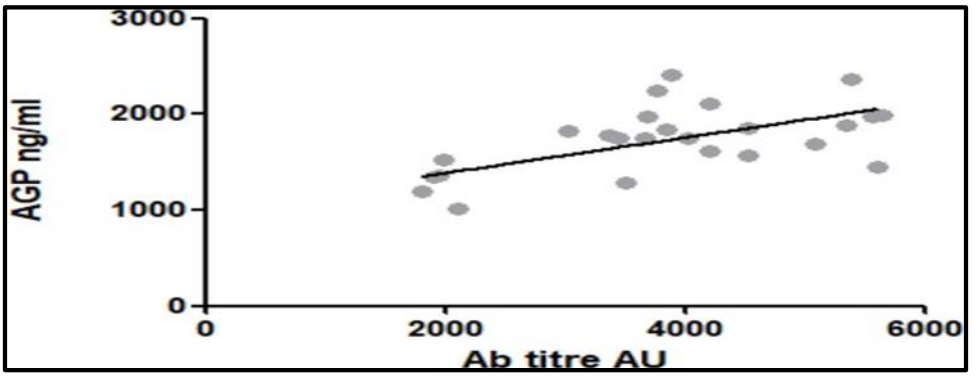

Figure 3: The AGP level correlated to the Ab titer. The correlation of the early innate responses with the late antibody titer in broiler chickens vaccinated with two types of Gumboro vaccine. The correlation was significant (P<0.05) and positive

The SAA response to the vaccine increased in both groups of vaccinated chickens with significant difference compared to the control group at (P<0.05) at each time points, also the increment was significant at 12 and 24 hours also. Figure-4 shows the SAA level of the intermediate plus vaccinated group was higher than the intermediate vaccinated group but the increment was not significant among groups and / or among time points for the same group.

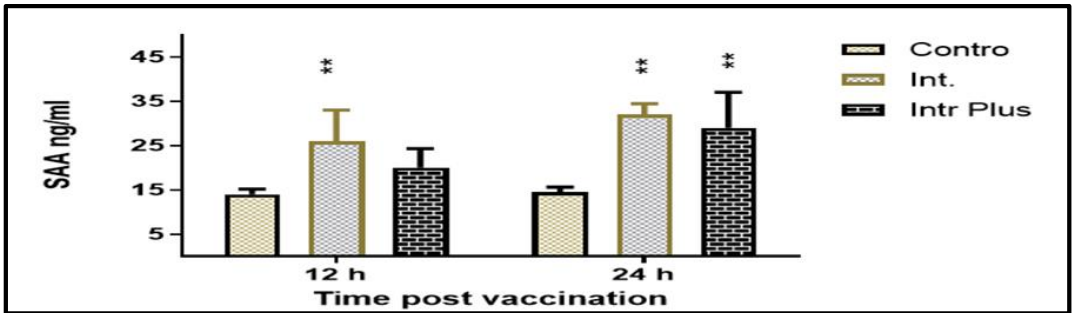

Figure 4: Reveals the SAA level responses post vaccination with two types of Gumboro vaccine(intermediate and intermediate plus) at first 24 hours post vaccination. The response of the SAA acute phase protein at 12- and 24-hours post-vaccination was significant differ from control at (P<0.05).

Discussion

The current project is conducted to find an early parameter that could help to evaluate the vaccine responses in chickens vaccinated with Gumboro disease virus. The conventional method for evaluation the immune response is by measure the antibody titer after ten days post vaccination. This period in terms of the chicken's industry especially broiler chickens considered a long period. Therefore, looking for an earlier Parameter within 24 to 48 hours post vaccination

will spare time that essential to provide aid to protect the flock from diseases in case need it. Previous studies indicated that(Corley, Giambrone and Dormitorio, 2001; Kaab, Bain and Eckersall, 2018). The current study aims to measurement of antibody titer and acute phase proteins (AGP and SAA) in broiler chickens subjected to the Gumboro vaccine to explore the intensity of the vaccine strain and the response of both the early innate immune response and late humoral immune responses (antibody titers) raised against the vaccine. The current results of the monitoring APPs within reported the responses of APP at twelve and twenty-four hours post vaccination. Where the vaccinated chicks had revealed a high concentration of AGP after 12- and 24-hours vaccine (Figure-2), significantly higher at P< 0.05 than the control group. This could be related to the cytokines activities as a response to the vaccine stimulation. Previous studies in poultry indicated that cytokines such as IL-1, IL-6, IL-17, and tumor necrotic factor (TNF-) are produce (Franchini *et al.*, 2004; Giansanti, Giardi and Botti, 2007). Injecting partially purified versions of these cytokines into hens has been shown to cause numerous APP effects as well as pyrexia and anorexia. IL- 6, for example, has been found to trigger the production of APP in the liver (K.C. and B.J., 1990; Juul-Madsen *et al.*, 2007) TNF- has been demonstrated to promote tumor cytolysis and cartilage resorption (Juul-Madsen *et al.*, 2007). This increment could a consequence to pro-inflammatory cytokines such as IL-1, IL-6, and tumor necrosis factor. They are released as a result of tissue infection or trauma, led to altering a number of proteins in the blood generated predominantly in the liver (APP) (Abd, 2014). Investigation the response of the APP has been monitor in the inflammatory illnesses for diagnosis and the inflammation progress (Nazifi *et al.*, 2010). There was a clear difference in the level of answers and the timing for both types of APPs employed. In comparison to another APP, AGP changed the most and offered the earliest reactions to stimuli. Agree with findings that AGP is a significant APP in chickens (Cerón, Eckersall and Martínez-Subiela, 2005; Ceciliani *et al.*, 2012). Following the give of Gumboro Intermediate and Intermediate plus vaccines, AGP increased by 1.56 and 2-fold, respectively (Figure-2). The fact that varied quantities of cytokines were generated as a result to vaccine reactions, as well as the host's response to these immunizations, could explain these disparities (Jergens *et al.*, 2003) This, in turn, would have an impact on the level of APP reaction (Eckersall, 1995; Cerón, Eckersall and Martínez-Subiela, 2005). There are a variety of reasons why the APP's reactions may change. First, different co- stimulatory cytokines could be released depending on the underlying stimuli (Higano *et al.*, 1997; Changes, 1999; Bode *et al.*, 2012). This could result in variable hepatic APP expression rates and APP production. Differences in AGP and SAA responses to immunizations suggest that cytokine- driven APP response mechanisms vary not only by individual but also by immunological state (Eckersall *et al.*, 2008). There are variances in APP compatibility between mammals at the gene and amino acid sequence level; for example, SAA in horses has 80.6 percent homology with a dog, 76.9% with a human, and 71.9 percent with a chickens (Albert, 2000). This homologous diversity could explain changes in APP levels response. Second, not only gene expression, but all subsequent steps, such as post-transcriptional regulation, have a significant role in the induction of some, but not all, APP like AGP (Hussain *et al.*, 1995). Various triggers may also affect secretion efficiency (Changes, 1999; Aljanaby and Al-Faham, 2022). The uneven metabolism and elimination of APP may represent a third pathway (Schrödl *et al.*, 2016). In such

instances, the clearance process is variably governed by the half-life of the APP (Lannergård *et al.*, 2003). A fourth consideration is whether alternative extrahepatic tissue origins should be considered as additional sources of circulating APP. The widely reported variability of circulating APP (glycosylation, isoelectric points,and chromatographic distribution), and also their association to numerous sources, are likely to be useful as biomarkers of inflammation (Schrödl *et al.*, 2016). The dualsecretion of APP could interconnect local or systemic responses, which may be of functional value. Many types of APP appear to be expressed in healthy and pathological individuals, both focally and extra-hepatic (Kalmovarin *et al.*, 1991; Berg *et al.*, 2011; Marques *et al.*, 2017). Extra-hepatic expression rates are usually low in healthy people compared to the liver, but they skyrocket when there is a specific damage (Reinhardt *et al.*, 2013). As a marker for the innate immune system's early response to stimuli like immunization within a few hours (5 to 6 hr. in humans) of an inflammatory event, AGP commonly increases 10- to 100-fold and then declines after 48 hours (Kushner and Rzewnicki, 1994; Larson *et al.*, 2005). The current experiment, two types of Gumboro vaccines, medium strength (intermediate) and strong (intermediate plus).The antibodies titer raised against the intermediate and intermediate plus Gumboro vaccines at 10 days post vaccination with significantly higher than the control group.The antibody level was the highest in group vaccinated with intermediate plus compared to the other groups. The statistical analysis illustrates the correlation between the APPs (early response)with the late antibody titer raised against the Gumboro vaccine (Figure-3). The correlation of the early AGP response, at the first-day post-vaccination, (intermediate and intermediate plus Gumboro vaccine) with the Ab titer raised against the vaccine after 10 days post-vaccination showed a significant correlation ($P < 0.05$). This correlation is important from the clinical view which could help to the early assessment of the vaccine efficacy as early as possible.

Conclusion

1: The current study concludes the following the use of this protocol because it is faster and does not require long periods to examine the immunity of birds after long periods of vaccination.
2: The current results suggested that the acute phase response was mild and significant increase with AGP and correlated positively significant with the late humoral immune responses.

Recommendation

1: Conducting more research to reach the desired goals as well as urging the provision of the appropriate vaccine depending on the type of chicken as well as the origin of the vaccine manufacturer
2: Work to educate field owners about the need for laboratories to carry out the necessary laboratory tests in order to obtain sufficient information to reach good production.

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