1. Introduction
Pathogenic Avian Influenza subtype H5N1 is one of the zoonotic diseases which attracts worldwide attention. According to WHO, up to 2019, there were 861 cases of avian influenza A (H5N1) in humans with 455 fatal cases ended with death [1]. Until today several reports on the incidence of avian influenza due to the existing H5N1 viruses in poultry. Therefore, the avian influenza H5N1 to remain to threaten Indonesia. Following WHO recommendations, the drug to treat patients in AI cases in humans is neuraminidase inhibitor drugs. It includes oseltamivir and zanamivir or amantadine and rimantadine instead of neuraminidase inhibitors [2]. However, amantadine and rimantadine tend to be resistant to some strains of the H5N1 virus [3].

The active immunization against AI has not been possible, because AI H5N1 vaccine for humans is still not available in Indonesia. Therefore, passive immunity using hyperimmune bovine colostrum which contains anti-AI H5N1 antibodies may serve as an alternative in controlling bird flu. Bovine colostrum is the initial secretion present in the mammary gland of cows at or near the time of parturition before actual milk appears [4]. Bovine colostrum is rich in immunoglobulins (Igs), mainly immunoglobulin G (IgG), designed to protect the neonatal calf from environmental pathogens. Frequent,
repeated inoculation of a gestating dairy cow may stimulate increased production of high levels of colostral immunoglobulin against a targeted antigen, resulting in hyperimmune bovine colostrum [5].

Hyperimmune bovine colostrum which contains anti-AI H5N1 antibodies/IgG could be produced by vaccination of a cow during dry periods using commercial killed Avian Influenza (AI) H5N1 vaccine. It has a potential or prospect for passive immunotherapy purposes on controlling avian influenza. Specific antibodies obtained from passive immunity through the administration of hyperimmune colostrum (colostrum which contains anti-AI H5N1 IgG) may be able to inhibit the attachment of AI viruses on the surface of host cells. Specific antibodies can bind the AI virus circulating in the blood will be neutralized [6].

The vaccine is an antigenic material used to produce active immunity against diseases. Vaccination is the administration of antigenic material (a vaccine) to stimulate an individual’s immune system to develop protection (adaptive immunity) against a pathogen/disease or diseases [7, 8]. The exposure of a dry cow against the antigen (vaccine) will produce a specific antibody in their blood circulation. Antibodies, proteins that combat foreign substances in the body, are associated with the globulin fraction of the immune serum [4]. The contribution of blood globulin concentration, i.e. γ-globulin, to the total concentration of protein is vital, so the measurement of total protein concentration can be used as an indicator of the immunoglobulin concentration in the serum [6]. Total protein concentration may serve as a reference to the success of antibody formation [9]. Observation of the protein fraction profile in this study is to evaluate whether the administration of avian influenza H5N1 vaccine would affect the total protein profile in the blood circulation.

2. Materials and methods

2.1. Hyperimmunization of pregnant Holstein Cows
Pregnant Holstein cows, 2nd-3th lactation, clinically healthy were used in this experiment and divided into two groups, control (n=3) and treatment (n=7). The treatment group received the injection of commercial killed Avian Influenza (AI) H5N1 vaccine subcutaneously, four times doses, three times every two weeks.

2.2. Blood samples collection and analysis
Blood samples were collected from the coccygeal vein, starting before the 1st vaccination, every two weeks until four weeks after the 3rd vaccination for serum total protein, albumin, and globulin analysis. Serum total protein and albumin concentration were analyzed using photometer methods. The known total protein and albumin concentration in the serum may reflect the globulin concentration in the serum.

3. Results and discussion
Protein is the most significant main ingredient in blood plasma, whose main fraction consists of albumin and globulin. Changes in total protein concentration can occur when one of the main fractions, i.e. albumin or globulin changes [10]. Tables 1, 2, 3. presented the concentration of total protein, albumin, and globulin in pregnant cows in the final trimester of the control and the vaccinated group.

Table 1. Mean values of total protein (g/dL) serum concentrations of pregnant cows in the last third-part of pregnancy of vaccinated and control groups.

| Group       | 0               | 2nd Week       | 4th Week       | 6th Week       |
|-------------|-----------------|----------------|----------------|----------------|
| Control     | 6.60±0.20       | 6.27±0.31      | 7.20±0.80      | 6.93±0.61      |
| Vaccinated  | 6.80±0.90       | 6.57±1.00      | 6.69±0.92      | 6.54±0.98      |

The observations showed that the average of total protein concentration in the last part of pregnancy of Holstein cows between the control and the vaccinated group were not significantly different (P>0.05) from the day of vaccination until two weeks after the third vaccination. In general, the average total protein concentration profile in the two groups had almost the same pattern throughout the time of
observation. However, the mean total protein concentration in the vaccinated group seemed to be stable throughout the observation.

The mean values of serum total protein concentration in the vaccinated group were lower if compared to the referential values reported by [11], which range from 6.74-7.46 g/dL. Many factors affect the total concentration of protein in blood circulation. According to [9], the total concentration of protein in serum is not only influenced by pathological conditions (disease), but influenced by age, development, hormones, pregnancy, lactation, nutrition, dehydration, and stress. In pregnant cows, the concentration of total serum proteins starts to increase two months before calving, reaching its maximum concentration a month before calving and then suddenly decreases at just before calving.

Table 2. showed the mean albumin concentrations in the final part of pregnancy Holstein cows of the control and the vaccinated group. The results showed that the mean albumin concentration in the third part of pregnancy between the control and the vaccinated group were not significantly different (P> 0.05) throughout the observation. In general, the mean albumin concentration in both groups tended to decrease at the end of the observation.

Table 2. Mean values of serum albumin (g/dL) concentrations of pregnant cows in the last third-part of pregnancy of vaccinated and control groups

| Group      | 0             | 2nd Week      | 4th Week      | 6th Week      |
|------------|---------------|---------------|---------------|---------------|
| Control    | 2.99±0.14     | 2.74±0.15     | 2.84±0.32     | 2.87±0.37     |
| Vaccinated | 2.74±0.33     | 2.64±0.28     | 2.69±0.24     | 2.73±0.33     |

Albumin is the main protein produced in the liver. It usually accounts for 35-50% of the total plasma protein. Albumin is chiefly responsible for the colloid osmotic pressure of the blood. It binds and transports many substances (including unconjugated bilirubin, free fatty acids and thyroxine (in part), and many drugs) [12]. According to [10], physiologically, there are no factors that could increase albumin synthesis, where the increased albumin concentration caused by fluctuations in blood volume [13]. The decrease in the concentration of albumin in the blood is not only caused by a decrease in its synthesis but involves a multifactorial process which includes albumin damage, its leakage to extravascular and protein intake [14]. The decrease of albumin concentrations may occur in the case of chronic dehydration, malnutrition (protein deficiency), protein-losing enteropathy, and failure of liver function [7]. In the pregnant cows, albumin concentration decreases and reaches its lowest level in the mid-pregnancy. The concentration then gradually increases to such a level within the standard value limits, where it remains until calving [9].

Table 3 present the mean globulin concentrations in the third part of the pregnancy of the control and the vaccinated group. Globulins are a heterogeneous group of abundant serum proteins other than albumin. Based on protein electrophoretic fractionation, serum globulin comprises a various type of faction such as alpha, -beta, and -gamma globulin fractions. These include clotting proteins, complement, many acute-phase proteins, immunoglobulins, and lipoproteins. Immunoglobulins (Igs), primarily IgG, migrate in the gamma globulin fraction [9].

Table 3. Mean values of globulin (g/dL) serum concentrations of pregnant cows in the last third-part of pregnancy of vaccinated and control groups

| Group      | 0             | 2nd Week      | 4th Week      | 6th Week      |
|------------|---------------|---------------|---------------|---------------|
| Control    | 3.61±0.27a    | 3.53±0.46     | 4.36±1.05     | 4.07±0.87     |
| Vaccinated | 4.06±0.97     | 3.93±1.01     | 3.99±1.03     | 3.82±1.18     |

The results showed that the mean globulin concentration in the third part of pregnancy between the control and the vaccinated group were not significantly different (P>0.05) throughout the observation. In general, the average globulin concentration in both groups tended to fluctuate in the same pattern as the total protein concentration throughout the observation period. The mean globulin concentration in the vaccine group tended to decrease at the end of the observation with a concentration tended to be
lower than the control. However, the concentration is still in the normal range, according to [9], which ranges from 3.24-4.25 g/dL. According to [13], decreased serum globulin concentration may also be associated with a decreased total protein concentration, depending on the severity of the globulin change.

Pregnancy significantly influences the proteinogram of serum proteins in all animal species [9, 10]. During pregnancy, there is a tendency for albumin concentration to decrease on the one hand and a globulin concentration increase on the other. The globulin concentration increases until the eighth month of pregnancy and then starts to decrease, due to the accumulation of immunoglobulin in the mammary gland, it suddenly decreases immediately before calving [9].

4. Conclusion
There were no differences in total protein, albumin, and globulin concentration between groups and time of vaccination (P>0.05). The vaccination on dry Holstein cows using commercial killed avian influenza H5N1 vaccine subcutaneously, four times doses, three times every two weeks before calving does not influence the profiles of protein fraction.

Acknowledgments
The experiment was supported by The Ministry of Research, Technology and Higher Education through research grant in FY 2017-2019 (“Penelitian Terapan Unggulan Perguruan Tinggi”).

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