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308 Lasani Town, Sargodha Road, Faisalabad - Pakistan
Mob: +92 300 3008585, Fax: +92 41 8815544
E-mail: editorijps@gmail.com

Serum Protein Profiles of Juvenile Ring-Necked Pheasants Vaccinated or Not Against Newcastle Disease

Elizabeth Moreira dos Santos Schmidt^{1,2,3}, Antonio Carlos Paulillo^{3,4}, Rosangela Locatelli-Dittrich⁵,
Olair Beltrame⁵ and Janine Denadai^{2,3,6}

¹Post-doctorate Researcher in Veterinary Medicine of FCAV-Unesp, Jaboticabal, Brazil

²Bolsista FAPESP, Brazil

³Departamento de Patologia Veterinária, FCAV-Unesp, Jaboticabal, Brazil

⁴Research Fellow-CNPq, Brazil

⁵Departamento de Medicina Veterinária, UFPR, Curitiba, Brazil

⁶Curso Pós-graduação em Medicina Veterinária, FCAV, Unesp, Jaboticabal, Brazil

Abstract: The aim of this study was to investigate blood protein parameters in juvenile ring-necked pheasants using Ulster 2C, B1 and LaSota vaccines strains of the Newcastle disease virus. Total serum protein, albumin and globulin concentrations showed significant differences among vaccinated and non-vaccinated birds. Significant variations were not observed in the analyses in relation to protein electrophoresis profile.

Key words: Newcastle disease, *Phasianus colchicus*, serum proteins, albumin, globulins, Ulster 2C, B1

INTRODUCTION

Protein is the most abundant component of plasma. However, this large mass of protein consists of many different individual protein molecules. The main functions of protein in blood are in blood coagulation, in host defenses against pathogens (immunoglobulins), in transport of metabolites, in regulation of cellular metabolism and in maintaining osmotic pressure (Eckersall, 2008). Blood proteins are important complementary constituents in the diagnosis of gastrointestinal, hepatic, renal or infectious diseases. Determination of these proteins seldom leads to a specific diagnosis but will help to evaluate the nature, severity and progress of a disease (Lumeij, 2008). Moreover, there are no experimental studies that interpret bird's proteins responses after antigen exposition and immune stimulation. Thus, this study aimed to evaluate the blood proteinogram profile in ring-necked pheasants vaccinated or not against Newcastle disease.

MATERIALS AND METHODS

The juvenile ring-necked pheasants were allocated in experimental floor-pen housed, receiving water and food *ad libitum*. The feed was formulated with corn and soybean according with NRC (1994) recommendations. Forty-eight pheasants (24-94 day-old) were distributed in a completely randomized experimental design into four different treatments of 12 birds each. Birds were designated to treatments, according to vaccination strain as G1 (Ulster 2C), G2 (B1), G3 (LaSota) and to treatment G4 (control group-not vaccinated). Commercial live NDV vaccines (Ulster 2C, B1 and LaSota) were administered

to each experimental group, as described by Paulillo *et al.* (1996). Birds were vaccinated at 10 days of age and revaccinated at 38 and 66 days of age with the same vaccine strain that was applied in the first vaccination. Birds were vaccinated and revaccinated by eye drop. Blood samples of ring-necked pheasants were collected from seven to 94 days of age, at regular fourteen day intervals. Sera samples were submitted to inhibition of hemagglutination (HI) test, according to Cunningham (1971).

Aliquots of each blood sample were transferred immediately to a 10-mL plain glass tube containing no anticoagulant for serum protein analyses. Blood samples of the ring-necked pheasants were collected from the jugular and ulnar superficial vein, from these birds on days 24, 38, 52, 80 and 94 for the determination of serum total protein and albumin concentrations, with an automated (spectrophotometer) chemistry analyzer. The globulin value was determined by the difference between serum total protein and albumin. Serum albumin, alpha-1 globulin, alpha-2 globulin, beta-globulin and gamma-globulin fractions were determined by agarose gel electrophoresis and serum total protein was determined by the biuret method, on 52 and 80 days of age. The control of the chemical analysis was made using Qualitrol-N.

The data were analyzed by ANOVA and those with statistical differences were submitted to the Tukey's test at 0.05%, using Statview® (version 5.0).

RESULTS AND DISCUSSION

Mean antibody titres against NDV from ring-necked pheasants are shown in Table 1. As the control group

Table 1: Mean antibody titres measured by HI test (\log_2) of ring-necked pheasants (*Phasianus colchicus*) submitted to different vaccination programs against ND

Group	Vaccine	Mean antibody titers measured by hi test (\log_2)						
		Ring-necked pheasants age (days)						
		7	24	38	52	66	80	94
G1	Ulster 2C	0,0	5,0 ^a	4,4 ^a	4,4 ^a	4,3 ^a	4,7 ^a	4,8 ^a
G2	B ₁	0,0	4,2 ^b	4,8 ^a	5,0 ^b	5,0 ^b	4,9 ^a	4,6 ^{ab}
G3	LaSota	0,0	4,6 ^{ab}	4,2 ^a	4,4 ^a	4,0 ^a	4,8 ^a	4,3 ^b
G4*	-	0,0	0,0 ^c	0,0 ^b	0,0 ^c	0,0 ^c	0,0 ^b	0,0 ^c

*Control group-not vaccinated against ND. 1-Means followed by the same letter, in the same column, are not different at 5% of probability by Tukey test ($p>0.05$)

Table 2: Serum protein values in juvenile ring-necked pheasants (*Phasianus colchicus*) (24, 38, 52, 80 and 94 days old). Vaccinated or not against Newcastle disease (Mean \pm SD)

Mean and standard-deviation Total Serum Proteins (Pst) (g/dL), albumin (Alb) (g/dL) and globulins (Glo) (g/dL)									
Ring-necked pheasants age (days)									
	24			38					
Group	Pst	Alb	Glo	Pst	Alb	Glo			
I	3.5±0.6 ^a	1.9±0.3 ^a	1.5±0.5 ^a	4.7±0.1 ^a	2.5±0.2 ^a	2.3±0.1 ^a			
II	3.1±0.4 ^a	1.8±0.2 ^a	1.3±0.3 ^a	3.6±0.6 ^a	2.0±0.1 ^a	1.6±0.6 ^a			
III	3.2±0.1 ^a	1.8±0.1 ^a	1.4±0.1 ^a	3.8± 0.7 ^a	2.1±0.4 ^a	1.7±0.3 ^a			
IV*	3.2±0.4 ^a	1.8±0.2 ^a	1.4±0.2 ^a	3.8±0.3 ^a	2.1±0.2 ^a	1.7±0.1 ^a			
Mean and e standard-deviation Total Serum Proteins (Pst) (g/dL), albumin (Alb) (g/dL) and globulins (Glo) (g/dL)									
Ring-necked pheasants age (days)									
	52			80			94		
Group	Pst	Alb	Glo	Pst	Alb	Glo	Pst	Alb	Glo
I	3.2±0.2 ^c	1.7±0.2 ^{ab}	1.4±0.1 ^{ab}	3.8±0.3 ^{ab}	1.8±0.2 ^a	2.0±0.2 ^{ab}	3.9±0.8 ^a	1.7±0.2 ^a	2.2±0.6 ^a
II	3.7±0.4 ^b	2.2±0.3 ^c	1.5±0.1 ^b	4.1±0.3 ^a	1.9±0.1 ^a	2.2±0.2 ^a	3.9±0.3 ^a	1.8±0.2 ^a	2.1±0.2 ^a
III	3.5±0.2 ^{bc}	2.0±0.2 ^{bc}	1.5±0.1 ^b	3.5±0.3 ^b	1.7±0.2 ^a	1.7±0.3 ^b	3.9±0.1 ^a	1.9±0.4 ^a	2.1±0.3 ^a
IV*	2.8±0.2 ^a	1.6±0.2 ^a	1.3±0.2 ^a	4.1±0.4 ^a	1.9±0.2 ^a	2.3±0.3 ^a	4.0±0.4 ^a	1.9±0.5 ^a	2.1±0.6 ^a

*Control group - not vaccinated against NDV. Means followed by the same letter, in the same column are not different at 5% of probability by Tukey test ($p>0.05$)

(G4) was not vaccinated, its antibody titres were null from seven to 94 days of age.

The blood proteinogram values for juvenile ring-necked pheasants are shown in Table 2. There were no statistical differences in the total protein, albumin and globulin concentrations among the different groups of 24 and 38 day-old ring-necked pheasants, vaccinated or not against NDV (Table 2). At 52 days of age (Table 2), the total protein, albumin and globulin concentrations were significantly higher between pheasants vaccinated with Ulster 2C, B1 and LaSota strains and the control group. Such fact may suggest that immune stimulus caused by the vaccination with any of NDV the strains was responsible for this situation. According to Rivetz *et al.* (1977) protein changes occur in birds infected by NDV. Also, in acute or chronic inflammatory conditions, a rise in total protein caused by elevated globulin fractions may occur (Lumeij, 2008), since the chronic antigenic stimulation leads to production of immunoglobulins by B lymphocytes, plasma cells, or both (Campbell, 2004).

Washburn and Eidson (1970) found globulin increase in fowls exposed to Marek's disease virus.

Ring-necked pheasants (80 day-old) (Table 2) vaccinated with LaSota strain showed significant differences for total protein and globulin concentrations in relation to the control birds and to B1 vaccinated group. There were no significant differences for albumin concentrations between these groups. It seems reasonable to speculate that vaccination with LaSota strain decreased these parameters, even though there were no statistical differences for the HI test among the vaccinated groups (Table 1). However, these results are not compatible with the great diffusion potential of LaSota strain (Winterfield *et al.*, 1957), because often globulin concentrations are elevated in antigenic stimulation (Lumeij, 2008). Thus, the great diffusion potential of LaSota strain (Winterfield *et al.*, 1957) probably caused a depression of the immune response, observed by the decrease of globulin concentrations 14 days after the last revaccination against NDV. In this

Table 3: Electrophoretic protein profile of juvenile ring-necked pheasants (*Phasianus colchicus*) (52 day-old). Vaccinated or not against Newcastle disease (Mean±SD)

Group	Total serum protein (g/dL)	Albumin (g/dL)	Alpha-1 globulin (g/dL)	Alpha-2 globulin (g/dL)	Beta-globulin (g/dL)	Gamma-globulin (g/dL)
I	2.5±0.7 ^a	1.3±0.4 ^a	0.09±0.04 ^a	0.4±0.1 ^a	0.4±0.1 ^a	0.2±0.08 ^a
II	2.6±0.3 ^a	1.5±0.1 ^a	0.09±0.03 ^a	0.5±0.08 ^a	0.4±0.06 ^a	0.1±0.02 ^a
III	1.8±0.7 ^a	1.0±0.4 ^a	0.07±0.03 ^a	0.3±0.1 ^a	0.3±0.09 ^a	0.1±0.05 ^a
IV*	2.3±0.4 ^a	1.3±0.2 ^a	0.07±0.03 ^a	0.5±0.09 ^a	0.4±0.07 ^a	0.1±0.04 ^a

*Control group-not vaccinated against NDV. Means followed by the same letter, in the same column are not different at 5% of probability by Tukey test (p>0.05)

Table 4: Electrophoretic protein profile of juvenile ring-necked pheasants (*Phasianus colchicus*) (80 day-old). Vaccinated or not against Newcastle disease (Mean±SD)

Group	Total serum protein (g/dL)	Albumin (g/dL)	Alpha-1 globulin (g/dL)	Alpha-2 globulin (g/dL)	Beta-globulin (g/dL)	Gamma-globulin (g/dL)
I	3.1±0.4 ^a	1.7±0.1 ^a	0.1±0.03 ^a	0.5±0.08 ^a	0.7±0.1 ^a	0.2±0.04 ^a
II	2.5±0.3 ^a	1.5±0.1 ^a	0.08±0.02 ^a	0.4±0.09 ^a	0.6±0.2 ^a	0.1±0.02 ^a
III	3.2±0.7 ^a	1.7±0.4 ^a	0.1±0.05 ^a	0.5±0.09 ^a	0.7±0.1 ^a	0.2±0.03 ^a
IV*	3.1±0.4 ^a	1.6±0.2 ^a	0.09±0.03 ^a	0.5±0.09 ^a	0.8±0.2 ^a	0.2±0.06 ^a

*Control group - not vaccinated against NDV. Means followed by the same letter, in the same column are not different at 5% of probability by Tukey test (p>0.05)

context, Schultz *et al.* (1970) observed a reduction in total protein concentrations in turkeys with blue comb. Chicken infected with NDV also showed a decrease in total protein concentrations, although it is not clear if the globulin concentrations were also affected (Squibb *et al.*, 1971). On the other hand, Rivetz *et al.* (1977) found significant differences in total protein concentrations in birds inoculated with a virulent strain of NDV, with a reduction in total protein and albumin concentrations but without any alterations in globulin concentrations. Talebi (2006) also reported significant differences in total protein and albumin concentrations in broiler chickens vaccinated against NDV, infectious bronchitis and infectious bursal disease. Another important possibility is a decrease of serum proteins caused by a reduction in food intake due to anorexia in ring-necked pheasants vaccinated with the LaSota strain. These results might indicate that this situation is due to the organ tropism of each strain (Rivetz *et al.*, 1977).

Because of the above discussed results on the blood proteinogram of ring-necked pheasants vaccinated or not against NDV, with the main differences in 52 and 80 day-old birds, we chose to determine the electrophoretic protein profile of these birds. According to Lumeij (1987, 2008) and Rosenthal (2000) total protein determinations in birds (by biuret method) without information on protein electrophoresis have limited value to evaluate the health status.

The electrophoretic serum protein concentrations of ring-necked pheasants, vaccinated or not against NDV are shown in Tables 3-4. Five protein fractions were obtained: albumin, alpha-1 globulin, alpha-2 globulin, beta-globulin and gamma-globulin. This electrophoretic protein profile was similar to broiler chickens (Filipovic *et al.*, 2007), ostriches (Polat *et al.*, 2004), rhea (Conrado *et al.*, 2007) and ring-necked pheasant's protein

electrophoretic pattern (Müller, 1995). However, the pre-albumin fraction was not observed, even though adult broiler breeders (Hasegawa *et al.*, 2002), ducks (Green *et al.*, 1982) and psittacine birds (Lumeij and Overduin, 1990) often demonstrate this fraction. Although protein electrophoresis estimates the albumin, alpha-1 globulin, alpha-2 globulin, beta-globulin and gamma-globulin concentrations, no statistical difference was found between the vaccinated and non-vaccinated groups (Tables 3-4). These results are not compatible with information available in literature, especially in relation to gamma-globulins concentrations. This fraction was not significantly different between vaccinated or not vaccinated ring-necked pheasants (Tables 3-4). The gamma-globulin fraction mainly consists of immunoglobulins (IgA, IgM, IgG or IgY and IgE) that are produced by the immune system in response to an antigenic stimulus. Thus, if there is an immune response with antibody production, the gamma-globulin fraction is elevated (Rosenthal, 2000), often with a decrease in albumin concentrations in these cases (Lumeij, 2008). In this context, in NDV infected birds, Clark and Foster (1968) found decreased albumin concentrations and elevated gamma-globulin concentrations. However, albumin and globulin concentrations that are derived using this method (electrophoresis) do not necessarily match those that are derived using spectrophotometric (biuret) methods (Campbell, 2004). On the other hand, some immunoglobulins may migrate to beta-globulin fraction (Hochleithner, 1994). As the electrophoretic protein profile did not clearly indicate the immune responses or the antigenic stimulus in the present study, it would be interesting and even necessary to determine, separately, the immunoglobulin fractions, such as IgG, IgM and IgA, to evaluate the specific IgG concentrations in birds vaccinated or not against NDV.

Conclusions: Juvenile ring-necked pheasants showed alterations on total serum protein, albumin and globulin concentrations suggesting that the blood proteinogram profile was affected by vaccination against NDV, especially with LaSota strain at 52 and 80 days of age, although no difference was observed in the electrophoretic protein profile.

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