

Evaluation of Experimental Vaccination Against Newcastle Disease and the Blood Proteinogram in Ring - Necked Pheasants (*Phasianus colchicus*) During Breeding Season

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Abstract: This study aimed to evaluate humoral immune responses and the blood proteinogram of ring-necked pheasants (*Phasianus colchicus*), vaccinated against Newcastle disease, during breeding season. Eighty adult birds (52 to 60 week-old) were distributed into four different groups: G1- vaccinated with NDV Ulster 2C strain, G2- vaccinated with NDV B1 strain, G3- vaccinated with NDV LaSota strain and G4- not vaccinated (control). The immune response was evaluated by the HI test. The blood proteinogram of these birds also determined. The vaccination programs with Ulster 2C, B1 and LaSota strains were equally efficient to stimulate humoral immune responses. Vaccinated ring-necked females with LaSota strain showed significantly ($p < 0,05$) alterations on serum total protein and albumin concentrations.

Key words: Ring-necked pheasant, serum total protein, Newcastle disease, Ulster 2C, B1 and LaSota strains, albumin and globulins

Introduction

Newcastle disease (ND) remains as the most important poultry virosis with highly infectious ability, affecting domestic and wild birds. Routine vaccination combined with sacrifice of affected birds has helped to control virulent disease caused by the ND virus (NDV), classified as *Avian parainfluenzavirus* type 1. This disease is the main health barrier for the international trade of poultry and its products between countries (OIE, 1996) and affects several birds species (Kaleta and Baldauf, 1988). The commercial production of the ring-necked pheasant is distributed in several countries around the world, specially in Europe for shooting as a sport on game sport estates (Aldous and Alexander, 2007). Also the potential of these birds to produce high nutritive meat is increasing in many countries. However, there is little information available on health control programs in this species.

Blood proteins play roles in the maintenance of colloid osmotic pressure, as a rapid substitute for indispensable amino acids, assuring glucose through gluconeogenesis, in transport of minerals and hormones, in build of enzymes and immune system in the organism. Thus, the blood proteinogram has an exceptional significance in homeostasis maintenance. Therefore, the present study aimed to evaluate vaccination programs against Newcastle disease and

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

Group	Mean antibody titers measured by HI test (\log_2)					
	Ring-necked pheasants age (weeks)					
	52	54	56	58	60	
I*	Ulster 2C	0.0	6.7a	6.1a	4.2a	4.6a
II*	B ₁	0.0	7.4a	6.2a	3.4a	4.7a
III*	LaSota	0.0	7.5a	5.9a	4.2a	5.0a
IV*	-	0.0	0.0b	0.0b	0.0b	0.0b

*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 ($p > 0,05$).

the blood proteinogram of adult ring-necked pheasants during breeding season.

Materials and Methods

Experimental birds and management: A total number of 80 (52 to 60 week-old) adult ring-necked pheasants were distributed into four different treatments of 20 pheasants each, as shown in Table 1. Each group was divided into four repetitions with five pheasants each, 1 male: 4 females, allocated in experimental floor-pen housed during breeding season, receiving water and feed *ad libitum*. The feed was formulated with corn and soybean according with NRC (1994) recommendations. Birds were designated to treatments, according to

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Table 2: Total serum protein, albumin and globulin in adult Ring-necked pheasants (*Phasianus colchicus*) females, during breeding season, vaccinated or not against Newcastle disease (Mean±SD)

Vaccination		Total serum protein (Tsp) (g/dL), albumin (Alb) (g/dL) and globulin (Glo) (g/dL) concentrations														
52 weeks		Ring-necked pheasants age (weeks)														
Group	Revaccination	52			54			56			58			60		
		Tsp	Alb	Glo	Tsp	Alb	Glo	Tsp	Alb	Glo	Tsp	Alb	Glo	Tsp	Alb	Glo
I	Ulster 2C	5.1± 0.4a	3.1± 0.1a	2.1± 0.3a	6.4± 0.8a	3.3± 0.3a	3.1± 0.5a	6.1± 0.7a	3.3± 0.3a	2.8± 0.7a	5.4± 1.0a	2.8± 0.2ab	2.6± 0.7a	5.6± 0.6ab	2.9± 0.3a	2.6± 0.4ab
II	B ₁	5.1± 0.4a	3.1± 0.5a	2.1± 0.4a	6.0± 0.6a	2.9± 0.1a	3.1± 0.4a	5.9± 0.1a	3.0± 0.1a	2.9± 0.1a	5.2± 0.7a	2.6± 0.2b	2.5± 0.5a	5.7± 0.3ab	2.9± 0.2a	2.7± 0.2ab
III	LaSota	5.2± 0.5a	3.1± 0.3a	2.1± 0.3a	5.7± 0.5a	3.1± 0.1a	2.6± 0.4a	6.2± 0.5a	3.2± 0.1a	3.2± 0.6a	6.2± 0.7a	2.9± 0.2a	3.3± 0.6a	6.2± 0.4b	2.9± 0.2a	3.2± 0.3b
IV*	-	4.5± 0.7a	2.6± 0.2a	1.9± 0.6a	5.5± 0.9a	3.1± 0.5a	2.4± 0.4a	5.9± 1.0a	3.1± 0.3a	3.0± 0.9a	5.8± 0.4a	2.7± 0.7ab	2.7± 0.4a	5.4± 0.6a	2.8± 0.2a	2.5± 0.4a

*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)

vaccination strain as G1 (Ulster 2C), G2 (B1), G3 (LaSota) and G4 - control (not vaccinated).

Vaccines: Recently manufactured live NDV vaccines (Ulster 2C, B1 and LaSota) were applied to each experimental group, according to Paulillo *et al.* (1982) and Paulillo *et al.* (1996). Birds were vaccinated at 52 weeks of age and revaccinated at 56 weeks of age with the same vaccine strain that was applied in the first vaccination. Vaccine titers were obtained by determining 50% of the embryo-infecting dose in embryonated eggs of specific-pathogen-free breeders at 8 and 10 days of incubation. Titers of live vaccine strains Ulster 2C, B1 and LaSota were 7.15 log₁₀/0.1mL, 7.2 log₁₀/0.1mL, and 7.35 log₁₀/0.1mL, respectively. Birds were vaccinated and revaccinated by eye drop.

Serology: Blood samples of the ring-necked pheasants were collected from the ulnar superficial vein, from 52 to 60 weeks of age, at regular fourteen-day intervals. Sera were inactivated at 56°C for 30 minutes, frozen and stored at -20°C. Sera samples were submitted to inhibition of hemagglutination (HI) test, according to Cunningham (1971).

Blood proteinogram: Serum total protein and albumin concentrations were determined with an automated chemistry analyzer. The globulin value was determined by the difference between serum total protein and albumin. The control of the chemical analysis was made using Qualitrol-N.

The dates 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

Ring-necked pheasants from all groups, vaccinated or not against ND, did not show any clinical signs of post-vaccinal reactions. Mean antibody titers against NDV from ring-necked pheasants are presented in Table 1. At 52 weeks of age, none of the birds showed maternally-

derived antibodies against NDV, as breeders were not submitted to any vaccination programs against this disease. As the control group (G4) was not vaccinated, its antibody titers were null from 52 to 60 weeks of age. At 54 weeks of age, antibody titers against NDV were detected in the vaccinated groups. This active immunity was induced by vaccination at 52 weeks of age.. Ring-necked pheasants were revaccinated at 56 weeks of age and this procedure maintained antibody titers against NDV up to 60 weeks of age. The low invasion capacity of the B1 strain (Hofstad, 1951) and the low diffusion potential of the Ulster 2C strain (McFerran and Nelson, 1971), may explain the low to moderate antibody titers (G1 and G2) detected by HI in vaccinated ring-necked pheasants. On the other hand, the moderate to high antibody titers detected for the ring-necked pheasants vaccinated with the LaSota strain (G3) are compatible with the great diffusion potential of this strain (Winterfield *et al.*, 1957). The Tukey test did not demonstrate significant differences among groups vaccinated with Ulster 2C, B1 and LaSota strains. This suggests that vaccination, independent of the vaccination program used, can efficiently produce antibody titers against NDV.

The blood proteinogram values for adult ring-necked pheasants females and males are shown in Tables 2 and 3. The results of males and females are separated because Schmidt *et al.* (2007) found significant differences in serum total protein and albumin among males and females during breeding season. Which could be due to the egg production that may affect the concentration of the blood proteins. According to Thrall (2004) and Lumeij (1997), the hyperproteinemia is associated with an increase in vitellogenin and lipoproteins which is induced by estrogens because they are necessary for yolk production.

There were no statistical differences in the total protein, albumin and globulin concentrations among the different groups of ring-necked males, vaccinated or not against NDV (Table 3).

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Table 3: Total serum protein, albumin and globulin in adult Ring-necked pheasants (*Phasianus colchicus*) males, during breeding season, vaccinated or not against Newcastle disease (Mean±SD)

Group	Vaccination	Total serum protein (Tsp) (g/dL), albumin (Alb) (g/dL) and globulin (Glo) (g/dL) concentrations														
		Ring-necked pheasants age (weeks)														
52 weeks	Revaccination	52			54			56			58			60		
		Tsp	Alb	Glo	Tsp	Alb	Glo	Tsp	Alb	Glo	Tsp	Alb	Glo	Tsp	Alb	Glo
I	Ulster 2C	5.0± 0.9a	2.9± 0.4a	2.0± 0.5a	4.1± 0.4a	2.4± 0.2a	1.6± 0.2a	4.3± 0.7a	2.5± 0.1a	1.9± 0.5a	3.7± 1.0a	1.9± 0.4a	1.8± 0.6a	4.4± 0.7a	2.5± 0.2a	1.9± 0.5a
II	B ₁	3.5± 0.0a	2.2± 0.0a	1.3± 0.0a	4.2± 0.1a	2.4± 0.0a	1.7± 0.0a	4.6± 0.3a	2.4± 0.2a	2.1± 0.3a	3.9± 0.4a	2.1± 0.1a	1.8± 0.2a	4.9± 0.4a	2.6± 0.2a	2.2± 0.3a
III	LaSota	4.0± 0.1a	2.3± 0.0a	1.6± 0.0a	4.4± 0.3a	2.5± 0.1a	1.8± 0.4a	4.3± 1.0a	2.4± 0.3a	1.9± 0.7a	3.8± 0.4a	2.0± 0.1a	1.7± 0.4a	4.8± 0.6a	2.6± 0.1a	2.1± 0.5a
IV*	-	4.3± 0.7a	2.5± 0.1a	1.8± 0.5a	4.7± 0.6a	2.7± 0.2a	2.0± 0.4a	4.7± 0.7a	2.7± 0.3a	1.9± 0.4a	4.0± 0.3a	2.2± 0.1a	1.8± 0.2a	4.7± 0.4a	2.6± 0.1a	2.1± 0.3a

*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)

Female ring-necked pheasants of 60 weeks of age, vaccinated with LaSota strain showed significant difference in relation to the control group for total protein and globulin concentrations. However, no statistical difference was found for these parameters among the vaccinated groups (Ulster 2C, B1 and LaSota strains). There was also no statistical difference between vaccinated birds with Ulster 2C and B1 (G1 and G2) strains and the control group (G4). This suggests that vaccination with LaSota strain was responsible for the hyperproteinemia due to hyperglobulinemia because globulin concentrations may rise with antigenic stimulus (Lumeij, 1997). These results are compatible with the great diffusion potential of LaSota strain (Winterfield et al., 1957). According to Rivetz et al. (1977), protein concentrations are altered in viral infections of birds. In general, in acute or chronic inflammatory conditions a rise in total protein caused by elevated globulin concentrations may occur (Lumeij, 1987). Washburn and Eidson (1970) also found elevated globulin concentrations in chickens exposed to Marek's disease.

Conclusions: Adult ring-necked pheasants showed an equally efficient and moderated antibody response when vaccinated with commercially available live ND vaccines for chickens, without any clinical signs of post-vaccinal reaction. Total serum protein and globulin concentrations of female ring-necked pheasants of 60 weeks of age were affected by ND vaccination with LaSota strain.

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