

The haematological profile of female bronze turkeys (*Meleagris gallopavo*) vaccinated with various commercial strains of Newcastle disease

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The effects of vaccination on avian blood parameters are poorly understood. The present study was designed to evaluate whether different strains (Ulster 2C, B1, live LaSota and inactivated LaSota) of Newcastle disease vaccines had an effect on the haematological profile of female turkeys. Seventy-five female turkeys were allocated to treatment groups according to vaccination strain. All the birds, except those in the control group, were vaccinated at 32 weeks of age and revaccinated at 40 and 48 weeks of age. Blood samples were obtained for haematological analyses and serum samples for the haemagglutination inhibition test. Haemoglobin concentration was significantly lower ($p < 0.05$) in vaccinated female turkeys than in the control birds 28 days after vaccination. Monocytes were significantly higher ($p < 0.05$) in 44-week-old female turkeys vaccinated with inactivated LaSota strain compared with the other groups. Turkeys vaccinated with the B1 strain showed significantly higher ($p < 0.05$) total white blood cell counts compared with the other groups vaccinated with various commercial strains of the Newcastle disease virus. In conclusion, female turkeys showed significant differences in haemoglobin concentrations, monocytes and white blood cell counts when vaccinated against Newcastle disease.

Introduction

The bronze turkey (*Meleagris gallopavo*, Linnaeus, 1758) is the result of a cross between domestic turkeys brought from Europe and the wild turkey. They are selected to produce highly nutritive meat and feathers and for breeding flocks in United States, France, Italy, Chile, Brazil, Germany, United Kingdom, Portugal and Mexico (Brant 1998; Windhorst 2006).

Haematology is becoming a valuable routine diagnostic tool in the evaluation of avian patients and may also provide information that identifies diseases that affect the cells in the peripheral blood of birds (Campbell 2004). However, avian clinical pathology is poorly understood because there is a lack of information about reference values and haemogram responses after antigen exposure and immune stimulation. This study was conducted to determine whether different Newcastle disease (ND) vaccine strains (Ulster 2C, B1, live LaSota and inactivated LaSota) had an effect on the haematological profile of female turkeys vaccinated with various commercial strains of ND, as the effects of vaccination on avian haematology are poorly understood.

Material and methods

Seventy-five female bronze turkeys (*M. gallopavo*, Linnaeus, 1758) were used in the study. They were kept in a floor-pen house and received water and food *ad libitum*. The feed was formulated with corn, soybean, lysine, methionine, methionine + cystine and threonine according to specific National Research Council (NRC) recommendations for turkeys (NRC 1994). The hens were laying eggs for 20 weeks, from 32 to 52 weeks of age.

The 75 female turkeys were assigned in a completely randomised experimental design into five different treatment groups of 15 birds each. They were designated according to vaccination strain as G1 (Ulster 2C), G2 (B1), G3 (live LaSota), G4 (inactivated LaSota) and G5 (control group – not vaccinated). Commercial line ND vaccines (Ulster 2C, B1 and LaSota strains) were used (Paulillo *et al.* 1996). All the birds except those in the control group were vaccinated at 32 weeks of age and revaccinated at 40 and 48 weeks of age with the same vaccine strain that was applied in the first vaccination. Birds in G1 to G3 were vaccinated by eye drop using the Ulster 2C, B1 and live LaSota strains respectively and birds in G4 were vaccinated subcutaneously with inactivated LaSota strain.

Blood samples were collected from the superficial ulnar vein into a 2 mL glass tube containing ethylenediaminetetraacetic acid (EDTA) at 32, 36, 38, 44, 48 and 52 weeks for haematological

analyses and serum samples were obtained and submitted for the inhibition of haemagglutination (HI) test (ed. Cunningham 1971).

Thin blood smears were made immediately after blood collection to avoid any interference with cell structure and the haematological analyses were performed. Total red blood cell (RBC) and white blood cell (WBC) counts were performed by a manual method using a haemocytometer with blood diluted with 0.01% of toluidine blue (Zinkl 1986).

The haemoglobin concentration was measured by the cyanmethaemoglobin method. The mixture of cyanmethaemoglobin reagent and blood was centrifuged prior to analysis to remove the free nuclei from lysed erythrocytes to avoid an overestimation of the haemoglobin. The haematocrit (HT) was determined by the microhaematocrit method in capillary tubes and centrifuged at 12 000 g for 5 min. Differential WBC counts, using an average of 200 cells, were made from blood films stained with Wright's stain (ed. Jain 1986).

The data was analysed by analysis of variance (ANOVA) and those with statistical differences were submitted to Tukey's test at 0.05% using Statview® (version 5.0).

Ethical considerations

The study was conducted according to the Ethical Principles of Animal Experimentation adopted by the Brazilian College of Animal Experimentation (COBEA) and was approved by the Ethics Committee of Animal Welfare (CEBEA) with protocol number 005624-08.

There were no physical or psychological risks involved in the experimental trials.

Results

The birds were observed daily for mortality; none was recorded. Egg production and weekly weights were not recorded because they were not the focus of the study.

Mean antibody titres against ND from female turkeys are presented in Table 1. As the control group (G5) was not vaccinated, its antibodies titres were nil from 32 to 52 weeks of age.

The RBC, HT, haemoglobin (HB), WBC, heterophils (Het.), lymphocytes (Lymp.), eosinophils (Eosin.), monocytes (Mon.) and basophils (Bas.) results are presented in Tables 2–7. There were no significant differences for RBC and HT amongst the different groups of female turkeys vaccinated against ND or not (Tables 2–7). At 36 weeks of age, the haemoglobin concentration was significantly lower ($p < 0.05$) in female turkeys vaccinated with Ulster 2C, B1, live LaSota and inactivated LaSota strains compared with the control birds (G5) 28 days after vaccination (Table 3). At this age, these turkeys showed high mean antibody titres (Table 1).

No significant differences were found in this study for heterophils, lymphocytes, eosinophils and basophils of female turkeys vaccinated with various commercial strains of ND. Monocytes were significantly higher ($p < 0.05$) in 44-week-old female turkeys vaccinated with inactivated LaSota strain in relation to other groups, showing the highest antibody mean levels.

At 52 weeks of age, the WBC count was significantly higher ($p < 0.05$) for B1-vaccinated female turkeys compared with the other groups vaccinated with various commercial strains of ND, although no significant differences were observed for lymphocytes.

Discussion

The results from Tables 2 to 7 suggest that vaccinated birds may have developed anaemia. According to Dein (1986), viral infections in birds (chicken anaemia virus) have resulted in non-regenerative anaemia. In these cases, the bone marrow was hypoplastic, suggesting a severe inhibition of haematopoiesis. Campbell (2004) and Morrissey (2000) also observed anaemia caused by decreased production of RBC in inflammatory diseases of infectious aetiology. Also, 38-week-old and 44-week-old female turkeys vaccinated against ND with Ulster 2C strain showed significant decreases ($p < 0.05$) in haemoglobin concentrations, suggesting a prolonged anaemia in turkeys, probably caused by the vaccine antigens. On the other hand, the stress of vaccination can decrease food consumption and therefore cause anaemia (ed. Jain 1986).

Juvenile ring-necked pheasants also showed decreased haemoglobin concentrations when vaccinated against ND with Ulster 2C strain (Schmidt *et al.* 2008).

Lymphocytes were the major circulating leukocyte in these female turkeys vaccinated with various commercial strains

TABLE 1: Mean antibody titres measured by the haemagglutination test (\log_2) of female bronze turkeys (*Meleagris gallopavo*) vaccinated with various commercial strains of Newcastle disease.

Group	Vaccine	Female turkeys' age (weeks)										
		32	34	36	38	40	42	44	46	48	50	52
1†	Ulster 2C	0.0	6.7 ^a	7.4 ^a	6.8 ^a	7.5 ^a	10.4 ^a	6.8 ^a	6.3 ^a	5.7 ^a	6.9 ^a	5.9 ^a
2†	B1	0.0	7.7 ^b	7.0 ^a	7.4 ^a	7.0 ^a	8.6 ^{bc}	6.0 ^a	6.8 ^a	6.9 ^a	7.2 ^a	7.1 ^{bc}
3†	LaSota (live)	0.0	7.2 ^{ab}	7.2 ^a	8.0 ^a	8.3 ^a	7.5 ^b	6.6 ^a	6.4 ^a	6.8 ^a	7.9 ^a	6.1 ^{bc}
4†	LaSota (inactivated)	0.0	0.0 ^c	10.7 ^b	10.5 ^b	10.4 ^b	9.2 ^{bc}	9.7 ^b	8.1 ^b	9.1 ^b	10.2 ^b	8.9 ^d
5‡	Control	0.0	0.0 ^c	0.0 ^c	0.0 ^c	0.0 ^c	0.0 ^d	0.0 ^c	0.0 ^c	0.0 ^c	0.0 ^c	0.0 ^e

HI, haemagglutination.

†, Groups were also vaccinated with LaSota strain at 10, 35, 90 and 140 days of age.

‡, Control group – not vaccinated against Newcastle disease.

^{a, b, c, d, e}, Means followed by the same letter, in the same column, are not different at 5% probability by Tukey's test ($p > 0.05$).

TABLE 2: Haematological values in female bronze turkeys (*Meleagris gallopavo*) (32 weeks of age) vaccinated with various commercial strains of Newcastle disease (mean \pm s.d.).

Group	RBC (/mm ³)	HT (%)	HB (g/dL)	WBC (/mm ³)	Het. (/mm ³)	Lymp. (/mm ³)	Eosin. (/mm ³)	Mon. (/mm ³)	Bas. (/mm ³)
1†	1 061 600 \pm 174 704	38.4 \pm 5.6	16.5 \pm 2.2	8100 \pm 3143	35.7 \pm 2.9	52.8 \pm 15.4	0.5 \pm 0.7	4.4 \pm 3.5	6.6 \pm 3.1
2†	1 068 800 \pm 352 937	36.5 \pm 3.6	16.1 \pm 1.8	6200 \pm 2044	38.2 \pm 16.0	50.5 \pm 17.5	3.2 \pm 3.9	2.7 \pm 2.1	5.3 \pm 1.8
3†	1 152 253 \pm 196 800	37.2 \pm 3.4	17.4 \pm 1.5	7466.6 \pm 3583	27.6 \pm 9.8	60.3 \pm 10.9	1.0 \pm 0.9	4.0 \pm 2.4	7.0 \pm 3.2
4†	1 006 923 \pm 147 446	38.0 \pm 2.4	17.3 \pm 2.0	6000 \pm 2309	32.2 \pm 7.8	56.2 \pm 9.4	0.5 \pm 0.6	2.9 \pm 2.1	8.0 \pm 2.7
5‡	1 065 090 \pm 329 057	35.9 \pm 3.8	17.0 \pm 2.2	6363.6 \pm 2730	36.0 \pm 13.5	47.9 \pm 14.3	2.9 \pm 2.8	5.0 \pm 3.4	8.0 \pm 3.7

RBC, red blood cells; HT, haematocrit; HB, haemoglobin; WBC, white blood cells; Het., heterophils; Lymp., lymphocytes; Eosin., eosinophils; Mon., monocytes; Bas., basophils.

†, Groups were also vaccinated with LaSota strain at 10, 35, 90 and 140 days of age.

‡, Control group – not vaccinated against Newcastle disease.

TABLE 3: Haematological values in female bronze turkeys (*Meleagris gallopavo*) (36 weeks of age) vaccinated with various commercial strains of Newcastle disease (mean \pm s.d.).

Group	RBC (/mm ³)	HT (%)	HB (g/dL)	WBC (/mm ³)	Het. (/mm ³)	Lymp. (/mm ³)	Eosin. (/mm ³)	Mon. (/mm ³)	Bas. (/mm ³)
1†	1 193 090 \pm 200 200	36.6 \pm 5.4	20.6 \pm 2.6 ^a	6363.6 \pm 2063	31.0 \pm 12.9	60.1 \pm 8.3	1.0 \pm 1.2	2.5 \pm 2.4	5.2 \pm 2.1
2†	1 313 333 \pm 132 272	35.6 \pm 3.0	20.5 \pm 1.6 ^a	6777.7 \pm 1856	38.2 \pm 16.0	65.2 \pm 7.0	0.8 \pm 0.8	3.2 \pm 2.4	5.1 \pm 2.7
3†	1 122 000 \pm 271 949	34.9 \pm 2.7	22.2 \pm 2.4 ^a	6500 \pm 1716	27.6 \pm 9.8	59.9 \pm 6.6	0.5 \pm 0.5	2.3 \pm 1.5	8.3 \pm 3.5
4†	1 135 600 \pm 191 011	37.1 \pm 2.1	21.0 \pm 2.1 ^a	7000 \pm 2108	32.2 \pm 7.8	64.0 \pm 3.1	0.4 \pm 0.7	3.1 \pm 1.3	6.0 \pm 3.2
5‡	1 136 444 \pm 214 759	35.7 \pm 3.0	29.4 \pm 3.1 ^b	7000 \pm 2449	36.0 \pm 13.5	58.5 \pm 6.5	0.4 \pm 0.5	3.0 \pm 2.4	5.3 \pm 2.2

RBC, red blood cells; HT, haematocrit; HB, haemoglobin; WBC, white blood cells; Het., heterophils; Lymp., lymphocytes; Eosin., eosinophils; Mon., monocytes; Bas., basophils.

†, Groups were also vaccinated with LaSota strain at 10, 35, 90 and 140 days of age.

‡, Control group – not vaccinated against Newcastle disease.

^{a,b}, Means followed by the same letter, in the same column, are different at 5% probability by Tukey's test ($p < 0.05$).

TABLE 4: Haematological values in female bronze turkeys (*Meleagris gallopavo*) (38 weeks of age) vaccinated with various commercial strains of Newcastle disease (mean \pm s.d.).

Group	RBC (/mm ³)	HT (%)	HB (g/dL)	WBC (/mm ³)	Het. (/mm ³)	Lymp. (/mm ³)	Eosin. (/mm ³)	Mon. (/mm ³)	Bas. (/mm ³)
1†	1 295 000 \pm 38 557	35.2 \pm 3.5	17.7 \pm 2.8	8000 \pm 5416	29.5 \pm 9.2	65.5 \pm 9.5	0.5 \pm 1.0	1.2 \pm 0.9	3.2 \pm 2.2
2†	1 256 800 \pm 192 528	38.0 \pm 2.9	17.2 \pm 0.9	6000 \pm 2345	31.0 \pm 9.1	60.4 \pm 6.5	0.8 \pm 1.0	2.0 \pm 1.5	5.8 \pm 3.5
3†	1 279 000 \pm 346 107	32.5 \pm 2.5	18.6 \pm 4.4	6250 \pm 3304	31.2 \pm 7.2	57.5 \pm 5.5	1.0 \pm 1.4	2.2 \pm 1.5	8.0 \pm 4.6
4†	1 146 400 \pm 223 617	38.2 \pm 3.1	19.3 \pm 2.5	5400 \pm 2302	36.4 \pm 1.3	54.8 \pm 2.3	1.0 \pm 0.7	1.8 \pm 0.4	6.0 \pm 2.5
5‡	1 158 666 \pm 267 906	38.0 \pm 2.0	20.4 \pm 1.7	5000 \pm 2000	31.1 \pm 6.3	62.8 \pm 8.6	0.5 \pm 0.5	1.6 \pm 1.2	3.6 \pm 1.9

RBC, red blood cells; HT, haematocrit; HB, haemoglobin; WBC, white blood cells; Het., heterophils; Lymp., lymphocytes; Eosin., eosinophils; Mon., monocytes; Bas., basophils.

†, Groups were also vaccinated with LaSota strain at 10, 35, 90 and 140 days of age.

‡, Control group – not vaccinated against Newcastle disease.

TABLE 5: Haematological values in female bronze turkeys (*Meleagris gallopavo*) (44 weeks of age) vaccinated with various commercial strains of Newcastle disease (mean \pm s.d.).

Group	RBC (/mm ³)	HT (%)	HB (g/dL)	WBC (/mm ³)	Het. (/mm ³)	Lymp. (/mm ³)	Eosin. (/mm ³)	Mon. (/mm ³)	Bas. (/mm ³)
1†	1 125 555 \pm 303 133	34.8 \pm 4.3	15.9 \pm 2.5	6556 \pm 2698	34.4 \pm 13.2	57.7 \pm 13.8	0.4 \pm 0.5	3.3 \pm 2.1 ^a	4.0 \pm 2.1
2†	1 375 555 \pm 259 348	37.0 \pm 2.3	19.5 \pm 4.4	5444 \pm 1509	29.2 \pm 11.7	59.7 \pm 12.8	0.6 \pm 1.0	3.5 \pm 1.3 ^a	6.7 \pm 2.6
3†	1 162 285 \pm 250 650	33.1 \pm 4.0	19.7 \pm 2.1	7714 \pm 4112	42.8 \pm 13.7	48.2 \pm 12.8	0.4 \pm 0.7	2.0 \pm 1.5 ^a	6.4 \pm 2.3
4†	1 187 200 \pm 222 764	38.7 \pm 5.2	21.2 \pm 2.0	5500 \pm 1841	34.4 \pm 9.3	53.8 \pm 8.0	0.9 \pm 1.2	5.2 \pm 2.5 ^b	5.7 \pm 4.9
5‡	1 284 285 \pm 216 201	36.1 \pm 4.5	22.0 \pm 6.9	7214 \pm 2939	35.6 \pm 7.5	56.6 \pm 9.0	0.4 \pm 0.5	2.2 \pm 1.4 ^a	5.0 \pm 3.1

RBC, red blood cells; HT, haematocrit; HB, haemoglobin; WBC, white blood cells; Het., heterophils; Lymp., lymphocytes; Eosin., eosinophils; Mon., monocytes; Bas., basophils.

†, Groups were also vaccinated with LaSota strain at 10, 35, 90 and 140 days of age.

‡, Control group – not vaccinated against Newcastle disease.

^{a,b}, Means followed by the same letter, in the same column, are different at 5% probability by Tukey's test ($p < 0.05$).

TABLE 6: Haematological values in female bronze turkeys (*Meleagris gallopavo*) (48 weeks of age) vaccinated with various commercial strains of Newcastle disease (mean \pm s.d.).

Group	RBC (/mm ³)	HT (%)	HB (g/dL)	WBC (/mm ³)	Het. (/mm ³)	Lymp. (/mm ³)	Eosin. (/mm ³)	Mon. (/mm ³)	Bas. (/mm ³)
1†	1 169 000 \pm 166 493	38.0 \pm 4.0	16.1 \pm 1.3	7000 \pm 2138	40.8 \pm 11.0	48.75 \pm 14.0	0.37 \pm 0.7	3.7 \pm 2.3	6.3 \pm 3.5
2†	1 172 000 \pm 303 255	37.3 \pm 34.5	16.6 \pm 1.9	5888 \pm 1536	35.5 \pm 8.4	55.0 \pm 7.6	0.88 \pm 0.6	3.0 \pm 1.5	5.7 \pm 4.5
3†	1 119 333 \pm 162 469	37.3 \pm 3.7	18.2 \pm 3.1	6000 \pm 1897	42.6 \pm 9.2	45.3 \pm 13.8	1.16 \pm 0.7	5.2 \pm 5.4	5.6 \pm 2.8
4†	1 044 000 \pm 245	37.4 \pm 4.4	19.3 \pm 2.8	5727 \pm 1902	38.3 \pm 11.2	51.0 \pm 10.9	0.72 \pm 1.0	3.3 \pm 2.4	6.4 \pm 3.2
5‡	1 149 142 \pm 330 621	39.0 \pm 3.4	18.1 \pm 2.6	6428 \pm 2765	40.5 \pm 14.7	51.4 \pm 15.3	0.64 \pm 0.8	1.7 \pm 1.3	5.7 \pm 2.9

RBC, red blood cells; HT, haematocrit; HB, haemoglobin; WBC, white blood cells; Het., heterophils; Lymp., lymphocytes; Eosin., eosinophils; Mon., monocytes; Bas., basophils.

†, Groups were also vaccinated with LaSota strain at 10, 35, 90 and 140 days of age.

‡, Control group – not vaccinated against Newcastle disease.

TABLE 7: Haematological values in female bronze turkeys (*Meleagris gallopavo*) (52 weeks of age) vaccinated with various commercial strains of Newcastle disease (mean \pm s.d.).

Group	RBC (/mm ³)	HT (%)	HB (g/dL)	WBC (/mm ³)	Het. (/mm ³)	Lymp. (/mm ³)	Eosin. (/mm ³)	Mon. (/mm ³)	Bas. (/mm ³)
1†	1 308 000 \pm 233 009	37.0 \pm 5.8	18.7 \pm 4.2	5000 \pm 1414 ^a	34.0 \pm 15.5	54.7 \pm 20.0	0.85 \pm 0.9	4.4 \pm 5.3	6.0 \pm 2.5
2†	1 336 000 \pm 210 902	37.3 \pm 4.5	20.5 \pm 3.1	7250 \pm 2052 ^b	39.2 \pm 10.8	52.6 \pm 10.4	0.87 \pm 0.9	3.3 \pm 2.5	3.8 \pm 2.4
3†	1 092 000 \pm 293 557	30.8 \pm 1.9	17.1 \pm 1.4	4000 \pm 707 ^a	36.4 \pm 16.4	57.0 \pm 15.9	0.80 \pm 1.3	1.6 \pm 0.8	4.2 \pm 2.7
4†	1 132 888 \pm 180 441	33.1 \pm 4.8	19.5 \pm 3.5	5555 \pm 726 ^a	35.0 \pm 11.4	56.6 \pm 12.4	0.33 \pm 0.7	3.0 \pm 2.2	5.0 \pm 3.4
5‡	1 180 333 \pm 147 201	34.3 \pm 3.7	18.1 \pm 3.2	5250 \pm 1712 ^a	34.4 \pm 9.8	58.0 \pm 10.5	0.75 \pm 0.6	1.7 \pm 2.0	5.0 \pm 2.4

RBC, red blood cells; HT, haematocrit; HB, haemoglobin; WBC, white blood cells; Het., heterophils; Lymp., lymphocytes; Eosin., eosinophils; Mon., monocytes; Bas., basophils.

†, Groups were also vaccinated with LaSota strain at 10, 35, 90 and 140 days of age.

‡, Control group – not vaccinated against Newcastle disease.

^{a,b}, Means followed by the same letter, in the same column, are different at 5% probability by Tukey's test ($p < 0.05$).

of ND, irrespective of the age at which they were evaluated. Haematological studies in chickens, bronze and wild turkeys showed similar results, although heterophils are the most abundant leukocyte in peripheral blood of most species of birds (Bounous & Stedman 2000; Bounous *et al.* 2000; Dein 1986; Latimer & Bienzle 2000; Maxwell & Robertson 1998; Schmidt *et al.* 2009). However, the predominance of lymphocytes was also found by Schmidt *et al.* (2007) and Schmidt *et al.* (2008) in ring-necked pheasants vaccinated with various commercial strains of ND.

The monocytes were significantly higher ($p < 0.05$) in female turkeys aged 44 weeks vaccinated with inactivated LaSota strain – this group of turkeys also had the highest antibody mean levels in relation to other groups. It seems reasonable to suggest that vaccination with inactivated LaSota strain elevated the number of monocytes, demonstrating the participation of these cells in the immune response, as they are one of the major effector cells of non-specific immunity in birds (Klansing 1991).

It is possible to speculate that the leukocytosis may be present during one phase of the immune response (Dein 1986) because no significant differences were observed for lymphocytes, but the WBC count was significantly higher ($p < 0.05$) at 52 weeks of age for B1-vaccinated female turkeys when compared with the other groups vaccinated with various commercial strains of ND.

Conclusion

Female bronze turkeys showed significant differences in haemoglobin concentrations, monocytes and WBC when vaccinated against ND, especially with Ulster 2C, inactivated LaSota and B1 strains.

Acknowledgements

Competing interests

The authors declare that they have no financial or personal relationship(s) which may have inappropriately influenced them in writing this article.

Authors' contributions

E.M.d.S.S. (São Paulo State University) was responsible for the experimental and project design and was the project leader. A.C.P. (São Paulo State University) was also responsible for the project design. I.F.C.S. (Eduardo Mondlane University), G.R.V.M (São Paulo State University), J.D. (São Paulo State University) and I.M.L. (São Paulo State University) made conceptual contributions.

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