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THE INTERACTION EFFECT OF ANTIGEN TYPE AND ADMINISTRATION ROUTE ON IMMUNE RESPONSE IN RELATION TO SOME BLOOD CONSTITUENTS IN CHICKEN.

By

G.M.Gebriel, A.A.El-Fiky and Hind.A.A.Khedr

Dept. of Poultry Production, Faculty of Agric., Shibin El-Kom, Minufiya univ., Egypt.

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Abstract: *A total number of 243 of Norfa chicken at 25-wk of age were used to study the interaction effect of antigen types (SRBCs and BSA) and administration routes (IV and IM) on the antibody response in relation to some blood constituents in chickens. Traits studied were antibody titers (high, control and low responses), plasma total proteins (TP), albumin (Alb), globulin (Glob), red blood cells count (RBCs),white blood cells count (WBCs) and hematocrit value (before and after separation).*

The results showed that the injection of SRBCs antigen significantly increased the antibody (Ab) titers (10.429 vs 9.613), the RBCs counts (3.530 vs 2.701 $10^6/mm^3$) and hematocrit values (2.908 vs 2.23 pcv) when compared with the injection of BSA antigen. Also, the intravenous (IV) administration route of antigen , as a direct effect, had significantly higher Ab-titers (10.005 vs 9.549) , RBCs (3.150 vs 2.920 $X10^6/mm^3$) WBCs (57.854 vs 52.608 $X\ 10^3/mm^3$) and hematocrit value (2.381 vs 1.173pcv) when compared with the intramuscular (IM) administration route. In general, the injection of SRBCs,as a natural antigen and using the IV administration route enhanced the immune response and improved most of the blood constituents in chickens.

INTRODUCTION

The avian immune system is a defense system that protect the bird from foreign organisms (antigens). Structurally, the immune system can be classified as specific or non-specific systems, and cells that participate in the immune response and their products **(Kuby, 1992)**. Different natural antigens are used in the divergent selection experiments for enhancement of antibody response. Some of these antigen types are SRBCs **(Siegel and Gross, 1980, Van der Zijpp, 1983 and Gebriel, 1991)** and bovine serum albumin (BSA) **(Paramentier et al., 1998)**. **Gross and Siegel (1990)** found that immunization of different antigen types as SRBCs and BSA induced differences in Ab-titers level in chickens.

Kreukniet et al (1992) reported that the effects of both intramuscular (IM) and intravenous(IV) administration routes with the T- dependent antigen or sheep red blood cells (SRBCs) were studied in two chicken lines selected for either high (H) or low (L) antibody response. Intravenous (IV) and (IM) immunization with the T-dependent antigen bovine serum albumin (BSA) showed line differences similar to those found after I.M. or I.V. immunization with SRBCs. It is postulated that differences in antibody production between the selected lines might be attributed to the differences in T-cell activity. However, **Siegel and Gross (1980), Van der Zijpp et al (1986)** found that the intravenous (IV) and intramuscular (IM) administration

routes induced differences in Ab-titer levels in chickens.

Recently, **Gebriel et al. (2010)** found that some blood constituents concentrations differed greatly in chickens according to the effect of breed, antigen types, antigen administration routes, antibody response and sex. They reported that the total plasma proteins concentration in Norfa chickens ranged from 3.04 to 11.06 g/100 ml with total average of 5.59 g/ 100 ml , total plasma albumin concentration ranged from 1.07 to 6.04 g/100 ml with total average of 2.68 g/ 100 ml , concentration of plasma globulin ranged from 1.97 to 5.02 g/100 ml with total average of 2.91 g/ 100 ml , counts of red blood cells ranged from 1.12 to 4.81X 10^6 /mm^3 with total average of 3.18 X 10^6 /mm^3 and counts of white blood cells ranged from 21.34 to 39.13X 10^3 /mm^3 with total average of 29.89 X 10^3 /mm^3. On the other hand , **Ahmed Nagwa et al., (1997)** concluded that the change in the hematocrit value (PCV) pre and at sexual maturity can be used as indicator for the prediction of egg productivity stage since, the PCV decreased significantly with the progressing of egg production.

Therefore, the aim of the present work was to study the interaction effect of antigen type and administration route on antibody response in relation to some blood constituents in Norfa chickens.

MATERIALS AND METHODS

The present study was carried out at the Poultry Research Farm, Department of Poultry Production, Faculty of Agriculture, Minufiya University, Shibin El- Kom, Egypt, during the period of 2008 to 2009.

1- Chicken Stock:

Norfa strain was used in the present study as a synthetic local strain of chicken developed at the Poultry Research Farm, Department of Poultry Production, Faculty of Agriculture, Minufiya University, Shibin El-Kom. **Abdou, (1996).**

2- Experimental design and treatments:-

A total number of 243 Norfa chicken at 25-wk of age were divided into two groups according to the antigen type. The first group was injected with sheep red blood cells (SRBCs) antigen whereas, the second group was injected with bovine serum albumin (BSA) antigen. Each group was sub divided- according to the antigen administration-route into two subgroups: the first was injected with (SRBCs) and (BSA) intravenously (IV), while the second subgroup was injected with (SRBCs) and (BSA) intramuscularly (IM). Birds of each group were classified into three classes according to their immune response level (high, low and control responses). All birds were fed a starter diet containing 18.05% crude protein till 8^{th} week of age and from 9^{th} to 16^{th} week of age, chickens were fed a growing diet containing 14.01% CP.

2.1. Blood samples collection and determinations:-

Blood samples were collected into heparinized test tube from the wing vein of 243 birds for all groups of chickens. Plasma was separated by centrifugation at 3000 rpm for 15 minutes and stored frozen at -20°C until chemical analysis. Red blood cells and white blood cells counts were determined according to the method of **Coffin (1955) and Schalm (1965).**

Plasma total proteins concentration was quantitatively measured based on colorimetric determination, as described by **Merck (1974).** Albumin concentration was determined by Bio-ADWIC according to

Doumas et al. (1971). Plasma globulin value was obtained by subtracting the value of albumin from the corresponding value of total proteins. Hematocrit value (before and after separation) was determined and expressed as a percentage of packed cell volume (PCV %) according to **Hunsaker (1969).** Thantibody titers of each chicken were determined using the hemagglutination test according to **Siegel and Gross (1980).** Chickens were divided into three groups according to their Ab-titers as LSM ± one S.D including high response (HR), low response (LR) and control response (CR).

3. Statistical analysis:-

Data were analyzed according to SPSS program (SPSS, **1993**). The application of the least means significance test for the differences among the different treatments were done according to **Duncan (1955).** Least- squares analysis of variance was conducted for purposes of separate the gross effects of treatments. (**Snedecor and Cochran, 1980**)

RESULTS AND DISCUSSION

1. Effect of antigen types on high and low primary Ab- titers in Norfa Chickens.

The primary antibody titers against SRBCs and BSA were increased after the primary injection and reached its maximum level in high response chickens (HR), while, the Ab-titers had the lowest level in low response chickens (LR).The control response chickens (CR) had intermediate level for both antigen types **(Table 1)** . The LSM ± S.E of Ab-titers against SRBCs were 19.003, 8.291 and 3.696 for high, control and low responses, respectively. While, the LSM ± S.E against BSA at 7-day post- immunization were 19.003, 7.360 and 2.178 for high, control and low Ab-responses, respectively. The SRBCs significantly enhanced ($P \leq 0.05$) the Ab-titers higher than the BSA antigens. The total average of Ab-titers were 10.429 and 9.613for the SRBCs and BSA, respectively. The present results are similar to the result reported by **Gross and Siegel (1990) who** found that SRBCs had high Ab-response in chickens selected for high or low Ab-titers as compared to BSA antigen.

2. Effect of antigen administration route on high and low primary Ab- titers in Norfa chickens.

The (LSM ± S.E) of Ab-titers against intravenous (IV) injection were 19.003, 8.054 and 2.661 for high, control and low Ab-responses, respectively. The (LSM ± S.E) against intramuscular (IM) administration route at 7-d post- immunization were 19.003, 7.543 and 2.103 for high,

Control and low Ab-responses, respectively **(Table 2)**. The total average of Ab-response was 10.005 and 9.549 for IV and IM administration routes, respectively, with significant differences ($P \leq 0.05$). Similar results were

Observed by **Siegel and Gross (1980)** who used intravenous immunization (IV) for production of antibody to SRBCs antigen. Later, **Van der Zijpp et al., (1986)** utilized intramuscular injection (IM) with 2.5% SRBCs in phosphate buffer saline (PBS) for production of antibody response in chickens. They reported that good immune response to SRBCs from utilizing these two methods of injection. But they observed that IV had significantly (0.05) higher Ab-titers over IM administration routes in Ab-response.

3. The interaction effect of antigen types

(SRBCs and BSA) and primary Ab-titers on some blood constituents in Norfa chickens.

The LSM ± S.E of some blood constituent parameters of Norfa chicken serum and colostrums as affected by the Ab-response to SRBCs and BSA antigens at 7-day post- immunization are presented in Table (3). The control immune response chicken (CR) had higher means of total plasma proteins (TP) and total globulin (Glob) than both high (HR) and low (LR) immune response chickens. The means of total plasma proteins were 5.818, 5.282 and 5.069 g /dl plasma for control, high and low immune response chickens, respectively. The means of total globulin were 3.254, 2.844 and 2.612 g /dl plasma for control, high and low immune response chickens, respectively. The present results cleared that the total protein and total globulin were increased with BSA antigen than SRBCs antigen, but the statistical differences were not significant. While the differences among immune response (HR, LR and CR) chickens were highly significant ($P \leq 0.01$). However, the injection of SRBCs significantly increased ($P \leq 0.01$) the RBCs counts (3.530 $X10^6/mm^3$) than BSA antigens (2.701 $X10^6/mm^3$). While, both SRBCs and BSA antigens had almost similar counts of WBCs (55.400 and 55.827 $X10^3/mm^3$), respectively. The present results are almost similar to the results reported by **Eid (2010),** who found that control line for antibody response to SRBCs in broiler chickens had higher values of most blood constituents than selected lines. On the other hand, **Hanalon et al. (1997) and Kucharska et al. (1999)** found that the high immune response line(HL) had higher values of most of the blood constituents than the low one(LL) and both SRBCs and BSA had different effects on the concentration of some blood constituents within different immune response chickens.

4. The interaction effect of antigen types (SRBCs and BSA) and primary Ab-titers on hematocrit values in Norfa chickens.

The results explained that the SRBCs antigen had significantly higher hematocrit values before separation than BSA antigen (Table 4). The hematocrit values were 2.908 vs2.231 before separation and1.333 vs1.209 for hematocrit value after separation due to immunization with SRBCs and BSA, respectively. The present results were similar to the results reported by **Van der Zijpp et al. (1986)** who found that SRBCs antigen had higher concentrations of most blood constituents including hematocrit value.

5. The interaction effect of antigen administration route and primary Ab- titers on some blood constituents in chickens.

The LSM ± S.E of the blood constituents of Norfa chicken serum and colostrums as affected by the antigen administration routes (IV and IM) and Ab-titers to SRBCs and BSA antigen at 7-d post- immunization are presented in Table (5). The results showed that both IV and IM administration routes had similar concentrations for TP, Alb and Glob, and the differences between IV and IM were not significant. While, IV- administration route had higher counts of RBCs and WBCs than IM- administration. The differences between IV and IM administration route for RBCs and WBCs counts were highly significant ($p \leq 0.01$). These results are in good agreement with the results reported by **Donker (1989) , Donker and Beauving, (1989) and Dunnington et al., (1992)** who found that line differences in both IV and IM immunization were not significant for most blood constituents in chickens except the blood cells .

6. The interaction effect of antigen administration route and primary Ab-titers on hematocrit values in Norfa chickens.

The data showed that the IM administration route of different antigens had higher concentration of hematocrit value ,than IM administration, but, the differences were not significant before separation and were highly significant ($p \leq 0.01$) after separation (Table 6).In this respect, **Kreukniet et al (1992)** found that IV immunization of SRBCs had hematocrit value similar to the IM immunization before separation, but the differences were highly significant ($p \leq 0.01$) after separation.

It can be concluded that, both of the injection of SRBCs antigen and using intravenous adminstration route significanly increase the antibody titers and enhance the immune response,which in return increases the resistance against diseases and improve chicken productivity.

Table (1). Effect of antigen types on the level of primary Ab- titers in Norfa chickens.-

Antigen type	Immune response	n	Primary Ab. titers (LSM ± S.E)
RBC's	HR	40	19.003 ± .000
	CR	55	8.291 ± 0.291
	LR	25	3.696 ± 0.259
	Total average	120	10.429^{a} ± 0.183
BSA	HR	36	19.003 ± 0.000
	CR	60	7.360 ± 0.312
	LR	27	2.178 ± 0.091
	Total average	123	9.613^{b} ± 0.134

a, b values having different superscripts in the same column are significantly different at ($p \leq 0.05$).
HR= High response, CR= Control response and LR= Low response.

Table (2): Effect of antigen administration route on level of primary Ab- titers in Norfa chickens :-

Antigen Administration route	Immune response	n	Primary Ab- titers (LSM ± S.E)
Vein	HR	40	19.003 ± .000
	CR	59	8.054 ± 0.295
	LR	23	2.661 ± 0.226
	Total average	122	10.005^{a} ± 0.174
Muscle	HR	36	19.003 ± 0.000
	CR	56	7.543 ± 0.320
	LR	29	2.103 ± 0.242
	Total average	121	9.549^{b} ± 0.187

a,b values having different superscripts in the same column are significantly different at ($p \leq 0.05$).
HR= High response, CR= Control response and LR= Low response.

Table (3): The interaction effect of antigen types (SRBCs and BSA) and primary Ab- titers on some blood constituents in Norfa chickens.

Traits	Antigen Type	Immune response(Ab-titers) (LSM ± S.E)			Total Average
		HR	CR	LR	
TP (g/dl)	SRBCs	5.395 ± .115	5.816 ± .568	4.530 ± .221	5.247 ± .301
	BSA	5.168 ± .359	5.820 ± .448	5.607 ± .843	5.532 ± .55
	Total Average	5.282 ± .237	5.818 ± .508	5.069 ± .532	5.389 ± .426
Alb (g/dl)	SRBCs	2.770 ± .381	2.582 ± .177	2.303 ± .239	2.552 ± .266
	BSA	2.105 ± .062	2.546 ± .118	2.610 ± .217	2.420 ± .132
	Total Average	2.438 ± .222	2.564 ± .148	2.457 ± .228	2.486 ± .199
Glob (g/dl)	SRBCs	2.625 ± .255	3.234 ± .427	2.227 ± .455	2.695 ± .379
	BSA	3.063 ± .377	3.274 ± .336	2.997 ± .673	3.112 ± .462
	Total Average	2.844 ± .316	3.254 ± .382	2.612 ± .564	2.903 ± .421
RBCs(10^6/mm^3)	SRBCs	3.380 ± 0.85	3.830 ± 0.49	3.390 ± 0.66	3.530^a ± 0.67
	BSA	2.380 ± 0.28	2.680 ± 0.56	3.070 ± 0.38	2.701^b ± 0.41
	Total Average	2.880 ± 0.27	3.260 ± 0.53	3.230 ± 0.52	3.120 ± 0.54
WBCs(10^3/mm^3)	SRBCs	61.425 ± 5.746	58.666 ± 5.679	46.110 ± 3.868	55.400 ± 5.098
	BSA	53.343 ± 4.925	52.720 ± 2.269	61.417 ± 2.744	55.827 ± 3.313
	Total Average	57.384 ± 5.336	55.693 ± 3.974	53.764 ± 3.306	55.614 ± 4.206

a,b values having different superscripts in the same column are significantly different at ($p \leq 0.01$).
HR= High response, CR= Control response and LR= Low response.

Table (4): The interaction effect of antigen types and primary Ab titers on hematocrit value (before and after separation) in Norfa chicken

Traits	Antigen Type	Immune response(Ab-titers) (LSM ± S.E)			Total Average
		HR	CR	LR	
HB	SRBCs	2.563 ± .123	2.466 ± .097	3.696 ± .259	2.908^a ± .159
	BSA	2.225 ± .112	2.152 ± .072	2.315 ± .155	2.231^b ± .113
	Total Average	2.394 ± .118	2.309 ± .085	3.006 ± .207	2.569 ± .137
HA	SRBCs	1.395 ± .115	1.293 ± .078	1.312 ± .134	1.333 ± .109
	BSA	1.150 ± .099	1.107 ± .055	1.370 ± .138	1.209 ± .097
	Total Average	1.273 ± .107	1.2 ± .067	1.341 ± .136	1.271 ± .103

a,b values having different superscripts in the same column are significantly different at ($p \leq 0.01$).
HR= High response , CR= Control response and LR= Low response.

Table (5): The interaction effect of antigen administration routes (IV and IM) and primary Ab- titers on some blood constituents in Norfa chickens.

Traits	Antigen Type	Immune response(Ab-titers) (LSM ± S.E)			Total Average
		HR	CR	LR	
TP (g/dl)	Vein	5.128 ± .459	5.323 ± .213	5.413 ± .627	5.288 ± .433
	Muscle	4.863 ± .409	6.148 ± .525	4.380 ± .280	5.130 ± .405
	Total Average	4.996 ± .434	5.736 ± .369	4.897 ± .454	5.209± .419
Alb (g/dl)	Vein	2.484 ± .322	2.488 ± .089	2.478 ± .203	2.483 ± .205
	Muscle	2.360 ± .310	2.615 ± .161	2.415 ± .365	2.463 ± .279
	Total Average	2.422 ± .316	2.552 ± .125	2.447 ± .284	2.474 ± .242
Glob (g/dl)	Vein	2.644 ± .344	2.835 ± .220	2.935 ± .480	2.805 ± .348
	Muscle	2.503 ± .554	3.533 ± .372	1.965± .645	2.667 ± .524
	Total Average	2.574 ± .449	3.184 ± .296	2.45 ± .563	2.736 ± .436
RBCs(10^6/mm^3)	Vein	3.220 ± .0641	2.870 ± .0439	3.370 ± .0399	3.150^a ± .0493
	Muscle	2.300 ± .0554	3.510 ± .0621	2.960 ± .0860	2.920^b ± .0678
	Total Average	2.760 ± .0598	3.190 ± .053	3.170 ± .0629	3.040 ± 0.586
WBCs(10^3/mm^3)	Vein	56.956 ± 5.466	59.120 ± 6.673	57.485 ± 4.384	57.854^a ± 5.508
	Muscle	58.097 ± 6.028	53.408 ± 2.731	46.320 ± 6.690	52.608^b ± 5.149
	Total Average	57.527 ± 5.747	56.264 ± 4.702	51.903 ± 5.537	55.231 ± 5.329

a ,b values having different superscripts in the same column are significantly different at ($p \leq 0.05$).
HR= High response, CR= Control response and LR= Low response.

Table (6): The interaction effect of antigen administration routes (IV and IM) and primary Ab- titers on hematocrit value (before , after separation) in Norfa chickens.

Traits	Antigen Type	Immune response(Ab-titers) (LSM ± S.E)			Total Average
		HR	CR	LR	
HB	Vein	2.586 ± .126	2.425 ± .095	2.255 ± .163	2.422 ± .128
	Muscle	2.238 ± .112	2.185 ± .076	2.400 ± .143	2.274 ± .110
	Total Average	2.412 ± .118	2.305 ± .086	2.328 ± .153	2.348 ± .119
HA	Vein	1.506 ± .124	1.288 ± .078	1.348 ± .149	1.381^a ± .117
	Muscle	1.075 ± .084	1.109 ± .054	1.335 ± .112	1.173^b ± .083
	Total Average	1.291 ± .104	1.199 ± .066	1.342 ± .131	1.277 ± .1

a ,b values having different superscripts in the same column are significantly different at ($p \leq 0.01$).
HR= High response, CR= Control response and LR= Low response.

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الملخص العربي

تأثير التداخل بين نوع المستضد (الأنتيجين) وطريقه الحقن علي الاستجابة للمناعة وعلاقتها ببعض مكونات الدم في الدجاج.

جوده محمد جبريل ، عبد المنعم عبد الحليم الفقي ، هند عطيه أحمد خضر

كليه الزراعة - جامعة المنوفية- شبين الكوم - قسم إنتاج الدواجن

استخدم عدد243 من دجاج النورفا عند عمر٢٥ أسبوع لدراسة تأثير التداخل بين نوع المستضد (SRBCs and BSA) وطريقه الحقن (الوريد IV ، العضل IM) علي الاستجابة للمناعة وعلاقتها ببعض مكونات الدم في الدجاج. الصفات التي درست هي الأجسام المضادة Ab-titers (high, control and low responses)، مجموع بروتينات البلازما(TP) الالبيومين(Alb) الجلوبيولين (Glob) وعدد كرات الدم الحمراء(RBCs) عدد كرات الدم البيضاء (WBCs) والهيماتوكريت قبل وبعد الفصل.

أوضحت النتائج أن حقن المستضد SRBCs أدى إلي زيادة معنوية في مستوى الأجسام المضادة (Ab titers١٠.٤٢٩ ضد ٩.٦١٤) وعدد كرات الدم الحمراء(3.530 ضد 2.701 X 106/mm3) ونسبه الهيماتوكريت (2.38 ضد 1.173 pcv) عن حقن المستضد عن طريق العضل (IM) على التوالي. وعموما ، يؤدي حقن المستضد SRBCs كمستضد طبيعي عن طريق (IV) إلى زيادة الاستجابة للمناعة والي زيادة معظم مكونات الدم في الدجاج .