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# EFFECT OF DOSE AND ROUTE OF ANTIGEN ADMINISTRATION ON ANTIBODY RESPONSE OF LOHOMAN SELECTED LEGHORN (LSL) AND FAYOUMI CHICKS

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## SUMMARY

The effect of three doses of sheep red blood cells (SRBC) 10 , 1 and 0.1 % and three routes of antigen administration, intravenous (i.v.), intramuscullar (i.m.), and subcutenous (s.c.) were compared in two experiments. The first experiment was conducted to measure the primary antibody titer in LSL and Fayoumi chicks after immunization with each of the three previous doses of SRBC. The second experiment were conducted to measure the secondary antibody titer in Fayoumi chicks after immunization with 1 or 0.1 % SRBC. The results of the first experiment indicate that lower dose of SRBC was followed by a smaller anti-SRBC response for all different routes. Fayoumi chicks yielded higher primary antibody titer than LSL chicks.

In both experiments, the i.v. immunization produced significantly higher antibody titer than i.m. or s.c. immunization. However there were no differences observed between i.m. or s.c. immunization in both primary and secondry antiboday titers.

**Keywords** : Chicken , antibody production , injection routes , SRBC doses

## INTRODUCTION

The main variables that influence immune responses to different antigens are route of adminstration (van der Zijpp *et al.*,1986 and Donker *et al.*,1990), immunization schedule (Davis and Glick, 1988), antigen dose and time of response testing (Kreukniet and van der Zijpp, 1990 and Gross, 1986).

Each antigen has a certain optimal dose at which it produces the highest response, too low of a dose fails to activate lymphocytes. Conversely, an excessively high dose also fail to induce a response because it causes lymphocytes to enter a nonresponsiveness state (Klein, 1991and Kuby,1992 ).

Antigen administered intravenously travel first to the spleen, whereas antigen administered subcutaneously, intramuscularly or intraperitoneally moves first to local lymph nodes. Differences in the lymphoid cells populating these organs generate differences in the quality of the subsequent immune response (Klein 1991 and Kuby,1992).

The genetic constitution of an immunized animal influences the degree of the immune response. Differences in antibody production within lines selected for high (H) or low (L) antibody production to sheep red blood cells (SRBC) could be attributed to a difference between these lines in terms of antigen handling by macrophages (Biozzi *et al.*, 1979 and 1984), in the multiplication rate of B-lymphpocytes (Biozzi *et al.*, 1984) and in the number of immunocompetent cells in the spleen (Donker, 1989).

The purpose of this study was to determine the effect of breed, dose and route of antigen adminstration on the antibody response to SRBC.

## MATERIALS AND METHODS

Two experiments were conducted using male chicks of Lohman Selected Leghorn (LSL) and Fayoumi. The chicks were housed in battery cages. Commercial starter feed (calculated 2800 Kcal ME per Kg, 20% crude protein) with water available *ad libitum*. Birds were exposed to continous light throught the experiments. The chicks were vaccinated against Newcastle disease at 7, 21 and 50 days of age and against Infection Bursal disease at 15 days of age.

Sheep red blood cells were obtained in a haparin solution from three sheeps and washed twice in physiological saline (0.9% NaCl) before being diluted to 0.1, 1.0, or 10% suspensions.

Antibody titer were determined, using a microtiter procedure (van der Zijpp and Leenstra, 1980). The number of titers was expressed as the log 2 of the highest serum dilution giving total agglutination.

The first experiment was conducted to determine the interaction between breed of chicks, three doses of SRBC, and routes of antigen adminstration on primary antibody response. At 6 weeks of age 90 male chicks from each breed (LSL and Fayoumi) were divided into three groups (30 chicks per group). The first group was immunized intravenously (i.v.) in the brachial vein (cutanea ulnaris), second group was immunized intramuscularly (i.m.) in the chick's thighs, whereas the third group was immunized subcutenously (s.c.) in back of the neck. Each group was divided into three subgroups( 10 chicks per subgroup), each subgroup was immunized with 0.5ml of either 0.1, 1.0 or 10.0% dilutions of SRBC. Sera were collected for antibody titer determination on the seventh day after antigen administration .

Based on results from the first experiment, the second experiment was conducted. Since there was no difference among breeds, only Fayoumi chicks were used in the second experiment. The second experiment was conducted to determine the interaction between two doses and three routes of antigen on secondary (anamestic) antibody response of Fayoumi chicks. Forty two chicks were injected at 6 weeks of age via i.v. with 0.5ml of 1.0% SRBC. Blood samples for antibody titer were drawn from brachial vein at day 7 later. At 10 weeks of age (four weeks after the first immunization) the chicks were divided into three groups (14 chicks per group) according to the route of antigen adminstration as in the first experiment, each group divided into three subgroups (7 chicks per subgroup), each was reimmunized with 0.5ml of either 0.1, or 1.0% dilution of SRBC. Sera were collected for antibody titer determination on days 3, 7, and 10 after injection of antigen.

The data were analyzed using The SAS package (1988). In the first experiment,

General Linear Models procedure with a three way ANOVA model using effect of breed, dose, and route of antigen adminstration as main effects, whereas two-way ANOVA model in the second experiment using dose and route of antigen admintration as main effect. Where appropriate means were separated using Duncan's multiple range test.

## RESULTS

### Experiment one

Differences between doses and routes of antigen administration on antibody titer presented in both breeds (Table 1). Generally, the level of response was influenced by the SRBC dose administred: a lower dose of SRBC was followed by a smaller anti-SRBC response in all different routes (Fig 1). For i.v. route, the antibody titer decreased significantly when the dose of SRBC decreased, whereas in both i.m. or s.c. routes the dose of 10 % SRBC only caused higher significant antibody titer than those of 1 or 0.1 % SRBC, where the last doses gave a simillar antibody titer.

Table 1. Primary antibody titer of LSL and Fayoumi chicks immunized by different route admninistration and different doses of sheep red blood cells (SRBC).

| BREED | DOSE | Route of antigen administration | | |
|---|---|---|---|---|
| | | I.V. | I.M. | S.C. |
| LSL | 10.0% | 8.6+0.45ab | 6.9+0.48cd | 5.2+0.53efg |
| | 1.0% | 5.6+0.45ef | 3.6+0.31i | 3.0+0.37i |
| | 0.1% | 4.0+0.26ghi | 3.5+0.22i | 2.7+0.37i |
| Fayoumi | 10.0% | 9.6+0.22a | 9.2+0.25a | 7.8+0.51bc |
| | 1.0% | 6.2+0.55de | 3.9+0.48hi | 3.8+0.42hi |
| | 0.1% | 4.9+0.35fgh | 3.2+0.57i | 3.3+0.40l |

Values with different letters are significantly different from each other (P<0.05).

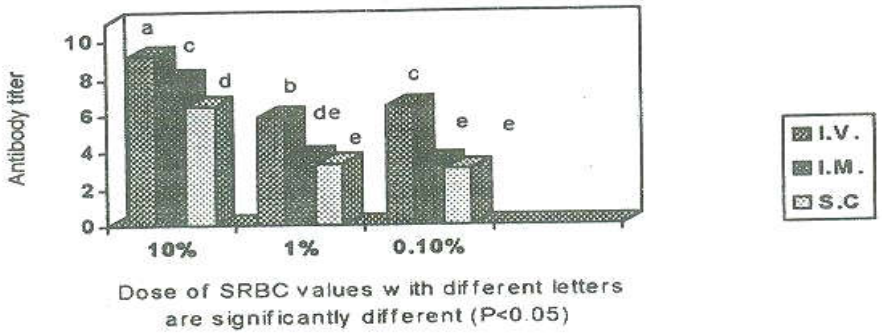
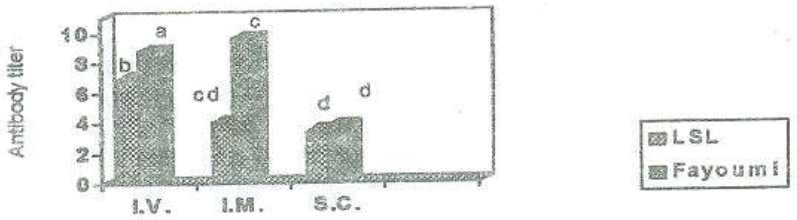


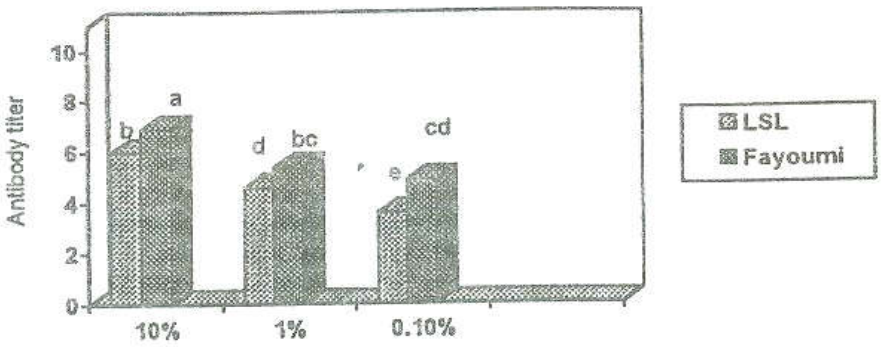

Fig. 1: Effect of dose and route of anyigen administration on antibody titer against SRBC

Regardless of the breed or dose, the i.v. immunization produced significantly higher antibody titer followed by i.m., where s.c. was last (Fig 2). When Fayoumi chicks immunized i.v. or i.m. with dose of 10 % SRBC, they produced simillar antibody titer with highly significant than those counterpart of s.c. route. On the other hand, the LSL chicks produced only highly significant antibody titer when immunized via I.V. with dose of 10% SRBC than the counterpart which immunized via i.m. Whereas the last route of administration are significantly higher than that of S.C.




Route of adminstration
values with different letters are
significantly different (P<0.05).

Figure 2. Antibody titer against SRBC in LSL and Fayoumi chicks immunized with different route of antigen adminstration.




Dose of SRBC

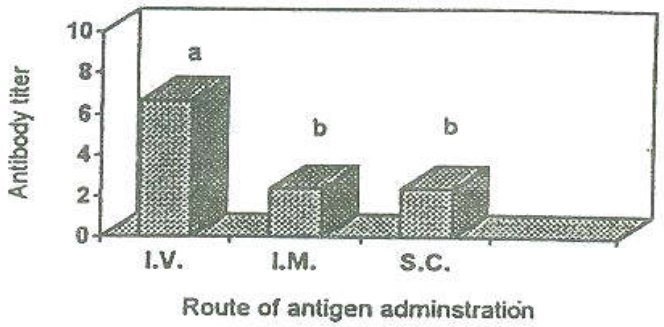
values with different letters are significantly different from each other (P<0.05).

Figure 3. Antibody titer in LSL and Fayoumi chicks immunized with different doses of SRBC (all routes combined).

Irrespective of the route of antigen administration, Fayoumi chicks responded better to SRBC at dose of 1 and 10 % and produced significantly higher antibody titer SRBC than those of LSL (Fig 3). However, the significantly differences were clearly observed between breed when injected with 10 % SRBC via i.m. or s.c. (Table 1).

**Experiment two**

As expected, since all chicks firstly were immunized via i.v. with 1 % SRBC. So all groups had simillar primary antibody titer ( Table 2 ). As it is shown in Table 2, the secondary antibody titer were affected by dose and route of antigen administration (Fig. 4 and 5). In general, the antibody titer were significantly higher at all days after i.v. immunization than after i.m. or s.c. immunization. However both i.m. or s.c. immunization yielded simillar and low secondary antibody titer. At the same time, there were no influences of SRBC dose in secondary antibody titer within each route. In general, the peak titers for all groups were reached on day 7 post immunization.




values with different letter are significantly different from each other (P<0.05)

Figure 4. Secondary antibody titer level influnced by different route of antigen adminstration (all dose combined).

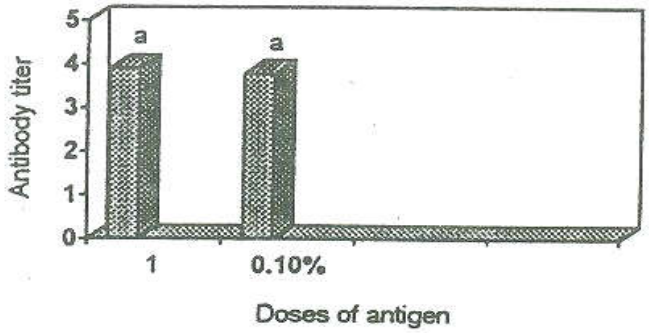



Figure 5 Secondary antibody titer level influnced by two doses of sheep red blood cells (SRBC) (all routescombined).

Table 2: Secondary antibody titer in Fayoumi chicks immunized with two different doses and three routes of antigen administration.

| Route | Dose(%) | Primary Titer | Secondary Titer | | |
|---|---|---|---|---|---|
| | | Days post-immunization | | | |
| | | 7 | 3 | 7 | 10 |
| i.v. | 1.0 | 5.3±0.4 | 6.00±1.03$^{a}$ | 7.67±0.67$^{ab}$ | 7.00±0.63$^{ab}$ |
| | 0.1 | 5.1±0.6 | 5.71±0.61$^{a}$ | 8.00±0.49$^{a}$ | 6.14±0.86$^{b}$ |
| i.m. | 1.0 | 5.0±0.5 | 1.83±0.31$^{bc}$ | 3.50±0.34$^{c}$ | 2.33±0.33$^{cd}$ |
| | 0.1 | 5.4±0.8 | 1.33±0.21$^{c}$ | 2.83±0.31$^{cd}$ | 3.00±0.69$^{cd}$ |
| s.c. | 1.0 | 5.2±0.3 | 2.33±0.21$^{bc}$ | 2.71±0.29$^{cd}$ | 2.86±0.34$^{cd}$ |
| | 0.1 | 5.6±0.7 | 1.50±0.22$^{c}$ | 3.00±0.44$^{cd}$ | 1.86±0.26$^{cd}$ |

Means with different letters within each days post-immunization are significantly different ( $P<0.05$ ).

## DISCUSSION

The route of antigen adminstration led to differences in antibody production. The i.v. injection route produce significantly higher primary and secondary antibody titer to SRBC than other injection routes, these results in consistent with those reported by van der Zijpp *et al.* (1986) and Donker *et al.* (1990). These finding may be due to that antigen administrated via i.v. is carried first to the spleen, whereas antigen administrated i.m. or s.c. moves first to local lymph nodes, and the differences in the lymphoid celis populating these organs generate differences in the quality of the subsequent immune response (Klein,1991 and Kuby,1992). This interpretation is consistent with the opinion of van der Zijpp *et al.* (1986) who theoirzed that the ellipsoid-associated cells and dendritic cells, that bind antigen are more effective in presenting SRBC to immunocompetent cells than that of the antigen-presenting cells of the tissue or peritoneal cavity.

The present results indicate that 10 % of SRBC was obviously the optimum dose of SRBC that produce high level of antibody titers specially when concurrent in i.m. and s.c. immunizations.

The positive relation between the dose of antigen and the level of antibody production was consistant with the results of Kreukneit and van der Zijpp (1989) who reported that antibody production is dependent on the level of antigen dose.

The differences in antibody production against SRBC between LSL and Fayoumi chicks in the present study confirms the previous observation of Atta (1990) that Fayoumi chicks responded better to antigen than Leghorn chicks.

The superiority of Fayoumi chicks in antibody production over LSL has been shown to be due to the high activity of lymphocytes (Atta,1996). It is also possible that this superiority could be due to better handling of antigen by macrophages as reported by Biozz *et al.* (1979 and 1984).

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# تأثير تركيز الأنتيجين وطريقة التطعيم على الإستجابة المناعية فى كتاكيت اللجهورن المنتخب والفيومى

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أجريت تجربتين لدراسة تأثير إستخدام ثلاث تركيزات من أنتيجين كرات الدم الحمراء للغنم وهى ١٠٪، ١٪، ٠,١٪، وثلاث طرق للتطعيم وهى : الحقن فى الوريد، الحقن فى العضل و الحقن تحت جلد الرقبة، ولقد أجريت التجربة الأولى لتقدير مستوى الأجسام المناعة (الإستجابة المناعية الأولية) الناتجة من تطعيم كتاكيت اللجهورن والفيومى لأول مرة بأنتيجين كرات الدم الحمراء للغنم بالتركيزات الثلاث وطرق التطعيم السابقة الذكر. بينما أجريت التجربة الثانية لتقدير مستوى الأجسام المناعة (الإستجابة المناعية الأولية) الناتجة من تطعيم كتاكيت الفيومى لثانى مرة بنفس الأنتيجين بتركيزات ١٪ و ٠,١٪ وبطرق التطعيم السابقة. وأوضحت نتائج التجربة الأولى أن التطعيم بتركيز منخفض يكون متبوعا باستجابة مناعية منخفضة لكل الطرق المستخدمه فى التطعيم. كما أوضحت النتائج أن كتاكيت الفيومى بوجه عام تنتج أجسام مناعية أكثر من كتاكيت اللجهورن. وفى كلتا التجربتين فان التطعيم بطريق الحقن فى الوريد أعطت أفضل استجابة مناعية عن التطعيم بطريقة الحقن فى العضل أو تحت جلد الرقبة. ومن ناحية أخرى لم تتضح أى إختلافات فى الإستجابة المناعية الأولية والثانوية الناتجة من الحقن فى العضل أو تحت جلد الرقبة.