Effects on some immunological traits after crossing three rabbit breeds in Egypt

Mahmoud S. El-Tarabany & Khairy M. El-Bayoumi

Summary
The authors evaluate the effect of crossing three purebred rabbit breeds in a complete 3 × 3 diallel crossbreeding experiment (New Zealand White, V-line and Gabali) on certain immunological traits, including IgG titre. Sheep red blood cells (SRBCs) were administered at 8 weeks of age and boosted at 10 weeks of age. V-line/Gabali crossbreeds gave the highest significant (p<0.05) level of serum total protein at 8 and 12 weeks of age (4.71 and 7.70 g/dl, respectively). Its reciprocal crossbreed Gabali/V-line resulted in the highest estimate of serum globulin at 8 weeks of age (2.07 g/dl). V-line/Gabali crossbreeds showed the highest positive heterosis percentage (H%) of total protein, albumin and globulin at 8 weeks of age (27.98, 30.28 and 25.00, respectively). However, its reciprocal crossbreeds, Gabali/V-line, revealed negative estimates for total protein and albumin at the same age (−4.07 and −29.80). V-line/Gabali crossbreeds gave the highest titre (3.167) and positive H% (0.69) of IgG to the second dose of SRBCs. On the contrary, the highest negative estimate to the second dose was recorded in Gabali/New Zealand White crossbreeds (−2.49). Using the Gabali breed as the female line gave superior results for most immunological parameters.

Keywords
Breed, Breeding, Crossbreed, Egypt, Heterosis, IgG, Immunity, Rabbit.

Gli effetti di alcuni tratti immunologici a seguito di incrocio di tre razze di coniglio in Egitto

Riassunto
Gli autori studiano l’effetto dell’incrocio di tre razze pure di conigli in un esperimento di incrocio 3 × 3 (New Zealand White, V-line e Gabali) su alcuni tratti immunologici, compresi il titolo IgG. Sono state somministrate le cellule di globuli rossi di una pecora (SRBCs), a 8 settemani e ripetute a 10 settemani di età. L’incrocio delle razze V-line/Gabali ha dato il massimo risultato (p<0.05) nei livelli sierici di proteine totali sia a 8 che a 12 settimane di età rispettivamente (4.71 e 7.70 gr/dl). Il reciproco incrocio delle razze Gabali/V-line ha portato al più alto livello di globulina di siero a 8 settimane di età (2.07 gr/dl). L’incrocio V-line/Gabali ha mostrato una maggiore concentrazione di eterosi (% H) delle proteine totali, albumina e globulina a 8 settimane di età (27.98, 30.28 e 25.00, rispettivamente). Tuttavia, il reciproco incrocio V-line/Gabali, ha mostrato le titolazioni negative per proteine totali e albumina alla stessa età (−4.07 e −29.80). L’incrocio Gabali/V-line ha mostratol il titolo più alto (3.167) ed eterosi positiva % (0.69) ad IgG nei confronti della seconda dose di SRBCs. Al contrario, la titolazione negativa più alta sulla seconda dose è stata registrata nell’ incrocio Gabali/New Zealand White (−2.49). Utilizzando la razza Gabali come la linea femminile, si dimostrano risultati superiori rispetto ai parametri immunologici.
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Mahmoud S. El-Tarabany & Khairy M. El-Bayoumi

Parole chiave

Introduction
In recent years, domestic rabbits have been recommended as a good alternative source of dietary protein for the increasing human populations of developing countries (10). Genetic variations of antibody response to sheep erythrocytes have been demonstrated in several species, including mice (3), chickens (20) and rabbits (5).

The immune response to a natural, non-specific, non-pathogenic, multi-determinant and T-cell-dependent antigen, such as sheep red blood cells (SRBCs), provides an indication of the status of natural immunity (13). A study of antibody response in various age categories of rabbits showed that even day-old rabbits are able to form specific antibodies of isotype IgG (8). Different reactivity in the haemagglutination (HA) test and formation of antigen variant was detected in a study designed to analyse selected parameters of non-specific humoral immunity in rabbits (11)

The objective of our work was to investigate the effects of purebred rabbits in comparison to crossbreeds and their reciprocal crosses on antibody response (IgG) to sheep red blood cells SRBCs and some blood parameters including total protein, albumin, globulin, albumin globulin ratio, as well as estimation of heterosis of such traits.

Materials and methods
This study was conducted at the rabbit farm of the Department of Animal Wealth Development at the Faculty of Veterinary Medicine of Zagazig University, Egypt.

Experimental colony
The three pure rabbit breeds were used in this study were as follows:
- New Zealand White
- V-line
- El-Gabali.

Two bucks and five does of 6 months of age from each breed were used in the experiment. Bucks and does were apparently healthy and vaccinated against pasteurellosis and viral haemorrhagic disease of rabbits (17) at 6 and 10 weeks of age, respectively.

Colony management and mating plan
Rabbits were housed in an open-sided hutch. Breeding animals were kept individually in triangular galvanised wire cages (40 × 60 × 50 cm) provided with a nipple system for watering and a manual feeder. A metal nest box was attached to the doe’s cage.

Litters were weaned, ear tagged and separated in cages at 4 weeks of age. They were raised in identical management and nutritional conditions. Individual records were established for each breeding animal. Rabbits of all ages were fed commercial pelleted ration containing crude protein (not less than 18.5%), crude fibre (11.5%) and lipids (2.5%). Clean fresh water was available at all times to all rabbits. The environmental temperature was maintained at approximately 27°C. Mature bucks and does were mated in a full 3 × 3 diallel design. Two bucks and five does from each of the above three breeds were used.

Traits studied
Blood parameters
Blood samples were collected from randomly assigned animals (purebred, crossbred and reciprocal crossbred) for serological examination. Blood samples were collected from the ear vein by puncture. Serum samples were taken in clean, sterilised and labelled tubes and were frozen at −20°C until assay (16). Specific diagnostic kits for total protein and albumin were used for colorimetric evaluation and the globulin concentration (g/d) was obtained as a total protein-albumin concentration (4, 12). The source of the diagnostic kits was BK8145C (CinnaGen Inc. Shahrak Ekbatan, Teheran).

Immunological traits
Each animal received an intramuscular injection of 0.5 ml of 10% suspension of SRBCs at two months of age; this was repeated two weeks after the first injection (21). The blood
samples from each genotype were collected one week after each injection; the serum was separated and frozen at −20°C until assay.

The enzyme-linked immunosorbent assay (ELISA) was used to detect antibodies (IgG) as a response to SRBCs (Bethyl rabbit IgG ELISA quantitative kit: E120-111, Bethyl Laboratories, Montgomery, Texas).

**Data handling and statistical analysis**

All available data were analysed using the SPSS Statistical Analysis System package (14). Individual titres were transformed to log₁₀ and the log geometric mean (log GM) was calculated for each genotype in accordance with the following formula:

\[
\text{Log } GM = \frac{1}{n} \sum \text{Log } X_i
\]  

(22)

Least squares means (LSM) ± standard errors (SE) were calculated and tested for significance using the 'T' test (15).

The data were analysed using different statistical models as following:

\[
Y_{ijk} = \mu + B_i + C_j + e_{ijk}
\]

\[
\text{Y}_{ijk} = \text{any observed value}
\]

\[
\mu = \text{overall mean}
\]

\[
B_i = \text{effect of breed (i = 1, 2, and 3, i.e. New Zealand White, V-line and El-Gabali)}
\]

\[
C_j = \text{effect of crossing (j = 1, 2, 3… 6)}
\]

\[
e_{ijk} = \text{random deviation due to unexplained source.}
\]

**Heterosis (hybrid vigor)**

Heterosis or hybrid vigor is estimated for the different crossbreds and reciprocal crossbreds based on the following formula (9):

\[
\text{H%} = \left(\frac{\bar{X}_{F_1} - 1/2 (\bar{X}_{P_1} + \bar{X}_{P_2})}{1/2 (\bar{X}_{P_1} + \bar{X}_{P_2})}\right) \times 100
\]

\[
\bar{X}_{F_1} = \text{actual heterozygote mean of the first generation}
\]

\[
\bar{X}_{P_1}, \bar{X}_{P_2} = \text{means of the parents breed.}
\]

**Results**

LSM ± SE, as well as heterosis percentage (H%) of some blood parameters (serum total protein, albumin, globulin and albumin/globulin [A/G] ratio) due to crossing New Zealand White, V-line and Gabali rabbits at 8 and 12 weeks of age are summarised in Tables I and II.

The log GM ± standard deviations (SD) of antibody titre (IgG) to SRBCs as well as the H% due to crossing of New Zealand White, V-line and Gabali rabbits at 8 and 12 weeks of age are presented in Tables II and III.

**Discussion**

**Blood parameters**

There were non-significant differences among different pure lines for most parameters at either 8 or 12 weeks of age. Similar results were recorded in other similar studies (2).

The V-line genotype had significantly (p<0.05) higher total protein and globulin (4.10 and 1.99 g/dl) at 8 weeks of age. The overall mean of crossbred animals was significantly higher than that of purebreds for serum albumin at 8 weeks of age (2.35 vs 2.06 g/dl).

The V-line/Gabali crossbred recorded the highest significant (p<0.05) level of serum total protein at either 8 or 12 weeks of age (4.71 and 7.70 g/dl). In addition, Gabali/New Zealand White genotype gave the highest level of serum albumin at 8 and 12 weeks of age (2.79 and 4.11 g/dl). Meanwhile, V-line/Gabali genotype had the highest estimate of serum globulin at 8 weeks of age (2.07 g/dl).

Significant differences among crossbreds at 12 weeks were reported (6). The author found that Flander × New Zealand White crossbred demonstrated high and significant levels of serum total protein at 12 weeks of age.

Gabali/V-line crossbreds showed the highest positive H% of total protein, albumin and globulin at 8 weeks of age (27.98, 30.28 and 25.00, respectively). However, its reciprocal crossbreds Gabali/V-line, recorded negative estimates for total protein and albumin at the same age (−4.07 and −29.80). These results support those reported by Abdel-Hamid (1). Abdel-Hamid reported high positive estimates for Gabali/V-line crossbreds, while its reciprocal reported contradicted estimates, as it had high negative results. High negative H% of total protein were reported for Californian/
### Table I
Least square means ± standard errors of some immunological traits at different age intervals due to crossing of New Zealand White, V-line and Gabali breeds

<table>
<thead>
<tr>
<th>Trait</th>
<th>Total protein</th>
<th>Serum albumin</th>
<th>Serum globulin</th>
<th>Albumin/globulin ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>Genotype No.</td>
<td>8 weeks</td>
<td>12 weeks</td>
<td>8 weeks</td>
<td>12 weeks</td>
</tr>
<tr>
<td>NN 20</td>
<td>3.31±0.20</td>
<td>6.38±0.30</td>
<td>2.03±0.15</td>
<td>3.99±0.40</td>
</tr>
<tr>
<td>VV 20</td>
<td>5.80±0.20</td>
<td>7.56±0.15</td>
<td>2.11±0.41</td>
<td>3.51±0.32</td>
</tr>
<tr>
<td>GG 17</td>
<td>7.56±0.15</td>
<td>7.56±0.17</td>
<td>2.05±1.07</td>
<td>4.43±0.42</td>
</tr>
<tr>
<td>VN 21</td>
<td>4.10±0.20</td>
<td>6.58±0.35</td>
<td>2.06±0.09</td>
<td>3.98±0.37</td>
</tr>
<tr>
<td>GN 21</td>
<td>5.87±0.27</td>
<td>7.56±0.17</td>
<td>2.05±0.10</td>
<td>4.03±0.16</td>
</tr>
<tr>
<td>GV 17</td>
<td>5.30±0.18</td>
<td>7.56±0.17</td>
<td>2.05±0.10</td>
<td>4.03±0.16</td>
</tr>
<tr>
<td>NV 21</td>
<td>6.54±0.40</td>
<td>7.56±0.17</td>
<td>2.05±0.10</td>
<td>4.03±0.16</td>
</tr>
<tr>
<td>NG 16</td>
<td>6.87±0.27</td>
<td>7.56±0.17</td>
<td>2.05±0.10</td>
<td>4.03±0.16</td>
</tr>
<tr>
<td>VG 17</td>
<td>7.56±0.17</td>
<td>7.56±0.17</td>
<td>2.05±0.10</td>
<td>4.03±0.16</td>
</tr>
<tr>
<td>Mean</td>
<td>57</td>
<td>5.89±0.14</td>
<td>3.59±0.35</td>
<td>3.98±0.37</td>
</tr>
</tbody>
</table>

Means of purebreds, and crossbreds and its reciprocal within the same category having different superscripts (lower case) are significantly different (p<0.05). Means of purebreds versus crossbreds and its reciprocal within the same column having different superscripts (upper case) are significantly different (p<0.05). N New Zealand White V V-line G Gabali

### Table II
Heterosis percentages of some immunological traits for crossbreds and reciprocal crossbreds due to crossing of New Zealand White, V-line and Gabali rabbits

<table>
<thead>
<tr>
<th>Trait</th>
<th>Total protein</th>
<th>Serum albumin</th>
<th>Serum globulin</th>
<th>Enzyme-linked immunosorbent assay (IgG)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Genotype</td>
<td>8 weeks</td>
<td>12 weeks</td>
<td>8 weeks</td>
<td>12 weeks</td>
</tr>
<tr>
<td>VN 6.34</td>
<td>31.2</td>
<td>-48.0</td>
<td>0.26</td>
<td>22.15</td>
</tr>
<tr>
<td>GN 18.11</td>
<td>31.2</td>
<td>36.76</td>
<td>2.37</td>
<td>15.62</td>
</tr>
<tr>
<td>GV -4.07</td>
<td>-5.68</td>
<td>-29.80</td>
<td>-9.31</td>
<td>29.37</td>
</tr>
<tr>
<td>NV -4.09</td>
<td>-6.48</td>
<td>-27.77</td>
<td>2.46</td>
<td>-38.92</td>
</tr>
<tr>
<td>NG 3.50</td>
<td>-15.78</td>
<td>6.86</td>
<td>-22.32</td>
<td>-4.68</td>
</tr>
<tr>
<td>VG 27.98</td>
<td>15.26</td>
<td>30.28</td>
<td>1.25</td>
<td>25.00</td>
</tr>
</tbody>
</table>

N New Zealand White V V-line G Gabali
Purebred New Zealand White rabbits had the highest significant IgG titre to the first dose of SRBCs (3.02). Non-significant differences were detected among purebred rabbits to the second dose of SRBCs. These results confirmed those obtained in a study to evaluate antibody response among temperate rabbit breeds by Thomas and Nandakumar (18) who found that the effect of breed was not significant on the antibody response at 0, 7 and 14 days post immunisation.

Non-significant differences were observed among overall purebreds and crossbreds at either first or second dose against SRBCs. The highest titre to the first dose was recorded for the Gabali/New Zealand White crossbreed (3.02), while the same crossbreed demonstrated the lowest estimate after the second dose (3.052). V-line/Gabali crossbreds gave the highest IgG titre (3.167) to the second dose of SRBCs. Significant variations among crossbreeds were recorded by Hassan (6) who found that Flander × New Zealand White crossbred showed the highest antibody titre to the first dose of SRBCs.

Gabali/New Zealand White crossbreeds and their reciprocal New Zealand White/Gabali provided the highest positive H% of IgG titre to the first dose of SRBCs (2.75 and 1.02), while the superior estimate to the second dose was demonstrated by Gabali/V-line crossbreeds (0.69). On the contrary, the highest negative estimate to the second dose was recorded by Gabali/New Zealand White crossbreeds (−2.49). These results were in agreement with those obtained in trials to evaluate genetic variation in immune response of rabbits to certain antigens (7). Hemeda obtained positive H% to CN crossbreds at 7 days of injection (3.4) and 21 days (4.3). A negative value to Californian × Baladi crossbreds (−8.6) at 21 days post-immunisation was observed. Similar negative results to the first and second dose of immunisation among New Zealand White × V-line crossbreeds results were recorded (1). In an experiment performed to evaluate the systemic antibody response of New Zealand White rabbits, anti-SRBC immunoglobulins (IgG)

New Zealand White crossbreds (−16.00) at three months of age (7).

**Antibody titre (IgG) against sheep red blood cells**

The log GM ± SD of antibody titre (IgG) to SRBCs as well as H% due to crossing of New Zealand White, V-line and Gabali rabbits at 8 and 12 weeks of age are given in Tables II and III.
were studied. The IgG titre increased sharply up to 7-14 days post immunisation (19).

**Conclusion**

From the above results, the Gabali breed is used as the female line at crossing, demonstrating the superiority of most immunological parameters, especially the serum globulin and IgG titre, to SRBCs.

**References**


