

# THE EFFECT OF BURSECTOMY ON THE PRODUCTION OF SERUM ANTIBODY AGAINST ND VACCINE IN THE INDONESIAN NATIVE CHICKENS AND WHITE LEGHORN

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## ABSTRACT

Two groups of chickens were surgically bursectomized at 3 days of age. One week after the operation the experimental chickens were injected i.m. with ND vaccine strain La Sota. The 2nd injection was given 4 weeks after the 1st injection. All injected chickens showed an increase in HI-serum antibody titer. The response to the 2nd injection was higher than the 1st. The bursectomized animals showed lower HI antibody titer than the non bursectomized after the 1st injection, but a reverse response was observed after the 2nd injection. Lymphocyte count showed a similar pattern as the HI antibody titer. Better responses were observed in the Indonesia native chicken compared to the white Leghorn. The increase of the serum globulin levels in both groups after the 1st injection was mainly due to the increase of the alpha globulin fraction, and 2nd injection, the beta and gamma serum globulin levels in almost all injected chickens were elevated, except for the bursectomized White Leghorns, where the gamma globulin fraction was not increased.

## INTRODUCTION

It is well known that the bursa of Fabricius of poultry plays an important role in the production of antibody (Glick, 1987). Several experiments have been done to study the role of the bursa of Fabricius by bursectomy at different ages and observe the immune responses to different kind of antigens. Variation in results are apparently due to the stage of the bursal growth at which the bursectomy are performed and the type of antigens used in the experiments (Sato, 1966; Matsuda *et al.*, 1973). The strain of the chicken and the conditions of the experiment have to be accounted for the variety of the results obtained (Heller *et al.*, 1981).

Literature on the immune system of the Indonesia native chickens is very limited. The purpose of this experiment is to study the effect of bursectomy at 3 days of age on the serum antibody response to the ND vaccine injection of the Indonesian native chickens compared to the White Leghorns.

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## MATERIALS AND METHODS

Two groups of chickens, the Indonesian native chickens (I.N.) and the Hy-Line White Leghorns (W.L.) were used in this experiment. The fertilized I.N. eggs were obtained from the Laboratory of Physiology, Departement of Physiology and Pharmacology, Faculty of Veterinary Medicine, Bogor Agricultural University, and the W.L. from a Hy-Line Commercial Farm (W36) in Bogor, Indonesia. The eggs were incubated in the laboratory. Each group of the day old chicken was divided into 4 subgroups of 5 chickens, and kept in the experimental cages separately. The chickens were fed a commercial feed and water *ad libitum*. Two subgroups of each group of chickens were surgically bursectomized at 3 days of age by the technique described by Glick *et al* (1956) and the other subgroup served as bursectomy controls and were sham operated. One week after the operation, one of the bursectomized and one of the non-bursectomized subgroup (sham operated) were injected with 80 HAU/0.2 ml ND vaccine strain La Sota, intramuscularly. The other subgroup which served as the antigen controls were injected with the same volume and the same route of injection with saline. The 2nd injection was given 4 weeks after the 1st injection with the same materials, dose and route of injection.

The blood samples were obtained from the jugular vein of each chicken before the operation, before the injection, 2 times a week during 2 weeks, followed by once for 2 weeks after the 1st injection and 2 times a week for 2 weeks after the 2nd injection for serological and hematological analysis.

The antibody titration was performed on antisera of the individual chicken and was done by a conventional HI-method (Anonymus, 1971). The titer of the serum antibody is expressed as the reciprocal of the highest serum dilution inhibiting hemagglutination with the ND vaccine.

The total serum protein was determined by the Biuret method using the Spectronic 70 (B & L) apparatus at 545 m $\mu$  wavelength. The serum protein fractions of the individual serum were analyzed using the Gelman Electrophoresis apparatus. Separation was conducted on the cellulose polyacetate support media (sephapore III strip) with Tris barbital buffer pH 8.8, ionic strength 0.05 as the bathing solution, and using an electric current of 250–300 volt, 2–3 m.a./strip for 40 minutes.

The total leucocytes count in the blood was obtained using the hemacytometer counting chamber and the modified Rees and Ecker blood diluting solution (Sikar *et al*, 1984). The differential leucocyte count was done by the conventio-

nal method on the Giemsa stained blood smear.

## RESULTS AND DISCUSSIONS

**The serum antibody production.** The HI-serum antibody titers following the first and 2nd ND vaccine injections of the experimental chickens are presented in fig. 1 (I.N.) and fig. 2 (W.L.).

Before the ND vaccine was injected to the experimental chickens, all d.o. W.L. chickens showed a remarkable HI positive reaction against the ND vaccine used in this experiment (fig. 2), but the serum of the d.o. I.N. chicken gave a negative result. The titer of the W.L. pullets during the period of egg collection for the experiment was quite high (GMT: 640) and of the d.o. I.N. was low (GMT : 20). The d.o. W.L. chickens got the antibody against the ND vaccine from their mother; a passive transfer of the maternal antibody. The titer of this antibody in the baby chick was lower than that of their mothers. This fact is in agreement with the general consensus of opinion of previous research workers (Ronohardjo, 1972; Partadiredja, 1978). In the serum of the d.o. chickens of this experiment, the maternal antibody in the serum of the non ND vaccine injected W.L. chickens decreased gradually, and disappeared from the serum of the chickens (HI titer = 0) at 24 days of age. It means

that the maternal antibody of the baby chick is completely catabolized and excreted from the blood around 3 weeks of age.

Seven days after the 1st ND vaccine injection, the HI serum antibody of all injected chickens started to rise. The titer of the bursectomized chicken was lower than the nonbursectomized animals and was lower in the W.L. than the I.N. chickens. The bursectomized W.L. subgroup nearly did not show an increase in the antibody titer. A negative reaction was observed in the bursectomized antigen-injected W.L. subgroup at days 21 after the 1st injection, while the non-bursectomized subgroup still gave a positive result, although it was very low as compared to the I.N. nonbursectomized subgroup. (fig.1, 2).

All the control subgroups (saline injection) were negative. Following the 2nd ND vaccine injection with the same dose and the same route of injection, a remarkable increase of the HI-serum antibody titers were observed in all injected chickens. The 2nd injection gave a better antibody response than the 1st, the titer of the antibody was higher and the response was faster. The antibody titer of the bursectomized chickens were higher than the nonbursectomized subgroup. The I.N. chickens gave better antibody response than the W.L. The presence of the maternal anti-

body and strain of the chickens are apparently the cause of the difference in the antibody response to the ND vaccine injection in this experiment.

**The Leucocytes** (fig. 3a, b and fig. 4a, b). The pattern of the total leucocytes in the blood following the 1st ND vaccine injection in almost all experimental animals (the I.N. and W.L. non-bursectomized subgroups and W.L. bursectomized subgroup) except the bursectomized I.N. subgroup, were similar. The total leucocytes in the blood were increased at 3 days and 14 days after the 1st injection. In general, the increase was followed by a decrease of the total leucocytes count in the blood, and was mainly due to the lymphocytes. The bursectomized I.N. chickens started with a decrease in the total leucocytes count mainly caused by a decrease in lymphocytes too at 3 days after the 1st injection and was gradually elevated afterwards until it reached a peak at 14 days after the injection. Observations on the 21st and 28th days, the total leucocytes count and the lymphocyte count in the blood of all injected chickens were decreased. The lymphocyte count in the blood of the bursectomized chickens was higher than the non-bursectomized. An increase followed by a decrease of the lymphocyte count in the blood is usually followed by the increase

of the titer of the HI-serum antibody. Following the 2nd injection, the total leucocytes and mainly the lymphocytes in the blood of all injected subgroups were elevated again. Generally, all the Indonesian native chickens gave higher lymphocyte count in the blood than the White Leghorns following the ND vaccine injections in this experiment.

**The serum proteins.** The results of the observations on the serum protein fractions in the blood of the experimental chickens are presented in fig. 5a, b (I.N.) and fig. 6a, b. (W.L.).

**The Indonesian native chicken** (fig. 5a, b). There was no significant rise in the total serum protein level of all chickens after the 1st ND vaccine injection, but the total globulin fraction was increased, and the HI-test was positive. This indicated that immunoglobulin was produced and secreted in the blood of the antigen injected chickens. The alpha, beta and gamma proteins, especially the alpha protein level were increased in the bursectomized chickens, and in the non-bursectomized chickens the alpha and beta globulin fractions were increased too, but not the gamma globulin. After the 2nd injection, the gamma globulin fraction in all injected subgroups was increased, and was higher in the non-bursectomized chickens than the bursectomized. The alpha glo-

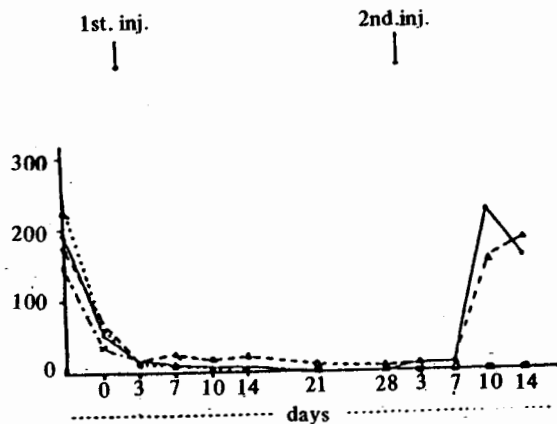
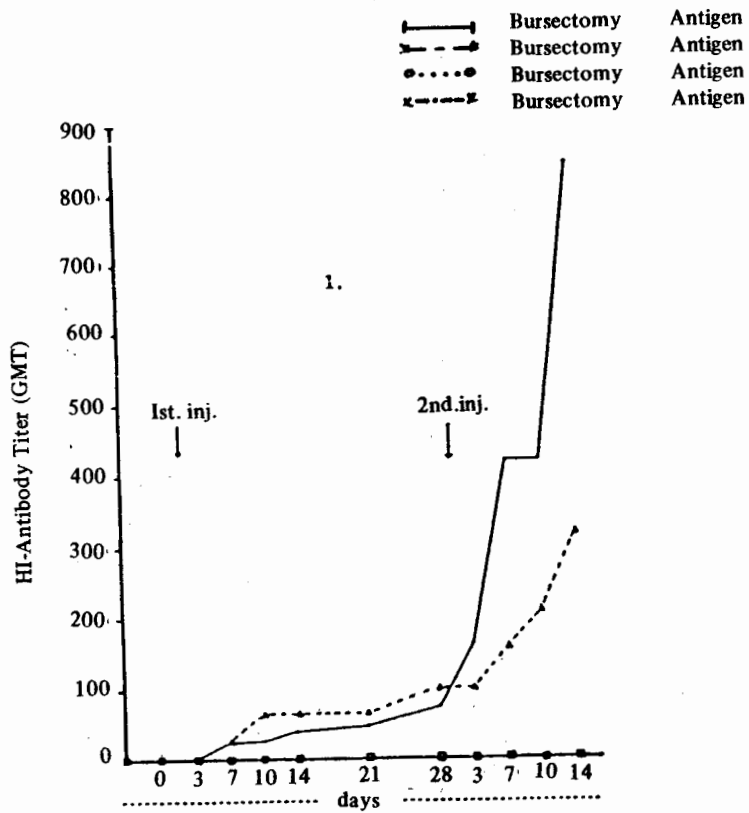
bulin level of the bursectomized chicken was higher than the non-bursectomized subgroup.

**The White Leghorn chicken** (fig. 6a, b). The total serum protein of all ND vaccine injected chickens was increased. Only a slight increase of the serum globulin fraction was observed 7 days after the 1st injection, and was decreased afterwards in all subgroup.

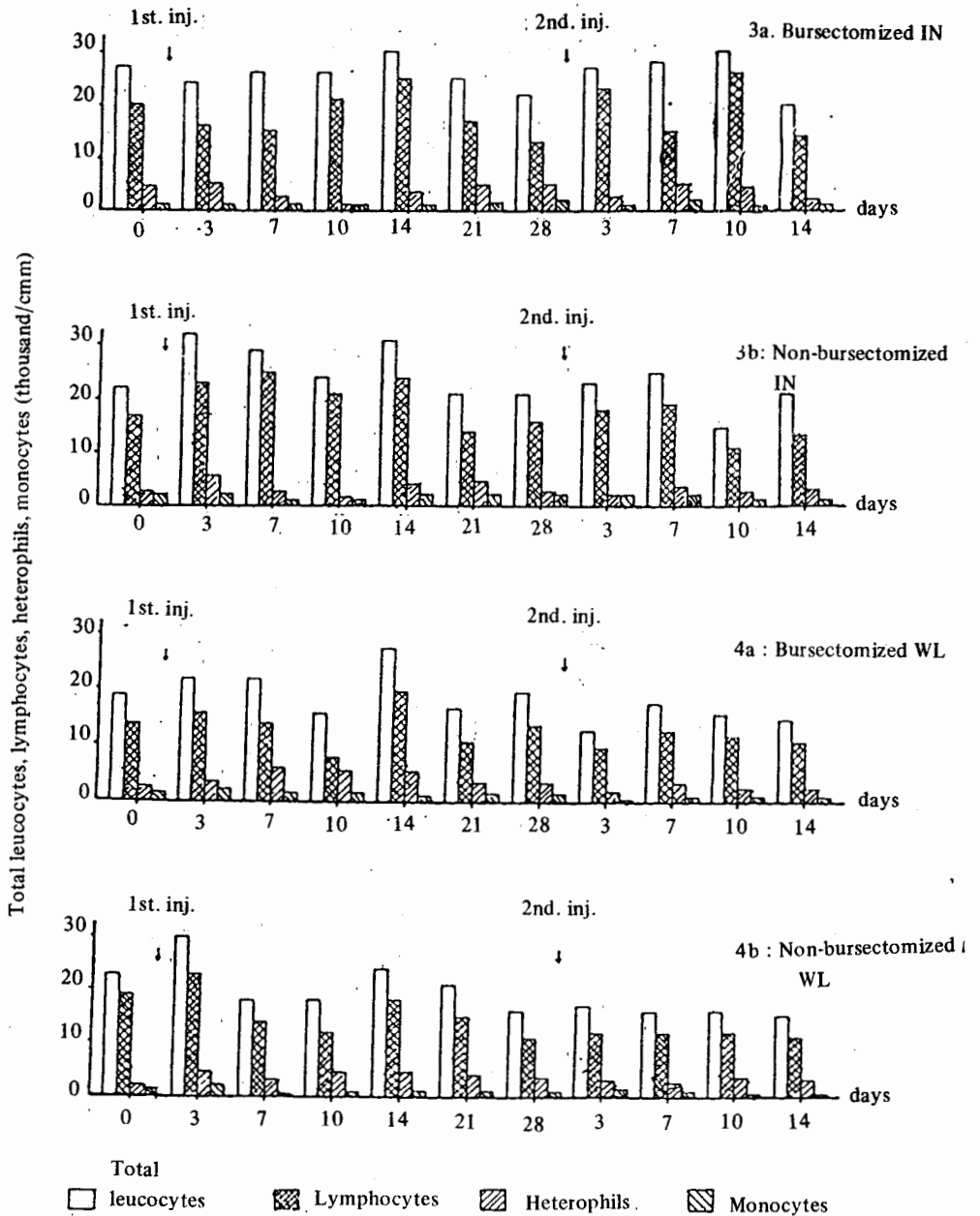
The beta and gamma globulin fractions were gradually decreased, and the alpha globulin was increased in the bursectomized subgroup. In the non-bursectomized chickens besides the alpha, the beta globulin was also increased. Following the 2nd antigen injection

the total globulin fraction was not increased. The beta and especially the gamma fraction in the blood of the non-bursectomized chickens were increased, but not the gamma globulin. The alpha globulin concentration in the blood of the bursectomized chickens was higher than the non-bursectomized chickens.

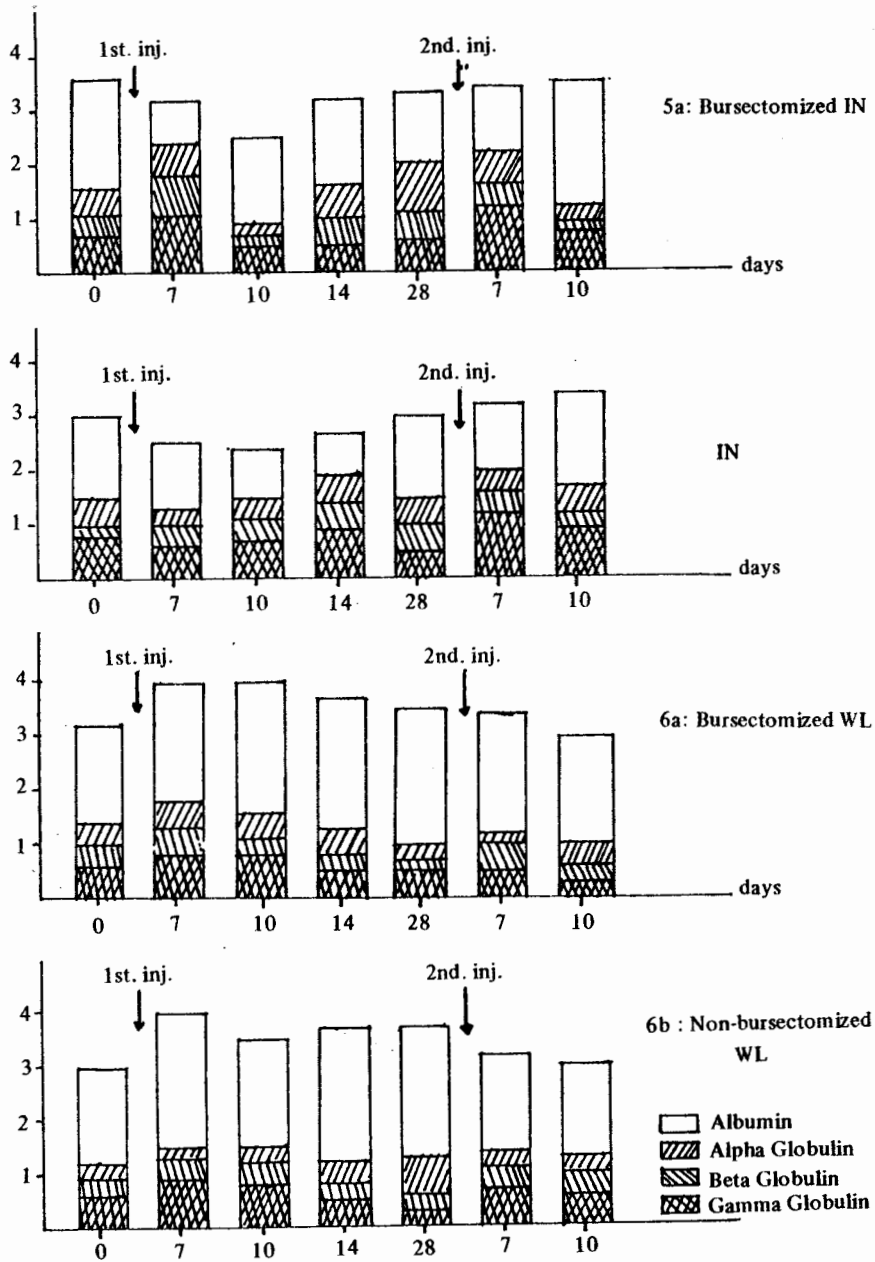
From over findings it is apparent that bursectomy at 3 days of age reduces the antibody production to ND vaccine injection in the Indonesian native chickens and the White Leghorns. The different response of the serum protein fraction against the antigen injection in the Indonesian native chicken and White Leghorn needs further study.



Figures 1 and 2. HI antibody production following injections of ND vaccine in the Indonesian native chickens (fig. 1) and White Leghorns (fig. 2) bursectomized at 3 days of age.



Figures 3a, 3b, 4a and 4b.: Total leucocytes, lymphocytes, heterophils and monocytes following injections of ND vaccines in the Indonesian native chickens (fig. 3a and 3b) and White Leghorns (fig. 4a and 4b) bursectomized at 3 days of age.



Figures 5a, 5b.: Serum protein fractions following injections of ND vaccine in the Indonesian native chickens (fig. 5a and 5b) and White Leghorn (fig. 6a and 6b) bursectomized at 3 days of age.



# PENGARUH BURSEKTOMI PADA PRODUKSI SERUM ANTIBODI TERHADAP VAKSIN ND PADA AYAM KAMPUNG DAN WHITE LEGHORN\*

Oleh

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## RINGKASAN

Dua kelompok ayam kampung dan White Leghorn dibursektomi pada umur tiga hari. Satu minggu kemudian anak ayam disuntik i.m. dengan vaksin ND galur La Sota. Suntikan kedua diberikan empat minggu setelah suntikan pertama. Semua anak ayam yang disuntik antigen menunjukkan kenaikan titer HI antibodi di dalam serum darahnya. Respons terhadap suntikan kedua lebih tinggi daripada yang pertama. Titer antibodi pada yang dibursektomi pada respons pertama lebih rendah daripada kontrol, tetapi terhadap suntikan kedua kebalikannya. Ayam kampung berespons lebih baik dibandingkan dengan White Leghorn. Gambaran hitung limfosit sejalan dengan titer HI-antibodi. Peningkatan konsentrasi globulin di dalam serum darahnya, terutama akibat peningkatan fraksi alfa globulin; yang dibursektomi lebih tinggi dari pada kontrol. Respons terhadap suntikan kedua menunjukkan peningkatan konsentrasi beta dan gama serum globulin pada semua ayam, kecuali pada White Leghorn yang dibursektomi, fraksi gama globulinnya tidak meningkat. Perlu diteliti lebih lanjut adanya perbedaan ini.

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