BIORHYTHMIC VARIATIONS OF WHITE FORMED ELEMENTS IN BROILER CHICKENS

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Abstract: *it is well-known that variations in the environment have given a certain biorhythm to all functions of animal bodies. In this context, it is important to find the correlation between biorhythmic variations and the most favourable moments in a nychthemeron for applying treatments and performing prevention activities through vaccination. The experiment studies nychthemeral variations of white formed elements, especially the variation of lymphocyte count in broiler chickens. The chickens were observed weekly, since they were one day old until they were 21 days old, before and after being vaccinated against Newcastle disease. The experiment involved 10 broiler chickens, since they were one day old until they were 21 days old. When the chickens were one day, 7 days, 14 days and 21 days old, blood samples were taken for the preparation of smears (at 7 o'clock, 1 pm, 7 pm and 1 am) which were stained through the Diff Quick method. The smears were examined by reading 200 leucocytes/smear. The chickens were vaccinated when they were one day old and then again when they were 14 days old, with La Sota live attenuated vaccine. Blood samples were taken from 6 chickens at 21 days, for the determination of vaccine antibodies. The experimental data were processed statistically with the help of the non-parametric Mann Whitney U Test. When the broiler chickens were one day old, the highest value of lymphocyte count during a nychthemeron was recorded at 7 o'clock in the morning (40.45±3.36%). Statistically, this value was insignificantly bigger than the values obtained at 1 pm, 7 pm and 1 am. When the chickens were 7 days old, statistically significant differences were recorded in the lymphocyte count, with bigger values in the morning than at night ($p<0.05$). In the same way, the values were bigger at noon than in the evening ($p<0.05$) and at night ($p<0.001$), respectively. When the chickens were 14 and 21 days old, no statistically significant differences were found among the values recorded during a nychthemeron. The average proportion of lymphocytes in one day-old chickens during one nychthemeron is significantly lower ($p<0.0001$) than the average values of 7, 14 and 21 days, after the vaccination against Newcastle disease. The statistically significant increase of the lymphocyte count in broiler chickens until 14 days old is the effect of the vaccination they received when they were one day old. The booster vaccination received at 14 days does not statistically change the nychthemeral average proportion of lymphocytes. Similarly, from a statistical point of view it changes nothing in the average lymphocyte count in the different sampling hours when the chickens were 14 and 21 days old.*

Key words: *biorhythm, nychthemeron, broiler chicken, leucocytes, lymphocytes*

INTRODUCTION

Living organisms developed under diurnal, nocturnal, nyctemeral, selenar, seasonal, annual and multiannual variations of the environment, variations that have induced a certain biorhythm to all the functions of the animal body. Any functional manifestation of an organism that reappears at regular time intervals points to the existence of a biorhythm. At organism level, the best-studied biorhythm is the circadian one (diurnal or nyctemeral) with a period of 24 hours and that involves physiological, metabolic, biochemical and behavioural biorhythms.

Chronobiology, the branch of medicine that studies the temporal characteristics of biological phenomena, and particularly chronopharmacology, can provide alternative solutions for the application of curative and preventive treatments by correlating biorhythmical variations with the most favourable moments of a nyctemer for vaccination or for administration of medicine.

They studied widely the existence of biorhythms in cardiac activity both in humans (2, 3, 4) and domestic animals (7, 9, 8, 10), as well as in haematology, in cattle, rabbits, and dogs (1, 6, 11).

MATERIALS AND METHODS

The experiment was carried out on 10 broiler chickens aged between 1 day and 21 days. The broiler chickens were housed in a battery ensuring 0.15 m^2/individual; they were fed on standard combined feed corresponding to their age category. The microclimate conditions were 28-32ºC according to age and a light regime of 24 hours.

At the age of 1, 7, 14 and 21 days, we sampled blood from the crest at 7, 13, 19 and 01 o'clock. We prepared smears coloured through the Diff Quick method, and then examined them under the microscope. Establishing the number of white elements was done by reading 200 leukocytes/smear. The number of white elements identified was then divided by two to get the percentage corresponding to each white element.

Vaccinating broiler chicken against avian pseudo pest was done at the age of 1 and 14 days with the live attenuated vaccine La Sota. At the age of 21 days, we sampled blood from six broiler chickens to dosage vaccine antibodies.

Statistic comparison of experimental data regarding the share of leukocytes during nyctemer at different ages pre- and post-vaccine was processed statistically with the non-parametric Mann Whitney U Test.

RESULTS AND DISCUSSION

Mean values of the share of leukocytes during nyctemer and per broiler chicken age are shown in Table 1. We could see that at the age of 1 day, the largest share of leukocytes was during nyctemer at 7:00 a.m. This corresponds to the moment the broiler chicken vaccination (Figure 1).

Mean value of the share of leukocytes per nyctemer was 38.46±1.37% at the age of 1 day. After vaccination, at the age of 7 and 14 days, the share of leukocytes increased as a response to vaccine antigens to 52.42±1.45% at the age of 7 days and to 60.10±0.40% at the age of 14 days (Figure 2). In percentage, compared to the share of leukocytes at the age of 1 day, their number increased with 36.30% at the age of 7 days post vaccination and with 56.24% at the age of 14 days after vaccination pointing out the increase of the immune response through lymphocyte activation (Figure 3).

Table 1

Mean share of leukocytes during nyctemer and in evolution from 1 day to 21 days in broiler chickens

Hour	Age (days)				Mean
	1	7	14	21	
7	40.45	53.35	59.65	60.20	**53.41±9.18**
13	37.50	53.95	59.90	61.05	**53.10±10.85**
19	37.60	51.50	60.30	59.35	**52.26±10.49**
01	38.3	50.90	60.55	59.65	**52.56±10.32**
Mean	**38.46±1.37**	**52.42±1.45**	**60.10±0.40**	**60.06±0.74**	**52.83±10.19**

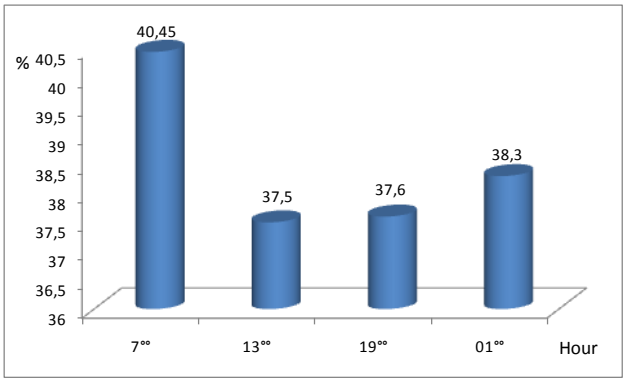

Figure 1. Mean share of leukocytes during nyctemer in broiler chickens aged 1 day

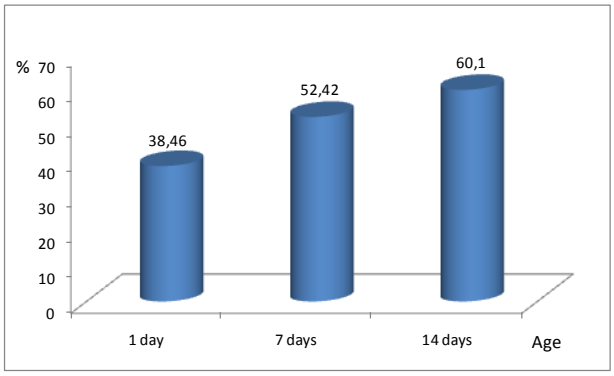

Figure 2. Mean nyctemeral values of the share of leukocytes in broiler chickens

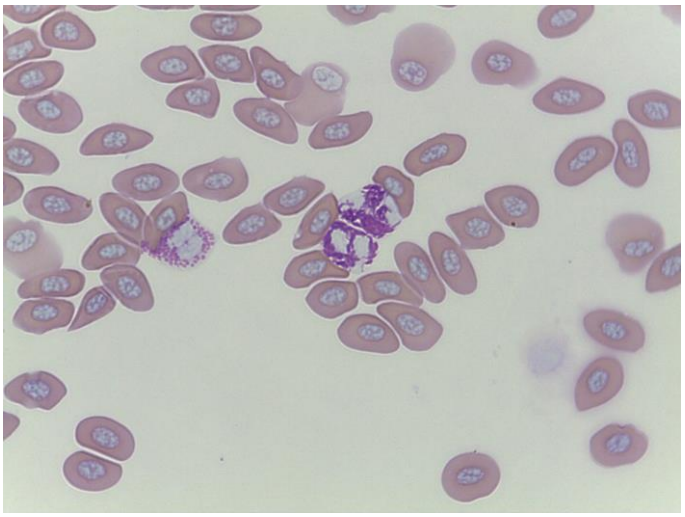

Figure 3. Aspect of white elements in broiler chickens at the age of 14 days after vaccination

After vaccination at the age of 14 days, we could see an insignificant statistically increase of the share of lymphocytes until the age of 21 days after vaccination (Figure 4).

In broiler chickens, mean nyctemeral values of the population of lymphocytes from the age of 1 day to the age of 21 days point out that the share of lymphocytes varies insignificantly depending on sampling time; the mean value is 52.83±10.19% (Figure 5), except for the mean nyctemeral values that was lower at the age of 7 days ($p<0.0001$).

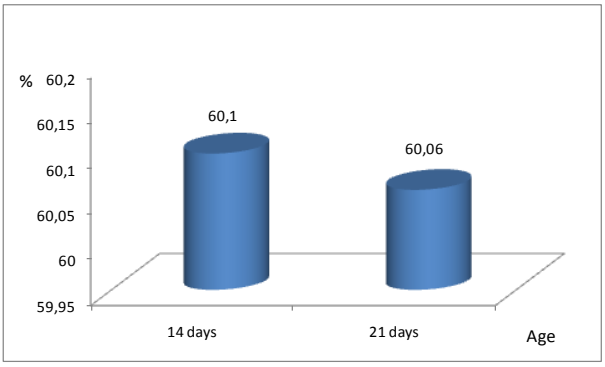

Figure 4. Mean nyctemeral share of lymphocytes in broiler chickens at the age of 14 and 21 days after vaccination against avian pseudo pest

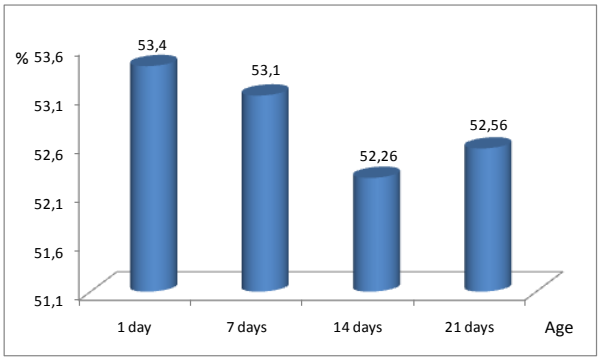

Figure 5. Mean nyctemeral values of the share of lymphocytes in broiler chickens between the age of 1 day and 21 days

Immune response to vaccine antigens at the age of 21 days shows a high antibody titre (Table 6). This antibody titre may be correlated with the time of vaccination during nyctemer, i.e. at the time of the maximum share of lymphocytes (7:00 a.m.).

Table 2

Anti pseudo pest vaccine antibody titre in broiler chicken aged 21 days

Broiler chickens number	**Anti pseudo pest vaccine antibody titre**
1	1/2048
2	1/1024
3	1/1024
4	1/2048
5	1/1024
6	1/1024
Mean	**1/1365,33**

CONCLUSIONS

1. At the age of 1 day in broiler chickens, the highest value of the share of lymphocytes during nyctemer was in the morning at 7:00 a.m. (40.45±3.36%), a value that is statistically insignificant higher compared to the values recorded at 13:00 and 19:00 p.m. and at 1:00 a.m.
2. At the age of 7 days, there were statistically significant differences between leukocyte shares, with higher values in the morning than in the evening ($p<0.05$) and in the afternoon than in the evening ($p<0.05$) or at night ($p<0.001$). These differences can be the response of the body to vaccine antigens.
3. At the age of 14 and 21 days, there were no statistically significant differences between the values measured during the nyctemer.
4. The mean share of leukocytes during nyctemer at the age of 1 day is significantly smaller ($p<0.0001$) than the mean values at 7, 14 and 21 days after vaccination against avian pseudo pest.
5. At the age of 7 days, the mean share of lymphocytes during nyctemer was statistically significantly smaller ($p<0.0001$) than the mean values at the age of 14 days and 21 days. There were no statistically significant differences between the mean share of the lymphocytes during nyctemer at the age of 14 and 2 days.
6. The statistically significant increase of the share of lymphocytes in broiler chickens up to the age of 14 days is the effect of vaccination at the age of 1 day. The vaccination at 14 days according to the vaccination protocol does not change significantly the mean nyctemeral share of lymphocytes or between the different sampling times at the age of 14 and 21 days.

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