DETERMINATION OF DOPAMINE AND SEROTONIN WITH IMMUNE STATE CD4 AND CD8 OF THE NEWCASTLE INFECTION IN BROILERS

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ABSTRACT

This study was aimed to describ the course of Newcastle disease at 24 and 72 hr. of infections in broilers flocks within the period (1-December-2021 to 1- Jun- 2022) and the study aimed to determine the immunological (CD4, CD8) response and changes in neurotransmitter hormones. The blood samples were collected and prepared then examined by ELISA kits test and were worked under the protocols of companies. The result of immunological response showed the response to cellular immunity (CD8) in all cases increasing within 24hr.with statistically represented levels while, the result of humoral immunity (CD4) increasing in all cases except two cases within 72hr. with statistically significant levels as well as the results of (CD4/ CD8) ratio. while the results neurotransmitter hormones concentration revealed all cases statistically decreased represented levels at 72hr. The study concluded the 1st immune response within 24hr. showed elevation with cellular immunity then humoral immunity after 48-72hr. Also the ND infections caused down-regulation of neurotransmitter hormones by decreasing to serotonin and dopamine levels during all periods of infections.

Key words: Neurotransmitter hormones, neurochemical responses.

تحديد مستويات الدوبامين والسيروتونين مع الحالة المناعية CD4 و CD8 لمرض النيوكاسل في الدجاج اللاحم

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المستخلص

وصفت الدراسة مسار مرض نيوكاسل في ال 24 و72 ساعة من الإصابات في قطعان الدجاج اللاحم خلال للفترة بين 1 ديسمبر – 2021 إلى 1 يونيو – 2022.هدفت الدراسة إلى تحديد الاستجابة المناعية (CD4 و CD8) والتغيرات في هرمونات الناقل العصبي (الدوبامين والسيروتونين). تم جمع عينات الدم وتحضيرها بعد 24 ساعة و72 ساعة من الإصابة، تم فحصها بواسطة اختبار ELISA وعمل الفحوصات بموجب بروتكول الشركات المصنعة. أظهرت نتائج الاستجابة المناعة الخلوية (CD8)زيادة في جميع الحالات خلال ال 24 ساعة مع تمثيل إحصائيًا عند مستويات. ($P\leq 0.05$) بينما أظهرت نتيجة المناعة الخلطية (CD4) زيادة في جميع الحالات ما عدا حالتين عند 72 ساعة مع تمثيل إحصائي عند مستويات ($P\leq 0.05$) ،كذلك نتائج نسبة(CD4 و CD8) في حين سجلت نتائج أن تركيز هرمونات النواقل العصبية (الدوبامين، والسيروتونين) انخفاض في جميع الحالات مع تمثيل إحصائي عند مستويات ($P\leq 0.05$) عند 72 ساعة من الإصابة خلصت الدراسة إلى أول استجابة مناعية خلال 24 ساعة من المرض ارتفاع مع المناعة الخلوية تلتها المناعة الخلطية بعد 48–72 ساعة من المرض، بينما تسبب مرض النيوكاسل في انخفاض تنظيم هرمونات الناقل العصبي عن طريق الانخفاض إلى مستويات السيروتونين والدوبامين خلال جميع فترات العدوى.

الكلمات المفتاحية: هرمونات الناقل العصبي، الاستجابات الكيميائية العصبية.

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INTRODUCTION

Newcastle disease (ND) is one of the major viral contagious diseases in poultry, causing heavy economic losses in poultry production and causing retard growth with high morbidity and mortality and a decrease in egg production (10,31). The (NDV) classified to family *Paramyxoviridae*, type one (PMV-1) negative-sense RNA, single-stranded, non-segmented (24). The ND disease remains the main problem in the poultry industry because caused damage to nervous, respiratory, and GIT systems (7). Also caused failure to the immune response to ND vaccine by causing disturbance to humoral and cellular immunity (27,30). The immune response after six hours' post-infection to ND splenic cell proliferation and produce alpha and beta interferon with (IL-6) to activate cell-mediated immunity (CMI) (15,23). Also, the T-lymphocyte through T helper cell type 1 especially Cytotoxic T lymphocytes (CD8) immediately activated after several hours' post-infection with ND (18). Then activation became more advance through stimulation other of cytokines and T helper cell type 2 (CD4) within 2-3 days of ND infections (29). The infection with ND will cause a challenge to the immune response and activation of the (hypothalamic-pituitary-adrenocortical) center, as well as the concentrations of corticosterone and hypothalamic will increase after exposure to lento-mesogenic (NDV) that will cause down release to serotonin and dopamine levels to change neurochemical responses (1,2,9). Dopamine and serotonin (neurotransmitter hormones) in the brain are responsible for several critical functions (19). These hormones act on the regulation immune system in addition to CNS processes, gastrointestinal motility, blood pressure, and neuroimmune communication (8,23).

MATERIALS AND METHODS

Sampling and Preparation: Thirty samples were collected from Broilers infected flocks during the course of Newcastle disease within 1st and 3rd days of infections between the period (1-December-2021 to 1-Jun-2022) (14). Blood samples were collected in a gel tube and then exposed to a centrifuge (1500/ rpm × 15mint) serum was collected in an Eppendorf tube and stored in a freezer (-20) until ELISA measuring (15)

ELISA Kit: Chicken cluster of differentiation 4(CD4): Procedure of kit according to the company protocol (CUSABIO®) with detection range (3.125ng/ml-800 ng/ml.).

Chicken cluster of differentiation 8(CD8): Procedure of kit according to the company protocol (CUSABIO®) with detection range (3.125ng/ml-800 ng/ml.).

ST/5-HT(Serotonin/5Hydroxytryptamine): Procedure of kit according to the company protocol (Elabscience®) with detection range (15.63-1000 ng/ml.) and Sensitivity (9.38 ng/ml.).

DA(Dopamine): Procedure of kit according to the company protocol (Elabscience®) with detection range (31.25-2000 pg/ml.) and Sensitivity (18.75 pg/ml.).

Statistical analysis

The program of Statistical Analysis System, SAS was used different study parameters (Chi-square) to show Least significant difference between transactions and (LSD test) to find significant contrast between variable means (26).

RESULTS AND DISCUSSION

The results show in **Table-1** immunological response to humoral immunity (CD4) and cellular immunity (CD8) during the course of Newcastle disease infections result of humoral immunity (CD4) in all cases except two cases revealed increasing levels at 72hr. In comparison with 24hr. with statistically significant at ($P \leq 0.05$) levels meaning the humoral immunity will be activated after 24-72hr.while the response of cellular immunity (CD8) showed all cases increasing of CD8 levels at 24hr. In comparison with 72hr. with statistically significant at ($P \leq 0.05$) to ND infection represented the cellular immunity and 2nd response humoral immunity. These results of immune response to cellular and humoral immunity agree with other studies that represented the CMI firstly activated and rapidly expand T-lymphocyte in the thymus gland and spleen especially (CD8) T lymphocytes after mints of exposure to NDV antigen (17,18). Activated T-cell helper (CD4) to activate and differentiate B-lymphocytes into plasma cells to produce antibodies to

neutralize specific NDV antigens (16,17). Also, the infection with a lentogenic strain of NDV caused T cells activation and proliferation in vivo and in vitro (12). As well as the immune response to infection with NDV immediately develop T- cell through activation of cytotoxic T cells CD8 a regulatory role and follow by helper T cells to express CD4 on the surface (4,17). The challenge by field strains of NDV caused a significant increase ($p \leq 0.001$) in the T-cell population specifically (CD8 and CD4) in the intestinal and respiratory tissue in broilers chicken and in the sera (13).

Table 1. CD4 and CD8 concentration level at 24 and 72 hr. against NDV

CD4	24 hr. (ng/L)	72 hr. (ng/L)	LSD value	CD8	24 hr. (ng/L)	72 hr. (ng/L)	LSD value
1	115.701	242.266	73.29 *	1	249.382	107.984	77.02 *
2	109.334	200.993	66.42 *	2	285.955	112.322	69.45 *
3	123.958	261.784	75.39 *	3	209.272	98.455	77.33 *
4	179.320	288.532	61.28 *	4	337.358	194.947	79.45 *
5	201.009	321.097	69.04 *	5	348.818	210.817	66.92 *
6	89.991	123.209	43.59 NS	6	322.572	213.026	63.88 *
7	142.305	265.233	83.24 *	7	300.719	189.926	72.06 *
8	185.509	302.126	71.22 *	8	194.947	89.279	53.48 *
9	151.577	251.701	65.09 *	9	287.626	112.006	73.17 *
10	93.828	167.543	59.44 *	10	285.095	121.234	84.91 *
11	111.644	196.992	64.26 *	11	311.136	172.721	65.43 *
12	113.811	206.345	70.66 *	12	346.545	206.878	71.08 *
13	126.089	249.284	91.03 *	13	340.175	196.929	85.33 *
14	221.134	346.027	74.58 *	14	321.033	193.426	70.96 *
15	173.588	284.602	64.98 *	15	297.962	167.733	63.29 *
16	151.542	256.533	62.9 *	16	255.921	123.834	66.84 *
17	206.101	331.875	78.65 *	17	234.452	104.691	61.02 *
18	210.234	299.843	61.02 *	18	304.745	177.024	82.46 *
19	182.305	161.606	39.85 NS	19	199.828	88.235	59.77 *
20	92.911	248.092	72.36 *	20	279.080	117.266	65.14 *
21	134.678	330.126	95.43 *	21	269.311	109.488	57.22 *
22	201.271	304.221	76.82 *	22	350.722	208.301	71.09 *
23	192.444	304.221	69.73 *	23	367.900	211.427	82.35 *
24	160.378	277.424	63.22 *	24	328.316	169.928	77.93 *
25	164.590	280.611	78.09 *	25	298.911	142.735	70.23 *
26	136.590	240.001	60.55 *	26	344.622	200.644	85.06 *
27	179.701	279.999	58.94 *	27	276.842	191.765	72.19 *
28	177.542	273.829	62.03 *	28	210.936	101.013	79.67 *
29	185.976	301.661	73.98 *	29	193.792	96.924	63.22 *
30	220.358	348.288	64.10 *	30	361.212	211.106	71.09 *

*** (P≤0.05).**

The result shows **Table-2** show (CD4/ CD8) ratio between time 24hr. and 72hr. during the course of Newcastle disease infections showed all cases increasing of (CD4/ CD8) ratio levels at 72hr. In compression with 24 hr. with statistically represented at ($P \leq 0.05$) levels meaning development of both cellular and humoral immune responses. The results of the (CD4/ CD8) ratio also agrees with the study that revealed the administration of the different commercial vaccines to ND days old in broiler flocks increased the concentration of (CD4-CD8) ratio with high significant levels ($p \leq 0.001$) levels when detected by ELISA (4,8,17), and immunohistochemistry in the each of intestine, trachea, and serum (3,5). While the Bar-Shira *et al.*, rvealed no significant difference in the average (CD4/CD8) ratio after both 1st and 2nd vaccination with the B1 strain of ND vaccine (6).

Table 2. CD4 and CD8 ratio at 24 and 72 hr. against NDV

Time	CD4 ng/L	CD8 ng/L	CD4/CD8 ratio
24hr.	157.84 ± 7.29	290.50 ± 9.55	0.54
72hr.	264.86 ± 10.08	154.73 ± 8.40	1.71
LSD value	57.97 *	72.83 *	0.448 *

* (P≤0.05).

The result (**Table-3**) of levels neurotransmitter hormones (Dopamine, Serotonin) during the course of Newcastle disease infections shown are as follows: The result of Dopamine and Serotonin concentration revealed all cases decreased with statistically significant at (P≤ 0.05) levels at 72hr. in compression with 24hr.ND infections. The result of dopamine and serotonin concentration during the course of Newcastle disease infections was compatible that revealed the following. The neurotransmitter hormones especially dopamine and serotonin levels had a relationship with immunological state because play a major role in body activities (appetite, weight gain, energy expenditure, mediatory role of ingestion, and animal behavior) because act a stimulatory, modulatory or inhibitory role in avian species (21,25). The challenge of NDV showed an increase in plasma corticosterone hormone that will lead to a decline in the concentration of dopamine and serotonin levels in mice (1,10). The deficiency in serotonin and dopamine levels caused cannibalism and feather and vent picking and the deficiency of neurotransmitter hormones had a close relationship with neurological signs (4,11). Also, the levels of dopamine and serotonin were significantly decreased (p≤0.05) in broiler chicken feeding a contaminated diet with mycotoxin when compared with groups feeding a diet containing anti-mycotoxin or free mycotoxin (22). In general, the immune and nervous system participate in extensive pathways mediated by a wide of hormones, cytokines, and neurotransmitters (20,23), and elicits immune responses that will lead improve immunity and healthy performance (8,28).

Table 3. Dopamine and serotonin levels at 24 and 72 hr. against NDV

DOP	24hr. (ng/ml)	72hr. (ng/ml)	LSD value	SERO	24hr. (pg/ml)	72hr. (pg/ml)	LSD value
1	6.469	2.142	3.59 *	1	18.528	9.602	5.41 *
2	6.528	1.367	3.77 *	2	18.578	9.830	5.75 *
3	5.733	0.706	3.09 *	3	14.243	4.904	6.33 *
4	4.999	0.191	3.28 *	4	17.158	6.002	5.04 *
5	6.665	1.408	4.02 *	5	19.520	10.127	5.28 *
6	6.450	1.815	3.55 *	6	14.138	5.553	5.61 *
7	6.454	1.885	3.52 *	7	14.122	5.479	5.44 *
8	5.818	0.908	3.59 *	8	19.719	9.972	6.83 *
9	6.453	1.912	3.64 *	9	15.882	6.911	5.04 *
10	4.726	0.177	2.98 *	10	17.326	7.813	6.19 *
11	5.907	0.969	3.02 *	11	16.699	5.641	4.98 *
12	5.676	0.733	3.55 *	12	18.906	9.648	5.66 *
13	6.376	1.698	3.65 *	13	14.273	5.374	5.52 *
14	6.382	1.467	3.29 *	14	14.479	5.855	6.42 *
15	6.009	1.316	4.01 *	15	14.137	5.257	6.29 *
16	6.309	1.403	3.55*	16	17.006	7.361	5.98 *
17	5.483	0.736	3.02 *	17	15.371	5.902	5.44 *
18	6.138	1.199	3.26 *	18	15.462	6.004	5.06 *
19	5.844	0.978	2.88 *	19	16.877	6.789	4.89 *
20	6.670	2.261	2.94 *	20	14.913	5.912	5.39 *
21	5.322	0.386	3.44 *	21	14.296	4.332	5.09 *
22	5.910	1.176	2.91 *	22	14.770	4.838	4.81 *
23	5.819	1.100	3.05 *	23	15.221	5.839	5.32 *
24	6.254	1.399	3.95 *	24	18.710	9.686	5.49 *
25	4.220	0.349	2.69 *	25	14.731	5.618	5.87 *
26	5.627	0.931	3.42 *	26	14.091	5.322	5.61 *
27	6.551	1.792	3.64 *	27	15.515	6.131	5.33 *
28	5.370	0.614	3.08 *	28	15.388	4.999	5.18 *
29	5.713	0.922	3.51 *	29	17.202	7.387	6.21 *
30	6.788	2.317	2.86 *	30	14.211	5.413	5.38 *

* (P≤0.05).

CONCLUSIONS

The conclusion of the study of the Newcastle disease infection course has two immune responses the primary response activation of cellular immunity through increasing of (CD8) T- cell levels during the 1st 24 hr. of infection then, activation of the secondary immune response by humoral immunity through increasing of (CD4) T- cell levels within 72hr., as well as the ND infections caused down-regulation to neurotransmitter hormones through decreasing to serotonin and dopamine levels in all period of infections.

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