Zag. Vet. J.(2001) Vol. 29, No. 1 pp. 61-81.

Biochemical, Immunological And Blood Haematological Changes In Thymectomized Japanese Quail Vaccinated With Newcastle Disease Virus

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ABSTRACT

The aim of the present study was to investigate the possible effects of total bilateral thymectomy on some hematological parameters, immune responses and on the level of serum free amino acid pattern as well as serum protein and its components in the blood of Japanese quail vaccinated with Newcastle disease virus vaccine .A total number of 180 Japanese quails (Coturnix coturnix japonica) were used in this study. The experimental work was designed into two experiments. Experiment I: comprised 100 birds of both sexes (50 male and 50 female). At 10^{th}.day old, birds were equally divided into 4 groups (2 groups of male and 2 groups of female), and the total bilateral surgical thymectomy was performed for one group of male and one group of female birds. Two weeks post-thymectomy period blood samples were taken every week from control and thymectomized group for 5 successive weeks for determination of hematological parameters. Experiment II: included 80 male and female birds. A total bilateral thymectomy was performed for 40 birds at 10 days of age and the 40 kept as intact control. Two weeks post-thymectomy period the intact and thymectomized birds were divided into four equal groups. GroupI: intact control (non-vaccinated). GroupII: thymectomized birds (non-vaccinated). Group III: intact birds (vaccinated). GroupIV: thymectomized birds (vaccinated). Two weeks later post-vaccination blood samples were collected from all animal groups. Serum was separated and used for determination of serum protein and its fractions, free amino acids and haemagglutination inhibition antibody titer. Also, heparinized blood was taken for lymphocyte blastogenesis micro-assay. Two weeks post-vaccination all groups were challenged with a velogenic viscerotropic NDV strain via i.m route. The obtained results revealed that, thymectomy caused significant decrease in erythrocytic count in both male and female group, whereas PCV was significantly decreased in female group. Total leukocytic count was markedly reduced in both male and female groups. Lymphocyte percentage was significantly decreased, whereas heterophils percentage was increased in both male and female after thymectomy. Immunological study revealed that thymectomy caused marked reduction in the level of blastogenic capacity of peripheral blood lymphocytes induced by phytohaemagglutinin and by NDV antigen in both vaccinated and non-vaccinated thymectomized group compared with control one. Antibodies were markedly reduced after thymectomy in vaccinated groups determined by hemagglutination inhibition antibody titer. Challenge with VVNDV two weeks post-vaccination resulted in 75% protection rate in vaccinated intact control group and thymectomized one compared with a protection rate 50% in intact and 25% in thymectomized non-vaccinated group. The biochemical changes after thymectomy showed a significant decrease in the value of serum glutamic, proline, methionine and arginine concentrations, whereas serum cysteine, isoleucine and tyrosine levels revealed a significant increase in thymectomized quail compared with intact control (groupI). There was a significant decrease in the concentrations of serum glutamic and proline values in intact birds vaccinated with lasota strain of Newcastle disease virus (NDV) compared with intact non-vaccinated (groupI). A significant decrease in serum glycine, valine, isoleucine, tyrosine and lysine levels in thymectomized vaccinated quail with NDV. Meanwhile, serum aspartic, proline and arginine concentrations showed a significant increase when compared with thymectomized non-vaccinated (groupII). The values of serum total protein, albumin and gamma globulin levels showed a significant decrease, whereas serum alpha-globulin level showed a significant increase in thymectomized quails compared with intact control (groupI). Serum alpha-globulin level exhibited a significant increase in intact quails vaccinated with NDV. However, serum protein and its fractions showed no significant changes in thymectomized vaccinated quails compared to thymectomized non-vaccinated (groupII). The results of this study indicate that, thymus gland seems to be important for erythropoiesis, leukopoiesis, and lymphocyte number and its activity in cell mediated immune response as well as antibody formation. Also, from biochemical investigations thymus gland seems to be participates in regulation of protein and amino acids metabolism.

INTRODUCTION

Poultry species including quails constitute a major source of meat and eggs. Quails become mature within six weeks and in full egg production by 50 days of age and their meat and eggs are considered of high protein content and of good taste and delicacy **(1)**.

Thymus glands is considered a primary lymphoid organ, a variety of polypeptides known as cytokines, lymphokines and thymic hormones are produced by epitheliolymphoid cells of the thymus **(2)**. These humoral agents seem to be important in differentiation, immune response, endocrine functions, calcium regulation, cell growth and metabolism. All of these functions disappear from the circulation following thymoctomy **(3)**.

The homeostatic thymic hormone **(4)** is a relatively small molecule (mol. Wt. about 2000) that contains amino acids, amino sugars and

possibly a nucleotide. This substance appears to play a permissive role in the negative feedback effects of several hormones on the hypothalamo-hypophysial axis as well as being immunologically active itself **(3)**. Moreover, thymosin (protein extracted from the thymus) alleviates leukopenia and provide some improvement in lymphoid histology in thymectomized mice **(5)**.

Thymectomy in neonatal mice results in marked reduction in lymphocytes in the blood, lymphoid hypoplasia of bone marrow resulting mild anemia **(6)**.

Numerous experiments have identified the role of the avian thymus and bursa of Fabricius in the immune response and the interrelation of these glands and their cellular products with the neural endocrine system. Steroids influence the growth pattern of the thymus and bursa. Increases in thymi growth appear to be controlled, in part, by fluctuations in thyroid activity **(7)**. Moreover, the thymus probably presents some kind of relation with the hypothalamus through a positive feed-back, stimulating secretion of substances that would act on the adenohypophysis rising the secretion of growth hormone. In addition thymus playing a role in maintaining the serum levels of growth hormone and thyroxine necessary for the growth and development of bones**(8)**.

Vaccination is the main routes of combating or controlling viral diseases of chickens, as they stimulate the defense mechanisms to produce antibodies. Since antibodies are consists of proteins which are composed of amino acids, supplementation of amino acids in particularly the essential ones plays a main role in combating such diseases **(9)**.

Serum amino acids of chickens are affected with viral and bacterial diseases **(10)**. Also, the essential amino acids (phenylalanine, lysine, methionine, valine, leucine, isoleucine, threonine, arginine, histidine and proline) were decreased in the serum of chickens after vaccination against Newcastle and Gumboro diseases. As these amino acid play an important role in the production of antibodies which are responsible for defense mechanisms **(11)**.

To our knowledge the changes taking place in the biochemical pattern of serum free amino acids and electrophoretic profiles of serum protein under the effect of thymus deficiency (thymectomy) are not previously recorded.

Accordingly, the objective of this experiment to investigate the possible effect of thymectomy on the hematological picture, immune response and some biochemical parameters such as electrophoretic pattern of serum protein and free amino acids in Japanese quail vaccinated with Newcastle disease virus vaccine.

MATERIAL AND METHODS

One hundred and eighty, one day old Japanese Quails *(Coturnix coturnix japonica)* were used in the experimental investigation of this study. The birds were obtained from a private farm at Kalubia province. They were kept at a constant environmental and nutritional condition throughout the period of the experiment, and housed in wire cages. Continuous light program (23 hours light:1 hour dark) were used **(12)**. The birds were grown up on a formulated commercial balanced broiler starter ration containing 28% protein and various necessary ingredients for optimal growth. Food and water were supplied ad-libitum.

*** Newcastle disease lives vaccine: -**

Delvax ND Lasota vaccine with a titer of 10^6 ELD_{50} of virus / dose.

*** Challenge ND virus: -**

The velogenic viscerotropic Newcastle disease virus (VVNDV) containing $10^6 ELD_{50}$ was used as challenge virus for all birds.

***Design and procedural steps for experimental work: -**

The present study was designed into two experiments.

Experiment I: -

One hundred birds of both sexes (50 males and 50 females) were used in this experiment. At 10^{th}. day old, birds were divided equally according to their sex into four groups, each one consisting of 25 birds (2 groups of males and 2 groups of females).

A total bilateral thymectomy was performed surgically **(13)** at 10 days of age for one group of male and one group of female birds and other groups were kept intact as a control group.

Two weeks post-thymectomy blood samples were taken from all animal groups (thymectomized and normal intact quails) after sacrificing of birds 5 times and periodically every 3-week for a duration of six weeks. Heparinized blood samples were collected and gently mixed for hematological parameter determinations.Counting of erythrocytes and leukocytes were performed using **(14)** method.4- Packed cell volume was determined by the microhematocrit method. Blood smears were prepared and stained by Giemsa stain for relative distribution of leukocytes.

Experiment II: -

A total number of eighty males and females quails were used in this experiment. A total bilateral thymectomy was performed by surgical removal of thymus gland from 40 birds at 10 days of age and the other 40 kept as intact control. Two weeks post-thymectomy both thymectomized and normal intact quails were divided into four equal groups each one consisting of 20 birds, placed in individual cages and classified as follows: -

Group I: - Intact control (non-vaccinated).

Group II: - Thymectomized (non-vaccinated).
Group III: - Intact (vaccinated).
Group IV: - Thymectomized (vaccinated).

All birds in-group III and group IV were vaccinated with live lasota Newcastle disease vaccine (NDV) through ocular route.

Blood samples: -

Blood samples for serum separation were obtained from all groups (control and experimental groups) by heart puncturing at two weeks post-vaccination, then allowed to coagulate at room temperature for 30 minutes, then centrifuged at 3000 r.p.m for 10 minutes. The clean serum was separated and kept in a deep freeze at –20°C until used for subsequent biochemical analysis. Another blood samples were taken in heparinized tube for lymphocyte blastogenesis micro-assay **(15)**.

Biochemical analysis: -

1- Total protein was determined according to **(16)**.
2- Serum protein fractions, were quantitatively estimated by using polyacrylamide gel electrophoresis **(17).** Quantitations of the different protein fractions were performed by using modified Beckman scanner.
3- Free amino acids were estimated chromatographically **(18)** using Beckman Amino Acid Analyzer Model 119/CL. preparation of serum sample for quantification of amino acids were carried out **(19).**
4- Haemagglutination inhibition test of Newcastle disease virus was performed **(20)**.

Two weeks post-vaccination all groups were challenged i.m. With velogenic viscerotropic Newcastle disease virus (VVNDV). The virus was prepared locally in Vet. Serum and Vaccine Research Institute, Abbasia, Cairo.

Also three weeks post-challenge all birds were kept under observation, clinically symptoms and mortality were recorded.

Statistical analysis: -

The obtained data were statistically analyzed and the significant difference between groups was evaluated **(21).**

RESULTS

Effect of thymectomy on some hematological parameters was given in table 1. Red blood cells (RBCs) count in male group was significantly decreased ($P<0.05$ after two weeks, $P<0.01$ after 3 weeks) after thymectomy at 2 and 3 weeks. This decrease became non-significant at 4,5 and 6 weeks. While, in female group, thymectomy caused significant decrease ($P<0.05$) in RBCs count after 2, 3, 4, 5 and 6 weeks. Packed cell volume (PCV) was non-significantly decreased in male group during all experimental period. While, in female group PCV was significantly decreased after 2, 5, 6 weeks, whereas at 3 and 4 weeks there was non-significant decrease. Total leukocytic count in male group was significantly ($P<0.05$ for 2, 4, 6 weeks; $P<0.01$ for 3 and 5 weeks) decreased during all experimental period. In female group, total leukocytic count was also significantly ($P<0.05$ at 6 weeks, $P<0.01$ at 2, 3 weeks, $P<0.01$ at 4, 5 weeks) decreased during all experimental period.

Table 2 showed effect of thymectomy on relative distribution of leukocytes in male group. It revealed that, lymphocytes percentage was significantly ($P<0.001$ for 2 weeks, $P<0.01$ for 3

weeks) decreased after 2, 3 weeks. This decrease became non-significant after 4, 5 and 6 weeks. While, heterophilis percentage was significantly ($P<0.001$) increased after 2 weeks. This increase became non-significant after 3, 4, 5 and 6 weeks. Eosinophilis, monocytes and basophilis showed non-significantly change after thymectomy.

Table 3 showed effect of thymectomy on relative distribution of leukocytes in female group. It revealed that, lymphocyte percentage was significantly ($P<0.001$) decreased after 2 and 3 weeks, then non significant decrease was noticed after 4, 5 and 6 weeks. Heterophilis percentage was significantly ($P<0.01$) increased after 2 and 3 weeks. This increase became non-significant after 4, 5 and 6 weeks. Eosinophilis, monocytes and basophilis percentage showed non-significantly change after thymectomy.

Results of cellular immunity using phytohaemagglutination and NDV antigen lymphocyte blastogenesis test as shown in table (4) indicated a positive response in the lymphocyte activity in group (III) intact thymus and vaccinated with a mean 1.760975 ± 0.06 and to NDV antigen 2.2150 ± 0.11 compared with thymectomized vaccinated which showed 1.180 ± 0.04 and 1.2933 ± 0.05 to NDV antigen.

No antibodies were detected by the haemagglutination inhibition test before vaccination. Antibody titers in group (III) intact vaccinated showed Log^2HI antibody titer (4). In group (IV) Thymectomized and vaccinated quails showed Log^2HI antibody titer (1). After challenge group (III) and group (IV) showed a protection rate 75% while in group (I) 4 bird out of (8) died and these birds showed a 50% protection while birds in group (II) showed 25% protection as showed in table (5). Results of both humeral and cellular immunity comes in contact with that of challenge test against ND in-group (III) represented in table (5).

The obtained results table (6) and Fig. (2) showed a significant decrease in the value of serum Glutamic, proline, methionine and arginine concentrations in thymectomized Japanese quial. Meanwhile, there was a significant increase in the levels of cysteine, isoleucine and tyrosine. A non-significant decrease was recorded in the levels of Aspartic, serine, glycine, alanine, phenylalanine, histidine and lysine. On the other hand, the value of serum threonine, valine and leucine concentrations showed a non significant increase compared with intact control values (group I).

The obtained data table (6) and Fig. (3) revealed that, there was a significant decrease in the concentrations of serum glutamic and proline values. However, the levels of serum threonine, serine, glycine, phenylalanine and lysine revealed a non-significant decrease. Meanwhile, serum aspartic, alanine, cysteine, valine, methionine, isoleucine, leucine, tyrosine, histidine and arginine concentrations showed a non significant increase in normal intact (control) quail vaccinated with Lasota strain of Newcastle disease virus vaccine in comparison with intact control values (group I).

The obtained results table (6) and Fig. (4) showed a significant decrease in the value of serum glycine, valine, isoleucine, leucine, tyrosine and lysine levels in thymectomized quail vaccinated with lasota strain of Newcastle disease virus vaccine. Meanwhile, serum aspartic, proline and arginine concentration showed a significant increase. A non-significant decrease was observed in the levels of threonine, serine, alanine, cysteine and histidine. However, serum glutamic, methionine and phenylalanine concentrations showed a non significant increase when compared with thymectomized quail values (group II).

The obtained results table (7) and Fig. (5) showed a significant decrease in the value of serum total protein, albumin and gamma globulin concentrations. On the other hand, serum alpha-globulin level showed a significant increase while total globulin and beta-globulin levels exhibited a non significant decrease in thymectomized Japanese quail compared with intact control values (group I).

A non-significant decrease in serum total protein, albumin and beta globulin concentrations was reported in normal intact (control) quail vaccinated with lasota strain of Newcastle disease virus vaccine. However, serum total globulin and gamma globulin levels showed a non significant increase. On the other hand, the value of serum alpha-globulin level exhibited a significant increase in comparison with (control) values (group I).

A non-significant increase in the value of serum total protein, albumin, total globulin and

alpha-globulin concentrations in thymectomized Japanese quail vaccinated with Newcastle disease virus. However, serum gamma-globulin level showed a non significant decrease, while serum beta-globulin level exhibited no significant change when compared with thymectomized quail values (group II).

DISCUSSION

Thymectomy caused a significant reduction in the RBCs count. Similarly, athymic and thymectomized mice showed mild anemia and the author attributed this result to erythroid hypoplasia of the bone marrow as a result of lymphoid hypoplasia, due to the absence of thymocyte-derived factors promoting increase in erythrocyte production **(6)**. Moreover, in the fetus and the mature animals the lymphoepithelial thymus serves as a primary lymphoid organ for T lymphopoiesis. The activated T lymphocytes produce interleukin-3 that greatly increases the proliferation of bone marrow stem cells, which differentiated to granulocytes, erythrocytes and megakaryocytes **(2)**.

In male group, thymectomy caused significant reduction in RBCs count for 3 weeks after thymectomy, thereafter this reduction in RBCs count became non-significant, where as in female group the reduction in RBCs count continued to the end of the experiment. These results could be attributed to the interaction of sex hormones that begin to increase, as the animal became mature at age of 42 day in male and 50 day in female **(22).** The hematopoiesis action of the androgen may be reverse the effect of thymectomy in the male when the animal became mature. However, the thymectomized males are still lower than the intact one.

Packed cell volume was non-significantly decreased in male group and in female group (after 3, 4 weeks of thymectomy). This result could be attributed to increase in the size of the erythrocytes because the erythroblastic cells of bone marrow fail to proliferate rapidly and the cells become larger than normal ***(23)*.**

Total leukocytic count was markedly reduced in both male and female after thymectomy. In the same aspect, thymectomy in the mouse caused leukopenia and lymphopenia ***(5)***. He also reported that thymosin (is protein extracted from the thymus) alleviate leukopenia slightly in thymectomized mice. The relative distribution of the leukocytes in the present study revealed decrease of lymphocyte percentage and increase in the heterophilis percentage in thymectomized groups. From these results, thymectomy caused reduction in the circulating lymphocytes. Similar results were observed in neonatal mice after thymectomy ***(5)*** **and** ***(6)***. Moreover, thymus produces lymphocyte-stimulating hormone that increases the proportion of lymphocytes among white blood cells ***(3)***. Also, helper T lymphocytes cells produce several lymphokines, which affect the proliferation, differentiation and maturation of lymphocytes and hematopoietic cells ***(2)***.

Blastogenesis microassay employing 3-(4,5- dimethylthiazol-2-yl) 2,5 diphenyl Titrazolium bromide (MTT) was adapted to measure blastogenic responses of lymphocytes from quail blood to T. lymphocytes mitogens. Lymphocytes isolated from peripheral blood from group (III) intact thymus and vaccinated had highly significant mitogenic responses to T- cell mitogen (25ugm/ml) and to N.D soluble antigen at 25ugm/ml compared with lymphocyte from intact thymus non vaccinated (control), thymectomized and thymectomized- vaccinated ones. This was primarily due to the low yield of lymphocytes. The ability of the (MTT) blastogenesis microassay to detect blastogenic responses of the thymus gland to mitogenesis may be indicative of its important (useful) for measuring cell mediated immunity responses to other antigens. The importance of cell mediated immunity (CMI) in resistance to velogenic NDV in ova-bursectomized chickens using blastogenic capacity of peripheral blood lymphocytes induced by phytohemagglutination was proved by ***(24)***. Moreover, T cell responsible for delayed hypersensitivity required the presence of the thymus in their development that those responsible for cytotoxicity or antibody production ***(25)***. Immunocompetent B cells had not developed in thymectomized chickens ***(26)***. Moreover, chickens subjected to surgical thymectomy before vaccination the production of agglutinin and immunoglobulin was unimpaired but the production of resistance against challenge was diminished ***(27)***. Protection therefor thymus

dependent. Quail vaccinated with Lasota followed by VVND virus 2 weeks later are protected against N.D in presence or absence of thymus gland.

It is concluded that blastogenic capacity of peripheral blood lymphocytes induced by phytohaemagglutinin and by NDV antigen showed higher cell mediated immunity in presence of thymus gland and this affected on the immune response of quails against VVND virus.

Specific derangement of thyroid and gonadal function were observed in athymic nude and neonatal thymectomized mice. Such endocrine alterations are already established during the prenatal period and maintained through adult life. Thus the thymus may well have a basic role in the organization of the adult hypothalamus-pituitary axis for thyroid and sexual functions ***(28)***.

It is evident from the present study that, there was a significant decrease in the value of serum total protein, albumin and gamma globulin fraction concentrations as well as in the level of serum glutamic, proline, methionine and arginine amino acids in thymectomized Japanese quail. Meanwhile, the value of alpha-globulin level and serum cysteine , isoleucine and tyrosine concentrations showed a significant increase compared with intact control values.

Studies by many investigators demonstrated that, the immune system is subjected to regular circadian fluctuation. Some rhythms that have been reported include circadian changes in components of the immune system, e.g. lymphocytes, and circadian variation in primary and secondary immune responsiveness. The observation that many of these rhythms are inversely correlated to the glucocorticoid rhythm has led to the suggestion that fluctuations in the immune system may be a result of the glucocorticoid circadian rhythm ***(29)***. Moreover, the thymosine-alpha 1, a 28-amino acid polypeptide isolated from bovine thymus undergoes circadian rhythm persisted in thymectomized Cs7BL/6 or Swiss Webster mice. A significant increase in the amplitude of the corticosterone rhythm in the thymectomized mice relative to controls was also observed ***(29)***.

The reduction in serum total protein level and its component in thymectomized. Quail might be attributed to the catabolic effect of corticosterone on muscle protein ***(30)*** as well as due to the suppressor effect of thymectomy on growth hormone secretion ***(31)***. The later authors reported that, glucocorticoids were known to cause inhibition of growth and reduction of body weight in animals. Also, glucocorticoids reduce serum somatomedin by inhibiting the effect of growth hormone on the generation of somatomedin ***(32)***. Moreover, congenital athymia in the mouse is associated with decreased basal levels of serum thyroid stimulating hormone and growth hormone in the presence of a normal somatotroph and thyrotroph morphology ***(33)***. Furthermore, oral administration of thymus extract (1ml/kg) markedly and significantly increased the total protein, albumin, globulin, Tri-iodothyronine (T3), Thyroxine (T4) and the body weight gain in one-day-old chick ***(34)***.

Corticosterone is a major glucocorticoid in avian species and in rodents. The mechanism by which corticosterone increase muscles protein breakdown in poultry is thought to be similar to those in rats and mice. However, chicken muscle is more sensitive to corticosterone than rat and mouse muscle ***(35)***.

The obtained results revealed a significant decrease in serum glutamic, proline, methionine and arginine concentrations in thymectomized quail. Such decrease in serum amino acids could be attributed to the fact that, cortisol enhances transport of amino acids into the hepatic cells and could also account for enhanced utilization of amino acids by the liver to cause such effect as (1) increased rate of deamination of amino acids by the liver (2) increased protein synthesis in the liver (3) increased formation of plasma protein by the liver, and (4) increased conversion of amino acids to glucose ***(36)***. On the other hand, the significant increase in serum cysteine, isoleucine and tyrosine concentrations in thymectomized quail might be attributed to releases of amino acids from tissue protein due to the direct catabolic effects of corticosteroid on muscle protein degradation. This suggestion was confirmed by ***(37)***. Corticosterone directly affects chick muscle protein degradation in incubated chick muscle. The data also indicates that, tyrosine and N-methylhistidine releases, as indices of total muscle and myofibrilar

proteolysis, were increased by corticosterone, indicating an independent regulation of myofibrilar and non-myofibrillar protein degradation ***(37)***. In addition, tyrosine and N-methylhistidine releases reflect total muscle protein (myofibrillar + non-myofibrillar protein) and myofibrillar protein degradation, respectively ***(38)***.

Furthermore, recent studies in isolated tissue have demonstrated that, cortisol depresses amino acid transport into muscle cells and perhaps into other extrahepatic cells. Obviously, the decreased transport of amino acids into extrahepatic cells decreases their intracellular amino acid concentrations and as a consequence decreases the synthesis of protein. Yet catabolism of proteins in the cells continues to release amino acids from the already existing proteins, and these diffuse out of the cells to increase the plasma amino acid concentration. Therefore, cortisol mobilizes amino acids from the non-hepatic tissues which diminishes the tissue stores of protein ***(36)***.

On the other hand, corticosterone is fully exert its effect on myofibrillar protein degradation in the presence of other hormones such as thyroid hormone. Indeed, ***(39)*** have reported a synergistic effect of corticosterone and thyroid hormone on muscle proteolysis.

The obtained results showed a significant decrease in gamma-globulin level in thymectomized quail. These finding were in agreement with the results obtained by ***(26)***. In chickens which had been thymectomized only, the serum IgA level was reduced. Moreover, immunocompetent B cells had not developed in thymectomized chickens ***(26)***. The reduction in gamma-globulin level in thymectomized quail could be attributed to deficient protein synthesis and immune suppression ***(40)***.

The obtained results revealed that, there was a significant decrease in the concentrations of serum glutamic and proline values in normal intact quail vaccinated with lasota strain of Newcastle disease virus vaccine. Similar results were reported by ***(11)***. Chickens vaccinated with Lasota strain (live, lentogenic Newcastle disease vaccine) showed a highly significant decrease of glutamic and proline levels. The recorded significant decrease of amino acids detected in particular the essential one might be attributed to their incorporation for biosynthesis of immune proteins (antibodies) towards the vaccination ***(11)***, this explanation was confirmed by ***(41)*** who reported that, threonine is one of the major amino acid components of gamma-globulin fraction of chicken plasma. Also the obtained results revealed a non-significant decrease of threonine concentration. Moreover, the obtained findings concerning serum protein fractionation in Japanese quail vaccinated with Lasota strain of Newcastle disease virus vaccine revealed a significant increase in α-globulin and a non significant increase in the gamma-globulin fraction levels, which augment the observation of ***(41)*** and the recorded non significant decrease occurred for threonine as essential amino acid.

Likewise, some of these amino acids either the essential or non-essential ones were involved in tissue protein synthesis allover the quail body, since quail in this experimental study were in the growing period .This fate might be in part implicated for the decreased free amino acids detected in serum of quail particularly, threonine, serine, glycine, phenylalanine and lysine. Moreover, amino acids play great important role in physiological functions and growth of chicks ***(42)***. Also, glycine or serine has been considered essential for optimum growth of chickens ***(43)***.

Furthermore, chickens could produce high levels of antibodies following injections of serum proteins ***(44)***. Similarly, the level of antibody production is influenced by nutritional factors as proteins and amino acids, and optimal antibody production may not be obtained at the same nutrient level as optimum growth ***(9)***.

The present study revealed that, there was a significant decrease in the value of serum glycine, valine, isoleucine, leucine, tyrosine and lysine levels in thymectomized quail vaccinated with Lasota strain of Newcastle disease virus vaccine. Similarly, a decrease in serum amino acid leucine, valine, lysine and tyrosine concentration was noticed in chickens vaccinated with lasota strain of Newcastle disease virus vaccine after 14 days post vaccination ***(11)***. Moreover, the author also added that, a decrease in serum glycine and isoleucine with minimal (non-significant) was observed in chickens vaccinated with Hitchner B1 vaccine of

Newcastle disease virus after one day post vaccination.

In addition, glycine, cysteine, in the serum of chickens vaccinated with mesogenic (Komarov) vaccine strain of Newcastle disease were insignificantly decreased and might be due to incorporation for antibody production ***(45)***. Such decrease in serum amino acids level might be due to their participation for building up of body tissues and/or to carry certain physiological functions ***(43)***. Furthermore, the starvation of chickens due to lowered feed intake resulted as one of constellated symptoms of post vaccinal reactions, might be in part implicated for the decrease in amino acids, as augmented by ***(46)***. Also, the decrease of amino acids might be in part to their use for different physiological functions and growth of chickens ***(42, 43* and *47)***.

Regarding serum aspartic, proline and arginine concentrations, the obtained data demonstrated its significant increase in thymectomized vaccinated quail. The observed increase in such serum amino acid concentrations may be contributed to the effect of vaccination since vaccine caused a post vaccinal reaction from which, reduced food intake, so this was considered a nutritional emergency state, consequently hypoglycemia, the body reacted to meet such state via mobilization of plasma albumin ***(48)*** resulted in the increase level of glucogenic amino acids to be changed to glucose compensating hypoglycemia might be happened, although some of those acids are essential. On contrary, serum arginine, proline and aspartic acid concentrations were decreased in chickens vaccinated with Hitchiner B1 strain of Newcastle disease virus vaccine at 10 days post vaccination ***(11)***. The differences in dose and type of vaccine strain; animal species, pattern of samples post vaccination as well as duration of treatment may be the cause of inaccordance.

It is evident from the present study that, there was a significant increase in the value serum alpha-globulin level in normal intact quail vaccinated with Lasota strain of Newcastle disease virus. However, serum total globulin and gamma globulin concentrations showed a non significant increase. On the other hand, serum protein, albumin and beta globulin levels revealed a non-significant decrease. Similarly, chickens vaccinated with Lasota vaccine of Newcastle disease virus showed a highly significant increase in serum alpha-globulin level at 14 days post-vaccination ***(11)***. Also, an increase in gamma globulin, and alpha-globulin concentration and a decrease in beta globulin and albumin levels were observed in adult birds vaccinated with R_2B (Mukteswar) and F strain of Newcastle disease virus ***(49)***. Moreover, serum alpha- and gamma globulins were increased while serum albumin was decreased in chickens vaccinated with drinking water vaccine of Newcastle disease virus ***(50)***. The recorded increase in alpha and gamma globulin levels might be attributed to vaccine application which began to stimulate immune system consequently antibodies were produced ***(44)***. Moreover, the gamma-globulin fraction and the titers rise as established through the hemagglutination-inhibition reaction in bird treated with the Lasota vaccine ***(51)***. This is reasonable to believe that the reticuloendothelial system was favorably activated as regards the immunogenesis against the Newcastle disease virus. Also, vaccine had reacted through β-globulin's rather than α-globulin. Thus caused a shift to increased synthesis of β-and α-globulin's ***(52)***. In the same aspect, characteristic changes in the serum protein profile were established in broilers following immunization with Newcastle disease vaccine. Moreover, there was decrease in the total serum protein, increase in the globulin and particularly in the fractions the peak level of which corresponded to the period of most strongly expressed immunity ***(53)***. Also, the intra-ocular vaccination of chicks with F strain of NDV resulted in the highest titer of haemagglutination inhibition antibodies in the tears ***(54)***. Furthermore, antibodies increased in bird after 23 day post-vaccination with Newcastle disease vaccine ***(55)***. On contrary, there was a decrease of serum alpha-globulin level in chickens after vaccination with mesogenic Newcastle virus ***(45)***.

The non significant decrease of serum total protein level in normal intact quails vaccinated with NDV might be attributed to the reduced food intake as a symptoms of post-vaccinal reaction, or it might be due to the recorded decrease of serum albumin ***(44)***. Moreover, inoculation of chickens with Newcastle disease virus caused reduction in

serum total protein level which mostly due to decrease in the level of albumin ***(56)***.

Serum albumin level showed a non-significant decrease in vaccinated quail with NDV. Similarly, serum albumin and beta globulin concentrations were decreased in Newcastle vaccinated chickens ***(45)***. Such non-significant decrease in albumin fraction may be due to the response to vaccine, since at that day the antibody level reached a maximum ***(57)***. Also, albumin was believed to act as a protein reserve and a protein source for amino acids to time of subnormal intake of food ***(48)***.

On the other hand, the concentrations of total protein, albumin, total globulin's and alpha-globulin fraction showed a non significant increase in thymectomized quails vaccinated with Lasota strain of Newcastle disease virus (NDV). However, serum gamma-globulin level showed a non significant decrease similar results were reported by ***(58)*** in vaccinated chickens with NDV. Also, in chickens which had been thymectomized only, the serum IgA level was reduced on average only by about 60% while serum IgG and IgM concentrations were not reduced ***(26)***. The non significant decreased of gamma-globulin level in thymectomized vaccinated quails might be attributed to the direct effect of thymectomy as immunosuppressive agent rather than vaccine effects which induced an effect either by direct inhibition of plasma cells, lymphocytes, and macrophages or by indirect action through the stimulation of the adrenal gland to stimulate the synthesis of secretion of adrenocorticosteroid, which in turn suppress, or lyse antibody forming cells ***(59)***.

From the obtained results it could be concluded that, thymus gland seems to be important for erythropoiesis, leukopoiesis, and lymphocytes and its activity in cell mediated immune response in addition to antibody formation .Moreover, alterations in serum protein and its components as well as free amino acids pattern was related to ablation of thymus gland; this suggests that, thymus could participates in control of protein metabolism. Further studies were needed to explore the exact role of thymus gland in control protein metabolism.

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Table (3) : Effect of thymectomy on relative distribution of leukocytes in female Japanese quail

Parameters / Post-thymectomy / week	Lymphocytes %		Heterophils		Eosinophils		Monocytes		Basophils	
	Control	Thymectomized	Control	Thymectomized	Control	Thymectomized	Control	Thymectomized	Control	Thymectomized
Two weeks	60.40±2.88	47.00±1.96***	36.80±2.67	49.75±1.55**	1.40±0.51	1.50±0.50	1.60±0.64	2.00±0.58	0000	00.00
Three weeks	59.60±2.56	47.00±2.49***	36.60±2.92	49.00±1.17**	1.80±0.19	1.25±0.75	1.80±0.58	1.50±0.29	0.20±0.20	00.00
Four weeks	58.16±2.60	56.00±2.76	37.83±2.76	41.40±2.83	1.84±0.47	1.20±0.37	1.80±0.60	1.40±0.50	00.00	00.00
Five weeks	52.50±2.10	51.25±1.32	42.75±1.60	46.25±1.31	2.00±0.41	1.75±0.63	2.50±0.87	1.00±0.41	0.25±0.25	00.00
Six weeks	52.50±1.33	51.20±2.25	44.00±1.08	46.20±1.96	1.50±0.29	1.60±0.68	2.00±0.41	1.20±0.58	00.00	00.00

The values are significantly different at ** P < 0.01 and *** P < 0.001.

Table (4) : Mean values of PHA and NDV antigen in intact, thymectomized and vaccinated Japanese quails with Lasota strain of ND virus vaccine.

Parameters / Animal groups	PHA	NDV antigen
Intact (non-vaccinated)	1.3670±0.05a	1.2198± 0.06a
Thymectomized (non- vaccinated)	0.9815± 0.03b	1.1185±0.02ac
Intact (vaccinated)	1.7609± 0.06c	2.2150± 0.11b
Thymectomized (Vaccinated)	1.1808 ± 0.04ad	1.2983± 0.05ad

The values have different letters in the same column are significantly different at (P<0.05)
PHA: Phytohaemagglutinin.
NDV: Newcastle Disease virus

Table (5) Challenge test against VVNDV two weeks post-vaccination with Lasota strain of N.D v thymectomized and intact control quails.

Animal groups	Number of birds	Mortality rate	Protection rate
Intact (non-vaccinated)	8	4/8	50%
Thymectomized (non vaccinated)	8	6/8	25%
Intact (vaccinated)	8	2/8	75%
Thymectomized (Vaccinated)	8	2/8	75%

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Table (6) : Mean values of serum free amino acids pattern of normal intact, thymectomized and vaccinated Japanese quail with Lasota strain of Newcastle disease virus vaccine in mg/dl.

Animal groups / Amino acids detected	GroupI Intact non vaccinated	Group II Thymectomized non vaccinated	Group III Intact vaccinated	Group IV Thymectomized vaccinated
Aspartic	4.30 ± 0.29 ^{ab}	3.63 ± 0.01 ^{ac}	4.46 ± 0.25 ^{bdf}	5.38 ± 0.64 ^{beg}
Threonine	13.37 ± 0.78 ^{a}	14.40 ± 1.39 ^{a}	12.52 ± 0.61 ^{a}	12.28 ± 0.09 ^{a}
Serine	7.39 ± 0.20 ^{a}	7.09 ± 0.53 ^{a}	7.25 ± 0.27 ^{a}	7.01 ± 0.09 ^{a}
Glutamic	11.28 ± 0.75 ^{a}	8.89 ± 0.22 ^{b}	7.95 ± 0.28 ^{c}	9.20 ± 0.18 ^{b}
Proline	15.69 ± 0.43 ^{a}	8.48 ± 0.35 ^{b}	13.33 ± 0.40 ^{c}	11.03 ± 0.47 ^{d}
Glycine	8.75 ± 0.38 ^{a}	7.95 ± 0.14 ^{a}	7.85 ± 0.31 ^{a}	6.13 ± 0.03 ^{b}
Alanine	8.71 ± 0.49 ^{ac}	7.83 ± 0.31 ^{ac}	8.87 ± 0.32 ^{a}	7.09 ± 0.46 ^{bc}
Cysteine	1.95 ± 0.23 ^{ac}	2.96 ± 0.19 ^{b}	2.21 ± 0.06 ^{ac}	2.55 + 0.05 ^{b}
Valine	5.05 ± 0.25 ^{ac}	6.03 ± 0.36 ^{ac}	5.43 ± 0.20 ^{ac}	4.19 ± 0.22 ^{b}
Methionine	2.46 ± 0.17 ^{a}	1.93 ± 0.03 ^{bc}	2.64 ± 0.10 ^{a}	2.10 ± 0.26 ^{ac}
Isoleucine	4.34 ± 0.14 ^{a}	6.38 ± 0.20 ^{b}	4.97 ± 0.26 ^{a}	4.08 ± 0.28 ^{a}
Leucine	4.60 ± 0.26 ^{a}	5.35 ± 0.22 ^{ac}	4.70 ± 0.10 ^{dc}	3.78 ± 0.14 ^{b}
Tyrosine	4.33 ± 0.20 ^{a}	5.69 ± 0.27 ^{b}	4.45 ± 0.09 ^{ac}	3.99 ± 0.25 ^{ad}
Phenylalanine	3.00 ± 0.13 ^{ad}	2.50 ± 0.17 ^{ad}	2.93 ± 0.06 ^{ab}	2.66 ± 0.07 ^{cd}
Histidine	8.29 ± 0.26 ^{ad}	7.99 ± 0.16 ^{ad}	8.76 ± 0.38 ^{ab}	7.05 ± 0.53 ^{cd}
Lysine	14.92 ± 0.87 ^{a}	13.80 ± 0.02 ^{ac}	13.82 ± 0.03 ^{ac}	10.63 ± 0.68 ^{bd}
Arginine	11.72 ± 0.69 ^{a}	8.71 ± 0.27 ^{b}	11.94 ± 0.5 ^{ad}	9.80 ± 0.25 ^{c}

Data are presented as Mean ± S.E
S.E = (Standard error). The values with different superscripts in the same line are significantly differents at ($P < 0.05$).

Table (7): Mean values of serum total protein and protein fractions in normal intact, thymectomized and vaccinated Japanese quail with Lasota strain of Newcastle disease virus vaccine in g/dl.

Parameters / Animal groups	Total protein	Albumin	Total globulins	Globulin fractions		
				α- globulin	β-globulin	γ-globulin
Group I: Intact non vaccinated	7.52 ± 0.21 ^{a}	3.70 ±0.11 ^{ab}	3.64 ± 35 ^{a}	0.75 ± 0.08 ^{a}	1.28 ± 0.30 ^{a}	1.61 ± 0.01 ^{a}
Group II: Thymectomized non vaccinated	5.99 ± 0.14 ^{b}	2.89 ± 0.03 ^{b}	3.05 ± 0.13 ^{ab}	1.03 ± 0.07 ^{a}	1.14 ± 0.12 ^{a}	0.88 ± 0.12 ^{b}
Group III: Intact vaccinated	7.25 ± 0.81 ^{ab}	3.20 ± 0.17 ^{ab}	3.99 ± 0.08 ^{ab}	1.23 ± 0.12 ^{a}	1.10 ± 0.11 ^{a}	1.66 ± 0.13 ^{a}
Group IV: Thymectomized vaccinated	6.44 ± 0.22 ^{ab}	3.08 ± 0.25 ^{ab}	3.39 ± 0.30 ^{a}	1.44 ± 0.17 ^{a}	1.14 ± 0.07 ^{a}	0.80 ± 0.11 ^{b}

Data are presented as Mean ± S.E S.E = (standard error)
The values with different superscripts in the same column are significantly differents at ($P < 0.05$).

Fig. (5) Electrophoretic profiles of serum proteins in intact, thymectomized and vaccinated Japanese Quail with NDV.
Lane 1, La ne 2 and Lane 3 (Thymectomized vaccinated group)
Lane 4, Lane5 and Lane6 (Thymectomized non vaccinated group)
Lane 7, Lane 8 and Lane 9 (Intact non vaccinated group)
Lane 10, Lane 11 and Lane 12 (Intact vaccinated group)

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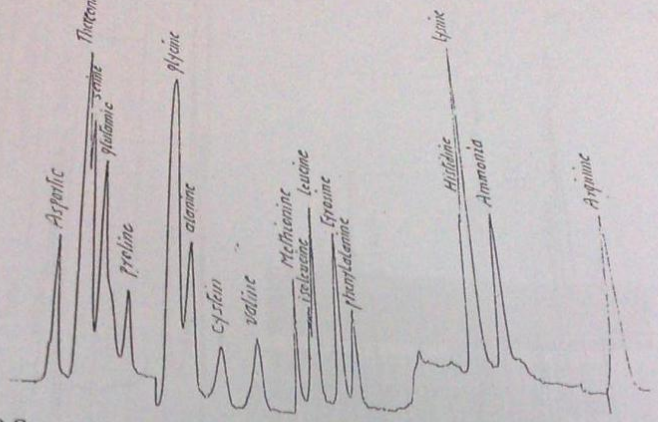

Figure (1) Serum free amino acids pattern of intact non-vaccinated Japanese quail

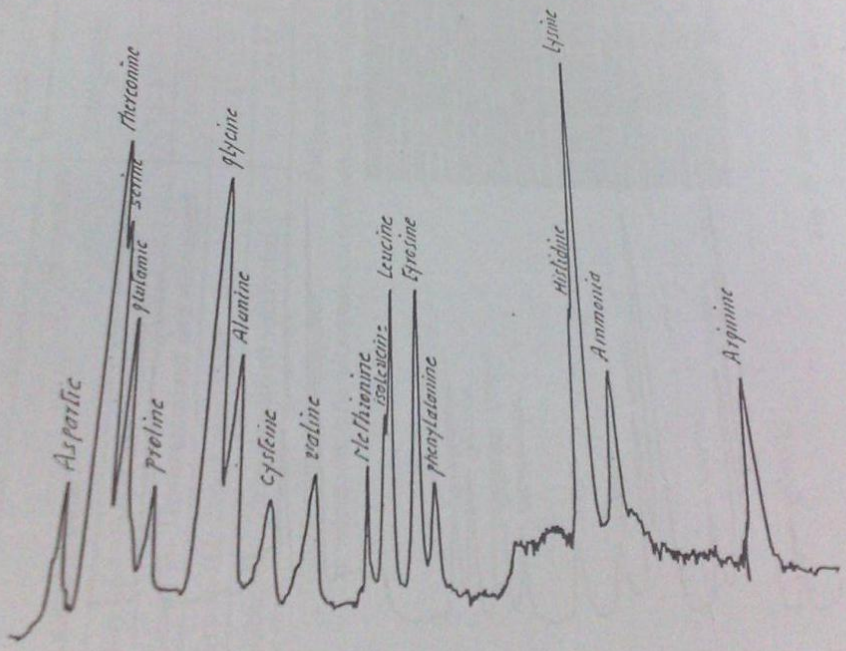

Figure (2) Serum free amino acids pattern of thymectomized non-vaccinated Japanese quail .

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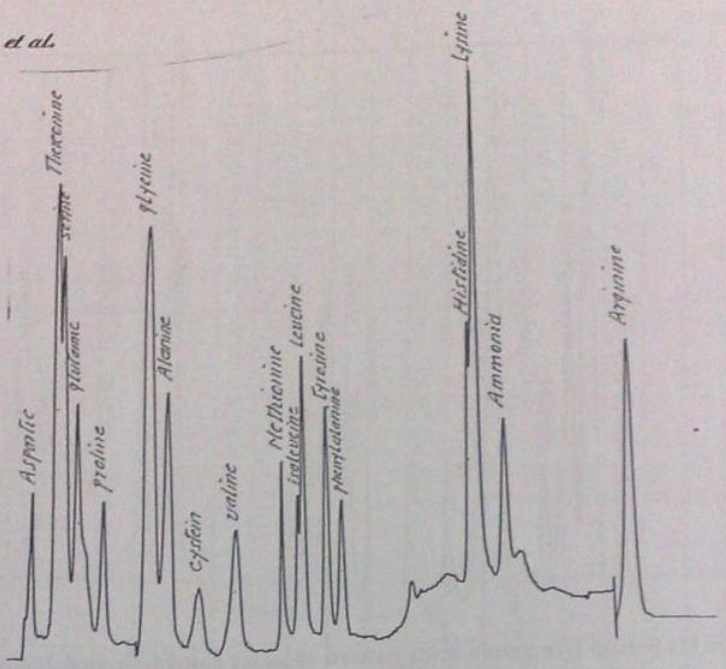

Figure (3) Serum free amino acids pattern of intact vaccinated Japanese quail

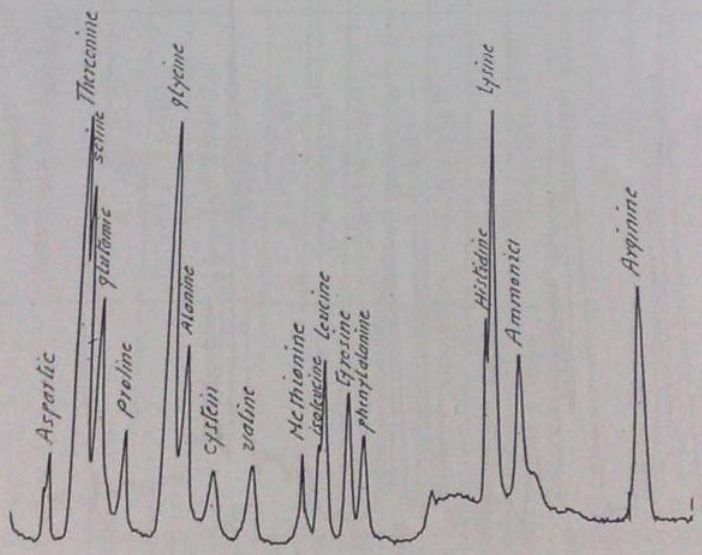

Figure (4) Serum free amino acids pattern of thymectomized vaccinated Japanese quail

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الملخص العربى

التغيرات البيوكيميائية، المناعية والدموية في دم السمان الياباني المستأصل منه غدة التوثة والمحصن ضد مرض النيوكاسل.

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أجريت هذه الدراسة لتوضيح تأثير إستئصال غدة التوثة على بعض القياسات الدموية ، الاستجابة المناعية ومستوى الأحماض الأمينية الحرة وبروتين المصل ومشتقاته في طيور السمان الياباني المحصن والغير محصن ضد فيروس مرض النيوكاسل وقد أستخدم لأجراء هذا البحث عدد ١٨٠ من طيور السمان الياباني عمر يوم. وقد تم تصميم هذه الدراسة في تجربتين :

التجربة الأولى :

أستخدم في هذه التجربة عدد ١٠٠ طائر (٥٠ ذكور ، ٥٠ إناث). عند ١٠ أيام من العمرتم تقسيم الطيور إلى أربعة مجموعات متساوية في العدد من الذكور والإناث ثم تم استئصال غدة التوثة جراحيا لطيور مجموعة من الذكور ومجموعة من الإناث وتركت مجموعة ضابطة لكل من الذكور والإناث. بعد أسبوعين من إجراء إستئصال الغدة جراحيا تم أخذ عينات الدم أسبوعيا ولمدة ستة أسابيع متتالية لتقدير القياسات الدموية .

التجربة الثانية:

أستخدم لأجراء هذه التجربة عدد ٨٠ طائر ذكور وإناث . وتم استئصال غدة التوثة لعدد ٤٠ طائر عند عمر ١٠ أيام واستخدمت مجموعة ضابطة عدد ٤٠ طائر . تم تقسيم الطيور السليمة والمستأصل منها غدة التوثة بعد أسبوعين من إجراء العملية إلى أربع مجموعات متساوية . مجموعة ١ طيور سليمة غير محصنة و مجموعة٢ (طيور مستأصل منها غدة التوثة غير محصنة) و مجموعة٣ (طيور سليمة محصنة) ومجموعة ٤ (طيورمستأصل منها غدة التوثة ومحصنة) . تم تحصين جميع طيور المجموعة الثالثة والرابعة ضد مرض النيوكاسل وتم تجميع عينات الدم من كل المجموعات السابقة بعد أسبوعين من التحصين ثم تم فصل مصل الدم وذلك لتقدير البروتين الكلى في المصل ومشتقاته بالإضافة آلي الأحماض الأمينية الحرة في المصل وتقدير الأجسام المناعية في الدم باستخدام اختبار مانع التلازن الدموي. كما تم تجميع عينات دم على هيارين وذلك لتقدير النشاط

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البلاستوجينى للخلايا الليمفاوية(المناعة الخلوية) . بعد أسبوعين من التحصين أيضا تم عمل اختبار تحدى بالعترة المعوية لمرض النيوكاسل(VVNDV) لكل المجموعات السابقة وذلك عن طريق الحقن بالعضل.

وقد أسفرت الدراسة على النتائج الآتية :

أدى استئصال غدة التوتة إلى نقص معنوي في عدد كرات الدم الحمراء في كل من مجموعات الإناث والذكور ، أما الهيماتوكريت قد نقصت معنويا في مجموعة الإناث. كما أدى استئصال الغدة إلى نقص ملحوظ في العدد الكلى لكرات الدم البيضاء في مجموعات الذكور والإناث ، أما بالنسبة للتوزيع النسبي للخلايا البيضاء فقد أدى استئصال غدة التوتة إلى نقص في نسبة الخلايا الليمفاوية وزيادة في نسبة الهيتروفيل في الدم. كما أظهرت الدراسة المناعية الخلوية انخفاض في النشاط البلاستوجينى للخلايا الليمفاوية في المجموعات المحصنة والغير محصنة المستأصل منها الغدة بالمقارنة بالمجموعات السليمة المحصنة والغير محصنة بقياس الأجسام المناعية في مصل الدم باستخدام اختبار مانع التلازن الدموي. وأظهرت النتائج عن وجود أجسام مناعية Log2 Hi titer (4) في الطيور السليمة والمحصنة ضد مرض النيوكاسل بالمقارنة بالمجموعة المستأصل منها الغدة والمحصنة والتي أظهرت النتائج وجود أجسام مناعية تساوى Log2 Hi titer (1) . كما أسفرت نتائج عمل التحدي بالعترة المعوية لمرض النيوكاسل (VVNDV) أن نسبة الوفيات في كلاً من الطيور السليمة والمستأصل منها الغدة والمحصنة أقل من كلا الطيور الغير محصنة السليمة المستئصل الغدة منها. أظهرت النتائج أن نسبة التحدي كانت ٧٥% حماية في كلا من الطيور السليمة والمستأصل منها الغدة والمحصنة وحماية قدرها ٥٠% في الطيور السليمة الغير محصنة و ٢٥% في الطيور المستأصل منها الغدة والغير محصنة.

وقد أظهرت الدراسات البيوكيميائية أن استئصال غدة التوتة أدى إلى انخفاض معنوي في مستوى حمض الجلوتاميك واللين والميثونين والارجنين في المصل بينما أرتفع مستوى حمض السيستين والأيزوليوسين والتيروزين بالمقارنة بالمجموعة الضابطة (مجموعة١). أدى التحصين ضد مرض النيوكاسل في الطيور السليمة إلى انخفاض في تركيز حمض الجلوتاميك والبرولين بينما أدى التحصين ضد مرض النيوكاسل في الطيور المستأصل منها غدة التوتة إلى نقص فى مستوى حمض الجليسين ، الفالين ، الأيزوليوسين ، الليوسين ، التيروزين والليثين بينما أزداد مستوى الأسبارتك والبرولين والارجنين وذلك بالمقارنة بالطيور المستأصل منها غدة التوتة والغير محصنة (مجموعة ٢).

كما أظهرت النتائج أن استئصال غدة التوتة أدى إلى نقص معنوي في مستوى البروتين الكلى والألبومين والجاما جلوبيولين ، بينما ازداد مستوى الألفا جلوبيولين . أدى التحصين ضد مرض النيوكاسل إلى زيادة في مستوى الألفا جلوبيولين في المجموعات السليمة بينما أدى التحصين في المجموعة المستأصل منها غدة التوتة إلى تغيرات غير معنوية في مستوى البروتين الكلى ومشتقاته وذلك بالمقارنة بالطيور المستأصل الغدة منها والغير محصنة.

من النتائج السابقة يمكن استخلاص الآتي :

أن غدة التوتة تبدو أن لها أهمية في تكوين خلايا كرات الدم الحمراء وكرات الدم البيضاء وعدد الخلايا الليمفاوية في الدم ونشاطها في الاستجابة المناعية للخلية بالإضافة إلى تكوين الأجسام المضادة.واتضح من الدراسات البيوكيميائية وجود تغيرات في مستوى البروتين الكلى ومشتقاته وكذلك بعض الأحماض الأمينية الحرة بعد استئصال غدة التوتة مما يدل على أن لها دور مشارك في أيض البر وتينات والأحماض الأمينية بالجسم.مما يتطلب المزيد من الدراسة لمعرفة تأثير غدة التوتة على أيض البروتينات والأحماض الأمينية.