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EFFECT OF SELECTION FOR HIGH AND LOW ANTIBODY RESPONSE AGAINST SHEEP RED BLOOD CELLS ON HORMONAL PROFILE DURING IMMUNE RESPONSE IN CHICKENS

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SUMMARY

Two experiments were conducted in this study using two lines of Fayoumi chickens divergently selected for high (H) and low (L) antibody response against sheep red blood cells (SRBC) for four generations. The objectives of this study are to compare humoral and cell mediated immune responses between the lines, as well as to monitor the T_3 and corticosterone hormone profile during the initiation of the immune responses.

In the first experiment one hundred male chicks of each line were primary injected intramuscular with either 1 ml 25% SRBC suspension or saline at 50 and secondary reinjected at 80 days of age. Blood samples were collected at 0, 1, 3, 6, 9, 12 and 24 h and at 3, 7 and 10 days following primary and secondary injection. The results showed that H line produced significantly higher primary and secondary antibody titer than L line at 7 and 10 days post injection. The results showed that T_3 levels decreased significantly at 3 h in H line and at 3, 6 and 9h in L line, then remained at low levels till 10 days post primary SRBC injection as compared to 0 h. Within both H and L lines, the T_3 level showed insignificant differences between 0 hr and at any time post secondary SRBC or saline injection (except that in H line at 3h post SRBC injection). In both line there was a significant increase in corticosterone levels at 24h as well as at 3, 7 and 10 days post primary, and at 3,7 and 10 days post secondary SRBC injection as compared to 0 h.

In the second experiment, one-hundred and twenty male chicks of each line were injected intradermally into wattle with either 100 μg PHA-P or saline at 56 days of age to monitor the delayed type hypersensitivity (DTH) reaction. Blood samples were collected at 0, 3, 6, 9, 12 and, 24 hs post injection. The results indicate that H line had higher DTH value at 12 and 24 h than that in L line at the same time point. There was no remarked change in the level of T_3 or corticosterone between PHA-P and saline injected birds at any time point following injection within both lines.

In conclusion, the results of T_3 or corticosterone levels in the current study may explain that the initiation of humoral immune response involves the activation of HPT- and HPA-axis. While this axis is not involved in delayed type hypersensitivity reaction.

Keywords: Chicken, immune response, divergent selection, T_3, corticosterone

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INTRODUCTION

Disease resistance in animals is a result of the interaction of the animal's environment and its genetic component, so the relationship between genetic make-up and immunity has been studied extensively (Gehad *et al.,* 1999; Ahmed,2004). Biozzi *et al*., (1982) indicated that the different branches of avian immune system are under independent genetic control. So it has been common practice now to enhance disease resistance through genetic improvement represented by divergent selection for antibody production against polyvalent non-pathogenic T-dependent antigen (such as SRBC).

The immune system is a system that is very much integrated into the total physiology of the animal (Marsh, 1992). From this integration, is the bi-directional communication between the neuro-endocrine and the immune system that allow the organism to withstand immunologic challenges without disturbance of body homeostasis (Davis, 1998). Kruger (1996) has demonstrated that the immuno-regulatory effects of the hypothalamus-pituitary-thyroid (HPT) axis is quite diverse and influence different aspects of the immune system physiology. Thyroid hormones exert a regulatory effect on humoral immunity more than a stimulatory one (Trout *et al*., 1988 and Marsh, 1992). Moreover there is a strong hypothesis that thyroid hormones exert effect on cell-mediated immunity through their action on the maturation and function of thymus-derived lymphocytes and this action is mediated by genetic background of the animal (Marsh, 1992).

Similarly the communication between the immune system and hypothalemo-pituitary-Adrenal axis (HPA axis) seems crucial to the proper function of immune system. Mashaly *et al*. (1998) found that the first step in the initiation of humoral immunity is the release of IL-1 by macrophages which in turn stimulates CRF by hypothalamus, then CRF stimulates ACTH production by anterior pituitary and / or Leukocytes; additionally CRF will directly enhance lymphocyte activities in the spleen. Then corticosteroid production will be stimulated by ACTH and will cause redistribution of lymphocytes from circulation to secondary lymphoid organs such as the spleen for antigen processing and eventual production of antibodies against the invading antigens.

Finally, both ACTH and corticosterone will interact in a negative feed back manner to regulate and control the process of antibody production by inhibiting lymphocyte activity and/ or reducing the responsiveness to different stimuli. Glucocorticoids also limit cell- mediated immune response in chickens (Murray *et al.,* 1987).

The objectives of this study are:

1- To evaluating the humoral as well as the cell-mediated immune parameters between two lines of Fayoumi chickens divergently selected for high (H) and low (L) humoral immune response against SRBC for four generations.
2- To detect the tri-iodothyronine (T_3) and corticosterone hormone profile within lines during primary and secondary immune response as well as along different phases of delayed type hypersensitivity (DTH).

MATERIALS AND METHODS

Two experiments were conducted and carried out at Poultry Research Center, Animal Production Department, Faculty of Agriculture, Cairo University, Giza. Egypt. The experimental flock compared two lines of Fayoumi chickens selected for high (H) and low (L) antibody response against sheep red blood cells (SRBC) for four generations.

General Management

At day of hatch, all chicks were wing banded; then placed intermingled in floor brooder pens which contained wood shaving litter, hanging tube feeder and plastic drinker. The birds were exposed to continuous light during the first three days of age and thereafter they were exposed to natural day light. They were fed commercial starter ration (20% crude protein and 2800 K. cal ME/Kg). All chicks were vaccinated against Newcastle disease virus at 7, 22 and 57 days of age; against infection bursal disease at 14 and 24 days and against infection fowl box disease at 35 days of age.

Experimental Protocol

The first experiment was conducted to monitor the early events during the initiation of the primary and secondary humoral immune response following the injection of SRBC antigen in both H and L lines which is the release kinetics of the T_3 and corticosterone hormone following the injection of SRBC antigen to determine the possible differences between lines.

The second experiment was conducted to investigate the changes in circulating levels of T_3 and corticosterone during the development of the delayed type hypersensitivity (DTH) response.

First Experiment

One hundred chicks of each line were used in the first experiment. At 50 days of age, chicks of each line were divided into two groups (50 birds for each group). The first group was injected intramuscular with 1 ml 25% SRBC suspension to determine primary response, whereas the second group was injected with 1 ml 0.9% saline to be served as a control group. To determine the secondary immune response, the same birds of each group were injected at 80 days of age with the same schedule followed in the primary response.

Five birds from each group of each line were bled just before injection (zero h). Five additional birds from each group of each line were bled at 1, 3, 6, 9, 12 and 24 h and at 3, 7 and 10 days following primary and secondary injection. No bird was bled more than once to ensure that handling the birds would not affect corticosterone levels. Approximately 3 ml of blood was drawn from the brachial vein of each bird. The samples were allowed to clot to provide serum for antibody titer and hormone assays.

Antibody titer

The samples of 3, 7 and 10 days post SRBC injection were used to determine primary and secondary immune response using micro titer technique described by Van Der Zijpp and Leenstra (1980).

Hormonal profile changes during humoral immune responses

Concentration of circulating T_3 and corticosterone were measured in all blood samples collected at the different times following SRBC or saline injections. The hormones were measured using radio immunoassay (RIA) kits purchased from Cambridge Medical Diagnostic Lab (Billeezica MA).

Second Experiment

At 56 days of age, one-hundred and twenty birds of each line were used in this experiment to study DTH reaction. The birds within each line were divided into two equal groups (60 birds for each one). The first group was injected intradermally into the right wattle using 100μg PHA-P prepared in 1 ml sterile saline. The second group was injected intradermally into the right wattle using 0.1 ml of sterile saline. The wattle thickness was measured at 0, 3, 6, 9, 12 and 24 hr following PHA-P or saline injection using a digital micrometer. The percentage increase in wattle thickness was calculated according to the following equation:

(Wattle thickness after injection - initial wattle thickness)/ initial wattle X 100.

Hormonal profile changes during DTH reaction

Six blood samples were drawn from both PHA-P and saline injected groups at the same time of measuring wattle thickness. The concentrations of T_3 and corticosterone were measured in all serum samples collected at the different times following PHA-P or saline injections using Radio-Immunoassay (RIA) kits and the technique was as previously mentioned.

Statistical analysis

Data were analyzed using three-way analysis of variance (general liner models procedure, SAS Institute, 2000). The sampling time, injection treatment (SRBC, PHA-P or saline) and line, were used as the main effects in statistical analysis of hormonal profile changes. While one-way analysis was used through out antibody titer and DTH using the line as a main effect. Mean values were compared according to Duncan (1955).

RESULTS AND DISCUSSION

First Experiment

Antibody Titer against SRBC

The results in Table 1 showed that H line produced significantly higher primary and secondary antibody titer than L line at 7 and 10 days post injection. However, no significant differences were found between the two lines at 3 day post injection. These results are in agreement with those reported by Kreukneit *et al.* (1996); Parmentier *et al.* (1996); and Ahmed (2004). They found differences between lines in antibody response against SRBC (T-dependent antigen). The difference is also in agreement with Scott *et al.* (1994) and Nelson *et al.* (1995), who reported a similar difference in antibody titer against *Brucella abortus* (T-independent antigen).

Table 1. Means ± SE of primary and secondary antibody titer against sheep red blood cells of high and low lines of Fayoumi chickens

Line	Days post immunization		
	3	7	10
	Primary immune response		
High	3.66± 0.67 [a]	5.66±0.67 [a]	3.00±0.00 [a]
Low	2.33± 0.33 [a]	3.00±0.30 [b]	2.00±0.00 [b]
	Secondary immune response		
High	4.00±0.58 [a]	6.66±0.33 [a]	5.00±0.00[a]
Low	2.66±0.33 [a]	3.33±0.33 [b]	3.00±0.00 [b]

[a] and [b] Values with different superscripts within days and within immune response are significantly different ($P < 0.05$) .

Several investigations have been done to explain the immune differences between lines. Van Der Zijpp *et al.* (1989) and Scott *et al.* (1994), have demonstrated that the variation between lines in antibody production could be attributed to macrophages's ability to identify and present antigens on their membranes where the antigen is rapidly catabolized in low line and vice versa in high line. Moreover Siegel *et al.* (1992) and Kreukniet *et al.* (1996), have confirmed that a higher percentage of CD4+ cells (T-helper cells) are present in the spleens of H lines, while higher percentage of CD8+ cells (cytotoxic T-cells) are located in L line's spleen.. The previous observation has been confirmed by Siegel *et al.* (1992), who have found that there was higher percentage of B-cells in the spleens and blood of H line than L line of chickens.

Hormonal profile changes associated with humoral immune response:

A) Profile of Serum T_3 Hormone

The results in Table 2 show that T_3 levels of H line decreased significantly at 3h and remain low till 10 days post primary and secondary SRBC injection as compared to 0 h. Moreover, within saline injected birds, the results didn't have a clear pattern or any significant change (except that at 9 h pos primary injection) . The results of SRBC injected birds of L line showed a significant decrease in T_3 level at 3, 6 and 9 h post primary SRBC injection (Table 2). On the other hand, no significant differences were observed in T_3 levels of saline injected birds of L line as compared to 0 h.

With respect to secondary immune response within L lines, the T_3 level showed no significant differences between 0 h and any other time post-SRBC injection (Table 2). Regardless the interline difference, there were no significant differences between SRBC and saline injected birds at any sampling time point of primary and secondary immune response. Additionally, no differences were observed between pooled means of T_3 levels of H and L lines during either primary or secondary immune response (Table 3).

Trout *et al*. (1988) recorded a decrease in T_3 during the first day and then an increase by day 2 post-antigen injections. Furthermore, Atta (2002) revealed that T_3 level decreased significantly at 3 h post SRBC injection compared to 0 h in White Leghorn chicken. The study of Martin and Siegel (1988) explained a magnitude

difference between H and L lines in T_3 concentration, which may explain the association between thyroid hormone levels and antibody titers.

Table 2. Change in serum T_3 level (n mol/L) in SRBC and saline injected of of high and low lines of Fayoumi chickens

Line	Treatment	Primary immune response									
		Time post injection (Hours)							Time post injection (days)		
		0	1	3	6	9	12	24	3	7	10
	SRBC	4.2	3.9	2.2	2.4	2.6	2.9	2.8	3.8	3.2	3.0
High		±.6	± .4	±.7*	± .8	± .4	± .2	±.2	±.7	±.2	±.6
	Saline	4.2	2.8	2.9	3.3	2.7	3.3	3.7	2.9	4.3	4.4
		±0.6	±0.3	±0.5	±0.2	±0.4*	±0.4	±0.3	± .6	±0.1	±0.3
	SRBC	5.0	4.8	2.2	2.4	2.5	3.2	3.3	5.0	4.6	4.6
Low		±0.5	±0.1	±0.4*	±0.2*	±0.1*	±0.5	±0.7	±0.3	±0.6	±0.7
	Saline	5.0	5.0	2.9	3.0	3.4	2.5	4.9	3.0	4.1	3.0
		±0.5	±0.1	±0.3	±0.6	±0.3	±1.0	±0.2	±0.7	±0.0	±0.3
		Secondary immune response									
	SRBC	3.4	1.7	0.6	1.1	1.6	1.8	2.0	2.0	1.4	3.4
High		±0.2	±0.7	±0.3*	±0.2	±0.4	±0.3	±0.5	±0.3	±0.2	±0.7
	Saline	2.5	1.4	2.0	1.4	1.4	1.9	2.1	2.2	1.4	2.5
		±0.1	±0.2	±0.4	±0.9	±0.1	±1.2	±0.1	±0.1	±0.2	±0.1
	SRBC	2.6	2.0	1.2	1.4	1.6	1.9	1.9	3.0	2.0	2.6
Low		±0.1	±0.1	±0.2	±0.9	± 0.3	±0.2	±0.7	±0.9	±0.2	±0.1
	Saline	3.6	1.7	1.6	1.3	2.3	2.1	0.8	2.6	1.9	3.6
		±0.6	±0.7	±0.6	±0.5	± 0.9	±0.3	±0.3	±0.2	±0.8	±0.7

* Indicate significance (P<0.05) compared to 0 hr. within each treatment.
+ No significant differences was observed between SRBC and saline treatment at any specific time point.

Table 3. Variations in serum T_3 level (n mol/L)of high and low lines of Fayoumi chickens over the time of primary and secondary immune response

Line	Primary immune response									
	Time post injection (Hours)							Time post injection (days)		
	0	1	3	6	9	12	24	3	7	10
High	4.2	3.9	2.2	2.4	2.6	2.9	2.8	3.8	3.2	3.0
	±0.6	±0.4	±0.7	±0.8	±0.2	+0.2	±0.2	±0.7	±0.2	±0.6
Low	5.0	4.8	2.2	2.4	2.5	3.2	3.3	5.0	4.6	4.6
	±0.5	±0.1	±0.4*	±0.2*	±0.1*	+0.5	±1.7	±0.3	±0.6	±0.7
	Secondary immune response									
High	2.9	1.6	1.3	1.3	1.0	1.9	2.0	2.1	1.4	35.1
	±1.0	±0.5	±0.3	±0.4	±0.3	+0.5	±0.6	±0.5	±0.1	±0.1
Low	3.1	1.9	1.4	1.3	1.9	2.0	1.4	2.8	2.0	30.2
	±0.6	±0.5	±0.3	±0.3	±0.4	+0.9	±0.4	±0.6	±0.7	±4.9

* Indicate significance (P<0.05) compared to 0 hr. within each line and immune response.
+ No significant differences were observed between lines at any specific time point.

On the other hand, Gehad (2000) explained that the level of T_3 is affected by the nature of antigen stimulating specific humoral immune response. He observed that injection of lipopolysaccharide (LPS) antigen (T-independent antigen) causes significant decrease of T_3 and T_4 levels during the first day post-antigen injection. They in turn cause releasing of cytokines (IL-1 and TNF-α) from macrophages with subsequent inhibition of thyrotropin release from the pituitary gland (Dibuis *et al.*,

1988 and Pange *et al.,* 1989). Moreover, Kahl *et al.* (2000) clarified that injection of LPS reduces the activity of the enzyme 5-diiodinase that converts T_4 to T_3 in the liver. On the contrary to LPS antigen, Gehad (2000) found that bovine serum albumin (BSA) (T-dependent antigen) injection causes no significant change in level of T_3 in the plasma because BSA does not stimulate the secretion of neither IL-1 nor TNF-α.

B) Profile of serum corticosterone hormone.

The change in corticosterone levels during primary and secondary immune response within H and L lines are shown in Table 4. The results showed that in both line, SRBC injected birds had no significant change at any sampling time along the first 12 h comparing to that at 0 h (except that at 6 h post secondary injection of L line). On the other hand, there was a significant increase in corticosterone level at 24 h as well as at 3, 7 and 10 days post primary injection, ant at 3,7 and 10 days post secondary injection as comparing with 0 h. Within saline injected group and during primary response, there was a significant increase in corticosterone level within H line throughout the first 12 h and at 3 and 6 h in L line as compared to 0 h. In respect of secondary immune injection the high level of corticosterone was remarked in both line at 6 h only as comparing with 0 h.

However, corticosterone level of SRBC injected birds were significantly higher at 3, 7 and 10 days than that of saline injected birds at the same time point. Regardless the type of injection, the current results showed that the pooled means of corticosterone levels of H line are significantly higher than L line at 1 h post primary injection and at 3 h post secondary injection. On the other hand, the vise versa was observed post secondary injection. However, no differences were observed between serum corticosterone of H and L lines at the remaining time point post primary or secondary injection (Table 5).The results in both line showed that pooled mean of corticosterone level were significantly higher at 3,7 and 10 post primary injection, and at 6h as well as7d in L line and at 7d in H line post secondary injection.

Additionally, the significant change of corticosterone level along 24 hrs is partially in agreement with Gehad (2000), who observed that BSA antigen (T-dependent and non-pathogenic) does not produce a significant change of corticosterone level along 24 hrs post antigen injection on contrary to lipopolysaccharide (LPS) antigen that induces significant rise of corticosterone level at 3 and 6 hrs post antigen injection. He concluded that LPS stimulates production of IL-1 that in turn stimulates HPA-axis.

Besedovsky *et al.* (1977) found when rodents injected with SRBC's plasma cortricosterone elevated at 5-7 days after immune stimulation. Moreover, Stenzel-Poore *et al.* (1993) reported that the maximum significant increase of corticosterone level at 7 days associated with maximum level of antibody production. Mashlay *et al.* (1998) reported that the rise of corticosterone level may resulted from direct stimulation of antigen (SRBC) on leucocytes to produce CRF, which in turn stimulate ACTH production from anterior pituitary and/or leucocytes. Additionally it was previously mentioned that T-lymphocytes are capable of producing CRF (Kravchenco and Furalev, 1994) and ACTH (Lynos and Blalock, 1995) as well as macrophages produce ACTH (Hendricks and Mashaly, 1998).

Second Experiment

Delayed type Hypersensitivity Test (DTH)

The current results showed a time effect in both lines (Table 6). Within H line the reaction started as early as 3 hrs post injection, increased markedly with the time, peaked at 12 hrs and slightly decreased at 24 h. However, No significant differences were recorded during the first 6 hrs post injection. On the other hand, DTH response at 9,12 and 24 h occupied first with significant differences than at any other time. While within low line, the increase in wattle thickness was not recorded at 3 hrs post injection, while it delayed till 6 hrs and increased gradually, peaked significantly at 12 h. Gotto *et al.* (1977); Parmentier *et al*. (1996) and Gehad (2000), found that the DTH post injection characterized by rapid acute response that had started as early as 3 h and had peaked at 6 h, where it showed a higher magnitude and still elevated till 24 h, then the response ceased at 48 h. This difference could be attributed to the genetic differences of birds that used in the experiments. The genetic control of delayed type hypersensitivity response was assigned to a locus linked to the B-complex (Rejci *et al*., 1974 and Taylor *et al*., 1987).

Table 6. Values of delayed type hypersensitivity test over the time post injection of high and low lines of Fayoumi chickens

Line	Time post injection (hours)					
	Zero	**3**	**6**	**9**	**12**	**24**
High	100.0±0.0	150.0±23.8	166.6±33.3	233.3±33.3*	300.0±0.0*+	277.0±25.0*+
Low	100.0±0.0	100.0±00.0	133.3±33.3	166.6±33.3	200.0±0.0*	166.6±33.3

* Indicate significance (P<0.05) compared to 0 hr. within each treatment.
+ Indicate significance differences (P<0.05) between lines.

The early response to PHA-P is attributed to the direct effect of T-cell mitogen on the immune cells, where the sensitization period is not needed (Ptak *et al*., 1991). This mitogen activates macrophages, leading to release of IL-1 and TNF-Ɉ that result in expression of adhesiveness followed by extra-vascularization of leucocytes (Binns *et al*., 1990 and 1992). Moreover local oedema in response to PHA-P could also be attributed to slow degranulation of vasoactive amines from stimulated basophiles that accumulate around the injected area (Dvorak *et al.,* 1981 and Ptak *et al.,* 1991).

Gehad (2000) observed that injection of PHA-P into the wattle decreased the percentage of CD3+ cells (all T lymphocytes) in the circulation within 2 hrs and CD8+ cells by 2 and 6 hrs post injection; as well as CD4+ cells showed a decrease in the circulation by 2 hrs post injection. All these observations have been account for migration of CD3+, CD8+, CD4+ from the peripheral circulation to the wattle; as PHA-P can initiate this migratory effect from the circulation through the induction of homing receptors on the tissues that can be recognized by peripheral lymphocytes (Binns *et al.,* 1990). Gehad's observations was previously reported by Parmentier *et al.* (1998), who found that the entry of blood lymphocytes to the skin sites injected with PHA-P was first detect by 2 hrs following injection, was maximal by 6 hrs and disappeared by 48 hrs following injection. Moreover, Van Loveren *et al.* (1983) found that CD4+ cells were recruited to the local sites of antigen injection in the early phases of DTH responses; where in the spleen there was a significant increase in splenic CD4+ cells by 2 hrs post injection. All the previously mentioned findings

indicate the morphological changes in wattle's skin along 24 hrs post injection in our study.

The comparison between line in DTH response explain that H line had higher value at 12 and 24 h than that in L line at the same time point. these results are in agreement with the findings of Scott *et al*. (1991) and Parmentier *et al*. (1993), who have found that the *in vivo* mitogen responses to both con-A and PHA-P were higher in H line than those of L line. Parmentier *et al.* (1993) have explained his results on the basis of lower T-cell activity in the L line and higher CD4+ / CD8+ T-cell ratio in the high line. On the other hand, Ahmed. (2004) showed no significant differences between wattle thickness of high and low lines of Fayoumi chickens. These differences may be attributed to the difference in antigen used; since he used a bovine serum albumin not PHA-P.

The previous findings indicate that the divergent selection for humoral immune responsiveness in poultry will positively related and improve cell-mediated immune parameters. Also the acute intermediate wattle swelling in response to PHA-P can be used as an informative parameter for divergent selection for humoral immunity.

Hormonal Profile Changes During Delayed Type Hypersensitivity

A. Profile of serum T_3 hormone

The results in Table 7 indicate that there was no change remarked in the level of T_3 between PHA-P and saline injected birds at any time point following injection within both lines. However within PHA-P injected birds, the hormone level decreased at 6 hrs post injection as compared with 0 h without significance difference, then the level of hormone returned gradually to the base level at 9, 12 and 24 hrs., Irrespective the type of injection the results indicated that, the profile of T_3 within L line was identical to that of H line (Figure1).

The present results are in agreement with those of Weetman *et al.* (1984); Bachman and Mashaly (1987) and Gehad (2000), who found no significant effect of PHA-P injection on the level of circulating T3. Bachman and Mashaly (1987) concluded that T_3 level is lower than normal physiological level might be sufficient to maintain normal cell mediated immune response.

Table 7. Change in serum T_3 level (n mol/L) in PHA-P and saline injected birds of high and low lines of Fayoumi chickens

Line	Treatment	Time post injection (hours)					
		Zero	3	6	9	12	24
High	PHA	3.1± 0.5	2.9 ± 0.1	2.2 ± 0.4	3.4 ± 0.9	3.1 ± 0.8	3.0 ± 0.3
	Saline	3.1± 0.5	3.1 ± 0.1	3.2 ± 0.3	3.9 ± 1.6	4.0 ± 0.9	4.6 ± 0.5
Low	PHA	4.2± 0.5	3.2 ± 0.1	2.9 ± 0.0	3.1 ± 0.5	3.8 ± 0.1	4.0 ± 0.5
	Saline	4.2± 0.5	2.9 ± 0.5	3.4 ± 0.2	3.5 ± 0.2	4.6 ± 0.2	3.2 ± 0.2

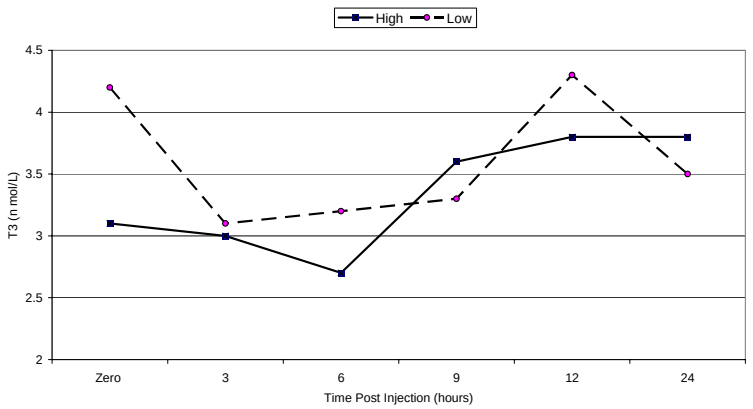

Figure (1): Variations in serum T3 (n mol/L) of high and low lines of Fayoumi chickens over the time of delayed type hypersensitivity.

B. Profile of serum corticosterone hormone

The results of corticosterone level indicated that there were no significant changes between PHA-P and saline injected birds at any sampling time within both H and L lines (Table 8). Additionally, within PHA-P or saline injected birds, no differences were observed in corticosterone levels at any sampling time point. Also, no differences between corticosterone concentration of H and L lines (Figure 2). The insignificant changes of corticosterone hormone following PHA-P injection are in agreement with those of Binns *et al.* (1992); Chrsousos (1995) and Gehad (2000), who found that DTH in chicken injected with PHA-P can proceed without actual stimulation of HPA-axis. This is due to absence of pro-inflammatory cytokines in mitogenic reaction.

Table 8. Change in serum corticosterone level (ng/ml) of PHA-P and saline injected birds of high and low lines of Fayoumi chickens

Line	Treatment	Time post injection (hours)					
		Zero	**3**	**6**	**9**	**12**	**24**
High	PHA	507.5 ±4.6	534.1 ±41.2	568.6 ±26.5	510.8 ±8.5	508.0 ±26.2	507.5 ±4.6
	Saline	507.5 ±4.6	513.6 ±11.4	482.3 ±18.9	527.0 ±50.2	509.0 ±5.8	519.6 ±11.4
Low	PHA	513.6 ±11.4	527.1 ±50.0	524.8 ±34.1	509.6 ±5.0	482.3 ±78.0	510.0 ±8.5
	Saline	513.68 ±11.4	524.8 ±34.1	527.1 ±50.2	514.0 ±11.1	508.0 ±26.2	566.8 ±14.3

In conclusion, the current study may explain that the initiation of humoral immune response involves the activation of HPT- and HPA-axis, which results in a decrease in T_3 levels and an increase in serum corticosterone levels post primary and

secondary SRBC injection. While this axis is not involved in delayed type hypersensitivity reaction.

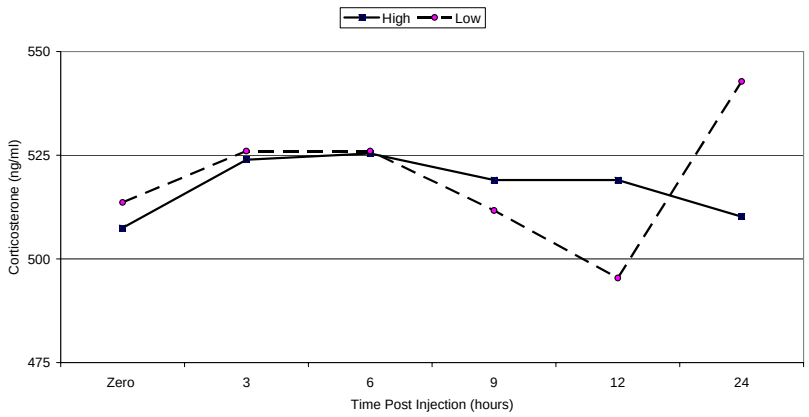

Figure (2): Variations in serum corticosterone (ng/ml) of high and low lines of Fayoumi chickens over the time of delayed type hypersensitivity.

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تأثير الإنتخاب للإستجابة العالية والمنخفضة للأجسام المناعية المضادة لكرات الدم الحمراء للغنم على التغيرات الهرمونية المصاحبة للاستجابة المناعية في الدجاج

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في هذه الدراسة تم إجراء تجربتان أستخدم فيها خطين من الدجاج الفيومي، أحدها منتخب للاستجابة المناعية المصلية العالية والآخر للاستجابة المناعية المصلية المنخفضة. وكان الغرض من هذه الدراسة هو مقارنة الاستجابة المناعية المصلية والخلوية بين هذين الخطين، فضلا عن معرفة التغير في مستوي هرمون T_3 والكورتيكوستيرون المصاحب لبدء هذه الاستجابة .

ولقد استخدم في التجربة الأولى مائة طائر من كل خط حيث حقن نصفهم بـ 1 سم ^{3} من معلق كرات الدم الحمراء للغنم (تركيز 25%) ، أما النصف الآخر فحقن بمحلول فسيولوجي . أخذت عينات الدم عند صفر، 1 ، 3 ، 6 ، 9 ، 12 ، 24 ساعة و 3 ، 7 ، 10 يوم بعد الحقن لأول وثاني مرة . هذا وقد أوضحت النتائج أن الخط العالي أكثر كفاءة في الاستجابة المناعية الأولية والثانوية مقارنة بالخط المنخفض وذلك عند 7 ، 10 يوم بعد الحقن . من ناحية أخرى لوحظ أن مستوى هرمون T_3 انخفض بصورة معنوية بعد 3 ساعات من الحقن لأول مرة بكرات الدم الحمراء للغنم في الخط العالي وبعد 3 ، 6 ساعات من الحقن بكرات الدم الحمراء للغنم في الخط المنخفض مقارنة بمستوى الهرمون عند صفر ساعة . بينما زاد تركيز هرمون الكورتيكستيرون في كلا من الخطين عند 3 ، 7 ، 10 يوم بعد الحقن بكرات الدم الحمراء للغنم لاول وثاني مرة مقارنة بمستوى الهرمون عند صفر ساعة .

في التجربة الثانية استخدم مائة وعشرون طائر من كلا الخطين حيث حقن نصفهم بمادة PHA-P بمعدل 100 ميكروجرام بينما حقن النصف الآخر بمحلول فسيولوجي وذلك لغرض قياس فرط الحساسية المتأخر. أخذت عينات الدم عند صفر ، 3 ، 6 ، 9 ، 12 ، 24 ساعة بعد الحقن ولقد أوضحت التجارب أن الخط العالي أبدى قدرة عالية في اختبار فرط الحساسية بعد 12 ، 24 ساعة بعد الحقن مقارنة بالخط المنخفض.

من ناحية أخرى لم يلاحظ أي تغيير في مستوى هرمون T_3 والكورتيكسيرون مصاحب لاختبار فرط الحساسية المتأخر في كلا الخطين .

Table 4. Change in serum corticosterone level (ng/ml) in SRBC and saline injected of high and low lines of Fayoumi chicken

Line	Treatment	Primary immune response									
		Time post injection (Hours)							Time post injection (days)		
		0	1	3	6	9	12	24	3	7	10
High	SRBC	25.4 ±2.4	35.1 ±16.0	164.9 ±58.9	120.6 ±48.6	91.6 ±26.7	38.9 ±11.0	275.2 ±13.2*	484.5 ±28.0+*	527.8 ±45.0+*	480.9 ±69.2+*
	Saline	25.4 ±2.4	162.2 ±81.7*	207.6 ±35.0*	183.2 ±44.9*	175.3 ±20.1*	172.3 ±35.2*	87.8 ±19.4	59.6 ±5.2	45.3 ±9.2	37.3 ±6.6
Low	SRBC	29.2 ±1.3	30.1 ±1.16	115.4 ±39.4	97.3 ±22.7	87.8 ±19.4	37.7 ±9.9	172.3 ±35.7*	444.8 ±21.0+ *	458.8 ±20.0+*	373.5 ±39.3+*
	Saline	29.2 ±1.3	38.9 ±11.02	172.3 ±35.7*	120.6 ±48.6*	91.6 ±26.7	87.3 ±19.4	73.8 ±19.9	59.4 ±5.0	35.14 ±3.6	60.1 ±12.2
		Secondary immune response									
High	SRBC	23.5 ±4.8	30.7 ±12.8	88.8 ±12.4	84.9 ±20.4	75.6 ±8.6	74.9 ±36.2	37.3 ±6.6	207.6 ±35.0+*	458.5 ±45.0+*	275.2 ±29.5+*
	Saline	23.5 ±4.2	30.6 ±3.4	89.9 ±58.9	176.9 ±24.2*	91.6 ±26.7	69.7 ±21.7	52.7 ±3.9	59.4 ±5.1	37.3 ±6.6	35.1 ±16.0
Low	SRBC	28.4 ±6.4	45.3 ±9.2	91.6 ±26.7	165.5 ±10.4*	81.2 ±28.5	75.6 ±8.5	55.5 ±14.2	180.1 ±50.0+*	458.5 ±48.0+*	190.6 ±54.2+*
	Saline	28.4 ±6.49	87.8 ±19.4	120.8 ±19.4	164.9 ±58.9*	75.6 ±8.5	62.9 ±28.0	51.1 ±14.2	63.01 ±31.9	61.6 ±9.4	30.2 ±4.9

* Indicate significance (P<0.05) compared to 0 hr. within each treatment.

+ Indicate significance differences (P<0.05) between treatment (SRBS or saline) within immune response at any specific time point.

Table 5. Variations in serum corticosterone levels (ng/ml) of high and low lines of Fayoumi chickens over the time of primary and secondary immune response

Line	Primary immune response									
	Time post injection (Hours)							Time post injection (days)		
	0	**1**	**3**	**6**	**9**	**12**	**24**	**3**	**7**	**10**
High	25.4	98.6	186.3	151.9	133.4	105.6	181.5	272.1	286.6	259.1
	±2.4	±33.3^{+}	±32.1	±32.1	±24.0	±34.1	±19.4	±95.9*	±10.9*	±10.4*
Low	29.2	34.5	151.6	118.0	89.7	62.7	123.1	252.1	246.0	373.1
	±1.3	±5.3	±19.1	±28.0	±14.8	±14.8	±28.2	±86.7*	±96.8*	±39.3*
	Secondary immune response									
High	23.5	30.7	165.0	128.6	83.6	72.3	47.5	133.5	274.9	155.2
	±2.9	±1.6^{+}	±37.2^{+}*	±26.8	±13.0	±18.9	±3.9	±36.7	±96.4*	±52.7
Low	28.4	66.6	106.1	165.2	78.4	64.2	55.5	121.6	266.1	110.4
	±4.1	±13.5	±12.5	±23.7*	±13.4	±13.4	±9.0	±37.3	±9.4*	±43.3

***** Indicate significance ($P<0.05$) compared to 0 hr. within each line and immune response.

+ Indicate significance differences ($P<0.05$) between lines within immune response at any specific time point.