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Relationship Between Immune Response and Some Reproductive Hormones in Chickens

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Abstract
Aiming to study the relation between immune response to sheep red blood cells antigen (SRBC) and reproductive female hormones (FSH and LH), and their relation with some productive performance in Norfa chickens. The present research was carried out at the Department of Poultry and Fish Production, Faculty of Agriculture, Shebin El-Kom, Menoufia University, Egypt. The experiment was extended from March, 2018 to July, 2019. Experimental birds injected with SRBC antigen at 20 weeks of age, and antibody titer measured 7 days post immunization. Birds were divided according to their immune response to three groups; control, high and low immunity groups. Productive traits were individually recorded and collected data were statistically analyzed. Results showed that, serum FSH and LH levels not differed significantly between experimental groups, and recorded a range of 1.09-1.14 mIU/ml and 0.644-0.737 mIU/ml, respectively. Both FSH and LH positively and significantly correlated to immune response in different groups, except the correlation between antibody titer and LH in low immunity group was negative (-0.233) but not significant.
The high immunity group of chickens had significantly higher percentages of fertility, hatchability, livability, early age at sexual maturity, and higher egg production traits either at 90 day of laying or at 42-wk of age, as compared to control and low immunity groups of chickens. According to recent study, enhancement of reproductive performance may be achieved by investment in immune response.
Key Word*: Immune response, chickens, reproductive hormones, FSH, LH.*

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I. Introduction

The immune system is the first line of defense of the organism against pathogens. The immune system of all vertebrates including the avian species are like mammals, but birds have a different repertoire of organs, cells and molecules compared to mammals. Birds have Bursa of Fabricius and Thymus gland[1]. Avian immune system includes specific (Primary lymphoid organs, i.e., thymus and bursa of Fabricius) and non-specific system (including Secondary lymphoid organs). Cooperation between B (from bursa) and T lymphocytes (from thymus) is often necessary for antibody response[2]. The immune system is regulated by the gonadal steroids estrogen, androgen and progesterone[3,4], but the circulating levels of these steroids can also be affected by immune system function. Such interactions appear to be mediated through the hypothalamic-pituitary-gonadal-thymic axis and depend on pituitary luteinizing hormone released by thymic factors under the control of the gonadal steroids.

Decreasing or enhancement reproductive performance may be achieved by investment in immune response[5]. They found that immunization with sheep red blood cells (SRBC) antigen increased mortality of great tit birds comparing with control nests. Immune system efficiency depressed by higher reproductive performance, and birds with high immune response predicted to lowering its reproductivity[5,6]. Humoral immune response in birds play a key role in minimizing effect of diseases and prevent reinfection[7]. SRBCs considered as non-pathogenic antigen that is very suitable for testing the immune challenge effects on performance[5]. This antigen stimulates humoral immunity by activation of B lymphocytes by T lymphocytes helping, resulting in antibody production.

Gonadotrophs in pituitary gland synthesizes and secretes both luteinizing (LH) and follicle stimulating (FSH) hormones under controlling of hypothalamus which control secretion of gonadotropin releasing hormone (GnRH – the main hormone regulates reproduction)[8]. Reproductive performance then controlled by hypothalamic pituitary gonadal axis. Receptors of GnRH are found outside pituitary tissues, these receptors were detected in immune cells (spleen and thymus of rates), revealing the possible relationship within the function of immune system[9].

Both of LH and FSH are glycoproteins synthesized and secreted by the gonadotropic cells of the anterior pituitary gland. LH essential to provide the androgen substrate for estrogen synthesis, which in turn contributes to oocyte maturation and may play a relevant role in optimizing fertilization and embryo quality[10,11].

Also, FSH regulates the development, growth, sexual maturation, and reproductive processes of the body[12], normal ovarian physiology[13] and playing a key role in follicular growth and maintenance of the follicular hierarchy[14,15].

Little information is available regarding the relation between immune response to SRBC antigen and reproductive hormones. Therefore, the aim of the present study was designed to examine the relationship between immune response to SRBC antigen and some reproductive hormones in Norfa chickens. In addition, effect of immune response to SRBC antigen on productive performance was studied.

II. Material and Methods

The current study was conducted at the Department of Poultry and Fish Production, Faculty of Agriculture, Shebin El-Kom, Menoufia University, Egypt. The experiment extended from March, 2018 to July, 2019, in order to investigate the relationship between immune response and some reproductive hormones in Norfa chickens.

Chickens stock: Norfa chicken used in recent study is a local Egyptian strain that was produced according to the Egyptian Norwegian project for improving Egyptian layer strains. The aim of the project was to improve the egg production of the well-adapted indigenous chickens at the farmer and small chicken holder level in the Menoufia governorate. The Norfa strain produced far more eggs than domestic birds, also egg fertility, hatchability and viability were good. Norfa layers have proven to be very suitable for villages where poor management generally prevails, due to their high ability to adapt to the local environment. On average, Norfa layers exceeded domestic birds in egg production by about 25%[16].

Mating system: The artificial insemination was used as a mating system for reproducing the next generation. Each family contained 3 dams that assigned at random to one sire. Fertile eggs were collected three times a day and numbered according to their dams. Dirty, cracked, and misshapen eggs were removed. Then, eggs were stored in egg storage room at 16 – 18 °C for 7 days, with 75 % of relative humidity. For incubation, all collected eggs were moved to the incubation room and left for at least 12 hours at room temperature. Then, the eggs were set with wide end up in the setting trays according to their dams and incubated in a forced draft incubator at 37.8 °C with a relative humidity of 65 %. Egg was turned every 2 hours from the 2nd to 18th day of incubation. All eggs were transferred to a separate hatcher in pedigree baskets according to their dams at 36.5°C and 80% relative humidity. On the day of hatching, chicks were wing banded, weighed and moved to brooding room.

Experimental stock management: The starting brooder temperature was 34 °C during the first week, then the brooder temperature was decreased gradually from 2-3 °C every week to reach 20- 24 °C at almost 42 days of age. The chicks were moved to rearing house at eight weeks of age. All birds were exposed to continuous artificial light for 24 hours during the first week of age, and then the artificial light was decreased gradually to reach the natural light by about 8 weeks of age. All chickens were received only natural day light from 9 to 17 weeks of age. At 18 weeks of age, birds were moved to individual cages in laying house, where the hens were kept until 42 weeks of age with 16 hour of light a day.

All chicks were fed *ad libitum* diet 1 (containing 2889 Kcal ME/Kg and 19.88% crude protein) during brooding and rearing periods. Whereas, chickens were fed *ad libitum* diet 2 (containing 2739 Kcal ME/Kg and 17.51% crude protein) during production until the end of experiment.

Experimental design and treatments:

Data from a total number of 75 layers of Norfa chickens were used in this experiment. At age of 20 weeks, the primary antibody response was determined for each pullet at 7 days post-immunization against SRBCs antigen. Chickens were divided into 3 groups based on the level of primary antibody titers against SRBC antigen. General mean ($\bar{X}$±SD) of antibody titer was (5.29±1.91) for all data, then experimental birds were divided into three groups (i.e., Control, low and high groups) according to their response against SRBC antigen.

Antibody response determination:

The primary antibody titers to SRBCs were determined for all birds (n = 75) as the following steps:

- **Sheep red blood cells antigen preparation:** The sheep red blood cells (SRBC) were chosen as natural, nonspecific, non-pathogenic and multi-determinant immunizing antigen to elicit the antibody response in the chickens[17].The SRBC were obtained in a heparin solution from Ossimi sheep breed and washed 3 times in phosphate buffer saline (PBS). After final wash, the packed SRBC were brought to a 2.5% V/V solution in the PBS and used for immunization.

- **Antigen immunization:** At age 20 weeks, each chicken was received an intravenous immunization via the wing vein with 0.1 ml of 2.5% SRBC suspension to induce the primary antibody response.

- Blood samples collection and serum preparation: Blood samples were taken from the wing vein at 7 days post immunization by syringe. About 2.5-3 ml of blood was taken from each chicken. Blood serum was collected, placed in disposable tubes and frozen for subsequent laboratory analysis.

- Determination of antibody titers: Total antibody titers to SRBC were determined by agglutination according to Van der Zijpp and Leenstra[18] in serum. Antibody titers measured against SRBC were expressed as the $\log_2$ of the reciprocal of the highest serum dilution giving complete agglutination. Titrations were assessed the same day in 96-well microtiter plates, using SRBC from the same stock as used for the immunizations.

Antibody titer concentrations in control and experimental groups:

The obtained results in Table (1) showed the concentrations of antibody titer in control and experiment groups of Norfa chickens. Results indicated that, the antibody titer concentration at the high response group of Norfa chicken was increased by 46.3 % compared to control group, while it was only 60.6 % at the low response group. Differences between groups were highly significant (P<0.01). The highest mean value was recorded at high Norfa group (7.752), followed by control group (5.296), while low Norfa group showed the least value (3.212).

Table (1): Antibody titer concentrations ($\bar{X}$±SE) in control and experimental groups of Norfa chickens.

Traits		Immunity groups			Sig.
		Control (n=25)	High (n=24)	Low (n=26)	
Ab titer	$\bar{X}$ ± SE	5.296 ± .382^{b}	7.752 ± .352^{a}	3.212 ± .210^{c}	**
	RC%	100	146.374	60.649	

a, b, c: Means in the same row bearing different superscripts are significantly different.
RC%: relative change %.

Determination of FSH and LH hormones levels:

At 24 weeks of age, the levels of both hormones (LH and FSH) determined for each individual. Serum was isolated and stored in 1.5-mL Eppendorf tubes at -17°C until analyses. Serum FSH, LH levels were analyzed using RIA kits (Hengyuan Biological Technology Co., Ltd, Shanghai, China) by radio immunoassay, according to the recommendations of the manufacturer. For measurement of FSH concentration, the assay sensitivity, measuring range, intra- and inter-assay CVs were 0.4 mIU/ml, 1.5 mIU/ml to 100 mIU/ml, <10% and <15%, respectively. For measurement of LH concentration, the assay sensitivity, measuring range, intra- and inter-assay CVs were 0.02, 0.5to 20 ng/mL, <10% and <15%, respectively. The primer sequences of follicle-stimulating hormone receptor, luteinizing hormone receptor (LHR), used for quantitative real time PCR were synthesized by Generay Biotech Co., Ltd (Shanghai, China).

Studied traits:

The following traits were studied (for all individuals in different experimental groups) during the experimental period:

1. The concentration of both LH and FSH hormones in serum.
2. Body weights (g) at 4, 6, 8, 12 and 16 weeks of age.
3. Body weight at sexual maturity (BW_{SM}) and body weight at maturity (i.e. 36 wks of age – BW_M).
4. Age at sexual maturity (ASM) days.
5. Egg production: Individual egg number was recorded as the number of eggs laid during the first 90 days of laying (EN90), as well as during the 42 weeks of age (EN42).
6. Egg weight: Average egg weight was measured in grams as an average weight of all eggs laid during the first 90 days of laying (EW90), as well as the average weight of first five eggs laid after reaching 42 weeks of age (EW42).
7. Egg mass: Egg mass was calculated by multiplying the number of eggs per laying hen times the average egg weight in grams during the first 90 days of laying (EM90), as well as during the 42 weeks of age (EM42).
8. Fertility and hatchability percentages: Fertility and hatchability were determined for each hen at 36-wk of age. Numbers of fertile eggs were determined by candling of all eggs on the 18th day of incubation and cracking the remaining eggs after hatching. The percentage was calculated for each hen as follows:

$$Fertility\ (\%) = \frac{Number\ of\ fertile\ eggs}{Total\ number\ of\ incubated\ eggs} \times 100$$

Whereas, hatchability percentage was calculated for each hen as follows:

$$Hatchability\ (\%) = \frac{Number\ of\ hatched\ chicks}{Total\ number\ of\ fertile\ eggs} \times 100$$

9. Livability percentage: The livability percentage of hatched chicks was determined during the brooding period for one month by using the following formula:

$$Livability\ (\%) = \frac{Number\ of\ alive\ chicks}{Total\ number\ of\ hatched\ chicks} \times 100$$

Statistical analysis:

Least square means and their standard errors ($\bar{X}$±SE) for each studied trait were calculated for each group. Data obtained were statistically analyzed using SPSS[19]program version 26.0. Probability value, ($P<0.05$) was considered for significant. All percentages data were converted to the corresponding arcsine prior statistical analysis. Duncan's multiple range tests was used for the multiple comparisons of means[20]. One-way classification statistical fixed model was used for statistical analysis as the following:

$$\mathbf{Y_{ij} = \mu + G_i + e_{ij}}$$

Where:

Y_{ij} = The value of the trait (observation).
μ = The common mean.
G_i = The fixed effect i^{th} group of antibody response.
e_{ij} = Experimental error.

III. Results and Discussion

Effect of immunity levels on the concentration of some reproductive hormones in Norfa chickens:

Both LH and FSH are gonadotropins developed by anterior pituitary gland[21]. The key factor for releasing these hormones is GnRH or LHRH which released from hypothalamus, and play a major role in sexual maturity, ovulation rate, spermatogenesis, incubation and general reproductive performance. During the ovulation cycle, the pituitary gland releases LH and FSH to stimulate the growth and development of follicles, where the progesterone synthesis gradually increases[22].

LH and FSH concentrations in experimental groups as affected by antibody titer were represented in Table (2). Although LH increased by 14.44 and 3.88% in high and low immunity groups, respectively, comparing with control group, the differences between groups were not significant. Regarding FSH, a decrease in both high and low groups was recorded (-0.702 and -4.650%, respectively) comparing with control, but also with no significancy. Dai et al.,[22] reported that, fluctuation in LH, FSH and progesterone may be led to passing of avian leukosis virus (sup-group J) viremia in chickens with discontinuous viremia. On the other hand, reduction of plasma concentrations of LH and testosterone in birds infected by Newcastle disease virus were observed by Rehman et al.,[23] revealing the relationship between immune challenge and levels of reproductive hormones. They argued that this correlation tends to depress reproductive performance. They also found that the viral load in infected birds decreased expression of luteinizing hormone and androgen receptors.

Table (2): LH and FSH concentrations ($\bar{X}$±SE) in control and experimental groups of Norfa chickens as affected by immunity levels.

Traits		Immunity groups			Sig.
		Control (n=25)	High (n=24)	Low (n=26)	
LH (mIU/ml)	$\bar{X}$ ± SE	0.644 ± .027	0.737 ± .022	0.669 ±.019	NS
	RC%	100	114.44	103.881	
FSH (mIU/mL)	$\bar{X}$ ± SE	1.140 ± .027	1.132 ± .023	1.087±.022	NS
	RC%	100	99.298	95.35	

RC% = relative change % of control; NS = non-significant

Results showed that, there were positive correlations between immune response (Ab-titer) and both FSH and LH hormones of serum concentrations in all experimental groups, except only the correlation with LH in low immunity group (-0.233) as represented in Fig. 1 (c). These correlations were significant in control group, and only for FSH in high group, but not significant for low immunity group (Fig. 1).

In human and animals' reproduction controlled mainly by gonadotropins, these gonadotropins activities may controlled by modulating antibodies. Antibodies predicted to inhibit the antigen activity by reducing interaction of these antigens with its receptors. While, in some work, some antibodies found to activate this interaction[24]. They also added, immunoglobulins my by inhibit or activate signaling pathways according to binding site (epitope) on the antigen. Su et al.,[25] reported that, serum LH and FSH decreased by immunization against GnRH. A substantial decreasing (negative correlation) in testosterone was detected in mallard[26] and chickens[27] males that produce more antibodies when immunized by SRBC. Reduction in androgen levels may be occurred by increasing humoral immunity (response), in peafowl, negative correlations has been reported between androgens and either leukocytes or SRBC response[28]. Indicating the negative relationship of immune system and hypothalamus-pituitary gonadal axis. SRBC challenge led to reducing testosterone levels as reported by Garamszegi *et al.*,[29] (in flycatchers) and Peters *et al.*,[26] (in mallards).

Immune system affected by multiple factors including neuroendocrine peptides, sex hormones and some metabolites. Sex hormones considered as important regulators of the immune system, their concentrations fluctuated according to the levels of LH and FSH hormones[30,31].

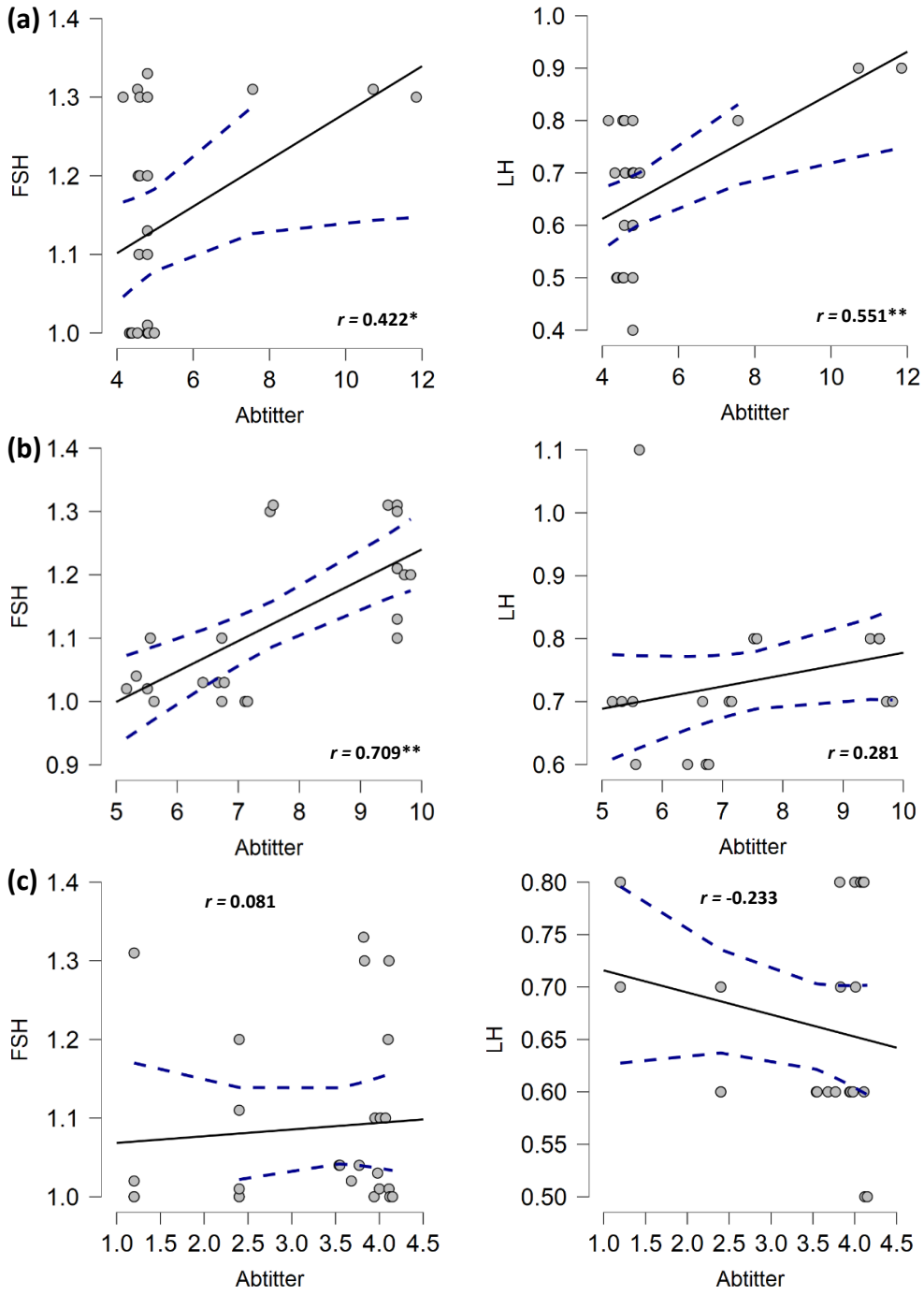

Fig (1): Scatterplot with confidence intervals (at 95%) and correlations between antibody titer (Abtiter) and the studied reproductive hormones (FSH and LH) in different experimental groups: (a) = control; (b) high and (c) low immune response groups.

Fertility and hatchability percentages: The fertility and hatchability percentages of Norfa chicken groups as affected by immune response levels are presented in Table (3). The statistical analysis revealed that the high immunity group of Norfa chickens was recorded the highest significant percentage of fertility and hatchability (88.50 and 82.33%, respectively) when compared with control group (84.04 and 75.00%, respectively) with increasing about 5.30% and 9.77%, respectively, as a relative change compared to control group. While, low immunity group recorded the least one (82.69% and 74.61%, for fertility and hatchability, respectively) with decreasing about (-1.61% and -0.52%, respectively) as relatively compared to control group. Similar findings

have been reported by Gebriel*et al.*[32], they found that, the high IgY antibody had significantly higher percentage of either fertility or hatchability than the control and low IgY antibody lines in both Silver Montazah and Sinai strains of chickens (P≤0.05). Moreover, Abd El-Naby[33] reported the same trend in relation between immune response and both fertility and hatchability in Norfa chickens.

Livability percentages as affected by immune response:

The livability percentages of Norfa chicken groups during the brooding period as affected by antibody titer concentrations were presented in Table (3). Results showed that, there were highly significant differences (P≤ 0.01) in livability percentages between studied experimental groups, the high immunity group of Norfa chickens was recorded the highest significant percentage of livability (89.92%) when compared with control group (87.14%) with increasing about (3.18%), while low group recorded the least value (86.83%) with decreasing about (-0.36%) compared to control group.

It was reported that newly hatched chicks started to produce antibodies on their own immune system differs markedly between species[34]. In some birds, production of antibodies may constitute an important addition to the ability of newly hatched chicks to take care of pathogens in order to have good livability and low mortality[35]. Current results are of one accord with Gebriel *et al.*[32] who investigated the livability associated with the IgY antibody concentration in layers. High IgY lines had higher percentages of livability. High IgY lines increased livability percentages by 15.09 to 17.70%, where low IgY lines decreased livability percentages by 16.95 to 21.16% as compared to control lines of Montazah and Sinai strains of chickens (P<0.01).

Recently, Abd El-Naby[33] found that, the high group of immunity of Norfa chickens was recorded the highest significant percentage of livability (90.68%) when compared with control group (88.65%), while low group recorded the least one (85.86%). Livability positively correlated to immune response (antibody titer) as illustrated by Abd El-Naby[33] and Leitner *et al.,*[36].

Table (3): Fertility, hatchability and livability percentages ($\bar{X}$±SE) in control and experimental groups of Norfa chickens as affected by immunity levels.

Traits		Immunity groups			Sig.
		Control (n=25)	High (n=24)	Low (n=26)	
Fertility %	$\bar{X}$ ± SE	84.04 ± .83^{b}	88.50 ± .63^{a}	82.69 ± .49^{b}	**
	RC%	100.00	105.30	98.39	
Hatchability %	$\bar{X}$ ± SE	75.00 ± 1.37^{b}	82.33 ± 1.07^{a}	74.61 ± .34^{b}	**
	RC%	100.00	109.77	99.48	
Livability %	$\bar{X}$ ± SE	87.14 ± .58^{b}	89.92 ± .30^{a}	86.83 ± .18^{b}	**
	RC%	100.00	103.18	99.64	

a, b, c: Means in the same row bearing different superscripts are significantly different.
RC%: relative exchange %.

Effect of immunity levels on body weight at different ages:

The body weight during growing period at 4, 6, 8, 12, and 16-wk of age of Norfa chicken groups as affected by immunity levels, are presented in Table (4). There were highly significant differences (P≤0.01) in body weight at different ages, the low immunity group of Norfa chickens was recorded the highest body weight at all studied ages followed by control group, while, highest immunity group recorded the lowest one. Similar results were observed by Martin *et al.*[37], Parmentier *et al.*[38] and Yang *et al.*[39]. They reported that chickens selected for low antibody response against SRBC, were significantly heavier at 2, 4, 5, 17 and 24-wk of age than those selected for high antibody response. In addition, the present results are in good agreement with the results reported by Abou-Elewa[40] who studied the effect of antibody titers on BW of both Norfa and White Leghorn chickens. She found that chickens of the low antibody titers line were significantly (P≤0.01) heavier in body weight at 16-wk than their high or control antibody titers line. Similarly, Abd El-Naby[33], found that the low immunity group of Norfa chickens was recorded the highest significant percentage of the body weight at 16-wk of age (847.94g), when compared with control group (822.48g), while highest group recorded the lowest one (762.30g).

Body weight at both sexual maturity and at maturity as affected by immunity levels:

The BW_{SM} and BW_{M} of Norfa chicken groups as affected by antibody titer concentrations, are presented in Table (4). Similar trend as in growing period was recorded at production period, the statistical analysis revealed that, the low immunity group of Norfa chickens recorded the highest significant body weight at sexual maturity (1025.50g) and at maturity (1174.84g), compared with control group (973.080 and 1123.28g), while high immunity group recorded the lowest one (939.333 and 1076.33g, respectively) at (P≤0.05). Current results fully agreed with those reported by Abd El-Naby[33]; Martin *et al.,*[37]and Abou-Elewa[40] regarding the body weight as affected by immune response level.

Table (4): Body weight (g) at different ages (4, 6, 8, 12, 16-wk of age, at sexual maturity and at maturity) as affected by immunity response in control and experimental groups.

Traits		Immunity groups			Sig.
		Control (n=25)	High (n=24)	Low (n=26)	
BW4	$\bar{X} \pm SE$	175.08 ± 4.04^{a}	159.79 ± 5.90^{b}	182.59 ± 2.45^{a}	**
	RC%	100.00	91.26	104.29	
BW6	$\bar{X} \pm SE$	285.08 ± 7.96^{a}	261.25 ± 10.38^{b}	305.92 ± 5.54^{a}	**
	RC%	100.00	91.64	107.31	
BW8	$\bar{X} \pm SE$	398.36 ± 10.94^{ab}	370.66 ± 13.48^{b}	420.00 ± 6.55^{a}	**
	RC%	100.00	93.04	105.43	
BW12	$\bar{X} \pm SE$	641.16 ± 14.99^{a}	$600.37 \pm 16.20_{b}$	658.92 ± 6.84^{a}	**
	RC%	100.00	93.63	102.77	
BW16	$\bar{X} \pm SE$	815.48 ± 13.77^{ab}	784.04 ± 18.98^{b}	851.69 ± 9.51^{a}	**
	RC%	100.00	96.14	104.44	
BW_{SM}	$\bar{X} \pm SE$	973.08 ± 19.08^{ab}	939.33 ± 26.22^{b}	1025.50 ± 15.15^{a}	*
	RC%	100.00	96.53	105.38	
BW_{M}	$\bar{X} \pm SE$	1123.28 ± 17.60	1076.33 ± 25.03	1174.84 ± 12.93	*
	RC%	100.00	95.82	104.59	

a, b, c: Means in the same row bearing different superscripts are significantly different.
RC%: relative exchange %.

Age at sexual maturity and egg production traits as affected by immunity levels:

Age at sexual maturity (ASM): Results in Table (5) showed the average age at sexual maturity according to immunity groups (control, high and low). Highly significant differences ($P \leq 0.01$) were observed in average ASM between control and other two high and low Norfa chicken groups, high immunity group reached sexual maturity earlier (167.87d) than other experimental groups followed by control (171.44d) then low (178.15d) immunity groups. Current results are in agreement with those previously indicated by Abou-Elewa[40] and El-Mougy[41] and more recently Abd El-Naby[33], they reported that the bullets highly responded to immune challenge with SRBC reached sexual maturity earlier than those recorded lower immunity response and control groups.

Egg number at 90 days of laying (EN90): The high immunity group of Norfa chickens recorded the highest significant ($P \leq 0.01$) egg number during the first 90 days of production cycle (51.50 eggs) compared with control group (46.60 eggs), while low immunity group recorded the lowest one (38.08 eggs) as shown in Table (5).Results also indicated that the egg number at 90 days of high immunity group of Norfa chickens was increased by 10.51 % compared to control group, while it was decreased by 18.29% at the low immunity group (Table, 5). The same trend was recorded byAbou-Elewa[40] in a study of the relation between antibody response and egg production traits at 90d of laying in both White Leghorn and Norfa layers. She reported that the high immune response line had the highest significant means of egg number (59.30 and 55.19 egg) and the low immune response line had the lowest significant means of egg number (48.15 and 45.82 egg), at 90 days of laying in the third generation in both White Leghorn and Norfa layers, respectively. The present results are in good agreement with those reported by Abd El-Naby[33] and El-Mougy[41]who observed greater egg number in the high immune response line than the low immune response line of Norfa chickens.

Egg number at 42-wk of age (EN42): The same direction of egg production as at 90d of laying was observed at 42-wks of age. The high immunity group laid the highest number (79.42) of eggs followed by control (70.60) and finally low (59.69) immunity group. Differences between groups were highly significant ($P \leq 0.01$) as shown in Table (5). The obtained results are in agreement with those reported byAbd El-Naby[33], in Norfa chickens.

Egg production and body weight reported to be negatively correlated with antibody titer in chickens[37]. While, Abd El-Naby[33] indicated that immune response to SRBC challenge positively correlated with egg production (number of eggs). These differences between workers may be attributed to the differentiation of experimental conditions, environments, strains, designs and antigens used.

Egg weight (g): Low immunity group recorded the heaviest egg weight at 90d of laying and also at 42-wks of age comparing with control and high immunity groups. The lightest eggs laid by the high immunity chickens (Table 5). Statistical differences were detected ($P \leq 0.01$) between the investigated groups at both 90d of laying and at 42-wks of age. Results, also indicated that the egg weight (g) at 42-wk of age in the low immunity group of Norfa chickens was increased by 1.96 % compared to control group, while it was decreased by -3.96 % in the high immunity group. The obtained results are in agreement with those reported by Abd El-Naby[33] and El-Mougy[41], they found that the low immune response lines had the highest egg weight at 90 days of laying, where the high immune response level of antibody titer had the lowest egg weight at 90 days of laying.

Egg mass (g): The egg mass at 90d of laying and 42-wks of age in Norfa chicken groups as affected by antibody titer concentrations were presented in Table (5). The statistical analysis of the obtained data (Table 5) revealed that the high immunity group of Norfa chickens was recorded the highest significant value of egg mass at 90d of laying (1991.29g) when compared with control group (1894.68g), while low group recorded the lowest one (1616.92g). At 42-wk of age, the same trend was observed, and the high response group recorded the highest egg mass comparing with control and low immunity groups, the differences between groups were highly significant ($P \leq 0.01$) as represented in Table (5). Similar results were recorded byAbou-Elewa[40]and recently byEl-Mougy[41] who found that the high immunity line had the largest significant ($P \leq 0.05$) means of egg mass and the low immunity line had the lowest significant means of egg mass in Norfa, Leghorn, Saini and Silver Montazah chicken strains.

Table (5): Age at sexual maturity and egg production traits as affected by immunity response in control and experimental groups.

Traits		Experimental groups			Sig.
		Control (n=25)	High (n=24)	Low (n=26)	
ASM	$\bar{X} \pm SE$	171.4 ± 1.64^{b}	167.8 ± 2.70^{b}	178.1 ± 1.57^{a}	**
	RC%	100.00	97.90	103.90	
EN90	$\bar{X} \pm SE$	46.60 ± 2.67^{a}	51.50 ± 3.32^{a}	38.08 ± 2.13^{b}	**
	RC%	100.00	110.51	81.71	
EN42	$\bar{X} \pm SE$	70.60 ± 3.37^{a}	79.42 ± 4.94^{a}	59.69 ± 2.53^{b}	**
	RC%	100.00	112.49	84.55	
EW90	$\bar{X} \pm SE$	$41.44 \pm .580^{b}$	$39.92 \pm .852^{ab}$	$42.96 \pm .365^{a}$	**
	RC%	100.00	96.34	103.67	
EW42	$\bar{X} \pm SE$	$50.96 \pm .50^{a}$	$49.08 \pm .80^{b}$	$51.96 \pm .32^{a}$	**
	RC%	100.00	96.31	101.96	
EM90	$\bar{X} \pm SE$	1894.68 ± 95.35^{a}	1991.29 ± 101.43^{a}	1616.92 ± 75.04^{b}	*
	RC%	100.00	105.09	85.34	
EM42	$\bar{X} \pm SE$	3556.96 ± 147.81^{a}	3809.13 ± 195.57^{a}	3082.00 ± 110.40^{b}	**
	RC%	100.00	107.02	86.59	

a, b, c: Means in the same row bearing different superscripts are significantly different.
RC%: relative exchange %.

Immunization of avian species reflect negative effects on some productive performance traits (i.e., egg production, egg weight, feeding rates, chick quality and reproductivity[5,42,43]. On the other hand, literature reviewed didn't indicated any negative effects of immune challenge on reproductivity[42]. These differences could be caused by the variations in experimental design, sampling method, numbers tested, environmental and genetical factors and/or their interactions.

IV. Conclusion

It can be concluded that, response to SRBC antigen challenge in the current research didn't affect reproductive hormones serum concentrations (i.e., LH and FSH) significantly. In addition, the highly responded (high immunity response group) Norfa chicken group showed superiority in most studied traits comparing with other experimental groups (control and low), reflecting the positive relationship between immune response and productive performance in recent study. Finally, enhancement of reproductive performance may be achieved by investment in immune response using SRBC antigen as non-pathogenic humoral immunity activator.

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