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RESPONSES OF HUMORAL AND CELL-MEDIATED IMMUNITY IN RELATION TO THE REPRODUCTIVE PERFORMANCE OF FAYOUMI MALE CHICKENS

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ABSTRACT: The present experiment was conducted to investigate the relationship between immune-related parameters and reproductive traits in Fayoumi cocks. Three lines low; high and control were used to evaluate the association between immune responses, selected blood hormones, semen quality traits, and morphological measurements (comb and wattle growth). Antibody titers against NDV, H9, and H5 were not significantly affected by line. Plasma testosterone concentrations were significantly higher in the high and control lines compared with the low line. The plasma concentrations of T3 and T4 (ng/ml) were significantly higher in the low line compared with the high line, while no significant differences were observed between the control line and either the low or high lines. The T3/T4 ratio was not significantly affected. The greatest semen volume was observed in the low line compared with the high line. The control line did not differ from the high line, but it was significantly higher ($P \leq 0.05$) than the low line. Similarly, sperm motility was significantly greater in the low line, followed by the control line, which did not differ from the high line. Comb width (cm), wattle width (cm), and wattle length (cm) were not significantly influenced by line. However, comb length (cm) was significantly improved in the control line, followed by the low and high lines. These findings suggest that careful selection of poultry breeds, suited to rearing conditions, production systems, and breeding objectives, along with balanced selection programs, is essential to maximize immune competence and reproductive efficiency.

Keywords: Fayoumi chicken, Immune, PHA-P, Reproduction, Semen, measurements.

INTRODUCTION

Poultry industry plays a critical role in global food security, particularly in developing countries where indigenous and resilient chicken breeds are highly valued. Among these, the Fayoumi chicken, an Egyptian breed, is renowned for its hardiness, disease resistance, and adaptability to harsh environments (Hassan *et al.*, 2019). As selection for enhanced productivity continues, the relationship between immune competence and reproductive performance has emerged as a significant area of interest in avian biology. Immune responses in poultry can be broadly categorized into humoral and cell-mediated immunity. Humoral immunity, primarily driven by B lymphocytes, involves the production of antibodies that neutralize extracellular pathogens, whereas cell-mediated immunity, governed by T lymphocytes, targets intracellular infections and plays a crucial role in immune regulation (Abbas *et al.*, 2017). These immune mechanisms are not only vital for survival but may also influence reproductive traits such as semen quality, testicular development, and hormonal profiles (Chand *et al.*, 2016). In the present study, we aimed to test the hypothesis that selection for stronger cell-mediated immunity reduces semen quality (volume, motility, concentration) and alters endocrine profiles (testosterone, corticosterone, and thyroid hormones) through allocation trade-offs.

The trade-off between immune function and reproduction is a well-documented concept in evolutionary biology, with energy and physiological resources often allocated preferentially depending on environmental pressures (Sheldon and Verhulst, 1996). In poultry, it has been suggested that elevated immune activity could either enhance reproductive outcomes by reducing disease burden or, conversely, impair reproduction through resource diversion (Lwelamira *et al*., 2009). However, such interactions may vary across breeds, and data on indigenous lines like Fayoumi chickens remain limited. Understanding how humoral and cell-mediated immunity influence the reproductive performance of Fayoumi males could provide insights for breeding strategies that optimize their health and fertility. Therefore, this study aims to evaluate the immune responses in Fayoumi roosters and their association with key reproductive parameters.

MATERIALS AND METHODS

This study was conducted at Fayoum Poultry Breeding Research Station, Animal Production Research Institute, Agricultural Research Center, Ministry of Agriculture, Egypt.

Experimental birds

A pedigreed second-generation Fayoumi flock was used in this study. Birds were selected based on primary antibody titers against PHA-P antigen, injected intramuscularly at 56 days of age, and measured 7 days later. The high-responder line (HR) included males with titers ≥278.73, whereas the low-responder line (LR) included males with titers ≤171.6. Males with intermediate titers (≈219.30) were randomly assigned to establish the control line (CL).

Experimental stock management

Every chick was wing-banded and pedigreed by family at hatching. They were reared in floor pens under a semi-open system until 18 weeks, and then transferred to wire cages. Brooding started at 33°C and was gradually reduced to 24–25°C. Chicks had continuous light for the first 3 days, then natural daylight until 17 weeks, followed by 14 hours of light daily at 18 weeks, and gradually increased to 17 hours using artificial lighting. Birds received clean water and feed *ad libitum* based on age-specific diets formulated according to Egyptian Feed Composition Tables (2001).

I. Studied traits

I.1. Immunization schedule and humoral immune response assessment

All birds received vaccinations against the AI viruses, H5N1 at 12 and 37 days, and H9N1 at 9 and 30 days, as well as the ND virus at 7, 18, and 21 days. At 56 days, birds were intramuscularly injected with 0.1 ml of PHA-P suspension prepared from SRBCs in PBS (Ghaffari Laleh *et al.*, 2008). After 7 days, 4 ml of blood was collected from the brachial vein, and serum was separated. Antibody titers against (ND) and (AI) viruses, and SRBCs were assessed via hemagglutination inhibition test and reported as $\log_2$ of the highest dilution showing hemagglutination.

I.2. Cell-mediated immunity assay

Cell-mediated immune response was assessed by intradermal injection of 1 mg PHA-P in 1 ml sterile saline into the right wattle and 0.1 ml sterile saline into the left wattle of 10 randomly selected males at 36 weeks of age for each line. Using a digital micrometer, the thickness of the wattle was measured before injection and 24, 48, and 72 hours after injection. The thickness increase as a percentage was then computed.

$$= \frac{\text{wattle thickness after injection} - \text{Initial wattle thickness}}{\text{Initial wattle thickness}} \times 100$$

Comb (width and length) and wattle (width, length, and thickness) were measured individually for each selected male, with linear dimensions recorded to the nearest centimeter and relative values expressed as percentages.

Immunoglobulin concentrations (IgG and IgM) were determined by enzyme-linked immune immunosorbent assays using commercial chicken kits.

I.2. Reproductive performance: Semen characteristics and measurements

At 60 weeks of age, 15 cocks (3 cocks of each treatment) had their semen samples randomly taken using the massage technique. The following features of semen samples were analyzed, per Kalamah *et al*. (2000).

1) A 1 ml tuberculin syringe was used to measure the ejaculate volume to the closest 0.01 ml.

2) Score for mass motility (grades 1–5).

3) Following iosine and nigrosine staining, the percentage of living and aberrant sperm was ascertained.

4) The Thomes–Zeis hemocytometer was used to measure the concentration of sperm.

5) Total sperm/ejaculate $\times 10^9$ = (ejaculate volume × sperm conc.).

6) Total abnormal sperm/ejaculate $\times 10^9$ = (abnormal sperm% × total sperm/ejaculate/100).

7) Total live sperm/ejaculate $\times 10^9$ = (live sperm% × total sperm/ejaculate/100).

The aforementioned attributes were established in accordance with Kalamah *et al*. (2000).

I.3. Hormonal profile

Five randomly chosen cocks per line had their blood samples taken at 36 weeks. Testosterone levels were measured using RIA kits from Beckman Coulter (France), while T_3, T_4 and corticosterone levels were assessed using RIA kits from Cambridge Medical Diagnostic Lab (Billerica, MA.

I.4. Statistical analysis

Data were analyzed using one-way ANOVA via the GLM procedure in SAS (2000), with mean comparisons conducted using Duncan's multiple range test (Duncan, 1955). Percentage values were arcsine-transformed before analysis;

actual means are reported. The model used was:

$$Y_{ij} = \mu + L_i + e_{ij}$$

Where: Yij = observation, μ = overall mean, Li = line effect (i = 1, 2, 3), and eij = random error.

RESULTS AND DISCUSSION

The Immune Responses

The data in the Table 2 show the effects of selection for immune response to PHA-A (phytohemagglutinin-A) on comb and wattle traits in Fayoumi cocks at 36 weeks of age.

Wattle thickness %

The average wattle thickness percentage varied significantly, as seen by the data in Table 2; the low line bird had the lowest values and the high line had the greatest.

Comb width

There were no significant differences among the lines for comb width, although the control line (13.48 cm) recorded the highest mean, followed closely by the high (13.23 cm) and low (12.75 cm) lines. This agrees with the findings of Zuk *et al*. (1995), who reported that comb size is influenced by genetic background and hormone levels but may not always respond directly to immune selection pressure. Comb size is also under sexual selection and often reflects testosterone levels, which were higher in the high and control lines in this study.

Comb length

Comb length differed significantly between lines, with the control line showing the longest combs (8.10 cm), which was significantly greater than both the low (7.25 cm) and high (7.32 cm) lines. This suggests that random breeding may have preserved a broader genetic variation that favored comb development. Comb length is known to be testosterone-dependent and is often associated with reproductive fitness (Johnsen and Zuk, 1996). However, extreme immune selection may reduce resource allocation to secondary sexual traits like comb length, supporting the immunocompetence handicap hypothesis (Folstad and Karter, 1992).

Wattle width and length

No significant differences were found in wattle width or wattle length across lines, though the high line recorded slightly higher values. Wattle size is, like comb traits, an indicator of health and sexual fitness, but these specific traits may be less sensitive to immune-based selection or may require longer-term selection to show differences. Studies such as Ligon *et al*. (1990) note that both comb and wattle sizes are influenced by androgen levels and nutritional status, which may explain why subtle, non-significant trends were observed here.

The findings suggest that selection for immune response in Fayoumi cocks does have some measurable effects on comb length, a trait closely tied to reproductive signaling. However, the lack of consistent changes in other morphological traits (comb width, wattle size) may indicate that these features are more resilient to immune selection pressures or influenced by other non-immune factors such as environmental and nutritional status. The control line often showed better or comparable traits, suggesting that non-directed (random) selection can maintain or even enhance morphological features.

Table 3 shows the correlation between the immune response to ND and avian influenza in 20-week-old Fayoumi males and selection for line immunological response to PHA-P. It is clear from the table that ND, H9 and H5 did not significantly influence by the Fayoumi males selected lines.

Plasma testosterone levels

Figure 1 illustrates the relationship between plasma testosterone levels and selection for the immunological response to PHA in 36-week-old Fayoumi males.

The results clearly showed that plasma testosterone concentrations in the high and control lines were significantly higher than those in the low line. However, no significant differences were detected between the high and control lines.

Table 4 shows the connection between the thyroid activity of 36-week-old Fayoumi boys and selection for immunological response to PHA. The findings unequivocally demonstrated that the low line had much higher levels of T3 and T4 (ng/ml) than the high line. The levels of the control line were in between those of the low and high lines. There was no discernible change in the T3/T4 ratio. Our study's findings concur with those of Bachman and Mashaly (1987), Weetman *et al*. (1984), and Gehad (2000), who discovered no impact of PHA-P injection on the amount of T3 in the blood. According to Bachman and Mashaly (1987), a low T3 level in comparison to a normal physiological level may be enough to sustain a typical cell-mediated immune response. Following a PHA injection, our study's negligible corticosterone hormone alterations are consistent with those of Binns *et al*. (1992), Chrsousos (1995), and Gehad (2000). They discovered that DTH in PHA-injected hens can proceed even in the absence of true HPA-axis stimulation. This is because the mitogenic reaction does not contain pro-inflammatory cytokines. Plasma corticosterone hormone (ng/ml) of different lines of Fayoumi males after 10 days from injection of PHA-A are shown in Table 4. Data clearly showed that the average of plasma corticosterone concentration for males was significantly higher for the high line, followed by the control with the low line recorded.

Reproductive traits

Semen quality

The current study highlights significant differences in semen quality traits among Fayoumi male chickens from different immune response-selected lines (High, Control, and Low) in response to PHA-A stimulation at 36 weeks of age (Table 5).

Ejaculate volume

The low line showed significantly higher ejaculate volume (0.604 ml) than the high line (0.364 ml), with the control line (0.590 ml) being statistically similar to the low line (Table 5). This suggests a potential trade-off between immune responsiveness and semen volume. Selection for high immune response may lead to reduced semen output, possibly due to energy reallocation from reproductive to immune function, supporting the immunocompetence handicap hypothesis (Folstad and Karter, 1992).

Sperm motility and live sperm %

Motility and percentage of live sperm were significantly higher in the low line (4.00 and 89.70%) compared to the high line (3.10 and 81.90%), while the control line showed intermediate values (3.40 and 85.90%) as shown in Table 5. This aligns with studies indicating that enhanced immune activation can negatively impact sperm function due to oxidative stress and altered hormonal balances (Verhulst *et al*., 1999).

Sperm concentration and total sperm count

The low immune responsive line again outperformed others in terms of sperm concentration (4.026×10^9/ml) and total sperm per ejaculate (2.416×10^9) as shown in Table 5. These findings suggest that selecting for high immune responsiveness might compromise reproductive output, consistent with previous literature documenting reproductive costs of immune activation in poultry (Zuk and Stoehr, 2002).

Total live and abnormal sperm counts

Total live sperm per ejaculate was highest in the low immune responsive line (3.617 × 10^9); significantly surpassing both high and control lines (Table 5). There were no appreciable differences in the proportion or overall quantity of aberrant sperm across the groups, though. This suggests that while overall sperm viability is reduced in high responders, sperm morphology remains relatively unaffected.

Semen pH

As shown in Table 5, semen pH did not differ significantly between lines, maintaining values around neutral (7.49–7.60), indicating that immune selection did not influence this physiological parameter.

These findings collectively indicate a negative correlation between selection for strong immune response and reproductive traits, especially semen quality, in Fayoumi cocks. The results align with the theoretical framework, suggesting physiological trade-offs between immune function and reproduction due to resource allocation limits (Sheldon and Verhulst, 1996). Therefore, while immune competence is crucial for disease resistance, over-emphasis on immune traits in breeding programs should be balanced with reproductive efficiency.

CONCLUSION

The findings of this study demonstrated that Fayoumi males across three different studied lines based on primary antibody titers against PHA antigen at 56 weeks of age had significant variation in their physiological and reproductive performance traits and immune responses, providing valuable insights for breeders aiming to enhance physiological and productivity in rural and small-scale poultry systems.

Table (1): The composition of the experimental basal diet.

Ingredients	**Percentage (%)**
Yellow corn	61.57
Soya bean (44%)	17.00
Wheat bran	6.70
Corn gluten (60%)	4.50
Di Ca P export	1.39
Limestone	8.16
Salt (NaCl)	0.37
*Premix	0.30
L Methionine	0.01
Total	100.00
Calculated values (%)	
Crude Protein	16.5
Metabolizable energy (M.E.) kcal/kg	2699
Crude fiber (C.F.)	3.468
Ether extract	2.964
Calcium	3.399
Available Phosphorous	0.397
Total Phosphorous	0.61
Sodium	0.164
Arginine	1.28
Lysine	0.73
Methionine	0.335
Methionine and cysteine	0.619

*Premix added to the 1 kg of diet, including Vit.A 10000 I.U; vit. D3 2000 I.U; vit. E 15 mg; vit K3 1 mg; vit B1 1mg; vit. B2 5 mg; vit. B12 10 µg; vit B6 1.5mg; Niacin 30mg; Pantothenic acid 10mg; folic acid 1mg; Biotin 50 µg; choline 300 mg; zinc 50mg; copper 4mg; iodine 0.3 mg; iron 30mg; selenium 0.1mg; manganese 60mg; cobalt 0.1mg and carrier CaCo3 up to 1kg.

Table (2): Relationship between selection to immune response to PHA-A and comb and wattle traits of El-Fayoumi cocks at 36 weeks of age.

Item	**Line**			**SEM**
	Low	**Control**	**High**	
Means (%) of wattle thickness (24 hrs after injection PHA-P)	171.60[c]	219.30[b]	278.73[a]	2.764
Comb width (cm)	12.75	13.48	13.23	±0.250
Comb length (cm)	7.25 [b]	8.10 [a]	7.32 [b]	±0.091
Wattle width (cm)	5.18	5.27	5.38	±0.240
Wattle length (cm)	5.31	5.12	5.39	±0.322

[a, b]means that within each row, have no similar letter (s) are significantly different (*P* ≤ *0.05*)

Table (3): Relationship between selection for immune response to PHA-A and the immune response to ND and Avian Influenza of Fayoumi males at 56 days of age.

Item	Line			SEM
	low	Control	high	
ND	5.400	5.610	5.933	±0.555
H9	3.933	4.407	4.267	±0.258
H5	3.533	3.017	3.067	±0.194

Table (4): Relationship between immune response to PHA and thyroid activity of El-Fayoumi males at 36 weeks of age.

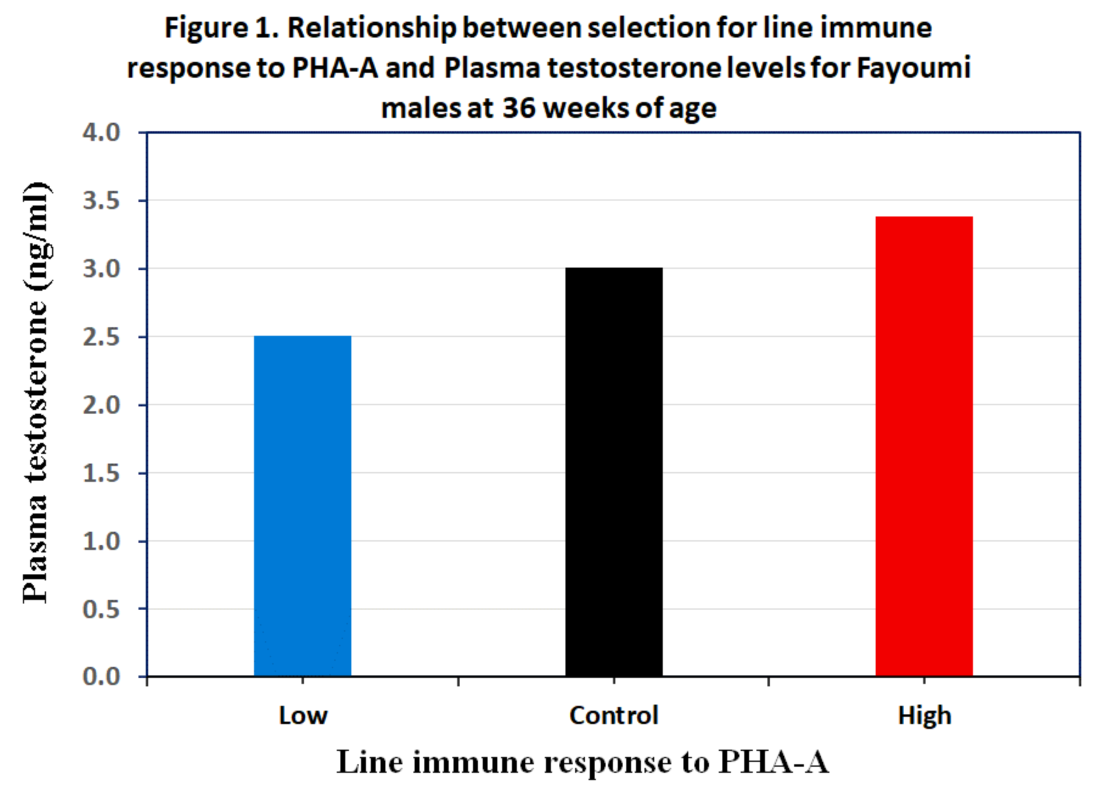
Item	Line			SEM
	High	Control	Low	
T_3 (ng/ml)	2.973[b*]	3.450[ab]	3.760[a]	±0.147
T_4 (ng/ml)	14.453[b]	15.753[ab]	16.687[a]	±0.484
T_3/T_4 ratio	0.205	0.219	0.226	±0.009
Corticosterone concentration	222.2[a]	185.0[b]	147.6[c]	±4.01

[a, b] Means within each row have no similar letter (s) are significantly different ($P \leq 0.05$)

Table (5): Selection for semen quality features in 36-week-old Fayoumi cocks and their immunological response to PHA-A.

Item	Line			SEM
	High	Control	Low	
Ejaculate volume (ml)	0.364[b]	0.590[a]	0.604[a]	±0.064
Semen pH	7.60	7.59	7.49	±0.162
Sperm motility (1-5)	3.10[b]	3.40[ab]	4.00[a]	±0.267
Live sperm %	81.90[b]	85.90[ab]	89.70[a]	±1.951
Abnormal sperm %	14.50	14.00	12.30	±1.162
Sperm concentration ($\times 10^9$)	3.344[b]	3.467[b]	4.026[a]	±0.159
Total sperm/ejaculate ($\times 10^9$)	1.221[b]	2.080[a]	2.416[a]	±0.252
Total live sperm/ejaculate ($\times 10^9$)	1.011[b]	1.783[a]	2.166[a]	±0.184
Total abnormal sperm/ejaculate ($\times 10^9$)	0.168[b]	0.297[a]	0.294[a]	±0.032

[a, b] Means within each row have no similar letter (s) are significantly different ($P \leq 0.05$)



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Fayoumi chicken, Immune, PHA-P, Reproduction, Semen, measurements.

الملخص العربى

الارتباط بين درجة الاستجابات المناعية الخلطية والخلوية في سلالة الفيوم وكفاءة الأداء التناسلي لذكور دجاج الفيومي

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أُجريَت هذه التجربة لدراسة العلاقة بين بعض المؤشرات المناعية والصفات التناسلية في ديوك الفايومي. استُخدمت ثلاثة خطوط وراثية—منخفض، مرتفع، وضابط لتقييم الارتباط بين الاستجابات المناعية وبعض الهرمونات الدموية وصفات جودة السائل المنوي والقياسات المورفولوجية (نمو العُرْف واللَّغْد). لم تتأثر عيارات الأجسام المضادة ضد NDV وH9 وH5 تأثراً معنوياً بالخطوط. وُجد أن تركيز هرمون التستوستيرون في البلازما كان أعلى معنوياً في الخط المرتفع وخط الضابط مقارنة بالخط المنخفض. أما تركيز هرموني T3 و T4 (نانوغرام/مل) في البلازما فقد كان أعلى معنوياً في الخط المنخفض مقارنة بالخط المرتفع، في حين لم تُسجل فروق معنوية بين خط الضابط وكل من الخطين المنخفض والمرتفع. ولم يتأثر معنوياً معدل .T3/T4 سُجل أكبر حجم للسائل المنوي في الخط المنخفض مقارنة بالخط المرتفع، بينما لم يختلف خط الضابط عن الخط المرتفع، لكنه كان أعلى معنوياً ($P \leq 0.05$) من الخط المنخفض. وبالمثل، كانت حركة الحيوانات المنوية أعلى معنوياً في الخط المنخفض، تلاه خط الضابط الذي لم يختلف عن الخط المرتفع. لم تتأثر معنوياً قياسات عرض العُرْف (سم) وعرض اللَّغْد (سم) وطول اللَّغْد (سم) باختلاف الخطوط. في المقابل، تحسّن طول العُرْف (سم) معنوياً في خط الكنترول، تلاه الخط المنخفض ثم الخط المرتفع. تشير هذه النتائج إلى أن الاختيار الدقيق للسلالات الداجنة المناسبة لظروف التربية وأنظمة الإنتاج والأهداف الإنتاجية، إلى جانب برامج انتخاب متوازنة، يُعد ضرورياً لتعظيم الكفاءة المناعية والكفاءة التناسلية.