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PHENOTYPIC VARIATION BETWEEN TWO EGG-TYPE CHICKEN STRAINS FOR RESIDUAL FEED CONSUMPTION AND CELL-MEDIATED IMMUNITY

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SUMMARY

An experiment was conducted to estimate the residual feed consumption (RFC), cell mediated immunity and their relationship in Hy-line (commercial) and Norfa (synthetic Egyptian) strains. Sixty laying hens (30 each) were randomly chosen and individually housed in cages from 46 to 54 weeks of age. The present results revealed that the Hy-line had significantly higher egg mass and better feed conversion ratio compared to Norfa one. Opposite trend was observed for eggshell quality. With respect to cutaneous basophil hypersensitivity (CBH) response, the Norfa was hyper responder to Phytohemagglutinin-P (PHA-P) injection compared to Hy-line. The observed feed consumption values were somewhat close to their expected values in Hy-line. Swelling measured at 24hrs post PHA-P injection was significantly negatively correlated with RFC in Hy-line. Similar trend was noticed between swelling measured at 48hrs post PHA-P injection and RFC in Norfa . It could be concluded that the Hy-line strain had better egg production traits and RFC; however, the Norfa had better eggshell quality and was a higher responder to PHA-P injection.

Keywords: Residual feed consumption, immunity, laying hens

INTRODUCTION

Laying hens can be considered as being genetically programmed for efficient egg mass production (Dunnington, 1990). However, there is a lot of variation in efficiency between individuals within a population, as shown by variation in feed consumption that cannot be explained by metabolic body weight, body weight gain, and egg mass. This variation in feed consumption is reflected in differences in residual feed consumption (RFC) (Bentsen, 1983; Luiting and Urff, 1991). RFC is defined as the difference between observed feed consumption (OFC) and FC predicted from metabolic BW (representing maintenance) and BW gain, egg mass (both representing production). RFC is, thus, a measure for feed efficiency: chickens with low RFC (R−) need less feed to reach the same BW and production level and are, therefore, more efficient producers than chickens with high RFC (R+).

Selection for general immune response in poultry has been proposed as a sustainable alternative to selection for resistance against specific diseases. Progress with selection for resistance approach might be hindered by interactions between host and pathogen which would lead to continuous adaptability on both sides. In addition, it would not be feasible to select for disease resistance against the tremendous number of different pathogens that an animal could face in its entire life cycle.

Several general immune traits were experimentally selected for in chicken lines. Lamont *et al*. (2003) and Pinard (2002) revealed that the different immune response mechanisms may have different genetic components.

Phytohemagglutinin is a lectin,a mixture of four subunits isolated from *Phaseolus Vulgaris* (red kidney bean), its seeds rich with proteins and glycoprotein, and is considered (PHA-P) a good *in vivo* measure T-lymphocyte function (Qureshi *et al*., 1997). The PHA intradermal reaction, a T-lymphocyte-dependent response, has been well researched and shown to be a reliable indicator of *in vivo* cellular immunity in poultry (Goto *et al.,* 1978 and McCorkle *et al.,* 1980). The skin response reflects a complex series of physiological events such as mitogen-receptor and lymphocyte-macrophage interactions, release of chemical mediators, cellular proliferation, and changes in vascularity (Chandra and Newberne, 1977). Histologically, PHA is a strong mitogenic to T-lymphocytesa, and intradermal injections elicit macrophage infiltration and dense perivascular accumulations of lymphocytes 24h post-injection in chickens (Goto *et al.* 1978 and McCorkle *et al.,* 1980). The increased infiltration by basophils and eosinophils 24h post-injection has been described as a cutaneous basophil hypersensitivity response (Stadeckerm *et al*., 1977).

Previous studies on commercial chickens differing in residual feed consumption (RFC) demonstrated no difference between the efficient and inefficient poultry in specific antibody production to *M. Butyricum* (van Eerden *et al*., 2004) and confirming that antibody production is not an energy demanding process (Klasing, 1998 and Parmentier *et al*., 2002). The objective of the present experiment was to investigate the residual feed consumption, cell-mediated immunity and their relationship in two genetic egg-type chicken strain layers.

MATERIALS AND METHODS

Genetic flocks and husbandry

This experiment was carried out at the poultry breeding farm, Poultry Production Department, Faculty of Agriculture, Ain Shams University. Sixty 46-wk-old, white Hy-line (commercial) and Norfa (synthetic Egyptian) strains (30 in each) were housed in individual cages, on a 16-hour light schedule. Chickens in both genetic groups were reared under the same environmental, managerial and hygienic conditions. Feed and water were supplied *ad libitum*. The hens received a typical layer diet containing 2800 kcal ME/kg and 18% CP to meet or slightly exceed the nutrient requirement recommended by NRC (1994). The average high and low ambient temperatures recorded during the experimental period (February – March, 2008) were 22.6 and 15.8°C, respectively.

Measurements and observations

Body weights were individually recorded at 46 and 54 weeks of age. Numbers and weights of egg were recorded daily from 46 to 54 weeks of age. At 54 weeks of age, sixty eggs (30 from each strain) were chosen to determine the internal and eggshell quality. The egg length (long axis) and width (short axis) were measured with an electronic caliper. The width to length ratio was shown in percentage points and constituted the egg shape index. The height of thick albumen (H) and the egg weight (W) were used to calculate Haugh units according the formula of Williams (1997): $HU = 100 \log (H + 7.7 - 1.7 W^{0.37})$, where H= thick albumen height, W= egg weight. The eggshell, after the removal of the egg content, was dried. Subsequently the eggshell was weighed to the nearest 0.01g. Eggshell thickness, without membranes, was measured (mm) with a micrometer. The albumen weight was calculated from the difference between the entire egg weight and the yolk and eggshell weights. Yolk, albumen and the eggshell were expressed as percentages from the weight of the fresh egg. The breaking strength was measured according to the method Fathi and El-Sahar (1996) which assessed the resistance of the egg to crushing.

Hematological parameters

At 54 weeks of age, blood samples were taken from the brachial vein into heparinzed tubes from all birds. Plasma was obtained from the blood samples by centrifugation for 10 min. at 4000 rpm and stored at -20°C until the time of analysis. The frozen plasma was allowed to thaw at room temperature prior to analysis. Plasma total protein, albumin, calcium, phosphorus and cholesterol were determined by enzymatic colorimetric methods using available commercial kits. The plasma globulin was calculated as the difference between plasma total protein and albumin.

In vivo cell-mediated immunity

A phytohemagglutinin-P (PHA-P) injection assay was used to evaluate *in vivo* T-cell-mediated immune response of 30 Hy-line and 30 Norfa hens according to Cheng and Lamont (1988). At 54 weeks of age, birds were injected intradermally in the wattle with 100 µg of PHA-P (Sigma Chemical Co., St. Louis, Missouri) in 0.1 ml of phosphate buffered saline (PBS) after marking the injection site. The thickness of wattle was measured (to nearest 0.01mm) at 0, 24, 48 and 72 hrs post PHA-P injection. Wattle swelling was calculated as the difference between the thickness of the wattle prior to and after injection of PHA-P.

Heterophils and lymphocytes

At 54 week of age, blood samples were obtained from 60 hens (30 each strain) for heterophil (H) and lymphocyte (L) enumeration based on the procedures of Gross and Siegel (1983). Briefly, one drop of blood was smeared on each glass slide. The smears were stained using Wright's stain. Two hundred leukocytes, including granular (heterophils) and nongranular (lymphocytes) ones, were counted on different microscopic fields representing 200 cells, and the heterophil to lymphocyte ratio was calculated.

Computing data and statistical analysis

The feed consumption for hens was predicted by a regression equation for each strain. Residual feed consumption (RFC) was calculated as the difference between observed (OFC) and expected feed consumption (EFC) for each experimental hen. Each strain had its own regression coefficients according to the following equation (Flock 1998):

$$EFC = aBW_i^{0.75} + bEM_i + c\Delta W_i + d$$

Where: EFC = expected feed consumption of hen I (g);

$BW_i^{0.75}$ = mean metabolic body weight of hen I ($kg^{0.75}$);

EM_i = egg mass production of hen I (grams);

ΔW_i = body weight change;

a, b and c = partial regression coefficients;

d = intercept.

All calculations and analyses were made using General Linear Models (GLM) procedure of SAS User's Guide, 2001. Correlation coefficients of RFC with productive traits and immune responses were estimated for each strain using the “PROC CORR” procedure of SAS.

RESULTS AND DISCUSSION

Productive parameters

Egg production, egg quality and some hematological traits of Hy-line and Norfa strains are summarized in Table (1). The present results revealed that there was no significant difference between strains for body weight. However, the Hy-line hens produced significantly higher number of eggs (19.3 eggs) and heavier egg weight (11.9g) compared to Norfa ones. Consequently, the Hy-line hens had significantly consumed more feed compared to Norfa ones. Feed conversion ratio of Hy-line was significantly better than that of Norfa ones. With respect to internal egg quality, it could be noticed that the percentage of albumen for Hy-line was significantly higher than that of Norfa ones, consequently, the eggs produced from Norfa hens had significantly higher yolk percentage compared to those produced from Hy-line hens. There was no significant difference between strains for Haugh unit. Concerning eggshell quality, the results indicated that the Norfa had significantly higher eggshell percentage, shell thickness and eggshell breaking strength compared to Hy-line ones. This improvement in eggshell quality could be attributed to lower egg number and smaller egg size associated of Norfa compared to Hy-line. Finally, it could be concluded that the Hy-line had better egg production parameters and feed conversion; however, the Norfa had better eggshell quality.

There was no significant difference between strains for plasma total protein. However, the Hy-line hens had significantly higher plasma albumen compared to Norfa hens. Opposite trend was noticed for plasma globulin, whereas the Norfa had significantly higher plasma globulin compared to Hy-line . It has been shown that the levels of plasma calcium are related to the number of eggs laid by different species of birds (Kosin, 1972). Our results showed that both plasma calcium and phosphorus of Hy-line were significantly higher than that of Norfa . Inversely, the Norfa had significantly higher plasma cholesterol compared to Hy-line .

Heterophils and Lymphocytes count

Lymphocytes and heterophils are the two principle leukocytes, which exert dominance on other leukocytes. Data presented in Figure (1) show that Norfa had significantly higher lymphocytes percentage and lower heterophils percentage compared to Hy-line. The H/L ratio is a recognized measure of stress in birds (Davison *et al*., 1983; Gross and Siegel, 1983; Maxwell, 1993; Al-Murrani *et al*., 2002) that has became a valuable tool in stress research especially when combined with the convenience and repeatability of automated blood cell counts (Post *et al*., 2003). The present results revealed that heterophil to lymphocyte ratio was significantly affected by strain, whereas Norfa had significantly lower ratio compared to Hy-line.

Table 1. Egg production parameters, egg quality and some hematological traits of Hy-line and Norfa strains

	Strain			
Trait	**Hy-line**	**Norfa**	**Prob.**	**Difference**
Body weight, g (46 wk)	1357.8±14.75	1324.4±15.05	NS	+33.42
Body weight, g (55 wk)	1445.5±24.51	1412.6±23.37	NS	+32.94
Egg number	51.13±0.11	31.82±0.34	0.001	+19.31
Egg mass, g	3179.23±12.21	1602.00±18.36	0.001	+1577.23
Egg weight, g	62.18±0.17	50.33±0.17	0.001	+11.85
Feed consumption, g	7211.37±57.08	5174.23±66.94	0.001	+2037.14
Feed conversion ratio	2.27±0.02	3.23±0.01	0.001	-0.96
Egg quality				
Albumen, %	60.80±0.12	59.68±0.02	0.01	+1.12
Yolk, %	29.51±0.08	30.17±0.02	0.01	-0.66
Haugh units, %	85.36±1.89	85.12±2.24	NS	+0.24
Shell, %	9.68±0.08	10.15±0.02	0.01	-0.47
Shape index, %	75.14±1.23	76.11±1.05	NS	-0.97
Shell thickness, mm	0.322±0.002	0.356±0.002	0.01	-0.034
Breaking strength kg/cm^2	3.24±0.03	3.58±0.03	0.05	-0.34
Hematological parameters				
Plasma total protein, g/dl	5.86±0.10	5.84±0.14	NS	+0.02
Albumen, g/dl	3.51±0.11	3.14±0.09	0.02	+0.37
Globulin, g/dl	2.35±0.07	2.70±0.04	0.01	-0.35
Plasma calcium, g/dl	21.15±0.92	19.25±0.86	0.001	+1.90
Plasma phosphorus, g/dl	10.14±0.42	9.10±0.51	0.01	+1.04
Cholesterol, mg/dl	130.15±1.12	134.17±1.30	0.01	-4.02

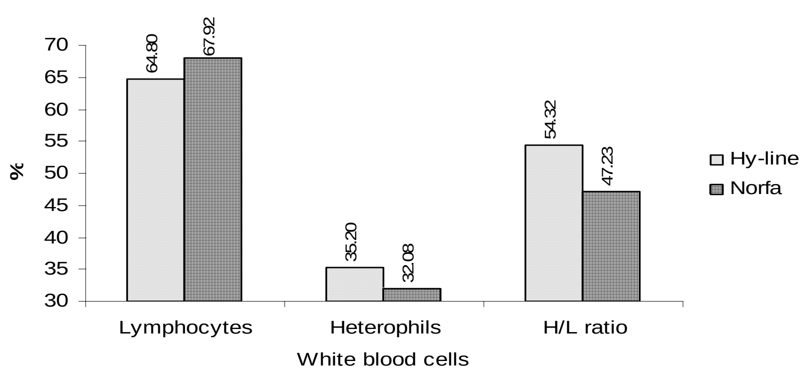

Fig. 1. Lymphocytes, heterophils percentages and H/L ratio of Hy-line and Norfa strains

In vivo cell-mediated immunity

In vivo cell-mediated immune response as measured by PHA-P stimulation (wattle) is presented in Figure (2). The present results revealed that the specific responses to PHA-P injection at all times showed marked differences between strains. Norfa had significantly hyper response to PHA-P injection compared to Hy-line at all measured times. The difference between strains for response to PHA-P injection could be attributed to the presumed polygenic effect of the lymphoblastogenic response to PHA-P (Morrow and Abplanalp, 1981). Also, T-cell mediated immune response of chicken has significant variation among birds of different genetic lineage (Miggiano *et al*., 1976; Lassila *et al*., 1979; Lamont and Smyth, 1984; Cheng and Lamont, 1988). Successful divergent selection of chickens for various T-cell functions suggested that many of these functions are highly heritable, and are often negatively correlated with body weight (Yamamoto and Okada, 1990; Afraz *et al*., 1994). There is good evidence that cell-mediated immunity plays an important role in controlling and clearing intracellular bacteria (Kogut *et al*., 1995; Sarker *et al*., 2000). In addition, selection on cellular responsiveness might add to enhancement of resistance to coccidiosis (Parmentier, *et al*. 2001).

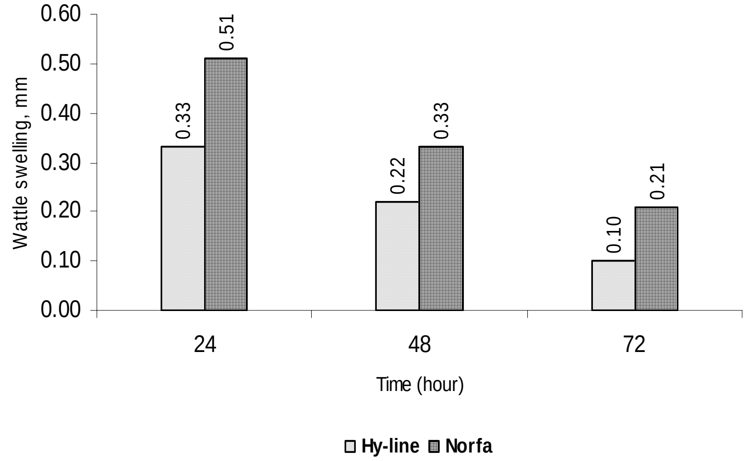

Fig. 2: Wattle swelling of Hy-line and Norfa strains

Multiple regression equation of feed consumption

Observed feed consumption, egg mass, change in body weight and metabolic body weight for each hen within each strain that were used to estimate the regression coefficients are listed in Table (2). The results revealed that the equation calculated for Hy-line had better rate of determination (R^2 = 0.91) compared to Norfa (R^2 = 0.66) . This means that the figures of RFC calculated from these equations are more reliable and have a highly applicable prospective. Pirchner (1985) reported that differences in observed feed consumption (OFC) and expected feed consumption (EFC) are caused by variability in several factors, such as composition of product (eggs), body weight change, food spillage, metabolic rate and in the ability to synthesis egg and body constituents. Figure (3) illustrates the observed against expected feed consumption for each hen within strain. While Figure (4) depicts the residual feed consumption value for each hen.. The negative values of RFC are desirable rather than positive ones. The efficient hens, which have negative RFC figures were more frequent than inefficient ones in the Hy-line strain. The results of Morrison and Leeson (1978); El-Sayed and El-Hakim (1994) and Hussein *et al.* (2000) confirmed that the efficient birds were less active, less heat producing, spent more time resting and less time standing than inefficient bird. van Eerden *et al.* (2004) reported that selection for higher efficiency of feed used by laying hens might result in birds that are less frustrated prior to laying and are less stressed. If reduction in feed intake is an indicator of stress, it was observed that feed intake of the R+ (eat more than expected) chickens decreased considerably after transportation, whereas feed intake of R- (eat less than expected) chickens remained almost unaffected.

Table 2. Coefficients of partial regressions and constants for Hy-line and Norfa strains

Strain	Constant	EM	ΔW	$BW^{0.75}$	R^2
Hy-line	1460.89	-0.74	-4.34	34.56	0.91
Norfa	-1132.23	3.45	0.75	3.14	0.66

EM = egg mass ΔW = body weight change
$BW^{0.75}$ = metabolic body weight R^2 = rate of determination

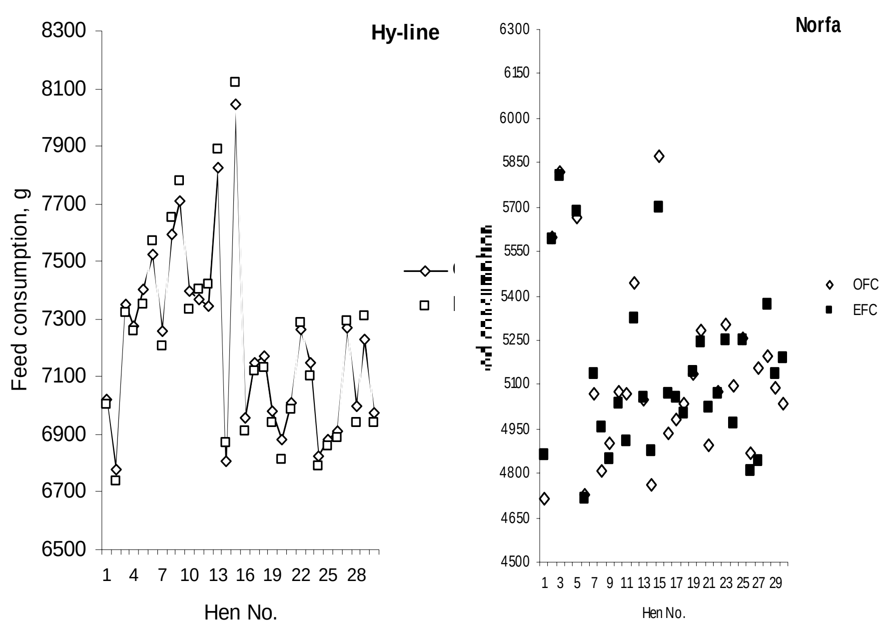

Fig. 3. Observed against expected feed consumption in Hy-line and Norfa strains

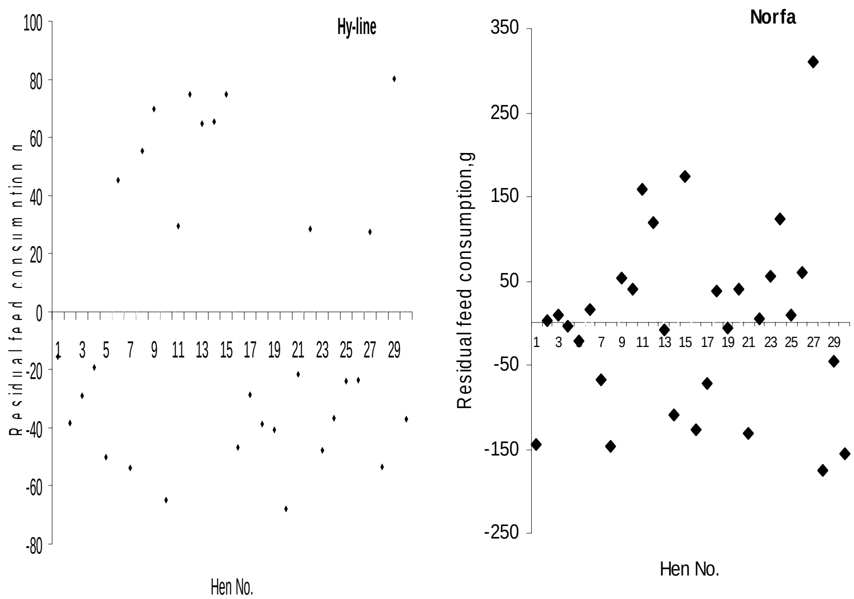

Fig. 4. Residual feed consumption of Hy-line and Norfa strains

Phenotypic correlation coefficients

Calculation of RFC by phenotypic multiple regression analysis is an acceptable alternative in a breeding program if no reliable estimates of genetic correlations are available (Luiting and Urff, 1991). This suggestion was reported because the authors found that the estimates of genetic correlation of RFC with the economic traits did not clearly differ from zero. Also, Tixier-Boichard *et al.* (1995) found that the genetic correlation between RFC and the independent variables used in the prediction equation (metabolic body weight, change in body weight and egg mass) were generally low, which confirms the validity of the selection on a phenotypic assessment of RFC. Correlation coefficients for some quantitative traits and cell-mediated immunity with RFC are presented in Table (3). Egg number was positively (but not significantly) correlated with RFC in Hy-line strain. Tixier-Boichard *et al.* (1995) reported positive correlation between RFC and egg number. There was highly significant relationship (r_p= 0.92) between RFC and feed consumption in Hy-line strain, however, this correlation was moderate (r_p= 0.30) and not statistically significant in Norfa strain. Feed conversion ratio was highly significantly correlated with RFC in both strains.

Significant positive relationship between yolk percentage and RFC was observed in both strains. Similar result was obtained by Fathi and Galal (2007). They reported that the yolk percentage was significantly positively correlated with RFC in both brown and white egg-type strains. El-Sayed and El-Hakim (1994) reported significant and high positive correlation between RFC and the yolk percentage in full-sib normal and dwarf hens. They indicated that the positive correlation probably reflects the increase in dry matter percentage and energy content in the egg when the proportion of yolk increases. Shell thickness was significantly negatively correlated with RFC in Hy-line strain. Inversely, there was a significant positive relationship between shell thickness and RFC in Norfa strain.

Table 3. Phenotypic correlation coefficients between residual feed consumption (RFC) and both productive and immunity traits

	Strain	
Trait	**Hy-line**	**Norfa**
Egg number (No.)	0.29	0.01
Egg weight (g)	-0.23	-0.05
Feed consumption	0.92***	0.30
Feed conversion ratio	0.77**	0.96***
Albumen, %	0.05	0.08
Yolk, %	0.49*	0.61**
Shell, %	-0.08	-0.29
Shell thickness, mm	-0.39*	0.41*
Breaking strength, kg/cm2	-0.21	-0.08
Wattle swelling at 24h post PHA-P injection	-0.39*	0.15
Wattle swelling at 48h post PHA-P injection	-0.21	-0.54**
Wattle swelling at 72h post PHA-P injection	-0.20	-0.11

* P< 0.05 ** P< 0.01 *** P< 0.001

The wattle swelling measured at 24hrs post PHA-P injection was significantly negatively correlated with RFC in Hy-line (Table 3). However, the RFC was significantly negatively correlated with wattle swelling measured at 48h post PHA-P injection in Norfa. Generally, it could be noticed that the responses to PHA-P injection were negatively correlated with RFC in both strains. Van Eerden *et al.* (2004) reported considerable variation in RFC in a population of chickens from a commercial breed. Specific antibody production against Keyhole Limpet Hemocyanin (KLH), *M. butyricum*, and *S. enteritidis* lipopolysaccharide, however, is not influenced by efficiency in terms of RFC. R+ animals may have a higher level of non-antigen specific antibodies, as indicated by their higher antibody response to *Salmonella* protein.

Phenotypic correlation coefficients between wattle swelling and productive traits are summarized in Table (4). The present results showed that egg weight was significantly positively correlated with swelling measured at 24hrs post PHA-P injection in Hy-line strain. Inversely, the relationships between swelling measured at all times and egg weight were weak and negative in Norfa. Negative relationships between feed consumption and response to PHA-P injection at all times were observed in Hy-line. However, these relationships were weak in Norfa. Feed conversion ratio was significantly negatively correlated with swelling measured at 24hrs post PHA-P injection in Hy-line strain. Similar trend, but at 48hrs post PHA-P injection, was observed between swelling and feed conversion ratio in Norfa.

Table 4. Phenotypic correlation coefficients between wattle swelling and productive traits

Trait	Wattle swelling (mm) 24h post PHA-P injection	48h post PHA-P injection	72h post PHA-P injection	Strain
Egg number (No.)	0.24	-0.07	0.24	Hy-line
	0.05	0.19	0.17	Norfa
Egg weight (g)	0.43*	0.08	0.28	Hy-line
	-0.24	-0.07	-0.20	Norfa
Feed consumption	-0.38*	-0.25	-0.21	Hy-line
	0.02	-0.003	0.10	Norfa
Feed conversion ratio	-0.50**	-0.22	-0.32	Hy-line
	0.11	-0.46**	0.08	Norfa
Albumen, %	0.01	0.38*	0.31	Hy-line
	-0.24	-0.18	-0.11	Norfa
Yolk, %	0.01	-0.29	-0.47**	Hy-line
	0.19	0.20	0.24	Norfa
Shell, %	-0.03	-0.30	-0.01	Hy-line
	0.17	0.07	-0.06	Norfa
Shell thickness, mm	0.51**	0.01	0.39*	Hy-line
	-0.18	-0.29	-0.15	Norfa
Breaking strength, kg/cm^2	0.43**	-0.18	0.18	Hy-line
	-0.13	-0.16	-0.17	Norfa

* P< 0.05   ** P< 0.01

Albumen percentage was significantly positively correlated with swelling at 48hrs post PHA-P injection in Hy-line. Opposite trend was noticed between yolk percentage and swelling measured at 72hrs post PHA-P injection. Significant positive relationships between eggshell thickness and swelling measured at 24 and 72hrs post PHA-P injection were observed in Hy-line strain. Also, swelling measured at 24hrs post PHA-P injection was significantly positively correlated with eggshell breaking strength. However, low and negative relationships was observed in Norfa.

Finally, it could be concluded that the Hy-line strain had better egg production traits and residual feed consumption; however, the Norfa strain had better eggshell quality and higher response to PHA-P injection.

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التباين المظهرى بين سلالتين لإنتاج البيض بالنسبة لكمية العلف المتبقى والإستجابة المناعية الخلوية

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صممت هذه التجربة لتقدير المتبقى من العلف (RFC) والمناعة الخلوية والعلاقة بينهما فى سلالتى الهاى لاين التجارية والنورفا المصرية. أخذ 60 دجاجة بياضة (30 طائر/سلالة) وسكنت فى أقفاص فردية من عمر 46 الى 54 اسبوع. أوضحت النتائج تفوق سلالة الهاى لاين بصورة معنوية فى كتلة البيض المنتجة ومعامل التحويل الغذائى مقارنة بسلالة النورفا، وعلى العكس من ذلك فقد تفوقت سلالة النورفا فى جودة قشرة البيض. سجلت سلالة النورفا إستجابة معنوية أعلى عند الحقن بمادة PHA-P مقارنة بسلالة الهاى لاين. كما أشارت النتائج أن كمية العلف المستهلكة المشاهدة تقترب نوعاً ما من كمية العلف المتوقعة وخصوصا فى سلالة الهاى لاين. وجد أرتباط معنوى سالب بين الاستجابة للحقن بمادة PHA-P بعد 24 ساعة وكمية العلف المتبقى فى سلالة الهاى لاين، بينما شوهد نفس الاتجاة بعد 48 ساعة من الحقن مع كمية العلف المتبقى فى سلالة النورفا. والخلاصة فقد أوضحت التجربة تفوق سلالة الهاى لاين فى مقاييس إنتاج البيض ومعدل التحويل الغذائى بينما تفوقت سلالة النورفا فى مقاييس جودة القشرة والاستجابة المناعية للحقن بمادة PHA-P.