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Immunological Parameters and Laying Performance of Naked Neck and Normally Feathered Genotypes of Chicken under Winter Conditions of Egypt

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Abstract: The study was undertaken to evaluate and measure some immunological traits and laying performance for two genotypes (heterozygous naked neck and normally feathered) of chicken under low ambient temperature. The Nana hens had heavier body weight and slightly higher body temperature as compared to nana one. According to H/L ratio and leucocyte percentages, it could be seen that the nana birds were more stressed than Nana counterparts. The results of PHA-P assay showed that the Nana hens had a significantly greater dermal swelling compared to normally feathered ones. Additionally, the normal plumage hens had a higher mortality and culling rate than heterozygous naked neck hens. Concerning egg production and eggshell quality measurements, the nana hens had a better performance than Nana ones. In naked neck hens, there was a positive relationship among egg mass, egg number and cell mediated response occurs at 48 and 72h post-injection. While, in normally feathered genotype, there was a highly positive correlation between egg weight and cell-mediated response at 72h post-injection.

Key words: Naked neck gene, immunoresponse, laying performance

Introduction

The breeding strategies in developing countries should be focused on the genetic potential of the breeds (Yalcin *et al*., 1997). The improving genetically resistance to disease and therefore immune capacities of animals is more important and desirable, so that it is important to develop genetic tools in this concern. Low ambient temperature is of great concern in all genotypes of chickens bearing genes reducing feather coverage (naked neck, frizzle, slow feathering and scaleless). As known, the major genes (e.g. naked neck and frizzle) are used to improve heat endurance and are often implemented in breeding programs with local chickens to increase poultry production (Garces *et al*., 2001). Worthy mentioning, characterization and evaluation of immune parameters in various genotypes can offer knowledge that can be incorporated into breeding programs for enhancing the natural resistance to disease in tropical and subtropical environment. Alvarez *et al*. (2003) stated that the indigenous naked neck and normally feathered chickens seem to have better immune response than the commercial chicken line. Additionally, results showed that the heterozygous naked neck (Nana) chickens developed the best immune responses against *Salmonella* infection. Host resistance to pathogens is complicated and involved both specific and nonspecific resistance factor. Likewise, the humoral immune is the principle specific immunity against extracellular bacteria, while cell-mediated immunity plays a major role in responses against intracellular bacteria and virus (Li *et al*., 1999).

Many authors have been noted that the naked neck gene had an advantage on laying performance under either hot or moderate ambient temperature (Fathi, 1987; Mérat, 1990; Fathi *et al*., 1994; Galal, 1995; Singh *et al*., 2001). On the other hand, under low ambient temperature, the nana genotype produced higher egg mass and egg number when compared with that of Nana genotype (Galal *et al*., 2000; Galal and Fathi, 2002). Previous work has demonstrated that, the H/L ratio is recognized measure of stress in birds (Maxwell, 1993; Al-Murrani *et al*.,1997, 2002) that has become 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 experiment was conducted to evaluate and measure the laying performance and some immunological traits of two genotypes of chicken. Also, the phenotypic correlations between them were computed.

Materials and Methods

Birds and husbandry: A total of 120 heterozygous naked neck (Nana) and normal plumage (nana) laying hens (60 each) were used in this experiment. All hens were housed for 56 weeks in standard cages, starting at 18 weeks of age. During the laying stage, they were fed a laying diet containing 16% crude protein, 2924 Kcal ME/Kg, 3.4% calcium and 1.01% available phosphorus. Hens were placed on an artificial lighting program of 16L:8D. The mean of high and low ambient temperatures recorded during the trial period (mid laying period, from 36 to 56 weeks of age) inside the house were 19 ±1 and 12 ±1^{0}C, respectively.

Measurements

Body weight and rectal temperature: Body weight (in grams) was recorded for each hen within the two

Table 1: Means ± SE of body weight, rectal temperature and blood constituents for naked neck and normal plumage laying hens

Trait	Nana	nana	Probability
Body weight (g)	1584.3±72.2	1453.1±68.1	NS
Rectal temperature (°C)	41.8±17.7	41.6±17.6	NS
Hematocrite	48.7±1.0	45.9±0.98	NS
Total protein (mg/100ml)	3.62±0.19	3.2±0.29	NS
Albumen (mg/100ml)	2.05±0.16	2.19±0.19	NS
Globulin (mg/100ml)	1.57^{a}±0.20	1.01^{b}±0.16	0.04

^{a} and ^{b} means with no common superscript in each row differ significantly.

genotypes at 34 weeks of age. Also, rectal temperature was measured at the same age by inserting the rode of digital thermometer to approximate 3 cm in cloaca.

***In vivo* cell-mediated immunity assay:** The cutaneous basophil hypersensitivity test, a measure of T-cell mediated response, was quantitated against phytohemagglutinin-P (PHA-P) using a wattle injection technique. Twenty three mature hens aged 34 weeks were used (12 nana and 11 Nana). Each hen was intradermally injected in the left wattle with 100 µg (PHA-P) in 0.1 ml sterile saline. The thickness of the wattle was measured with a constant tension caliper preinjection and at 24, 48 and 72 h postinjection. The wattle swelling was calculated as the difference between the thickness of the wattle before and after injection.

Blood parameters: Using the same birds, a total of 23 females (12 nana and 11 Nana) were assigned to determine some blood traits. A 3.0 ml blood sample was withdrawn from brachial vein and obtained by using heparinized needles and syringes. A portion of the blood was used for hematocrit determination using heparinized capillary tubes and microhematocrit centrifuge. The hematocrit figures were measured after spinning microhematocrit for 12 min. The heparinized blood samples were then centrifuged at 5000 rpm for 10 min. Plasma was collected from each sample and frozen at -20^{0}C till analysis. The frozen plasma was allowed to thaw prior to analysis. Total protein and albumen were determined by enzymatic methods using available commercial kits (Bio Merieux Sa 69280 Marcy l Etoile-France). Globulin level was calculated as the difference between total protein and albumen. One drop of blood from each individual sample was obtained and placed on duplicate glass microscope slides and then smeared with the canted edge of a third slide. After drying, the slides were stained using Wright's stain (Shen and Patterson, 1983). Leucocytes, including granular (heterophils, eosinophils and basophils) and non-granular (lymphocytes and monocytes) were counted at x1000 (oil immersion lens) until a total of 250 cells per slide was achieved. Heterophil/lymphocyte ratio (H/L) were determined by dividing the number of heterophils by that of lymphocytes for each slide.

Mortality and culling rate: Dead and culled hens were recorded daily per genotype during the mid laying period.

Egg production traits: Egg number was recorded over the period of 20 weeks of laying period (from 36 to 56 weeks of age). The eggs were individually weighed to the nearest 0.1g for each genotype throughout the experimental period. Using the previous figures, egg mass was also determined during the same period. Likewise, the percentage of both cracked and double-yolked eggs was also calculated. At 36 weeks of age, an eggshell quality assessment (shell weight, shell% and shell thickness) was done (30 egg from each genotype). The strength of eggshell and eggshell area was determined according to Fathi and El-Sahar (1996).

Statistical analysis: Data were subjected to a one-way analysis of variance with genotype effect using the General Linear Model procedure (Proc GLM) of SAS Institute, 2001. Means were separated for significant by Duncan's multiple range test. Correlation coefficients were computed using the PROC CORR procedure. The statistical model used in this study was as follows;

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

Where;

μ = Overall mean,

G_i= Genotype effect,

e_{ij}= experimental error.

Results and Discussion

Body weight, body temperature and some blood traits for heterozygous naked neck and normally feathered hens are presented in Table 1. It could be stated that there was no significant difference between the two genotypes for body weight trait at 34 weeks of age, but Nana hens were heavier body weight as compared to nana ones. The previous result may be attributed to the rearing period for these hens which were exposed under hot environmental conditions, such kind of circumstances led to a higher performance of Nana hens rather than nana ones. In terms of body temperature, the results showed that the Nana females were slightly higher body temperature compared to nana ones. This observation was coincident with the finding of Pinard-van der Laan (2002), who found a higher body temperature for birds have a good cell-mediated immune response. Moreover, under the environmental temperature of this trial the Nana hens need to be wormed comparatively to nana counterparts. Regarding the blood parameters, it could be noted that the higher hematocrit value sticking with naked neck gene (Na). This result is in agreement with the previous study by Yahav *et al.* (1998) and Galal (1999). They reported a higher hematocrit may have enhanced oxygen delivery to the tissues at the lower temperature. The heterozygous hens had a slightly higher plasma total protein when compared with the

Table 2: Means±SE of H/L ratio and leucocyte percentages in naked neck and normally feathered laying hens

Trait	Nana	nana	Probability
H/L ratio	0.22±0.05	0.35±0.08	NS
Lymphocytes	77.8±3.04	71.2±3.6	NS
Heterophils	13.9^{b}±2.5	22.5^{a}±3.1	0.05
Monocytes	3.8±0.79	3.3±0.65	NS
Eosinophils	1.1±0.19	1.9±0.50	NS
Basophils	1.5±0.31	1.3±0.25	NS

$^{a\ and\ b}$ means with no common superscript in each row differ significantly.

Table 3: Means ± SE of egg mass, egg number and egg weight for naked neck and normal plumage hens during mid-laying period

Trait	Nana N=41	nana N=40	Probability
Egg mass (g)	3065^{b}±64.3	3266.9^{a}±62.2	0.03
Egg number	61.8±1.3	64.6±1.6	NS
Egg weight (g)	49.7±0.35	50.6±0.36	NS
Cracked-eggs (%)	4.6^{a}±0.48	3.2^{b}±0.34	0.01
Double-yolked eggs (%)	0.33^{b}±0.14	1.2^{a}±0.42	0.05

$^{a\ and\ b}$ means with no common superscript in each row differ significantly.

Table 4: Means± SE of eggshell measurements for naked neck and normally feathered genotypes

Trait	Nana	nana	Probability
Egg weight (g)	53.0±0.82	55.3±0.95	NS
Shell weight (g)	4.6^{b}±0.09	5.1^{a}±0.12	0.005
Shell (%)	8.7±0.18	9.2±0.20	NS
Shell thickness (mm)	0.325^{b}±0.005	0.343^{a}±0.006	0.01
Breaking strength (Kg/cm^{2})	4.37±0.35	4.62±0.36	NS
Eggshell area (cm^{2})	65.5^{b}±1.19	67.5^{a}±0.80	0.05

$^{a\ and\ b}$ means with no common superscript in each row differ significantly.

normal plumage ones. This result might be attributed to the acute phase of an immune response (hyper active of immunity system) , where the liver cells produce and secrete Acute Phase Protein (APP), which gives protection to birds against infection or any invasion. The opposite trend was observed in plasma albumen figure, but the difference was not significant. With respect to plasma globulin, a significant difference was observed between two genotypes, the Nana females had a higher globulin level compared with the nana ones. These results sustained the important role of globulin in the terms of immunity.

Summarized results in Table 2 revealed that the means of H/L ratio and leucocyte percentages for naked neck and normal plumage hens. There was no significant difference between the two genotypes for H/L ratio, but increased H/L ratio was associated with the normally feathered genotype. In this context, there was a significant difference between genotypes for heterophil %, where the nana hens had a higher percentage as compared to Nana ones. Concerning other leucocytes, the results did not show significant difference among them in the two genotypes. According to previous results, it could be stated that the nana birds were more stressful than Nana counterparts, consequently the heterozygous naked neck hens had a good state of health comparatively to nana ones. Lane (1987) sustained the previous observation. Who found that the avian heterophil leucocytes are an indicator of state of health and respond to problems associated with diet, chronic bacterial infections, stress, light and trauma.

The results of cutaneous basophilic hypersensitivity (CBH) response are shown in Fig. 1. The responses were measured at 24, 48 and 72h post PHA-P injection into the wattle. The Nana hens had a significantly greater dermal swelling response to PHA-P injection compared to nana ones at both 24 and 48 h post-injection. The same trend was observed at 72h after injection, but the difference was not significant. In congruent with this finding, Alvarez *et al.* (2002) and Patra *et al.* (2004). They stated that the heterozygot genotype (Nana) had the best cellular and humoral response as compared to the normally feathered genotype (nana).

Fig. 2 gives mortality and culling percentages of normally feathered and naked neck genotypes during the mid laying period. The normal plumage hens had a higher mortality and culling rate than heterozygous naked neck hens. This result is logic according to the previous results of immunity.

Egg production measurements for Nana and nana genotypes are shown in Table 3. Total egg mass laid during the experimental period (mid laying period) differed significantly between genotypes. However, the nana hens produced heavier egg mass when compared with those of Nana ones. The same trend was realized for both egg number and egg weight traits, but the differences were not significant. The results are in agreement with the finding of (Galal *et al.*, 2000; Galal and Fathi, 2002). They reported that under low ambient temperature, the nana genotype produced higher egg mass and egg number when compared with that of Nana genotype. Eggs produced from naked neck hens appear to crack more readily rather than those produced from normally feathered counterparts. Also, double-yolked eggs production was higher in nana hens as compared to Nana ones. These results may be due to the atmosphere of the experimental period, which led to deteriorate in productive performance of naked neck birds.

Regarding eggshell measurements Table 4, it could be noticed that the nana genotype had a significantly heavier shell weight than that of Nana one. The same trend was observed for shell%. As known, eggshell thickness was a considered function to fracture strength within laying strains. Results elucidates that the nana genotype was higher shell thickness than Nana one and the difference was highly significant, Abdel-Rahman (1990) sustained the previous results. With respect to the terms of breaking strength, the normal plumage genotype had a slightly stronger eggshell than that of Nana genotype. Furthermore, eggs produced from nana hens had higher eggshell area comparatively to Nana ones.

Table 5: Phenotypic correlation coefficients between egg production traits and PHA-P mediated swelling response for normally and naked neck hens

Trait	A	A1	A2	A3	A4	A5	A6	A7	A8	A9	A10	G.
EM (A)	--	0.92***	0.32*	0.25	0.12	0.14	-0.13	-0.12	-0.27	-0.25	0.35	na
	--	0.95***	0.17	0.17	0.03	0.04	0.24	-0.14	-0.01	0.44	0.36	Na
EN (A1)		--	-0.07	0.21	0.12	0.17	-0.18	0.008	-0.28	-0.26	0.01	na
		--	-0.27	-0.02	0.01	0.01	0.25	-0.13	-0.10	0.40	0.53	Na
EW (A2)			--	0.14	0.05	-0.02	0.09	-0.33	-0.03	-0.05	0.76***	na
			--	0.27	0.09	0.09	-0.06	-0.003	0.23	0.02	-0.51	Na
SW (A3)				--	0.70***	0.85***	-0.03	0.06	0.17	-0.03	0.37	na
				--	0.77***	0.84***	0.35	-0.04	-0.61	-0.22	-0.16	Na
S% (A4)					--	0.86***	0.21	0.12	-0.06	-0.18	0.16	na
					--	0.89***	0.42*	-0.07	-0.44	-0.27	0.002	Na
ST (A5)						--	0.05	0.15	0.26	0.14	0.23	na
						--	0.49**	0.11	-0.60*	-0.20	0.08	Na
BS (A6)							--	-0.34	-0.36	-0.25	-0.21	na
							--	0.26	-0.49	-0.25	0.19	Na
EA (A7)								--	0.34	0.47	-0.40	na
								--	-0.32	-0.60*	-0.07	Na
24h (A8)									--	0.69**	0.19	na
									--	0.53	0.19	Na
48h (A9)										--	0.16	na
										--	0.67*	Na
72h (A10)											--	na
											--	Na

*P< 0.05 **P< 0.01 ***P< 0.001 EM: Egg mass EN: Egg number EW: Egg weight SW: Shell weight S%: Shell % ST: Shell thickness BS: Breaking strength EA: Eggshell area 24h: At 24 48h: At 48 72h:At 72. G: Genotype

Table 6: Phenotypic correlation coefficients among body temperature, body weight, some hematological parameters and PHA-P mediated swelling response for normally and naked neck hens

Trait	B	B1	B2	B3	B4	B5	B6	B7	B8	B9	G.
RT (B)	--	-0.26	0.36	-0.22	-0.13	-0.23	-0.37	-0.10	-0.17	0.36	na
	--	0.34	0.45	-0.06	0.20	-0.22	-0.21	0.06	-0.11	0.29	Na
BW (B1)		--	-0.45	-0.11	0.26	-0.49	-0.27	0.39	0.20	-0.17	na
		--	0.34	-0.14	0.38	-0.45	-0.08	-0.01	-0.44	-0.14	Na
Ht (B2)			--	0.11	-0.003	0.21	-0.01	0.03	-0.09	0.62*	na
			--	-0.29	0.24	-0.52	0.51	-0.37	-0.11	0.11	Na
TP (B3)				--	0.84***	0.77***	0.26	0.61*	0.27	0.18	na
				--	0.38	0.69**	-0.29	-0.15	-0.20	0.14	Na
AL (B4)					--	0.31	-0.01	0.77***	0.47	0.19	na
					--	-0.40	-0.28	-0.22	-0.35	0.22	Na
GL (B5)						--	0.47	0.17	-0.08	0.09	na
						--	-0.07	0.02	0.07	-0.05	Na
H/L (B6)							--	0.21	0.34	-0.18	na
							--	-0.03	0.74**	0.51	Na
24h (B7)								--	0.69**	0.19	na
								--	0.53	0.19	Na
48h(B8)									--	0.16	na
									--	0.67*	Na
72h(B9)										--	na
										--	Na

*P< 0.05 **P< 0.01 ***P< 0.001 RT: Rectal temperature BW: Body weight Ht: Hematocrit TP: Total protein AL: Albumen GL: Globulin H/L: H/L ratio 24h: At 24 48h: At 48 72h:At 72. G: Genotype

The possible phenotypic correlation coefficients between egg production traits and cellular response to phytohemagglutinin (PHA-P) injection assay in normally and naked neck laying hens are presented in Table 5. Correlations were stronger between egg mass and egg number in two genotypes, also there was a highly positive correlations among shell weight, shell% and shell thickness in the two genotypes. Whereas, in naked neck hens, there was a significant positive correlation among shell%, shell thickness and breaking strength. With respect to the relationship between cell-mediated response and productive traits, it could be noted that, there was a positive correlation among egg mass, egg number and swelling occurs at 48 and 72 hrs post-injection in naked neck laying hens. Also, there was a highly negative correlation among shell thickness, egg area and the dermal swelling after 48h post-injection. Positive relationship was existed between dermal

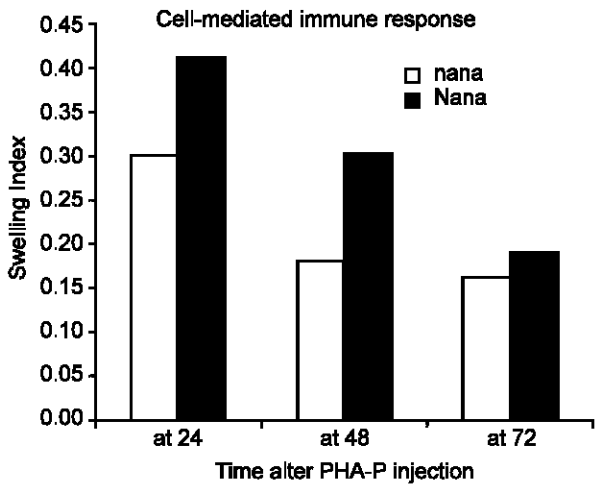

Fig. 1: PHA-P cell-mediated swelling index of the wattle for normal and naked neck genotypes

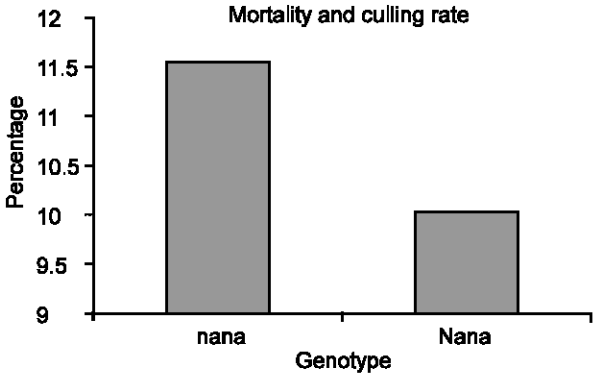

Fig. 2: Mortality and culling rate of normally feathered and naked neck genotypes

swelling occurs at 48h and at 72h after injection. On the other hand, in normally feathered genotype, there was a highly positive correlation between egg weight and cell-mediated response at 72h post-injection. This result is in concordance with the observations by Chao and Lee (2001). They found a positive correlation between egg weight and immune response to PHA-P in Taiwan country chickens (nana). The same trend was existed among shell thickness, egg area and immune response. Likewise, there was a significantly positive correlation between the dermal swelling response to PHA-P injection measured at 24h and the swelling after 48h.

The phenotypic correlations among body temperature, body weight, some hematological parameters and immunological traits are presented in Table 6. In both genotypes, there was a positive correlation between body temperature and dermal swelling response to PHA-P at 72h after injection. In normal plumage hens, the positive relationship was observed between body weight and cellular immune response at 24 and 48h post-injection. The previous result is fully in agreement with the results of Pinard-van der Laan (2002), who reported that the cellular response was positively correlated with body weight. The same trend was realized between hematocrit and swelling response at 72h post injection. Likewise, there was a highly positive correlation among total protein, globulin and dermal swelling at 24h post-injection. As expected, in naked neck hens there was a highly positive correlation between H/L ratio and swelling response to PHA-P, especially at 48h after injection. The relationships among total protein, albumen and globulin were high and positive in the two genotypes.

In conclusion, under low ambient temperature, the results suggest that the naked neck laying hens showed superior immunoresponsivness and less productive performance than the normally feathered hens.

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