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Laying performance, immune response and antioxidant properties of hens segregating for naked neck and frizzle genes under low ambient temperature

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Abstract: Major genes could be introgressed into laying hens to attenuate heat stress. However, under cold and/or moderate ambient temperature, these genes might possess different behaviour. The main objective of this study was to evaluate laying performance, immune response, and antioxidant status of native laying hens segregating for naked neck (*Na*) and frizzle (*F*) genes under low ambient temperature. Five genotypes were studied: homozygous naked neck (*NaNaff*), heterozygous naked neck (*Nanaff*), homozygous frizzle (*nanaFF*), heterozygous frizzle (*nanaFf*), and normally feathered (*nanaff*). The hens were raised under temperature range 22.2–16.7°C. No adverse effect due to ambient temperature was detected in laying performance for naked neck genotypes. Significant decrease in egg weight was recorded in *nanaFF* genotype compared to the other genotypes leading to significant decrease in egg mass. Significant improvement in shell thickness was associated with *Na* and *F* genes. *NaNaff* genotype had a significantly higher eggshell strength compared to *nanaff*. Furthermore, *Na* and *F* genes improved cellular mediated immune responsiveness, whereas this improvement did not extend to humoral immunity. Birds carrying *F* gene in homozygous state had a higher total antioxidant activity compared to the remaining genotypes. It could be concluded that the presence of *Na* and *F* genes in laying hens raised under low ambient temperature significantly increased shell thickness and, in turn, improved shell strength. Moreover, they greatly enhanced cellular immunity, particularly in heterozygous naked neck status.

Keywords: laying hens; naked neck gene; frizzle gene; immunocompetence; low temperature

The associated deleterious effects of birds raised under high ambient temperature may result in huge economic losses for poultry projects. On the other hand, productive performance of chicken goes down at low ambient temperature (El-Safty et al. 2006). The main problem related to low environmental temperatures when poultry are maintained in open-sided houses is increasing feed intake as a

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natural response to compensate the energy released to maintain the essential body functions (Alves et al. 2012). The thermoregulatory effects of naked neck (*Na*) gene were intensively investigated in egg type chickens (Bordas et al. 1992; Chen et al. 2004; Fathi et al. 2013; Zerjal et al. 2013) and in broiler chickens (Cahaner et al. 2008). There is no doubt that the birds carrying reducing plumage genes (i.e. *Na* and *F* (frizzle) genes) have better productive performance under high ambient temperature, particularly those bearing *Na* gene. It is well known that the naked neck and frizzled birds are more tolerant to heat stress compared to their normally feathered siblings because of reduced feather mass leading to better heat dissipation (Patra et al. 2002; Rajkumar et al. 2011; Fathi et al. 2013). These genes are often referred to as tropical relevant major genes (Fathi et al. 2013; Nwachukwu and Ogbu 2015).

Conversely, under low ambient temperature, the performance of chickens barring *Na* or *F* genes was dramatically affected. At moderate temperature equal to or less than 20°C, the *Na* gene deteriorates feed efficiency for growing chicks (Monnet et al. 1979) and laying hens (El-Safty et al. 2006). Under ambient temperature (19°C), there were no differences in laying performance and fertility between both *NaNaff* and *Nanaff* genotypes (Sharifi et al. 2010). The dressing percentage was significantly higher in naked neck birds in both winter and summer season because of reduced plumage (Rajkumar et al. 2011).

It could be hypothesized that under low and/or moderate ambient temperatures, major genes might offer other properties and benefits to laying hens. In this context, a dropped performance in chickens carrying *Na* and/or *F* gene raised under low or moderate temperature during fall and winter seasons will be expected. Therefore, the performance of naked neck and frizzle chickens was evaluated with respect to egg production, egg quality, antioxidant properties and immune response under low or moderate environmental temperature.

MATERIAL AND METHODS

Experimental design, genotype and management. Five genotypes were used in the current experiment including homozygous naked neck (*NaNaff*), heterozygous naked neck (*Nanaff*), homozygous frizzle (*nanaFF*), heterozygous frizzle (*nanaFf*), and normally feathered (*nanaff*). A total of 90 laying hens aged 36 weeks were assigned representing five genotypes (*NaNaff n* = 20, *Nanaff n* = 20, *nanaFF n* = 10, *nanaFf n* = 20 and *nanaff n* = 20). The hens originated from the same parental population segregating for both naked neck and frizzle mutations. They were individually raised in wire cages in semi-closed house for an eight-week experimental period during the laying cycle. The average temperatures during the whole experimental period were between 22.2 ± 0.3°C and 16.7 ± 0.2°C. The hens were placed in wire cages (45 × 45 × 43 cm, L × W × H) under lighting schedule of 17 h/day light cycle. The birds were fed a standard layer diet (17% crude protein; 2850 kcal metabolizable energy per kg diet). Feed and water were provided *ad libitum* throughout the experimental period. All hens were kept under similar environmental, managerial and hygienic conditions. The care and handling of the birds were in accordance with regulations of animal care committee of Qassim University.

Laying performance. Body weight of laying hens was recorded at the beginning of the experiment. Egg production was recorded daily for each laying hen over the whole eight-week experimental period. The laying rate was calculated from the number of eggs laid divided by the number of days of the experimental period. All eggs (intact or damaged) were individually collected and weighed. Egg mass was calculated for the whole experimental period. Feed intake was individually recorded for the whole experimental period. Feed conversion ratio (FCR) was calculated at the end of the experiment based on the amount of feed consumed (in g) divided by egg mass (in g). Damaged eggs including broken, cracked and shell-less eggs were visually recorded as they occurred. Rectal temperature was measured at 10.00 h in the morning by inserting a digital thermometer probe approximately 3 cm into the cloaca and kept there until the reading was stable.

Egg quality traits. Starting from 40 weeks of age and onward, 120 intact eggs/genotype were collected to assess internal and external egg quality characteristics. Egg width and egg length were measured in mm using electronic digital Vernier caliper (± 0.01 mm). Egg shape was calculated according to the formula: Egg shape = (egg width/egg length) × 100.

Following collection, the breaking strength for intact eggs was determined in kg/cm^2 using an Egg Force Reader™ (Orka Food Technology Ltd, USA). Also, egg weight, Haugh unit and yolk colour were

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measured automatically using Egg Analyzer™ (Orka Food Technology Ltd). The liquid contents were put aside and the shell plus membranes were washed under running water to remove adhering albumen. The wet eggshell was left for 24 h for drying and then weighed to the nearest 0.01 g (Fathi et al. 2018). The relative weight of dry eggshell was calculated based on egg weight. To measure shell thickness, pieces from three different regions (two poles and equator) of each eggshell with intact membranes were measured with a dial gauge micrometer (Ames, USA) to the nearest 0.01 mm.

Immune response

Assay for cell-mediated immunity. The *in vivo* response induced by injecting a mitogen was evaluated by injecting PHA-P into the left wattle. At 42 weeks of age, 10 birds from each genotype were randomly chosen and injected intradermally with 100 µg phytohaemagglutinin-P (PHA-P) (Sigma Chemical Co., USA) in 0.1 ml of sterile saline. Upon injection, the site of needle was marked with permanent black ink to facilitate further measuring. The swelling resulting from accumulation of lymphocytes which stand for cell-mediated immune response in the wattle site was measured with a constant tension dial micrometer (Ames) before injection and at 24, 48 and 72 h after PHA-P injection. The wattle swelling was expressed as the difference between the thickness of the wattle before and after injection.

Humoral immunity. Detection of antibodies against *Newcastle disease virus* (*NDV*) in serum of immunized chickens was performed by ELISA using ND antibody commercial test kit (BioChek, the Netherlands). The assay was carried out as described by the manufacturer's instructions. Briefly, chicken serum samples were diluted to 1 : 500 and added to the microtiter wells and incubated at room temperature for 30 min. Upon aspiration and washing, anti-chicken IgG labelled with enzyme alkaline phosphatase was then added to the wells and incubated at room temperature for additional 30 min. After another wash to remove unreacted conjugate, substrate was added to the appropriate wells in the form of pNPP (p-nitrophenyle phosphate) chromogen and incubated at room temperature for 15 min. Stop solution was added to stop the reaction. Finally, the absorbance of samples was recorded by microtiter plate reader at 405 nm.

Blood biochemical and hematological parameters. At the end of the experimental period, 10 blood samples from each genotype were withdrawn for blood biochemical analysis. Plasma total protein, albumen, total cholesterol, and triglyceride were spectrophotometerically determined using commercial kits (Stanbio Laboratory, USA). The globulin was calculated as the difference between the total protein and albumen. Serum triiodothyronine (T_3) and thyroxine (T_4) concentrations were measured using commercial ELISA kits (BioCheck®, USA). The hematological parameters were assessed in the same blood samples by using an Automatic Fully Digital Hematology Analyzer BC-3000 Plus (Mindray, China).

Antioxidative properties. Total antioxidant capacity (TAC, mmol/l) was determined using a commercial kit (Biodiagnostic for diagnostic and research reagents, Egypt; www.bio-diagnostic.com). This method exploits the ability of antioxidants to reduce hydrogen peroxide (H_2O_2). Determination was performed by the reaction of antioxidants in the sample with a defined amount of exogenously provided H_2O_2. The antioxidants in the sample eliminate a certain amount of the provided H_2O_2. The residual of H_2O_2 is quantified colorimetrically by an enzymatic reaction which evolves the conversion of 3,5-dichloro-2-hydroxybenzenesulphonate to a coloured product. Glutathione peroxidase (GSH-Px) was determined in erythrocytes. The red blood cells were collected from blood samples and washed with saline solution 3 times. Cold deionised water (4°C) was added to lyse the cells. The resulting clarified supernatant was used in GSH-Px assay (Biodiagnostic for diagnostic and research reagents). Enzyme activity (reduction of organic peroxide) was spectrophotometrically monitored by decreasing absorbance at 340 nm.

Statistical analysis. Data were subjected to one-way ANOVA using JMP of SAS (Version 11, 2013) with genotype as a fixed effect. The results are presented as the mean and the pooled standard error of the mean. The significance of difference among the genotypes was assessed using Duncan's new multiple range test. Significance was set as $P < 0.05$.

RESULTS

Productive performance. Laying performance of five different chicken genotypes is presented in Table 1. No adverse effect due to raising hens under low/moderate temperature was detected in body weight among genotypes. However, a numerical decrease was found in *nanaFF* genotype compared

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Table 1. Laying performance as affected by *Na* and *F* genes of laying hens under low ambient temperature

Trait	Genotype					Deviation[1] (%)				SEM	Probability
	NaNaff	*Nanaff*	*nanaFF*	*nanaFf*	*nanaff*	*NaNa*	*Nana*	*FF*	*Ff*		
Body weight (g)	1048.7	1076.0	953.4	1034.4	1039.1	+0.9	+3.6	−8.3	−0.5	12.3	ns
Egg weight (g)	45.0[a]	45.5[a]	41.3[b]	45.0[a]	44.5[a]	+1.1	+2.3	−7.2	+1.1	0.35	0.02
Laying rate (%)	56.4	62.5	55.6	53.6	61.7	−8.6	+1.3	−9.9	−13.1	1.84	ns
Egg mass (g)	1429.5[b]	1580.2[a]	1281.2[c]	1348.8[c]	1534.8[a]	−6.9	+3.0	−16.5	−12.1	46.7	0.05
FCR	3.6	3.5	3.2	3.5	4.1	−12.2	−14.6	−22.0	−14.6	0.19	ns
Damaged eggs (%)	0.65	0.74	1.56	1.13	2.67	−75.7	−72.3	−41.6	−57.7	0.33	ns
Rectal temperature (°C)	41.3	41.5	41.4	41.2	41.4	−0.2	+0.2	0.0	−0.5	0.04	ns

FCR = feed conversion ratio, SEM = standard error of the means, ns = non-significant, *NaNaff* = homozygous naked neck, *Nanaff* = heterozygous naked neck, *nanaFF* = homozygous frizzle, *nanaFf* = heterozygous frizzle, *nanaff* = normally feathered

[1]deviation from *nanaff* genotype (gene effect) = ((*Na*/–) or (*F*/–) – *nanaff*))/*nanaff* × 100

[a–c]values with different superscripts within the same row are statistically different

to *nanaff* one (deviation= −8.3%). Homozygous frizzled hens produced significantly lighter eggs ($P < 0.02$) compared to the other groups (deviation = −7.2% compared with *nanaff*). Although, there was no significant difference among genotypes for laying rate percentage, both frizzled hens (*nanaFF* and *nanaFf*) produced significantly lower egg mass ($P < 0.05$) compared to normal plumage sisters. Numerical reduction in the percentage of damaged eggs was observed in naked neck and frizzled genotypes compared to normally feathered genotype. This reduction as expressed in gene effect ranged from −41.6 to −75.7% as a deviation from *nanaff*. Also, the *Na* gene reduced damaged eggs more than the *F* gene. Under low ambient temperature of the current experiment, rectal temperature did not significantly differ among genotypes.

Egg quality. The results of egg quality characteristics are presented in Table 2. It could be noticed that the egg shape index was similar in all genotypes. Haugh unit significantly decreased ($P < 0.05$) by about −5.5% in *nanaFF* laying hens compared to *nanaff* counterparts. The value for yolk colour was significantly higher ($P < 0.02$) in *nanaFf* genotype compared to *nanaff* one. A significant superiority of yolk colour in *nanaFf* genotype (+17.7%) if compared to *nanaff* genotype was found. Whereas *Na* gene (both in homozygous or heterozygous state) did not significantly affect yolk colour compared with normally feathered. In terms of eggshell quality, it could be noticed that the shell percentage did not differ among genotypes. Concerning shell thickness, the naked neck and frizzle genotypes (in both states) had a significantly increased ($P < 0.01$) thickness compared to a normal plumage genotype.

Table 2. Egg quality traits as affected by *Na* and *F* genes of laying hens under low ambient temperature

Trait	Genotype					Deviation[1] (%)				SEM	Probability
	NaNaff	*Nanaff*	*nanaFF*	*nanaFf*	*nanaff*	*NaNa*	*Nana*	*FF*	*Ff*		
Egg shape index	76.7	75.7	76.1	75.8	76.5	+0.3	−1.1	−0.5	−0.9	0.25	ns
Haugh unit	63.4[a]	61.6[ab]	57.0[b]	61.6[ab]	60.3[ab]	+5.1	+2.2	−5.5	+2.2	0.61	0.05
Yolk colour	3.29[ab]	3.25[ab]	3.54[ab]	3.73[a]	3.17[b]	+3.8	+2.5	+11.7	+17.7	0.05	0.02
Shell (%)	10.0	9.99	9.88	10.1	9.65	+3.6	+3.5	+2.4	+4.7	0.07	ns
Shell thickness (μm)	396.5[a]	396.1[a]	392.9[a]	393.8[a]	378.9[b]	+4.7	+4.5	+3.7	+3.9	1.25	< 0.01
Breaking force (kg/cm^2)	4.29[a]	4.03[ab]	3.77[b]	3.96[ab]	3.63[b]	+18.2	+11.0	+3.9	+9.1	0.06	< 0.01

SEM = standard error of the means, ns = non-significant, *NaNaff* = homozygous naked neck , *Nanaff* = heterozygous naked neck, *nanaFF* = homozygous frizzle, *nanaFf* = heterozygous frizzle, *nanaff* = normally feathered

[1]deviation from *nanaff* genotype (gene effect) = ((*Na*/–) or (*F*/–) – *nanaff*))/*nanaff* × 100

[a,b]values with different superscripts within the same row are statistically different

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Table 3. Cell mediated response for PHA-P injection and humoral immunity in different genotypes of laying hens under low ambient temperature

Trait		Genotype					Deviation[1] (%)				SEM	Probability
		NaNa	*Nana*	*FF*	*Ff*	*nanaff*	*NaNa*	*Nana*	*FF*	*Ff*		
Post injection	24 h	0.63[c]	0.78[a]	0.77[a]	0.70[b]	0.58[c]	+8.6	+34.5	+32.8	+20.7	0.03	0.05
	48 h	0.42[b]	0.63[a]	0.56[ab]	0.50[ab]	0.40[b]	+5.0	+57.5	+40.0	+25.0	0.03	0.05
	72 h	0.19[b]	0.41[a]	0.30[ab]	0.30[ab]	0.13[b]	+46.2	+215.4	+130.8	+130.8	0.02	< 0.01
NDV titre		6013.8	5844.2	6081.8	5935.3	6023.7	−0.16	−2.98	+0.96	−1.47	65.48	ns

NDV = *Newcastle disease virus*, SEM = standard error of the means, ns = non-significant, *NaNaff* = homozygous naked neck, *Nanaff* = heterozygous naked neck, *nanaFF* = homozygous frizzle, *nanaFf* = heterozygous frizzle, *nanaff* = normally feathered

[1]deviation from *nanaff* genotype (gene effect) = ((*Na*/–) or (*F*/–) – *nanaff*))/*nanaff* × 100

[a–c]values with different superscripts within the same row are statistically different

Cell-mediated immunity. As shown in Table 3, heterozygous naked neck and homozygous frizzled hens had significantly greater dermal swelling response to PHA-P compared to the remaining genotypes at 24 h post injection. This improvement was +34.5 and +32.8%, respectively, as a deviation from normal plumage. At the later times, the heterozygous state (*Nanaff*) still gained the best cell mediated immunity compared with normally feathered one.

Humoral immune response. The antibody titres against *NDV* in hens of different genotypes are presented in Table 3. There was no significant difference detected among genotypes. However, numerical decrease was noticed in heterozygous genotypes (*Nanaff* and *nanaFf*) compared to normally the feathered one (*nanaff*).

Blood biochemical and hematological parameters. Table 4 shows that both genotypes of naked neck had a significantly lowered ($P < 0.01$) total protein level compared to the rest of genotypes. The albumin level was not affected in all genotypes. A reduction in globulin was detected in all genotypes compared to *nanaff* but and this difference was significantly lower in heterozygous naked neck genotype. There were no significant

Table 4. Blood biochemistry and hematological parameters of naked neck and frizzled laying hens under low ambient temperature

Trait	Genotype					Deviation[1] (%)				SEM	Probability
	NaNaff	*Nanaff*	*nanaFF*	*nanaFf*	*nanaff*	*NaNa*	*Nana*	*FF*	*Ff*		
Total protein (g/dl)	6.37[b]	6.33[b]	7.51[a]	7.32[a]	7.27[a]	−12.4	−12.9	+3.3	+0.7	0.12	< 0.01
Albumin (g/dl)	3.21	3.45	3.71	3.39	3.26	−1.5	+5.8	+13.8	+4.0	0.07	ns
Globulin (g/dl)	3.16[ab]	2.88[b]	3.80[ab]	3.94[a]	4.01[a]	−21.2	−28.2	−5.2	−1.8	0.12	< 0.01
Cholesterol (mg/dl)	143.9	131.8	170.1	141.7	152.3	−5.5	−13.5	+11.7	−7.0	3.99	ns
Triglycerides (mg/dl)	1436.3	1233.6	1224.5	1221.8	1084.9	+32.4	+13.7	+12.9	+12.6	56.56	ns
T3 (ng/ml)	2.17	2.37	1.63	1.80	1.76	+23.3	+34.7	−7.4	+2.3	0.13	ns
T4, (µg/ml)	4.98	5.02	5.47	5.97	6.16	−19.2	−18.5	−11.2	−3.1	0.29	ns
RBCs (10^6)	2.6	2.4	2.3	2.4	2.4	+8.3	0.0	−4.2	0.0	0.05	ns
Hemoglobin	15.5[a]	14.5[ab]	13.5[b]	14.4[ab]	13.6[b]	+14.0	+6.6	−0.7	+5.9	0.23	0.05
Hematocrit	33.1	30.8	28.6	31.0	29.7	+11.5	+3.7	−3.7	+4.4	0.50	ns
Thrombocytes	7.6[b]	6.92[b]	13.3[a]	5.75[b]	7.1[b]	+7.0	−2.5	+87.3	−19.0	0.67	0.04

RBCs = red blood cells, SEM = standard error of the means, ns = non-significant, *NaNaff* = homozygous naked neck, *Nanaff* = heterozygous naked neck, *nanaFF* = homozygous frizzle, *nanaFf* = heterozygous frizzle, *nanaff* = normally feathered

[1]deviation from *nanaff* genotype (gene effect) = ((*Na*/–) or (*F*/–) – *nanaff*))/*nanaff* × 100

[a,b]values with different superscripts within the same row are statistically different

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Table 5. Antioxidant status as affected by *Na* and *F* genes of laying hens under low ambient temperature

Trait	Genotype					Deviation[1] (%)				SEM	Probability
	NaNaff	*Nanaff*	*nanaFF*	*nanaFf*	*nanaff*	*NaNa*	*Nana*	*FF*	*Ff*		
TAC (mM/l)	2.46	2.16	2.95	2.27	2.61	–5.8	–17.2	+13.0	–13.0	0.11	ns
GSH-Px (U/ml)	1675.5[a]	1681.7[a]	1625.9[b]	1649.5[ab]	1649.2[ab]	+1.6	+2.0	–1.4	0.0	6.12	0.02

TAC = total antioxidant capacity, GSH-Px = glutathione peroxidase, SEM = standard error of the means, ns = non-significant, *NaNaff* = homozygous naked neck , *Nanaff* = heterozygous naked neck, *nanaFF* = homozygous frizzle, *nanaFf* = heterozygous frizzle, *nanaff* = normally feathered

[1]deviation from *nanaff* genotype (gene effect) = ((*Na*/–) or (*F*/–) – *nanaff*))/*nanaff* × 100

[a,b]values with different superscripts within the same row are statistically different

differences among genotypes for the concentration of cholesterol, triglycerides and thyroid hormones.

Respecting to blood hematology, *Na* gene increased hemoglobin level compared to normal genotype. This increase was significant in *NaNaff* genotype. Thrombocytes significantly increased ($P < 0.04$) in *nanaFF* genotype (+87.3% deviation from *nanaff*) compared to the remaining genotypes.

Antioxidative properties. As shown in Table 5, there was no significant difference among genotypes in TAC. However, a slight decrease of TAC in both naked neck genotypes was observed compared to the normal plumage one and this may be due to increasing the effect of lower temperature on the wide featherless area in such genotypes. The GSH-Px level was significantly increased ($P < 0.02$) in naked neck genotypes (in both states) compared to the other genetic groups.

DISCUSSION

Productive performance. As shown in Table 1, no adverse effect due to low ambient temperature was detected in body weight among genotypes. However, a numerical decrease was found in *nanaFF* genotype compared to *nanaff* one (deviation –8.3%). Conversely, Almeida and Zuber (2010) reported that the cold stress condition reduced body weight in naked neck chicken if compared to their normally feathered sibs. Homozygous frizzled hens produced significantly lighter eggs ($P < 0.02$) compared to the other groups (deviation –7.2% compared with *nanaff*). This might be acceptable because the *nanaFF* genotype had lighter body weight. Although, there was no significant difference among genotype for laying rate percentage, both frizzled hens (*nanaFF* and *nanaFf*) produced significantly lower egg mass ($P < 0.05$) compared to normal plumage sisters. Contrary to our findings, Galal and Fathi (2002) reported that the *nanaff* genotype produced higher egg mass than the *Nanaff* genotype at low ambient temperatures. Generally, several authors have reported that the naked neck gene had an advantage in laying performance under either hot or moderate ambient temperatures (Galal 1995; Singh et al. 2001). Under tropical conditions, the frizzled pullets (*nanaFf*) were superior to their normal counterparts (*nanaff*) in terms of egg mass and rate of lay (Adomako et al. 2014). Feed conversion ratio was not affected by genotype, while under high environmental temperature, Mahrous and El-Dlebshany (2011) found an improvement in feed conversion sticking with *Na* gene in a single manner or in combination with *F* gene.

Under low ambient temperature of the current experiment, rectal temperature did not significantly differ among genotypes (Table 1). Similar results were reported by Deeb and Cahaner (1999) who stated that there was no difference in body temperature between the different naked neck genotypes at moderate temperature (24°C). Conversely in hot climate, the naked neck broilers had significantly lower body temperature and better heat dissipation capabilities as compared to normal broilers (Patra et al. 2002; Fathi et al. 2013), while El-Safty et al. (2006) stated that the *Nanaff* laying hens had a slightly higher body temperature compared to *nanaff* counterparts under low environmental temperature.

Egg quality. Haugh units significantly decreased ($P < 0.05$) by about –5.5% in *nanaFF* laying hens compared to *nanaff* counterparts (Table 2). Contrary to our findings, an advantage in Haugh unit was observed in the homozygous and heterozy-

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gous frizzles compared to the normal feathered ones kept at temperature 21.6–25.9°C (Adomako et al. 2014). Eggshell quality differs according to genotype, lines, and families of the laying hens. Therefore, it is important to select an appropriate genotype and/or to improve eggshell quality through genetic selection (Ledvinka et al. 2011; Tumova et al. 2011; Ketta and Tumova 2016). Regarding to eggshell quality, it could be noticed that the shell percentage did not differ among genotypes (Table 2). Concerning shell thickness, the naked neck and frizzle genotypes (in homozygous or heterozygous state) had a significantly increased ($P < 0.01$) thickness compared to the normal plumage genotype. The advantage of these genes on shell thickness is consistent with findings of Chen et al. (2002) who reported that the *Na* gene had a positive effect on shell thickness trait in the two genotypes (*NaNaff* and *Nanaff*) at low ambient temperature (22°C). El-Safty et al. (2006) concluded that laying hens carrying *Na* gene had a superior shell thickness and strength compared with normally feathered hens. In contrast, Abdel-Rahman (1990) stated that normally feathered laying hens produced significantly thicker eggshells compared to heterozygous birds (*Nanaff*) raised under low ambient temperatures. The superiority in shell thickness of the frizzled hens in our study is contrary to the findings of Missohou et al. (2003) who studied the performance of fizzled and dwarf laying hens under Senegalese conditions and found that the *F* gene did not significantly influence egg quality. Referring to eggshell strength, we found that both genes had a favourable effect on breaking force and this was more pronounced in *Na* gene compared to *F* gene. The deviation from normal plumage was +18, +11, +4 and +9 for *NaNaff*, *Nanaff*, *nanaFF*, and *nanaFf*, respectively. This improvement was reflected in the diminishing percentage of damaged eggs (Table 2) in laying hens carrying these genes. However, the favourable effect due to *Na* and *F* genes on eggshell strength and ultrastructure is well established (Mahrous et al. 2003; El-Safty et al. 2006; Chen et al. 2009; Fathi et al. 2013).

Immune response

Cell-mediated immunity. Results of the present study provided an evidence that heterozygous naked neck and homozygous frizzled hens had significantly greater cell-mediated immunity compared to the remaining genotypes (Table 3). The current findings are in consistency with the study of El-Safty et al. (2006), who concluded that the *Na* gene significantly improved cell mediated immunity in chickens raised under winter conditions. Additionally, in agreement with the previous results, Alvarez et al. (2002, 2003) reported that the *Nanaff* genotype is a higher responder to cell-mediated immunity and the most resistant to *Salmonella gallinarum* infection than its normally feathered (*nanaff*) and homozygous (*NaNaff*) genotypes. Regardless of prevailing ambient temperature, several reports stated that the presence of *Na* and/or *F* gene increased cell mediated responses. Fathi et al. (2008) and Galal (2008) found that a higher non-specific (cell-medaited) immune response was evoked in *Na* gene bearing birds. Likewise, a significantly higher cellular immunity in naked neck (*Nanaff*) and frizzled (*nanaFf*) genotypes at all tested times compared to normally feathered (*nanaff*) birds was observed (Fathi et al. 2005). In broiler breeds, Patra et al. (2004) reported significantly higher cell mediated immunity estimates in *Nanaff* and *NaNaff* genotypes compared to *nanaff*. On the other hand, Rajkumar et al. (2011) reported that there was no variation in cell mediated immune and antibody response to *NDV* between the naked neck and normal chicken in both summer and winter seasons. However, a slightly numerical increase was recorded for naked neck genotype.

Humoral immune response. No significant difference in the antibody titres against *NDV* was detected among genotypes (Table 3). However, a numerical decrease was noticed in heterozygous genotypes (*Nanaff* and *nanaFf*) compared to normally feathered one (*nanaff*). In agreement with our findings, no significant variation in antibody response to *NDV* between the naked neck and normal chicken in both summer and winter seasons was detected (Rajkumar et al. 2011). Humoral assays have indicated that there were no significant differences for antibody response to a multi-determinant antigen, such as sheep red blood cells (SRBC), in heterozygous naked neck and frizzled birds when compared with normally feathered counterparts. However, the titres were lower in heterozygous naked neck birds (Haunshi et al. 2002). On the other hand, the majority of relevant reports have shown that naked neck and frizzle genotypes have higher antibody production against SRBC under high ambient temperatures (Kundu et al. 1999;

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Fathi et al. 2005, 2014; Rajkumar et al. 2010). A higher titre against *NDV* was observed in naked neck genotypes (*NaNaff* and *Nanaff*) compared to normal plumage (*nanaff*) under tropical climatic conditions (Reddy et al. 2015).

Blood biochemical and hematological parameters. Results of the present study illustrated that both genotypes of naked neck had a significantly lowered ($P < 0.01$) total protein level compared to the rest of genotypes (Table 4). Conversely, under moderate temperatures (27°C), Mahrous et al. (2008) reported that the presence of the *Na* or *F* gene in single status or in combination significantly increased plasma total protein. The albumin level was not affected by genotype. A reduction in globulin was detected in all genotypes compared to *nanaff* but and this difference was significantly lower in heterozygous naked neck genotype. A superiority of globulin and cholesterol levels was found in normal plumage hens compared to frizzled and naked neck genotypes (Peters et al. 2011). Conversely, Mahrous et al. (2008) reported that the *Nanaff*, *nanaFf* genotypes had significantly higher plasma globulin compared to normal genotype under moderate temperatures. There were no significant differences among genotypes for the concentration of cholesterol, triglycerides and thyroid hormones. In consistency with our findings, Mahrous and El-Dlebshany (2011) stated that plasma total cholesterol content did not change by naked neck and frizzled genes. Conversely, significantly lower cholesterol concentration in naked neck chicken was reported by Patra et al. (2002) and Rajkumar et al. (2010, 2011). However, a numerical increase in triglycerides (ranging 12.6–32.4 as a deviation from *nanaff*) was detected in naked neck and frizzled genotypes.

Regarding to blood hematological parameters, *Na* gene increased the hemoglobin level compared to normal genotype. This increase was significant in *NaNaff* genotype. Thrombocytes significantly increased ($P < 0.04$) in *nanaFF* genotype (+87.3% deviation from *nanaff*) compared to the remaining genotypes. Peters et al. (2011) found that the normally feathered birds had a higher packed cell volume and hemoglobin concentration compared to frizzled and naked neck genotypes.

Antioxidative properties. Total antioxidant capacity contributes to the balance of active oxygen and a potent parameter reflects the status of all antioxidants in plasma and body fluids (Rajani et al. 2011). As shown in Table 5, there was no significant difference among genotypes in TAC. However, a slight decrease of TAC in both naked neck genotypes were observed compared to the normal plumage one and this may be due to increasing the effect of lower temperature on the wide featherless area in such genotypes. In this context, Yang et al. (2014) reported that low ambient temperature significantly decreased TAC in the serum. GSH-Px concentration was significantly increased ($P < 0.02$) in naked neck genotypes (in both states) compared to the other genetic groups. The increased activity of the antioxidant enzymes has been considered as the protective response against the oxidative stress. In contrast with our findings, Rajkumar et al. (2011) reported that the GSH-Px activity was significantly higher in stressed birds (normal plumage) in both winter and summer seasons. Additionally, lipid peroxidation was higher in normal birds in summer indicating the increased lipid oxidation under stress condition resulting in the higher concentration of malondialdehyde in blood (Rajkumar et al. 2011).

CONCLUSION

In conclusion, raising naked neck and frizzled laying hens in open sided houses under low/moderate ambient temperature still has an advantage on immune response and eggshell quality, particularly shell thickness and breaking force without penalizing egg production performance.

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