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Impact of Naked Neck (Na) and Frizzle (F) Genes on Growth Performance and Immunocompetence in Chickens

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Abstract: Growth performance and immune response of naked neck, frizzle and double segregation chickens were evaluated under moderate temperature. Four genetic groups originated from the same genetic origin (normally feathered, frizzled, naked neck and naked neck-frizzled) were used in the current study. Body weight and feed conversion ratio were determined from 4-12 weeks of age. Humoral immunity, phagocytic ability, cell-mediated immunity and relative weight of lymphoid organs were determined. The frizzled (nanaFf), naked neck (Nanaff) and naked neck frizzled (NanaFf) genotypes had significantly heavier body weight compared to normally feathered (nanaff) counterparts. The presence of Na gene in a single state or interacted with F gene significantly improved feed conversion ratio compared to nanaff sibs. With respect to immunocompetence measurements, it could be noticed that the Na, F and double segregation genes significantly increased total antibody titer against sheep red blood cells (SRBCs) compared to nanaff genotype. Concerning cautenous basophilic hypersensitivity (CBH), it could be speculated that the naked neck, frizzle and naked neck frizzle birds were hyper responder to phytohemagglutinin-P (PHA-P) injection compared to normally feathered sibs. Likewise, naked neck, frizzled and naked neck-frizzled birds had significantly higher carbon clearance index (lower carbon particles in their blood circulation) compared to normally feathered counterparts. Negative relationship between body weight and relative weight of lymphoid organs was observed in all genetic groups. Also, there was significantly negative correlation between body weight and total antibody against SRBCs in all genotypes. The phagocytic ability measured at all times was negatively correlated with body weight in all genetic groups. It could be concluded that the naked neck, frizzled genes in a single state or in combination significantly increased immune response of chicken under prevailing conditions of Egypt. Therefore, introducing naked neck (Na) and frizzle (F) genes in selection programs for disease-resistance must be taken into consideration, particularly in unfavorable environments.

Key words: Naked neck gene, frizzle gene, humoral immunity, phytohemagglutinin-P, phagocytic ability

Introduction

The birds immune system is a complex network of specialized organs, glands and cells which when working properly protect the body from pathogens such as virus, bacteria and fungus. This system is composed of three basic sub-systems, the humoral, cellular and phagocytic. These sub-systems have different methods of defending the body from diseases. Lymphoid organ weights are easily measured and reflect body's ability to provide lymphoid cells during an immune response (Heckert *et al.,* 2002). The spleen and bursa are the important lymphoid organs involved in the development and differentiation of T or B lymphocytes (Eerola *et al*., 1987; Toivanen *et al*., 1987). Major genes of chickens are believed to confer not only adaptability to the tropical climate, but also resistance to diseases (Haunshi *et al.,* 2002). Reports on the influence of major genes such as naked neck and frizzle on immunocompetence are few. Some other major genes such as slow feathering and dwarfism have been evaluated for their possible influence on immune competence in chicken (Klingensmith *et al.*, 1983; Bacon *et al.,* 1986). Significantly higher cell-mediated immune (CMI) estimate was observed in Nana and NaNa broilers as compared to nana (Patra *et al.,* 2004). Martin *et al.* (1989), Kundu *et al*. (1999) and Haunshi (1999) reported that there was no significant effect of naked neck and frizzle gene on cell mediated (CMI) response to Con-A. Inversely, Alvarez *et al.* (2002) found that the heterozygous naked neck (Nana) genotype had a better cellular and humoral response than their normally feathered (nana) and homozygous naked neck (NaNa) genotypes. Also, El-Safty *et al*. (2006) observed that the Nana hens had a significantly greater dermal swelling (cell mediated) compared to normally feathered ones. Additionally, the normal plumage hens had a higher mortality and culling rate than heterozygous naked neck hens. This experiment was designed to study the effects of naked neck, frizzle and double segregation genes on immunocompetence of chickens under moderate temperature.

Materials and Methods

Genetic flock and husbandry: This experiment was carried out at poultry breeding farm, Poultry Production Department, Faculty of Agriculture, Ain Shams University.

Heterozygous naked neck frizzled (NanaFf) males were artificially inseminated with normally feathered (nanaff) females. According to the previous mating, four genetic groups {231 normally feathered (nanaff), 89 frizzled (nanaFf), 155 naked neck (Nanaff) and 98 naked neck frizzled (NanaFf)} were obtained. At hatching, all chicks were wing-banded and brooded in electrical brooding batteries till they were reached 4 weeks of age. Then, they were transferred to floor pens. All chicks were reared under similar environmental, managerial and hygienic conditions. The feed and water were supplied *ad libitum*. The average high and low ambient temperatures recorded during experimental period were 28.7 and 22.6EC, respectively.

Measurements and observations

Body weight, feed consumption and feed conversion ratio: Body weight was individually recorded for each genotype at 0, 4, 8 and 12 weeks of age. The feed consumption was measured from 4 to 12 weeks of age. Also, feed conversion ratio was calculated. Also, mortality rate was recorded within each genotype through the experimental period

Sheep red blood cells (SRBCs): At 4 weeks of age, 10 chicks per genotype were randomly assigned for assessing humoral immunity response. The sheep red blood cells (SRBCs) were collected and washed 3 times in phosphate–buffer saline (PBS). After that, the packed cells were brought to a 7% vol/vol solution in the PBS. At 2 wks of age, chicks were injected into thigh muscle with SRBC (3% suspension in PBS, 1 ml/chick) followed by a booster injection of SRBC suspension at 4 wks (at 14 days of the first injection). Blood samples were drawn at 7, 14 for days first and second injection. Plasma was stared at –20EC until tested. The antibody levels against SRBC were measured by hemagglutination test using 2% SRBCs suspension. Plasma was heat inactivated at 56EC for 30 min and then analyzed for total, mercaptoethanol-sensitive (Presumably IgM) and mercaptoethanol-resistant (IgG) anti-SRBCs antibodies as previously described (Yamamoto and Glick, 1982; Qureshi and Havenstein, 1994). Briefly, 50 µL of plasma was added in an equal amount of PBS in the first column of a 96-well V-shaped bottom plate, and the solution was incubated for 30 min at 37EC. A serial dilution was then made and 50 µl of 2% SRBC suspension was added to each well. Total antibody titers were then read offer 30 min of incubation at 37EC. The well immediately preceding a well with a distinct SRBC button was considered as the endpoint titer for agglutination. For MER (IgG) response, 50 µl of 0.01 M mercaptoethanol in PBS was used instead of PBS alone, followed by the pervious mentioned procedure. The difference between the total and IgG response was considered to be equal to the IgM antibody level.

Phytohemagglutinin-P injection (In vivo cell-mediated immunity assay): Response induced in vivo by mitogen was evaluated by injection of phytohemagglutinin-P (PHA-P) into the wattle. At 12 weeks of age, 15 chicks from each genotype were used. Each chick was intradermally injected in the wattle with 100 µg PHA-P (Sigma Chemical Co., St. Louis, MO 63178) in 0.1 ml of sterile saline measured with a constant tension caliper before injection and at 24, 48 and 72 hr after PHA-P injection. The wattle swelling was calculated as the difference between the thickness of the wattle before and after injection.

Carbon clearance (mononuclear phagocytic system function assay): The phagocytic ability of chicks was determined by the carbon clearance assay (CCA) based on the method of Cheng and Lamont (1988) and modified by Fathi *et al*. (2003). Briefly, the supernatant fraction of Black India ink (Design/Hggins, 4415, Sanford, Bellwood, Illinios 60104) was obtained through centrifugation (5000 rpm for 30 min). At 7 weeks of age, 5 chicks from each genotype were injected with ink at the rate of 1 ml/kg body weight into the left wing vein. The blood samples at 0, 3 and 15 min after ink-injection were taken from the opposite wing and immediately transferred into 2 ml of 1% sodium citrate. The samples were then centrifuged at 5000 rpm for 4 min. The relative amount of carbon particles remaining in the supernatant was measured spectrophotometrically at a wave length of 640 nm using samples at zero min as the zero value.

Blood parameters: Total protein and albumen were determined in plasma by enzymatic methods using available commercial kits SCLAVO INC., 5 Mansard Count Wayne NJ07470 USA. The globulin was calculated as the difference between the plasma total protein and albumen.

Lymphoid organs weight and some organs: After completion of immunocompetence measurements assay, chicks were slaughtered and lymphoid organs (bursa, spleen and thymus, all lobes from left side of the neck) and some organs (heart and liver) were removed and weighed to the nearest milligram.

Statistical analysis: Data were subjected to a one-way analysis of variance with genotype effect using the General Linear Model (GLM) procedure of SAS User's Guide, (2001). Correlation coefficients (PROC CORR) were calculated to analyze the relationships between immunocompetence measurements, body weight and relative lymphoid organs weight.

Results and Discussion

Body weight, feed consumption and feed conversion ratio: Data presented in Table 1 showed that the

Table 1: Body weight, body weight gain, feed consumption and feed conversion ratio of chicken as affected by naked neck (Na), frizzle (F) and double segregation genes

	Genotype					
Age (wk)	nanaff	nanaFf	Nanaff	NanaFf	Pooled SE	Prob.
	Body weight, g					
0	28.25[b]	28.0[b]	30.26[a]	29.63[a]	0.44	0.01
4	274.36[b]	275.5[b]	281.0[a]	289.27[a]	6.21	0.05
8	661.96[b]	695.61[a]	695.36[a]	698.9[a]	13.50	0.02
12	965.66[b]	1036.77[a]	1057.5[a]	1099.3[a]	22.43	0.03
	Body weight gain, g					
4-8	387.6[b]	420.1[a]	414.4[a]	409.3[a]	5.17	0.01
8-12	303.7[b]	341.16[ab]	362.1[ab]	400.7[a]	4.56	0.01
4-12	691.3[b]	761.3[a]	776.5[a]	810.0[a]	5.12	0.01
	Feed consumption, g					
4-8	980.6[b]	1054.5[a]	1002.8[a]	974.2[b]	7.21	0.01
8-12	810.9[b]	880.2[ab]	894.5[ab]	977.7[a]	8.50	0.05
4-12	1791.5[b]	1934.7[a]	1897.2[a]	1951.9[a]	11.12	0.04
	Feed conversion ratio					
4-8	2.53[a]	2.51[a]	2.42[b]	2.38[b]	0.06	0.05
8-12	2.67[a]	2.58[ab]	2.47[b]	2.44[b]	0.08	0.02
4-12	2.59[a]	2.54[a]	2.44[b]	2.41[b]	0.07	0.05

[a, b] Means with the same letters at the column did not significantly differed

Table 2: Total antibody titer against SRBCs, immunoglobulin-G and immunoglobulin-M as affected by naked neck, frizzled and double segregation genes

	Time			
	7PPI	14PPI	7PSI	14PSI
Genotype	-------- Total anti-SRBCs antibody titer -------			
nanaff	5.10[b]	4.73[b]	5.87[b]	4.02[b]
nanaFf	5.71[a]	5.22[a]	6.15[a]	4.61[a]
Nanaff	5.83[a]	5.32[a]	6.32[a]	5.10[a]
NanaFf	6.04[a]	5.63[a]	6.80[a]	4.62[a]
Pooled SE	0.02	0.04	0.05	0.03
Prob.	0.01	0.01	0.01	0.01
Genotype	---------- Immunoglobulin-M ----------			
nanaff	3.17[b]	2.67[b]	2.92[b]	1.15[b]
nanaFf	3.52[a]	3.10[a]	3.23[a]	1.32[a]
Nanaff	3.67[a]	3.15[a]	3.35[a]	1.48[a]
NanaFf	4.00[a]	3.62[a]	3.72[a]	1.57[a]
Pooled SE	0.03	0.02	0.03	0.04
Prob.	0.01	0.01	0.01	0.05
Genotype	---------- Immunoglobulin-G ----------			
nanaff	1.93[b]	2.06	2.95	2.87[b]
nanaFf	2.19[a]	2.12	2.92	3.29[a]
Nanaff	2.16[a]	2.17	2.97	3.62[a]
NanaFf	2.04[a]	2.01	3.08	3.05[a]
Pooled SE	0.01	0.03	0.02	0.01
Prob.	0.05	NS	NS	0.01

[a,b]Means with the different letters at the column are significantly differ. NS = non-significant
7PPI = at 7 days post primary SRBC-injection
14PPI = at 14 days post primary SRBC-injection
7PSI = at 7 days post secondary SRBC-injection
14PSI = at 14 days post secondary SRBC-injection

presence of Na gene in a single state or combined with F gene significantly increased body weight of chicks at hatch compared to nanaff genotype. Similar trend was noticed at 4 weeks of age. The body weight of nanaFf, Nanaff and NanaFf genotype was significantly heavier than that of nanaff ones at 8 and 12 weeks of age. The presence of Na gene significantly reduced feather coverage by about 30% in Nana and 40% in NaNa. The naked neck chickens (NaNa or Nana) had heavier body weight compared to normally feathered sibs (Patra *et al*., 2002; Lin *et al*., 2006). With respect to feed consumption, it could be observed that the birds carrying naked neck (Na), frizzle (F) and double segregation genes significantly consumed more feed compared to nanaff counterparts. In accordance of feed conversion ratio, the presence of Na gene in a single state or interacted with F gene significantly improved feed conversion ratio compared to nanaff sibs. However, there was no significant difference between nanaFf and nanaff genotypes. Under high ambient temperature, Galal and Fathi (2001) concluded that the naked neck gene was associated with higher feed consumption compared to fully feather one. Concerning the term of feed conversion ratio, the same authors found that the Na allele had a better effect on feed conversion ratio, where the Nana genotype had significantly lower fed conversion ratio as compared to nana one. Also, Alvarez *et al*. (2002) found that the feed conversion ratio was 2.42 in nana, 1.84 in Nana and 1.92 for NaNa hens under moderate ambient temperature. Under the high ambient temperature (34EC), Jianxia (2002) reported that male broilers with frizzle and naked neck genes increased feed intake by 6.0% an average when compared to the normally feathered broilers. Concerning mortality rate, the data illustrated in Fig. 3 showed that the nanaFf, Nanaff and NanaFf genotypes had lower mortality rate compared to nanaff ones.

Humoral immunity: Sheep red blood cells (SRBCs) have been chosen in this study as non-pathogenic antigen for

Table 3: Plasma total protein, albumen and globulin as affected by naked neck, frizzled and double segregation genes

	Time			
	7PPI	14PPI	7PSI	14PSI
Genotype	---------- Plasma total protein (g/dl) ----------			
nanaff	5.84[b]	4.72[b]	5.32[b]	4.10[b]
nanaFf	6.06[a]	4.88[ab]	5.61[a]	4.73[a]
Nanaff	6.15[a]	4.92[a]	5.73[a]	4.82[a]
NanaFf	6.19[a]	5.03[a]	5.81[a]	4.65[a]
Pooled SE	0.05	0.04	0.06	0.03
Prob.	0.02	0.05	0.03	0.01
Genotype	---------- Albumen (g/dl) ----------			
nanaff	4.39[a]	4.00[a]	3.82[a]	3.21[a]
nanaFf	3.91[a]	3.32[ab]	3.71[a]	3.50[a]
Nanaff	3.22[b]	2.90[b]	3.20[b]	3.00[b]
NanaFf	3.13[b]	2.87[b]	3.15[b]	2.88[b]
Pooled SE	0.05	0.03	0.04	0.02
Prob.	0.01	0.01	0.01	0.01
Genotype	---------- Globulin (g/dl) ----------			
nanaff	1.45[b]	0.72[b]	1.50[b]	0.89[b]
nanaFf	2.15[a]	1.56[a]	1.90[b]	1.23[a]
Nanaff	2.93[a]	2.02[a]	2.53[a]	1.82[a]
NanaFf	3.06[a]	2.16[a]	2.66[a]	1.77[a]
Pooled SE	0.02	0.05	0.03	0.01
Prob.	0.01	0.01	0.01	0.01

[a,b]Means with the different letters at the column are significantly differ

7PPI = at 7 days post primary SRBC-injection

14PPI = at 14 days post primary SRBC-injection

7PSI = at 7 days post secondary SRBC-injection

14PSI = at 14 days post secondary SRBC-injection

Table 4: Lymphoid organs weight and some organs of chicken as affected by naked neck (Na) and frizzle (F) genes.

	---------- Genotype ----------					
Trait	nanaff	nanaFf	Nanaff	NanaFf	Pooled SE	Prob.
Bursa,%	0.23[c]	0.22[c]	0.42[a]	0.35[b]	0.01	0.01
Spleen,%	0.22[b]	0.35[a]	0.24[b]	0.36[a]	0.02	0.01
Thymus,%	0.18[b]	0.19[b]	0.28[a]	0.26[a]	0.01	0.01
Liver,%	3.34[b]	3.45[b]	3.56[ab]	3.67[a]	0.03	0.01
Heart,%	0.51[b]	0.58[ab]	0.57[ab]	0.61[a]	0.02	0.01

[a, b, c]Means with the different letters at the column are significantly differ

stimulating T-cell dependant response (Saxena *et al.,* 1997; Kundu *et al.,* 1999). With respect to primary immune response, data presented in Table 2 showed that the naked neck (Nanaff), frizzle (nanaFf) and naked neck-frizzle (NanaFf) chicks had significantly higher total anti-SRBCs antibody titer. Similar trend was noticed for secondary immune response. The last result could be indicated that the presence of Na, F and double segregation genes improved the immunological memory compared to nanaff genotype. Boa-Amponsem *et al.* (1999) concluded that immunological memory would to be influenced by genetic selection. The present result also may indicate that the birds carrying Na, F and double segregation genes were more resistant to parasites and viruses diseases. Lines of chickens selected for their ability to produce high antibody to SRBCs exhibited higher antibody to Newcastle disease virus, were more resist to *Mycoplasma gallisepticum* (van der Zijpp 1983; van der Zijpp *et al.*, 1983) and lower mortality rate when they were exposed to Marek's disease virus (Pinard *et al*., 1993) than the chicken lines that produced low antibody. Therefore, disease resistance may be indirectly improved by selection for immune parameters. The higher secondary response in Nanaff, nanaFf and NanaFf genotypes might positively affect the effectiveness of vaccination. Parmentier *et al.* (1996) found that a line of chicken selected for humoral response to SRBCs antigen responded better to vaccination with viral antigens than a line selected in the opposite direction.

The IgM anti-SRBCs antibody titer measured at all times in both primary and secondary immune response of Nanaff, nanaFf and NanaFf genotypes was significantly higher than that of nanaff counterparts. With respect to IgG anti-SRBCs antibody titer, it could be speculated that the IgG anti-SRBCs antibody titer measured at 7 days post primary injection and 14 days post secondary SRBCs injection of Nanaff, nanaFf and NanaFf genotypes was significantly higher than that of nanaff ones. Inversely there was no significant difference between strains for IgG anti-SRBCs antibody measured at 14 days PPI and 7 days post secondary injection (PSI). Okada and Yamamoto (1987) demonstrated that the high IgG level was associated with high antibody response to SRBCs and lipopolysaccharides. Also, Martin *et al.* (1989) reported that IgG level was higher for high antibody level than low antibody level.

Phagocytic activity: The defensive functions of phagocytosis come into effect immediately upon the invasion by the foreign materials, whereas the T cells needs time to be stimulated and proliferate before they respond to the invasion (Lamont, 1986). The Phagocytic activity was measured by injection of India ink into the birds for all four genetic lines and comparing their ability to clear the injected carbon from blood circulation over a period of time. An increase in the percentage of optical density (OD) value would be indicative of more carbon present in the sample at the time of quantification. Data presented in Fig. 1 showed that the naked neck (Nanaff) birds had significantly lower levels of carbon in their blood circulation by about 24.4 and 42.3 at 3 and 15 minutes, respectively as compared to normally feathered (nanaff) genotype. Similar trend was observed in frizzled (nanaFf) genotype when compared with nanaff sibs. Moreover, the phagocytic ability of naked neck-frizzled (NanaFf) birds was more efficient compared to other genetic lines. These result indicated that the mononuclear Phagocytic index for naked neck, frizzle and naked neck-frizzled birds chicks were more efficient than those for normally feathered genotype. The last results may be indicated that the birds carrying the Na or

Table 5: Correlation coefficients among body weight, relative lymphoid organs weight and some organs.

	Bursa%	Spleen%	Thymus%	Liver%	Heart%	Genotype
Body weight, g	-0.50*	0.15	-0.73**	-0.64**	-0.79**	nanaff
	-0.42	-0.44	-0.01	-0.75**	-0.53*	nanaFf
	-0.54*	-0.35	-0.38	-0.82***	-0.70**	Nanaff
	-0.74**	0.68*	-0.65*	0.16	-0.20	NanaFf
Bursa, %		0.53*	0.76**	0.74**	0.85***	nanaff
		0.44	0.71**	0.17	0.55*	nanaFf
		0.46	0.64**	0.78**	0.81***	Nanaff
		0.61*	-0.24	-0.24	-0.08	NanaFf
Spleen, %			0.66*	0.65*	0.45	nanaff
			0.90***	0.50*	0.75**	nanaFf
			0.54*	0.42	0.59*	Nanaff
			0.41	0.45	0.38	NanaFf
Thymus, %				0.98***	0.95***	nanaff
				0.26	0.61*	nanaFf
				0.23	0.26	Nanaff
				0.07	0.60*	NanaFf
Liver, %					0.86***	nanaff
					0.86***	nanaFf
					0.97***	Nanaff
					0.72**	NanaFf*

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

Table 6: Correlation coefficients among body weight, relative weight of lymphoid organs and wattle swelling of naked neck, frizzled and double segregation genes.

	D24	D48	D72	Genotype
Body weight, g	0.27	0.21	0.12	nanaff
	0.70**	0.52*	0.51*	nanaFf
	0.61**	0.58*	0.61**	Nanaff
	0.67**	0.81**	0.52*	NanaFf
Bursa,%	-0.25	-0.30	-0.51*	nanaff
	-0.62**	-0.51*	-0.50*	nanaFf
	-0.42	-0.51*	-0.34	Nanaff
	-0.51*	-0.70*	-0.41	NanaFf
Spleen,%	0.22	0.52*	0.27	nanaff
	0.41	0.40	0.38	nanaFf
	0.25	0.23	0.61*	Nanaff
	0.57*	0.45	0.36	NanaFf
Thymus, %	0.45	0.32	0.51*	nanaff
	0.40	0.52*	0.33	nanaFf
	0.32	0.27	0.39	Nanaff
	0.35	0.25	0.30	NanaFf
D 24		0.77**	0.81**	nanaff
		0.82**	0.81**	nanaFf
		0.85**	0.90**	Nanaff
		0.72**	0.78**	NanaFf
D 48			0.88**	nanaff
			0.82**	nanaFf
			0.92**	Nanaff
			0.90**	NanaFf

D24 = wattle swelling measured at 24h post PHA-P injection
D48 = wattle swelling measured at 48h post PHA-P injection
D72 = wattle swelling measured at 72h post PHA-P injection
* P<0.05 ** P<0.01 *** P<0.001

F genes in a single state or interact had more resistance for bacterial, viral and parasitic infections. Qureshi *et al.* (2000) reported that birds with higher macrophage phagocytic potential and nitrite production could protect against bacterial, viral, and parasitic infections. Cheng and Lamont (1988) suggested that phagocytosis may be under the influence of *B* complex. Under Egyptian environmental conditions, Nazmi (2006) found that the Nana genotype had significantly lower levels of carbon in their circulation as compared to nana genotype. Conversely, Haunshi *et al.* (2002) did not detect a difference between naked neck and normally feathered genotypes for phagocytic ability.

Cell-mediated immunity: Phytohemagglutinin-P (PHA-P), a T-cell mitogen, induces proliferation in T-lymphocytes. Injection of PHA-P at a selected site in chickens can be considered as an inducer of localized *in vivo* T-lymphoproliferative response (Cheema *et al.,* 2003). This response was measured at 24, 48 and 72h post PHA-P injection into the wattle, and showed in Fig. 2. It could be speculated that the naked neck (Nanaff), frizzle (nanaFf) and naked neck-frizzle (NanaFf) genotypes had a significantly hyper responder to PHA-P injection compared to normally feathered (nanaff) counterparts. Similar results were obtained by Fathi *et al*. (2005) and El-Safty *et al.* (2006). Also, Patra *et al.* (2004) reported that significantly higher cell-mediated immunity (CMI) estimates were observed in Nana and NaNa genotypes compared to nana counterparts. There was a good indication that cell-mediated immunity plays an important role in controlling and clearing intracellular bacterium (Kougt *et al.,* 1994, 1995). Also, selection on cellular responsiveness might add to enhancement of resistance to coccidiosis (Parmentier *et al.,* 2001). Therefore, the naked neck and frizzled birds may be more resistance to coccidiosis than that of normally feathered ones.

Blood constituents: Data presented in Table 3 showed that the effect of naked neck, frizzle and double

Table 7: Correlation coefficients among body weight, relative weight of lymphoid organs and carbon clearance assay.

	Body weight(g)	Bursa(%)	Spleen(%)	Thymus(%)	Liver(%)	Heart(%)	Genotype
OD3	-0.53*	0.08	-0.40	0.19	-0.01	0.12	nanaff
	-0.16	0.76**	-0.53*	0.75**	-0.39	-0.53*	nanaFf
	-0.38	0.73**	0.91***	0.78**	0.49	0.63*	Nanaff
	-0.20	0.43	0.21	0.67**	-0.50	0.14	NanaFf
OD15	-0.78**	0.18	-0.30	0.17	-0.30	0.40	nanaff
	-0.80**	0.63**	-0.32	0.62**	-0.84***	-0.75**	nanaFf
	-0.08	0.61**	0.85***	0.76**	0.23	0.42	Nanaff
	-0.11	0.50*	0.14	0.70**	-0.14	0.51*	NanaFf

OD increase% = {(OD reading at a considered time - OD reading at zero min)/OD reading at zero min}*100.

Table 8: Phenotypic correlation coefficients between immunocompetence parameters and some productive traits of naked neck, frizzled and double segregation genes.

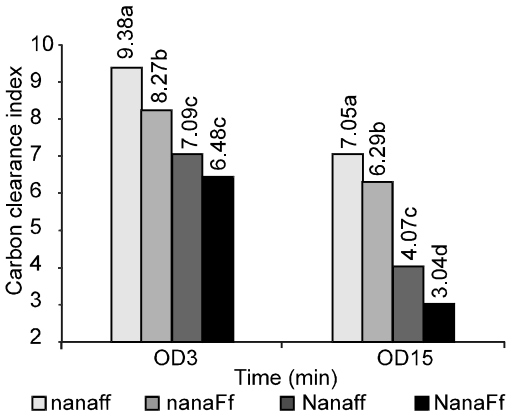
	7PPI	14PPI	7PSI	14PSI	Genotype
Body	-0.67**	-0.58*	-0.81**	-0.68**	nanaff
weight	-0.72**	-0.70**	-0.84**	-0.73**	nanaFf
	-0.57*	-0.63**	-0.75**	-0.60**	Nanaff
	-0.82**	-0.75**	-0.67**	-0.65**	NanaFf
Bursa, %	-0.43	-0.57*	0.60**	0.52*	nanaff
	0.61*	0.42	-0.55*	-0.43	nanaFf
	0.66**	0.75**	0.68**	0.58*	Nanaff
	-0.37	-0.52*	-0.50*	-0.42	NanaFf
Spleen,%	-0.21	-0.42	-0.28	-0.31	nanaff
	0.38	0.47	0.30	0.37	nanaFf
	0.42	0.35	0.28	0.19	Nanaff
	0.31	0.47	0.25	0.30	NanaFf
Thymus, %	0.17	0.08	0.15	0.11	nanaff
	-0.38	-0.40	-0.31	-0.47	nanaFf
	-0.51*	-0.67**	-0.41	-0.53*	Nanaff
	-0.35	-0.54*	-0.51*	-0.48	NanaFf
Plasma-	0.42	0.35	0.36	0.25	nanaff
Globulin	0.51*	0.48*	0.52*	0.57*	nanaFf
	0.67**	0.23	0.45*	0.61**	Nanaff
	0.48*	0.52*	0.56*	0.61**	NanaFf

7PPI = at 7 days post primary SRBC-injection
14PPI = at 14 days post primary SRBC-injection
7PSI = at 7 days post secondary SRBC-injection
14PSI = at 14 days post secondary SRBC-injection

segregation genes on some blood parameters of chickens. It could be noted that the presence of Na or F gene in single manner or interacted significantly increased plasma total protein measured at 7 day post primary SRBCs injection compared to nanaff sibs. At 14 days post primary SRBC injection, both Nanaff and NanaFf genotypes had significantly higher plasma total protein compared to nanaff ones. However, the nanaFf genotype was intermediated. With respect to secondary immune response, the present result showed that the plasma total protein measured at all times for Nanaff, nanaFf and NanaFf genotypes was significantly higher than that of nanaff ones. Concerning plasma albumen, it could note that the nanaff and nanaFf genotypes had significantly higher plasma albumen measured at all times in both primary and secondary SRBC injection compared to Nanaff and NanaFf counterparts. Inversely, the Nanaff, nanaFf and NanaFf genotypes had significantly higher plasma globulin compared to remaining genotypes.

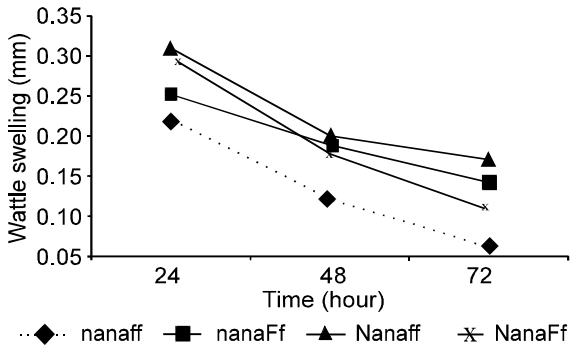
Relative lymphoid organs weight and some organs: The bursa of Fabricius is a key lymphoid organ that is responsible for the development and maturation of B-lymphocytes, and the humoral antibody response is dependent on this central organ (Zhang *et al.,* 2006 and Cheema *et al.,* 2007). For, example, a high antibody response to SRBC has been associated with a larger bursa size in White Leghorn chicken strains (Ubosi *et al.,* 1985). Furthermore, Zhang *et al.* (2006) showed a clear association between non-MHC genes and changes in the size of lymphoid organs by using highly inbred parental and recombinant congenic chicken lines. Data presented in Table 4 showed that the Nanaff genotype had significantly higher relative bursa weight compared to other genetic groups. Also, the NanaFf genotype had significantly higher relative bursa weight compared to nanaff and nanaFf ones. The size of the spleen of avian species may be influenced by genotype (Ubosi *et al.,* 1985). The presence of F gene in single manner or interacted with Na gene significantly increased relative spleen weight compared to nanaff genotype. Concerning relative thymus weight, the presence of Na gene in a single state or interacted with F gene significantly increased relative thymus weight compared to normal type. The NanaFf genotypes had significantly higher relative weight of both liver and heart compared to other genetic groups.

Phenotypic correlations: Correlation coefficients among body weight, relative lymphoid organs weight and some organs are summarized in Table 5. Significantly negative relationship between body weight and relative bursa weight was observed in all genetic groups, but the relationship was not significant in nanaFf genotype. Muir and Jaap (1967) reported that bursa weight at hatching was negatively associated with post-hatching body weight. A similar relationship was observed for turkeys (Li *et al.,* 2001). The relationship between body weight and relative spleen weight was moderately negative in both nanaFf and Nanaff genotypes. Conversely, this relationship was significantly high positive (rp= 0.68) in NanaFf counterparts. However, the body weight was low correlated with relative spleen in nanaff genotype. There was a significantly negative correlation between body weight and relative thymus weight of nanaff and NanaFf genotypes. Similar trend, but not statistically significant



	Gene effect		
Time (Min.)	Na	F	Na-f
OD3	-24.41	-11.83	-30.91
OD15	-42.27	-10.78	-56.88

Fig. 1: Carbon clearance for naked neck, frizzle and double segregation genes.
OD increase% = {(OD reading at a considered time - OD reading at zero min) / OD reading at zero min}*100.
Gene effect was calculated as a deviation from normally feathered (nanaff) genotype.



	Gene effect		
Time (hr)	Na	F	Na-f
24	+40.91	+13.64	+36.36
48	+66.67	+58.33	+50.00
72	+183.33	+133.33	+83.33

Fig. 2: Effect of naked neck, frizzle and double segregation genes on wattle swelling.
Gene effect was calculated as a deviation from normally feathered (nanaff) genotype.

was observed in Nanaff genotype. Significantly negative relationships between body weight and relative both liver and heart weights were observed in nanaff, nanaFf and Nanaff genotypes. Relative bursa weight was

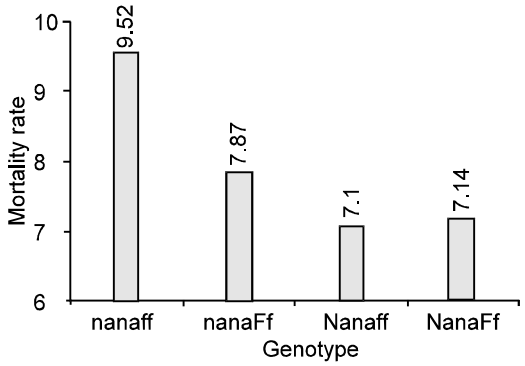


Fig. 3: Mortality rate of naked neck, frizzled and naked neck frizzled birds.

significantly positive correlated with relative spleen weight in both nanaff and NanaFf genotypes. Similar trend, but not statistically significant, was observed in remaining genetic groups. Significantly Positive relationships between relative bursa weight and relative thymus, liver and heart weight were observed in nanaff, nanaFf and Nanaff genotypes. However, these relationships were negative and weak in NanaFf genotype. Relative spleen weight was positively correlated with relative thymus, liver and heart weight in all genetic groups. Significantly positive relationship between relative thymus weight and relative liver weight was noticed in nanaff genotype. However, these relationships were low in remaining genetic lines. Relative heart weight was significantly positive correlated with both thymus and heart percentages in all genetic groups.

Data presented in Table 6 showed that the relationships among cell-mediated immunity, body weight and relative lymphoid organs weight. Highly significant positive relationship between body weight and toe-web swelling measured at all times was noticed in nanaFf, Nanaff and NanaFf genotypes. However, this relationship was low in nanaff counterparts. Negatively relationship between relative bursa weight and toe-web swelling measured at all times was noticed in all genetic groups. Conversely, there was positive correlation between toe-web swelling measured at all times and relative weight of both spleen and thymus in all genotypes. There was highly significant positive correlation between toe-web swelling measured at all times in all genetic groups.

Correlation coefficients among body weight, relative lymphoid organs and carbon clearance assay are presented in Table 7. It could be noticed that the phagocytic activity measured at 3 and 15 min. post Indian Ink injection was negatively correlated with body weight in all genetic groups. Significantly high positive correlations between phagocytic activity and relative bursa weight were observed in both nanaFf and Nanaff genotypes. Similar trend, but moderate, was noticed in

NanaFf genotype. However, this relationship was low in nanaff sibs. Conversely, Al-Rishan (2006) found that the phagocytic index was negatively correlated with relative bursa weight in three broiler strains. The conflicting results may be due to the different between both local and commercial strains. Negative relationships between phagocytic activity and relative spleen weight were observed in nanaff and nanaFf genotypes. Conversely, the phagocytic activity was significantly positive correlated with relative spleen weight in Nanaff genotype. However, this relationship was low in NanaFf counterparts. Except of nanaff genotype, significantly high positive correlations between phagocytic activity and relative thymus weight were observed in all genetic groups. Relative liver weight was negatively correlated with phagocytic activity in all genetic groups, except of Nanaff genotype. Relative heart weight was positively correlated with phagocytic activity measured at all times was observed in nanaff, Nanaff and NanaFf genotypes. Inverse relationship was noticed in nanaFf genotype.

Phenotypic correlation coefficients between some productive and humoral immune response of chickens are summarized in Table 8. Highly significant negative relationship between body weight and total anti-SRBC antibody titers measured at all times was observed in all genetic groups. Immunocompetence and growth are influenced by genetic and non-genetic factors. There is evidence in the literature regarding negatively correlation between growth and anti-SRBC antibody response in Leghorn (Siegel *et al*., 1982), broilers (Qureshi and Havenstein, 1994), brown egg-layers (Kruekniet *et al.,* 1994) and Egyptian native breeds (Yakoub *et al.,* 2005). Negative correlation between body weight and level of antibody response based on pleiotropic effects for genes associated with immunoresponsiveness (Martin *et al*., 1989). On the other hand, arguments for resource allocation for prioritization of resource use for various demands by chickens artificially selected for body growth (Dunnington and Siegel, 1996). Also, this inverse relationship could be because utilization of resources such as energy and protein might be diverted toward the support of the production of immune products and away from stimulated growth (Mashaly *et al.,* 2000). Benson *et al.* (1993) reported that stimulation of immune response resulted in decreased chick growth.

Significantly positive relationship between relative bursa weight and total anti-SRBC antibody titer was observed in Nanaff genotype. Inversely, this relationship was negative in NanaFf genotype. With respect to normally feathered genotype, it could be noticed that the there was negative correlation between body weight and total anti-SRBC antibody titer measured at 7 and 14 days post primary SRBC injection. The opposite trend was noticed at 7 and 14 days post secondary SRBCs injection. In contrary nanaff genotype, there was positive relationship between body weight and total anti-SRBC antibody titer measured at 7 and 14 days post primary injection. Opposite trend was noticed at secondary immune response. These results indicated that the bursa size may not necessarily be associated with antibody titer. Yamamoto and Glick (1982) found that a chicken line selected for small bursa size had higher total and 2-mercaptoethanol-resistant antibody titers in the primary response to SRBC and also had higher total antibody titers in the line selected for large bursa size. Ubosi *et al.* (1985) observed that a chicken line selected for high response to SRBC had a larger bursa size than the line selected for low response. There was low and negative relationship between relative spleen weight and total anti-SRBC antibody measured at all times in nanaff genotype. Similar trend, but positive, was noticed in remaining genotypes. There was inverse relationship between relative thymus weight and total anti-SRBC antibody titer in all genetic groups, except of nanaff genotype. This suggests the size of thymus did not affect the antibody immune response. Significantly positive relationship between total anti-SRBC antibody titer and plasma globulin was noticed in nanaFf, Nanaff and NanaFf genotypes. Similar relationship, but not statistically significant, was noticed in nanaff genotype.

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