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Immunocompetence and Some Hematological Parameters of Naked Neck and Normally Feathered Chicken

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An experiment was conducted to evaluate the effect of naked neck (*Na*) gene, sex and their interaction on immunocompetence of chickens. Three genotypes (*NaNa, Nana* and *nana*) were the offspring of heterozygous naked neck (*Nana*) Fayoumi (Egyptian breed) males and females. They were reared under similar environmental, managerial and hygienic conditions. The present results showed that the *NaNa* birds had lower mortality rate as compared to *Nana* and *nana* counterparts. The *NaNa* genotype had significantly heavier 8wk-body weight and higher relative weights of bursa, spleen, heart and liver compared to nana sibs. With respect to cutaneous basophilic hypersensitivity (CBH) response, the presence of *Na* gene in a double state significantly increased dermal swelling response to phytohemagglutinin-P (PHA-P) injection compared to nana counterparts. The *Nana* genotype was intermediated in the most cases. Dermal swelling response to PHA-P, relative bursa weight and relative spleen weight were significantly affected by interaction between *Na* gene and sex. Concerning correlations between productive traits and immunocompetence, it could be speculated that the body weight was negatively correlated with relative lymphoid organs weight in all genetic groups. However, the body weight was significantly positive correlated with toe-web swelling measured at all times in *NaNa* and *Nana* genotypes. In conclusion, the difference among genetic groups in response to PHA-P and in some physiological response suggest that the *NaNa* genotype had higher cell-mediated immune response followed by *Nana* compared to *nana* genotype. Accordingly, introducing *Na* gene in susceptible strains may be benefit to increase immunocompetence of chicken.

Key words: Fayoumi chicken, immunocompetence, naked neck gene

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Introduction

The major current goals of poultry breeding programs industry is improving poultry health, which can be achieved by selection for the components of the immune system. This system is composed of three basic subsystems, the humoral, cellular and phagocytic. It is of interest to know that the genetic control of these components may be independent from each other (Cheng and Lamont, 1988; Sarker *et al.,* 2000; Li *et al.,* 2001; Yunis *et al.,* 2002). T-cell mediated immune response of chicken has significantly variation among birds of different genetic lineage (Lamont and Smyth, 1984; Cheng and Lamont, 1988). Successful divergent selection of chickens for various T-cell functions suggests that many of these functions are highly heritable, and are often negatively correlated with body weight (Yamamoto and Okado, 1990; Afraz *et al.,* 1994). The difference among lines for response to PHA-P injection could be attributed to the lymphoblastogenic response to PHA-P is presumed to be polygenic (Morrow and Abplanalp, 1981). Major genes are believed to confer not adaptability to the tropical climate, but also resistance to diseases. Significantly higher cell-mediated immune (CMI) estimate were observed in *NaNa* and *Nana* broilers as compared to *nana* ones (Patra *et al.,* 2004). Martin *et al.* (1989), Kundu (1999) and Haunshi (1999) reported that the naked neck and frizzle genes did not significantly effect on cell-mediated immunity (CMI) response to Concanavalin A (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, Alvarez *et al.* (2003) showed that the *Nana* chickens are the most resistant to *Salmonella Gallinarum* (SG) infection and the best responder to vaccination with SG antigens compared to *NaNa* and *nana* sibs. El-Safty *et al.* (2006) observed that the *Nana* hens had a significantly greater dermal swelling response to phytohemagglutinin-P (PHA-P) compared to normally feathered ones. This experiment was designed to evaluate the effect of *Na* gene in a single or double state, sex and their interaction on immunocompetence of Fayoumi chicken under prevailing conditions of Egypt.

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Materials and Methods

Genetic flock and management

This experiment was carried out at Poultry Breeding Farm, Poultry Production Department, Faculty of Agriculture, Ain Shams University. This experiment was run from June to July, 2006. Heterozygous naked neck (*Nana*) Fayoumi males were artificially inseminated with *Nana* Fayoumi females. According to the previous mating, three genotypes were produced as follows, homozygous naked neck (*NaNa*), heterozygous naked neck (*Nana*) and normally feathered (*nana*). At hatching, all chicks were wing banded and brooded in electrical brooding batteries till reached 4 weeks of age. Then, they were transferred to rearing batteries till reached 8 weeks of age. All chicks were reared under similar environmental, managerial and hygienic conditions. Feed and water were provided *ad libitum*. They were fed a diet containing 20% crude protein and 3200 kcal ME/kg. The average high and low daily ambient temperatures recorded from 4 to 8 weeks of age are 31.8±1.4 and 29.1±1.1℃, respectively.

Measurements and observations

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 two-web between the second and the third digits of chicks. 60 chicks (10 chick/sex/genotype) at 8 weeks of age were used. Each chick was intradermally injected in the toe-web of the left foot with 100 μg phytohemagglutinin-P (Sigma Chemical Co., St. Louis, MO, USA) in 0.1 m*l* of sterile saline. The swelling response was measured with a constant tension caliper before injection and at 24, 48 and 72 hr after PHA-P injection. The toe-web swelling was calculated as the difference between the thickness of the toe-web before and after injection.

Lymphoid organs, some organs and some blood constituents

After completion of phytohemagglutinin-P (PHA-P) assay, the same chicks were weighed and slaughtered. The bursa of Fabricius, spleen, thymus (all lobes from left side of the neck), heart and liver were removed and weighed to the nearest milligram. Concerning blood constituents, 2.0 m*l* blood sample was withdrawn from the jugular vein during slaughtering. A portion of blood was used for hematocrit level determination using capillary tubes and a microhematocrit centrifuge. The hematocrit figures were measured after spinning microhematocrit for 12 min. The resulting plasma was stored at −20℃ for later analysis. The frozen plasma was thaw prior to analysis. Total protein and albumen levels were determined in plasma by enzymatic methods using available commercial kits (SCLAVO INC., Wayne NJ, USA). The globulin level was calculated as the difference between the total plasma protein and albumen levels.

Mortality rate

Cumulative mortality rate for each genotype was calculated from hatching time to 8 weeks of age.

Statistical analysis

Data were subjected to a two-way analysis of variance with genotype and sex effects using the General Linear Model (GLM) procedure of SAS User's Guide (2001). When significant differences among means were found, means were separated using Duncan's multiple range tests. Correlation coefficients (PROC CORR) were calculated to analyze the relationship between some traits.

Results and Discussion

Lymphoproliferative response to phytohemagglutinin-P (PHA-P)

Phytohemagglutinin-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 72 h post PHA-P injection into the toe web, and is reported in Figure 1 and Table 1. The maximum cutaneous basophilic hypersensitivity (CBH) swelling response in all genetic groups occurred at 24 h after PHA-P injection and decreased by 48 and 72 h. The *NaNa* and *Nana* genotypes had significantly higher dermal swelling response at 24 hr post PHA-P injection than that of *nana* ones. Similar trend was noticed for *NaNa* genotype at 48 and 72 h post PHA-P injection. However, the *Nana* was intermediated. 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)

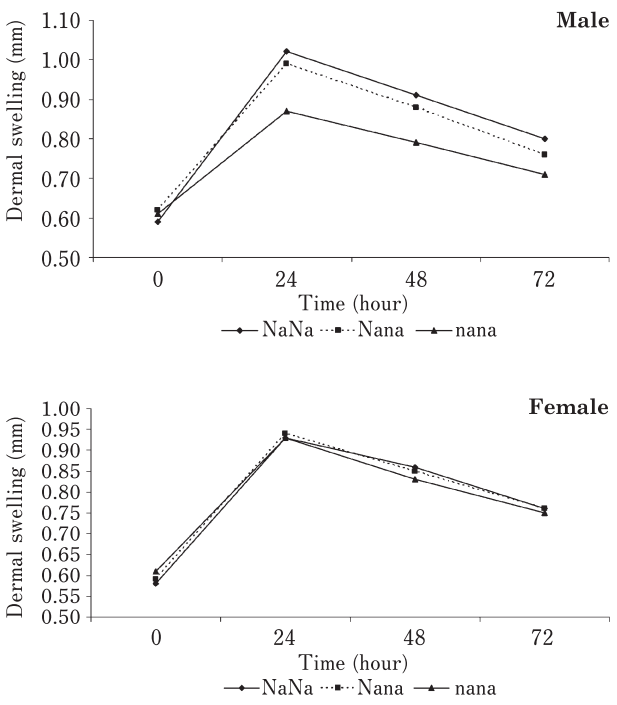

Fig. 1. Effect of naked neck (*Na*) gene on dermal swelling response to PHA-P injection of chicken.

Table 1. Dermal swelling response (difference) to phytohemagglutinin-P (PHA-P) injection as affected by naked neck (*Na*) gene, sex and their interaction

	Time (hour)		
	24	48	72
Genotype (G)			
NaNa	0.39[a]	0.30[a]	0.20[a]
Nana	0.36[a]	0.26[ab]	0.16[ab]
nana	0.29[b]	0.20[b]	0.12[b]
Probability	0.001	0.01	0.01
Sex (S)			
Male	0.35	0.25	0.15
Female	0.34	0.25	0.16
Probability	NS	NS	NS
G*S			
NaNa-male	0.43±0.03	0.32±0.04	0.21±0.02
Nana-male	0.37±0.02	0.26±0.03	0.14±0.01
nana-male	0.26±0.02	0.18±0.01	0.10±0.01
NaNa-female	0.35±0.04	0.28±0.02	0.18±0.03
Nana-female	0.35±0.01	0.26±0.03	0.17±0.02
nana-female	0.34±0.02	0.22±0.04	0.14±0.03
Probability	0.001	0.001	0.001

24, 48 and 72: toe-web swelling measured at 24, 48 and 72 hrs post PHA-P injection, respectively.
Na: incomplete dominant gene of naked neck.
na: recessive gene of naked neck.
[a, b] Means with the same letters did not significantly differed.
NS: not significant.

estimates were observed in *Nana* and *NaNa* genotypes compared to *nana* counterparts. Klingensmith *et al.* (1983) reported higher cell-mediated response of major genes (dwarf) in comparison to the normal birds. 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 *NaNa* followed by *Nana* birds may be more resistance to coccidiosis than that of *nana* ones. With respect to sex effect, there was no significant difference between sexes for dermal swelling response to PHA-P injection measured at all times. Conversely, the dermal swelling response to PHA-P injection was significantly affected by interaction between *Na* gene and sex. The last result could be attributed to the effect of naked neck (*Na*) gene on dermal swelling response to PHA-P was more pronounced in male chickens rather than female ones.

Body weight, relative lymphoid organs weight, heart and liver weights

Data presented in Table 2 showed that the *NaNa* genotype had significantly ($P<0.001$) heavier body weight by 9.4% compared to *nana* ones. However, the *Nana* genotype was intermediated. The presence of *Na* gene, especially in a double state, may result in an increase in the heat dissipation, which is directly related to the reduced feather coverage associated with this gene (about 30% in *Nana* and 40% in *NaNa*). The naked neck chickens (*NaNa* or *Nana*), compared to normally feathered sibs (*nana*), have heavier body weight (Patra *et al.,* 2002; Lin *et al.,* 2006).

Primary and secondary lymphoid organs provide the site for maturation lymphocytes, and for the interaction between lymphocytes and antigens. 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). Data summarized in Table 2 indicated that the presence of *Na* gene in a double state significantly increased relative bursa weight by about 29.3% compared to nana counterparts. However, the *Nana* genotype was intermediated. With respect to relative spleen weight, the spleen is the major organ involved in immune responses to some antigens (White *et al.,* 1975). On the basis of the results presented in the previous table, it could be showed that the *NaNa* genotype had significantly higher relative spleen weight by about 28.6% compared to *nana* sibs. Ubosi *et al.* (1985) found that the size of the spleen of avian species may be influenced by genotype. There was no significant difference among genotypes for relative thymus weight. The heart and liver play important roles in metabolic activity of poultry. The

Table 2. Body weight, relative lymphoid organs weight and some organs weight of chicken at 8 weeks of age as affected by naked neck (*Na*) gene, sex and their interaction

	Phenotypic parameters					
	Body weight (g)	Bursa (%)	Spleen (%)	Thymus (%)	Heart (%)	Liver (%)
Genotype (G)						
NaNa	731.0[a]	0.40[a]	0.36[a]	0.39	0.52[a]	3.44[a]
Nana	694.4[ab]	0.35[ab]	0.30[b]	0.34	0.49[a]	3.31[a]
nana	668.3[b]	0.31[b]	0.28[b]	0.33	0.39[b]	2.99[b]
Probability	0.001	0.01	0.001	NS	0.01	0.01
Sex (S)						
Male	761.0	0.37	0.32	0.37	0.45	3.07
Female	634.6	0.30	0.30	0.33	0.48	3.41
Probability	0.001	NS	NS	NS	NS	0.03
G*S						
NaNa-male	806.5± 9.86	0.44±0.08	0.37±0.04	0.42±0.02	0.51±0.02	3.27±0.12
Nana-male	760.0±11.10	0.37±0.05	0.30±0.05	0.35±0.02	0.48±0.05	3.10±0.16
nana-male	717.0±14.16	0.30±0.07	0.28±0.03	0.33±0.01	0.37±0.01	2.85±0.20
NaNa-female	655.5± 8.90	0.35±0.04	0.35±0.04	0.35±0.03	0.53±0.06	3.60±0.17
Nana-female	628.7± 9.86	0.33±0.03	0.29±0.03	0.33±0.04	0.50±0.04	3.52±0.15
nana-female	619.6±10.17	0.31±0.05	0.27±0.02	0.32±0.02	0.41±0.05	3.12±0.21
Probability	0.05	0.01	0.01	NS	NS	NS

Na: incomplete dominant gene of naked neck.
na: recessive gene of naked neck.
[a, b] Means with the same letters did not significantly differed.
NS: not significant.

proportions of heart and liver weights are shown in Table 2. The results showed that the presence of *Na* gene in a single or double manner significantly increased proportion of heart (33.3 and 25.6%, respectively) and liver (15.1 and 10.7%, respectively) compared to *nana* ones. The higher heart and liver proportion associated with naked neck (*NaNa* and *Nana*) birds may suggest greater cardiac output and liver activity to support the higher metabolic rate resulting in higher growth recorded for these genotypes. With respect to sex effect, there was no significant difference between sexes for relative lymphoid organs and heart weight. However, the female chickens had significantly higher relative liver weight compared to male ones. Concerning *Na* gene by sex interaction, it could be observed that both relative bursa and spleen weight was significantly affected by interaction between *Na* gene and sex. This result could be attributed to the effect associated with *Na* gene on these organs was more pronounced in male chickens rather than female ones.

Blood constituents

Data presented in Table 3 showed that the *NaNa* genotype had significantly higher hematocrit level compared to *nana* sibs. However, there was no significant difference between *Nana* and *nana* genotypes. The higher level of hematocrit may have enhanced oxygen delivery to the tissue (Zongo and Petitjean, 1990). Also, this increment is supposed to be a factor for increased blood volume as a reaction to increase body oxygen requirement. There was significant difference among genotypes for plasma total protein and globulin, whereas the *NaNa* genotype had significantly higher plasma total protein and globulin by about 5.7 and 19.8%, respectively compared to *nana* sibs. However, the *Nana* genotype was intermediated. Inversely, the plasma albumen did not significantly affected by genotype. With respect to sex effect, the plasma total protein, albumen and globulin of male chicks were significantly higher than that of female ones. Both plasma total protein and globulin was significantly affected by interaction between genotype and sex.

Mortality rate

Under high ambient temperature (summer season), data presented showed that the *NaNa* birds (Total no. 98) had lower mortality rate (7.14%) as compared to *Nana* (Total no. 145) (8.28%) and *nana* (Total no. 187) (9.63 %) ones. However, the difference did not statistically significant. Patra *et al.* (2004) found that the *NaNa* broiler chicks (11.7%) had lower mortality percentage as compared to *Nana* (12.28%) and *nana* (13.59%) counterparts.

Phenotypic correlations

Phenotypic correlation coefficients among relative lymphoid organs weight, some blood parameters and dermal swelling response to PHA-P injection are presented in Table 4. Significantly negative relationship between body weight and relative both bursa and thymus weight was observed in all genetic groups. Similar relationship, but not statistically significant, was noticed between body weight and relative spleen weight. Generally, the relative lymphoid organs weight is negatively correlated with body weight. The smaller bursa of Fabricius

Table 3. Some blood parameters of chicken as affected by naked neck (*Na*) gene, sex and their interaction

	Hematocrit level (%)	Total protein (mg/dl)	Albumen (mg/dl)	Globulin (mg/dl)
Genotype (G)				
NaNa	33.1[a]	4.70[a]	2.03	2.72[a]
Nana	32.5[b]	4.61[ab]	2.10	2.52[ab]
nana	32.3[b]	4.43[b]	2.16	2.27[b]
Probability	0.05	0.02	NS	0.01
Sex (S)				
Male	32.2	4.93	2.29	2.63
Female	33.0	4.24	1.90	2.37
Probability	NS	0.01	0.001	0.05
G*S				
NaNa-male	32.7±1.12	5.12±0.14	2.12±0.08	3.00±0.05
Nana-male	32.2±1.10	5.00±0.10	2.31±0.05	2.96±0.06
nana-male	31.8±1.25	4.66±0.12	2.45±0.04	2.21±0.10
NaNa-female	33.5±1.17	4.28±0.13	1.95±0.06	2.43±0.04
Nana-female	32.8±1.15	4.22±0.11	1.88±0.07	2.34±0.06
nana-female	32.7±1.10	4.20±0.10	1.87±0.03	2.33±0.05
Probability	NS	0.01	NS	0.05

Na: incomplete dominant gene of naked neck.
na: recessive gene of naked neck.
[a, b] Means with the same letters did not significantly differed.
NS: not significant.

weight and higher ratio of spleen to bursa weight may reflect the effect of growth on the lymphoid tissues, and these changes in weight possibly resulted in some changes in the lymphocyte subpopulations. In chickens, Muir and Jaap (1967) reported that bursa of Fabricius weight at hatching was negatively associated with post-hatching BW. A similar relationship was observed for turkeys (Li *et al.,* 2001). Body weight was significantly positive correlated with plasma total protein in both *NaNa* and *Nana* genotypes. Similar trend, but not statistically significant, was noticed in nana genotype. The relationship between body weight and plasma albumen was positive and weak in all genetic groups. However, the body weight was positively moderate correlated with plasma globulin in all genotypes. Immunocompetence and growth are influenced by genetic and non-genetic factors. Significantly positive relationship between body weight and toe-web swelling measured at 24 and 72 hr post PHA-P injection was noticed in *NaNa* and *Nana* genotypes. Similar correlation, but not statistically significant, was noticed at 48hr post PHA-P injection. However, inverse relationship was noticed in *nana* genotype. Relative bursa weight was negatively correlated with relative both spleen and thymus weight in all genotypes. Inversely, significantly positive relationship between relative bursa weight and total plasma protein was observed in both *NaNa* and *Nana* genotypes. However, this relationship was negative and weak in *nana* genotype. There was significant positive association between relative bursa weight and plasma globulin in *NaNa* genotype. Similar trend, but not statistically significant, was observed in *Nana* and *nana* genotypes. The relationship between relative bursa weight and dermal swelling response to PHA-P injection measured at all times was negative and weak in all genetic groups. This result was confirmed with Fathi *et al.* (2003) and Yakoub *et al.* (2005). They reported that negative relationship between the relative bursa weight and the swelling of toe web measured at 24, 48 and 72 hrs post PHA-P injection. This suggested that the size of bursa did not affect the cell-mediated immune response. Relative spleen weight was positively correlated with relative thymus weight and plasma total protein in all genotypes. However, the relationship between relative spleen weight and both plasma albumen and globulin was positive and weak. The relative spleen weight was positively correlated with toe-web swelling measured at all times in all genotypes. Yakoub *et al.* (2005) showed that positive relationships between relative spleen weight and toe-web swelling measured at all time were observed in Fayoumi breed. Conversely, Fathi *et al.* (2003) reported that a pronounced negative relationship between relative spleen weight and swelling of toe-web for all different strain. Plasma total protein was significantly positive correlated with plasma albumen and negatively correlated with plasma globulin in both *NaNa* and *Nana* genotypes. Opposite trend was noticed in *nana* genotype. Positively relationship between plasma total protein and dermal swelling response to PHA-P injection measured at all times was observed in all genotypes, with statistically significant at 48 hr post PHA-P injection in *NaNa* and

Table 4. Phenotypic correlation coefficients among relative lymphoid organs weight, blood parameters and dermal swelling response to PHA-P injection

	B%	S%	TH%	PTP	AL	G	D1	D2	D3	Genotype
BW	−0.62**	−0.33	−0.54*	0.46*	0.12	0.40	0.61**	0.42	0.54*	*NaNa*
	−0.55*	−0.24	−0.50*	0.47*	0.18	0.33	0.66**	0.38	0.60**	*Nana*
	−0.43*	−0.35	−0.48*	0.36	0.16	0.28	−0.59*	−0.29	−0.51*	*nana*
B%	⋯	−0.25	−0.12	0.55*	0.12	0.48*	−0.18	−0.15	−0.22	*NaNa*
	⋯	−0.41	−0.24	0.51*	0.24	0.33	−0.24	−0.16	−0.19	*Nana*
	⋯	−0.36	−0.15	−0.19	0.16	0.40	−0.13	−0.18	−0.15	*nana*
S%		⋯	0.25	0.22	0.12	0.10	0.42	0.51*	0.30	*NaNa*
		⋯	0.26	0.25	0.08	0.16	0.33	0.43	0.35	*Nana*
		⋯	0.31	0.32	0.14	0.13	0.27	0.25	0.18	*nana*
TH%			⋯	0.33	0.41	0.21	0.57*	0.62**	0.58*	*NaNa*
			⋯	0.36	0.22	0.33	0.72**	0.63**	0.50*	*Nana*
			⋯	0.45	0.14	0.15	0.54*	0.71**	0.63*	*nana*
TP				⋯	0.51*	−0.42	0.40	0.51*	0.42	*NaNa*
				⋯	0.56*	−0.45	0.36	0.59*	0.33	*Nana*
				⋯	−0.45	0.33	0.28	0.45	0.24	*nana*
AL					⋯	−0.45*	0.12	0.19	0.14	*NaNa*
					⋯	−0.52*	0.18	0.22	0.15	*Nana*
					⋯	−0.66**	0.10	0.13	0.08	*nana*
G						⋯	0.44	0.55*	0.42*	*NaNa*
						⋯	0.54*	0.57*	0.39	*Nana*
						⋯	0.67**	0.36	0.44*	*nana*
D1							⋯	0.74**	0.84**	*NaNa*
							⋯	0.75**	0.90**	*Nana*
							⋯	0.81**	0.83**	*nana*
D2								⋯	0.81**	*NaNa*
								⋯	0.84**	*Nana*
								⋯	0.78**	*nana*

BW: 8wk-body weight, B%: relative bursa weight, S%: relative spleen weight, TH%: relative thymus weight, TP: plasma total protein, AL: plasma albumen, G: plasma globulin.
D1, D2 and D3: toe-web swelling measured at 24, 48 and 72 hrs post PHA-P injection, respectively.
*$P<0.05$, **$P<0.01$.

Nana genotypes. Plasma albumen was significantly negative correlated with plasma globulin in all genetic groups. Significantly positive relationship between plasma globulin and toe-web swelling measured at all times was observed in all genotypes. The relationships between dermal swelling responses to PHA-P injection measured at all times were significantly positive in all genetic groups.

In conclusion, the difference among genetic groups in response to PHA-P and in some physiological response suggest that the *NaNa* genotype had higher cell-mediated immune response followed by *Nana* genotype when compared with *nana* ones. Accordingly, introducing some single genes in susceptible strains may be benefit to increase immunocompetence of chicken.

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