

IMPACT OF NAKED NECK AND FRIZZLE GENES ON CELL-MEDIATED IMMUNITY OF CHICKENS

By

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Abstract: *An experiment was conducted on naked neck and frizzled genotypes compared to normally feathered ones to determine the immunocompetence measurements under winter season of Egypt.*

- With respect to cutaneous basophil hypersensitivity (CBH) response, the results showed that the naked neck chickens had a greater dermal swelling response to phytohemagglutinin-P (PHA-P) followed by frizzled ones when compared to normally feathered sibs.

- Frizzle genotype exhibited greater bursa and thymus (as a percentage of live body weight) compared to the remaining genotypes.

- Introducing either naked neck or frizzled gene into normally feathered birds significantly increased the plasma globulin.

- Negative relationship between the relative bursa weight and the swelling of toe web was observed at all times for normally feathered genotypes. Opposite trend was observed for naked neck and frizzled genotypes.

- These findings show that the naked neck or frizzled chickens are hyper responder compared to normally plumage even though their lymphoid organs growth lagged behind normal counterparts in some organs.

INTRODUCTION

Commercial poultry breeding has amongst its objectives, the improvement of production potential and disease resistance. Over the years there has been much emphasis on growth improvement that is negatively associated with some aspects of immunological performance of poultry as reported by Qureshi and Havenstein (1994); Rao *et al.* (1999); Yunis *et al.* (2000); Cheema *et al.* (2003); Fathi *et al.* (2003). While genetic make up of the birds has been clearly shown to have a significant impact on disease resistance and/or susceptibility (Lamont *et al.*, 1987; Lakshman *et al.*, 1997), limited information is available (Yonash *et al.*, 2001; Cheema *et al.*, 2003) that relates to the effects of particular genes, gene loci or genetic markers with particular immune response parameters in chicken lines. However, some major and marker genes, such as naked neck (Na) and

frizzle (F) are believed to control not only adaptability to the tropical climates, but also resistance to diseases. Some other major genes such as slow feathering (K) and dwarfism (dw) have been evaluated for their possible influence on immune competence in chicken (Klingensmith *et al.*, 1983; Bacon *et al.*, 1986) and shown no adverse effect. Such reports on naked neck and frizzle genes are scanty in the literature. Demey *et al.* (1996) observed no consistent trend for influence of naked neck on antibody response to sheep red blood cells (SRBC's). On the other hand, Haunshi *et al.* (2002) concluded that the major genes such as naked neck and frizzling do not have a negative effect on most of the immune competence measures rather having some positive effects on IgM antibody titers and serum complement levels. In addition, Alvarez *et al.* (2002) found that Nana chickens were the best responders to immunization with *Salmonella gallinarium* antigens and they also showed a good innate immune response against *Salmonella* infection.

Under Egyptian conditions, Mohamed (2002) sustained that the naked neck birds produced higher antibody titer against SRBC compared to normally feathered sibs. Also, amongst the Indian native breeds, Kundu *et al.* (1999) found that the naked neck birds had the highest titer to sheep erythrocytes on day 5 postimmunization. To remove some mystery of immunocompetence for such genes, an experiment was conducted to evaluate immune performance of naked neck, frizzled and normally feathered birds issued from the same genetic origin.

MATERIALS AND METHODS

Genetic flocks and husbandry

Ten heterozygous naked neck frizzled (NanaFf) males were artificially inseminated 50 normally feathered (nanaff) females. The offspring obtained were wing-banded and brooded in electrical brooding batteries. At 4 weeks of age, they were transferred to rearing batteries. All chicks were brooded and reared under similar environmental, managerial and hygienic conditions. Feed and water were provided *ad libitum*. They were fed a diet containing 18% crude protein and 2870 kcal ME/kg. The average high and low ambient temperatures recorded from 4 to 6 weeks of age were 20 and 13C, respectively.

Phytohemagglutinin Injection (In vivo cell-mediated immunity assay)

Response induced in vivo by mitogen was evaluated by injection of phytohemagglutinin-P (PHA-P) into the toe web between the second and the third digits of male chicks. Ten male chicks from each genotype, except of

NanaFf genotype, at 6 weeks of age were used. Because of a limited number from NanaFf genotype, the last genotype was excluded from the PHA-P test. Each chick was intradermally injected in the toe web of the left foot with 100 µg phytohemagglutinin-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 toe web swelling was calculated as the difference between the thickness of the toe web before and after injection.

Relative weight of lymphoid organs

After completion of phytohemagglutinin assay, the same chicks were weighed and slaughtered. The bursa of Fabricius, spleen and thymus (all lobes from left side of the neck) were removed and weighed to the nearest milligram.

Hematological parameters

At 6 weeks of age and after completion of phytohemagglutinin-P assay, a 2.0 ml blood samples was withdrawn from the jugular vein during the slaughtering. The resulting plasma was stored at -20C for latter analysis. The frozen plasma was thaw prior to analysis. Total plasma protein and albumen were determined in plasma by enzymatic methods using available commercial kits SCLAVO INC., 5 Mansard Count., Wayne NJ 07470, USA. The globulin was calculated as the difference between the total plasma protein and albumen.

Heterophil / Lymphocyte ratio

At the same time, blood was obtained from each genotype for heterophil (H) and lymphocyte (L) enumeration based on the procedures of Gross and Siegel (1983). Briefly, two drops of blood were taken from a small puncture in the wing vein, placed on a slide and stained with Wright's stain. All slides were coded and heterophils and lymphocytes were counted to a total of 100 cells per slide by the same individual. H/L ratio was calculated by dividing the number of heterophils by the number of lymphocytes.

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. When significant difference among means were found, means were separated using Duncan's multiple range test. Correlation coefficients (PROC CORR) were calculated to analyze the relationship between relative lymphoid organs weight and the response to phytohemagglutinin injection.

RESULTS AND DISCUSSION

Cell-mediated immunity (CMI)

Phytohemagglutini-P is a lectin isolated from red kidney bean and stimulates T-cell proliferation with minimal effect on B cells. It is considered a good *in vivo* measure of T-lymphocyte function (Qureshi *et al.*, 1997). CMI response was found significantly higher in naked neck (Nanaff) and frizzled (nanaFf) genotypes at all interval time as compared to normally feathered (nanaff) ones (Table 1 and Figure 1). These findings were in agreement with Patra *et al.* (2004), who reported significantly higher CMI estimates were observed in Nana and NaNa broilers compared to nana. In contrast, Martin *et al.* (1989), Kundu *et al.* (1999) and Haunshi (1999) reported non-significant effect of CMI response to Con-A on naked neck and frizzle gene. From genetic standpoint, Morrow and Abplanalp (1981) reported that at least two alleles control PHA-P response in birds, whereas a cross between PHA-P high responder and low responder lines was tested for mitogenic response to PHA-P, an intermediate result was seen. There was good indications that cell-mediated immunity plays an important role in controlling and clearing intracellular bacterium (Kougt *et al.*, 1994, 1995). The results reported herein pointed out the heterozygous naked neck and frizzled chickens has better immune responders than the normally feathered counterparts. Also, selection on cellular responsiveness might added to enhancement of resistance to coccidiosis (Parmentier *et al.*, 2001). Therefore, the naked neck and frizzled genotypes may be more resistant to coccidiosis than that of normally feathered ones.

Relative lymphoid organs weight

Body weight and relative lymphoid organs weight of normal, naked neck and frizzled genotypes are presented in Table (2). The body weight of naked neck males was significantly lighter than that of normally feathered ones by about 13.1%. Similar trend was noticed for frizzled males, but the difference was not significant. This result could be attributed to the lower ambient temperature (about 20C). At 20C and below, the differences between genotypes are small in most cases, although in two instances (Merat, 1979 for males; Monnet *et al.*, 1979 for females at the lower temperature) a slight handicap is suggested for the naked neck birds. Hanzl and Somes (1983) showed a marked inferiority of naked neck homozygous and heterozygous females and of heterozygous males as compared to the nana genotype.

Lymphoid organ weights are easily measured and reflect body's ability to provide lymphoid cells during an immune response (Heckert *et al.*, 2002). Primary and secondary lymphoid organs weights provide the site for

maturation lymphocytes, and for the interaction between lymphocytes and antigens. 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). The presence of naked neck gene significantly decreased the relative weight of both thymus and bursa by about 19.8% and 11.8%, respectively compared to normally feathered ones. Conversely, the presence of frizzled gene significantly increased the relative weights of both thymus and bursa by about 23.9% and 19.3%, respectively compared to normal type. With respect to relative spleen weight, the relative spleen weight of naked neck and frizzled genotypes was significantly higher than that of normally feathered ones by about 23.7% and 12.3%, respectively compared to normally feathered ones. The last observation could be attributed to the size of spleen may be influenced by genotype, whereas relative spleen weight was influenced mainly by additive genetic effects as well as sex-linked before infection (Boa-Amponsem *et al.*, 1998).

Hematological parameters

Improved disease resistance may be achieved without challenging animals with disease agents. Selection on the basis of serum immunoglobulin isotypes, which represents the response to a wide variety of unknown antigens, may be a useful tool in this regard (Sarker *et al.*, 1999). Total plasma protein, albumen and globulin as affected by naked neck and frizzled genes are listed in Table (3). It could be noticed that the total plasma protein, albumen and globulin of naked neck males were significantly higher than that of normal type by about 22.8, 31.6 and 10.4%, respectively. Similar trend was observed for frizzled gene, whereas the frizzled genes significantly increased total plasma protein, albumen and globulin by about 30.2, 26.3 and 35.6%, respectively compared to non-frizzled ones. The results indicated that introducing either naked neck or frizzle gene into normally feathered birds may increased the immune response through increased plasma globulin.

Heterophils : Lymphocytes ratio

Heterophils increase and lymphocytes decrease when chickens are stressed, so that the ratio between them is a good index of response to a stressor (Gross and Siegel, 1983; Siegel, 1995). There is a genetic component to heterophil and lymphocyte responses to stressor (Gross and Siegel, 1985) and their ratio has been used as a heritable selection criterion for heat resistance in chickens (Al-Murrant *et al.*, 1997). Data in Table (4) indicate that the heterophils and H/L ratio of naked neck genotype were significantly higher than that of normal type by about 7.2 and 14.2%, respectively. In contrast, the presence of Na

allele significantly decreased the lymphocytes by about 6.21% compared to normally feathered ones. Concerning the frizzle allele, the heterozygous frizzled genotype significantly increased heterophils and H/L ratio by about 3.5 and 7.5%, respectively compared to non-frizzled one. Also, the presence of frizzled gene significantly decreased lymphocytes by about 3.8% compared to normally feathered. These findings could be attributed to the low ambient temperature during the experimental period. Heterophils have been reported to phagocytosis and digest *Escherichia coli*, *Bacillus megaterium*, and *Staphylococcus aureus* (Gross, 1962). Heterophils are active phagocytic cells, and eosinophils play a major phagocytic role in the defense against parasitic organism (Glick *et al.*, 1964). Kreukniet *et al.* (1994) observed that the phagocytic activity was not correlated with the antibody response in the chicken lines.

Phenotypic correlation coefficients

The correlations between swelling toe web at different times and relative weight of lymphoid organs are summarized in Table (5). The correlation coefficient between the swelling in toe web measured after 48 hrs post injection and the swelling after 24 hrs in frizzled genotype showed significantly positive value ($r_p = 0.87$) and this value was higher than those in naked neck ($r_p = 0.54$) and normal type ($r_p = 0.57$).

Negative relationship between the relative bursa weight and the swelling of toe web was observed at all times for normally feathered genotypes. Opposite trend was observed for naked neck and frizzled genotypes, whereas there was positive relationship between the percent of bursa weight and the swelling of toe web at all times. Similar trend was noticed for the relationship between the relative thymus weight and the swelling of toe web for naked neck genotype. Conversely, the normally feathered and frizzled genotypes had a positive correlations. This suggests that the size of bursa and thymus did not affect the cell mediated immune response (Fathi *et al.*, 2003). Bursa of Fabricius size may not necessarily be associated with antibody titers, 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 secondary response compared to the counterparts in the line selected for large bursa size. Uboosi *et al.* (1985) reported that, in ninth generation, high antibody titer had smaller thymus and larger bursa than low antibody titer, relative to body weight, but there were no differences in relative spleen weight.

In conclusion, the results of the present study indicate that the naked neck or frizzled chickens are hyper responder compared to normally plumage even though their lymphoid organs growth lagged behind normal

counterparts in some organs. These results support the idea that introducing such genes could be considered in poultry breeding programmes against intracellular bacteria.

Table 1. Means \pm SE for PHA-P mediated swelling (difference) in the toe-webs of normal, naked neck and frizzled genotypes

Time	Genootype			Gene effect	
	nanaff	Nanaff	nanaFf	Na	F
24h	0.37b ± 0.038	0.53a ± 0.027	0.41b ± 0.029	43.24	10.81
48h	0.18c ± 0.021	0.35a ± 0.024	0.27b ± 0.020	94.44	50.00
72h	0.11b ± 0.017	0.19a ± 0.021	0.15b ± 0.018	72.73	36.36

a-c The means within rows with no common superscript differ significantly ($P < 0.01$).

n = 10 birds per genotype.

gene effect = $(\text{Nanaff or nanaFf} - \text{nanaff}) / \text{nanaff} * 100$

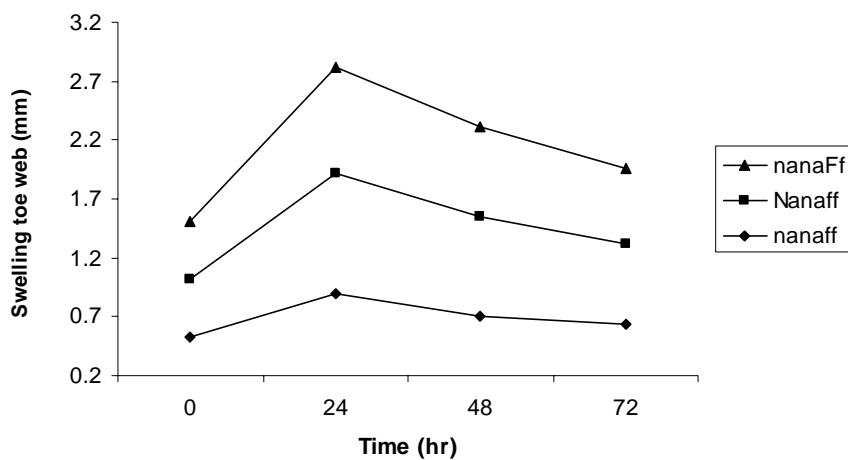


Fig. 1. PHA-mediated swelling in the toe-web of normal, naked neck and frizzled genotypes.

Table 2. Means±SE for body weight and relative weight of lymphoid organs of normal, naked neck and frizzled genotypes.

Trait	Genotype			Gene effect%	
	nanaff	Nanaff	nanaFf	Na	F
Body weight, g	453.00 ^a ±24.47	393.75 ^b ±20.94	434.13 ^a ±18.75	-13.08	-4.17
Thymus, g	1.12 ^b ±0.054	0.78 ^c ±0.070	1.33 ^a ±0.065	-30.36	+18.75
Thymus, %	0.247 ^b ±0.011	0.198 ^c ±0.018	0.306 ^a ±0.021	-19.84	+23.89
Bursa, g	1.27 ^b ±0.015	1.08 ^c ±0.020	1.45 ^a ±0.014	-14.96	+14.17
Bursa, %	0.280 ^b ±0.042	0.274 ^b ±0.031	0.334 ^a ±0.028	-11.79	+19.29
Spleen, g	1.07 ^b ±0.010	1.15 ^a ±0.014	1.15 ^a ±0.011	+7.48	+7.48
Spleen, %	0.236 ^c ±0.059	0.292 ^a ±0.074	0.265 ^b ±0.045	+23.73	+12.29
Bursa/spleen	1.187 ^a ±0.023	0.939 ^b ±0.018	1.261 ^a ±0.051	-20.89	+6.23

^{a-c}The means within rows with no common superscript differ significantly (P<0.01).

n = 10 birds per genotype

gene effect = (Nanaff or nanaFf - nanaff) / nanaff*100

Table 3. Means±SE for plasma total protein, albumen and globulin of normal, naked neck and frizzled genotypes.

Trait	Genotype			Gene effect%	
	nanaff	Nanaff	nanaFf	Na	F
Total plasma protein, mg/100ml	3.25 ^c ±0.108	3.99 ^b ±0.107	4.23 ^a ±0.201	+22.77	+30.15
Albumen, mg/100ml	1.90 ^b ±0.032	2.50 ^a ±0.027	2.40 ^a ±0.029	+31.58	+26.32
Globulin, mg/100ml	1.35 ^c ±0.011	1.49 ^b ±0.009	1.83 ^a ±0.020	+10.37	+35.56

^{a-c}The means within rows with no common superscript differ significantly (P<0.01).

n = 10 birds per genotype

gene effect = (Nanaff or nanaFf - nanaff) / nanaff*100

Table 4. Means±SE for Heterophils (H), and Lymphocytes (L) of normal, naked neck and frizzled genotypes.

Trait	Genotype			Gene effect%	
	nanaff	Nanaff	nanaFf	Na	F
Heterophils (H)	42.35 ^c ±2.24	45.38 ^a ±1.89	43.82 ^b ±2.31	+7.15	+3.47
Lymphocytes (L)	58.44 ^a ±2.15	54.81 ^c ±2.20	56.22 ^b ±2.33	-6.21	-3.80
H/L ratio	0.725 ^b ±0.098	0.828 ^a ±0.083	0.779 ^b ±0.085	+14.21	+7.45

^{a-c} The means within rows with no common superscript differ significantly (P<0.01).

n = 10 birds per genotype

gene effect = (Nanaff or nanaFf - nanaff) / nanaff*100

Table 5. Phenotypic correlations coefficient between relative lymphoid organs and toe web response for normal, naked neck and frizzled genotypes.

Variable	Th	B	S	T24	T48	T72	Genotype
Thymus % (Th)	1.00	0.16	0.21	0.25	0.57*	0.57*	nanaff
	1.00	0.14	0.63**	0.52*	-0.13	-0.44	Nanaff
	1.00	0.24	-0.22	0.41	-0.11	0.01	nanaFf
Bursa % (B)	1.00	1.00	0.33	-0.56*	-0.20	-0.18	nanaff
	1.00	1.00	0.68**	0.41	0.25	0.14	Nanaff
	1.00	1.00	-0.24	0.50*	0.59*	0.51*	nanaFf
Spleen % (S)	1.00	1.00	1.00	0.56*	0.17	0.19	nanaff
	1.00	1.00	1.00	0.58*	0.54*	0.42	Nanaff
	1.00	1.00	1.00	0.11	-0.10	-0.41	nanaFf
Toe web increase after 24h (T24)	1.00	1.00	1.00	1.00	0.57*	0.32	Nanaff
	1.00	1.00	1.00	1.00	0.54*	-0.09	Nanaff
	1.00	1.00	1.00	1.00	0.87**	0.71**	nanaFf
Toe web increase after 48h (T48)	1.00	1.00	1.00	1.00	1.00	0.40	nanaff
	1.00	1.00	1.00	1.00	1.00	0.29	Nanaff
	1.00	1.00	1.00	1.00	1.00	0.88**	nanaFf
Toe web increase after 72h (T72)	1.00	1.00	1.00	1.00	1.00	1.00	Nanaff
	1.00	1.00	1.00	1.00	1.00	1.00	Nanaff
	1.00	1.00	1.00	1.00	1.00	1.00	nanaFf

* P<0.05

** P<0.01

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الملخص العربي

تأثير العاملين الوراثيين عرى الرقبة والريش المجعد على المناعة الخلوية في الدجاج
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صممت هذه التجربة لدراسة تأثير العاملين الوراثيين عرى الرقبة والريش المجعد على المقاييس المقدره المناعية في الدجاج تحت ظروف الشتاء في مصر.
- سجل العامل الوراثي عرى الرقبة قيما أعلى للاستجابة للحقن بمادة PHA-P يليه عامل وراثي المسئول عن الريش المجعد وذلك مقارنة بالتركيب الوراثي الطبيعي الترييش.
- سجل العامل الوراثي مجعد الترييش قيما أعلى للوزن النسبي لكل من غدة البرسا والغدة التيموسية مقارنة بالتركيب الوراثية الأخرى.
- أدى إدخال العاملين الوراثيين عرى الرقبة ومجعد الريش في الطيور الطبيعية ألي زيادة مستوى الجلوبيولين في بلازما الدم.
- وجد ارتباط سالب بين الوزن النسبي لغدة البرسا والاستجابة للحقن بمادة PHA-P عند كل الأوقات وذلك في التركيب الوراثي الطبيعي الترييش. بينما شوهد عكس الاتجاه في التركيب الوراثية الأخرى.
- أوضحت النتائج أن الطيور عارية الرقبة والأخرى مجعدة الترييش كانت أعلى استجابة مناعية للحقن بمادة PHA-P مقارنة بالتركيب الوراثي الطبيعي الترييش على الرغم من أن معدل نمو بعض الأعضاء الليمفاوية سجلت قيما أعلى في التركيب الوراثي الطبيعي الترييش.