

## Serum Haemolytic Complement Levels in German Dahlem Red Chickens Are Affected by Three Major Genes (Naked Neck, Dwarf, Frizzled) of Tropical Interest

P. Dorny<sup>1</sup>, R. Baelmans<sup>1</sup>, H.K. Parmentier<sup>2\*</sup>, M.G.B. Nieuwland<sup>2</sup>, F. Demey<sup>1</sup> and D. Berkvens<sup>1</sup>

<sup>1</sup>*Department of Animal Health, Institute of Tropical Medicine, Antwerp, Belgium;*

<sup>2</sup>*Department of Animal Sciences, Adaptation Physiology Group, Wageningen University, Marijkeweg 40, 6709 PG Wageningen, The Netherlands*

\*Correspondence: E-mail: [Henk.Parmentier@WUR.nl](mailto:Henk.Parmentier@WUR.nl)

Dorny, P., Baelmans, R., Parmentier, H.K., Nieuwland, M.G.B., Demey, F. and Berkvens, D., 2005. Serum haemolytic complement levels in German Dahlem Red chickens are affected by three major genes (naked neck, dwarf, frizzled) of tropical interest. *Tropical Animal Health and Production*, **37**(1), 1–9

### ABSTRACT

German Dahlem Red chickens with three different major genes of tropical interest: *Nana*<sup>-</sup> (naked neck), *Ff*<sup>-</sup> (frizzled) and *dw*<sup>-</sup> (dwarf), respectively, were tested for serum haemolytic complement, which is essential in innate host defence against infectious agents. Eight different combinations of genes for body size and feather coverage were evaluated. Significant differences both for both the calcium-dependent (classical, CPW) and the calcium-independent (alternative, APW) complement titres were found between the phenotypes. Phenotype *nanaffDw*<sup>-</sup> showed the highest complement status. The frizzled (*Ff*<sup>-</sup>) gene had a negative influence on APW titres, whereas the dwarf (*dw*<sup>-</sup>) gene had a negative influence on CPW titres. The naked neck (*Nana*<sup>-</sup>) gene had various influences on the haemolytic complement status. All tested hens had MHC (B) 21 haplotypes, whereas the gene for dwarfism appeared to be linked with the B19 haplotype. It was concluded that introducing major genes (*Nana*<sup>-</sup>, *dw*<sup>-</sup>, *Ff*<sup>-</sup>) to conquer environmental stress in hot climates can have a negative impact on certain aspects of the innate immunity of poultry.

**Keywords:** complement, chicken, German Dahlem Red, naked neck, frizzled, dwarf; MHC (B complex)

**Abbreviations:** APW, calcium-independent (alternative) pathway; CPW, calcium-dependent (classical) pathway; *dw*<sup>-</sup>, dwarf gene; *Ff*<sup>-</sup>, frizzled gene; GDR, German Dahlem Red; MHC (B), major histocompatibility (B) complex; *Nana*<sup>-</sup>, heterozygous naked neck gene; TNF, tumour necrosis factor

### INTRODUCTION

The development of efficient, small-scale, extensive poultry production systems in developing countries has been described as an international priority for the world poultry science community (Sheldon, 2000). Breeding strategies in developing countries should be focused on the genetic potential of the breeds. High environmental temperatures constrain the performance of poultry in both intensive and extensive production systems (Yalcin *et al.*, 1997). It has been shown that improved breeds, such as Rhode Island Red, White Leghorn, Light Sussex, New Hampshire, etc. are not

superior to local chickens under semi-scavenging conditions because their genetic potential cannot be fully materialized owing to low management levels. Heat stress in particular has a strong negative effect on broiler growth, feed efficiency and meat yield (Beaumont *et al.*, 1998; Cooper *et al.*, 1998; May *et al.*, 1998). Egg-type layers selected for improved feed efficiency have a lower appetite at high ambient temperatures, with a drop in egg production (Bordas and Merat, 1984; Mathur and Horst, 1994). These problems cannot be completely eliminated by management practices, especially because these are too costly for developing countries (Mwalusanya *et al.*, 2002).

Indigenous chickens are well adapted to the adverse climatic conditions of the tropical environment. They contain a highly conserved genetic system, with high levels of heterozygosity, which may provide biological material for the design of genetic stocks with improved adaptability and productivity (Ponsuksili *et al.*, 1996; Wimmers *et al.*, 2000). However, indigenous breeds have significantly reduced productivity under these stress factors. Breeding and selection strategies can be exploited to achieve the best possible production in unfavourable tropical environments. Particular major genes ( $Na^-$ ,  $dw^-$  or  $Ff^-$ ) are known to improve heat endurance (Horst, 1983; Yunis and Cahaner, 1999). The naked neck gene reduces feather coverage in chickens by 20% and 40% in the heterozygous ( $Nana^-$ ) and homozygous ( $NaNa^-$ ) states, respectively (Deeb and Cahaner, 2001). Frizzle chickens ( $Ff^-$  gene) show a reduced density of feather coverage, which provides some heat tolerance to egg-type layers (Garces *et al.*, 2001). Feather reduction accumulates in combinations of naked neck with frizzled feathers ( $NanaFf^-$ ). The dwarf ( $dw^-$ ) gene causes about 30% reduction in body weight (Cole, 2000) to reduce the effect of high ambient temperatures (Khan *et al.*, 1987; Gowe and Fairfull, 1995).

A major goal in poultry immunogenetics is the enhancement of innate immunoresponsiveness and resistance to disease (Fussell, 1998). Genetic resistance to diseases is a multigenic trait governed mainly by the immune system and its interactions with many physiological and environmental factors (Gross and Siegel, 1988; Zekarias *et al.*, 2002). The major histocompatibility (MHC) B-complex is perhaps the best-characterized family of host genes that modulates response to a variety of antigens and pathogenic challenges. The association of different MHC (B) alleles with disease resistance has been known for decades (Lamont, 1994). However, few references exist for the association of major genes and the B-complex. Identification and monitoring of homeostasis of the immune system in response to major genes (Haunshi *et al.*, 2002) can help to reduce the inherent dangers in breeding programmes to upgrade indigenous breeds for higher productivity using chickens with high yielding exotic gene material. Such studies can assist in safeguarding genetic diversity in poultry (Dietert *et al.*, 1994).

In the present study, we assessed B-haplotypes and serum haemolytic complement titres of eight genotypes of the German Dahlem Red (GDR) line with combinations of three major genes of tropical interest. CPW and APW titres were measured since they have an essential role in innate host defence against infectious agents (Kinoshita, 1991; Ochs *et al.*, 1993),

## MATERIALS AND METHODS

### *Chickens*

Day-old chickens were kindly provided by Professor Dr P. Horst. Chicks were transported from the Institute of Animal Sciences, Humboldt University of Berlin, Germany to Antwerp. The pullets were the offspring of a Dahlem Red experimental male line heterozygous for the naked neck ( $Nana^-$ ), frizzle ( $Ff^-$ ) and dwarf ( $dw^-$ ) genes, and a Rhode Island White female line, homozygous for the normal alleles of the three genes. Eight different combinations of genes for body size and feather coverage, constituting eight different genetic groups, were segregated (Table I).

### *Study design*

A total of 128 hens were allocated to eight groups of 16 individuals. The pullets were housed in cages with free access to feed (commercial diets) and water. The birds were vaccinated for Marek disease at hatch. Blood samples were collected at the age of 8 weeks. Serum was collected and kept at  $-80^{\circ}\text{C}$  until testing for haemolytic complement activity.

### *Complement assays*

Complement activity was determined with a haemolytic technique as described elsewhere (Demey *et al.*, 1993) using an adapted light-scattering method. Briefly, sera were diluted serially in appropriate buffers in flat-bottomed 96-well microtitre plates and incubated with sensitized sheep erythrocytes to measure CPW or rabbit erythro-

TABLE I  
Phenotype characteristics of the major genes studied

Group	Major genes	Phenotype characteristics
1	$nanaffDw^-$	Control line Dahlem Red (exotic)
2	$nanaffdw^-$	Dwarf line Dahlem Red
3	$NanaffDw^-$	Naked neck Dahlem Red
4	$Nanaffdw^-$	Naked neck and dwarf
5	$nanaFfDw^-$	Frizzled Dahlem Red
6	$nanaFfdw^-$	Frizzled dwarf
7	$NanaFfDw^-$	Frizzled and naked neck
8	$NanaFfdw^-$	Frizzled, naked neck and dwarf

cytes to measure APW. The plates were shaken in a Titertek (Flow Laboratories) every 30 min during the period of incubation. The results (the amount of light-scattering by erythrocytes upon lysis) were read at 655 nm in a microplate reader (BioRad model 3505). Readings were log–log transformed, and the haemolytic activity was expressed as the titre that lysed 50% of the erythrocytes ( $CH_{50}$  U/ml).

#### *MHC (BG) typing*

All birds were serologically haplotyped for MHC (BG) Class IV by direct haemagglutination in 96-well round-bottomed plates (Parmentier *et al.*, 2002). Briefly, blood samples were washed three times in phosphate-buffered saline (PBS). Twenty-five  $\mu$ l of 2% packed red cells in PBS (pH 7.2) and 25  $\mu$ l of antiserum were used per well. Before scoring, plates were shaken three times for 1, 2 and 3 min followed by 5, 15 and 45 min of rest, respectively.

#### *Statistical analysis*

Statistical analyses were carried out using Stata 8. Mean CPW and APW values were compared using negative binomial regression. Probability levels were Bonferroni-adjusted to account for the large number of comparisons made. Clustering and dendrogram construction was done using Euclidean distance and hierarchical agglomerative average linkage (StataCorp., 2003).

## RESULTS

All GDR phenotypes showed the MHC (B-complex) haplotype 21. In the dwarf ( $dw^-$ ) lines, some birds had B21 in combination with B19. The dwarf ( $dw^-$ ) gene seemed to be linked with the B19 haplotype. The dwarf ( $dw^-$ ) in combination with frizzled ( $Ff^-$ ) also contained the B15 haplotype (Table II).

The highest haemolytic CPW titres were found in the  $Dw^-$  hens, all containing B21 haplotypes (possibly being homozygous genotypes). These CPW titres were significantly different from those in hens bearing the B19B21 genotypes (i.e.  $dw^-$  hens). The lowest CPW levels were found in the dwarf non-frizzled ( $ffdw^-$ ) hens. GDR chickens phenotyped for  $NanaFfdw^-$  and  $nanaFfdw^-$ , both containing a combination of MHC (B) genes B15, B19 and B21, showed intermediate CPW titres, being between GDR lines containing B19B21 and GDR hens with B21 genotypes. Within the same  $ff^-$  phenotypes, significant differences were found between normal ( $Dw^-$ ) and dwarf ( $dw^-$ ) gene combinations. No differences in CPW levels were found within the  $Ff^-$  lines.

The highest haemolytic APW titres were also found in the  $Dw^-$  hens, but now the lowest APW titre was found in the DW- (and  $dw^-$ ) hens with the frizzled ( $Ff^-$ ) gene, albeit these hens also showed the B21 haplotype. The frizzled ( $Ff^-$ ) gene has a significantly negative influence on APW titres (Table II).

TABLE II  
Mean complement titres ( $\text{CH}_{50}$  U/ml  $\pm$  SE) in GDR hens and B-haplotypes determined

GDR line	Haplotypes	CPW	GDR line	Haplotypes	APW
<i>nanaffdw</i> <sup>-</sup>	B21	204 $\pm$ 12 <sup>a</sup>	<i>NanaFfDw</i> <sup>-</sup>	B19 B21	131 $\pm$ 4 <sup>a</sup>
<i>Nanaffdw</i> <sup>-</sup>	B21	222 $\pm$ 12 <sup>ab</sup>	<i>nanaFfdw</i> <sup>-</sup>	B19 B21	135 $\pm$ 5 <sup>a</sup>
<i>NanaFfdw</i> <sup>-</sup>	B15 B19 B21	239 $\pm$ 8 <sup>abc</sup>	<i>nanaFfDw</i> <sup>-</sup>	B15 B19 B21	139 $\pm$ 5 <sup>a</sup>
<i>nanaFfdw</i> <sup>-</sup>	B19 B21	241 $\pm$ 11 <sup>abc</sup>	<i>NanaFfdw</i> <sup>-</sup>	B15 B19 B21	143 $\pm$ 3 <sup>a</sup>
<i>NanaFfDw</i> <sup>-</sup>	B19 B21	255 $\pm$ 19 <sup>bcd</sup>	<i>nanaffdw</i> <sup>-</sup>	B21	186 $\pm$ 5 <sup>b</sup>
<i>nanaFfDw</i> <sup>-</sup>	B15 B19 B21	277 $\pm$ 10 <sup>cd</sup>	<i>Nanaffdw</i> <sup>-</sup>	B21	225 $\pm$ 9 <sup>c</sup>
<i>NanaffDw</i> <sup>-</sup>	B21	283 $\pm$ 11 <sup>cd</sup>	<i>NanaffDw</i> <sup>-</sup>	B21	239 $\pm$ 7 <sup>c</sup>
<i>nanaffDw</i> <sup>-</sup>	B21	299 $\pm$ 14 <sup>d</sup>	<i>nanaffDw</i> <sup>-</sup>	B21	246 $\pm$ 6 <sup>c</sup>

<sup>a-d</sup>Means with the same letter in a column shows no significant difference

Measuring the dissimilarity of complement titres resulted in a phenotypic segregation of frizzled (*Ff*<sup>-</sup>) and non-frizzled (*ff*<sup>-</sup>) lines. Regarding the MHC (B) haplotypes, segregation was found for hens with B19B21 and B15B19B21 genotypes, and a dispersion of the B21 haplotype (Figure 1).

## DISCUSSION

Chickens suffer at high ambient temperatures because their feather coverage hinders internal heat dissipation. The special use of major genes in breeding programmes to improve productive adaptability has been demonstrated (Horst, 1983). In general, naked neck (*Na*<sup>-</sup>) chickens are preferred in hot climates, with or without the combination with the *Ff*<sup>-</sup> gene. The dwarfing (*dw*<sup>-</sup>) gene has lesser effect. However, an increase in the effectiveness of one factor may result in reduced effectiveness of another factor (Gross and Siegel, 1988). In the present study, we found that the frizzled (*Ff*<sup>-</sup>) gene has a negative influence on APW, whereas the dwarf (*dw*<sup>-</sup>) gene negatively influenced CPW titres. The naked neck (*Nana*<sup>-</sup>) gene showed alternating influences on haemolytic complement status in combination with *Ff*<sup>-</sup> and/or *dw*<sup>-</sup> gene. The phenotype *nanaffDw*<sup>-</sup>, the German Dahlem Red exotic line without major genes, showed the highest complement status.

Haemolytic complement activity is important in early (innate) host defence. The complement system provides protection against viruses in mammals (Hirsch, 1982) and in birds (Skeeles *et al.*, 1979; Pandit *et al.*, 1997) against some forms of parasites (Touray *et al.*, 1994) and against bacteria (Holmskov and Jensenius, 1993; Patel and Jaiswal, 1994; Saxena *et al.*, 2000; Nolan *et al.*, 2002). Apart from various functions in the initiation of and as an effector of the innate immune system, complement

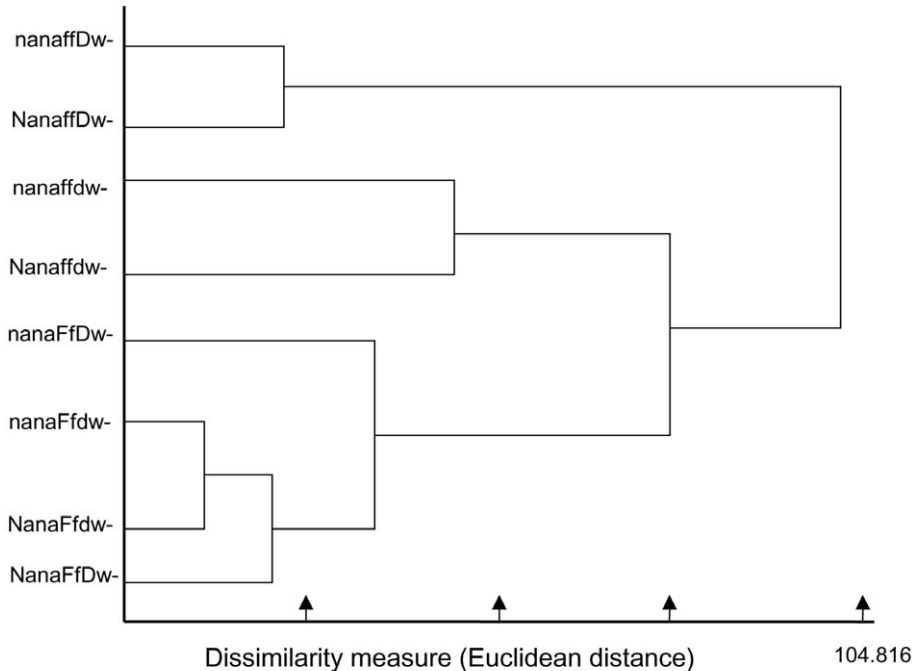


Figure 1. Dendrogram based on the complement titres of the eight GDR lines

components and receptors are involved in the initiation and regulation of the specific immune response of mammals (Thorbecke *et al.*, 1994) and probably of birds as well. Previously, we found statistical associations between CPW and APW titres, and the major histocompatibility (B) complex in eight pure-bred commercial Leghorn chicken lines and three ISA-Warren lines divergently selected for antibody responses to sheep red blood cells (Parmentier *et al.*, 2004). Significant differences in complement activities were found between pure-inbred lines and selection lines. Although effects of husbandry could not be excluded, we found high CPW and APW titres in chickens that were characterized by the B21 (and B2) haplotypes. Chickens with low CPW and APW corresponded with the B15 haplotype. The B15 was present in the *NanaFfdw*<sup>-</sup> and the *nanaFfdw*<sup>-</sup> GDR hens which showed low (nana) or intermediate (Nana) APW. Haplotypes B21 (and B2) are often found in birds with enhanced general immunity (Bacon and Witter, 1992, 1994a,b; Hepkema *et al.*, 1993), which may be related to the higher initial complement levels in birds with these haplotypes. The B15 haplotype is often related to a decreased immunocompetence (Bacon and Witter, 1992, 1993). This suggests that lower complement levels are related to lower disease resistance. In addition, we found a positive (cor)relation between APW and CPW in the commercial lines indicating (1) either common genetic or functional regulation and (2) that an

important part of the variety in complement levels between the several chicken breeds is related to unknown non-MHC ‘background’ genes, or alternatively is due to different husbandry conditions (Parmentier *et al.*, 2004). In the present study we found that major non-MHC related genes (*Nana*<sup>-</sup>, *dw*<sup>-</sup>, and *Ff*<sup>-</sup>) affected both CPW and APW. The frizzle gene negatively affected APW titres, but the CPW was negatively affected by the *dw*<sup>-</sup> gene. All chicks were characterized by the presence of the B21 haplotype. This implies that CPW and APW genes are not only different entities but are also regulated in different fashions.

The important role of complement in the initiation and regulation of specific immunity indicates the need for further studies to unravel functional and genetic relations between the B complex, complement levels and immune-mediated disease resistance in poultry. Identification of gene(s) encoding complement components, and studies on the expression of those genes, should reveal whether differences in disease resistance between poultry stocks are based on specific (antigen presentation, classical class I or II alleles) or non-specific innate (complement, TNF) parts of the chicken immune system, and interactions between these ‘immune response’ genes and major genes. The present study indicates that introducing major genes (*Nana*<sup>-</sup>, *dw*<sup>-</sup>, *Ff*<sup>-</sup>) of tropical interest, with the aim of overcoming environmental stress in hot climates for higher production, can have an impact on innate immunity and may thus have an influence on the immunocompetence of poultry.

## REFERENCES

- Bacon, L.D. and Witter, R.L., 1992. Influence of turkey herpesvirus vaccination on the B-haplotype effect on Marek’s disease resistance in 15.B congenic chickens. *Avian Diseases*, **36**, 378–385
- Bacon, L.D. and Witter, R.L., 1993. Influence of B-haplotype on the relative efficacy of Marek’s disease vaccines of different serotypes. *Avian Diseases*, **37**, 53–59
- Bacon, L.D. and Witter, R.L., 1994a. Serotype specificity of B-haplotype influence on the relative efficacy of Marek’s disease vaccines. *Avian Diseases*, **38**, 65–71
- Bacon, L.D. and Witter, R.L., 1994b. B haplotype influence on the relative efficacy of Marek’s disease vaccines in commercial chickens. *Poultry Science*, **73**, 481–487
- Beaumont, C., Guillaumin, S., Geraert, P.A., Mignon-Grasteau, S. and Leclercq, B., 1998. Genetic parameters of body weight of broiler chickens measured at 22°C or 32°C. *British Poultry Science*, **39**, 488–491
- Bordas, A. and Merat, P., 1984. Effects of the naked-neck gene on traits associated with egg laying in a dwarf stock at two temperatures. *British Poultry Science*, **25**, 195–207
- Cole, R.K., 2000. An autosomal dwarfism in the domestic fowl. *Poultry Science*, **79**, 1507–1516
- Cooper, M.A. and Washburn, K.W., 1998. The relationships of body temperature to weight gain, feed consumption, and feed utilisation in broilers under heat stress. *Poultry Science*, **77**, 237–242
- Deeb, N. and Cahaner, A., 2001. Genotype-by-environment interaction with broiler genotypes differing in growth rate. 1. The effects of high ambient temperature and naked-neck genotype on lines differing in genetic background. *Poultry Science*, **80**, 695–702
- Demey, F., Pandey, V.S., Baelmans, R., Agbede, G. and Verhulst, A., 1993. The effect of storage at low temperature on the haemolytic complement activity of chicken serum. *Veterinary Research Communications*, **17**, 37–40
- Dietert, R.R., Golemboski, K.A. and Austic, R.E., 1994. Environment–immune interactions. *Poultry Science*, **73**, 1062–1076
- Fussell, L.W., 1998. Poultry industry strategies for control of immunosuppressive diseases. *Poultry Science*, **77**, 1193–1196

- Garces, A., Casey, N.H. and Horst, P. 2001. Productive performance of naked neck, frizzle and dwarf laying hens under various natural climates and two nutritional treatments. *South African Journal of Animal Science*, **31**, 174–180
- Gowe, R.S. and Fairfull, R.W., 1995. Breeding for resistance to heat stress. In: N.J. Daghir (ed.), *Poultry Production in Hot Climates*, (Wallingford, Oxon, CAB International), 11–29
- Gross, W.B. and Siegel, P.B., 1988. Environment–genetic influences on immunocompetence. *Journal of Animal Science*, **66**, 2091–2094
- Haunshi, S., Sharma, D., Nayal, L.M., Singh, D.P. and Singh, R.V., 2002. Effect of naked neck gene (NA) and frizzle gene (F) on immunocompetence in chickens. *British Poultry Science*, **43**, 28–32
- Hepkema, B.G., Blankert, J.J., Albers, G.A.A., Tilanus, M.G.J., Egberts, E., Van der Zijpp, A.J. and Hensen, E.J., 1993. Mapping of susceptibility to Marek's disease within the major histocompatibility (B) complex by refined typing of White Leghorn chickens. *Animal Genetics*, **24**, 283–287
- Hirsch, R.L., 1982. The complement system: its importance in the host response to viral infection. *Microbiological Reviews*, **46**, 71–85
- Holmskov, U. and Jensenius, J.C., 1993. Structure and function of collectins: humoral C-type lectins with collagenous regions. *Behring Institute Mitteilungen*, **Dec(93)**, 224–235
- Horst, P., 1983. The concept of 'productive adaptability' of domestic animals in tropical and subtropical regions. *Journal of the South African Veterinary Association*, **54**, 159–164
- Khan, A.G., Tiwari, R.N., Baghel, K.K. and Gupta, R.D., 1987. Influence of the dwarfing gene dw on egg production and viability under summer heat stress. *British Poultry Science*, **28**, 541–546
- Kinoshita, T., 1991. Biology of complement: the overture. *Immunology Today*, **12**, 291–295
- Lamont, S.J., 1994. Poultry immunogenetics: which way do we go? *Poultry Science*, **73**, 1044–1048
- Mathur, P.K. and Horst, P., 1994. Genotype by environment interactions in laying hens based on relationship between breeding values of sires in temperate and tropical environments. *Poultry Science*, **73**, 1777–1784
- May, J.D., Lott, B.D. and Simmons, J.D., 1998. The effect of environmental temperature and body weight on growth rate and feed:gain of male broilers. *Poultry Science*, **77**, 499–501
- Mwalusanya, N.A., Katule, A.M., Mutayoba, S.K., Mtambo, M.M., Olsen, J.E. and Minga, U.M., 2002. Productivity of local chickens under village management conditions. *Tropical Animal Health and Production*, **34**, 405–416
- Nolan, L.K., Giddings, C.W., Horne, S.M., Doetkott, C., Gibbs, P.S., Wooley, R.E. and Foley, S.L., 2002. Complement resistance, as determined by viable count and flow cytometric methods, and its association with the presence of ISS and the virulence of avian *Escherichia coli*. *Avian Diseases*, **46**, 386–392
- Ochs, H.D., Nononyama, S., Zhu, Q., Farrington, M. and Wedgwood, R.J., 1993. Regulation of antibody responses: the role of complement and adhesion molecules. *Clinical Immunology and Immunopathology*, **67**, S33–40
- Pandit, F., Mishra, S.C. and Jaiswal, T.N., 1997. Effect of glucosaminyl muramyl dipeptide in hemolytic complement activity against fowl pox. *Indian Veterinary Medicine Journal*, **21**, 265–268
- Parmentier, H.K., Baelmans, R., Nieuwland, M.G., Dorny, P. and Demey, F. 2002. Haemolytic complement activity, C3 and Factor B consumption in serum from chickens divergently selected for antibody responses to sheep red blood. *Veterinary Immunology and Immunopathology*, **90**, 91–100
- Parmentier H.K., Baelmans, R., Savelkoul, H.F.J., Dorny, P., Demey, F. and Berkvens, D., 2004. Serum haemolytic complement activities in 11 different MHC (B) typed chicken lines. *Veterinary Immunology and Immunopathology*, **100**, 25–32
- Patel, S.M. and Jaiswal, T.N., 1994. Complement level in sera of chicks during immunization with oil adjuvant vaccine and challenge infection with *Pasteurella multocida*. *Indian Veterinary Journal*, **71**, 301–303
- Ponsuksili, S., Wimmers, K. and Horst, P. 1996. Valuation of different combinations of oligonucleotide probes and restriction enzymes to generate DNA fingerprints reflecting genetic variability in different strains of chicken. *Archiv für Geflügelkunde*, **60**, 227–235
- Saxena, V.K., Nath, M., Singh, H. and Dev-Roy, A.K., 2000. Immunocompetence based genetic divergence among guinea fowl varieties, desi fowl and commercial broilers. *Indian Journal of Poultry Science*, **35**, 236–239
- Sheldon, B.L., 2000. Research and development in 2000: directions and priorities for the world's poultry science community. *Poultry Science*, **79**, 147–158
- Skeeles, J.K., Lukert, P.D., De Buyssher, E.V., Fletcher, O.J. and Brown, J., 1979. Infectious bursal viral infections. II. The relationship of age, complement levels, virus-neutralising antibody, clotting and lesions. *Avian Diseases*, **23**, 106–117
- StataCorp., 2003. *Stata Statistical Software: Release 8.0*, (Stata Corporation, College Station, TX)



- Thorbecke, G.J., Amin, A.R. and Tsiagbe, V.K., 1994. Biology of germinal centers in lymphoid tissue. *FASEB Journal*, **8**, 832–840
- Touray, G.M., Douglas, C.S. and Miller, L.H., 1994. *Plasmodium gallinaceum*: differential lysis of two developmental stages of malaria sporozoites by the alternative pathway of complement. *Experimental Parasitology*, **78**, 294–301
- Wimmers, K., Ponsuksili, S., Hardge, T., Valle-Zarate, A., Mathur, P.K. and Horst, P., 2000. Genetic distinctness of African, Asian and South American local chickens. *Animal Genetics*, **31**, 159–165
- Yalcin, S., Settar, P., Ozkan, S. and Cahaner, A., 1997. Comparative evaluation of three commercial broiler stocks in hot versus temperate climates. *Poultry Science*, **76**, 921–929
- Yunis, R. and Cahaner, A., 1999. The effects of the naked neck (Na) and frizzle (F) genes on growth and meat yield of broilers and their interactions with ambient temperatures and potential growth rate. *Poultry Science*, **78**, 1347–1352
- Zekarias, B., Ter Huurne, A.A., Landman, W.J., Rebel, J.M., Pol, J.M. and Gruys, E., 2002. Immunological basis of differences in disease resistance in the chicken. *Veterinary Research*, **33**, 109–125

(Accepted: 9 February 2004)

**Les taux de complément hémolytique du sérum chez des poulets Dahlem rouges allemands sont affectés par trois gènes majeurs (cou nu, nain, frisé) d'intérêt tropical**

**Résumé** – Des poulets Dahlem rouges allemands ayant trois gènes majeurs d'intérêt tropical : *Nana*<sup>-</sup> (cou nu), *Ff*<sup>-</sup> (frisé) et *dw*<sup>-</sup> (nain), respectivement, ont été testés pour déterminer le complément hémolytique du sérum, qui est essentiel pour la défense innée de l'hôte contre des agents infectieux. Huit différentes combinaisons de gènes déterminant la taille du corps et la couverture en plumes ont été évaluées. Des différences significatives pour à la fois les titres dépendants du calcium (classique, CPW) et indépendants du calcium (alternatif, APW) ont été constatés entre les phénotypes. Le phénotype *nanaffDw*<sup>-</sup> a manifesté le statut de complément le plus élevé. Le gène frisé (*Ff*<sup>-</sup>) a eu une influence négative sur les titres APW, tandis que le gène nain (*dw*<sup>-</sup>) a eu une influence négative sur les titres CPW. Le cou nu (*Nana*<sup>-</sup>) a présenté diverses influences sur le statut du complément hémolytique. Tous les poulets testés avaient 21 haplotypes (B), tandis que le gène du nanisme a semblé être lié au phénotype B19. Il en a été conclu que l'introduction de gènes majeurs (*Nana*<sup>-</sup>, *Ff*<sup>-</sup> et *dw*<sup>-</sup>) pour conquérir le stress environnemental dans les climats chauds pouvait avoir un impact négatif sur certains aspects de l'immunité innée de la volaille.

**Los niveles de complemento hemolítico del suero en pollos rojos alemanes Dahlem están afectados por tres genes principales (cuello desnudo, enano, rizado) de interés tropical**

**Resumen** – Pollos de raza German Dahlem Red, con tres genes principales y distintos de interés tropical: *Nana*<sup>-</sup> (cuello desnudo), *Ff*<sup>-</sup> (rizado) y *dw*<sup>-</sup> (enano), fueron analizados para ver el complemento hemolítico del suero, el cual es esencial en la defensa innata del huésped contra agentes infecciosos. Se evaluaron ocho combinaciones diferentes de genes para tamaño corporal y cobertura de plumas. Se encontraron diferencias significativas entre los fenotipos para los títulos de complemento por vía clásica calcio-dependiente (CPW, en inglés) y para los títulos de complemento por vía alternativa calcio-independiente (APW, inglés). El fenotipo *nanaffDw*<sup>-</sup> mostró el estatus de complemento más alto. El gen rizado (*Ff*<sup>-</sup>) tenía una influencia negativa en los títulos de APW, mientras que el gen enano (*dw*<sup>-</sup>) tenía una influencia negativa en los títulos de CPW. El gen cuello desnudo (*Nana*<sup>-</sup>) tenía distintas influencias en el estatus del complemento hemolítico. Todas las gallinas analizadas tenían 21 haplotipos de complejo principal de histocompatibilidad (B) o MHC (B), mientras que el gen de enanismo aparecía ligado con el haplotipo B19. Se concluyó que el introducir genes importantes (*Nana*<sup>-</sup>, *dw*<sup>-</sup>, *Ff*<sup>-</sup>) para superar el estrés medioambiental en climas calurosos puede tener un impacto negativo en ciertos aspectos de la inmunidad innata de las aves de corral.