Morphological Characteristics and Growth Performance of F$_1$ Hybrids of Red Junglefowl Cocks Crossed with Fayoumi or H'mong Hens

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ABSTRACT: *Red Junglefowl (Gallus gallus) is considered as the direct ancestor of domesticated chickens. There is a wide speculation on the on-going gene flow between Red Junglefowls in the wild and some neigbouring native chicken populations in South and Southeast Asia. This may challenge the reliability of specific morphological markers to differentiate them and may have implications on the utility value of Red Junglefowls in the genetic improvement for disease resistance and adaptive traits of domestic chickens. The intensive monitoring and measurements of morphological and morphometric characteristics of 100 F$_1$ hybrid birds each from two mating groups involving Red Junglefowl cocks crossing with Fayoumi or H'mong hens were performed from day-old chicks to 12-week-old pullets in this study. The morphological data showed that the typical down feather pattern in striped phenotype as for camouflage, that is critical to the survival of Red Junglefowls in the wild, was not observed in any hybrid chick, suggesting that this specific phenotype is likely controlled by a recessive allele and thus a morphological marker for the identification of true Red Junglefowls in their early development stages or life. The high survival rate and improved growth performance of the two F$_1$ hybrid groups demonstrated the potential value of Red Junglefowls in reconstituting the genetic diversity and possibly improving the adaptability of domestic chickens. Therefore the unique wild relative resources that are able to survive in the increasingly fragmented jungles in South and Southeast Asia warrant further exploration and great attention for in situ conservation and sustainable exploitation.*

Keywords: Red Junglefowl, chicken, hybrid, morphology, performance.

INTRODUCTION

Molecular genetic evidence suggested Red Junglefowl (*Gallus gallus*) to be the direct ancestor of domesticated chicken (*Gallus gallus domesticus*) (Fumihito *et al*., 1994, 1996; Nishibori *et al*., 2005). Since all the four species of genus *Gallus* are exclusively distributed in South and Southeast Asia, the domestication of chicken was believed to have taken place in this region (Hillel *et al*., 2003; Sawai *et al*., 2010). Grey Junglefowl (*G. sonnerati*) and Sri Lankan Junglefowl (*G. lafayettei*) were also thought to have contributed to the gene pool of domestic chicken through inter-species hybridization (Nishibori *et al*., 2005), resulting in specific phenotypic traits, for example, the yellow skin found in some chicken breeds/populations (Eriksson *et al*., 2008). Based on the ancient mitochondrial DNA polymorphism which antedated the separation between Red Junglefowl and Green

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Junglefowl (*Gallus varius*) which is the last species in the genus *Gallus*, the latter was found not being involved in the process of chicken domestication (Fumihito *et al.*, 1994; Nishibori *et al.*, 2005).

Starting from domestication, there have been tremendous achievements in the genetic improvement of domestic chickens from primary breeds into modern commercial layer and broiler strains/lines in the second half of the 20th century through classical quantitative genetics techniques from the late 1940s' and by the advent of high speed computer in 1960' (Havenstein, 2006; Hunton, 2006). Around 85-90% of the improvement for growth rate in broilers could be attributed to genetic selection (Sherwood, 1977; Havenstein, 2006). The high reproductive rates and short generation times of chickens have enabled the rates of achievements observed over past 50 years (Zuidhof *et al.*, 2014). However, such improvements in broiler and layer productive performance have resulted in a negative adaptive immune response (antibody production) of the improved birds, though the cell-mediated and inflammatory responses were observed to have increased (Cheema *et al.*, 2003). The intensive genetic selection for increased reproductive and growth have also led to a significant decrease of 50% or more in the genetic diversity in commercial pure lines compared to their ancestors due to the limited number of incorporated primary breeds as well as increased inbreeding within the commercial lines (Muir *et al.*, 2008). This may compromise the future success in commercial poultry industry because non-commercial chicken breeds, which have very different economic trait profiles, are thus hardly used to reconstitute or restore the genetic variation that have been lost during pure line breeding by a small number of international companies (Muir *et al.*, 2008).

Because of the long-term interaction and evolutionary balance between disease causing agents and the hosts, there are low incidences of parasitic or infectious diseases in a population of free-ranging wild animals, thus leading to a general believe that compared to domestic breeds, wildlife has strong genetic resistance to diseases. It is understood that genetic variations in disease resistance of wildlife, as well as farm animals to some extent, are expressed at all levels of defense against infectious agents. However, genetic resistance/susceptibility to infections is a polygenic trait, hence is difficult to be measured and improved. Given the advent of new genomic tools, exploration of the genetic component of relevant wildlife species through smart hybridization to restore such resistance to diseases and to improve the adaptability to climatic changes through enhanced innate immunity in domesticated animals would be an option. This effort is not only important in sustaining and intensifying the smallholder livestock and backyard poultry production systems in developing countries but also very unique in the recognition of the additional value of wild relatives of livestock and poultry. Moreover, the domestication of wild relatives of livestock and poultry species can be considered as continuous and enhanced exploration and utilization of such species which were otherwise simply being hunted and extracted.

To investigate the changes in morphology and basic productive performance in F1 hybrid birds of wild Red Junglefowl and domesticated chicken, two experiments were conducted through hybridizing the Red Junglefowl cocks with the hens of two chicken breeds (Fayoumi and H'mong) with very different phenotypes and productive performance. The Red Junglefowl has a special value as ornamental bird as well as in traditional medicine where its legs and meat are commonly used to prepare therapeutic tonic for the treatment of tropical diseases such as cholera and dysentery as well as body weaknesses (Thieu *et al.*, 2011). The Fayoumi chicken is a very old and good white-egg-layer breed (170-180 eggs/hen/year) originated in Egypt. It has strong adaptability to hot climates and is suitable for backyard farming. A breedin flock of Fayoumi chicken was introduced to Vietnam in 1974.

The H'mong chicken is a local, indigenous breed raised by H'mong ethnic group in northern Vietnam. It has a popular flavor and the skin, meat and bones are all black in color. A high and distinct genetic diversity was detected in the H'mong chickens compared to other exotic and Vietnamese local chicken breeds (Granevitze et al., 2007; Berthouly et al., 2009; Cuc et al., 2010; Thuy et al., 2012). A large number of microsatellite alleles shared between the H'mong chickens and Red Junglefowls from Thailand and Vietnam, and a similar genetic admixture pattern observed in four out of the 30 local H'mong chicken populations with the Red Junglefowls suggested a frequent gene flow between them (Berthouly et al., 2009). This may explain the claim of genetic endangerment of wild Red Junglefowls based on the examination of 745 museum specimens, which suggested that most wild Junglefowl populations have been contaminated genetically by genes/alleles from domestic or feral chickens (Peterson and Brisbin, 1998). The current study was therefore undertaken to identify and confirm species specific m
orphological markers such as the eclipse plumage (Morejohn, 1968) in wild Red Junglefowl.

MATERIAL AND METHODOLOGY

The experiments were conducted at the National Institute of Animal Science, Hanoi, Vietnam under the experimental flock condition. Several Red Junglefowl cocks and hens were freshly captured from the mountains in northwestern Vietnam for the experiments. Two groups of 100 healthy F_1 hybrid chicks each were selected from two mating groups of Red Junglefowl cocks crossing with either Fayoumi (designated as G1) or H'mong (G2) hens. All F_1 birds were raised under the same intensive management conditions from day-old stage to 35 weeks of age.

The two F_1 groups were evaluated for morphological (appearance and color of plumage and skin) and morphometric (mortality and growth) characteristics using the standard parameters. The plumage morphology was observed by visual recording of the characteristics from day-old stage (down feathers) to the 12th week of age (after secondary feather development). Photographs were taken on weekly basis for easy reference of the characteristics. Survival rate of chicks was calculated on weekly basis and for the overall experimental period.

Body weight of individual birds was taken in the first day of every week starting from day-old stage to 12 weeks of age. The growth rates were estimated by calculating absolute and relative growth using the equations 1 and 2. Data were analysed using the Microsoft Excel 2010 program (Microsoft, Redmond, WA, USA).

Absolute growth (A) (g/chicken/day):

$$A = \frac{W_1 - W_0}{t} \quad \text{..} \quad \text{Eq 1}$$

where, W_1 = weight of a bird at the time t1 (g),
W_0 = weight of a bird at the time t0 (g),
t = time between two time scales (days).

Relative growth (R):

$$R = \frac{W_1 - W_0}{(W_1 - W_0)/2} \times 100 \, (\%) \qquad \text{…………………………..} \; Eq \; 2$$

where,

W_0 = beginning weight of a bird,
W_1 = ending weight of a bird.

RESULTS AND DISCUSSION

The plumage patterns and feather colors of the two F_1 groups could be clearly differentiated (Fig. 1 and Table 1), as previously observed by Morejohn (1955) in the hybrids resulting from the cross between Red Junglefowl and Brown Leghorns. In the present study, day-old F_1 chicks of G1 showed mainly white-spotted color pattern in down feathers followed by dark brown and other mixed colors. On the other hand, black down feathers were predominant in G2 chicks. It appears that the maternal contribution of two chicken breeds was heavily expressed in the down feathers which usually start to grow in eight-day-old embryos. However, it is worth noting that none of the chicks from these two mating groups expressed the camouflage down feather pattern typically observed in pure Red Junglefowls, suggesting that this particular trait in Red Junglefowl is controlled by a recessive allele and can be considered as a morphological marker for the identification of true Red Junglefowls (Morejohn, 1955; Choicharoen *et al.*, 2009).

Using the morphological characteristics of Red Junglefowls in South and Southeast Asia as described by Nishida *et al.* (1992, 2000), it could be suggested that the freshly captured Red Junglefowls in the present experiment from northwestern Vietnam demonstrated a genetic purity. Based on the external morphological characters, Condon (2012) once proposed bill length, comb height and comb length to be effective characters for identifying the introgression of domestic chicken in female Red Junglefowls and bill length and spur width to be most effective for male Red Junglefowls. However, these quantitative traits may not be readily applied in determining the level of introgression in Red Junglefowl. The search for the so-called genetically pure Red Junglefowl in the wild has therefore proven to be very challenging (Layler, 2012).

Fig. 1. **Morphology of the two groups of F_1 hybrid birds. A - G1 chicks; B – G2 chicks. Note: three artificially hatched Red Junglefowl chicks with a striped phenotype are also shown in the figure and indicated within red circles.**

At adulthoods, plumage color of chickens in G1 became further diversified with dark brown to be the major color in their feathers followed by black & white, blackish yellow, and yellowish brown. Blackish yellow color was predominant followed by black and dark brown colors in G2. It seems that the paternal influence of Red Junglefowl only started to be functional after the first juvenile molt in all chicks from one to five weeks of age.

Table 1. Percentage variations in feather colors of F_1 hybrid birds (numbers are given in parenthesis)

Group	Day-old chicks				Chickens at 12 weeks of age				
	White-dotted	Black	Deep brown	Others	Dark Brown	Black & white	Black & yellow	Yellow & brown	Black
G1	55% (55)	33% (33)		12% (12)	44% (38)	27% (23)	19% (16)	11% (9)	
G2		85% (85)	12% (12)	3% (3)	17% (14)	5% (4)	51% (43)	4% (3)	24% (20)

Based on the changes in plumage colors of birds from day-old to adult in this study, no correlation was observed in majority of the birds, implying that different sets of genes might be responsible for determining the feather colors and patterns at different ages of development of domestic chicken and Red Junglefowl.

Nevertheless, the skin colours of all F_1 hybrids showed a pattern of simple inheritance, and no change was observed from day-old chicks to 12-week-old stage. All birds in G1 carried only white skin color whereas two types of skin colors were present in G2 where 54% white skin and 46% black skin were observed. Given the skin colors of parental stocks (light pink or white in Red Junglefowl and Fayoumi; black in H'mong), a dominant (black) and recessive (white or pink) pattern of inheritance in skin color could be postulated assuming that H'mong was heterozygote to explain the observation of this study.

High survival rates (around 84%) were observed of two groups of F_1 birds despite slight variations during the twelve-week growing period (Fig. 2), indicating the strong adaptability to the management and feeding regimes of F_1 crossbreds. This finding is supporting the observation made by Mekchay *et al*. (1997, 1999) who determined the activity of alternative complement pathway (ACP50) in Red Junglefowl, Thai native chickens, and commercial layers and broilers. Their findings suggested that both Red Junglefowl and Thai native chickens showed higher activities in ACP50 and higher variations of natural immunity than commercial birds.
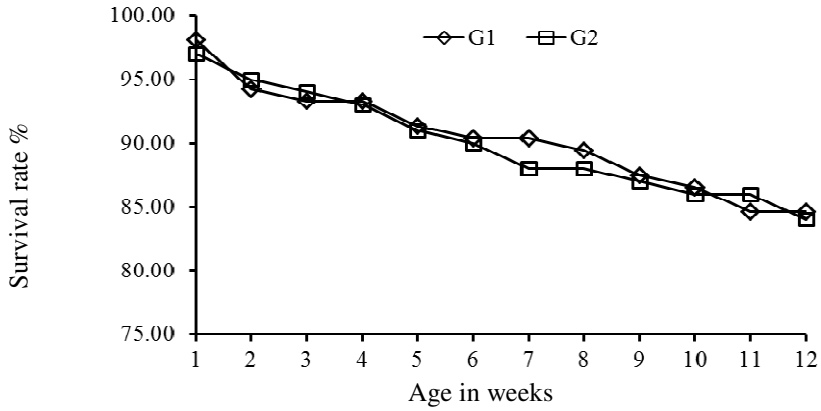
Fig. 2.   Survival rates of two groups of F$_1$ hybrid birds from the 1st to the 12th weeks of age.

The average body weights of day-old chicks of G1 and G2 were 29.2 and 28.0 g, respectively. At 12 weeks of age, the average weight of G1 chicken was 989.3 g which was approximately 30 grams higher than that of the G2 chickens (958.6 grams). These values were almost twice the weights of captive Red Junglefowls at respective ages (Choicharoen *et al.*, 2009). As shown in Fig. 3, the growth rates of the two groups of birds from their first to the sixth weeks of age were almost equal; however, they started to differentiate from the 7th week when G1 started to grow faster than G2. The relative growth rate significantly differed between the two groups in the first six weeks but more or less leveled out after the 6th week (Fig. 4).
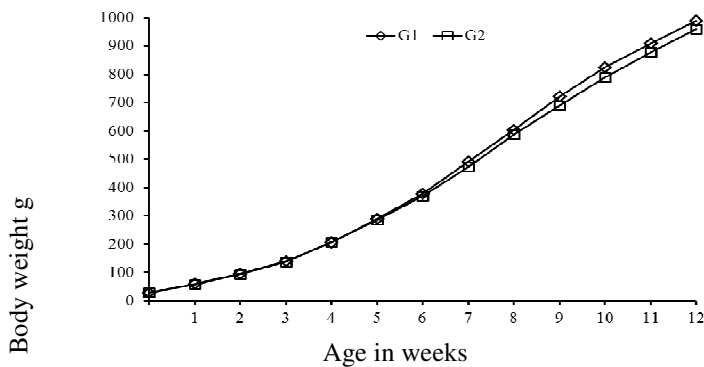

Fig. 3.   Changes in body weights (in grams on Y-axis) from day-old chicks to 12-week-old (X-axis) pullets.
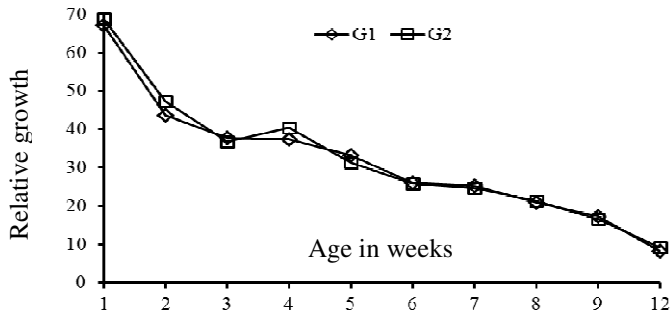
Fig. 4. **Relative growth rate of two groups of F_1 hybrid birds from the 1st to the 12th weeks of age.**

The absolute growth of the two groups increased from day-old stage to eight or nine weeks of age and then steadily declined. Nevertheless, the G1 birds grew faster than G2 birds between 11 and 12 weeks of age (Fig. 5), contributing to a relatively large body size of G1 birds at the end of the experimental period (Fig. 3).
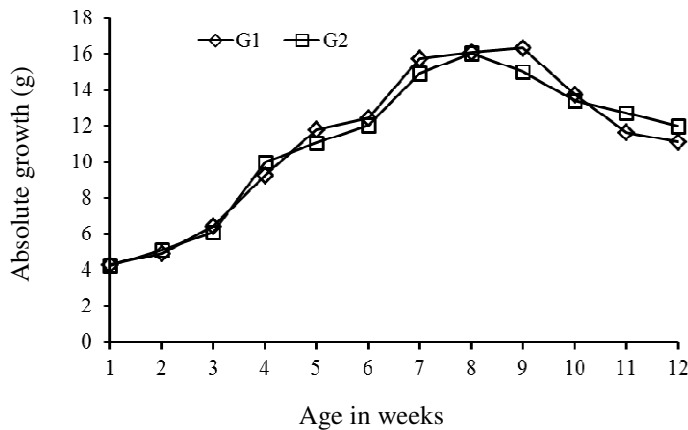

Fig. 5. **Changes of absolute growth of G1 and G2 birds with the age**.

Possible natural hybridization between Red Junglefowl and native chickens in South and Southeast Asia has been widely speculated and investigated (Nishida *et al*., 1992, 2000; Peterson and Brisbin, 1998; Brisbin *et al*., 2002; Berthouly *et al*., 2009; Condon, 2012). Molecular data also established a close genetic relationship between Red Junglefowl and some of the native chicken populations in the region compared to European breeds and commercial lines (Romanov and Weigend, 2001; Moiseyeva *et al*., 2003; Okumura *et al*., 2006; Berthouly *et al*., 2009; Akaboot *et al*., 2012). However, the results of the present study showed that none of the F_1 chicks produced by Junglefowl cocks mating with either Fayoumi or H'mong hens expressed the striped phenotype, which is likely to be determined by a recessive allele. This particular phenotype is a camouflage for chicks and is critical to the survival of Red Junglefowl in the wild. The survival rates of the two groups were high, and the growth performance of all F_1 pullets was significantly higher than that of the Red Junglefowl (data not shown). These findings clearly demonstrate the potential use and value of Red Junglefowl for the genetic enhancement of disease resistance and adaptive traits in domestic chicken.

CONCLUSIONS

The typical down feather pattern with striped phenotype which is critical for camouflage and the survival of Red Junglefowl in the wild was not observed in any F_1 chicks, indicating that this particular phenotype is controlled by a recessive allele. Thus, striped phenotype could be a reliable morphological marker for the identification of true Junglefowl in their early development. The high survival rates and significantly improved growth performance observed among F_1 birds in the present study proved the relative genetic integrity and potential value of Red Junglefowl to be explored for the genetic improvement of adaptive traits in domestic chicken.

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