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## Developmental Stability in Chickens Local to Warm Climate 1. Variation in Internal Organs and Bilateral Traits of Lines Selected Short-Term for Growth

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**Abstract:** This study aimed at adopting the developmental changes in the internal organs and bilateral traits of growing chickens selected five generations for increased 6-week body weight and genotyping for normal feathering (line F) or naked-necks (line N), compared to their corresponding genetic controls (lines CF and CN). Line N had the smallest weight percentages of total lungs and liver (0.54 and 2.38%) and biggest weight percentages of right and left shanks (2.46 and 2.48%). Line F had the smallest spleen weight percentage (0.20%) and tallest right and left shanks (8.50 and 8.57 cm) and widest right and left shanks (3.74 and 3.75 cm). Gizzard weight percentages of lines N and CN (3.37 and 3.55%) were significantly more than those of lines F and CF (2.74 and 3.00%). The results indicated that neither short-term selection nor *Na* allele have influenced the development of heart. *Na* allele showed variable pleiotropic effects, where the weight percentages of lungs and liver were decreased and the weight percentages of gizzard and spleen were increased. A reduction in lung and liver weight percentages characterized the naked-neck selected line, revealing an interaction between selection and *Na* allele. The pressure of short-term selection for body weight was early noticeable on the development of liver and spleen that could initiate possible metabolic disorders or susceptibility to disease infection later with the continuity of selection scheme. Short-term selection albeit boosted the bilateral shank measurements, but did not influence the bilateral developmental stability. The developmental stability characterized the bilateral characters in chickens carrying *Na* allele, suggesting a role of *Na* allele in the developmental stability of the birds pertaining the natural heating waves. The results of correlation coefficients indicated that the bilateral asymmetries of different characters are not significantly associated.

**Key words:** Bilateral asymmetry, developmental stability, selection, *Na* allele

### INTRODUCTION

All multi-cellular organisms undergo complex patterns of development. These patterns are especially noticeable in rapidly growing organisms, such as the broiler chickens, because growth is associated with continual remodeling in the proportions and functional importance of tissues (Tomas and Pym, 1995; Møller and Swaddle, 1997; Solomon *et al.*, 2002). The development could be perturbed at any stage if the organisms are stressed by genetic or environmental means (Yost, 1995). Therefore lines derived under stress of selection possibly endure disruption in the developmental homeostasis (Lerner, 1954; Fairfull, 1990), expressed in a variety of different phenomena. Selection for body weight in growing chickens has altered the expression of growth-related hormones (McGuinness *et al.*, 1995; Zhao *et al.*, 1995a; 1995b) and there has been an association of the proliferating muscle satellite cells of fast growing chicks and the great stimulation of DNA synthesis (Duclos *et al.*, 1995). Intensive selection for rapid growth and egg production have been contributed to skeletal, metabolic and reproductive disorders, bilateral trait asymmetries, susceptibility to disease infection, incidence of leg problems and ascites in meat stocks and osteomalacia

and fatty livers in layers (El-Gendy, 1992; Møller and Swaddle, 1997; Siegel and Dunnington, 1997; Yang *et al.*, 1997; Sewalem *et al.*, 1998; Sewalem and Wilhelmson, 1999; Hunton, 2006). Also, Sudden death syndrome and the development of adipose stores have been observed in rapidly growing broilers (Olkowski and Classen, 1995; Sizemore and Barbato, 2002). In growing turkeys, selection for body weight was negatively associated with the walking ability (Nestor, 1984) and this was accompanied by an increase of leg problems which could have also reduced potential genetic gain in body weight (Nestor and Anderson, 1998).

Both sides of a bilateral trait may lack identity when stressed, although they are anticipated to be symmetrical because they are products of the same genome (Leary and Allendorf, 1989). The degree of asymmetry in bilateral morphological characters may reflect perturbed development due to genetic or environmental stressors. Based on the distribution and significance of the differences between both sides, Van Valen (1962) categorized the asymmetry in bilateral traits into fluctuating asymmetry, directional asymmetry and anti-symmetry. In chickens, bilateral asymmetry and homozygosity of genes are positively correlated (Leary

and Allendorf, 1989; Parsons, 1992; Palmer, 1996; Palmer and Strobeck, 1986). In quail, however, bilateral asymmetry was little influenced by selection for increased or decreased body weight (Nestor *et al.*, 2002) and homozygosity did not influence the developmental stability.

The employment of local breeds in poultry production in some regions of the world is of growing concern. The information on developmental stability in local breeds in response to genetic stressors, although is greatly useful, is however limited. The objective of this study was to evaluate the developmental stability in chicken lines local to warm climate and genotyping for normal feathering or naked-necks and have undergone a short-term selection for growth.

## MATERIALS AND METHODS

**Genetic stocks:** Two lines of chickens selected five generations for increased 6-week body weight and their corresponding genetic control lines were used in this experiment. The selected lines were the normally Feathered (F) and Naked-neck (N) lines and the genetic controls were CF and CN lines. The base population from which the lines have been derived was formed by mating grandparent males of a fast growing normally feathered broiler strain to females of a naked-neck dual-purpose chicken population native to Egypt. The details of line formation is reported by El-Gendy (2009a).

**Management and measurements:** Chicks of the selected and control lines were hatched simultaneously and were placed in a floor pen in a conventional house at day old. All chicks received routine daily management and rations recommended for broilers. At 10 weeks of age, 16 males and 16 females of each line have been randomly selected, weighed, slaughtered by cutting the jugular vein and processed routinely. Weights of heart, liver, gizzard, spleen and right and left lungs were obtained for each chick. The weight, length and diameter (measured for the widest area) were also obtained for each of the right and left shanks. Weights of organs were then calculated as percentages of the live weight. Also, heart/lungs ratio was obtained as the ratio between heart weight and total lung weight. The developmental stability of the examined traits was assessed for the lines stressed by selection as the significant deviation from their corresponding control lines.

**Statistical analysis:** The general linear model (SAS, 1999) was applied to the data set to statistically analyze the effects of genetic line, sex and their interaction. When appropriate, the multiple range test of Duncan (1955) was used to assess the line differences. The differences in bilateral traits were examined for asymmetry according to Van Valen (1962) and t - test

was applied to examine the significance of deviation of mean differences between both sides from zero.

## RESULTS

Lines F and N weighed 1075 and 950 g respectively (Table 1) and were significantly heavier than their corresponding controls and lines F and CF were significantly heavier than lines N and CN. Heart weight percentage and heart/lungs ratio did not significantly differ among lines. The weight percentage of total lungs significantly differed among lines and was the biggest in line CF (0.70%) and the smallest in line N (0.54%). Liver weight percentage of line N (2.38%) was significantly less than that of line CN (2.78%) and this was not observed among lines F and CF. Gizzard weight percentages of lines N and CN (3.37 and 3.55%), were significantly more than those of lines F and CF (2.74 and 3.00%). Also, spleen weight percentage significantly differed among lines and was the biggest in line CN (0.25%) and the smallest in line F (0.20%). Except body weight, sex differences did not contribute to other trait variation.

No significant differences were observed between lines in any of the right and left lung weight percentages and in the difference between both sides (Table 2). The genetic lines significantly varied in the right and left shank weight percentages and line N was the heaviest in both parameters (2.46 and 2.48%) and line CF was the lightest (2.22 and 2.23%). Also lines significantly varied in right and left shank lengths and diameter. Line F had the tallest right and left shanks (8.50 and 8.57 cm, respectively) and widest right and left shank diameters (3.74 and 3.75 cm, respectively). But, the mean differences between both sides of shank weight, length and diameter were not significantly varied among lines. Aside from the measurements of shank length and diameter that were significantly higher in males than in females, sex effects on the other traits were not significant.

The bilateral traits were examined for asymmetry between both sides (Table 3). The differences between right and left lung weights were categorized as directional asymmetry in lines F and N, versus fluctuating asymmetry in line CF and anti-asymmetry in line CN. Also, directional asymmetry was scored for the differences in weight, length and diameter between right and left shanks of all lines, but the differences in shank weight of line CN was scored as anti-asymmetry. The correlation coefficients among the bilateral differences were in general insignificant, but the correlations among bilateral shank measurements in lines F and CF were significant and ranged between 0.42 and 0.54.

## DISCUSSION

A reduction in body weight was concomitant to *Na* allele, although the significant increases in body weights of the

Table 1: Effect of selection, *Na* allele and sex on the development of internal organs (LSM±SE)

Trait	Normally Feathered ( <i>na</i> ) Lines		Naked-Neck ( <i>Na</i> ) Lines		Sex Effect (p≤)
	Selected (F)	Control (CF)	Selected (N)	Control (CN)	
Body Weight, g	1075±39 <sup>a</sup>	890±33 <sup>b</sup>	950±46 <sup>c</sup>	848±37 <sup>d</sup>	0.0003
Heart, %	0.51±0.03 <sup>a</sup>	0.55±0.02 <sup>a</sup>	0.52±0.04 <sup>a</sup>	0.54±0.03 <sup>a</sup>	0.2841
Lungs, %	0.65±0.04 <sup>ab</sup>	0.70±0.04 <sup>a</sup>	0.54±0.06 <sup>b</sup>	0.65±0.05 <sup>ab</sup>	0.2377
Heart/Lungs	0.81±0.05 <sup>a</sup>	0.80±0.05 <sup>a</sup>	0.91±0.07 <sup>a</sup>	0.86±0.06 <sup>a</sup>	0.8248
Liver, %	2.43±0.13 <sup>ab</sup>	2.45±0.11 <sup>ab</sup>	2.38±0.15 <sup>b</sup>	2.78±0.12 <sup>a</sup>	0.8414
Gizzard, %	2.74±0.12 <sup>b</sup>	3.00±0.10 <sup>b</sup>	3.37±0.14 <sup>a</sup>	3.55±0.11 <sup>a</sup>	0.4426
Spleen, %	0.20±0.02 <sup>b</sup>	0.22±0.02 <sup>ab</sup>	0.23±0.02 <sup>ab</sup>	0.25±0.02 <sup>a</sup>	0.8830

<sup>a,b</sup>Means of different lines differ significantly (p<0.05)Table 2: Effect of selection, *Na* allele and sex on the development of bilateral traits (LSM±SE)

Trait	Normally Feathered ( <i>na</i> ) Lines		Naked-Neck ( <i>Na</i> ) Lines		Sex Effect (p≤)
	Selected (F)	Control (CF)	Selected (N)	Control (CN)	
Right lung, %	0.30±0.02 <sup>a</sup>	0.33±0.02 <sup>a</sup>	0.28±0.02 <sup>a</sup>	0.33±0.02 <sup>a</sup>	0.1706
Left lung, %	0.34±0.02 <sup>a</sup>	0.36±0.02 <sup>a</sup>	0.30±0.04 <sup>a</sup>	0.35±0.03 <sup>a</sup>	0.2116
Lung dif., g	0.79±0.21 <sup>a</sup>	0.22±0.20 <sup>a</sup>	0.59±0.29 <sup>a</sup>	0.46±0.24 <sup>a</sup>	0.4723
Right shank, %	2.39±0.07 <sup>ab</sup>	2.22±0.06 <sup>b</sup>	2.46±0.08 <sup>a</sup>	2.26±0.06 <sup>ab</sup>	0.4028
Left shank, %	2.39±0.07 <sup>ab</sup>	2.23±0.06 <sup>b</sup>	2.48±0.08 <sup>a</sup>	2.25±0.07 <sup>b</sup>	0.3753
Shank dif., g	0.37±0.14 <sup>a</sup>	0.26±0.12 <sup>a</sup>	0.54±0.17 <sup>a</sup>	0.27±0.14 <sup>a</sup>	0.1452
Right shank ln, cm	8.50±0.13 <sup>a</sup>	8.11±0.11 <sup>b</sup>	8.06±0.15 <sup>b</sup>	7.89±0.12 <sup>b</sup>	< 0.0001
Left shank ln, cm	8.57±0.13 <sup>a</sup>	8.13±0.11 <sup>b</sup>	8.03±0.15 <sup>b</sup>	7.85±0.12 <sup>b</sup>	< 0.0001
Shank ln dif., cm	0.06±0.02 <sup>a</sup>	0.07±0.02 <sup>a</sup>	0.06±0.02 <sup>a</sup>	0.06±0.02 <sup>a</sup>	0.1546
Right shank d., cm	3.74±0.06 <sup>a</sup>	3.54±0.05 <sup>b</sup>	3.68±0.06 <sup>ab</sup>	3.55±0.05 <sup>b</sup>	< 0.0001
Left shank d., cm	3.75±0.06 <sup>a</sup>	3.51±0.05 <sup>b</sup>	3.66±0.06 <sup>ab</sup>	3.57±0.05 <sup>b</sup>	< 0.0001
Shank d. dif., cm	0.05±0.02 <sup>a</sup>	0.06±0.01 <sup>a</sup>	0.06±0.02 <sup>a</sup>	0.07±0.02 <sup>a</sup>	0.0173

d. = diameter. <sup>a,b</sup>Means of different lines within rows differ significantly (p<0.05)

Table 3: Asymmetrical category and the correlation coefficients obtained for the bilateral traits

Trait	Normally Feathered ( <i>na</i> ) Lines		Naked-Neck ( <i>Na</i> ) Lines	
	Selected (F)	Control (CF)	Selected (N)	Control (CN)
<b>Asymmetrical category</b>				
L. wt dif, g	DA	FA	DA	AS
Sh. wt dif, g	---	---	DA	AS
Sh. ln dif, cm	DA	---	DA	DA
Sh. d. dif, cm	DA	DA	DA	DA
<b>Correlation coefficients</b>				
L. wt dif*Sh. wt dif	-0.26	-0.32	0.14	0.41
L. wt dif*Sh. ln dif	-0.24	0.06	-0.38	-0.32
L. wt dif*Sh. d. dif	-0.30	-0.01	0.08	0.32
Sh. wt dif*Sh. ln dif	0.32	0.29	-0.03	0.39
Sh. wt dif*Sh. d. dif	0.54*	0.43*	0.16	0.08
Sh. ln dif*Sh. d. dif	0.44*	0.42*	0.13	0.26

DA = directional asymmetry, FA = fluctuated asymmetry, AS = anti-asymmetry. L. = Lung, Sh. = Shank, d. = diameter, --- = type of asymmetry could not be obtained. \* = significant correlation coefficient (p&lt;0.05)

selected lines. The body weight responses to selection and *Na* allele are, however beyond the scope of this study. The results indicate that neither short-term selection nor *Na* allele have influenced the development of heart. The lung development seemed normal in all lines, although a reduction in lung weight percentage characterized the naked-neck selected line denoting that *Na* allele tends to suppress the development of lungs. Also, liver weight percentage of the naked-neck selected line was significantly less than that of the naked-neck control line and this was not observed among the normally feathered selected and control lines, revealing

an interaction between selection and *Na* allele on the development of liver. The *Na* allele was also associated with a significant increase in gizzard weight percentage. Selection has resulted in the suppression of spleen weight percentage in both selected lines, but with less magnitude in the naked-neck line. Thus the pressure of short-term selection for body weight was early noticeable on the development of liver and spleen. The early change in spleen size due to selection is possibly correlated with the susceptibility of selected chickens to disease infection. Ye *et al.* (1999) attributed the tumor development on the smooth muscles of quail selected

long term for body weight to the amplified genes or gene rearrangement occurred by selection. The *Na* allele showed variable pleiotropic effects on internal organ development, where the weight percentages of lungs and liver were decreased and the weight percentages of gizzard and spleen were increased. The pleiotropic effect of *Na* allele on the body composition and growth measurements were reported earlier by Mérat (1990). Selection, on short-term basis, obviously boosted the bilateral shank measurements and no evidence was shown for an effect of *Na* allele. The genetic association of selection, on long-term basis, for increased leg muscles and shank diameter with walking ability has been reported by Emmerson *et al.* (1991). Also, Ye *et al.* (1999) pointed out that the inferior walking ability in turkeys is associated with shorter and narrower shanks and wider breasts.

Directional asymmetry was scored for the differences between right and left sides of the examined bilateral traits of normally feathered and naked-neck selected lines. Also, directional asymmetry was scored for most of the bilateral shank measurements of both control lines. This concludes that directional asymmetry has an inheritance basis rather than being attributed to the genetic selection. The genetic bases of directional asymmetry and anti-asymmetry have been also suggested by Palmer and Strobeck (1992), Palmer (1996) and Møller and Swaddle (1997), so they are not as useful for the measurement of developmental stability. The results propose that short-term selection did not influence the developmental stability of bilateral traits. Since the base population, from which the control lines have been derived, was a randombred population. El-Gendy (2009b) estimated a heterozygosity level of 0.416 for that base population. So, the homozygosity is most likely high in the control lines. The fluctuating asymmetry scored for the weight differences between right and left lungs of the normally feathered control line is possibly associated with the high homozygosity. The positive association between fluctuating asymmetry and homozygosity of genes has been previously reported by Palmer and Strobeck (1986), Leary and Allendorf (1989), Parsons (1992) and Palmer (1996). The level of developmental stability was also positively correlated with the level of heterozygosity (Yang *et al.*, 1997) and negatively associated with body weight (Møller *et al.*, 1995). In turkeys, Nestor *et al.* (2000) observed that body weight had a greater influence on developmental stability than did heterozygosity. In quail, Nestor *et al.* (2002) reported little influence of the selection for increased or decreased body weight on the bilateral asymmetry and homozygosity did not influence developmental stability. Anti-asymmetry was scored for the weight differences between right and left lungs and shanks of the naked-neck control line, reflecting the developmental homeostasis of chickens carrying *Na*

allele. This may contribute to the role of *Na* allele in the adaptation of chickens to natural heating waves in warm regions. The estimated correlation coefficients indicate that the bilateral asymmetries of different characters are not significantly associated. Previous studies have reported poor correlations among the fluctuating asymmetry of different characters (Kimball *et al.*, 1997; and Clarke, 1998a), but a positive association was observed between the coefficient of variation of a character and the level of fluctuating asymmetry (Clarke, 1998b). Palmer (1996) and Møller and Swaddle (1997) suggested fluctuating asymmetry to be a good measure of environmental and genetic stresses. The effect of sex differences was limited to body weight and shank length and diameter. Liu *et al.* (2002) revealed the mechanism of sex differences and selection for increased growth to be explained by gene expression during skeletal muscle development and growth.

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