Associations among fluctuating asymmetry, duration of tonic immobility, heterophil-to-lymphocyte ratio, and one-legged standing, crooked toes, or footpad dermatitis in chickens

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ABSTRACT The purpose of this study was to analyze the associations among fluctuating asymmetry, duration of tonic immobility, heterophil-to-lymphocyte ratio, and one-legged standing, crooked toes, or footpad dermatitis in chickens. In experiment 1, cocks (n = 96; 36 wk old) from 11 Spanish breeds and a White Leghorn population that showed one-legged or normal standing were used. There were no significant differences for the relative fluctuating asymmetry, the duration of tonic immobility, and the heterophil-to-lymphocyte ratio between both groups of cocks, with mean values of one-legged standing birds being similar to those of normal birds. Thus, this pain-related behavior is not associated with some measures of well-being, fear, and stress. In experiment 2, cocks (n = 106; 36 wk old) from 9 Spanish breeds and the White Leghorn population that showed crooked or normal toes were used. Group effect was not significant for the relative fluctuating asymmetry and duration of tonic immobility. Heterophil-to-lymphocyte ratio and heterophil number were significantly greater ($P < 0.001$) in cocks with crooked toes and smaller in cocks with normal toes, with the opposite being true for lymphocyte number. Thus, this leg problem is associated with the stressfulness of birds and does not contribute to their well-being or fear levels. In experiment 3, cocks (n = 68; 36 wk old) from 5 Spanish breeds with or without footpad dermatitis were used. Group effect was significant for the relative asymmetry of toe length ($P < 0.05$), the relative asymmetry of cocks with footpad dermatitis being larger. Group × breed interaction was significant for the relative asymmetry of wattle length ($P < 0.001$), with the difference between cocks with footpad dermatitis and normal cocks being significant in one breed. In this breed (Red-Barred Vasca), fluctuating asymmetry of wattle length was significantly greater in cocks with footpad dermatitis, suggesting that this leg problem negatively affects the well-being of cocks.

Key words: fluctuating asymmetry, one-legged standing, crooked toe, footpad dermatitis, chicken

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INTRODUCTION

Freedom from pain is 1 of the 5 freedoms that have been devised for the assessment of animal well-being in farming systems (in addition to freedoms from hunger and thirst, discomfort, fear and distress, and the freedom to express normal behavior; Broom and Johnson, 1993). One-legged standing is a clear example of pain-related behavior in the chicken, and is probably caused by the deposition of uric acid in the joint cavity (Gentle and Wilson, 2004) that until recently has not been investigated. On the other hand, leg defects, including crooked toes and footpad dermatitis, have been a cause of concern in laying hens housed in cages or floor pens and in broiler chickens, and can compromise the well-being of the birds (Bradshaw et al., 2002). Both crooked toes and footpad dermatitis are relevant criteria to evaluate animal well-being (Sanotra et al., 2001), because they have a negative influence on the behavior of animals (walking, eating, drinking, scratching, and dust bathing). Management, genetics, environment, and nutrition all might influence the occurrence of leg disorders (Hester, 1994). Additionally, the risks of the occurrence of leg problems are significantly influenced by body weight and sex (Sanotra et al., 2001), males with high body weight exhibiting more leg disorders.

The relationship between one-legged standing or leg disorders and relative fluctuating asymmetry, a measure that has been reported to reflect well-being status and chronic stress (see Moller and Swaddle, 1997 for review), has received little attention. Sanotra et al. (2001) reported only weak correlations (ranging from 0.01 to 0.06) between crooked toes or footpad burns

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and the relative fluctuating asymmetry in the length, thickness, and diameter of the metatarsus.

The purposes of the present study were to analyze the associations among fluctuating asymmetry and one-legged standing, crooked toes, or footpad dermatitis in chickens (experiments 1, 2, and 3, respectively). Additionally, the associations among tonic immobility duration (a traditional measure of fearfulness in poultry; Gallup, 1979), heterophil-to-lymphocyte ratio (a reliable indicator of stress; Gross and Siegel, 1983), and either one-legged standing or crooked toes were also analyzed. We hypothesized that birds with one-legged standing, crooked toes, or footpad dermatitis would show more asymmetrical morphological traits, a longer duration of tonic immobility, and increased heterophil-to-lymphocyte ratios. The association of footpad dermatitis with tonic immobility duration and heterophil-to-lymphocyte ratio have been previously studied by Campo et al. (2005a), who found a significant effect of footpad dermatitis on tonic immobility but a nonsignificant effect on the heterophil-to-lymphocyte ratio.

MATERIALS AND METHODS

General Procedure

Different Spanish breeds of chickens (Blue Andaluza, Black-Barred Andaluza, Black-Red Andaluza, Black Castellana, Black Menorca, Buff Prat, White Prat, Birchen Leonesa, Red-Barred Vasca, and Red Villafranquina), 2 synthetic breeds (Quail Castellana, and Quail Silver Castellana), and a White Leghorn population (Campo and Jurado, 1982) were used in the current study. Andaluza and Castellana are white shell egg layers, whereas Prat is a tinted shell egg layer and Vasca and Villafranquina are brown and dark brown shell egg layers. Menorca is a classical ornamental breed, and Leonesa is used to produce hackles and saddles for fishermen. The synthetic breeds originated from an F_2 cross between Black Castellana and Buff Prat (Campo and Orozco, 1986; Campo, 1991, 1996). All these breeds are maintained at the Experimental Station of El Encín (Madrid, Spain) in a conservation program of genetic resources started in 1975 (Campo and Orozco, 1982) and have been described by Campo (1998).

Birds from all the breeds were reared together in an all-litter floor pen at a density of 10 birds/m^2 until 8 wk of age (mixed sexes). Artificial light (23L:1D) was provided only during the first week. Temperature was controlled with gas heaters (33–35°C at the chick level during the first week followed by a reduction of 3°C each week until the temperature reached 18–20°C at the sixth week of age). Birds were reared in another all-litter floor pen at a density of 6 birds/m^2 from 8 to 20 wk of age, where the lighting regimen was 8L:16D. Birds were fed standard rearing diets containing 19% CP, 2,800 kcal of ME/kg, 1% Ca, and 0.5% available P until 8 wk, and 15% CP, 2,700 kcal of ME/kg, 0.9% Ca, and 0.4% available P until 20 wk. Feed and water were supplied for ad libitum consumption. At 20 wk of age, cocks from each breed were housed separately in pens with a raised slatted floor covering a dropping pit and straw litter on the rest of the floor; the slatted area occupied approximately one-third of the floor. The number of cocks was 50 in each breed, and bird density was 4 birds/m^2. Room temperature was 16 to 20°C. Birds were fed a standard diet, containing 16% CP, 2,700 kcal of ME/kg, 3.5% Ca, and 0.5% available P. Feed and water were supplied for ad libitum consumption. Feeders and drinkers were in the slatted floor area.

The measured morphological traits were right (**R**) and left (**L**) middle toe length, leg (metatarsus) length, wing (radius) length, wattle length, and leg width at 36 wk of age. Right and left values of an animal were taken during the same session in random order. All 4 lengths and leg width were measured in millimeters using a digital caliper. Trait size was the mean of the right and left traits [(R + L)/2]. All traits showed normal frequency distributions. The fluctuating asymmetry for a trait was defined by the absolute difference between sides [|R − L|], equivalent to the mean deviation. A series of steps (Palmer, 1994; Knierim et al., 2007) was followed before identifying exhibited asymmetry as fluctuating asymmetry (normal distribution of signed R − L differences with a mean of zero), because there are several confounding factors that complicate the analysis of asymmetry: different types of bilateral asymmetry (fluctuating asymmetry, directional asymmetry, and antisymmetry), measurement error, and relation between fluctuating asymmetry and trait size (details can be found in Campo et al., 2008). Relative fluctuating asymmetry was used for all traits [2|R − L|/(R + L)]; it had distributions that were not normal and were transformed to arc-sin square root before analysis. Combined relative asymmetry was defined as the mean of the relative asymmetries of the different traits.

To obtain the heterophil-to-lymphocyte ratio (on the same day as morphological traits), birds were carried to a separate room and blood was collected immediately. Two drops of blood were taken from a small puncture in the comb of each bird and 1 drop was smeared on each of 2 glass slides. The smears were stained using May-Grünwald and Giemsa stains (Lucas and Jamroz, 1961), approximately 2 to 4 h after methyl alcohol fixation. One hundred leukocytes, including granular (heterophils, eosinophils, and basophils) and nongranular (lymphocytes and monocytes) cells, were counted on 1 slide of each bird (the other slide was supplementary), and the heterophil-to-lymphocyte ratio was calculated. Ratios were transformed to square root before analysis. All counts were made by M. T. Prieto.

Birds were tested for tonic immobility on the day following blood sampling. They were caught and carried in an upright position to a separate neighboring room. A few seconds after the bird was caught, tonic immobility was induced by placing the animal on its back with the head hanging in a U-shaped wooden cradle (Jones and Faure, 1981). The bird was restrained for

10 s. The observer sat in full view of the bird, about 1 m away, and fixed his eyes on the bird to give the fear-inducing properties of eye contact. If the bird remained immobile for 10 s after the experimenter removed his hands, a stopwatch was started to record latency (s) until the bird righted itself. If the bird righted itself in less than 10 s, it was considered that tonic immobility had not been induced, and the restraint procedure was repeated (a maximum of 3 times). If the bird did not show a righting response over the 10-min test period, a maximum score of 600 s was given for righting time. Thus, tonic immobility duration ranged from 0 to 600 s. Durations were logarithmically transformed before analysis.

Experiment 1: One-Legged Standing

Different cocks were used in each experiment. Cocks from 12 different breeds (Blue Andaluza, Black-Barred Andaluza, Black-Red Andaluza, Black Castellana, Black Menorca, Buff Prat, White Prat, Birchen Leonesa, Red-Barred Vasca, Quail Castellana, Quail Silver Castellana, and White Leghorn) were used. A total of 96 birds from 2 different replicates (hatches) separated by 14 d were sampled to study the association of one-legged standing and fluctuating asymmetry, duration of tonic immobility, and heterophil-to-lymphocyte ratio at 36 wk of age. Group 1 (one-legged standing) consisted of the 48 birds (25 and 23 in each replicate, respectively) that showed one-legged standing (and did not exhibit crooked toes or footpad dermatitis). The number of birds was 4, 5, 7, 3, 2, 5, 2, 3, 4, 4, 4, and 5 in the Blue Andaluza (1 and 3 in each replicate), Black-Barred Andaluza (2 and 3 in each replicate), Black-Red Andaluza (4 and 3 in each replicate), Black Castellana (2 and 1 in each replicate), Black Menorca (1 and 1 in each replicate), Buff Prat (2 and 3 in each replicate), White Prat (1 and 1 in each replicate), Birchen Leonesa (1 and 2 in each replicate), Red-Barred Vasca (3 and 1 in each replicate), Quail Castellana (2 and 2 in each replicate), Quail Silver Castellana (3 and 1 in each replicate) and White Leghorn (3 and 2 in each replicate) breeds, respectively. Mean percentages of one-legged standing cocks were 4, 5, 7, 3, 2, 5, 2, 3, 4, 4, 4, and 5% in each breed. Group 2 (normal standing) consisted of 48 additional birds (randomly selected) that did not show one-legged standing (and lacked crooked toes or footpad dermatitis). The number of sampled birds in each breed and replicate was equal to that of group 1.

Experiment 2: Crooked Toes

Ten breeds (Black-Barred Andaluza, Black-Red Andaluza, Black Castellana, Buff Prat, White Prat, Birchen Leonesa, Red-Barred Vasca, Red Villafranquina, Quail Castellana, and White Leghorn) were used. A total of 106 cocks from 2 different replicates (hatches) separated by 14 d were sampled to study the association of crooked toes and fluctuating asymmetry, duration of tonic immobility, and heterophil-to-lymphocyte ratio at 36 wk of age. Group 1 (crooked toes) consisted of the 53 birds (29 and 24 in each replicate, respectively) that showed crooked toes (and did not exhibit one-legged standing or footpad dermatitis); the crooked toes involved one or more of the outer, middle, and inner toes and were due to a deviation of the first phalanx. The number of birds was 6, 3, 2, 2, 3, 8, 7, 6, 12, and 4 in the Black-Barred Andaluza (3 and 3 in each replicate), Black-Red Andaluza (1 and 2 in each replicate), Black Castellana (1 and 1 in each replicate), Buff Prat (1 and 1 in each replicate), White Prat (1 and 2 in each replicate), Birchen Leonesa (5 and 3 in each replicate), Red-Barred Vasca (3 and 4 in each replicate), Red Villafranquina (4 and 2 in each replicate), Quail Castellana (7 and 5 in each replicate), and White Leghorn (3 and 1 in each replicate) breeds, respectively. Mean percentages of cocks showing crooked toes were 6, 3, 2, 2, 3, 8, 7, 6, 12, and 4% in each breed. Group 2 (normal toes) consisted of 53 additional birds (randomly selected) that did not show crooked toes (and lacked one-legged standing or footpad dermatitis). The number of sampled birds in each breed and replicate was equal to that of group 1.

Experiment 3: Footpad Dermatitis

The Black-Barred Andaluza, Birchen Leonesa, Red-Barred Vasca, Red Villafranquina, and Quail Castellana breeds were used. A total of 68 cocks from 2 different replicates (hatches) separated by 14 d was sampled to study the association of footpad dermatitis and fluctuating asymmetry at 36 weeks of age. Group 1 (footpad dermatitis) consisted of the 34 birds (16 and 18 in each replicate, respectively) that showed footpad dermatitis in legs (and did not exhibit one-legged standing or crooked toes). The number of birds was 5, 12, 7, 3, and 7 in the Black-Barred Andaluza (2 and 3 in each replicate), Birchen Leonesa (7 and 5 in each replicate), Red-Barred Vasca (3 and 4 in each replicate), Red Villafranquina (1 and 2 in each replicate), and the Quail Castellana (3 and 4 in each replicate) breeds, respectively. Mean percentages of cocks with footpad dermatitis were 5, 12, 7, 3, and 7% in each breed. Group 2 (normal legs) consisted of 53 additional birds (randomly selected) that did not show footpad dermatitis in legs (and lacked one-legged standing or crooked toes). The number of sampled birds in each breed and replicate was equal to that of group 1.

Statistical Analysis

To test the differences in fluctuating asymmetry, tonic immobility duration, and heterophil-to-lymphocyte ratio between groups of birds, a 3-way ANOVA (Sokal and Rohlf, 1981) was used with the statistical model

$$x_{ijkl} = \mu + G_i + B_j + GB_{ij} + r_k + Gr_{ik} + Br_{jk} + GBr_{ijk} + \varepsilon_{ijkl},$$

Table 1. Mean relative asymmetry (×100) of various morphological traits (%), tonic immobility duration (s), heterophil-to-lymphocyte ratio, heterophil number, and lymphocyte number in cocks showing one-legged standing or not (experiment 1; n = 96)

Standing	Toe length	Leg length	Wing length	Wattle length	Leg width	Combined	Tonic immobility	Heterophil: lymphocyte	Heterophil number	Lymphocyte number
One-legged	2.70	0.71	1.20	3.35	4.05	2.40	257	0.80	41	57
Normal	3.22	0.79	1.15	3.86	3.94	2.59	265	0.71	39	59
SEM	0.30	0.08	0.15	0.47	0.44	0.15	31	0.05	1	1

where x_{ijkl} = the analyzed measurement; μ = the overall mean; G_i = the effect of group (one-legged standing, crooked toes, and footpad dermatitis vs. normal, in experiments 1, 2, and 3, respectively); B_j = the effect of breed (j = 1…12, 1…10, and 1…5, in experiments 1, 2, and 3); r_k = the effect of replicate (k = 2); GB_{ij}, Gr_{ik}, Br_{jk}, and GBr_{ijk} = the interactions; and ε_{ijkl} = the residual (the number of birds in the individual subclasses was unequal, l ranging from 0 to 8). Group and breed were considered fixed effects and replicates were assumed to be a random effect. There were no significant differences among replicates or their interactions, and they were pooled with the residual to give a 2-way factorial model of group and breed effects ($x_{ijk} = \mu + G_i + B_j + GB_{ij} + \varepsilon_{ijk}$). Significant differences among breeds were estimated using the Student-Newman-Keuls multiple-range test (Snedecor and Cochran, 1980). The GLM procedure of the SAS statistical package (SAS Institute, Cary, NC) was used for data analysis.

RESULTS

Experiment 1: One-Legged Standing

Mean values indicating the effect of one-legged standing on fluctuating asymmetry are summarized in Table 1. Group effect was not significant for the relative asymmetry of toe, leg, wing, and wattle lengths, leg width, and the combined relative asymmetry of the 5 traits, the relative asymmetry of one-legged standing birds being similar to that of normal birds. Breed effect was significant ($P < 0.05$) for the relative asymmetry of toe length, the mean value being greater in the Quail Castellana Silver (5.23) than in the Black Menorca (1.17) and Red-Barred Vasca (1.15) breeds. There was no significant group × breed interaction for any relative asymmetry. Toe length, leg length, wing length, wattle length, and leg width did not differ significantly between the one-legged standing group and the normal group of birds.

Group, breed, and group × breed interaction were not significant sources of variation for the duration of tonic immobility (Table 1). There was no significant group effect for the heterophil-to-lymphocyte ratio. There were significant differences among breeds in terms of heterophil-to-lymphocyte ratio ($P < 0.01$), heterophil number ($P < 0.001$), and lymphocyte number ($P < 0.01$). The heterophil-to-lymphocyte ratio and heterophil number were greater for Red-Barred Vascas (1.16 and 51, respectively) than for Black-Barred Andaluzas (0.47 and 31), whereas lymphocyte number was smaller in Red-Barred Vascas (48 vs. 67).

Experiment 2: Crooked Toes

Mean values indicating the effect of crooked toes on fluctuating asymmetry are summarized in Table 2. Group effect was not significant for the relative asymmetry of toe, leg, wing, and wattle lengths, leg width, and the combined relative asymmetry of the 5 traits, the relative asymmetry of one-legged standing birds being similar to that of normal birds. Breed effect was not significant, and there was no significant group × breed interaction for any relative asymmetry. Toe length, leg length, wing length, wattle length, and leg width were not significantly different between the crooked toes group and the normal group of birds.

There was no significant group effect for duration of tonic immobility (Table 2). There were significant differences among breeds in terms of tonic immobility duration ($P < 0.05$). The duration of tonic immobility was longer for Black-Barred Andaluzas (547 s) than for Red Villafranquinas and Black-Red Andaluzas (227 and 205 s, respectively). Heterophil-to-lymphocyte ratio and heterophil number were significantly greater ($P < 0.001$) in birds with crooked toes and lower in nor-

Table 2. Mean relative asymmetry (×100) of various morphological traits (%), tonic immobility duration (s), heterophil-to-lymphocyte ratio, heterophil number, and lymphocyte number in cocks showing crooked toes or not (experiment 2; n = 106)

Toe	Toe length	Leg length	Wing length	Wattle length	Leg width	Combined	Tonic immobility	Heterophil: lymphocyte	Heterophil number	Lymphocyte number
Crooked	4.35	0.94	0.87	4.01	3.08	2.65	358	0.90^a	44^a	54^b
Normal	3.93	0.95	1.18	3.95	2.96	2.60	347	0.55^b	34^b	64^a
SEM	0.40	0.11	0.11	0.40	0.27	0.14	30	0.05	2	2

[a,b]Means within the same column with no common superscript differ ($P < 0.05$).

Table 3. Mean relative asymmetry (×100) of various morphological traits (%) in cocks showing footpad dermatitis or not (experiment 3; n = 68)

Footpad dermatitis	Toe length	Leg length	Wing length	Leg width	Combined
Yes	3.47[a]	0.86	1.00	3.04	2.37
No	2.44[b]	0.94	1.13	3.23	2.08
SEM	0.33	0.11	0.15	0.43	0.13

[a,b]Means within the same column with no common superscript differ ($P < 0.05$).

mal birds, with the opposite being true for lymphocyte number. Breed effect was not significant and there was no significant group × breed interaction for heterophil-to- lymphocyte ratio, heterophil number, or lymphocyte number.

Experiment 3: Footpad Dermatitis

Cocks with footpad dermatitis had significantly ($P < 0.05$) greater relative asymmetry than normal cocks for toe-length (Table 3). Group effect was not significant for the relative asymmetry of leg and wing lengths, leg width and the combined relative asymmetry, the relative asymmetry of birds with footpad dermatitis being similar to that of normal birds. There was no significant group × breed interaction for the relative asymmetry of toe, leg, and wing lengths, leg width, and the combined relative asymmetry. Breed effect was not significant.

Because group × breed interaction was significant for the relative asymmetry of wattle length ($P < 0.001$), subclass means are summarized in Table 4. The effect of footpad dermatitis varied from breed to breed, differences between groups being significant in the Red-Barred Vasca breed. In this breed, relative asymmetry of wattle length was significantly greater in birds with footpad dermatitis and smaller in normal birds. In the normal group of birds, there were no significant differences among breeds in terms of relative asymmetry, although the Red-Barred Vasca had significant greater relative asymmetry than the Birchen Leonesa, Black-Barred Andaluza, and Quail Castellana breeds in the group of birds with footpad dermatitis.

Leg length (116.66 ± 0.79 vs. 109.83 ± 0.79 mm), and leg width (22.16 ± 0.14 vs. 21.45 ± 0.14 mm) differed significantly ($P < 0.001$) between the groups of males with or without footpad dermatitis. Additionally, wattle length (58.87 ± 1.91 vs. 65.42 ± 1.91 mm) was significantly different ($P < 0.05$) between the groups of males with or without footpad dermatitis.

Table 4. Mean relative asymmetry (×100) of wattle length (%) in cocks from 5 breeds showing footpad dermatitis or not (experiment 3; n = 68)

	Footpad dermatitis	
Breed	Yes	No
Black-Barred Andaluza	2.72[y]	2.47
Red-Barred Vasca	5.77[a,x]	1.82[b]
Birchen Leonesa	2.78[y]	2.60
Quail Castellana	2.54[y]	4.09
Red Villafranquina	4.10[xy]	1.95

[a,b]Means within the same breed with no common superscript differ ($P < 0.05$).

[x,y]Means within the same column with no common superscript differ ($P < 0.05$).

DISCUSSION

We did not find significant associations between one-legged standing and fluctuating asymmetry, heterophil-to-lymphocyte ratio, and duration of tonic immobility in experiment 1. These findings disagree with those of Gentle and Wilson (2004), who include one-legged standing in the examples of pain-related behaviors. However, Gentle and Corr (1995) and Wylie and Gentle (1998) demonstrated the ability of birds to totally suppress such severe pain in birds tested in novel pens, during prelaying behavior, or with feeding motivation. Results in the current study suggest that the utilization of fluctuating asymmetry, duration of tonic immobility, and heterophil-to-lymphocyte ratios as biological indicators of well-being, fear, and stress may be not useful in all extreme conditions, especially in those including pain. There were no strong differences among breeds for the incidence of one-legged standing, mean percentages of one-legged birds ranging from 2 to 7% in the 12 analyzed breeds. These percentages are much lower than that reported by Chmielewski et al. (1993) in cocks from an inbred flock of the Brahma breed (69%), suggesting that the utilization of fluctuating asymmetry and some other indicators with low heritability (Campo et al., 2005b; 2007) should be much better in populations with genetic stress.

As would be expected, there were significant differences in the heterophil-to-lymphocyte ratio between the groups of birds with or without crooked toes (experiment 2), the mean value being significantly greater within the group with crooked toes. This result suggests that crooked toes could be a chronic stressor on heterophil-to-lymphocyte ratio. The ratio was approximately 64% greater in the birds showing crooked toes than that shown by the birds in the normal group. Birds that had crooked toes had significant heterophilia and lymphopenia, with the individual heterophil number being 29% greater than in normal birds. Lymphocytes represented 84% in the crooked toes group compared with those in the normal group. This fact agrees with the results of Sanotra et al. (2001) and Bradshaw et al. (2002), who indicated that crooked toes is a relevant criterion to evaluate animal well-being. However, there

were no significant differences in the duration of tonic immobility and fluctuating asymmetry between both groups of birds, suggesting that these stress indicators are not significantly associated with crooked toes in birds. Campo et al. (2007) indicated that fluctuating asymmetry was not associated with either heterophil-to-lymphocyte ratio or duration of tonic immobility in birds that had not been deliberately disturbed in any way. The lack of a crooked toe effect on fluctuating asymmetry agrees with the results of Sanotra et al. (2001), who found no significant correlations between the relative asymmetry in the length, thickness, and diameter of the metatarsus and the crooked toes (r = 0.06, r = 0.02, and r = 0.01, respectively). In the present study, there were strong genetic differences among breeds for the incidence of crooked toes, mean percentages of crooked toes birds ranging from 12% in the Black Castellana to 2% in the Quail Castellana. Eight of the 9 Spanish breeds that were used and the White Leghorn are light-weight breeds, whereas the Red-Barred Vasca is a medium-weight dual-purpose breed (brown eggs and meat). These percentages are very much lower than those reported by Sanotra et al. (2001) in commercial broilers (the proportion of broiler chickens with crooked toes varied between 16 and 56%), indicating that the mean prevalence of crooked toes is different between light- and heavy-weight breeds, and that selection for high meat production leads to a greater risk of crooked toes.

The main finding of experiment 3 was that footpad dermatitis affected fluctuating asymmetry. Cocks with footpad dermatitis had fluctuating asymmetry of middle toe length almost 42% greater than cocks without footpad dermatitis, whereas footpad dermatitis significantly increased the fluctuating asymmetry of wattle length more than 3-fold in the Red-Barred Vasca. The fluctuating asymmetry for toe length and wattle length was estimated confidently, and it was not confounded with measurement error (Campo et al., 2008). The significant group × breed interaction for the fluctuating asymmetry of wattle length suggests that the Red-Barred Vasca breed is more sensitive to footpad dermatitis and that fluctuating asymmetry is a useful indicator of stress in this breed. This finding agrees with Sanotra et al. (2001) and Bradshaw et al. (2002), who indicated that footpad dermatitis can compromise the well-being of birds. It also agrees with the results reported by Campo et al. (2005a) for the significant effect of footpad dermatitis on the duration of tonic immobility in Red-Barred Vasca and Black-Barred Andaluza cocks, although these researchers did not find a significant effect of footpad dermatitis on the heterophil-to-lymphocyte ratio. The lack of an association between footpad dermatitis and fluctuating asymmetry of leg length and leg width, which was observed in the current experiment, agrees with the results of Sanotra et al. (2001), who found only weak correlations between footpad burns and the relative asymmetry of leg length (r = 0.06) and leg width (r = 0.03) in broiler chickens. The significant variation among breeds for the fluctuating asymmetry of the wattle length found in the footpad dermatitis group (but not in the normal group of cocks) suggest that might be easier to detect breed differences for fluctuating asymmetry in stressed groups of birds. In the footpad dermatitis group, the fluctuating asymmetry of wattle length in the Red-Barred Vasca was almost 2-fold that in the Birchen Leonesa, Black-Barred Andaluza, and Quail Castellana cocks. In the present study, there were significant differences among breeds for the incidence of footpad dermatitis: mean percentage incidence of footpad dermatitis ranging from 12% in the Birchen Leonesa to 3% in the Red Villafranquina. These percentages are smaller than those reported by Sanotra et al. (2001) in commercial broilers (the mean proportion of broiler chickens with footpad dermatitis was 42%), indicating that the mean prevalence of footpad dermatitis is different between light and heavy weight breeds.

In conclusion, the results of the current study indicate that one-legged standing did not result in greater fluctuating asymmetry, heterophil-to-lymphocyte ratio, or duration of tonic immobility, suggesting that this pain-related behavior is not associated with some measures of well-being, stress, and fear. Crooked toes resulted in greater heterophil-to-lymphocyte ratio, suggesting that this leg problem is associated with the stressfulness of birds. Fluctuating asymmetry and duration of tonic immobility were not significantly greater in cocks with crooked toes, indicating that this condition did not contribute to the well-being or fear levels of birds. Footpad dermatitis resulted in greater fluctuating asymmetry of toe length, suggesting that it negatively affects the well-being of birds. The significant group × breed interaction for the fluctuating asymmetry of wattle length suggest that, depending on the genotype, cocks respond differently to footpad dermatitis, with the Red-Barred Vasca being more sensitive to this leg problem than other breeds.

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