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Broiler Walking Ability and Toe Asymmetry Under Harsh Rearing Conditions

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ABSTRACT

Morphological asymmetry has been described as a potential broiler welfare indicator, for interpreting the birds' ability to cope with the challenges that may affect its growth. The objective of this study was to evaluate the use of morphological asymmetry data to estimate broiler walking ability and welfare.dBroilers werefed diets supplemented or not with vitamin D. Toes were measured when birds were 42 and 49 days old using digital caliper.the left and right sides of the following four bilateral traits (tarsometatarsus length, outer toe length, mid toe length, and back toe length) were measured twice on intact alive birds by two different researcherh. Data from right and left sides were compared in the two treatments using the Student t-test, and Pearson's correlation was used to analyze the total asymmetry found as a result of the total sum of the differences in the measurements. Asymmetry data were comparedwith the total numberof leg lesions. Mid toe and tarsometatarsus asymmetry resultswere considered as actual fluctuating asymmetry, and presented normal distribution (Test of Kolmogorov-Smirnov, $p > 0.05$). However, back toe and outer toe measurements were not normally distributed, as determined by the test of Kolmogorov-Smirnov ($p < 0.05$), indicating anti-asymmetry; when comparing right with left limb,results were significantly different fron zero (t-Student, $p < 0.05$) indicating directional fluctuating asymmetry. The welfare of broilers withwalking difficulty due to the presence of severe asymmetry in limbs is poor.

INTRODUCTION

Morphological asymmetry has been describedas a potential broiler welfare indicator for interpreting birds' ability to cope with the challenges that may affect their growth (Tuyttens, 2003; Broom, 2006; Knierim, 2007). Fluctuating asymmetry is defined as random deviations from perfect growth symmetry that is generally expected in certain body parts when morphological development is successfully controlled, and it is the result of both genetic factors and environmental conditions.

Leg deformities are a common and severe problem in the broiler industry,and it is suggestedthat it is related to breeding (Kestin*et al*., 1992; Boekker & Koene, 2003), harsh rearing conditions (Gonzales & Macari, 2000; Dawkins *et al.*, 2004),and stocking density (Sorensen *et al.*, 2000; Hall, 2001). Leg disorders may reduce walking ability to walk, resulting in unnatural biomechanical forces, leg lesions, and ultimately gait changes that often lead to behavioral restriction, and,therefore, a welfare concern (Kestin*et al.*, 1999; Hall, 2001; Reiter & Kutriz, 2001; Weary *et al.*, 2006).

Lameness in broilers with poor gait scores has been extensivelystudied (McGeown *et al.*, 1999; Danbury *et al.*, 2000; Weary *et al.*, 2006; Nääs

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et al., 2009). Some authors propose the use o feed additives (Rath*et al*., 1998) or the dietary addition of vitamin D (Edwards Jr., 1989, Whitehead *et al*., 2004; Waldenstedt, 2006; Leeson, 2007) to alleviate this condition.

Thisstudy aimedat evaluating the use of morphological asymmetry data to estimate broiler walking ability and welfare.

MATERIALS AND METHODS

The housing and experimental procedures reported herein were approved by the Institutional Animal Care and Use Committee (CEEA 1664-1) of the State University of Campinas, Brazil.

Birds, housing and management

Thirty birds were selected from a flock of 300 one-day-old Cobb 500® male chicks weighing 0.47 kg ± 0.014.s Chicks wer reared in siebroiler houses (experimental small-scale houses measuring 3.0 2.0 x 1.4 m) located in an open area. Houses were built iEeastWwest directio,and open-sided with solid walls at the ends. Housed were equipped with yellow polypropylene (170 µ/mm) side curtains that could be opened when needed, and 50-mm deep wood-shaving litter on the concrete floor. Wood shavings were regularly replaced to maintaindproper litter conditions throughout the experiment. A brooder was placed in one corner of each house to provide supplemental heat during the first weeks.Each housewas equipped with atube feeder and bell drinker. Fresh water was supplied a plastic water tank located at the south end of each house. Broilers were fed a starter diet (ME = 3,125 kcal/kg, 22% CP) during the first two weeks, a growe'diet (ME = 3,150 kcal/kg, 20% CP) from 15 to 42 d of age, and a finisher diet (ME = 3,125 kcal/kg, 20% CP) thereafter. Feed and water were offered*ad libitum*. The birds in the flockwere reared to 49 days old with a maximum flock density of 30 kg/m^2 during the study.

Treatments

Two treatments were applied. Birds in treatmentA (control) was fed a placebo (0.2 kg/1,000 L), and those in treatmentB were fed 25-hydroxycholecalciferol (25-OH-D_3) in a soluble form (0.2 kg/1000 L, equivalent to 0.069 kg/ton of feed)diluted in the drinking water. Both groups were exposed during rearing to natural ventilation and weather conditions (temperature and relative humidity) as presented during the summer of 2008/09 in Campinas, Brazil (47°03' W, 22°54' S, 854 d altitudm). The local season average dry bulb temperature was 27.5°C with 83% relative humidity and SE prevailing wind.

Experimental procedure

Five birds from each housewere at randomselected daily, and their weights were recorded. Feed intake and water consumption were recordeddaily by weighing theamount offeredand the residues remaining both in the feeder and in the drinker. Ambient temperature, relative humidity, air velocity, and light intensity inside the houses were continuously recorded using a data logger placed in the center of the house at a height of 0.8 m. Fifteen broilers were randomly removed from groups A and B (five from each house) at 28, 35, 42, and 49 d of age for locomotionevaluation. Locomotion ability was evaluated by scoring each bird according to the subjective gait scoring system (GS) suggested by Dawkins (2004).Ten consecutive steps given by the birdwere observed. The following scale was used: 0 for birds that walked 10 steps normally; 1 for birds that walked 10 steps with some difficulty, showing unbalanced walking; and 2 for birds that could not walk more than four steps, and sat afterwards. The GS was evaluated by a trained observer.

Post-mortem examination and morphological asymmetry measurements

All birds were euthanized by cervical dislocation, and subsequently submitted to *post-mortem* examination. The following conditions were evaluated during the examination: physical abnormalities of the legs (FL), tibialdyschondroplasia (TD), valgus-varus deformities (VVD), angular bone deformities (ABD), spondylolisthesis (S), femoral head necrosis (FHN), curled toes (CT), and ruptured gastrocnemius tendon (RGT).

Toe measurements were taken twice in 60 live birds by two distinct persons (Van Nuffel*et al*., 2007; Van Pouckee*t al*., 2007), using a digital caliper (to the nearest 0.01 mm).The left and right sides of the following four bilateral traitswere measured:tarsometatarsallength; outer-toe length; mid-toe length and back-toe length.

Data analysis

Data were analyzed using one-way analysis of variance (ANOVA) at 95% statistical significance level. Paired test was used to compare the results. Effects were considered significant at$p < 0.05$ and, in some specified cases, at $p < 0.10$. All analyses were performed using a statistical software program (Minitab, 2007).

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The total amount of differences than sum of all the differences determined in the measurements, and it was used to analyze asymmetry (Palmer &Strobeck, 2003). The obtained asymmetry was comparedwith the total number of leg lesions observed.Right (R) and left (L) data were comparedbetween the two treatments using the Student'st-test and Pearson's correlation test. Kolmogorov-Smirnov's test was applied to test if data was normally distributed. All statistical analyses were carried out using the statistical software programMinitab® (2007).

RESULTS AND DISCUSSION

Table 1 and 2 show back toe, mid toe, outer toeand tarsometatarsus measurements,in mm, of the left and right legs of 42- and 49-day-old broilers submitted to treatments A and B. Student's t-test showed differences between the right and left legs in all measurements in 42-d-old broilers submitted to treatment A, while no differences were found in treatment B broilers.

Table 1 – Mean, standard deviation (SD)of back toe, outer toe, mid toe and tarsometatarsus length (mm)and Student t-test of comparing the right and left legs of 42-d-old broilers.

	Treatment	Leg	Mean±SD	p - value
Back toe	A	R	16.7 ± 1.1	0.01*
		L	17.7 ± 0.9	
	B	R	17.0 ± 1.0	0.06
		L	17.6 ± 0.8	
Outer toe	A	R	20.9 ± 1.1	0.03*
		L	21.5 ± 1.0	
	B	R	20.7 ± 1.2	0.06
		L	21.5 ± 1.0	
Mid toe	A	R	20.8 ± 0.9	0.03*
		L	21.3 ± 0.8	
	B	R	20.9 ± 0.9	0.60
		L	21.1 ± 0.8	
Tarsometatarsus	A	R	60.8 ± 1.8	0.01*
		L	59.3 ± 1.0	
	B	R	59.6 ± 1.0	0.76
		L	59.5 ± 0.6	

*Significant ($p < 0.05$).
Treatment A (control): broilersfed a placebo (0.2 kg/1000 L)
Treatment B broilers fed 25-hydroxycholecalciferol (25-OH-D3) in a soluble form (0.2 kg/1000 L, equivalent to 0.069 kg/ton of feed).

Table 2 – Mean, standard deviation (SD) of back toe, outer toe, mid toe and tarsometatarsus length (mm)and Student t-test of comparing the right and left legs of 49-d-old broilers.

	Treatment	Leg	Average	p-value
Back toe	A	R	17.5 ± 1.4	0.90
		L	17.7 ± 1.7	
	B	R	17.1 ± 1.9	0.34
		L	17.7 ± 1.6	
Outer toe	A	R	21.3 ± 1.5	0.35
		L	21.8 ± 1.8	
	B	R	21.9 ± 1.8	0.55
		L	22.3 ± 1.5	
Mid toe	A	R	21.4 ± 1.6	0.26
		L	21.9 ± 1.3	
	B	R	21.9 ± 1.6	0.76
		L	22.1 ± 1.4	
Tarsometatarsus	A	R	65.5 ± 2.2	0.33
		L	64.7 ± 2.6	
	B	R	64.9 ± 2.4	0.71
		L	64.4 ± 3.2	

Treatment A (control): broilers fed a placebo (0.2 kg/1000 L)
Treatment B broilers fed 25-hydroxycholecalciferol (25-OH-D3) in a soluble form (0.2 kg/1000 L, equivalent to 0.069 kg/ton of feed)

At 42 days of age, back toe, outer toe, and mid toe measurements were not significantly different between treatments ($p > 0.05$;Table 3).Tarsometatarsus was significantly longer in 41-d-old broilers submitted to treatment A ($p < 0.05$),as shown in Table 1. At 49 days of age, no significant difference was found between treatments (Table 3), possibly because the test did not use the same bird at both ages.

It is reported in literature that leg disorders are, in general, proportional to body weigh, and that younger birds (> 42d) are more sensitive to increase in body weight than older birds (Skinner *et al.*, 1992; Kerstin *et al.*, 2001). In the present experiment, birds were randomly selected for each age test, and the results showed that dietary supplementation with soluble 25-OH-D_3 can prevent the occurrence of leg disorders. This may be associated to better skeletal structure due to a better utilization of nutrients for growth during the first days of life (Applegate & Liburn, 2002). When ensuringaccess to vitamin D to young poultry, there is correct bone metabolism and bone abnormalitiesare reduced (Edwards *et al.*, 1992; Silva *et al.*, 2001; Whitehead *et al.*, 2004; Rennie& Whitehead, 1996, Mitchell *et al.*, 1997; Edwards, 1989, Zhang *et al.*, 1997). According to Bruno *et al.* (2007), there is rapid bone development in broilers up to 28 days of age; however, in this present study, it was observed that

Table 3 – Comparison of back toe, outer toe, mid toeand tarsometatarsus length in the treatments (42 and 49 days).

Days of age	Treatment	Back toe	Outer toe	Mid toe	Tarsometatarsus
42	A	17.1 ± 1.2	21.3 ± 1.0	21.0 ± 1.0	60.5 ± 2.0
	B	17.3 ± 0.9	21.1 ± 1.2	21.0 ± 0.8	59.6 ± 0.8
	p - value	0.40	0.64	0.87	0.02*
49	A	17.6 ± 1.5	21.6 ± 1.6	21.7 ± 1.4	65.1 ± 2.4
	B	17.4 ± 1.7	22.1 ± 1.6	22.1 ± 1.5	64.6 ± 2.8
	p - value	0.60	0.20	0.40	0.47

*Significant (p < 0.05).
Treatment A (control): broilers fed a placebo (0.2 kg/1000 L)
Treatment B broilers fed 25-hydroxycholecalciferol (25-OH-D3) in a soluble form (0.2 kg/1000 L, equivalent to 0.069 kg/ton of feed)

this development may continue. According Naas *et al*. (2009), broilers supplemented with vitamin$D_3$and showed significant differences in the highest vertical force between the right and the left leg at 28, 35 and 49 days of age, but no difference was foundwhen broiler were 42 days old.

In order to test possible asymmetry between the right (R) and left (L) limbs, measurements were submitted to Kolmogorov-Smirnov's normality test. Student'st-test was applied to verify if R and L values were significantly different from zero and represent an asymmetry (Table 4). Mid toe and tarsometatarsus asymmetry results were considered as actual fluctuating asymmetr, and presented normal distribution (Test of Kolmogorov-Smirnov, p > 0.05; Table 4).

Table 4 – Results of the Kolmogorov-Smirnov (K-S) test and Student's t-test

	K-S	Student's t-test	
	p - value	t	p - value
Back toe	0.039*	3.87	0.001*
Outer toe	0.048*	2.95	0.004*
Mid toe	> 0.15	2.28	0.024*
Tarsometatarsus	> 0.15	4.4	0.001*

*Significant non-normal distribution by Kolmogorov-Smirnov test (p < 0.05).
Treatment A (control): broilers fed a placebo (0.2 kg/1000 L)
Treatment B broilers fed 25-hydroxycholecalciferol (25-OH-D3) in a soluble form (0.2 kg/1000 L, equivalent to 0.069 kg/ton of feed)

Kolmogorov-Smirnov's test results of back toe and outer toe measurements indicated that these data did not present normal distribution (p < 0.05), leading to symmetry. Also, R and L value were significantly different from zero (t-Student, p <0.05). This directional fluctuating asymmetry is a tendency the limb has to be asymmetric in a specific direction (Ducher*et al.*, 2005; Van Valen, 1962). In some cases, directional asymmetry (Graham *et al*. 1993; Møller 1994) and asymmetry (Mckenzie& Clarke 1988; Leary & Allendorf, 1989) may be used to estimate homeostasis. According to Bizeray *et al*. (2000), asymmetrical bone development is naturally compensated by asymmetric gait,causing future lameness. This has a negative impact on welfare, because it makes it difficult for the bird to reach feeders and drinkers, and ultimately causes pain (Weeks *et al*., 2000; Kestin *et al*., 2001, Manning *et al*., 2007).

No significant correlations were found betweenback toe, outer toe, mid toe and tarsometatarsus asymmetry with gait scordo the presence of leg or spinal column lesions in 42-d-old birds from both treatments(Table 5).At 49 days of age (Table 6), there wasno correlation between general asymmetry, gait score or and leg lesions in birds submitted to both treatments. However, there was a positive correlation (p < 0.05) between back toe and outer toe asymmetry with the presence of leg lesions (0.63 and 0.55, respectively; Table 6) in treatment A. Tarsometa tarsus asymmetry was positively correlated (p < 0.05) with gait score in treatment A (0.60; Table 6).

This positive correlation indicates a possible negative effect on production, because it may compromise broiler walking ability and well being (Dawkins *et al*., 2003; Knowles *et al*., 2008). These results may have been influenced by environmental conditions and flock density, particularly during the last weeks of rearing due to increasing body weight and high stress levels (Ravindran*et al*., 2006). Elkin (1978) states that leg abnormalities in broilers are related to organic disorders in bone development in which the physical properties of collagen are altered during growth, leading to weak legs and gait problems, with a consequent reduction in feed intake and productivity (Onyango *et al*.,2003).

Table 5 – Pearson's correlation (p-value) between back toe, outer toe, mid toe and tarsometatarsus asymmetry

Treatment	Occurrence	Occurrence			
		Back toe	Outer toe	Mid toe	Tarsometatarsus
A	Gait score	-0.20	-0.25	-0.52	-0.05
		(0.48)	(0.38)	(0.05)	(0.85)
	Gait score	-0.07	0.02	-0.40	-0.24
		(0.80)	(0.95)	(0.14)	(0.39)
	Spine injury	0.02	0.08	-0.20	0.13
		(0.93)	(0.78)	(0.50)	(0.64)
B	Gait score	-0.20	0.09	-0.23	-0.18
		(0.96)	(0.74)	(0.41)	(0.51)
	Gait score	0.17	0.11	0.17	0.26
		(0.55)	(0.70)	(0.56)	(0.34)
	Spine injury	0.45	0.08	0.35	-0.52
		(0.09)	(0.78)	(0.20)	(0.05)

Treatment A (control): broilers fed a placebo (0.2 kg/1000 L)
Treatment B broilers fed 25-hydroxycholecalciferol (25-OH-D3) in a soluble form (0.2 kg/1000 L, equivalent to 0.069 kg/ton of feed).

CONCLUSION

The addition of vitamin D in the diet positively influenced the symmetry of right and left back toe, outer toe, and mid toe measurements. The tarsometatarsalasymmetry found in the present study was presented by broilers not supplemented with vitamin D and was positively correlated with high gait score values, leading to poor welfare.

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Table 6 – Pearson's correlation (p-value) between back toe, outer toe, mid toe and tarsometatarsus asymmetry

Treatment	Occurrence	Occurrence			
		Back toe	Outer toe	Mid toe	Tarsometatarsus
A	Gait score	0.48	0.29	0.23	0.60
		(0.07)	(0.29)	(0.41)	(0.02*)
	Gait score	0.63	0.55	0.36	0.50
		(0.01*)	(0.04*)	(0.19)	(0.06)
	Spine injury	0.26	0.16	0.40	-0.07
		(0.35)	(0.57)	(0.14)	(0.81)
B	Gait score	0.07	-0.50	-0.23	-0.40
		(0.80)	(0.06)	(0.40)	(0.14)
	Gait score	0.26	0.03	0.21	-0.05
		(0.35)	(0.92)	(0.46)	(0.86)
	Spine injury	-0.28	0.05	-0.04	-0.27
		(0.31)	(0.86)	(0.88)	(0.33)

*Significant ($p < 0.05$).
Treatment A (control): broilers fed a placebo (0.2 kg/1000 L)
Treatment B broilers fed 25-hydroxycholecalciferol (25-OH-D3) in a soluble form (0.2 kg/1000 L, equivalent to 0.069 kg/ton of feed).

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