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Behavioural responses of white and bronze turkeys (*Meleagris gallopavo*) to tonic immobility, gait score and open field tests in free-range system

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ABSTRACT

This study was carried out to investigate the behavioural responses of white and bronze turkeys to tonic immobility (TI), gait score (GS) and open field (OF) tests in a free-range system. 144 female turkeys (72 white and 72 bronze) were studied for 23 weeks. They were 32 weeks old. The stocking density was 2 birds/m^2 indoors and 0.66 birds/m^2 outdoors. Both bird genotypes were fed on a diet containing 16% crude protein and 11.7 ME MJ/kg. The birds were weighed in the 32nd, 35th, 48th and 55th week. The turkeys' behaviour was determined by TI, GS and OF tests. Behavioural parameters were established for each applied test. Although the mortality rates of white and bronze turkeys during the study were 6% and 3%, respectively, the white turkeys showed better results in the TI and OF tests suggesting that are more native breed than bronze ones. The results indicate that bronze turkeys are more suited for use in free-range systems than white turkeys with respect to GS and the consequent mortality rates in latter ones.

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KEYWORDS
Free-range; genotype; open field activity; tonic immobility; Turkey

1. Introduction

The interactions between housing conditions, management and animals are extremely complex. The impact of environmental conditions on animal welfare has to be considered in detail (Tuyttens et al. 2008). Numerous factors influence the ranging behaviour of poultry. Changing the environment of an animal to support its physical activity can fortify the structural integrity of its body (Balog et al. 1997). Environmental changes can contribute to the welfare of animals as well as their body health. There may be negative effects on animal health and welfare in intensive production (Maurice et al. 1990). In such environments, animals may be distressed, which in turn is associated to depressed growth (Schutz et al. 2004), increased injury (Reed et al. 1993), lower production (De Haas et al. 2013), feather pecking (De Haas et al. 2010) and a large number of similar negative effects related to productivity and welfare. Free-range systems are beneficial for animals in terms of their health and welfare. In this system, the outdoor shelter provides a wide free field with sunlight where animals are able to display their natural behaviour (Thiele and Pottgüter 2008; Turkoglu and Sarica 2009). Consumers are showing interest in poultry production systems that are semi-intensive, extensive or free-range. The products from these systems are more natural and healthy. They are produced in accordance with the accepted standards of animal welfare. Their popularity has, therefore, increased year by year (Sarica and Yamak, 2010).

Various indicators are available to assess the welfare conditions of animals, such as tonic immobility (TI), gait score (GS) and open field test (OF). Tonic immobility is connected to disposition and anti-predator behaviour, being a measure of courage toward predators (Edelaar et al. 2012). The longer a bird stays still, the higher its level of fear is (Moller and Szép 2011). Tonic immobility (TI) is a connatural reaction of animals in times of fear, manifesting itself with temporal petrification or paralysis. Tonic immobility can be observed in a variety of animals including domestic fowl (Gallup et al. 1972). In a study by Taskin (2009), adding thyme powder to the basal diets of broilers lowered the duration of tonic immobility (172 s) to a statistically significant extent ($P < .05$) when compared to the control group. It was evidenced that pharmacological activity of thyme powder had expectorant, antimicrobial, antiseptic, antioxidative, antivirotic, antihelminthic, sedative, antispasmodic, carminative, diaphoretic and antifungal effects (El-Hack et al. 2016). In a study conducted on free and intermittent feeding of turkeys, their tonic immobility duration was found at 327.6 and 427.3 s, respectively (Konca et al. 2004).

Improvements in detection of lameness bring about enhanced clinical results (Alawneh et al. 2012; Leach et al. 2012). Visual inspection of walking ability offers the advantage of allowing noninvasive evaluation of large numbers of birds in a short period of time (Webster et al. 2008). Customary procedure to assign gait scoring includes the manual scoring of animal behaviour in the poultry house (Aydin et al. 2010). Domestic fowls have been evaluated mostly by using the gait scoring system. Birds with a 3- or over gait scores experienced pain (Kestin et al. 1992; Ferket et al. 2009). Comprehensibility of the 3-point system currently used in commercial farms in the United States may encourage observer trustworthiness for gait scoring in commercial poultry flocks (Webster et al. 2008).

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In a study carried out on turkeys, the proportion of turkeys with a normal gait was found 56.67% and 64.71% in freely and intermittently fed groups. In turkeys with medium-level difficulties of walking, the rate was found as 20.0% and 20.59%. The percentage of the free feeding group with symptoms of severe walking difficulties was 23.33. This ratio was found as 18.75% in the intermittent feeding group (Konca et al. 2004).

The open-field test was used to investigate the emotional responsiveness and incentives of laboratory and poultry animals (Candland and Nagy 1969). Spontaneous locomotor activity of turkeys was assessed by the open field (OF) test (Belviranli et al. 2012). Activity of animals during the open-field tests is used to detect behavioural genetic differences between selected lines or strains (Koolhaas et al. 1999; Wahlsten et al. 2003; Lalonde and Strazielle 2008). Common fear-avoidance is associated with escaping, jumping and flight behaviours in an open field test (Melik et al. 2006; O'Brien and Sutherland 2007). Estimates of heritability (h^2) in open field behaviours have been observed in several studies and found to range from 0.08 to 0.49 for overall locomotion and from 0.06 to 0.10 for defecation (Boyer et al. 1970; Faure 1981; Webster and Hurnik 1989). Ambulation in the open field is also heritable (Forkman et al. 2007).

As they are more native breeds, bronze turkeys are more suitable for extensive production methods than white ones (Camci and Sarica 1991; Cevher and Turkyilmaz 1999; Turkoglu et al. 2005). For this reason, bronze turkeys are used as stocks in pasture-based, semi-intensive and extensive turkey farms.

Exogenous factors like feeding, housing and management or endogenous factors like genetic tendency (Hafez, 1999) have been propounded to explain the extremely aggressive behaviours of domesticated turkeys under commercial rearing conditions (Buchwalder and Huber-Eicher 2004). Fast-growing strains of turkeys such as B.U.T. BIG 6 are usually housed in large farms at stocking densities of up to 60 kg/m^2 (FAWC 1995).

There is a huge difference between white and bronze turkeys in terms of line weight. This affects their behavioural responses in a free-range system. It may be speculated or hypothesized that bronze turkeys feel themselves more comfortable than white ones since they have got bigger body sizes.

Unlike chickens and quails, turkeys have been under researched in terms of their welfare (Marchewka et al. 2013). There is not sufficient research as well to compare the behaviours and welfare of white and bronze turkey hens. As female turkeys are always in large numbers in breeding flocks, we felt the need to investigate their behavioural responses in a free-range housing condition. The present study, therefore, aimed at comparing white and bronze turkey hens in terms of their responses to TI, GS and OF tests. Comparisons include the advantage or disadvantage of body weight.

2. Material and methods

2.1. Animals and experimental design

The study was carried out with 144 white (72) and bronze (72) turkey pullets. They were 32 weeks old and each of them was designated with a numbered leg ring. It was conducted at the Poultry Unit of Agricultural Faculty of Ahi Evran University in Kirsehir province (39° 8′ 0″ N, 34° 10′ 0″ E) of Turkey. Meteorological data in experimental unit were collected by using data loggers (HOBO, Onset, Pocasset, MA). Outdoor climate data were obtained from the Kirsehir Meteorological Office. Environmental conditions of the free-range system are shown in Table 1. According to the table, average indoor temperature was between 17.9 °C and 22.5 °C during the 32nd and 55th weeks. Indoor relative humidity ranged between 57.1 and 69.9%.

In the free-range system, stocking density was 2 birds/m^2 indoors and 0.66 birds/m^2 outdoors. Feed and water were provided *ad libitum* indoors. The pens (indoors) were 2.0 × 6.00 m in size and bedded with fresh wood shavings. Possible incidences of mortality and any other abnormalities were observed daily. Both animal genotypes were fed on a diet containing 16% crude protein and 11.7 ME MJ/kg.). The pullets were housed in pens sized 2 × 6x2 m (height x length x width) indoors and having free access to the open field area sized 2 × 18 × 2 m (height x length x width). In open areas, the land is used in its natural state. In this study, round type plastic feeders and drinkers were used. All the birds were weighed on the 32nd, 35th, 48th and 55th weeks. For determining live weight changes of turkeys and collecting gait and open field test scores, all animals were used while only 12 animals from each genotype were used for TI, in order not to distress all animals.

2.2. Tonic immobility test

A tonic immobility (TI) test was conducted in the 32nd and 55th week according to the modified methods described by Noble et al. (1996). To measure TI, turkeys were gently taken from their pens at random and tested individually only once in a separate room isolated from other environmental events. Within a few seconds after the bird was caught, it was laid on a flat stand with fabric lamina. The observer restrained the turkey on its left side by placing his left hand over its right wing and tenderly grasped its legs with his right hand. He gradually kept his hands off the turkey nearly 15 s later. The duration of its laying position was counted in seconds with a chronometer. The turkey was observed from 1-m distance.).

The percentage of one induction (OI, percentage of animals exhibiting tonic immobility reaction in the first implementation of the test), vocalizations (V, percentage of sound-making during the test), defecations (D, percentage of animal feces during the test), full durations (FD, staying 600 s without standing up) and tonic immobility duration (TID, standing up willingly in 600 s without any enforcement) were registered in this study. These measurements were taken as values of behaviours exhibited by each animal (totally, twelve birds for each genotype). As the animals exhibited multiple behaviours, each behaviour was calculated in percentile values.

2.3. Open field test

For open field test, each turkey was singly transferred to an empty compartment bordering to the compartment in which they were housed for testing. The open field site consisted of a square land (3 m x 3 m). The testing site was covered by a black polycarbonate plate (1.50 m height). Blue plastic strips were used to form a net of 100 squares (each 0.09 m^2) on the

Table 1. Climate data of indoors and outdoors.

	Indoors		Outdoors				
Months	Temperature (°C)	Relative humidity (%)	Temperature (°C)	Relative humidity %	Areal rains (mm)	Max. Wind (m/s)	Sunbathe duration (hour)
April	18.1	66.3	13.0	58.1	20.2	19.2	247.7
May	19.6	68.9	16.4	60.8	46.6	20.7	266.7
Jun	19.6	65.4	20.0	53.6	36.0	18.1	294.9
July	22.4	57.1	26.3	38.4	13.0	18.8	362.7
August	22.5	59.4	26.8	39.4	17.0	23.6	339.5
September	19.6	63.1	19.9	51.2	30.4	16.4	261.0
October	17.9	69.9	13.5	67.0	31.6	14.6	218.6

ground. The birds were put in the centre of the site for 10 min and their behaviours were observed. The behaviours of standing, sitting, ambulation, vocalization, defecation, and escape were recorded. Since animals showed multiple behaviours, each behaviour was calculated in % values. The ethogram is described in Table 2.

2.4. Gait scores

The scoring system was established by using the system demonstrated by Ferket et al. (2009) with scores systematically arranged as following: 0 = no noticeable leg irregularities, 1 = mild lameness or wobbly leg, 2 = significant lameness and 3 = lame and lacking ability to move. Gait scores were determined using a scale ranging from 0 to 3. Gait scoring included the birds at the age of 32nd and 55th weeks. Their walking abilities were individually assessed by two estimators while they were walking within the pen. Each estimator scored the birds freely and an average score was computed in the sequel.

2.5. Statistical analysis

Bartlett's and Levene's tests were used to examine homogeneity of variance, and Anderson–Darling and Kolmogorov–Smirnov tests were used to examine normal distribution. These tests showed that the assumption of normality was met for the distribution of live weights. The repeated measures design was used, therefore, as a parametric statistical test. It was based on a two-factor experimental design in which one of the factors involved repeated measurement of levels. The turkeys were divided into groups on the basis of live weights and measurement times. Live weights were measured at five different times.

In what follows, y_{ijm}: m refers to the measurement value obtained from the experimental unit at the i_{th} level of factor A (group) and the j_{th} level of factor B (time). Considering the variance elements that could affect this measurement value, the following linear model was created (Gurbuz et al. 1999).

$$y_{ijm} : \mu + \alpha_i + \pi_{m(i)} + \beta_j + \alpha\beta_{ij} + \beta\pi_{jm(i)} + \varepsilon$$

μ: Overall mean value obtained from turkeys,
α_i: The effect of the ith level of live weight,
$\pi_{m(i)}$: The random effect of the mth experimental unit with a live weight of i,
β_j: The effect of the jth level of time,
$\alpha\beta_{ij}$: The effect of the interaction between live weight and time,
$\beta\pi_{jm(i)}$: The interaction between time and the experimental unit at the ith level of live weight,
$\varepsilon_{l(ijm)}$: The effect of random error.

To identify which group or groups were responsible for the inter-group differences found, we used Duncan's multiple comparison test (Gomez and Gomez 1984).

To see whether pretest and posttest measurements of independent variables, such as tonic immobility, gait score and open field test results differed from each other, the Mann Whitney U Test, which is a non-parametric statistical test, was used (Gamgam and Altunkaynak 2013). All data collected were subjected to Analysis by the Statistical Analysis System Institute (SAS, 1999).

Table 2. Description of the behaviours of turkeys.

Behaviours		Description
Standing	(ST)	Standing, feet or legs, but not belly, on the floor
Sitting	(SI)	Sitting with breast and belly on the floor
Ambulation	(A)	Two or more treads in swift progression.
Flying	(F)	Flapping wings, no contact with floor
Vocalization	(V)	Production of sounds by birds
Defecation	(D)	Defecating of the animals during the test
Escape	(E)	Endeavoring to leap out of the test stage

3. Results and discussion

3.1. Live weight changes

Weekly body weights of the turkey hens are given in Figure 1. As expected, there was a significant difference between white and bronze turkeys ($P < .05$). The variance analysis was carried out for bronze and white turkeys in accordance with the multiple comparison test; the average body weight changed according to the week ($P < .05$). In white turkeys, the average live weight was

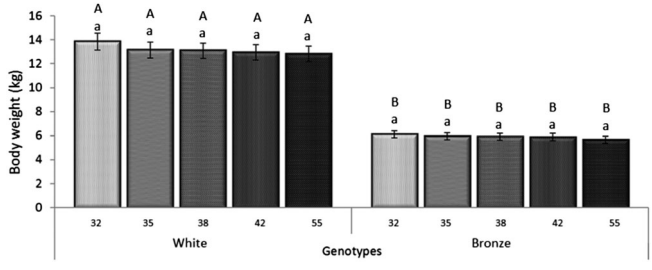

Figure 1. The changes in body weight (kg) of white and bronze turkeys during 32–55 weeks ($n = 72$). Capital letters show significant differences between genotypes while small letters show significant differences between ages at a significance level of $P < .05$.

13.85 kg in week 32, and 12.83 kg in week 55. In bronze turkeys, on the other hand, the initial weight was 6.11 kg, and the average weight in the final period was 5.65 kg. However, these differences were not found to be statistically significant within each genotype. Considering the average rearing period, the average weights of the white turkeys were 13.17 kg while those of the bronze turkeys were 5.89 kg. This difference between the genotypes was found to be statistically significant. Their live weights also are of significance when they are compared according to the weeks ($P < .05$).

3.2. Tonic immobility test

Some physiological changes observed throughout TI suggest that the autonomic nervous system is powerfully involved in this process (Alboni et al. 2008). Values for TI (OI, V, IFD and D) at 32 and 55 weeks of age in turkeys reared in a free-range system are presented in Figure 2.

In the first measurement (week 32), white turkeys' 66.66% showed OI behaviour ($P < .05$), 33.33% V behaviour ($P < .01$), 25% IFD behaviour ($P < .05$), and 75% D behaviour ($P < .01$). In the last measurement (week 55), these birds'58.83% exhibited OI behaviour ($P > .05$), 41.66% V behaviour ($P < .01$), 16.66% IFD behaviour, and 58.33% D behaviour. The significant changes ($P < .05$) were observed in these behaviours. Of the bronze turkeys, 66.66% exhibited OI behaviour in the first measurement, 75% V behaviour, 41.66% IFD behaviour, and 91.66% D behaviour. In the last measurement, on the other hand, 50% of them exhibited OI behaviour, 58.33% V behaviour, 25% IFD behaviour, and 83.33% D behaviour. In bronze turkeys, also, the significant decreases were observed in these behaviours ($P < .05$). In terms of vocalization behaviour, they showed less reaction than white turkeys. This could be a genotype-related characteristic. In this case, the white turkeys with an increasing age showed more vocalization behaviour during the TI test (when incurring the fear condition) compared to the bronze ones. The significance ($P < .05$) regarding those properties (OI, V, IFD and D) when the genotypes are compared in general and in weeks indicate its relevance to the genotypic differences.

Initial and final TI durations are shown in Figure 3**.** A significant difference was observed between the white and bronze turkeys in terms of TI durations. The bronze ones showed a significantly shorter duration than the white ones for 55th week. Considering TI behaviours, significantly lower TID (about 61 s) ($P < .01$) was observed in the white turkeys in the 32nd week. However, TID of bronze turkeys was significantly lower (approximately 33 s) ($P < .05$) than the white ones in the 55th week. A significant decrease in TID of both genotypes indicates that they were accustomed to free-range system. Significant decreases ($P < .05$) in TI in both genotypes from the 32nd to the 55th week may point to the positive effects of free-range system on animal welfare.

Bronze turkeys show less fear reactions, which can be interpreted as being less affected from free-range conditions. Fear reactions of turkeys are moderately correlated between days and weeks (Erasmus and Swanson 2014). Our study is in agreement with these findings. At 32-week old, the bronze turkeys showed more fear responses than the whites, but the older bronze turkeys lost this state of fear when compared to the white ones ($P < .01$). This might be attributed to the fact that white turkey is more domesticated than bronze one. However, it can be speculated that bronze turkeys are accustomed to handling at 55-week old. That was why they showed less fear. TI is used as a criterion to appraise fear (Villagra et al. 2011). A long duration of TI is mostly considered to be a sign of high levels of fearfulness (Reese et al. 1984). TI is involuntary and a reflexive state characterized by physical immobility, muscular rigidity, and suppressed vocal behaviour when confronted with inevitable and fear-inducing situations (Marx et al. 2008).

3.3. Open field test

Initial and final behavioural elements (ST, S, A, V, E, and D) of the bronze and white turkeys in the open field test are shown in

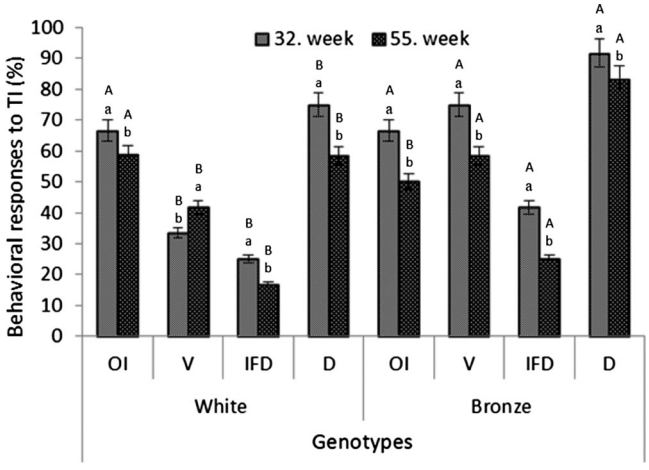

Figure 2. Behaviours of female turkeys (mean ± SE) during tonic immobility test change, ($n = 12$). OI (One induction); V (Vocalization); IFD (Immobile for full duration); D (Defecation). Capital letters show significant differences between genotypes while small letters show significant differences between ages at a significance level of $P < .05$.

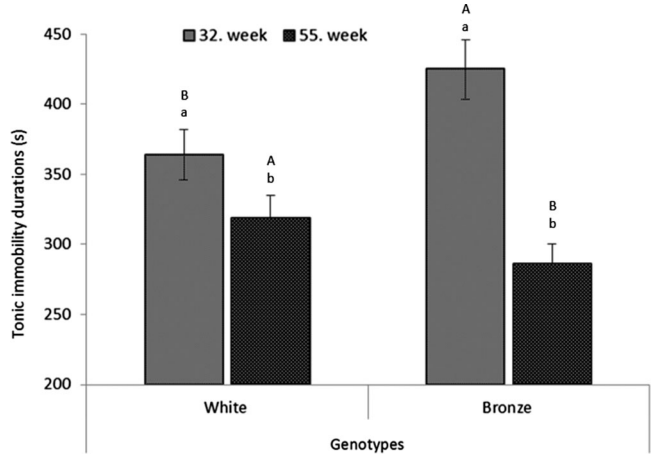

Figure 3. The average tonic immobility durations (s) of female turkeys ($n = 12$). $t = 5.16$ for white, $t = 8.91$ for bronze in 32–55 weeks, and $t = 4.5$ for between white and bronze in 32 week, $t = 2.83$ for between white and bronze in 55 week. Capital letters show significant differences between genotypes while small letters show significant differences between ages at a significance level of $P < .05$.

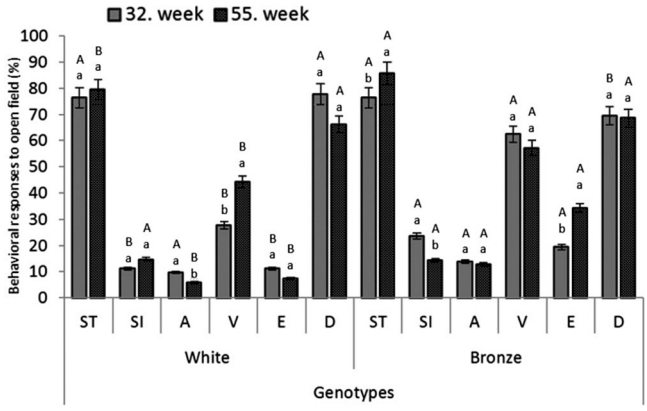

Figure 4. Open field scores (%) of female turkey ($n = 72$). ST (Standing); SI (Sitting); A (Ambulation); V (Vocalization); E (Escape); D (Defecation). Capital letters show significant differences between genotypes while small letters show significant differences between ages at a significance level of $P < .05$.

Figure 4**.** ST and SI values were found to be statistically non-significant in the white turkeys. A, V, E and D were found significant ($P < .05$). As for the bronze ones, A, V and D values were found nonsignificant while ST, S and E values were found to be significant ($P < .05$).

In the open field test, 76.38% of the white turkeys exhibited ST behaviour in the first test (week 32), 11.11% SI behaviour, 9.72% A behaviour, 27.77% V behaviour, 11.11% E behaviour, and 77.77% D behaviour. Conversely, in the last test (week 55), 79.41% of them displayed ST behaviour, 14.7% SI behaviour, 5.88% A behaviour, 44.11% V behaviour, 7.35% E behaviour, and 66.17% D behaviour. The difference between ST and SI behaviours was not statistically significant; however, there was a significant increase in V behaviour over time ($P < .01$) while the significant decrease was observed ($P < .05$) in the other behaviours in open field test. In the first measurement, 76.38% of the bronze turkeys exhibited ST behaviour, with 23.61% SE behaviour, 13.88% A behaviour, 62.5% V behaviour, 19.44% E behaviour, and 69.44% D behaviour. In the last measurement, however, 85.71% of them exhibited ST behaviour, 14.28% SI behaviour, 12.85% A behaviour, 57.14% V behaviour, 34.28% E behaviour, and 68,57% D behaviour. As for the bronze turkeys, differences between ST, SI and E behaviours were found to be both large and significant ($P < .01$); nevertheless, no significant differences were observed in terms of A, V and D behaviours ($P > .05$). The open field test scores of turkeys differed in terms of both weeks and genotypes. Both genotypes scored differently in the last week in proportion to the first week. As it can be seen in the groupings of turkeys according to the multiple comparison tests (Figure 4), those differences are statistically significant ($P < .05$), which shows that the reactions of both genotypes changed over time. Positive changes are important in terms of rearing. The most striking aspect here was the noise the turkeys made when they got outdoors. The bronze genotypes made more noise than the white ones did, possibly due to the fact that the former ones genetically have a more fierce temperament.

These differences between white and bronze turkeys are thought to stem from the relevant genotypes. In a study on enriched and non-enriched pens, there was no treatment effect on latency for birds to move out of the starting square in which they were placed, and no effect on the frequency of escape attempts or number of defecations (Hartcher et al. 2015).

3.4. Gait scores

Gait scores (%) of the bronze and white turkeys at the beginning and the end of the study (GS0, GS1, GS2 and GS3) are shown in Figure 5**.** Those scores (%) were significant ($P < .05$) in the 32nd and 55th weeks.

In the first measurement (week 32), 62.5% of the white turkeys displayed GS0, with 34.94% GS1, 5.56% GS2, and 0% GS3. In the last measurement (week 55), however, 55.88% of them displayed GS0, with 19.11% GS1, 13.24% GS2, and 11.77% GS3. Differences between behaviours were found to be large and significant ($P < .01$; $P < .05$ for GS0). In the bronze genotypes, 74.99% displayed GS0 in the first measurement, 20% displayed GS1, 5.71% displayed GS2, and 0% displayed GS3. In the last measurement, on the other hand, 85.71% displayed GS0, 11.43% displayed GS1, 1.43% displayed GS2, and 1.34% displayed GS3. Differences between the gait scores of bronze turkeys were found to be large and significant ($P < .01$; $P < .05$ for GS0). This indicates that the free-range system has eventually generated a positive impact on the gaits of bronze turkeys. In terms of gait scoring, the turkeys differed significantly in GS0, GS1, GS2, GS3 according to their genotypes. The fact that GS0 was higher among the bronze genotypes show that they did not have walking difficulties. GS1, GS2 and GS3 were also statistically lower among them, which indicates that the bronze turkeys can reap the benefits of free-range systems more actively than the white ones can. It needs to be further elaborated as this property also makes it possible to use feeding and housing in a more efficient way.

Exercises in free-range systems increase the metabolic activity and circulation, which may contribute to reducing the animal's stress (Kjaer 2004). It has been seen that there are fewer incidences of lameness in free-range chickens (Kestin et al. 1992). The increasing age negatively affected the gait of white turkeys, on the other hand. This effect stems from the high body weights depending on the genotype. Gait scores of the bronze turkeys improved in general. Examination of the heavier B.U.T. T9 and Big 6 strains revealed incidences of

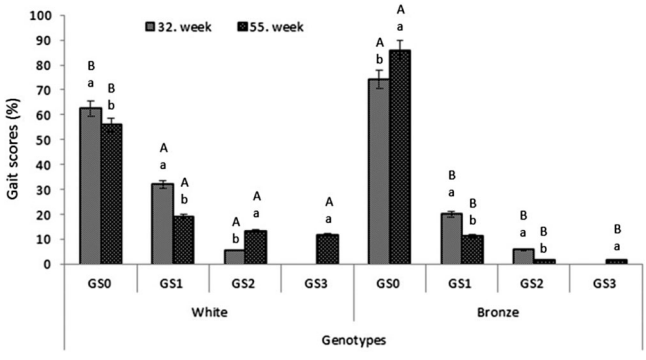

Figure 5. Gait scores (%) of female turkey ($n = 72$). Capital letters show significant differences between genotypes while small letters show significant differences between ages at a significance level of $P < .05$.

tibial dyschondroplasia with 88.2% and 90.5%, respectively (Reinmann 1999). The white turkeys were heavier than the bronze ones. At sexual development (27–34 weeks), all female turkeys of a heavy breeding line exhibit cartilage lesions (Hocking and Lynch 1991). This situation (genetic predisposition) can account for the worse gait scores of the white turkeys as well. Keeping commercial breeds under free-range conditions reduced, but did not eliminate lameness (Kestin et al. 1992). In some cases, the frequency of leg disorders can be high and are connected to elevated rates of mortality (Sanotra et al. 2002). In the present study, mortality rates were found higher among the white turkeys. The white turkeys showed a 6-percent mortality while that of the bronze ones was 3%. The results may be related to these rates.

4. Conclusions

The bronze female turkeys showed lower mortality compared to the white turkeys. The bronze ones responded with more natural behaviours to TI and OF tests, albeit not to GS. The results suggest that bronze turkeys are more comfortable than white ones in free-range systems. Considering their behavioural responses to ambient conditions, bronze turkeys may be more suitable than white turkeys for rearing in free-range systems.

Disclosure statement

No potential conflict of interest was reported by the authors.

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