ABSTRACT  In the laboratory and at a commercial farm in Argentina, chicks that showed a short latency to exit a T-maze (LEX) (HP, high performance) gained more weight than those with a longer LEX (LP, low performance). The present study re-examined this relationship and evaluated additional T-maze measures using broilers reared in quasi-intensive, environmentally controlled conditions. In the T-maze, a mirror strategically placed at the end of a corridor stimulates the test chick to leave the isolation chamber (start box) and to move toward its reflection. Having done so, it can then see other birds and thereby be further stimulated to completely exit the maze. However, when a test bird hesitates or stops at the mirror, its performance categorization may be confounded. Herein, the T-maze performance of 3-d-old broiler chicks was assessed using three different measurements: 1) the latency to exit the start box (LEB), 2) the latency to reach the mirror section of the maze (LRM), and 3) LEX. The fastest (top 25%) and slowest (bottom 25%) of the birds within a sex and within each of the three T-maze criteria were classified as HP and LP chicks, respectively. The relationships between these measures and body weight were examined in 4-, 42-, and 56-d-old males and females. Irrespective of the T-maze classification criteria used, HP and LP chicks had similar body weights at 4 d of age. However, chicks classified as HP according to LEB or LRM measures were heavier at 42 and 56 d of age than LP ones (P < 0.02 for all comparisons). These differences were apparent in both sexes. On the other hand, there were no detectable differences in body weight at the latter ages between chicks categorized as HP or LP according to their LEX scores. The present results indicate that 1) broiler chicks that exit the T-maze start box and reach the mirror quickly subsequently grow faster than their slower LP counterparts and 2) LEB and LRM are better predictors of growth than the LEX value used in previous studies.

(Key words: breeding program, body weight, broiler chicken, T-maze behavior)

INTRODUCTION

Many behavioral characteristics vary substantially between and within genetic strains of commercial poultry; these include aggression, mating, fearfulness, feather pecking, and sociality (Siegel, 1993; Jones et al., 1994; Jones, 1996; Muir, 1996; Craig and Muir, 1998; Faure and Mills, 1998; Jones and Hocking, 1999). By influencing the birds’ abilities to adapt to social and physical environments, variations in these traits can exert profound effects on the productivity and well-being of farmed poultry (Jones, 1996; Faure and Mills, 1998; Jones and Hocking, 1999).

A commonly held belief in the poultry industry is that selective breeding for improved performance implies selection for adaptability to the farm environment. However, changes in poultry husbandry practices over the past 60 yr might have been too rapid to allow bird behavior to evolve sufficiently as a correlated character (Faure, 1980). Because all the behavioral traits identified above are extremely sensitive to genetic manipulation, establishment of breeding programs for both performance- and welfare-friendly characteristics is a distinct possibility (Mench, 1992; Jones, 1996; Muir and Craig, 1998; Jones and Hocking, 1999). Indeed, such genetic selection may be the quickest and most reliable method of eliminating harmful characteristics while promoting desirable ones.

Herein, we extend our earlier study of T-maze behavior (Marin et al., 1999) and its potential use as a selection criterion for future breeding programs. The T-maze consists of a start box or isolation chamber opening to a corridor linked to two perpendicular arms. A mirror situ-
lated at the junction of the corridor and the perpendicular arms promotes movement of the chick out of the start box. Once it reaches this junction it can see its companions in a nearby brood area at the end of one of the arms. Substantial individual variation has been observed in the T-maze responses of individually tested, 2- to 3-d-old broiler chicks (Marin and Arce, 1996; Marin et al., 1997b; Jones et al., 1999). Laboratory and field trials conducted in Argentina also revealed that broiler chicks that traversed a T-maze quickly (HP, high performance) to reinitiate visual contact with their companions subsequently gained more weight, were more sociable, and showed a lower plasma corticosterone response and less behavioral inhibition to an acute stressor than their slower (LP, low performance) counterparts (Marin et al., 1997b; Jones et al., 1999; Marin and Jones, 1999; Marin and Martijena, 1999; Marin et al., 1999). Furthermore, a study of female Japanese quail from lines selected for reduced (LS, low stress) or exaggerated (HS, high stress) plasma corticosterone response to brief restraint (Satterlee and Johnson, 1988) revealed accelerated puberty and increased early hen-day egg production in those quail that negotiated the T-maze quickly (HP) with optimum performance evident in LS-HP quail (Marin et al., 2002). Collectively, the chicken and quail findings support the contention that T-maze behavior may have predictive value concerning the production potential and perhaps the adaptability and well-being of commercial poultry.

Our previous studies focused only on the time taken by the chick from its initial placement in the start box to exit the T-maze (Marin and Arce, 1996; Marin et al., 1997b; Jones et al., 1999). However, underlying sociality (motivation to be near social companions) is a particularly influential variable underpinning T-maze performance (Jones et al., 1999). Thus, high levels of this trait might accelerate movement toward the mirror as well as to the exit. Indeed, mirror-image stimulation is known to attract chicks placed in a novel environment (Gallup et al., 1972; Montevécheci and Noel, 1978). Furthermore, in a preliminary study as many of 30% of broiler chicks stopped for varying periods of time in front of the T-maze mirror (Marin and Jones, unpublished observations). Although these laggards reached the mirror quickly (<25 s), the time spent in front of it precluded their categorization as HP birds. Underlying fearfulness (predisposition to be easily frightened) may also influence T-maze behavior; fear generally inhibits activity (Jones, 1996) so high levels might delay departure from the start box. For these reasons, it may be important to measure the time taken to leave the start box and to reach the mirror as well as that to exit the T-maze. This issue was addressed in the present study (see below).

Our previous on-farm study in Argentina was conducted in open-sided houses with natural, high-intensity photoperiods, limited environmental control, and examination of BW at 7 wk only (Marin et al., 1999). Clearly, it is important to determine if the relationship between T-maze behavior and growth evident in Argentina generalizes to include more controlled rearing conditions. Therefore, conditions that more closely approximated modern U.S. broiler management practices, i.e., dark-out houses supplemented with continuous dim light and evaporative cooling, were used in the present study. We measured the T-maze performance of 3-d-old broiler chicks using three criteria; these were the latencies to 1) exit the start box (LEB), 2) reach the mirror (LRM), and 3) exit the maze (LEX). The relationships between T-maze performance and BW at 4, 42, and 56 d of age within each of these variables were then examined. The latter ages were chosen because broilers are commonly harvested between 6 to 8 wk of age in the U.S.

MATERIALS AND METHODS

Animals and Husbandry

Six hundred 1-d-old Cobb-500 broiler chicks (300 males + 300 females; vent sexed at the hatchery) were obtained from a commercial source. Upon receipt, males and females were marked faintly on the head with non-toxic paint (a different color for each sex) for further sex identification during the T-maze testing (see below). Sixty chicks (30 males + 30 females) were randomly allocated to each of 10 pens within a light-tight, fan-ventilated, evaporative cooled house at the Louisiana State University Poultry Farm. Pen size was 3 × 1.5 m (length by width) giving a stocking density of 0.075 m² per bird. Chicks were brooded using electric heat lamps at an initial temperature (bird head height) of 31°C with a weekly temperature decline of 3°C until ambient temperature (approximately 25°C) was achieved. Each pen was supplied with two manual bell-type drinkers and a cafeteria pan for initial daily feed issue. At 7 d of age, these were replaced by an automatic drip-nipple watering system (nine founts per pen) and by manual, tube-type feeders. Water and feed were supplied ad libitum throughout the experiment. A corn-soybean-based broiler starter ration (23% CP, 3,190 kcal ME/kg) was provided from 1 d to 3 wk followed by a grower ration (18% CP, 3,190 kcal ME/kg) thereafter. A 16-h light (approximately 280 lx);8-h dark cycle was used from 1 to 3 d of age (until completion of T-maze tests, see below), and a 24-h photoperiod (dim light, approximately 28 lx) was applied thereafter.

T-Maze Tests

Each chick was tested individually and once only in 1 of 10 identical T-mazes at 3 d of age (see below). These were situated in the central corridor of the broiler house. The basic prototype design of the T-maze apparatus is described in detail elsewhere (Gilbert et al., 1989; Marin et al., 1997a). Herein, each T-maze consisted of a start box or isolation chamber measuring 21 × 21 cm (length × width) opening to a 21 cm long × 7 cm wide corridor linked to two open-ended perpendicular arms, each measuring 7 × 7 cm and leading to equivalent open spaces within the T-maze section of the larger wooden box in which the T-maze was placed (see below). A mirror (10
the latter option (1997a, 1999; Jones et al., 1999) very infrequently chose study and in previous T-maze experiments (Marin et al., no companions. It is important to note that birds in this approach its companions in the brood area. If the bird looked down one of the two perpendicular arms. Exit it could see the brood area and its companions when it brooder box. When a test chick reached the mirror section, by incandescent lamps (100 W) situated 1.5 m above each freely available in the brood areas. Light was provided area that contained 19 other chicks. Food and water were freely available in the brood areas. Light was provided in each brood area had been tested. These chicks were

After each test. The floor of each T-maze was wiped clean later identification. Then, the bird was returned to the brood area. The head of the bird crossed an imaginary line at the end of the corridor leading to the test apparatus. A second mark was employed. After test, the chick was immediately removed from the test apparatus. A second mark was made on its head with nontoxic paint for distinction between tested and untested birds; it was also fitted with a numbered leg band (a different color for each sex) for later identification. Then, the bird was returned to the brood area. The floor of each T-maze was wiped clean after each test.

The above procedures were repeated until all 20 chicks in each brood area had been tested. These chicks were then returned to their home pens and another batch of 20 untested chicks from each pen was placed in the appropriate communal brood area. These procedures were repeated until all 60 birds in each of the 10 pens (total = 600 birds) had been tested. The experimenters moved slowly and steadily at all times to minimize alarm. The experimenters also synchronized their harvesting and return of batches of chicks. At 15 d of age, leg bands were replaced by wing bands.

The scores obtained by each of the 600 chicks were ranked within each of the three variables (LEB, LRM, and LEX) from the fastest chick to the slowest one. The quickest 75 and the slowest 75 males were classified within each variable as high performance (HP) and low performance (LP) chicks, respectively. This procedure was repeated in the females, thus effectively subdividing the overall population into the fastest and slowest quartiles. Thus, each bird was categorized according to each of the three measures (LEB, LRM, and LEX). It is important to note that the fastest and slowest performers according to a given variable (e.g., LEB) were not necessarily the fastest and slowest performers identified within each of the remaining two variables (e.g., LRM or LEX). Also, those birds with intermediate scores (medium performance) in each of the behavioral categories were not included in the analyses.

**Body Weights**

All birds were individually weighed to the nearest gram at 4, 42, and 56 d of age using a digital scale. Previous studies found no differences in BW of HP and LP chicks at 3 or 4 d of age when chicks were categorized by LEX (Marin et al., 1997a, 1999). However, because we measured BW at 4 d of age under different rearing conditions and using additional T-maze criteria (LEB and LRM), we wanted to ensure that subsequent differential growth between HP and LP birds categorized by any of the three T-maze behavioral measures did not simply reflect unconscious differences in weight at 3 d when the T-maze tests were conducted.

**Statistical Analysis**

Body weight data were averaged according to T-maze performance category and sex within each pen. Thus, the study was composed of 10 pen replicates. Separate repeated measures ANOVA were performed on each of the three variables used to assess T-maze performance (LEB, LRM, and LEX). In each analysis, we examined the main effects of T-maze performance category (HP or LP), sex (male or female), and age at weighing (the repeated measure, 4, 42, and 56 d of age) as well as their interactions. In order to fit ANOVA assumptions, BW of birds classified according to LEB and LRM scores were transformed by square root, and those of birds classified according to LEX were transformed to ranks (Shirley, 1987). Least significant difference (LSD) tests were used for post-hoc comparisons. Whenever there was a lack of significant
interaction between sex and T-maze category, the post-hoc analysis of the interaction between T-maze category \( \times \) age was performed by pooling data from males and females within the HP and within the LP categories at a given age.

**RESULTS**

When LEB was used to categorize the chicks as HP or LP at 3 d of age, neither the main effect of T-maze category nor the interactions of category \( \times \) sex or category \( \times \) sex \( \times \) age affected overall BW. As expected, males were consistently heavier \( (P < 0.0001) \) than females, and there was a significant interaction between sex and age \( (P < 0.0001) \). There was a marginal \( (P = 0.06) \) interaction between T-maze performance category and age. A post-hoc LSD test of this interaction (which used pooled data from males and females) revealed that although no differences existed between HP and LP chicks at 4 d of age, at 42 and 56 d of age HP chicks were heavier \( (P < 0.02) \) than LP ones (Table 1).

When broiler chicks were categorized as HP or LP at 3 d of age according to LRM, the main effect of T-maze category showed a marginal \( (P = 0.08) \) effect on BW (HP birds tended to be heavier than LP ones). There were no detectable influences of category \( \times \) sex or category \( \times \) sex \( \times \) age interactions on overall BW. Not surprisingly, males were again consistently heavier \( (P < 0.0001) \) than females, and the interaction of sex and age was significant \( (P < 0.0001) \). Importantly, there was a clear performance category by age interaction \( (P < 0.02) \). A post-hoc LSD test of this interaction (which used pooled data from males and females) revealed that, like LEB, although BW were similar in 4-d-old HP and LP chicks, the HP chicks were heavier than LP ones at 42 \( (P < 0.02) \) and 56 \( (P < 0.002) \) d of age (Table 2).

When LEX scores were used to categorize 3-d-old chicks into HP or LP T-maze categories, neither the main effect of T-maze category nor the interactions of category \( \times \) sex or category \( \times \) sex \( \times \) age affected overall BW. Once again, as was evident for LEB and LRM, males were consistently heavier \( (P < 0.0001) \) than females, and the interaction of sex and age was significant \( (P < 0.0001) \). Like the LEB results, the performance category \( \times \) age interaction for LEX classified birds approached significance \( (P < 0.07) \). Once again, LSD tests confirmed that HP and LP birds had similar BW at 4 d of age. However, unlike the significantly higher BW observed with HP than LP birds at 42 and 56 d of age in the LEB and LRM post-hoc results, BW data from chicks classified by LEX showed only numerical superiorities at these ages in HP birds (Table 3).

**DISCUSSION**

Chicks that had been classified at 3 d of age as HP or LP according to each of the T-maze test measures (LEB, LRM, and LEX) had similar BW 1 d later, i.e. at 4 d. Collectively, the findings suggest that early BW is unlikely to influence T-maze performance and that any differences in the subsequent growth rates of HP and LP chicks are not an artifact of variations in early weight. Previous studies of HP and LP birds classified by LEX showed similar results, that is, no differences in BW between HP and LP birds at 4 d of age (Marin et al., 1997b, 1999).

Broiler chicks that were classified as HP in the T-maze at 3 d of age according to their LEB or LRM had significantly higher harvest age BW than their LP counterparts. Specifically, at 42 and 56 d of age, HP birds classified by LEB had BW that was higher than LP birds by 2.1 and 1.9%, respectively. Similarly, HP birds classified by LRM had higher BW than LP ones by 2.0 and 2.3% at 42 and 56 d of age, respectively. However, there was no detectable relationship between T-maze performance category and growth in birds that had been classified as HP or LP according to their LEX scores. Conversely, rapidity of T-maze negotiation was associated with higher growth rates in previous laboratory and field trials (Marin et al., 1997b; Marin et al., 1999) conducted in Argentina (also with Cobb birds) and based solely on LEX scores. This apparent inconsistency may simply reflect differences between the Argentine and North American studies in housing, environmental control, photoperiod, or harvest age.

It was important to determine whether divergence in the growth rates of HP and LP broilers might only be evident (or more pronounced) when birds were reared in poultry houses with limited environmental control and

### TABLE 1. Body weights (g) in 4-, 42-, and 56-d-old broiler chicks categorized as high (HP) or low (LP) performers according to the latency to exit the start box of a T-maze (means ± SE)

<table>
<thead>
<tr>
<th>Age (d)</th>
<th>HP</th>
<th>LP</th>
</tr>
</thead>
<tbody>
<tr>
<td>4</td>
<td>87.6 ± 1.1</td>
<td>89.5 ± 1.1</td>
</tr>
<tr>
<td>42</td>
<td>2,069.8 ± 38.4</td>
<td>2,025.9 ± 36.9</td>
</tr>
<tr>
<td>56</td>
<td>2,861.7 ± 55.5</td>
<td>2,808.5 ± 54.7</td>
</tr>
</tbody>
</table>

*Means with no common superscript differ significantly \( (P < 0.02) \) by least significant difference test.

### TABLE 2. Body weights (g) in 4-, 42-, and 56-d-old broiler chicks categorized as high (HP) or low (LP) performers according to the latency to reach the mirror section of a T-maze (means ± SE)

<table>
<thead>
<tr>
<th>Age (d)</th>
<th>HP</th>
<th>LP</th>
</tr>
</thead>
<tbody>
<tr>
<td>4</td>
<td>88.4 ± 1.1</td>
<td>91.1 ± 1.3</td>
</tr>
<tr>
<td>42</td>
<td>2,056.6 ± 40.0</td>
<td>2,048.0 ± 43.7</td>
</tr>
<tr>
<td>56</td>
<td>2,850.9 ± 61.1</td>
<td>2,843.7 ± 64.0</td>
</tr>
</tbody>
</table>

*Means with no common superscript differ significantly \( (P < 0.02) \) by least significant difference test.
wherein they might be exposed to climatic extremes, such as in the previous farm study conducted in Argentina (Marin et al., 2000). However, the present results clearly demonstrate that this phenomenon generalizes to include well-insulated houses with environments controlled by fan ventilation and evaporative cooling.

Individual variation in underlying sociality is an important variable underpinning T-maze performance (Jones et al., 1999). Directed reinstatement responses are likely to have been evoked by two main visual features of the T-maze: 1) the chick’s image in the mirror (Thomson, 1964; Gallup et al., 1972) and, 2) the direct visual contact with stimulus chicks in the brood area gained when a chick reaches the mirror section. On the other hand, it can be argued that those test birds that choose to exit the T-maze through the arm nearest to the brood area do so because this choice visually offers a greater space than the arm leading to a blank wall. However, this explanation is not supported by reports that birds placed in novel environments are reluctant to enter larger exposed areas (Jones, 1996). Indeed, in such situations, the presence of other conspecifics in a nearby enclosure stimulates the test bird to move toward them and reinstate social contact (Marin et al., 2001).

Because chickens are social animals that are often housed in large groups, individual variation in underlying sociality (motivation to be near conspecifics) is likely to influence their welfare and productivity, particularly since this trait has been linked to fearfulness in certain circumstances in domestic chicks (Jones, 1996; Marin et al., 2001). Similarly, shyness and social withdrawal have been positively linked with circulating cortisol levels and general fearfulness in children (Kagan et al., 1988; Schmidt and Fox, 1998). Inappropriate levels of sociality could exert undesirable effects on all aspects of social interaction in birds, including affiliation, aggression, dispersal, and mating, as well as on their ability to cope with social disruption, such as isolation, exposure to strangers, or crowding (Vallortigara, 1992; Jones, 1996; Jones and Mills, 1999). Furthermore, a perceived mismatch between a bird’s underlying sociality and its social environment could elicit either a series of acute stress responses or chronic social distress with associated negative effects on performance (Mills et al., 1993; Jones and Hocking, 1999; Jones and Mills, 1999). Thus, because HP chicks were found to be more sociable than LP ones in the T-maze, as well as in other behavioral tests of sociality (runway and home-cage; Jones et al., 1999, 2002), HP-type birds may be better suited to rearing in very large or in high-density (crowded) groups. Indeed, this might at least partly account for the superior BW of HP broilers found here at 42 and 56 d of age. Comparisons of growth rates in HP and LP broiler chicks reared at different densities would provide a useful test of this hypothesis.

A collective consideration of the relationships found herein (i.e., between growth, LEB, and LRM scores) suggests that the presence of the mirror in the T-maze plays an extremely important role in the categorization of chicks and, thereby, in the ultimate prediction of their growth rates. Furthermore, approximately 24% of the birds classified as HP according to LRM scores in the present study would have been excluded from this category if we had only considered their LEX scores. Chickens are thought to respond to their reflections in mirrors as if they were conspecific (Thomson, 1964; Gallup et al., 1972; Montevettolini and Noel, 1978). Given the apparent importance of the mirror-image ‘social stimulus’ in eliciting social reinstatement behavior in the T-maze, it is conceivable that recording chicks’ responses in a simpler apparatus, such as a runway leading to a goal box containing visible chicks, might be as effective, if not more so, than the present apparatus. Indeed, chicks will readily negotiate runways to reinstate or to maintain contact with conspecifics (Vallortigara et al., 1990; Jones and Mills, 1999; Marin et al., 2001).

The precise influence of sex on the relationship between T-maze behavior and growth rate in broilers remains unclear. Higher growth rates in HP than LP broilers were apparent only in males when the birds were categorized according to LEX scores, reared in the laboratory, and weighed at 15 d of age (Marin et al., 1997b). On the other hand, improved growth was apparent at 7 wk in male and female HP broilers categorized according to LEX when they were reared at a commercial farm in Argentina (Marin et al., 1999). Herein, a divergence between the BW of HP and LP chicks was found in male and female broilers reared to 6 or 8 wk under commercial conditions that better exemplify those becoming increasingly extant in the U.S. poultry industry (i.e., dark-out houses with continuous dim light and improved environmental control). However, this phenomenon (superior BW in male and female HP broilers) was apparent only when birds were classified as HP or LP according to the new measures—LEX and LRM.

It is particularly noteworthy that the positive relationships among the LEB and LBM behaviors and harvest age BW are apparent in an animal that has undergone intense selection for rapid growth. Given the negative effects of corticosterone on BW (Bartov, 1982; Satterlee and Johnson, 1985; Puvadolpirod and Thaxton, 2000), the apparent reduction in adrenocortical stress responsiveness in HP birds (Marin and Jones, 1999) might also underpin their more rapid growth, i.e., in HP birds, bodily resources could be devoted to growth rather than stress-related defense mechanisms. But, regardless of the etiology of better growth rates in HP birds (greater sociability or reduced corticosterone stress responsiveness), the use of T-maze testing to identify superior genotypes would be easier to apply from a genetic selection standpoint than measurement of stress-induced blood corticosterone levels. Thus, our findings may have far-reaching implications for future breeding programs intended to improve broiler productivity if HP reflects a heritable trait that is not associated with undesirable characteristics. Additional advantages of the T-maze and other tests of sociality are that they are rapid, inexpensive, and noninvasive; they can be carried out on young chicks (allowing for
early culling of inferior genotypes); and they may be easily automated.

Because HP broilers show less anxiety-related behaviors when isolated (Marin et al., 1997b), lower adrenocortical responses and behavioral inhibition to acute stress (Marin and Jones, 1999; Marin and Martijena, 1999), and higher sociality (Jones et al., 1999), the use of T-maze behavior in future breeding programs might also lead to improved broiler welfare. However, it should be noted that high growth rate in broilers has also been associated with musculoskeletal and cardiovascular disease, e.g., ascites (Riddell, 1992; Julian, 1993, 1998; Lilburn, 1994). Thus, a conclusion that selection using T-maze performance might have a welfare advantage must remain guarded. It would be important to compare bone strength, skeletal deformities, and incidences of ascites in T-maze selected birds.

In conclusion, the present study showed that 3-d-old broiler chicks, when classified in a T-maze as HP by LEB or LRM, but not by LEX, had higher BW at 6 and 8 wk of age than did their LP counterparts. Thus, a clearer picture of the most appropriate T-maze behavioral measures to be used as predictors of harvest age BW in broilers is beginning to emerge.

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