

## Heritabilities of Tonic Immobility and Leucocytic Response in Sire and Dam Layer Lines

Özge ALTAN\*, Petek SETTAR, Yakut ÜNVER, Metin ÇABUK  
Department of Animal Science, Faculty of Agriculture, Ege University, 35100 İzmir - TURKEY

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**Abstract:** Fearfulness reaction was examined using tonic immobility (TI) response and differential leucocyte counts as physiological indicators of distress from sire and dam brown layer lines.

The study was performed on 20 male and 131 female chickens from the sire line and 24 male and 116 female chickens from the dam line. The duration of TI, the time interval until the bird righted itself, and the number of inductions (15 s periods of restraint) necessary to attain TI were recorded. If TI could not be induced after 5 attempts, a score of 0 was recorded.

After the TI test, blood samples were collected from 16 male and 45 female chickens in the sire line and 17 sires and 42 dams in the dam line and leucocyte parameters were examined.

After the normality test, TI, tonic immobility per number of inductions (TI/Ind) and the heterophil:lymphocyte (H/L ratio) showed deviations from normality. After applying the Box-Cox transformation all data were analysed by a general linear model using JMP. Heritabilities and phenotypic correlations were also obtained

Significant line differences were obtained from TI reactions. There were no significant differences in the leucocytic parameters between lines, except for eosinophils. The H/L ratio was significantly higher in males than in females.

Heritability estimates for the duration of TI and TI/Ind were low to moderate in the sire line, but moderate to high in the dam line. The results suggest that fearfulness could be controlled through selection.

**Key Words:** Tonic immobility, leucocytic traits, heritability, layer line

### Baba ve Ana Yumurtacı Hatlarda Tonik İmmobilite ve Lökosit Komponentlerinin Kalıtım Dereceleri

**Özet:** Baba ve ana kahverengi yumurtacı hatların tavuk ve horozlarında korkuya karşı gösterdikleri tepki, stresin fizyolojik indikatörü olarak tonik immobilite (TI) ve farklı lökosit komponentleri yardımı ile test edilmiştir.

Bu çalışma, baba hattından (L1 hattı) 20 erkek ve 131 dişi ve ana hattından 24 erkek ve 116 dişi ile yürütülmüştür. Hayvanların 15 s süreyle hareketsiz kalmaları sağlandıktan sonra beşikten kalkışına kadar geçen süre TI süresi (TI) olarak ölçülmüştür. Eğer beş uyarıma rağmen TI sağlanıyorsa bireye 0 değeri verilmiştir.

TI testinden sonra, baba hattından 16 erkek ve 45 dişi ve ana hattından 17 erkek ve 42 dişiden kan örneği alınmış ve lökosit parametrelerine bakılmıştır.

Normalite testi sonucunda, TI süresi, uyarım başına TI süresi ve H/L oranı değerlerinin normaliteden saptığı gözlenmiştir. Veri setlerine Box-Cox transformasyonu uygulanarak normalite sağlandıktan sonra JMP programı kullanılarak varyans analizi yapılmıştır. Ayrıca kalıtım derecesi ve fenotipik korelasyonlar elde edilmiştir.

TI reaksiyonları için hatlar arasındaki farklılık önemli bulunmuştur. Lökosit parametrelerinde ise, hatlar arasında eosinofiller dışında farklılık saptanmamıştır. Heterophil:lymphocyte oranı, erkeklerde dişilere göre önemli ölçüde yüksek bulunmuştur.

Kalıtım dereceleri, TI süresi ve uyarım başına TI süresi için baba hattında düşük-orta düzeylerde, ana hattında ise orta-yüksek düzeyde tahmin edilmiştir. Sonuçlar, korkaklığın seleksiyonla kontrol edilebileceğini göstermektedir.

**Anahtar Sözcükler:** Tonik immobilite, lökosit özellikler, kalıtım derecesi, yumurtacı hat

## Introduction

In recent years animal welfare has become one of the major contemporary issues of the livestock industry. There has been increasing public concern about the well-being of poultry. Producers have been under increasing pressure to provide certain basic environmental conditions that are beneficial to the welfare of animals, including protection against distress. As a consequence, several studies in recent years have focused on the stress and fear response of chickens. Prolonged and intense fear and distress may seriously harm the welfare, management and production performance, such as egg production, egg shell quality, body weight and food conversion, of poultry (1-5). These fear and stress responses may result in physical injury, impaired growth, reduced reproductive performance, the appearance of behavioural abnormalities and mortality.

Fear, an important component of stress, and duration of tonic immobility (TI) are considered to be a useful index of general fearfulness in fowl (6). TI is an unlearned state of reduced responsiveness to external stimulation and is induced by physical restraint. Several factors affect the duration of TI, such as previous handling, management, social factors, housing system and genetic background (5,7,8).

Leucocytic responses can be used as an indicator of stress status in poultry. Gross and Siegel (9) showed that the heterophil:lymphocyte (H/L) ratio is a reliable index of chronic stress. However, Maxwell et al. (10) suggested that heterophilia may be a response to moderate stress, but that heteropaenia and basophilia may develop during extreme stress conditions. The relationship between stressful and fearful states of hens is not clear.

The existence of breed and strain differences in fear behaviour (1,11-16) suggests that this characteristic may be subjected to genetic selection. Heritabilities for fearfulness measured as TI were reported to be in the low to moderate range (14-16).

In this study, stress and fear responses were examined in sire and dam layer lines. The objectives were to estimate the heritabilities of TI and other measured traits, to determine the relationship between fearfulness and leucocytic responses and to determine the response characteristics of sexes and lines to fear and stress.

## Materials and Methods

Stress and fearfulness reactions were examined by using TI and leucocyte counts as physiological indicators of distress. The experiment was performed in sire (L1) and dam (L55) brown layer lines from a commercial breeding company. These lines were selected by index values based on performance records for egg production traits in a hierarchical mating structure using artificial insemination (AI) for 12 generations. This experiment was performed using males and females at 58 weeks of age selected according to their egg production index in the 40<sup>th</sup> week of age in the 12<sup>th</sup> generation.

TI was induced by restraining the bird on its back in a specially constructed cradle for 15 s (17). The test was performed on 20 male and 131 female chickens from the sire line (14 sires mated by 42 dams) and 24 male and 116 female chickens from the dam line (11 sires mated by 31 dams). The duration of TI, the time interval until the bird righted itself, and the number of inductions (15 s periods of restraint) necessary to attain TI, were recorded. If TI could not be induced after 5 attempts, the bird was considered to be unsusceptible and a score of 0 was recorded. If the bird remained in TI after 15 min, the test was stopped and a maximum score of 900 s was given for duration.

After the TI test, blood samples were collected from 16 male and 45 female chickens in the sire line and from 17 sires and 42 dams in the dam line. Two drops of blood were collected from the branchial vein of each bird and smeared on each of 2 glass slides. The smears were stained with Wright stain for 15 min. One hundred leucocytes, including heterophils (H), lymphocytes (L), monocytes (M), basophils (B) and eosinophils (E), were counted on each slide and the H/L ratio was calculated by dividing the number of heterophils by that of lymphocytes. The means of the 2 slides were calculated for each bird.

All data were tested for normality using the Shapiro-Wilk test and by examining the residuals in the histogram. Duration of TI, tonic immobility per number of inductions (TI/Ind) and H/L ratio showed deviations from normality. In general, the Box-Cox transformation resulted in distributions closer to normality, and therefore this was applied to the TI, TI/Ind and H/L traits. Transformation was performed based on the procedure described by Sokal and Rohlf (18). Transformed data were used for analyses of variance, but actual mean values are

presented in the tables. Data of the 7 traits (TI, Ind, TI/Ind, H/L, E, B, and M) were analysed by general linear model ANOVA using JMP (19) according to the following linear model:

$$y = \mu + \text{line} + \text{sire:line} + \text{dam:sire:line} + \text{sex} + \text{line*sex} + e$$

Line, sex and their interaction term are considered fixed effects; sire within line and dam within sire and lines are considered random effects. The Duncan test was used to compare mean values by JMP (19). Heritabilities were estimated from sire ( $\sigma^2_s$ ), dam within sire ( $\sigma^2_{D:S}$ ), combined sire and dam ( $\sigma^2_{S+D:S}$ ) components obtained from sib analyses (20). Estimates of variance components were obtained within lines using the restricted maximum likelihood (REML) estimator by JMP (19). Heritabilities were estimated using both original and transformed data. Phenotypic correlations were calculated among all traits within lines and sexes.

## Results

Least square means for duration of TI, number of inductions, TI/Ind and leucocytic parameters of each line and sex group are given in Table 1. There were no line or line by

sex interaction differences for the number of inductions. Sex effect was significant ( $P < 0.03$ ) for duration of TI, and females ( $1.67 \pm 0.10$ ) required significantly higher induction numbers than males ( $1.14 \pm 0.19$ ). The dam line (L55) showed a significantly higher TI reaction ( $231.61 \pm 19.10$  s) and TI/Ind ( $209.94 \pm 24.11$  s) level than the sire line (L1) ( $169.82 \pm 20.23$  s and  $153.15 \pm 25.43$  s, respectively). Sex and line by sex interaction were not significant for the traits mentioned above.

There were no differences in the leucocytic parameters between sire and dam lines, except for eosinophils (Table 1). Eosinophils in the sire line (L1) ( $3.49\% \pm 0.16\%$ ) showed higher values than those in the dam line (L55) ( $2.32\% \pm 0.15\%$ ). The H/L ratio was significantly higher for males than for females, being  $0.42\% \pm 0.01\%$  and  $0.37\% \pm 0.01\%$ , respectively. There was a sex by line interaction for all leucocytic traits. For H/L ratio and eosinophils, males from the sire line (L1) showed higher values ( $0.45\% \pm 0.02\%$  and  $3.77\% \pm 0.27\%$ , respectively) than did others. Females showed higher values for basophils and monocytes ( $3.41\% \pm 0.18\%$  and  $3.90\% \pm 0.21\%$ ) than did males ( $1.77\% \pm 0.28\%$  and  $2.33\% \pm 0.32\%$ ) in the sire line. Contrasting, results were observed in the dam line.

Table 1. Means ( $\pm$  SD) of number of inductions (Ind), duration of tonic immobility (TI), tonic immobility per number of inductions (TI/Ind), heterophil:lymphocyte ratio (H/L), eosinophils (E), basophils (B) and monocytes (M) by lines (L1, sire and L55, dam) and sex (F, female and M, male) and significance levels of main effects and interaction.\*

		Ind (Number)	TI (s)	TI/Ind (s)	H/L	E (%)	B (%)	M (%)
L1	F	1.69 $\pm$ 0.14	174.84 $\pm$ 16.78	155.62 $\pm$ 39.55	0.34 $\pm$ 0.02 <sup>a</sup>	3.22 $\pm$ 0.18 <sup>a</sup>	3.41 $\pm$ 0.18 <sup>a</sup>	3.90 $\pm$ 0.21 <sup>a</sup>
	M	1.21 $\pm$ 0.31	164.81 $\pm$ 36.81	150.67 $\pm$ 55.42	0.45 $\pm$ 0.02 <sup>b</sup>	3.77 $\pm$ 0.27 <sup>b</sup>	1.77 $\pm$ 0.28 <sup>b</sup>	2.33 $\pm$ 0.32 <sup>b</sup>
	Average	1.45 $\pm$ 0.15	169.82 $\pm$ 20.23 <sup>a</sup>	153.15 $\pm$ 25.43 <sup>a</sup>	0.40 $\pm$ 0.01	3.49 $\pm$ 0.16 <sup>a</sup>	2.59 $\pm$ 0.17	3.12 $\pm$ 0.19
L55	F	1.65 $\pm$ 0.15	213.23 $\pm$ 17.97	183.61 $\pm$ 36.42	0.39 $\pm$ 0.02 <sup>c</sup>	2.57 $\pm$ 0.19 <sup>c</sup>	1.88 $\pm$ 0.20 <sup>b</sup>	2.43 $\pm$ 0.23 <sup>b</sup>
	M	1.07 $\pm$ 0.22	250.00 $\pm$ 33.71	236.27 $\pm$ 39.52	0.39 $\pm$ 0.02 <sup>c</sup>	2.08 $\pm$ 0.24 <sup>d</sup>	3.25 $\pm$ 0.24 <sup>a</sup>	4.16 $\pm$ 0.28 <sup>a</sup>
	Average	1.36 $\pm$ 0.13	231.61 $\pm$ 19.10 <sup>b</sup>	209.94 $\pm$ 24.11 <sup>b</sup>	0.39 $\pm$ 0.01	2.32 $\pm$ 0.15 <sup>b</sup>	2.56 $\pm$ 0.15	3.29 $\pm$ 0.18
F (L1 + L55)		1.67 $\pm$ 0.10 <sup>a</sup>	194.03 $\pm$ 12.29	169.62 $\pm$ 26.88	0.37 $\pm$ 0.01 <sup>a</sup>	2.89 $\pm$ 0.13	2.65 $\pm$ 0.13	3.16 $\pm$ 0.16
M (L1 + L55)		1.14 $\pm$ 0.19 <sup>b</sup>	207.40 $\pm$ 24.95	193.47 $\pm$ 34.03	0.42 $\pm$ 0.01 <sup>b</sup>	2.93 $\pm$ 0.18	2.51 $\pm$ 0.18	3.25 $\pm$ 0.21
P(F)								
Lines (L)		NS	0.03	0.01	NS	0.001	NS	NS
Sex (S)		0.03	NS	NS	0.01	NS	NS	NS
L x S		NS	NS	NS	0.01	0.01	0.001	0.001

\*Different letters indicate significant differences between means

Heritabilities ( $h^2$ ) from sire ( $h^2_s$ ), dam within sire ( $h^2_{D:S}$ ), and combined ( $h^2_{S+D:S}$ ) variance components for lines are presented in Table 2. In general, heritabilities were relatively higher in the dam line than in the sire line. Estimates computed from transformed data were compared with those based on original data to validate the effect of transformation.

In the sire line, heritability for the duration of TI was higher in dam components than in sire components in both original and transformed data (0.30 vs. 0.05 and 0.26 vs. 0.03, respectively). Similar differences were found for TI/Ind in original and transformed data (0.17 vs. 0.05 and 0.32 vs. 0.08, respectively). The heritability estimates from sire-plus-dam components were 0.07 and 0.10 for duration of TI and 0.05 and 0.21 for TI/Ind in original and transformed data, respectively. Heritability for H/L ratio was overestimated for all components in both the original and transformed data sets.

In the dam line, heritabilities estimated from dam components were higher than those from sire

components for all traits. The range of heritabilities for duration of TI was from 0.53 to 0.57 in original data and from 0.29 to 0.53 in transformed data. Similar results were obtained in TI/Ind and H/L ratio; original data ranged from 0.05 to 0.65 and transformed data ranged from 0.10 to 0.44 for TI/Ind and from 0.89 to 0.95 and from 0.86 to 0.89 for H/L ratio. The heritability estimates from sire-plus-dam components were 0.55 and 0.41 for duration of TI, 0.35 and 0.25 for TI/Ind and 0.92 and 0.88 for H/L ratio in original and transformed data, respectively.

Correlations for all traits were separately calculated for each line and sex. Correlation coefficients are given for females by sire and dam lines in Table 3 and for males by sire and dam lines in Table 4. The number of inductions was significantly ( $P < 0.05$ ) and negatively correlated with both duration of TI and TI/Ind for females in each line (Table 3). Correlation coefficients were -0.28 and -0.50 for the sire line and -0.39 and -0.64 for the dam line, respectively.

Table 2. Heritabilities ( $\pm$  SE) of duration of tonic immobility (TI) and tonic immobility per number of inductions (TI/Ind) in sire (L1) and dam (L55) lines from sire, dam and dam within sire variance components.

Line	Trait	Original data			Transformed data		
		sire	dam	dam:sire	sire	dam	dam:sire
L1	TI	0.05 $\pm$ 0.05	0.30 $\pm$ 0.20	0.07 $\pm$ 0.07	0.03 $\pm$ 0.03	0.26 $\pm$ 0.15	0.10 $\pm$ 0.08
	TI/Ind	0.05 $\pm$ 0.06	0.17 $\pm$ 0.16	0.05 $\pm$ 0.05	0.08 $\pm$ 0.07	0.32 $\pm$ 0.18	0.21 $\pm$ 0.12
L55	TI	0.53 $\pm$ 0.23	0.57 $\pm$ 0.25	0.55 $\pm$ 0.23	0.29 $\pm$ 0.12	0.53 $\pm$ 0.20	0.41 $\pm$ 0.15
	TI/Ind	0.05 $\pm$ 0.06	0.65 $\pm$ 0.30	0.35 $\pm$ 0.28	0.10 $\pm$ 0.07	0.44 $\pm$ 0.24	0.27 $\pm$ 0.20

Table 3. Correlation coefficients between number of inductions (Ind), duration of tonic immobility (TI), tonic immobility per number of inductions (TI/Ind) and heterophil:lymphocyte ratio (H/L) by sire (above diagonal) line and dam (below diagonal) line for females.

	Ind	TI	TI/Ind	H/L
Ind		-0.28*	-0.50*	-0.33*
TI	-0.39*		0.87*	-0.06
TI/Ind	-0.64*	0.96*		-0.22
H/L	-0.07	0.01	-0.01	

\*P < 0.05

Table 4. Correlation coefficients between number of inductions (Ind), duration of tonic immobility (TI), tonic immobility per number of inductions (TI/Ind) and heterophil:lymphocyte ratio (H/L) by sire (above diagonal) line and dam (below diagonal) line for males.

	Ind	TI	TI/Ind	H/L
Ind		0.10	-0.37*	0.25
TI	-0.18		0.89*	0.35*
TI/Ind	-0.49*	0.94*		0.19
H/L	-0.02	-0.35*	-0.32	

\*P < 0.05

Number of inductions and the duration of TI were not significantly correlated in either line (Table 4). There was a significant ( $P < 0.05$ ) and negative correlation between Ind and H/L ratio for females in the sire line only (-0.33, Table 3). For males, correlations between duration of TI and H/L ratio were significantly different from zero ( $P < 0.05$ ) in the sire and dam lines (0.35 and -0.35, respectively) (Table 4).

## Discussion

Chickens of the dam line remained immobile longer than did those of the sire line. The duration of TI per number of inductions in the dam line was longer than that of the sire line. These results suggest that chickens of the dam line were more fearful than those of the sire line. Consistent with our results, Beuving et al. (13) found no significant difference in susceptibility to TI between groups, but high fear responders remained immobile longer than lower fear responders did.

The birds were 58 weeks of age. Long-term exposure to care-takers and artificial insemination (AI) procedure was thought to reduce their susceptibility to TI. As reported in previous studies, several factors affect the duration of TI, such as frequency of handling, management, age, genetic and social factors and housing system (5,21). Repeated daily testing or regular handling of chicks also reduced susceptibility to and duration of TI (7,8,21).

There was no significant sex effect for duration of TI, but males were more susceptible to TI than were females. Males were reported to be more fearful than were females (16,17,22). However, no significant differences were found in Japanese quail between TI responses of females and males (23,24).

Gross and Siegel (9) reported that H/L ratios of about 0.2, 0.5 and 0.8 characterised low, optimum and high levels of stress, respectively. According to their results, chickens of the dam line were under mild to moderate stress, and males from the sire line were more sensitive to stress conditions.

Heritabilities for duration of TI and TI/Ind ratio were in low to moderate magnitudes. Heritability estimates of TI measured in the present study are fairly consistent with values reported by Craig and Muir (14) and Campo and Carnicer (16). The original data showed significant positive skewness and kurtosis after Box-Cox transformation yielded standard data in terms of

normality. Transformed data produced lower heritability estimates than did original data in most cases, but these were more reliable with lower standard errors. Heritabilities were overestimated in the sire line for H/L because, in the sire line, sire and dam components of variance were not significant for this trait. Heritability estimates in the dam line and those of Al-Murrani et al. (25) have suggested that the H/L ratio is highly heritable.

Number of inductions was negatively correlated with TI/Ind in all groups. These correlations indicated that chickens more susceptible to TI remained immobile longer per induction.

There were negative associations between duration of TI and number of inductions for females in both sire and dam lines. Consistent with these results, Jones et al. (21) obtained a negative correlation between duration of TI and induction number (-0.51). In this study, the correlation between duration of TI and H/L ratio was -0.35 for males in the dam line. This value is similar to Campo and Redondo's (4) result (-0.28). Conversely, this correlation coefficient was 0.35 and significantly different from zero for the sire line. This result agrees with the results reported by Beuving et al. (13) in which they obtained a positive correlation between duration of TI and H/L ratio. However, Zulkifli et al. (26) reported that the trend of H/L ratios did not correlate well with the duration of TI. Females in the sire line had a significant negative correlation (-0.33) between induction number and H/L ratios, suggesting that hens are susceptible to TI (high fear) and tend to be more stressful. Jones et al. (27) also reported that stress treatment increased susceptibility to TI but had no significant impact on duration of TI.

In conclusion, considerable differences in TI reaction were obtained between the 2 layer lines selected for increased part-year egg production. In addition, medium to high heritability values for TI in the dam line suggested that fearfulness could easily be reduced through selection. In agreement with previous studies, relationships between fearfulness and stressfulness of hens were inconsistent. However, more fearful females and males tended to be more stressful in the sire line.

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