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Evaluation of Welfare Traits in Hybrids Obtained from Reciprocal Crosses between Anadolu-T and Commercial Broiler Parent Stocks

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Abstract

This study aimed to evaluate the effects of genotype, sex, and their interaction on welfare-related traits, morphological characteristics, and developmental stability in reciprocal crosses between Anadolu-T and commercial broiler parent stocks at 3 and 6 weeks of age. Welfare indicators, including tonic immobility (TI) and rectal temperature (RT), were largely unaffected by genotype and genotype × sex interactions at both ages. At 3 weeks, sex had a significant effect on RT, with males exhibiting slightly higher values, suggesting early metabolic differences; however, this effect was not observed at 6 weeks. These findings indicate that early physiological welfare traits are relatively stable and largely independent of genetic background. Morphological traits, including comb length (CL), comb width (CW), and beak length (BL), were significantly influenced by sex at both ages, with males consistently showing higher values. Genotype effects were not evident at 3 weeks but became significant at 6 weeks, highlighting the increasing influence of genetic background on phenotypic expression as birds approached market age. Reciprocal cross differences suggested potential maternal and sex-linked genetic effects. Fluctuating asymmetry (FA) and relative asymmetry (RA) analyses revealed that developmental instability was generally low and not significantly affected by genotype at 3 weeks. However, at 6 weeks, genotype, sex, and genotype × sex interactions significantly influenced several traits, particularly in head, tarsometatarsus, and foot measurements. These results indicate that developmental instability increases with age and becomes more sensitive to genetic and physiological factors. Taken together, the findings demonstrate that early-stage traits are relatively stable across genotypes, whereas later developmental stages show increased variability driven by genotype, sex, and their interaction. These results emphasize the importance of considering age-dependent effects in broiler breeding and welfare evaluation.

Keywords: *Anadolu-T broiler, commercial broiler, reciprocal crossbreeding, developmental stability, fluctuating asymmetry*

Introduction

The global poultry industry has undergone substantial transformation over the past decades, largely driven by intensive genetic selection for rapid growth, improved feed efficiency, and high meat yield. While these advancements have significantly increased production efficiency, they have also raised concerns regarding potential trade-offs with animal welfare, physiological stability, and structural integrity (Scanes, 2015). In modern broiler production systems, understanding how genetic background and biological factors such as sex influence both performance and welfare-related traits is critical for sustainable breeding strategies.

Welfare-related physiological indicators, such as tonic immobility (TI) and rectal temperature (RT), are widely used to assess stress responsiveness and thermoregulatory capacity in poultry. TI is considered a reliable behavioral measure of fearfulness, while RT reflects metabolic activity and heat regulation (Dixon, 2020; Scanes, 2015). Previous research suggests that these physiological traits tend to be relatively stable during early development and may be less influenced by genotype compared to growth and morphological traits (Scanes, 2015). However, sex-related differences, particularly in metabolic rate and thermoregulation, may still be observed, especially during early growth phases.

Morphological traits, including comb length, comb width, and beak length, are important indicators of growth performance and developmental progression. These traits are strongly influenced by endocrine factors, particularly androgens, and often exhibit pronounced sexual dimorphism, with males generally showing greater development (Siegel, 2014; Siegel & Honaker, 2024). While early-stage morphological traits may show limited genotype effects, genetic differences tend to become more evident as birds approach later growth stages due to differential growth rates and metabolic demands (Zuidhof et al., 2014).

In addition to absolute trait measurements, developmental stability is an important aspect of animal performance and welfare. Fluctuating asymmetry (FA) and relative asymmetry (RA) are widely used indicators of developmental instability, reflecting an organism's ability to buffer against environmental and genetic perturbations during development (Klingenberg, 2015; Palmer & Strobeck, 1986). Increased asymmetry is often associated with stress, rapid growth, and reduced welfare, particularly in intensively selected broiler lines (Dixon, 2020).

Reciprocal crossbreeding between local and commercial genotypes provides an effective approach to investigate genetic, maternal, and heterosis effects on growth, morphology, and developmental stability. Such crosses can reveal differences arising from cytoplasmic inheritance, maternal effects, and sex-linked genes (Shen et al., 2016; Tuiskula-Haavisto & Vilkki, 2007). Moreover, crossbreeding strategies may contribute to improved developmental stability and performance by combining desirable traits from different genetic backgrounds.

Collectively, these considerations highlight the need to evaluate genotype, sex, and their interaction across different developmental stages. Therefore, the present study aimed to assess the effects of genotype, sex, and genotype × sex interaction on tonic immobility, rectal temperature, morphological traits, and developmental stability (FA and RA) in reciprocal crosses between Anadolu-T and commercial broiler lines at 3 and 6 weeks of age.

Material and Methods

Research Location and Animal Material

The study was conducted at the broiler research and development poultry house of the Animal Production Application and Research Center at Kahramanmaraş Sütçü İmam University. As animal material, broiler chicks belonging to four different genotypes, obtained from parents of the same age (46 weeks), were used. The permission numbered 2025/02 from the Kahramanmaraş Sütçü İmam University, Faculty of Agriculture Animal Experiments Local Ethics Committee (KSUZIRHADYEK). The crossbreeding combinations of the hybrids used in the study are presented in Table 1.

Table 1. The crossbreeding combinations of the hybrids

Genotype	Sire Line (♂)	Dam Line (♀)
Genotype 1 (AA)	Anadolu-T	Anadolu-T
Genotype 2 (CC)	Commercial Broiler	Commercial Broiler
Genotype 3 (AC)	Anadolu-T	Commercial Broiler
Genotype 4 (CA)	Commercial Broiler	Anadolu-T

Experimental Design and Housing Conditions

Each genotype constituted a treatment group, with four replicates per treatment and 24 chicks per replicate, resulting in a total of 4 × 4 × 24 = 384 chicks used in the study. The experiment was conducted using a completely randomized design, in which replicates were randomly assigned to the experimental treatments.

Environmental Conditions and House Management

The poultry house was a fully controlled, closed-type facility measuring 7 m × 19 m. The house was divided into pens using wire mesh partitions, and an area of 3 m² (2.0 m × 1.5 m) was allocated for each replicate, housing 24 birds per pen. The stocking density was 12 birds/m². Ventilation was controlled, and cooling of the poultry house was provided by evaporative cooling pads. As litter material, coarse pine wood shavings were used at a rate of 3 kg/m². A lighting program of 23 h light and 1 h dark was applied from the first day of the experiment. Lighting was provided using 15-W daylight-colored bulbs. Light intensity was measured at feeder level between two bulbs using a Lutron LX-101 lux meter and was maintained at 20 lux. Heating of the poultry house was ensured by air-blowing, thermostatically controlled electric heaters.

Feeding and Management Program

In the study, three commercial diets commonly used in the market and produced by a commercial feed company were used as feed material. The nutrient composition of the diets and the feeding periods are presented in Table 2. Feeding was carried out manually by weighing the feed, using one feeder per replicate. Feed was provided ad libitum throughout the experimental period. Water was supplied ad libitum through nipple drinkers, with five nipple drinkers installed in each replicate pen. A multivitamin supplement was added to the drinking water once a week at a rate of 20 g per 200 L. Live vaccines against Newcastle disease and Gumboro disease were administered via drinking water on days 6 and 13, respectively.

Table 2. Feed types and nutrient composition provided to chicks at different ages

Ingredients, %	*0-21 days*	*22-35 days*	*36-84 days*
Dry matter %	89.63	89.42	89.26
Metabolizable enerji MJ/kg	12.20	12.56	13.08
Crude Protein %	22.00	20.50	19.50

Data Collection and Measurements: The following parameters were recorded during the experimental period:

Morphological Measurements: In the study, a total of 384 broiler chickens from both genotypes were used. Morphological measurements were conducted on 64 chicks (16 males and 16 females from each genotype), and measurements were taken at 21 and 42 days of age, resulting in a total of 256 individual measurements across both time points, using a digital caliper (Van Nuffel et al., 2007). To minimize measurement error, all measurements were performed by the same researcher, and each measurement was taken three times on the same chick, with the average value used for analysis. In the study, each genotype consisted of four replicates, and four male and four female chicks were selected from each replicate. Measurements were taken at the 3rd and 6th weeks. In total, 64 chicks were evaluated (2 genotypes × 4 replicates × 16 chicks, comprising 8 males and 8 females per replicate). To evaluate the effects of genotype and sex on selected morphological traits and asymmetry, bilateral measurements were obtained from the head region of roosters on both the right and left sides. The traits measured included Comb length (CL), Comb width (CW), Eye width (EW), Beak length (BL), Facial width (FW), Wattle length (WL), Wattle width (WW) and nose Length (NL).

For the feet, morphological measurements were taken on both the right and left sides, including Joint width (JW), Tarsometatarsus length (TML Tarsometatarsus width at 1 cm above the spur (TMW-1), Tarsometatarsus width at the spur level (TMW-SL), Hind toe length (HTL), Outer toe claw length (OTCL), Fourth phalanx length of the outer toe (OP4L), Third phalanx length of the outer toe (OP3L), Middle toe claw length (MTCL), Fourth phalanx length of the middle toe (MP4L), and Third phalanx length of the middle toe (MP3L). The morphological characteristics evaluated in this study are illustrated in Figures 1 and 2 (Eleroğlu et al., 2020).

Fluctuating asymmetry (FA) was defined as the absolute difference between the right and left measurements, and was calculated as follows;

$$FA = |R - L| \quad \text{(Campo et al., 2002).} \quad (1)$$

Relative asymmetry (RA) was expressed as the ratio of the absolute asymmetry to the mean of the right and left trait values and was calculated as follows:

$$RA = (|R - L| / [(R + L)/2]) \times 100 \quad \text{(Yang, 1998).} \quad (2)$$

Relative asymmetry (RA) of the left and right bones (%) was calculated as $RA = |R - L| / ((R + L)/2) \times 100$, where R and L are the lengths of the right and left bones (mm), $|R - L|$ is their absolute difference, and $(R + L)/2$ is the average used to standardize the difference relative to bone size.

Physiological Measurements:

Rectal Temperature (RT): Cloacal temperature was measured by inserting the probe of a digital thermometer 2 cm into the cloaca.

Tonic Immobility (TI): Tonic immobility was used to evaluate fear responses in the broiler chicks. Chicks were gently placed on their backs on a padded surface, and light pressure was applied to the chest to induce immobility. The stopwatch was started after the pressure was released, and the duration until the bird attempted to stand up was recorded. Chicks that did not show a righting response within 10 minutes were excluded from the evaluation (Marx et al., 2008; Prieto & Campo, 2010).

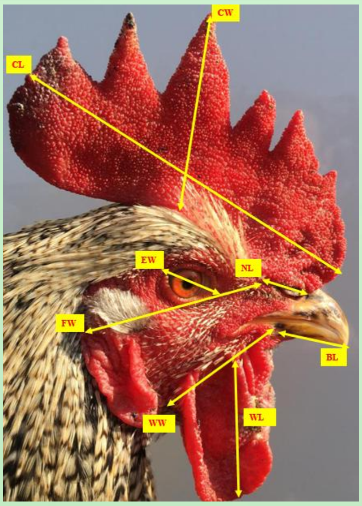

Figure 1. Head measurements

Comb length (CL), Comb width (CW), Eye width (EW), Beak length (BL), Facial width (FW), Wattle length (WL), Wattle width (WW) and nose Length (NL). (Eleroğlu et al., 2020).

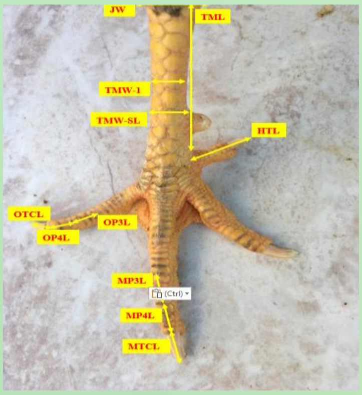

Figure 2. Foot measurements

Joint width (JW), Tarsometatarsus length (TML Tarsometatarsus width at 1 cm above the spur (TMW-1), Tarsometatarsus width at the spur level (TMW-SL), Hind toe length (HTL), Outer toe claw length (OTCL), Fourth phalanx length of the outer toe (OP4L), Third phalanx length of the outer toe (OP3L), Middle toe claw length (MTCL), Fourth phalanx length of the middle toe (MP4L), and Third phalanx length of the middle toe (MP3L). (Eleroğlu et al., 2020).

Statistical Analysis: At the end of each week, all birds were weighed in the afternoon, and average live weights were calculated. Data obtained for traits were evaluated using one-way analysis of variance (ANOVA), and comparisons among means were performed using Duncan's Multiple Range Test (Düzgüneş et al., 1987; Efe et al., 2000; Özdamar, 2002). Statistical analyses were carried out using the SPSS-22 statistical software package (SPSS-22, IBM Corp., USA)

Results are presented as means ± standard error of the mean (SEM).

Statistical model:

$$Y_{ij} = \mu + G_i + S_j + (G \times S)_{ij} + e_{ij}$$

Where

Y_{ij}= observed value,

μ= overall mean,

G_i= effect of genotype,

S_j= effect of sex,

$(G \times S)_{ij}$= interaction effect,

e_{ij}= random error.

Result and Discussion

The effects of genotype, sex, and their interaction on tonic immobility (TI) and rectal temperature (RT, °C) of reciprocal crosses between Anadolu-T and Commercial broiler parent stocks are summarized in Table 2. At 3 weeks of age, genotype did not significantly influence TI (P = 0.677) or RT (P = 0.441), while sex had a borderline significant effect on TI (P = 0.066) and a significant effect on RT (P = 0.024), with males exhibiting slightly higher rectal temperatures. The genotype × sex interaction was not significant for either trait (TI: P = 0.953; RT: P = 0.896). By 6 weeks of age, genotype and genotype × sex interaction remained non-significant for both TI (genotype P = 0.760; interaction P = 0.913) and RT (genotype P = 0.838; interaction P = 0.833). Sex effects on TI and RT were also non-significant at 6 weeks (TI: P = 0.486; RT: P = 0.677). These findings indicate that early welfare-related traits, as measured by TI and RT, are largely independent of genotype and genotype × sex interactions in these broiler crosses.

The current study demonstrates that tonic immobility and rectal temperature are largely unaffected by genotype and genotype × sex interactions in reciprocal crosses of Anadolu-T and Commercial broilers during early (3 weeks) and later (6 weeks) growth stages. This suggests that these welfare-related physiological traits are relatively stable across genotypes, consistent with prior reports on early broiler stress responses (Scanes, 2015; Dixon, 2020). The observed sex effect on rectal temperature at 3 weeks, with males showing slightly higher values, aligns with previous findings that male broilers often have higher basal metabolic rates and body temperatures during early development (Scanes, 2015).

The absence of significant genotype effects indicates that early thermoregulation and fear response, as measured by TI and RT, are largely independent of the genetic background, in contrast to morphological traits such as comb length and width, which show increasing genotype influence in later growth stages (Siegel & Honaker, 2024). Furthermore, the lack of genotype × sex interactions for TI and RT suggests that sex-specific responses do not differentially modulate these physiological parameters in early development. These results underscore the importance of considering both sex and genotype in morphological and growth studies, while early welfare-related traits may be more influenced by environmental and management factors than genetic selection (Dixon, 2020; Scanes, 2015; Siegel & Honaker, 2024).

Table 2. Effects of Genotype and Sex on Tonic Immobility Reaction and Rectal Temperature (°C) of Reciprocal Crosses between Anadolu-T and Commercial Broiler Parent Stocks

Items		*3. week*		*6. week*	
Genotype	Sex	TI±SEM	RT±SEM	TI±SEM	RT±SEM
AA	M	157.38±23.92	41.66±0.09	132.31±20.90	41.53±0.05
	F	127.19±22.81	41.54±0.08	138.44±20.33	41.49±0.06
	T	142.28±16.48	41.60±0.06	135.38±14.35	41.51±0.04
CC	M	143.06±22.81	41.70±0.06	155.38±24.96	41.54±0.06
	F	127.25±20.09	41.56±0.07	140.44±15.21	41.48±0.06
	T	135.16±15.02	41.63±0.05	147.91±14.44	41.51±0.04
AC	M	162.94±24.61	41.69±0.12	138.63±20.86	41.48±0.06
	F	136.13±23.74	41.61±0.09	121.94±8.61	41.51±0.05
	T	149.53±16.99	41.65±0.07	130.28±11.2	41.5±0.04
CA	M	144.25±19.79	41.63±0.07	137.81±8.41	41.47±0.04
	F	103.50±13.29	41.43±0.04	128.63±13.36	41.47±0.04
	T	123.88±12.28	41.53±0.04	133.22±7.81	41.47±0.03
Source of variations		Sig.	Sig.	Sig.	Sig.
Genotype		0.677	0.441	0.760	0.838
Sex		0.066	***0.024***	0.486	0.677
G × S		0.953	0.896	0.913	0.833

AA: Anadolu – T × Anadolu – T; CC: Commercial × Commercial; AC: Anadolu – T male × Commercial female; CA: Commercial male × Anadolu – T female; F = Female; M = Male; T: Total; TI = Tonic immobility; RT = Rectal temperatures; Genotype × Sex; SEM: The Mean of Standard Error

Table 3 presents the effects of genotype, sex, and their interaction on non-bilateral morphological traits—comb length (CL), comb width (CW), and beak length (BL)—at 3 and 6 weeks of age in reciprocal crosses between Anadolu-T and commercial broiler parent stocks.

At 3 weeks of age, genotype did not significantly influence CL, CW, or BL (P > 0.05), indicating that the early development of these morphological traits is relatively independent of genetic background. However, sex had a highly significant effect on all traits (P < 0.001), with males consistently showing higher values than females across all genotypes. This pattern suggests that sexual dimorphism in morphological characteristics begins to emerge early in broiler development. Similar findings have been reported in modern broiler lines, where males exhibit faster growth and more pronounced morphological development than females (Siegel & Honaker, 2024; Müsse et al., 2022).

The genotype × sex interaction was only significant for CW (P<0.05), suggesting that comb width development at this stage may be influenced by sex-specific genetic expression. This partial interaction effect implies that while overall genotype differences are not yet pronounced, certain traits may already show differential sensitivity to combined genetic and sex effects ().

At 6 weeks of age, genotype effects became significant for all traits (CL: P=0.002; CW: P=0.006; BL: P=0.012), indicating that genetic background plays a more prominent role as birds approach market age. Among genotypes, the CA group (Commercial male × Anadolu-T female) exhibited the highest total values for CL (42.47 mm), CW (15.35 mm), and BL (18.56 mm), followed by AA and CC groups, while the AC genotype generally showed lower values. These results suggest a possible maternal or reciprocal cross effect, where the direction of crossing influences phenotypic expression. Reciprocal cross differences have been widely documented in poultry due to maternal inheritance, cytoplasmic effects, and sex-linked genes (Shen et al., 2016; Tuiskula-Haavisto & Vilkki, 2007).

Sex remained a highly significant factor at 6 weeks (P<0.001), with males maintaining superior values for all traits. This is consistent with the continued influence of hormonal and growth-related differences between sexes, particularly in traits such as comb development, which are known to be androgen-dependent (Scanes, 2015). The persistence of sex effects across both ages reinforces the importance of considering sex as a key biological variable in morphological and production studies (Siegel, 2014; Dixon, 2020; FAO, 2022).

The genotype × sex interaction was significant only for BL (P<0.05) at 6 weeks, indicating that beak development may be differentially regulated depending on both genetic background and sex. Although few studies have specifically examined genotype × sex interactions on comb width, genotype × sex interactions have been widely documented for morphological and performance traits in chickens, indicating that combined genetic and sex-specific effects can differentially influence phenotypic expression (Ajayi & Ejiofor, 2009; El-Henfnawy et al., 2022).

The findings demonstrate that while early-stage (3-week) morphological traits are primarily influenced by sex, genotype effects become more pronounced with age (6 weeks). The superior performance of the CA genotype suggests that reciprocal crossing strategies can be utilized to enhance certain morphological traits. These results are consistent with recent research showing that genetic background increasingly influences growth performance at later stages and that sex consistently impacts broiler morphology and performance (England et al., 2022; Xie et al., 2026).

Table 3. Influence of Genotype and Sex on Non-Bilateral Morphological Characteristics of Reciprocal Crosses between Anadolu-T and Commercial Broiler Parent Stocks

Items		3. weeks			6. weeks		
Genotype	Sex	CL	CW	BL	CL	CW	BL
AA	M	36.47±0.86	14.31±0.51	16.08±0.15	50.33±1.59	20.59±1.14	18.22±0.18
	F	25.8±0.79	7.13±0.42	15.99±0.19	29.69±0.38	7.88±0.29	17.73±0.27
	T	31.14±1.12	10.72±0.72	16.04±0.12	***40.01±2.02^{b}***	***14.23±1.28^{ab}***	***17.97±0.17^{ac}***
CC	M	36.64±0.83	13.97±0.44	16.64±0.14	48.22±1.38	18.15±0.73	18.59±0.29
	F	26.33±0.63	7.45±0.4	15.79±0.16	30.65±0.51	8.76±0.43	18.35±0.23
	T	31.48±1.06	10.71±0.66	16.22±0.13	***39.43±1.74^{b}***	***13.45±0.94^{b}***	***18.47±0.18^{ab}***
AC	M	36.56±0.69	14.03±0.58	16.27±0.18	46.62±1.2	17.82±1.06	17.79±0.26
	F	25.93±0.5	6.87±0.28	15.64±0.17	31.14±0.35	8.13±0.36	17.89±0.31
	T	31.24±1.04	10.45±0.72	15.95±0.13	***38.88±1.52^{b}***	***12.97±1.03^{b}***	***17.84±0.2^{c}***
CA	M	36.78±0.75	12.35±0.49	16.35±0.2	51.27±0.88	20.73±0.39	19.25±0.24
	F	24.38±0.46	7.5±0.39	15.52±0.14	33.67±0.87	9.97±0.7	17.87±0.25
	T	30.58±1.2	9.93±0.53	15.93±0.14	***42.47±1.69^{a}***	***15.35±1.04^{a}***	***18.56±0.21^{a}***
Source of variations		Sig.	Sig.	Sig.	Sig.	Sig.	Sig.
Genotype		0.621	0.257	0.309	***0.002***	***0.006***	***0.012***
Sex		***0.000***	***0.000***	***0.000***	***0.000***	***0.000***	***0.007***
G × S		0.442	***0.034***	0.086	0.083	0.086	***0.033***

A: Anadolu – T × Anadolu – T; CC: Commercial × Commercial; AC: Anadolu – T male × Commercial female; CA: Commercial male × Anadolu – T female; F = Female; M = Male; T: Total; CL = Comb length; CW = Comb width; BL= Beak length; Genotype × Sex; SEM: The Mean of Standard Error

Fluctuating asymmetry (FA) and relative asymmetry (RA) indices at 3 weeks of age are presented in Table 4. The results showed that genotype had no significant effect on any of the FA traits, including wattle length (WL), wattle width (WW), eye width (EW), nose length (NL), and facial width (FW) (P>0.05). Similarly, genotype × sex interactions were not significant for these traits, indicating that developmental stability during early growth is largely independent of genetic background. This finding is consistent with previous studies indicating that early morphological development in broilers tends to be relatively uniform across genotypes and is largely shaped by environmental conditions and maternal influences, particularly during the embryonic and early post-hatch periods (Zuidhof et al., 2021; Dixon et al., 2016).

Sex effects on FA traits were generally limited at 3 weeks, with a significant effect observed only for WW (P<0.05). The lack of consistent sex-related differences at this stage suggests that sexual dimorphism has not yet fully manifested in early growth. Similar findings have been reported in broiler chickens, although sex-related differences in growth potential exist, their phenotypic expression is often limited during early developmental stages and becomes increasingly evident as birds approach later growth phases (An & Kong, 2025; England et al., 2023; Siegel, 2014).

For RA traits, genotype and genotype × sex interaction effects were also non-significant (P>0.05), reinforcing the notion that early developmental instability is not strongly genotype-dependent. However, sex had a highly significant effect on WW (P<0.001), indicating that even at an early age, certain traits may show sensitivity to sex-related physiological differences. This may be linked to early hormonal regulation and differences in tissue growth dynamics (Scanes, 2015).

Overall, the low and non-significant variation observed in both FA and RA traits at 3 weeks suggests that developmental stability is relatively high during early life. This supports the concept that fluctuating asymmetry tends to increase with age as organisms are exposed to cumulative environmental and physiological stressors (Palmer & Strobeck, 1986).

Fluctuating asymmetry (FA) and relative asymmetry (RA) indices at 6 weeks of age are presented in Table 5. Unlike the findings at 3 weeks, genotype had a significant effect on FA for wattle length (WL) (P<0.01) and nose length (NL) (P<0.01). These results indicate that genetic background becomes more influential on developmental stability as age progresses, which is consistent with previous reports highlighting that genetic effects on growth and morphology become more pronounced in later stages of broiler development (Zuidhof et al., 2014).

Sex effects were more pronounced at 6 weeks compared to 3 weeks. Significant sex effects were observed for WW (P<0.01) and NL (P<0.05), suggesting that sexual dimorphism increasingly influences asymmetry patterns with age. This may be explained by endocrine differences between males and females, particularly the role of sex hormones in regulating growth and secondary traits (Scanes, 2015).

Genotype × sex interactions were significant for several FA traits, indicating that the response of asymmetry traits to sex differs depending on genetic background. Similar interaction effects have been reported in broiler studies, where genotype and sex jointly influence growth patterns, carcass traits, and developmental stability (Siegel, 2014; Dixon, 2020).

For RA traits, genotype significantly affected WL, WW, and NL (P<0.05), while sex effects remained significant for WW and NL. The higher asymmetry observed in certain traits may reflect increased sensitivity of rapidly growing tissues to developmental perturbations, particularly under intensive selection for growth (Dixon, 2020).

When comparing the results between 3 and 6 weeks of age, a clear shift in the determinants of asymmetry is observed. At 3 weeks, both FA and RA traits were largely unaffected by genotype and genotype × sex interactions, indicating that early developmental stability is relatively uniform across genotypes. In contrast, at 6 weeks, genotype emerged as a significant source of variation, suggesting that genetic differences become more evident with age. This pattern aligns with studies showing that phenotypic divergence among broiler genotypes increases over time due to differential growth rates and metabolic demands (Zuidhof et al., 2014).

Similarly, sex effects, which were limited at 3 weeks, became more evident at 6 weeks. This supports the notion that hormonal influences intensify with age, contributing to differences in growth and morphology between males and females (Scanes, 2015).

The emergence of significant genotype × sex interactions at 6 weeks further indicates that developmental stability is shaped by a complex interplay of genetic and physiological factors. Previous research has emphasized that such interactions are particularly important in fast-growing broilers, where growth-related stress can differentially affect males and females (Dixon, 2020).

Across both ages, traits such as WL and WW exhibited higher asymmetry compared to more stable traits, suggesting that soft tissue structures are more susceptible to developmental instability. This is consistent with the general theory of fluctuating asymmetry, which states that rapidly growing or sexually dimorphic traits tend to show greater sensitivity to environmental and physiological stressors (Palmer & Strobeck, 1986).

At 3 weeks of age, fluctuating asymmetry (FA) values for tarsometatarsus traits (TML, JW, TMW-1, TMW-SL) did not differ significantly among genotypes (P>0.05). Similarly, sex and genotype × sex interaction effects were not significant for any FA trait, although sex approached significance for TML (P=0.085) and TMW-1 (P=0.103).

Descriptively, CC and AC genotypes showed relatively higher FA values for TML and TMW-SL, whereas CA males exhibited the lowest TML asymmetry; however, these differences were not statistically significant (P>0.05). Females in the CA genotype showed notably higher JW asymmetry compared to males, but this variation was not statistically significant. For relative asymmetry (RA), genotype had no significant effect on any trait (P>0.05). Sex significantly influenced TML (P=0.045), with females generally showing higher relative asymmetry than males. No significant genotype × sex interactions were detected.

Table 4. Fluctuating (FA) and Relative (RA) Asymmetry Indices Derived from Bilateral Head Measurements of Reciprocal Crosses between Anadolu-T and Commercial Broiler Parent Stocks at Week 3

Items		*3. Week Fluctuating Asymmetry (FA)*				
Genotype	Sex	WL±SEM	WW±SEM	EW±SEM	NL±SEM	FW±SEM
AA	M	2.87±0.53	0.85±0.16	0.34±0.05	0.25±0.06	1.03±0.22
	F	2.54±0.55	1.22±0.23	0.29±0.04	0.27±0.05	1.78±0.5
	T	2.7±0.38	1.04±0.14	0.32±0.03	0.26±0.04	1.4±0.28
CC	M	2.71±0.55	1.31±0.22	0.28±0.05	0.27±0.04	1.6±0.31
	F	1.2±0.23	1.13±0.19	0.3±0.04	0.29±0.06	0.91±0.26
	T	1.95±0.32	1.22±0.14	0.29±0.03	0.28±0.04	1.26±0.21
AC	M	2.23±0.42	1.0±0.21	0.28±0.05	0.2±0.05	1.54±0.26
	F	2.0±0.37	1.27±0.18	0.27±0.06	0.23±0.03	1.68±0.29
	T	2.12±0.27	1.13±0.14	0.28±0.04	0.21±0.03	1.61±0.19
CA	M	2.51±0.48	1.53±0.38	0.28±0.05	0.23±0.04	1.96±0.34
	F	1.61±0.28	0.84±0.12	0.27±0.06	0.18±0.04	1.44±0.3
	T	2.06±0.29	1.18±0.2	0.28±0.04	0.21±0.03	1.7±0.23
Source of variation		Sig.	Sig.	Sig.	Sig.	Sig.
Genotype (G)		0.324	0.859	0.845	0.340	0.502
Sex (S)		***0.019***	0.712	0.714	0.875	0.720
G × S		0.446	0.069	0.904	0.775	0.097
		3. Week Relative Asymmetry (RA)				
Genotype	Sex	WL±SEM	WW±SEM	EW±SEM	NL±SEM	FW±SEM
AA	M	3.75±0.72	1.51±0.27	0.67±0.1	1±0.22	0.64±0.14
	F	4.14±1	3.72±0.66	0.59±0.08	1.17±0.2	1.17±0.35
	T	3.95±0.61	2.61±0.4	0.63±0.06	1.08±0.15	0.9±0.19
CC	M	3.22±0.66	2.58±0.44	0.53±0.1	1.05±0.15	1±0.19
	F	1.68±0.32	3.54±0.5	0.61±0.08	1.24±0.29	0.57±0.16
	T	2.45±0.39	3.06±0.34	0.57±0.06	1.14±0.16	0.78±0.13
AC	M	2.83±0.49	1.92±0.41	0.55±0.1	0.77±0.18	0.96±0.16
	F	2.95±0.54	3.96±0.49	0.54±0.12	0.94±0.13	1.08±0.19
	T	2.89±0.36	2.94±0.36	0.54±0.08	0.85±0.11	1.02±0.12
CA	M	3.26±0.62	2.8±0.62	0.55±0.08	0.92±0.16	1.21±0.21
	F	2.56±0.41	2.74±0.41	0.56±0.12	0.75±0.15	0.91±0.19
	T	2.91±0.37	2.77±0.36	0.55±0.07	0.83±0.11	1.06±0.14
Source of variation		Sig.	Sig.	Sig.	Sig.	Sig.
Genotype (G)		0.112	0.812	0.826	0.248	0.533
Sex (S)		0.329	***0.000***	0.981	0.498	0.895
G × S		0.412	0.078	0.873	0.732	0.093

A: Anadolu – T × Anadolu – T; CC: Commercial × Commercial; AC: Anadolu – T male × Commercial female; CA: Commercial male × Anadolu – T female; F = female; M = male, T=Total; WL = Wattle length; WW = Wattle width; EW = Eye width; NL = Nose length; FW = Facial width; SEM: The Mean of Standard Error

The lack of significant genotype and interaction effects at week 3 indicates that developmental stability of tarsometatarsus traits is largely maintained across genetic backgrounds during early growth. This finding is consistent with the concept of developmental canalization, which describes the ability of organisms to buffer against genetic and environmental perturbations and maintain stable developmental trajectories (Klingenberg, 2015; Zakharov et al., 2020).

The limited sex effect observed only in RA for TML suggests that sexual dimorphism in developmental instability is still minimal at this stage. Previous studies in broilers have indicated that skeletal development and related morphometric traits show limited sexual dimorphism during early posthatch growth, whereas sex-related differences become more pronounced in later growth stages as divergence in growth rate increases (Rose et al., 1996; Charuta et al., 2013; Müsse et al., 2022).

Although some numerical differences were observed, particularly higher asymmetry in CC and reciprocal cross genotypes, these differences were not statistically significant, indicating that genetic influences on skeletal asymmetry are not yet strongly expressed at week 3. Taken together, these findings suggest a high level of developmental stability during early skeletal growth. This interpretation is consistent with previous broiler studies indicating that fluctuating asymmetry reflects developmental instability arising from genetic and environmental perturbations and can serve as an indicator of the organism's ability to cope with such factors (Van Nuffel et al., 2007; Buijs et al., 2012).

At 6 weeks of age, genotype significantly affected FA values for all traits: TML (P=0.004), JW (P=0.049), TMW-1 (P=0.024), and TMW-SL (P=0.011). The AA genotype showed the highest FA for TML (0.88±0.31), while CA exhibited the lowest (0.06±0.02). For other traits, CC and AC generally displayed higher asymmetry compared to CA.

Table 5. Fluctuating (FA) and Relative (RA) Asymmetry Indices Derived from Bilateral Head Measurements of Reciprocal Crosses between Anadolu-T and Commercial Broiler Parent Stocks at Week 6

Items		*6. Week Fluctuating Asymmetry (FA)*				
Genotype	Sex	WL±SEM	WW±SEM	EW±SEM	NL±SEM	FW±SEM
AA	M	2.27±0.51	1.96±0.32	0.3±0.06	0.22±0.04	2.16±0.23
	F	1.89±0.39	0.7±0.14	0.14±0.02	0.1±0.02	1.57±0.23
	T	***2.08±0.32[b]***	1.33±0.21	0.22±0.04	***0.16±0.02[b]***	1.87±0.17
CC	M	3.52±0.44	1.34±0.33	0.13±0.03	0.05±0.01	1.15±0.23
	F	2.85±0.26	1.58±0.26	0.41±0.13	0.22±0.06	1.99±0.23
	T	***3.18±0.26[a]***	1.46±0.21	0.27±0.07	***0.14±0.03[b]***	1.57±0.18
AC	M	1.53±0.23	1.72±0.29	0.22±0.03	0.15±0.02	2.26±0.22
	F	1.9±0.24	1.06±0.1	0.16±0.03	0.22±0.03	1.61±0.2
	T	***1.71±0.17[b]***	1.39±0.16	0.19±0.02	***0.18±0.02[b]***	1.93±0.16
CA	M	2.73±0.45	1.77±0.34	0.18±0.04	0.19±0.03	1.52±0.13
	F	1.98±0.27	0.85±0.19	0.26±0.05	0.4±0.11	1.45±0.15
	T	***2.35±0.27[b]***	1.31±0.21	0.22±0.03	***0.3±0.06[a]***	1.49±0.1
Source of variation		Sig.	Sig.	Sig.	Sig.	Sig.
Genotype (G)		***0.001***	0.937	0.537	***0.008***	0.085
Sex (S)		0.165	***0.001***	0.403	***0.022***	0.419
G × S		0.410	***0.031***	***0.002***	***0.005***	***0.001***
		6. Week Relative Asymmetry (RA)				
Genotype	Sex	WL±SEM	WW±SEM	EW±SEM	NL±SEM	FW±SEM
AA	M	2.14±0.48	2.27±0.35	0.53±0.11	0.76±0.12	1.16±0.12
	F	2.22±0.45	1.86±0.33	0.26±0.05	0.34±0.07	0.9±0.13
	T	***2.18±0.32[b]***	***2.06±0.24[c]***	0.39±0.06	***0.55±0.08[b]***	1.03±0.09
CC	M	3.72±0.47	1.8±0.44	0.23±0.05	0.18±0.04	0.61±0.12
	F	3.27±0.32	4.38±0.66	0.74±0.22	0.79±0.21	1.11±0.12
	T	***3.5±0.28[a]***	***3.09±0.46[a]***	0.48±0.12	***0.48±0.12[b]***	0.86±0.1
AC	M	1.56±0.25	2.52±0.44	0.38±0.06	0.49±0.08	1.23±0.12
	F	2.4±0.33	3.55±0.39	0.29±0.06	0.84±0.13	0.87±0.11
	T	***1.98±0.22[b]***	***3.03±0.3[ab]***	0.33±0.04	***0.67±0.08[b]***	1.05±0.08
CA	M	2.47±0.4	2.05±0.4	0.33±0.07	0.67±0.1	0.82±0.07
	F	2.37±0.34	2.25±0.51	0.48±0.09	1.48±0.4	0.81±0.09
	T	***2.42±0.26[b]***	***2.15±0.32[bc]***	0.41±0.06	***1.08±0.22[a]***	0.82±0.05
Source of variation		Sig.	Sig.	Sig.	Sig.	Sig.
Genotype (G)		***0.001***	***0.033***	0.568	***0.006***	0.090
Sex (S)		0.732	***0.009***	0.315	***0.009***	0.687
G × S		0.402	***0.008***	***0.002***	***0.006***	***0.001***

A: Anadolu – T × Anadolu – T; CC: Commercial × Commercial; AC: Anadolu – T male × Commercial female; CA: Commercial male × Anadolu – T female; F = female; M = male, T=Total; WL = Wattle length; WW = Wattle width; EW = Eye width; NL = Nose length; FW = Facial width; SEM: The Mean of Standard Error

The results of fluctuating (FA) and relative (RA) asymmetry analyses derived from bilateral tarsometatarsus measurements at weeks 3 and 6 are presented in Tables 6 and 7, respectively.

Sex had significant effects on TML (P=0.023), TMW-1 (P<0.001), and TMW-SL (P=0.049), indicating increased sexual divergence in asymmetry at this stage. Genotype × sex interaction was significant for JW (P=0.033), suggesting that sex-related differences varied depending on genotype.

For RA, genotype significantly influenced TML (P=0.005) and TMW-SL (P=0.023). The AA genotype showed the highest RA for TML, while CA consistently exhibited the lowest values. Sex significantly affected TML (P=0.019) and TMW-1 (P=0.003). A significant genotype × sex interaction was observed for JW (P=0.035).

The emergence of significant genotype effects across all FA traits at week 6 suggests that genetic background becomes an increasingly important determinant of developmental instability as skeletal growth progresses. In contrast to early developmental stages, later growth phases in broilers are more strongly influenced by genetic differences and the physiological costs associated with rapid growth and selection intensity (Van Nuffel et al., 2007; Buijs et al., 2012). The higher asymmetry observed in certain genotypes (AA for TML, CC and AC for other traits) may reflect genotype-dependent variation in the ability to maintain developmental stability under intensive growth conditions. Commercial broiler lines, which have been intensively selected for rapid growth and feed efficiency, are often associated with increased susceptibility to environmental and physiological stress. Recent experimental evidence in broilers has shown that heat stress and environmental conditions can increase fluctuating asymmetry as an indicator of developmental instability (Havenstein et al., 2003; Carvalho et al., 2026).

Significant sex effects further indicate that males and females differ in their response to developmental stress, likely due to differences in growth rate and hormonal regulation. The presence of genotype × sex interactions supports the idea that these effects are not uniform across genetic backgrounds.

Lower asymmetry in the CA genotype may suggest a potential heterosis effect, as fluctuating asymmetry is widely recognized as an indicator of developmental stability influenced by both genetic background and environmental stressors (Van Nuffel et al., 2007; Møller & Swaddle, 1997). Crossbreeding can enhance biological robustness through increased heterozygosity and favorable gene interactions (Falconer & Mackay, 1996; Wang et al., 2022). However, direct evidence linking heterosis to reduced asymmetry in poultry remains limited and inconsistent across studies (Van Dongen, 2006).

A clear ontogenetic shift in developmental stability is observed between weeks 3 and 6. At week 3, neither genotype nor sex had a significant effect on most FA and RA traits, indicating strong developmental buffering during early skeletal growth. In contrast, by week 6, genotype significantly influenced nearly all asymmetry traits, and sex effects became more pronounced.

This transition suggests that developmental instability increases with age, particularly as growth accelerates and metabolic demands rise. Early-stage canalization appears to protect skeletal development, but this stability diminishes over time, revealing underlying genetic differences.

Notably, the CA genotype consistently exhibited lower asymmetry at week 6, supporting the hypothesis that reciprocal crossbreeding may enhance developmental stability. Conversely, some pure and commercial genotypes showed increased asymmetry, possibly reflecting trade-offs between rapid growth and structural stability.

In summary, while week 3 results reflect uniform and stable skeletal development, week 6 findings highlight the increasing influence of genotype, sex, and their interaction on developmental instability, emphasizing the role of genetic selection and growth dynamics in shaping broiler welfare and morphology.

Table 6. Fluctuating (FA) and Relative (RA) Asymmetry Analyses Derived from Bilateral Tarsometatarsus measurements of Reciprocal Crosses between Anadolu-T and Commercial Broiler Parent Stocks at Week 3

Items		*3. Week Fluctuating Asymmetry (FA)*			
Genotype	Sex	TML±SEM	JW±SEM	TMW-1±SEM	TMW-SL±SEM
AA	M	0.41±0.11	0.42±0.11	0.3±0.07	0.26±0.04
	F	0.57±0.14	0.55±0.3	0.34±0.06	0.39±0.05
	T	0.49±0.09	0.48±0.16	0.32±0.05	0.32±0.03
CC	M	0.63±0.2	0.4±0.08	0.41±0.06	0.44±0.07
	F	0.6±0.14	0.24±0.07	0.28±0.05	0.42±0.08
	T	0.61±0.12	0.32±0.05	0.35±0.04	0.43±0.05
AC	M	0.41±0.13	0.45±0.12	0.48±0.06	0.45±0.08
	F	0.58±0.15	0.4±0.05	0.33±0.06	0.31±0.06
	T	0.49±0.1	0.42±0.07	0.41±0.04	0.38±0.05
CA	M	0.28±0.09	0.36±0.06	0.34±0.06	0.31±0.06
	F	0.68±0.16	0.77±0.3	0.31±0.05	0.31±0.06
	T	0.48±0.1	0.56±0.15	0.32±0.04	0.31±0.04
Source of variation		Sig.	Sig.	Sig.	Sig.
Genotype (G)		0.755	0.525	0.434	0.227
Sex (S)		0.085	0.483	0.103	0.874
G × S		0.505	0.360	0.347	0.233
Items		3. Week Relative Asymmetry (RA)			
Genotype	Sex	TML±SEM	JW±SEM	TMW-1±SEM	TMW-SL±SEM
AA	M	0.13±0.04	0.48±0.13	0.62±0.14	0.55±0.08
	F	0.19±0.05	0.64±0.33	0.75±0.13	0.84±0.11
	T	0.16±0.03	0.56±0.17	0.68±0.1	0.69±0.07
CC	M	0.19±0.06	0.43±0.09	0.78±0.11	0.86±0.13
	F	0.2±0.05	0.29±0.08	0.59±0.1	0.89±0.17
	T	0.19±0.04	0.36±0.06	0.68±0.08	0.87±0.11
AC	M	0.13±0.04	0.48±0.14	0.96±0.13	0.91±0.15
	F	0.19±0.05	0.47±0.06	0.7±0.13	0.69±0.13
	T	0.16±0.03	0.48±0.07	0.83±0.09	0.8±0.1
CA	M	0.09±0.03	0.4±0.06	0.68±0.13	0.65±0.14
	F	0.22±0.05	0.98±0.41	0.67±0.11	0.69±0.14
	T	0.15±0.03	0.69±0.21	0.68±0.08	0.67±0.1
Source of variation		Sig.	Sig.	Sig.	Sig.
Genotype (G)		0,796	0.444	0.519	0.400
Sex (S)		***0,045***	0.315	0.334	0.714
G × S		0,549	0.320	0.382	0.304

A: Anadolu – T × Anadolu – T; CC: Commercial × Commercial; AC: Anadolu – T male × Commercial female; CA: Commercial male × Anadolu – T female; F = female; M = male; T = Total; TML = Tarsometatarsus length; [3]JW = Joint width; TMW-1 = Tarsometatarsus width at 1 cm above the spur; TMW-SL = Tarsometatarsus width at the spur level; SEM: The Mean of Standard Error

Table 7. Fluctuating (FA) and Relative (RA) Asymmetry Analyses Derived from Bilateral Tarsometatarsus measurements of Reciprocal Crosses between Anadolu-T and Commercial Broiler Parent Stocks at Week 6

Items		*6. Week Fluctuating Asymmetry (FA)*			
Genotype	Sex	TML±SEM	JW±SEM	TMW-1±SEM	TMW-SL±SEM
AA	M	0.34±0.1	0.24±0.05	0.36±0.05	0.31±0.05
	F	1.41±0.6	0.43±0.06	0.12±0.02	0.18±0.04
	T	***0.88±0.31***[a]	***0.34±0.04***[b]	***0.24±0.03***[b]	***0.24±0.03***[b]
CC	M	0.27±0.08	0.8±0.19	0.5±0.11	0.44±0.06
	F	0.62±0.22	0.39±0.09	0.26±0.04	0.41±0.05
	T	***0.44±0.12***[ab]	***0.59±0.11***[a]	***0.38±0.06***[a]	***0.43±0.04***[a]
AC	M	0.2±0.04	0.71±0.2	0.42±0.07	0.5±0.1
	F	0.23±0.07	0.36±0.09	0.25±0.03	0.34±0.03
	T	***0.22±0.04***[b]	***0.54±0.11***[ab]	***0.34±0.04***[ab]	***0.42±0.05***[a]
CA	M	0.03±0.01	0.35±0.05	0.21±0.06	0.43±0.07
	F	0.09±0.03	0.32±0.07	0.23±0.06	0.39±0.08
	T	***0.06±0.02***[b]	***0.33±0.04***[b]	***0.22±0.04***[b]	***0.41±0.05***[a]
Source of variation		Sig.	Sig.	Sig.	Sig.
Genotype (G)		***0,004***	***0.049***	***0.024***	***0.011***
Sex (S)		***0,023***	0.067	***0.000***	***0.049***
G × S		0,094	***0.033***	0.104	0.674
Items		6. Week Relative Asymmetry (RA)			
Genotype	Sex	TML±SEM	JW±SEM	TMW-1±SEM	TMW-SL±SEM
AA	M	0.09±0.03	0.23±0.05	0.7±0.1	0.6±0.09
	F	0.41±0.18	0.48±0.07	0.24±0.04	0.38±0.08
	T	***0.25±0.09***[a]	0.36±0.05	0.47±0.07	***0.49±0.06***[b]
CC	M	0.07±0.02	0.75±0.18	0.89±0.19	0.81±0.12
	F	0.17±0.06	0.41±0.09	0.51±0.09	0.82±0.11
	T	***0.12±0.03***[ab]	0.58±0.1	0.7±0.11	***0.82±0.08***[a]
AC	M	0.05±0.01	0.67±0.19	0.77±0.14	0.94±0.2
	F	0.07±0.02	0.39±0.1	0.51±0.07	0.7±0.06
	T	***0.06±0.01***[b]	0.53±0.11	0.64±0.08	***0.82±0.1***[a]
CA	M	0.01±0	0.33±0.04	0.39±0.11	0.79±0.14
	F	0.02±0.01	0.35±0.07	0.47±0.12	0.84±0.16
	T	***0.02±0***[b]	0.34±0.04	0.43±0.08	***0.82±0.1***[a]
Source of variation		Sig.	Sig.	Sig.	Sig.
Genotype (G)		***0,005***	0.082	0.068	***0.023***
Sex (S)		***0,019***	0.253	***0.003***	0.269
G × S		0,082	***0.035***	0.105	0.558

A: Anadolu – T × Anadolu – T; CC: Commercial × Commercial; AC: Anadolu – T male × Commercial female; CA: Commercial male × Anadolu – T female; F = female; M = male; T = Total; TML = Tarsometatarsus length; JW = Joint width; TMW-1 = Tarsometatarsus width at 1 cm above the spur; TMW-SL = Tarsometatarsus width at the spur level; SEM: The Mean of Standard Error

The results of fluctuating (FA) and relative (RA) asymmetry analyses derived from bilateral foot measurements at weeks 3 and 6 are presented in Tables 8 and 9, respectively.

At 3 weeks of age, fluctuating asymmetry (FA) of foot traits was significantly affected by genotype only for hind toe length (HTL) (P=0.012), whereas no significant genotype effects were detected for the other traits (P>0.05). The CC genotype exhibited the highest FA values for HTL (1.61±0.18), while AA, AC, and CA genotypes showed lower and similar values.

Sex and genotype × sex interaction had no significant effects on any FA traits (P>0.05), indicating a lack of sexual dimorphism in foot asymmetry at this stage.

For relative asymmetry (RA), genotype significantly influenced HTL (P=0.013), with CC again showing the highest asymmetry. No significant effects of sex or genotype × sex interaction were observed for any RA traits.

Overall, asymmetry levels across most traits were moderate, with limited statistically significant variation.

The limited genotype effect observed only for HTL suggests that developmental instability in foot traits is generally low and relatively uniform across genotypes during early growth. This supports the concept that early morphological development is strongly buffered against genetic variation (Klingenberg, 2015).

The absence of sex effects further indicates that sexual dimorphism in foot asymmetry is not yet established at 3 weeks of age. Similar findings in broilers have shown that early skeletal and appendage traits develop under strong developmental control, minimizing variability between sexes (Charuta et al., 2013; Rose et al., 1996).

The higher asymmetry observed in the CC genotype for HTL may reflect early signs of growth-related stress associated with intensive selection; however, the lack of widespread significance suggests that these effects remain limited at this stage. Overall, the results indicate high developmental stability of foot structures in early life.

At 6 weeks of age, genotype significantly affected FA for outer toe claw length (OTCL) (P=0.019), while other traits were not significantly influenced by genotype. Sex significantly affected HTL (P=0.034) and OP4L (P=0.003), indicating increasing sexual divergence in asymmetry.

Significant genotype × sex interactions were observed for HTL (P<0.001), MTCL (P=0.001), and MP4L (P=0.013), demonstrating that the effects of sex varied depending on genotype.

For RA, genotype significantly influenced OTCL (P=0.008), with AA and CC genotypes generally showing higher asymmetry values compared to CA. Sex significantly affected OP4L (P=0.001), while genotype × sex interaction was significant for HTL (P<0.001), MTCL (P=0.001), and MP4L (P=0.021).

Compared to week 3, the emergence of significant genotype, sex, and interaction effects at week 6 may indicate increased developmental instability in foot traits as birds mature. This pattern is consistent with previous studies showing that rapid growth phases and adverse environmental or genetic factors can increase fluctuating asymmetry, reflecting reduced developmental stability (Møller & Manning, 2003; Van Nuffel et al., 2007)

The significant sex effects suggest that males and females differ in growth dynamics and structural development, leading to increased asymmetry in certain traits. This aligns with previous findings that sexual dimorphism in developmental instability becomes more evident at later growth stages (Klingenberg, 2015).

The presence of genotype × sex interactions highlights the complexity of developmental processes, where genetic background modulates the expression of sex-related differences. Additionally, the relatively lower asymmetry observed in some reciprocal crosses (particularly CA in certain traits) suggests potential heterosis effects improving developmental stability.

Table 8. Fluctuating (FA) and Relative (RA) Asymmetry Analyses Derived from Foot Measurements According to Genotype and Sex of Reciprocal Crosses between Anadolu-T and Commercial Broiler Parent Stocks at Week 3

Items		3. Week Fluctuating Asymmetry (FA)						
G	Sex	HTL±SEM	OTCL±SEM	OP4L±SEM	OP3L±SEM	MTCL±SEM	MP4L±SEM	MP3L±SEM
AA	M	0.9±0.16	0.4±0.12	0.38±0.07	0.85±0.14	0.7±0.12	0.5±0.08	0.84±0.15
	F	0.94±0.16	0.58±0.09	0.5±0.12	0.65±0.12	0.84±0.27	0.59±0.12	0.78±0.17
	T	***0.92±0.11^b^***	0.49±0.07	0.44±0.07	0.75±0.09	0.77±0.15	0.54±0.07	0.81±0.11
CC	M	1.52±0.25	0.68±0.12	0.47±0.07	0.86±0.15	0.49±0.09	0.5±0.12	0.71±0.14
	F	1.7±0.27	0.52±0.12	0.48±0.1	0.87±0.14	0.52±0.12	0.43±0.09	0.79±0.1
	T	***1.61±0.18^a^***	0.6±0.08	0.48±0.06	0.86±0.1	0.51±0.07	0.46±0.08	0.75±0.09
AC	M	1.12±0.21	0.92±0.14	0.43±0.09	0.88±0.11	0.52±0.13	0.65±0.15	0.95±0.18
	F	1.24±0.18	0.57±0.08	0.41±0.05	0.7±0.15	0.66±0.11	0.65±0.11	0.78±0.16
	T	***1.18±0.14^b^***	0.74±0.09	0.42±0.05	0.79±0.09	0.59±0.09	0.65±0.09	0.86±0.12
CA	M	1.08±0.21	0.58±0.14	1.12±0.5	0.49±0.12	0.58±0.12	0.52±0.09	0.71±0.13
	F	1.07±0.22	0.53±0.09	0.42±0.06	0.75±0.12	0.4±0.08	0.44±0.1	0.67±0.09
	T	***1.07±0.15^b^***	0.56±0.08	0.77±0.26	0.62±0.09	0.49±0.07	0.48±0.07	0.69±0.08
		Sig.	Sig.	Sig.	Sig.	Sig.	Sig.	Sig.
Genotype		***0.012***	0.163	0.242	0.321	0.179	0.324	0.662
Sex (S)		0.573	0.236	0.285	0.786	0.734	0.833	0.651
G × S		0.972	0.138	0.148	0.259	0.632	0.853	0.844
Items		3. Week Relative Asymmetry (RA)						
G	Sex	HTL±SEM	OTCL±SEM	OP4L±SEM	OP3L±SEM	MTCL±SEM	MP4L±SEM	MP3L±SEM
AA	M	1.1±0.19	1.09±0.31	1.46±0.26	1.68±0.29	1.62±0.28	1.61±0.24	1.11±0.21
	F	1.2±0.21	1.53±0.23	1.87±0.45	1.34±0.26	1.8±0.51	2.03±0.42	1.18±0.25
	T	***1.15±0.14^b^***	1.31±0.19	1.67±0.26	1.51±0.19	1.71±0.29	1.82±0.24	1.15±0.16
CC	M	1.78±0.29	1.93±0.36	1.63±0.25	1.53±0.28	1.1±0.2	1.64±0.41	0.9±0.18
	F	2.21±0.36	1.43±0.32	1.83±0.37	1.69±0.27	1.18±0.26	1.44±0.3	1.07±0.14
	T	***2±0.23^a^***	1.68±0.24	1.73±0.22	1.61±0.19	1.14±0.16	1.54±0.25	0.98±0.11
AC	M	1.35±0.26	2.53±0.38	1.56±0.33	1.64±0.22	1.17±0.29	2.1±0.51	1.22±0.23
	F	1.54±0.23	1.59±0.23	1.54±0.19	1.35±0.28	1.5±0.25	2.17±0.35	1.05±0.22
	T	***1.44±0.17^b^***	2.06±0.23	1.55±0.19	1.5±0.18	1.33±0.19	2.13±0.3	1.14±0.16
CA	M	1.31±0.27	1.66±0.38	3.21±0.96	0.91±0.23	1.27±0.26	1.73±0.3	0.91±0.17
	F	1.37±0.3	1.53±0.28	1.64±0.25	1.5±0.25	0.94±0.2	1.51±0.35	0.92±0.13
	T	***1.34±0.2^b^***	1.59±0.23	2.43±0.51	1.2±0.18	1.11±0.16	1.62±0.23	0.91±0.1
		Sig.	Sig.	Sig.	Sig.	Sig.	Sig.	Sig.
Genotype		***0.013***	0.130	0.198	0.444	0.163	0.382	0.549
Sex		0.296	0.212	0.433	0.889	0.750	0.951	0.881
[10]G × S		0.902	0.163	0.112	0.249	0.714	0.801	0.848

A: Anadolu – T × Anadolu – T; CC: Commercial × Commercial; AC: Anadolu – T male × Commercial female; CA: Commercial male × Anadolu – T female; F = female; M = male; T = Total; HTL = Hind toe length; OTCL = Outer toe claw length; OP4L = Fourth phalanx length of the outer toe; OP3L = Third phalanx length of the outer toe; MTCL= Middle toe claw length; MP4L=Fourth phalanx length of the middle toe; MP3L= Third phalanx length of the middle toe; SEM: The Mean of Standard Error

A clear developmental progression is evident when comparing Tables 8 and 9. At week 3, asymmetry in foot traits was largely unaffected by genotype and sex, with only HTL showing a significant genotype effect. In contrast, by week 6, multiple traits exhibited significant effects of genotype, sex, and genotype × sex interaction.

This shift indicates that developmental instability increases with age, particularly in appendage structures such as the foot. Early developmental stages are characterized by strong canalization, ensuring uniform growth, whereas later stages reveal underlying genetic and physiological differences.

The increasing influence of sex at week 6 suggests that differential growth rates and hormonal factors contribute to asymmetry. Moreover, genotype × sex interactions emphasize that these effects are not uniform across genetic backgrounds.

In general, week 3 results indicate a relatively stable and homogeneous developmental pattern, whereas week 6 findings reveal increased variability and greater sensitivity to genetic and biological factors, suggesting that growth intensity and selection pressures play a more pronounced role in shaping structural symmetry over time.

Table 9. Fluctuating (FA) and Relative (RA) Asymmetry Analyses Derived from Foot Measurements According to Genotype and Sex of Reciprocal Crosses between Anadolu-T and Commercial Broiler Parent Stocks at Week 6

Items		*6. Week Fluctuating Asymmetry (FA)*						
G	Sex	HTL±SEM	OTCL±SEM	OP4L±SEM	OP3L±SEM	MTCL±SEM	MP4L±SEM	MP3L±SEM
AA	M	0.86±0.1	0.67±0.09	0.35±0.06	0.54±0.13	0.91±0.15	0.33±0.05	0.49±0.11
	F	0.8±0.13	0.79±0.11	0.47±0.09	0.61±0.09	0.34±0.09	0.56±0.06	0.83±0.12
	T	0.83±0.08	0.73±0.07	0.41±0.05	0.58±0.08	0.62±0.1	***0.45±0.04***^{a}	0.66±0.09
CC	M	1.27±0.2	0.84±0.14	0.51±0.07	0.7±0.11	0.5±0.13	0.37±0.04	0.45±0.06
	F	0.75±0.09	0.73±0.15	0.52±0.09	0.36±0.07	0.47±0.07	0.28±0.05	0.44±0.11
	T	1.01±0.12	0.78±0.1	0.51±0.06	0.53±0.07	0.48±0.07	***0.33±0.03***^{b}	0.44±0.06
AC	M	1.99±0.38	0.83±0.14	0.31±0.04	0.84±0.09	0.41±0.06	0.32±0.06	0.52±0.07
	F	0.68±0.1	0.46±0.08	0.68±0.11	0.6±0.18	0.54±0.08	0.31±0.06	0.48±0.1
	T	1.33±0.22	0.65±0.08	0.5±0.07	0.72±0.1	0.47±0.05	***0.31±0.04***^{b}	0.5±0.06
CA	M	0.7±0.15	0.5±0.08	0.25±0.05	0.68±0.18	0.38±0.07	0.41±0.07	0.57±0.07
	F	1.38±0.25	0.39±0.09	0.41±0.1	0.69±0.09	0.48±0.08	0.32±0.05	0.57±0.1
	T	1.04±0.16	0.44±0.06	0.33±0.06	0.68±0.1	0.43±0.05	***0.37±0.04***^{ab}	0.57±0.06
		Sig.	Sig.	Sig.	Sig.	Sig.	Sig.	Sig.
Genotype		0.088	0.019	0.076	0.398	0.201	***0.090***	0.124
Sex (S)		0.034	0.146	***0.003***	0.151	0.183	0.775	0.287
G × S		***0.000***	0.220	0.165	0.331	***0.001***	***0.013***	0.163
Items		6. Week Relative Asymmetry (RA)						
G	Sex	HTL±SEM	OTCL±SEM	OP4L±SEM	OP3L±SEM	MTCL±SEM	MP4L±SEM	MP3L±SEM
AA	M	0.88±0.1	1.5±0.21	1.13±0.2	0.94±0.23	1.69±0.28	0.98±0.15	0.57±0.13
	F	0.89±0.14	1.84±0.26	1.6±0.29	1.15±0.17	0.62±0.16	1.72±0.18	1.03±0.14
	T	0.89±0.09	***1.67±0.17***^{a}	1.37±0.18	1.04±0.14	1.16±0.19	1.35±0.13	0.8±0.1
CC	M	1.26±0.2	1.96±0.32	1.56±0.21	1.11±0.17	0.94±0.25	1.09±0.14	0.51±0.07
	F	0.85±0.11	1.69±0.37	1.78±0.33	0.65±0.13	0.88±0.14	0.9±0.17	0.53±0.14
	T	1.06±0.12	***1.82±0.24***^{a}	1.67±0.19	0.88±0.11	0.91±0.14	1±0.11	0.52±0.08
AC	M	2.05±0.4	1.82±0.29	1.05±0.14	1.36±0.16	0.74±0.1	0.98±0.19	0.57±0.07
	F	0.78±0.11	1.01±0.17	2.39±0.39	1.12±0.36	0.98±0.14	0.97±0.17	0.59±0.12
	T	1.41±0.23	***1.42±0.18***^{ab}	1.72±0.24	1.24±0.19	0.86±0.09	0.97±0.13	0.58±0.07
CA	M	0.68±0.14	1.1±0.18	0.79±0.14	1.12±0.29	0.68±0.12	1.19±0.2	0.64±0.08
	F	1.47±0.27	0.85±0.19	1.4±0.35	1.25±0.16	0.92±0.16	0.99±0.17	0.7±0.12
	T	1.07±0.17	***0.97±0.13***^{b}	1.09±0.19	1.18±0.16	0.8±0.1	1.09±0.13	0.67±0.07
		Sig.	Sig.	Sig.	Sig.	Sig.	Sig.	Sig.
Genotype		0.089	***0.008***	0.085	0.377	0.213	0.119	0.070
Sex		0.135	0.182	***0.001***	0.570	0.214	0.498	0.079
[10]G × S		***0.000***	0.178	0.212	0.391	***0.001***	***0.021***	0.149

A: Anadolu – T × Anadolu – T; CC: Commercial × Commercial; AC: Anadolu – T male × Commercial female; CA: Commercial male × Anadolu – T female; F = female; M = male; T = Total; HTL = Hind toe length; OTCL = Outer toe claw length; OP4L = Fourth phalanx length of the outer toe; OP3L = Third phalanx length of the outer toe; MTCL= Middle toe claw length; MP4L=Fourth phalanx length of the middle toe; MP3L= Third phalanx length of the middle toe; SEM: The Mean of Standard Error

Conclusion

This study demonstrated that welfare-related physiological traits (tonic immobility and rectal temperature) were largely unaffected by genotype and genotype × sex interactions at both 3 and 6 weeks of age in reciprocal crosses between Anadolu-T and commercial broiler parent stocks. These results indicate that early physiological responses related to stress and thermoregulation are relatively stable and primarily independent of genetic background.

In contrast, morphological traits and developmental stability parameters showed a clear age-dependent pattern. While genotype effects were limited at 3 weeks, they became increasingly significant at 6 weeks, particularly for comb, beak, tarsometatarsus, and foot measurements. Similarly, fluctuating asymmetry (FA) and relative asymmetry (RA) analyses revealed low and non-significant variation at early age, whereas marked genotype, sex, and genotype × sex interaction effects emerged at later growth stages.

Overall, these findings indicate that developmental stability is strongly maintained during early growth but becomes progressively influenced by genetic background, sex, and their interaction as birds mature. The results also suggest that reciprocal crossbreeding may contribute to improved developmental stability in certain traits, highlighting the potential role of heterosis in reducing asymmetry.

Taken together, the study emphasizes that age is a critical factor in the expression of both morphological variation and developmental instability, and that genetic effects become more pronounced as broilers approach later growth stages. These insights are important for optimizing breeding strategies aimed at improving both productivity and welfare in broiler production systems.

Declarations

Ethical Approval Certificate

The experimental procedures of this study were approved by the Local Animal Care and Ethics Committee of Animal Experiments of Kahramanmaraş Sütçü İmam University (Approval date: 28 February 2025; Approval number: 2025/02)

Author Contribution Statement

The authors declare that they have contributed equally to the work.Conflict of InterestThe authors declare no conflict of interest.AcknowledgmentsIn this study, we would like to thank TAGEM for providing the animal material we used from project number TAGEM/HAYSÜT/G/22/A4/P4/6230

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