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Evaluation of Genotype-by-Environment Interaction in Poultry

Arícia Chaves Zanetti Reis
Doctor Scientiae

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Thesis submitted to the Animal Science Graduate Program of the Universidade Federal de Viçosa in partial fulfillment of the requirements for the degree of *Doctor Scientiae*.

Adviser: Renata Veroneze

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ABSTRACT

REIS, Arícia Chaves Zanetti, D.Sc., Universidade Federal de Viçosa, July, 2025. **Evaluation of Genotype-by-Environment Interaction in Poultry**. Adviser: Renata Veroneze. Co-advisers: Daniele Botelho Diniz Marques and Luiz Fernando Brito.

This thesis investigates genotype-by-environment interaction (GEI) in poultry, aiming to understand how different genotypes change across to environmental variation. GEI occurs when the performance of animals changes depending on the environment, which may lead to reranking of selection candidates. Although GEI has already been studied in commercial chickens, species such as quails and turkeys remain underexplored. This research aims to fill this gap by analyzing two important traits: body weight in meat quails and walking ability in turkeys, both influenced by environment and related to animal performance and welfare. The first study evaluated body weight from 7 to 42 days of age in meat quails raised under two classes of temperature-humidity index (THI). Genetic correlations between THI classes were low to moderate at early ages, indicating the presence of GEI and reranking of individuals. From 28 days, high genetic correlations were observed, suggesting more stable genetic expression under thermal stress. The second study assessed GEI for walking ability in turkeys using data from over 190,000 birds. Environmental gradients were defined based on contemporary group effects for body weight. Reaction norm models with different residual variances were compared. The best-fitting model indicated genetic variation in walking ability across environments and reranking of individuals across the environmental gradient. An inverse relationship between body weight and walking ability was also observed, reinforcing the importance of considering GEI in selection strategies. The results highlight the relevance of including GEI in genetic evaluations. This thesis contributes to expanding knowledge on GEI in poultry and supports the development of breeding programs focused on both productivity and animal welfare.

Keywords: genotype-by-environment interaction; poultry; quails; turkeys

RESUMO

REIS, Arícia Chaves Zanetti, D.Sc., Universidade Federal de Viçosa, julho de 2025. **Avaliação da Interação genótipo x ambiente em aves**. Orientadora: Renata Veroneze. Coorientadores: Daniele Botelho Diniz Marques e Luiz Fernando Brito.

Esta tese investiga a interação genótipo x ambiente (GEI) em aves, com o objetivo de compreender como diferentes genótipos respondem à variação ambiental. A GEI ocorre quando o desempenho dos animais varia conforme o ambiente, o que pode causar reclassificação dos candidatos à seleção. Embora a GEI já tenha sido estudada em frangos comerciais, espécies como codornas e perus ainda são pouco exploradas. Esta pesquisa busca preencher essa lacuna ao analisar duas características importantes: o peso corporal em codornas de corte e a habilidade de locomoção em perus, ambas influenciadas por fatores ambientais e relacionadas ao desempenho e bem-estar animal. O primeiro estudo avaliou o peso corporal entre 7 e 42 dias de idade em codornas de corte sob duas classes de índice de temperatura e umidade (THI). As correlações genéticas entre as classes de THI foram baixas a moderadas nas idades iniciais, indicando presença de GEI e mudanças no ranqueamento dos animais. A partir dos 28 dias, observaram-se correlações genéticas altas, sugerindo maior estabilidade na expressão genética frente ao estresse térmico. O segundo estudo avaliou a GEI para a habilidade de locomoção em perus, utilizando dados de mais de 190 mil aves. Os gradientes ambientais foram definidos com base nos efeitos de grupos contemporâneos para o peso corporal. Modelos de norma de reação com diferentes variâncias residuais foram comparados. O modelo com melhor ajuste indicou variação genética na habilidade de locomoção entre os ambientes e reclassificação de indivíduos ao longo do gradiente ambiental. Também foi observada uma relação inversa entre peso corporal e habilidade de locomoção, reforçando a importância de considerar a GEI nas estratégias de seleção. Os resultados obtidos destacam a relevância da inclusão da GEI nas avaliações genéticas. Esta tese contribui para ampliar o conhecimento sobre GEI em aves e apoia o desenvolvimento de programas de melhoramento focados tanto na produtividade quanto no bem estar animal

Palavras-chave: interação genótipo x ambiente; aves; codornas; perus

SUMMARY

CHAPTER 01

1.1 General Introduction

In animal breeding, genotype-by-environment interaction (GEI) has become an important topic, especially as livestock are raised under a wide range of environmental conditions (Falconer and Makay, 1996) and dramatically climate changes are expected. GEI occurs when the performance of genotypes changes depending on the environment, which can cause reranking of animals and reduce the accuracy of selection. Understanding and accounting for GEI is crucial to ensure that animals selected for high performance in one environment are also resilient and efficient in others (Poullet et al., 2022).

In poultry breeding, this concern is even more relevant. Birds are commonly exposed to diverse climates, feed resources, housing systems, and management strategies. Environmental stressors, such as extreme temperatures, high humidity, poor feed quality, or high stocking density, can directly affect growth, reproduction, welfare, and behavior. For example, high ambient temperatures can reduce feed intake and growth in broilers. Not all genotypes respond the same way to climatic stress. As a result, ignoring GEI in poultry breeding programs may lead to animals that perform well in controlled conditions but poorly in different systems.

To capture GEI in genetic evaluations, several statistical models have been developed (Song et al., 2020). These models vary in how they define the environment (as categories or continuous variables), how they model genetic effects, and whether they include genomic information. One of the ways to evaluate GEI is using the multi-trait models (MTMs), treating the same trait measured in different environments as separate but genetically correlated traits (Silva Neto et al., 2024). The presence of GEI is assessed through genetic correlations: values below 0.80 usually indicate meaningful GEI. MTMs are practical when the environments are clearly defined, such as organic vs. conventional systems or high vs. low biosecurity farms (Chu et al., 2019). However, these models are limited when environmental variation is continuous or difficult to classify. For those cases, we can use reaction norm models (RNM), which allow for a more flexible approach by modeling genetic merit as a function of an environmental gradient. These models estimate intercepts and slopes for each genotype, where the slope reflects the genotype’s sensitivity to environmental changes. Environments can be described using variables like temperature-humidity index (THI), contemporary group effects, or average group performance (Song et al., 2020).

RNMs are effective at studying phenotypic plasticity and identifying genotypes that are more stable across the environments. They also support modeling heterogeneous residual variances, which is important when environmental variability affects trait expression. Chen et al. (2021) showed that this approach improved prediction accuracy for reproduction and growth traits in pigs, and similar benefits are expected in poultry.

In poultry, GEI has been reported for many traits like body weight, feed conversion, egg production, and eggshell quality. There is also evidence of GEI for behavioral traits, including feather pecking and coping responses, which are directly linked to animal welfare (Chu, 2019). This highlights the need for breeding programs to select birds not only for productivity but also for robustness and welfare under diverse conditions.

Despite growing interest in GEI, some poultry species are lacking in scientific literature (Mulim et al., 2024). Most studies have focused on commercial chickens, particularly broilers and layers, due to their economic importance and large-scale production systems. However, species such as meat quails and turkeys have received less attention, despite their significant role in alternative and expanding poultry markets. These species may respond differently to environmental stressors, so their genetic evaluation requires approaches specific to their biology and production systems. Therefore, studying GEI in quails and turkeys is essential to expanding our understanding and developing more specific breeding strategies. In this context, this thesis investigates genotype-by-environment interaction in poultry species, the second chapter examines body weight in quails under cold stress, while the third chapter evaluates walking ability in turkeys across environments defined by body weight contemporary group effects.

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CHAPTER 02

Genotype-by-environment interaction across THI classes impacts the selection for body weight at young ages in meat quails

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2.1 Abstract

This study aimed to investigate the impact of genotype-by-environment interaction (GEI) on body weight (BW) in meat quails. BW data from ten generations, recorded on 7 (BW7), 14 (BW14), 21 (BW21), 28 (BW28), 35 (BW35), and 42 (BW42) days after hatching were used for the analyses. A temperature and humidity index (THI) divided into two classes (less or equal than 68 – THI1 and greater than 68 –THI2) was used as the environmental descriptor. Variance components and genetic parameters, including heritability and genetic correlation estimates, were computed for BW considering these two classes of THI. The rank correlation coefficients and percentage of individuals in common in the top 1% animals (classified by breeding values in THI2) for BW were calculated. Also, the top 10% with at least 13 offspring and the pattern of the top 10 breeding value animals were analyzed across THI classes. The heritability estimates ranged from 0.22 (for BW42 at THI1) to 0.49 (for BW28 and BW35 at THI1). In addition, the genetic correlations ranged from 0.16 (for BW7 between THI1 and THI2) to 0.98 (to BW42 between THI1 and THI2). The genetic correlation between THI1 and THI2 was low for BW7 (0.16), moderate for the two following weeks, i.e., BW14 and BW21 (0.65 and 0.55, respectively), and high for BW28, BW35, and BW42 (0.94, 0.98, and 0.96, respectively). The rank correlations indicated reranking of younger birds (BW7, BW14, and BW21), reflecting variations in breeding values across THI classes. The quails kept their ranking position from 28 days onwards when comparing the evaluated THI classes. However, younger birds, before 28 days, exhibited reranking across the different THI ranges. This study reinforces the relevance of the effect of THI on animal performance, highlighting the need to consider them in analyses for selecting superior and more resilient animals.

Key Words: animal adaptation, cold stress, poultry breeding, meat quail, Temperature-Humidity Index (THI)

2.2 Introduction

Global projections indicate an increase in demand for animal proteins (FAO, 2022), and although not a staple food, quail (*Coturnix coturnix*) meat offers a viable alternative resource due to small-scale and low-input production. Annually, 1.4 billion quails are reared for meat and egg production (Dalmau et al., 2024), yet data on global quail meat sales remain scarce.

The quail's rapid growth, early maturity, high productivity, low feed intake, and prolonged production period have driven its expansion. Quail carcasses yield 76% meat, 14% skin, and 10% bone, representing the highest meat and lowest bone content among poultry (Jahan et al., 2024). Additionally, their meat is rich in protein, essential fatty acids, and minerals such as sodium, potassium, and iron.

The expansion of quail consumption relies on the implementation of more efficient and enhanced production methods. Therefore, meat quail breeding programs have the potential to revolutionize quail production through the selection of lines that are more productive, efficient, resilient, and with higher meat quality.

One of the current challenges in animal production is global climate change. According to recent climate projections, some regions are expected to experience increased climate variability and more intense extreme weather events, including cold spells (IPCC, 2023; Piedra-Bonilla et al., 2025). For instance, cold weather conditions can result in significant adverse effects on the productive performance of livestock animals (Wang et al., 2023). Information on quail resilience to thermal stress, particularly under cold conditions, is limited (Dalle Zotte & Cullere, 2024). Evaluating the effect of induced cold stress in quails, Chunpeng et al. (2019) showed that cold stress caused oxidative stress, inflammatory injury in cecal tissues, and influenced cecal microbiota. Souza et al. (2014) observed higher mortality rates in quail groups exposed to cold environments.

Cold stress has an even greater impact on animals in the early stages of life because they are still growing, have small body sizes, have difficulties regulating their body temperature, and lack fully developed cover feathers (Beaulac., 2020). As addressed and explained by Collier and Collier (2012), many physiological systems in young animals are not yet fully functional or organized around a basal physiological level, which is particularly critical for the endocrine system and its roles in metabolism and stress regulation. Recent studies suggest that exposure to cold temperatures during the early stages of development can have long-lasting effects on the thermoregulatory phenotype of quails, despite their precocial nature and rapid feathering after hatching (Persson et al., 2024). Specifically, physiological alterations have been observed in cold-acclimated quail, including changes in basal metabolic rate and evaporative water loss. These findings raise the possibility of long-term or even irreversible consequences associated with early-life exposure to cold stress (Persson et al., 2024).

Studies defining thermal comfort zones in meat quails are scarce. Evaluating Japanese quail based on performance and egg quality, Castro et al. (2022) recommended THI between 68.4 to 76.2 for quail thermal comfort. Cold stress in quail is a significant challenge, as quails reared in cold environments must adapt their physiology to maintain a stable body temperature. Studying the impact of thermal stress on quails can fill the gap of knowledge about thermoregulation in these animals, and it is also a cheap way to improve our understanding of how to overcome climate change through genetics in poultry.

There is a demand for technologies capable of identifying and quantifying the level of adaptation of animals to adverse environmental conditions. A practical and efficient way is through the analysis of the genotype by environment interaction (GEI) (Poullet et al., 2022). GEI describes how genotypes react differently to environmental variations (Falconer & Makay, 1996), impacting genetic merit rankings. The temperature and humidity index (THI) is a widely used environmental variable for analyzing GEI in livestock (Carrara et al., 2023; Fodor et al., 2023; Neto et al., 2024), and it can be calculated using data from in-barn data loggers, nearby weather stations, or from NASA POWER (Rockett, 2021; Carrara et al., 2023; VanderZaag et al., 2023). However, GEI studies in quails using THI are limited, with most research focusing on nutritional levels as the environmental gradient (Mota et al., 2015; Taroco et al., 2018; Alcantara et al., 2019; Gouveia et al., 2019).

This study aimed to explore the GEI for the body weight measured at 7, 14, 21, 28, 35, and 42 days of age in quails, through the estimation of (co)variance components and genetic parameters for these traits measured in two THI classes. In addition, we evaluated the changes in rank correlation for the traits according to the THI class.

2.3 Material and methods

All animal procedures were approved in advance by the Animal Care and Use Committee of the Department of Animal Science at the Universidade Federal de Viçosa (UFV), Brazil (Protocol Number 73/2014 - CEUAP).

2.3.1 Data

A total of 85,170 records of body weight (BW) measured at 7 (BW7), 14 (BW14), 21 (BW21), 28 (BW28), 35 (BW35), and 42 (BW42) days of age in 9,159 birds were used in the analyses. The data belongs to a meat quail line from the Meat Quail Breeding Program at the Universidade Federal de Viçosa (UFV), Viçosa, Minas Gerais state, Brazil.

For each generation, 306 meat quails (102 males and 204 females) were mated in a ratio of one male to two females. All breeding animals were replaced by their offspring in each generation (i.e., there were no overlapping generations). The selection of replacement animals was based on BW28, given the strong genetic correlation between 4-week body weight (BW) and carcass traits, with the heaviest quails chosen as parents of the next generation. The selected birds were transferred to galvanized metal cages (0.90 × 0.44 × 0.23 m; length × width × height), which were equipped with linear feeders and drinkers supplied with running water. Eggs were collected for 10 consecutive days per reproductive cycle, incubated for 14 days, and transferred to a hatcher for three additional days until hatching. After hatching, the birds were identified with numbered tags on their legs for pedigree tracking. The quails were housed in stalls with concrete floors and wood-shaving beds, a protection circle, and heat brooders. Up to the 42nd day of age, diet was composed of 26% crude protein and 2,950 Kcal of metabolizable energy per kg. Feed and water were supplied *ad libitum*. The lighting program consisted of 24 hours of light up to the birds' 14th day of age, after which natural lighting was employed. The facilities were not climate-controlled, and temperature regulation was managed using curtains. Birds were weighed individually at 7, 14, 21, 28, 35, and 42 days of age (BW7 to BW42), with the last weight considered the slaughter weight. A detailed description of the data collection and the Meat Quail Breeding Program can be found in Paiva et al. (2018a, 2018b) and Silva et al. (2013).

The datasets were edited to eliminate records of quails with inadequate identification (such as missing IDs) and no information on sex or generation. Quality control was performed separately for each age group and different THI ranges. As a result, due to mortality and quality control procedures, the number of records varies across the different ages and THI ranges. The pedigree data included 14,195 animals, being 1,143 sires and 1,817 dams, spanning up to ten generations.

2.3.2 Temperature and humidity index (THI) definition

The data for calculating the THI came from the UFV climatic station located eight kilometers from the farm where the quails were raised. The database had information on precipitation, atmospheric pressure, dry bulb air temperature, dew point temperature, maximum and minimum temperatures, relative humidity, and wind speed. The information on dry bulb air temperature (Tbs) and dew point temperature (Tpo) was used to calculate THI. The equation proposed by Thom (1959) and used by Castro et al. (2022) for quails was used to calculate the THI:

$$\mathrm{THI} = \mathrm{Tbs} + 0.36 \times \mathrm{Tpo} + 41.5.$$

Temperatures from the quail weighing date and from the two previous days were collected. The average weather information from these three days was used to calculate THI values for 7, 14, 21, 28, 35, and 42 days of age. Two THI classes were defined (Sousa et al., 2014): THI values less than or equal to 68, which were considered as cold stress (THI1); and THI values higher than or equal to 69, which were considered thermal comfort (THI2).

2.3.3 Statistical modeling

Variance components and breeding values were estimated using the BLUPF90+ software (Misztal et al., 2014). A two-trait model was fitted considering weights at the same age recorded in different environments (THI1 and THI2) as distinct traits. Then, six analyses were performed. In matrix notation, the model can be described as:

$$\mathbf{y} = \mathbf{X}\boldsymbol{\beta} + \mathbf{Z}\mathbf{u} + \mathbf{e},$$

where **y** is the vector of phenotypic records; $\boldsymbol{\beta}$ is the vector of fixed effects which included only contemporary group defined by generation (with each generation belonging to a different batch) and sex; **X** is the incidence matrix of fixed effects; **u** is the vector of additive genetic random effects of the animals; **Z** is the incidence matrix of the additive genetic random effect; and **e** is the vector of random residual effect. The additive and residual genetic effects were assumed to follow a normal distribution: $\mathbf{u} \sim \mathrm{N}(0, \mathbf{A} \otimes \mathbf{G})$; and $\mathbf{e} \sim \mathrm{N}(0, \mathbf{I} \otimes \mathbf{R})$, where **A** is the pedigree-based relationship matrix; **G** and **R** are the additive genetic and residual (co)variances matrices, respectively; and **I** is an identity matrix.

The genetic correlation between the two THI classes was calculated as:

$$rg = \frac{cov_{1,2}}{\sqrt{\sigma_{a1}^2 \sigma_{a2}^2}}$$

where $cov_{1,2}$ represents the additive genetic covariance between the trait measured in the two THI classes, and σ_{a1}^2 and σ_{a2}^2 are the additive genetic variances for each THI class. Genetic correlations below 0.8 were considered an indication of significant GEI. This threshold was proposed by Robertson (1959), and it has been widely adopted in the literature (e.g., Shabalina et al., 2020; Hardie et al., 2021; Digesa, 2024).

We computed rank correlations (RC) and percentage (%) of individuals in common (IC) in the ranking of the best (based on THI2) 1% animals (including all animals), and 10% of the animals with at least 13 offspring (with phenotypic records) in each THI class for all evaluated traits. This analysis was also conducted separately for males and females. The threshold of 13 offspring was chosen to include animals with more accurate breeding values, and 13 represents the average number of offspring in the population. Furthermore, the pattern of the 10 animals with the highest breeding values across THI classes was plotted for each BW, also based on THI2 with at least 13 offspring. The ranking of the best animals was based on THI2 because it represents thermal comfort. The intent was to determine if the animals that ranked highest under thermal comfort conditions remained the best performers in a cold stress environment.

2.4 Results

The number of records and descriptive statistics used in the analysis for BW are presented in Table 1, including an overview of the BW at different ages (7, 14, 21, 28, 35, and 42 days) categorized by THI classes. The results show that at BW7, the mean at THI1 was 34.06 g (SD = 6.57), while at THI2 it was higher at 38.12 g (SD = 6.01). For the BW14, the average weights were 95.42 g (SD = 15.68) and 86.79 g (SD = 13.32) for THI1 and THI2, respectively. This pattern continued, with average weights consistently higher under THI1 at later ages: 165.07 g (SD = 20.48) vs. 156.82 g (SD = 21.53) at BW21, 225.66 g (SD = 23.48) vs. 213.68 g (SD = 27.65) at BW28, 270.94 g (SD = 27.56) vs. 249.46 g (SD = 22.44) at BW35, and 289.27 g (SD = 39.16) vs. 287.46 g (SD = 31.26) at BW42. Minimum and maximum values also highlight the differences between the THI classes.

Table 1. Number of observations (N), mean (M), minimum (Min), maximum (Max), and standard deviation (SD) for quail body weight (in grams) at 7, 14, 21, 28, 35, and 42 days of age for each THI class

	BW7		**BW14**		**BW21**		**BW28**		**BW35**		**BW42**	
	THI1	**THI2**	**THI1**	**THI2**	**THI1**	**THI2**	**THI1**	**THI2**	**THI1**	**THI2**	**THI1**	**THI2**
N	3391	2381	3457	2048	2828	2105	4206	1449	3782	1264	3074	739
M	34.06	38.12	95.42	86.79	165.07	156.82	225.66	213.68	270.94	249.46	289.27	287.46
Min	13.91	14.69	39.02	38.50	88.69	88.00	131.73	133.16	169.32	165.25	159.93	163.78
Max	52.16	58.74	137.93	132.82	225.95	220.78	302.54	308.61	364.68	321.36	418.15	376.34
SD	6.57	6.01	15.68	13.32	20.48	21.53	23.48	27.65	27.56	22.44	39.16	31.26

THI1: values less than or equal to 68 (cold stress); THI2: values greater than or equal to 69 (thermal comfort).

The estimates of variance components, heritabilities, and genetic correlations between the two THI classes are shown in Table 2. Heritabilities were of moderate to high magnitude, ranging from 0.22 (for BW42 at THI1) to 0.49 (for BW28 and BW35 at THI1). For some of the evaluated BW, notable differences in heritability were observed for the same BW in the different THI environment. The highest difference (from 0.42 at THI1 to 0.29 at THI2) between heritabilities for the two THI classes was for BW21. Genetic correlations ranged from 0.16 (for BW7 between THI1 and THI2) to 0.98 (to BW42 between THI1 and THI2), indicating strong GEI effects in younger ages, mainly on BW7.

Table 2. Variance components and heritabilities for body weight at 7, 14, 21, 28, 35, and 42 days of age for each THI range and genetic correlations for BW between the two THI classes.

	BW7		**BW14**		**BW21**		**BW28**		**BW35**		**BW42**	
Parameter	**THI1**	**THI2**	**THI1**	**THI2**	**THI1**	**THI2**	**THI1**	**THI2**	**THI1**	**THI2**	**THI1**	**THI2**
σ_a^2	11.36	14.09	36.88	59.42	143.39	80.44	225.21	216.20	285.18	252.75	178.55	209.09
σ_e^2	14.96	18.30	83.75	91.18	198.41	193.51	236.82	311.32	298.74	255.56	626.70	607.62
h^2	0.43	0.43	0.30	0.39	0.42	0.29	0.49	0.41	0.49	0.50	0.22	0.25
SE(h^2)	0.039	0.048	0.035	0.050	0.043	0.047	0.035	0.053	0.036	0.057	0.035	0.071
rg	0.16		0.65		0.55		0.94		0.98		0.96	

Direct additive genetic variance (σ^2_a); residual variance (σ^2_e); heritability (h^2), standard error of heritability (SE for h^2), genetic correlation (rg).

To better visualize the temperature during the days of data collection, Figure 2 shows a plot of the average temperature over the years in the region of Viçosa, MG, Brazil. The plot illustrates the daily maximum and minimum temperature trends over the study period, with the average daily minimum temperature tending to be around 20°C.

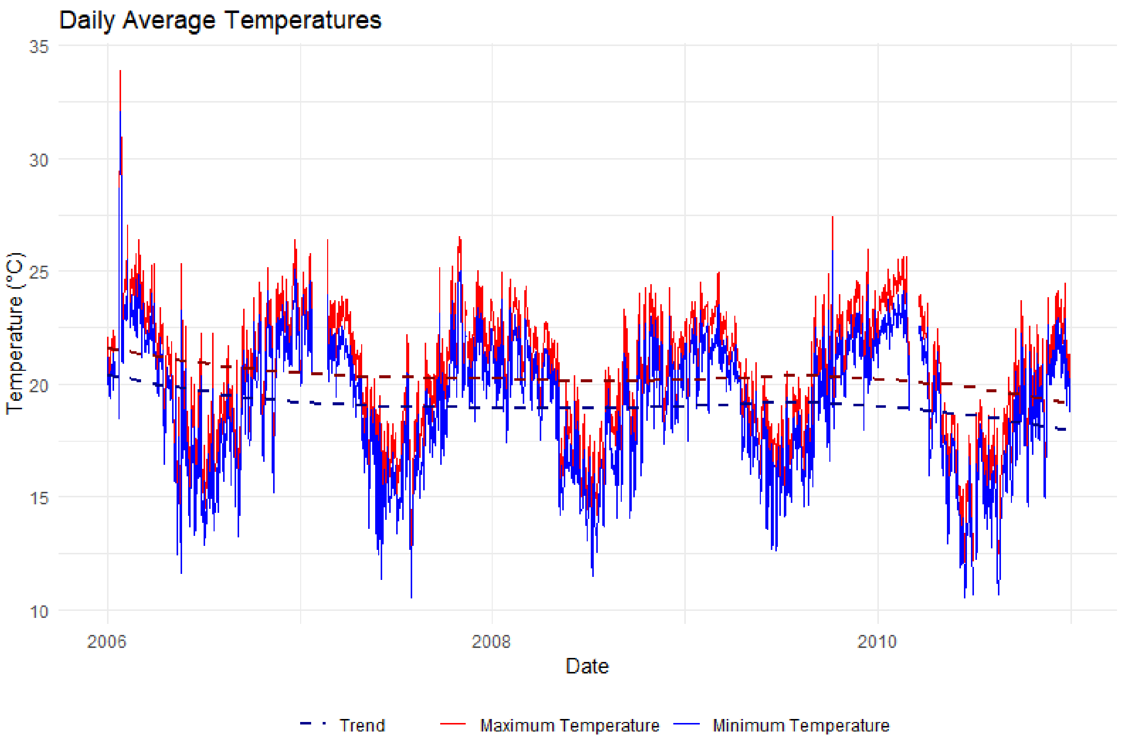

Figure 1. Daily maximum and minimum average temperatures (in °C) over the years (during which the data were collected) in the region of Viçosa, MG, Brazil.

The results presented in Tables 3 and 4 reveal how the THI influences the ranking of quails for BW across different ages. Notably, the RC and the IC between THI1 and THI2 varied across ages, showing the potential impact of GEI on quail selection. The estimates of RC and IC among the top 1% of animals ranked based on THI2 for body weight at 7, 14, 21, 28, 35, and 42 days of age showed that at younger ages (i.e., BW7, BW14, and BW21), significant changes would occur in the individuals selection decisions, potentially affecting genetic gain. For BW7, the RC was 0.36, and the IC was only 1.53%, indicating very low agreement in rankings across THI classes at this age. For BW14, the RC increased to 0.42, with IC of 50.77%. At BW21, the RC dropped slightly to 0.32, while the IC remained similar at 49.23%. From BW28 onwards, both RC and IC showed a substantial increase: the RC was 0.92, and the IC was 92.30% for BW28. At BW35, the RC reached 0.98, with the IC rising to 93.85%. Finally, for BW42, the RC remained high at 0.93, and the IC peaked at 95.38%.

Table 3 provides data on the top 1% (using all animals) and 10% of quails with at least 13 offspring with phenotypic records. The RCs for the top 10% increased after BW7, with strong correlations observed from BW14 (RC > 0.78). For those animals, environmental influences have a strong impact in the early stage but stabilize quickly, indicating that the best animals under one THI class remain among the top animals in the other class after 14 days of age.

Table 3. Estimates of rank correlations (RC) and percentage (%) of individuals in common (IC) in the ranking of the best 1% animals (using all animals) and 10% animals (based on THI2) with at least 13 offsprings for body weight at 7, 14, 21, 28, 35, and 42 days of age between THI classes

		BW7	**BW14**	**BW21**	**BW28**	**BW35**	**BW42**
1%	**RC**	0.36	0.42	0.32	0.92	0.98	0.93
	IC	1.53%	50.77%	49.23%	92.30%	93.85%	95.38%
10%	**RC**	0.33	0.81	0.78	0.99	0.99	0.99
	IC	96.29%	97.56%	100.00%	85.45%	77.05%	94.73%

Table 4 distinguishes between males and females. Females show a different trend, with a moderate RC at BW7 (0.51), but this decreases at BW14 (0.07), before stabilizing and increasing significantly after BW28. These patterns suggest that selection of males and females is more affected by THI differences at younger ages and both stabilize in ranking by BW28, with females showing slightly more variability across earlier ages than males.

Table 4. Estimates of rank correlations (RC) and percentage (%) of individuals in common (IC) in the ranking of the **best (10%) males and females with at least 13 offsprings** (based on THI2) for body weight at 7, 14, 21, 28, 35, and 42 days of age between THI classes.

		BW7	BW14	BW21	BW28	BW35	BW42
M	**RC**	-0.06	0.41	0.56	0.75	0.98	0.93
	IC	28.94%	71.05%	57.89%	63.16%	92.10%	92.10%
F	**RC**	0.51	0.07	0.38	0.83	0.94	0.87
	IC	29.16%	62.50%	45.83%	91.67%	95.83%	91.67%

M: males; F: females

High RC and IC for the top 1% animals at BW28, BW35, and BW42 were observed, which implies that the quails kept their ranking position from 28 days onwards in both environments. When a minimum number of offspring threshold is applied (for top 10% with at least 13 offspring), the RC values are substantially higher across all ages, indicating that the ranking of the top quails becomes more stable between the environments.

Figure 2 presents the differences in the ranking of the top 10 animals' breeding values, with a minimum of 13 offspring, across two distinct thermal environments (THI 1 and THI 2). The figure illustrates the changes in ranking for the following body weights at different ages: (A) BW7, (B) BW14, (C) BW21, (D) BW28, (E) BW35, and (F) BW42. These rankings provide insights into how THI variation influences the genetic evaluation of animals at different stages of life.

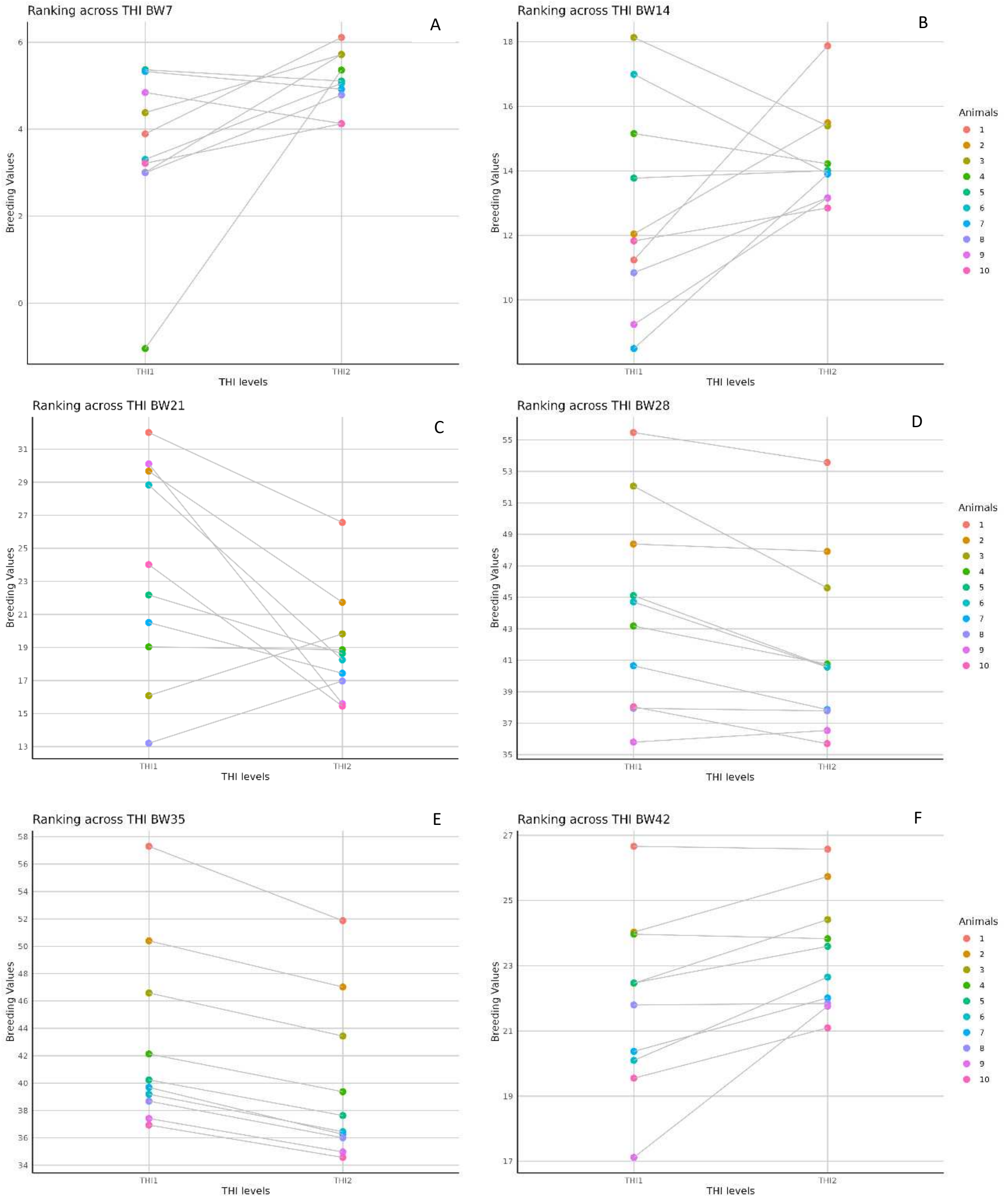

Figure 2. Ranking of the best 10 animals breeding values with at least 13 offsprings in THI1 and THI2 for (A) BW7, (B) BW14, (C) BW21, (D) BW28, (E) BW35, and (F) BW42.

2.5 Discussion

Quail breeding and production have received considerable attention due to their economic importance. Selection for desirable traits such as body weight, growth rate, and reproductive performance plays a key role in improving productivity. Understanding the factors influencing these traits is essential for advancing breeding programs. In this context, the results of the descriptive statistics show that the observed means for body weight were higher than those found in the literature (Khaldari et al., 2010; Souza et al., 2014). A possible explanation for this discrepancy could be that animals have been selected for body weight over the years.

The estimated heritabilities for BW in each THI class were moderate to high (Table 2). The highest change in heritability across the THI classes was for BW21, which appears to be primarily driven by changes in additive genetic variance. In contrast, for other BW traits, the variation in heritability was influenced by changes in both additive genetic and residual variances.

The estimated heritabilities found were close to those reported in previous studies for body weight in quails, ranging from 0.22 to 0.53 (Silva et al., 2013; Silva et al., 2021). These values may indicate moderate to high genetic gain for BW; however, we showed that in the selection for BW21, higher genetic gain is expected if the animals are selected based on THI1.

Our results suggest that, for certain body weights, the animals' breeding values vary depending on the environment in which the animals are selected. As proposed by Robertson (1959), genetic correlations below 0.8 were considered an indication of significant GEI. In our study, genetic correlations between environments below this threshold were found for BW7, BW14, and BW21. The lowest genetic correlation between THI1 and THI2 was for BW7 (Table 2). This finding is corroborated by the results for rank correlation based on the top 1% animals, which also showed a low RC and a reduced IC at the top animals at 7 days. This finding indicates a strong GEI in the early stages of quail development.

The low correlation results in the reclassification of animals across environments in the early growth stages. It highlights the importance of developing selection for the specific THI class. One possible explanation for the lowest genetic correlation between the THI classes at 7 days is that at this age, the quails are still in the development phase and are particularly more susceptible to climatic variations, suffering greater stress in cold conditions (Matsubara et al., 2016). Young chicks are unable to cope with cold stress because their thermogenic organs are still immature, which limits their ability to produce heat (Mujahid and Furuse, 2009). Cold stress causes oxidative damage in the heart, brain, and duodenum of chicks (Mujahid and Furuse, 2009; Zhang et al., 2011), which can impair their growth. Collier and Collier (2012) also described the responses of younger chickens to environmental challenges. Cold-stressed animals tend to expend more energy to generate body heat, resulting in lower fat accumulation needed for insulation and energy storage, which can have long-term effects. Young chickens also lack the fat reserves that adults use for warmth. In addition, their bodies are not yet fully developed for regulating temperature, as the integration of thermal signals in the hypothalamus and the body-to-brain temperature gradient takes up to 10 days to mature post-hatching (Arad and Itsaki-Glucklish, 1991). The occurrence of hypothermia in chicks exposed to cold after hatching has been documented (Mujahid and Furuse, 2009), which explains the provision of supplemental heat during the "brooding" period as a standard practice in poultry production.

The negative effects of cold stress in adult birds are relatively limited, mainly due to their established metabolism, skin fat deposits, and feather coverage. As long as they maintain adequate feed intake and their feathers remain intact, a slight reduction in the environmental temperature and consequently in the body temperature is not a major concern. However, like in younger chickens, the incomplete feather coverage can lead to significant heat loss when exposed to cold temperatures (Collier and Collier, 2012). Different sets of genes may regulate growth in young chicks exposed to cold versus thermoneutral environments, as metabolism shifts to cope with cold stress and dietary energy is redirected toward heat production. This difference is less evident at older ages, as thermogenic organs are mature and less energy is required for thermogenesis, allowing more to be allocated for growth.

Studies have examined indicators and methods for measuring and identifying thermal stress in birds. For example, Matsubara et al. (2016) examined the effects of acute cold stress on the hearts of seven-day-old neonatal chicks, which resulted in left ventricular hypertrophy, chamber dilation, and wall thickening. This could be a sign of adaptation to increase functional capacity or a risk of harmful cellular alterations. Gasparino et al. (2015), for example, investigated how thermal stress affects gene expression and blood parameters in meat-type quails with different feed efficiencies. Their results showed that thermal stress induces changes in the expression of genes related to energy metabolism and oxidative stress and affects hematological parameters. Regarding cold stress specifically, studies have demonstrated that it can induce the expression of heat shock proteins in the spleen of quails, resulting in oxidative stress and inflammatory lesions (Zhao et al., 2014).The changes in the animals ranking mainly for BW7, BW14, and BW21 are reflected in the variation in breeding value according to the THI class. The animals with better breeding values in the THI1 class will not necessarily be the best ones in the THI2, confirming the presence of a GEI. The existence of GEI in this population indicates that the outcomes of selection on early body weight when made in a specific environment, will not be the same in other environments.

Studies evaluating GEI in quails have been primarily focused on nutritional differences. For example, the studies conducted by Alcântara et al. (2019), Gouveia et al. (2019), Mota et al. (2015), and Tarôco et al. (2018) explored the significant influence of GEI in meat quails under varying dietary conditions, particularly for amino acid levels. An important finding across all studies is that quail lines exhibit differing sensitivity to nutritional changes, consistently showing substantial reranking and sensitivity to nutrient ratios.

Alcântara et al. (2019) and Mota et al. (2015) observed significant GEI effects on BW and carcass traits, particularly at slaughter weight. These studies, along with Gouveia et al. (2019), demonstrated how heritabilities and genetic correlations changed with different dietary nutrient levels. All studies emphasized the need for breeding programs to carefully align selection environments with production environments to maximize genetic gains and avoid performance losses due to changes in the environment. However, none of these studies evaluated GEI using THI as an environmental variable.

Purswell et al. (2012) evaluated BW, weight gain, feed consumption, and feed conversion ratio in broiler chickens under the effect of the THI. Their findings revealed a strong impact of THI variation on animal performance, demonstrating that when the animals are not in thermal comfort, their performance is significantly compromised.

The estimates of genetic correlations for BW14 and BW21 between THI1 and THI2 were moderate, indicating some degree of GEI in these traits. These results are consistent with RC with moderate magnitude and the IC which was 50.77% for BW14 and 49.23% for BW21. It can be observed that approximately half of the best animals in one THI class do not exhibit the same ranking in the other class. This changing in the ranking of the best animals between THI conditions substantiates the existence of GEI, as evidenced by the genetic correlation. It is therefore recommended that GEI should be considered when selecting this population of quails for BW. Given the re-ranking of animals between THI classes, selection strategies should account for environmental variability. To optimize genetic progress and maintain performance in different environments, selection should focus on robust genotypes with stable performance under all conditions.

From BW28, the genetic correlation estimates were high (Table 2), reaching values of 0.94, 0.98, and 0.96 between environments for BW28, BW35 and BW42, respectively. High genetic correlations indicate that common genes are responsible for the trait control in the two different environments and indicate that there is no GEI for BW at these ages. These correlations are supported by the estimates of the rank correlations and the percentages of the top individuals which were high for the three final weights, for both the top 1% of animals and the top 10% of animals with at least 13 offspring. This pattern can be attributed to the fact that animals at these later stages become less sensitive to the environment and consequently more resilient. It can thus be inferred that if an animal from the studied population is selected for BW28, BW35, or BW42 in THI1, it will likely perform well in THI2, and vice-versa.

This population is selected based on BW28, in this study we showed no evidence of GEI at this age. The absence of GEI at 28 days indicates that the same animals would be selected in cold or thermoneutral environments, making it an ideal time for selection. Furthermore, a relevant study by Khaldari et al. (2010) on body weight selection in 28-day old quails supports the effectiveness of this approach. Their results showed that selecting for four-week BW led to correlated responses, particularly in carcass weight components. These results demonstrate substantial genetic progress in the trait of interest and reinforce the suitability of selecting for BW28. However, the study also highlights the importance of considering resilience to thermal changes to improve productivity and animal welfare. Since, we showed that young animals can exhibit high temperature sensitivity.

Our findings corroborate with those reported by Chen et al. (2014) in the sense that cold stress is a physical environmental stress factor and negatively affects the growth performance and well-being of birds. The ability of birds to regulate their body temperature after hatching increases with age (Zhou et al., 2020). Therefore, birds in their first days of life are highly susceptible to low temperatures due to the immaturity of their thermogenic organs. Zhou et al. (2020) also reported that cold stress increases basal metabolic rate and energy metabolism, which are high-priority factors that require a lot of energy for homeotherms. This increased energy requirement to generate heat has become the main reason for the decrease in BW gain. Studies conducted on chicks (Chen et al., 2014; Yang et al., 2014) reported that cold stress significantly increased feed conversion ratio (i.e., lower feed efficiency) and reduced BW gain. Both studies confirmed that cold stress increased the feed conversion ratio, suggesting the redistribution of nutrients from growth to thermoregulatory responses.

We found a GEI associated with quail cold stress. However, it is important to highlight that heat stress can also have a significant negative impact on production. Since, when the temperature is outside an animal's thermoneutral zone, the animal has to adopt additional heat conservation or dissipation strategies. Unfortunately, our dataset did not allow the study of heat stress, as the region where the data was collected does not have extremely high temperatures across the years.

Cassuce et al. (2013) studied thermal comfort during the early stages of poultry development. They concluded that the environmental temperature values that promoted the greatest weight gain in broiler chickens during the initial growth period were 31.3°C, 25.5°C, and 21.8°C for the first, second, and third weeks of age, respectively. Similarly, Souza et al. (2014) investigated the thermal comfort ranges (THI) for meat-type quail during the initial growth period (1-21 days of age) in the same regions as this study. They found that the optimal thermal range for early-stage quail was achieved when the birds were exposed to temperatures of 36-39°C at 7 days of age, 27-30°C at 14 days, and 24°C at 21 days. This reinforces the idea that young birds require higher temperatures for better growth performance. As shown in Figure 2, the region of Viçosa, where the data for this study were collected, generally experiences temperatures below the thermal comfort range for young animals, with an average of about 20°C.

Temperature stress is a big concern in poultry production, as birds are generally inefficient at regulating their body temperature. Poultry are particularly vulnerable to heat stress due to their lack of functional sweat glands, high core body temperature, and high metabolic rate (Leishman et al., 2021). Poultry growth and productivity can be impacted by both heat and cold stress (Bilal et al., 2021; Nawaz et al., 2021; Biswal et al., 2022).

Considering the impact of heat/cold stress, it would be interesting to investigate the potential of THI as a means of assessing this stress factor in poultry. Understanding the role of THI can help us better understand how environmental conditions affect stress responses in poultry, making it easier to select more resilient animals. Applying this knowledge in breeding programs can improve genetic progress by making selection more accurate and focused, while also encouraging diversity and making the breeding process faster. This could lead to more adapted animals to different environments. Practical applications include improving management protocols with a focus on environmental control for young birds and developing selection indices that consider resilience to temperature changes. These findings can support decisions regarding strategic mating and the allocation of animals according to seasonal conditions. This contributes to improved productivity and reduced production costs. Furthermore, selecting animals that are better adapted to environmental challenges can promote animal welfare and advance initiatives to obtain animal welfare certifications, adding value to production systems.

2.6 Conclusions

The study confirmed the influence of the genotype by environment interaction in quail body weight, showing changes in the quail genetic classification, especially during the initial phases when the animals were more sensitive to temperature variations. This shows the importance of temperature and humidity indices in animal performance, emphasizing the need to consider them for selecting superior and more resilient animals. A limitation of this study was the lack of data from high THI, which did not allow us to evaluate the existence of genotype by environment interaction for body weight under heat stress in quails. Thus, further research on thermal stress in quails should be carried out.

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CHAPTER 03

Genotype-by-environment interaction for walking ability in turkeys

3.1 Abstract

This study evaluated genotype-by-environment interaction (GEI) for walking ability in turkeys using reaction norm models. Phenotypic records from 2010 to 2023 were used, including walking ability scores for 192,020 birds, body weight records for 240,985 birds, and a pedigree with 975,521 individuals. Walking ability was scored on a 1-to-6 scale at 20 weeks of age. A two-step approach was applied: first, environmental gradients (EG) were estimated based on contemporary group (CG) solutions from a single-trait animal model for body weight. Then, reaction norm models were used to assess GEI for walking ability, considering EG as a continuous covariate. Three models were compared: M1 (homogeneous residual variance), M2 (two residual variance classes), and M3 (three residual variance classes). The best fit was obtained with M3, which had the lowest AIC and significant likelihood ratio tests ($p < 0.001$). This model provided more stable heritability estimates, ranging from 0.425 to 0.472 across the environment. The residual variances estimated for each class under M3 were 0.531 for class 1, 0.416 for class 2, and 0.386 for class 3, reflecting decreasing residual variation across the environment. Genetic correlations across the environmental gradient range from 0.30 to 1.00. The lower correlations were found between more distant environmental segments, especially between the extremes of the gradient Rank correlations were also low between extreme environment. Thus, genetic correlations and ranking changes across environments confirmed the presence of GEI. An inverse relationship between body weight and walking ability was also observed, reinforcing the importance of considering GEI in turkey breeding programs.

3.2 Introduction

Worldwide meat consumption has grown in recent decades (Yalcin et al., 2019). Consumers have been looking for healthier food with lower fat content, which makes turkey meat one of the most demanded that meets these conditions (Baeza et al., 2022). The domesticated turkey (*Meleagris gallopavo*) has significant agricultural importance and is the second largest contributor to world poultry meat production (Barros et al., 2023). To supply consumer demands, turkey breeding programs have applied intense genetic selection pressure for fast growth and increased body size and meat yield.

Walking ability is one of the indicator traits used by the poultry industry to assess bird mobility and to correct some of the leg related abnormalities. Walking ability exhibited high genetic correlation with leg defects (-0.76) (Mulim et al., 2024). It is a complex trait caused by multiple factors. Birds with compromised mobility tend to have reduced ability to carry out routine functions, including the ability to reach feeders and drinkers in extreme cases. Consequently, the ability of the bird to move has a direct association with productivity and animal welfare. Walking ability is subjectively scored based on a six-grade scoring approach, with one being poor and six being good. Motion, pitch, balance, leg angulation, hock strength, hip strength, and leg structure are the seven mobility features used to subjectively assess walking ability scores. Quinton et al. (2011) presented a full description of the scoring system.

The increase in the body weight of commercial turkeys has had some impact on turkey mobility, which compromises the productivity and welfare of the animal. There is a consensus on the negative genetic relationship between growth and mobility (Soyalp et al., 2023). Evaluating body weight and leg defects in turkey, Mulim et al. (2024) reported heritabilities ranging from 0.45 to 0.57 for body weight at different ages, 0.12 for leg defects and 0.23 for walkability. Showing that high genetic gain can be obtained in the selection for body weight and moderate gain for walkability. The authors reported unfavorable genetic correlations between body weight and leg defects (from 0.26 to 0.32) and body weight and walkability (from -0.36 to -0.46). Thus, the selection for increased body weight results in a reduction of the bird's mobility, increasing leg defects, and decreasing walkability. Animals with locomotion problems and leg defects, in addition to having their welfare compromised, can cause economic losses due to the potential compromise of their carcasses. These disorders are a major poultry welfare issue as they cause pain and affect the bird’s ability to eat and drink, and it result in economic losses for farmers in terms of culled or downgraded birds. Quinton et al. (2011) state that selection and management have increased the growth rate in meat poultry lines have been associated with several structural disorders.

Evaluating walkability in Turkeys under different classes of growth rate, Soyalp et al. (2023) highlighted the need for further research using methodologies, such as random regressions, reaction norms, and changepoint (breakpoint) models as alternative approaches to managing the potential relationship between growth and mobility in turkeys. Reaction norm models have been proposed as a useful approach to study G×E by modeling genetic sensitivity to environmental gradients (Kolmodin et al., 2002; Su et al., 2006; Mulder & Bijma, 2005). One practical method to define the environmental gradients in livestock studies is through contemporary group (CG) solutions obtained from routine genetic evaluations. These solutions reflect the average environmental conditions experienced by animals in a group (defined by farm, year, season, etc.), and can be used to construct continuous environmental gradients (EG). Reaction norm models then allow the estimation of individual genetic responses along these gradients, providing insight into both performance potential and environmental sensitivity (Strandberg, 2006; López de Maturana et al., 2007).

This methodology has been successfully applied to traits such as growth, production, and reproduction in livestock. However, its use for welfare-related traits, particularly mobility traits in poultry, remains limited. In addition, usually the papers explore the CG solutions for the trait that will be evaluated under the random norm approach, which means that the same data will be used twice. To date, no published studies have explored walking ability in turkeys using reaction norm models with environmental gradients derived from contemporary group (CG) solutions for productivity traits, such as body weight. This represents a gap in the literature because mobility is likely affected by both genetics and the production environment in which birds are raised, particularly in systems with high variation in management quality, housing conditions, and nutrition.

To fill this gap, our study investigated potential genotype-by-environment interactions in turkeys for traits related to walking ability, using contemporary group solutions for body weight within a reaction norm model framework. This approach allows us to assess genetic variation in walking ability across environments and to identify robust genotypes with consistent performance under different conditions, enabling a more precise and biologically meaningful assessment of G×E effects, with potential applications in turkey breeding programs. In addition, it will improve our understanding on the relationship of walking ability and body weight.

3.3 Material and methods

Approval from the Animal Care Committee was unnecessary for this study since all analyses were conducted using pre-existing databases. Hendrix Genetics (Kitchener, ON, Canada) provided all the datasets analyzed in this study.

3.3.1 Data

Phenotypic records were collected from a female line of purebred Turkeys (*Meleagris gallopavo*), born from 2010 to 2023. The dataset included growth and walking ability traits. For the G×E analysis, walking ability (WALK) was used as the target trait, while body weight (BW), recorded from 19 to 23 weeks for males and 17 to 21 weeks for females. The data analyzed in the present study included body weight records from 240,985 birds, walking ability scores for 192,020 birds, and a pedigree comprising 975,521 individuals.

Walking ability was evaluated by trained technicians on a scale of 1 to 6 at 20 weeks of age using the approach described in Quinton et al. (2011). A score of 1 indicated poor walking ability (poor motion, pitch, and balance, severe inward leg angulation, weak hip or hock, bow/twisted leg) and a score of 6 indicated good walking ability (fluid motion, excellent pitch and balance, low outward leg angulation, strong hip and hock and no leg defects). The contemporary groups (CG) were formed by animals born in the same hatch (year and week). Outliers were discarded from CG if they deviated by more than 3.5 SD from the trait mean, and CG with fewer than 10 records were removed from the analyses.

3.3.2 Analysis

We used a two-step approach to evaluate the genotype by environmental interaction. The environmental gradient (EG) was estimated based on the CG solutions obtained from a traditional single-trait animal model for BW. The model was implemented using the BLUPF90 software (Misztal et al., 2014). The following single-trait animal model fitted can be described as:

$$\mathrm{y} = \mathrm{Xb} + \mathrm{Za} + \mathrm{e}$$

where y is the vector of phenotypic observations for BW; b is the vector of fixed effects including CG (hatch: week and year that the animal was born), sex, and age at measurement in weeks; a is the vector of random additive genetic effects; e is the vector of random residual errors; X, and Z are the incidence matrices associated with the fixed and additive genetic effects, respectively. The additive genetic effects were assumed to follow a normal distribution, a ~ N (0, $A\sigma^2_a$), while the residuals were assumed to be independently and normally distributed as e ~ N (0, $I\sigma^2_e$).

The estimated CG solutions for BW were obtained from this model. To construct a continuous environmental descriptor, the CG solutions were standardized (mean = 0, SD = 1), values lower than zero represents the least favorable environment and higher than zero the most favorable for body weight. The standardized solution of CG was used as the environmental gradient (EG) for the next step.

To investigate GEI, walking ability (WALK) was modeled using a reaction norm model (RNM), incorporating the EG as a continuous covariate. The simpler model (M1) was formulated as:

$$y = Xb + \beta(EG) + Z_0\, a_0 + Z_1\, a_1(EG) + e$$

where y is the vector of phenotypic observations for walking ability; b is the vector of fixed effects of CG, sex and age; β is the fixed regression coefficient for the population mean response to the EG; a_0 is the vector of random intercepts representing the additive breeding values of individuals at the average environmental condition; a_1 is the vector of random slopes representing the genetic sensitivity of individuals to changes across the EG; e is the random residual errors. X, Z_0, and Z_1 are the incidence matrices corresponding to the fixed effects, the additive genetic intercepts, and the additive genetic slopes, respectively.

Three models were fitted using different specifications for the residual variance structure based on the environmental gradient. In Model 1 (M1), a homogeneous residual variance was assumed across all environmental levels. In Model 2 (M2), heterogeneous residual variance was modeled using two classes, defined according to the sign of the environmental gradient: environments with gradient values less than 0 (i.e., less favorable) were assigned to one residual variance class, while environments with gradient values greater than or equal to 0 (i.e., more favorable) were assigned to a second class. In Model 3 (M3), heterogeneous residual variance was modeled using three classes, based on predefined intervals of the standardized environmental gradient:

–1.25 to –0.25 (least favorable environments), –0.25 to 0.75 (intermediate environments), and 0.75 to 1.75 (most favorable environments). This classification was based on the distribution of the environmental gradient, which was previously standardized and derived from the contemporary group (CG) solutions for body weight.

The optimal RNM was chosen based on the Akaike Information Criterion (AIC; Akaike, 1973) and Likelihood Ratio Tests (LRTs). AIC values were used to compare the models, balancing fit and complexity. The LRT statistic was computed as the difference in −2 log likelihood between models. Its significance was evaluated using a chi-square distribution with degrees of freedom corresponding to the number of additional parameters.

Based on the selected optimal model, heritability estimates and breeding values were plotted across the environmental gradient to visualize patterns of genetic variability and potential genotype-by-environment interaction. A heatmap was constructed using the genetic covariance matrix obtained from the random regression model to visualize the genetic correlations between environments. The heatmap was created using the ggplot2 package in R.

Additional analyses were performed to evaluate the stability of genetic rankings across environments. Rank correlation coefficients (RC) and the percentage of individuals in common (IC) were calculated for walking ability by comparing the estimated breeding values (EBVs) of animals across low, medium, and high environmental levels, as defined by CG solutions for body weight.

3.4 Results

Table 1 presents the descriptive statistics for body weight (BW) and walking ability (WALK) traits in turkeys. A total of 240,985 BW records were available, with values ranging from 7,750 to 20,600 grams (g) and an average of 12,523 g. For WALK, 192,020 records were analyzed, with scores ranging from 1 to 4 and an average score of 1.99. BW had a standard deviation of 3,598.20, while WALK had a standard deviation of 0.81.

Table 1. Descriptive statistics of body weight (BW) and walking ability (WALK) traits for a female line of turkey: number of observations (N), minimum (Min), maximum (Max), mean, and standard deviation (SD) for each trait.

	BW	**WALK**
N	240,985	192,020
Mean	12,523	1.99
Min	7,750	1
Max	20,600	4
SD	3,598.20	0.81

Three models were evaluated to verify the existence of residual variance heterogeneity, and their comparison was based on log-likelihood, AIC, and likelihood ratio tests (LRT), as shown in Table 2. Model M3, which assumed heterogeneous residual variance with three classes along the environmental gradient, exhibited the lowest AIC and was significantly better than both M1 and M2 according to the LRT. The LRT statistics for comparisons between models were all significant ($p < 0.001$), confirming that modeling heterogeneous residual variance substantially improved model fitness. Therefore, Model M3 was selected as the optimal model for reaction norm analysis.

Table 2. Comparison of models with different residual variance structures: log-likelihood (−2 LogL), Akaike Information Criterion (AIC), and Likelihood Ratio Test (LRT) statistics.

Model	−2 LogL	AIC	LRT Comparison	LRT Statistic	p-value
M1	428,934.06	428,942.06	M2 vs. M1	508.64	< 0.001
M2	428,425.42	428,435.42	M3 vs. M2	39.21	< 0.001
M3	428,386.21	428,398.21	M3 vs. M1	547.85	< 0.001

M1 = homogeneous residual variance; M2 = heterogeneous residual variance with two classes; M3 = heterogeneous residual variance with three classes.

Figure 1 shows estimate of additive genetic variance and heritability (h^2) for walking ability across the environmental gradient as defined by contemporary group solutions for body weight using a model with homogeneous residual variance (M1). In this model, heritability estimates varied substantially, with a mean value of 0.486 (SD = 0.132). Heritability was highest (0.708) in favorable environments and lowest (0.317) in challenging environments. For M1 the estimated residual variance was 0.415.

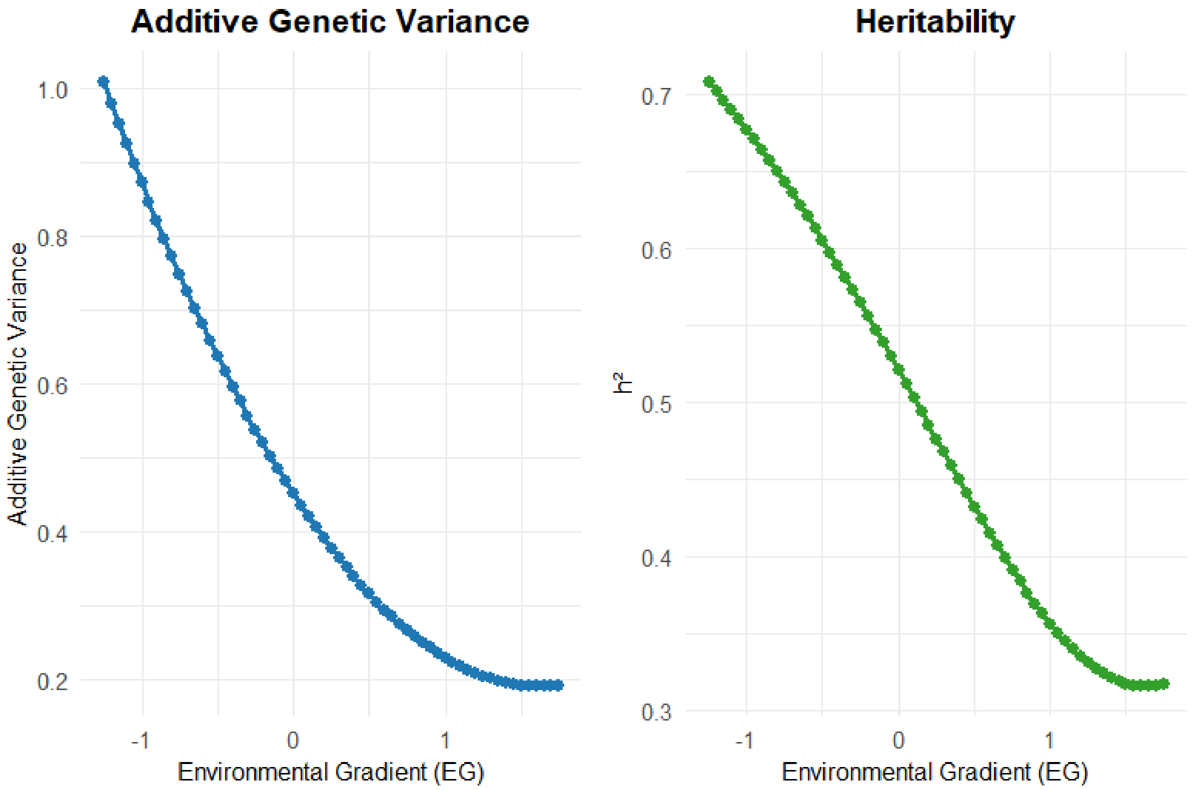

Figure 1. Additive genetic variance and heritability for walking ability in turkeys across the environmental gradient based on the contemporary group for body weight under homogeneous residual variance model.

Figure 2 illustrates the estimates of additive genetic variance and heritability for walking ability using the model with heterogeneous residual variance (M3), which was selected as the best-fitting model. This model considered three residual variance classes distributed along the environmental gradient. The results show more stable heritability estimates across environments compared to M1. The average h^2 per class was 0.426 (SD = 0.028) for class 1 (favorable environments), 0.408 (SD = 0.016) for class 2 (intermediate), and 0.407 (SD = 0.007) for class 3 (unfavorable). Despite the slight variation between classes, heritability estimates remained relatively consistent, ranging from 0.425 to 0.472. Compared to the homogeneous residual variance model (M1), the additive genetic variance estimates decreased under M3. The residual variances estimated for each class under M3 were 0.531 for class 1, 0.416 for class 2, and 0.386 for class 3, reflecting decreasing residual variation across the environment.

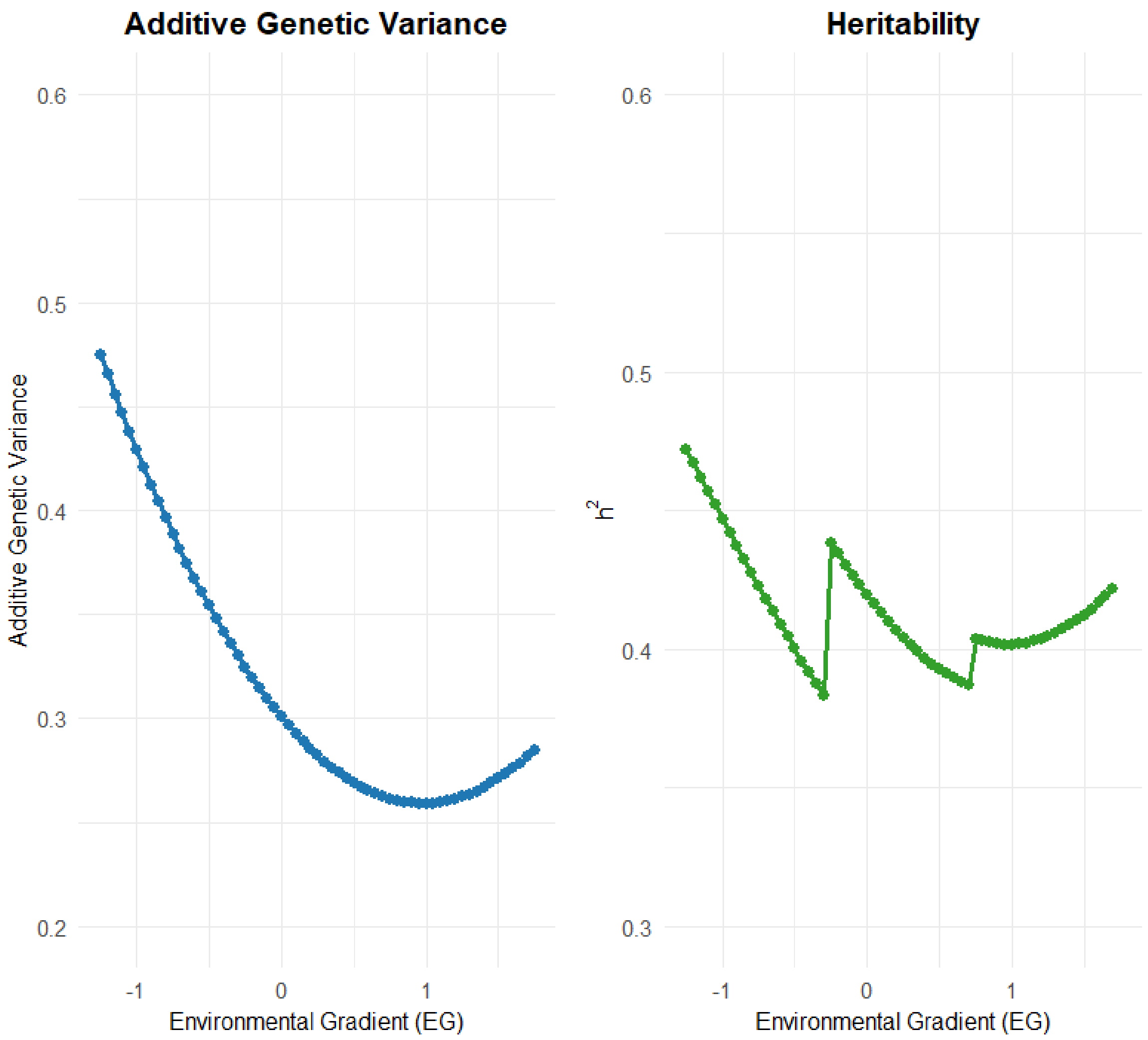

Figure 2. Additive genetic variance and heritability for walking ability in turkeys across the environmental gradient based on the contemporary group for body weight under heterogeneous residual variance model.

Genetic correlations estimated across the environmental gradient as a heatmap is presented in figure 3. In this gradient, a negative value represents the least favorable environment for body weight, and positive values represents the most favorable environments (optimal conditions for growth). The heatmap shows that the genetic correlations across the environmental gradient ranges from 0.30 to 1.00. Higher genetic correlations were observed between similar environment. However, lower correlations were found between more distant environmental segments, especially between the extremes of the gradient. These low genetic correlations indicate the presence of genotype by environment interaction, as they fall below the commonly used threshold of 0.80.

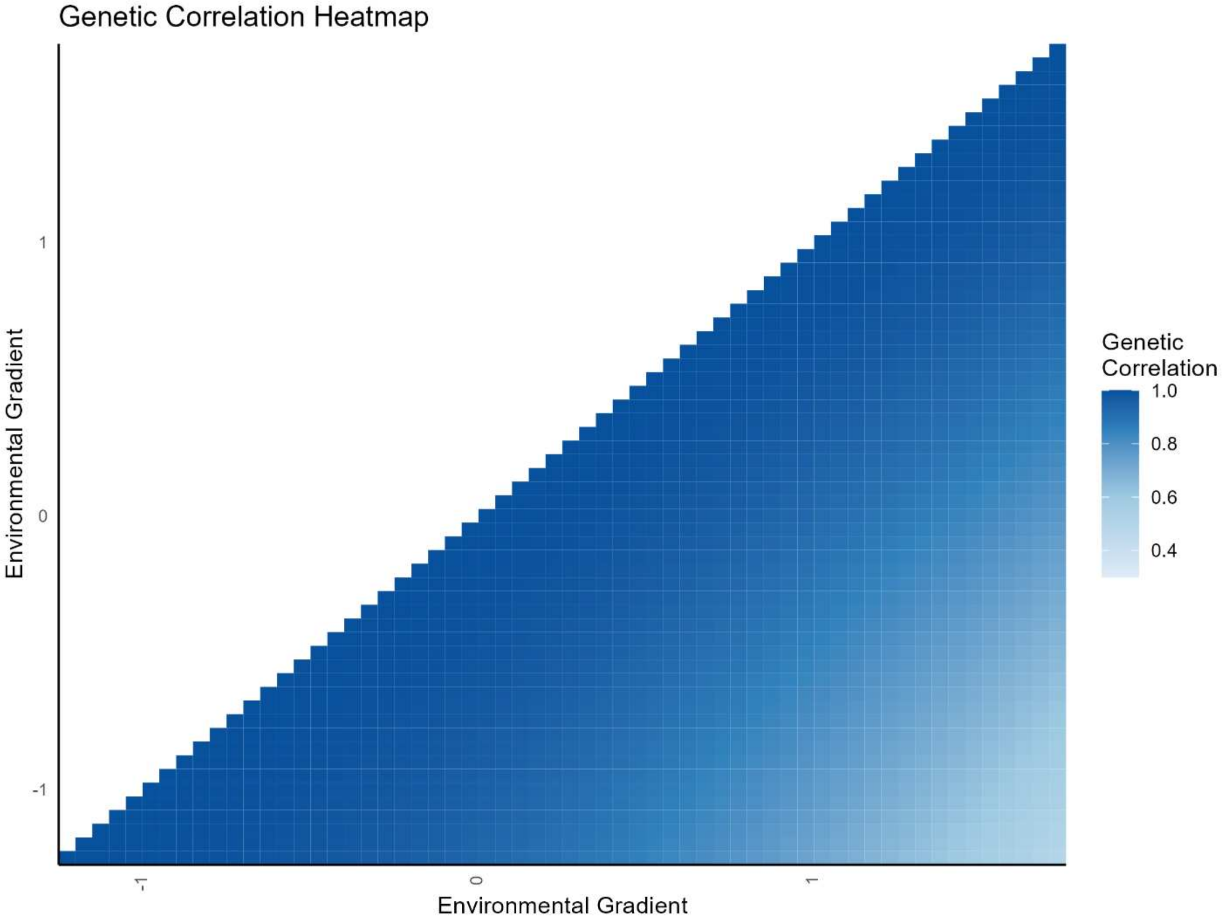

Figure 3. Heatmap showing genetic correlations across an environmental gradient based on the contemporary group solutions for body weight.

Using breeding values for walking ability obtained with M3 we computed rank correlations (CR) and the percentage of individuals in common (CI) among different environmental levels (low, medium, and high) for male turkeys that have produced at least 100 offspring (Table 3). Among the top 20 animals, the CR values were 0.88 (low vs. medium), 0.19 (low vs. high), and 0.56 (medium vs. high). These values indicate a decrease in correlation between the most extreme environments. The CI values followed a similar pattern: 90% between low and medium and low and high, and 95% between medium and high. These results reflect moderate to high consistency in rankings for similar environments, but significant re-ranking when comparing extreme conditions. In the Top 1% group included (n = 40) the CR between low and high environments was 0.35, indicating re-ranking across the environments. Between adjacent environments there are strong agreements in CR and CI (CR = 0.81; CI ≥ 90%). With greater selection thresholds (Top 5% and 10%), rank correlations and the number of common individuals across environments increased. CR values ranged from 0.39 to 0.89, and CI values were above 98% for all comparisons. These patterns suggest that selection decisions based on walking ability in males are sensitive to environmental conditions, particularly when selecting the top elite animals. However, the high CI indicates that basically the same animals will be selected across environments, mainly for the top 10%.

Table 3. Rank correlations (CR, above the diagonal) and percentages of individuals in common (CI, below the diagonal) for walking ability in turkeys, comparing rankings of males with at least 100 offspring across low, medium, and high environmental conditions, based on the contemporary group for body weight at 19 – 23 weeks.

	Top 20 animals			**Top 1% (N=40)**		
	Low	**Medium**	**High**	**Low**	**Medium**	**High**
Low	**1.00**	0.88	0.19	**1.00**	0.81	0.35
Medium	90.00%	**1.00**	0.56	90.00%	**1.00**	0.81
High	90.00%	95.00%	**1.00**	100.00%	100.00%	**1.00**
	Top 5% (N=200)			**Top 10% (N=400)**		
	Low	**Medium**	**High**	**Low**	**Medium**	**High**
Low	**1.00**	0.80	0.39	**1.00**	0.89	0.61

Medium	98.00%	**1.00**	0.82	98.75%	**1.00**	0.88
High	99.00%	98.50%	**1.00**	99.50%	99.5%	**1.00**

Evaluating breeding values (EBVs) for walking ability under different environmental conditions revealed changes in animal ranking, indicating genotype-by-environment interaction (G×E). Figures 4 shows the EBVs of the top 20 animals with at least 100 offspring. They are ranked along the environmental gradient. There was a clear re-ranking of males across environments, which is in agreement with the observed CR, mainly between low and high environmental conditions.

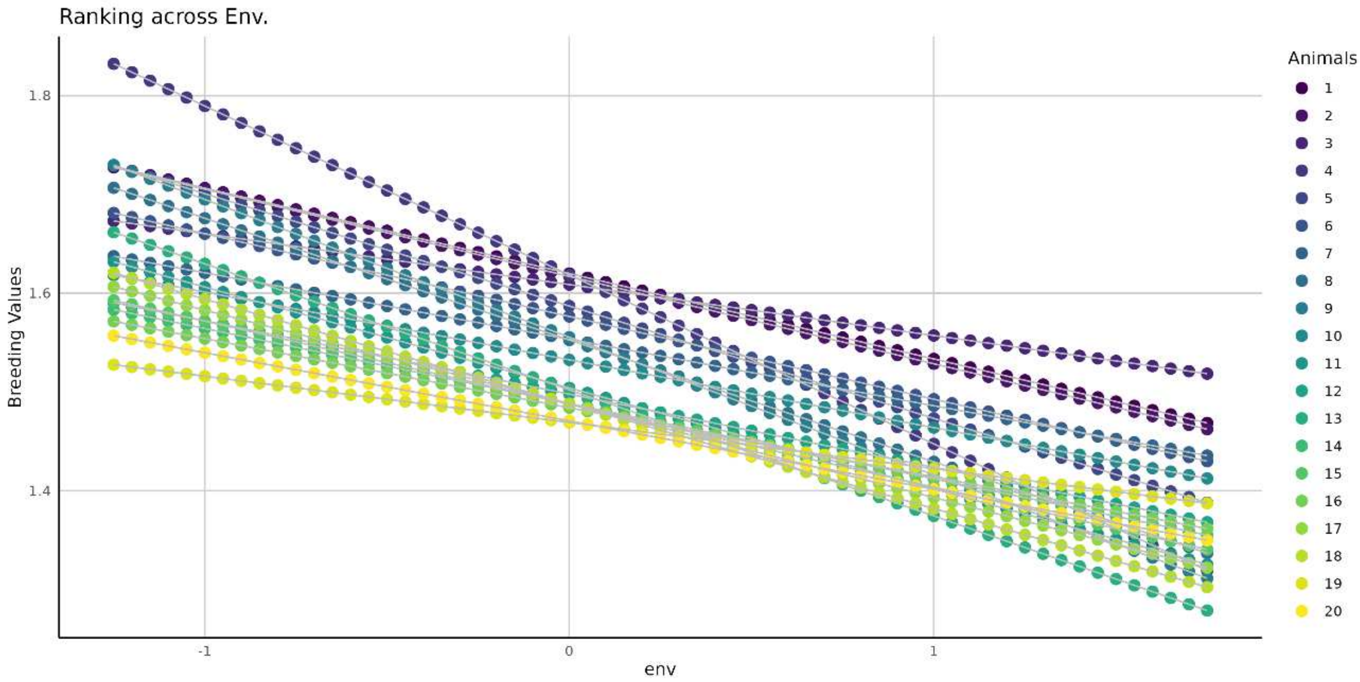

Figure 4. Breeding values for walking ability in turkeys for the top 20 males with at least 100 offspring across the environmental gradient based on the contemporary group for body weight at 19 – 23 weeks.

Table 4 shows rank correlations (CR) and percentages of individuals in common (CI) for female turkeys that had produced at least 50 offspring. For the top 20 animals, the CR was 0.88 between low and medium environments, dropping to 0.18 between low and high environments and 0.56 between medium and high, results that closely align with those found in males. The CI values were 90% (low vs. medium and low vs. high) and 95% (medium vs. high). For the top 1% (n = 94), we also observed the lowest rank correlation between the low and high environments (CR = 0.32), even though 100% of the selected individuals were the same in both environments (CI = 100%). In contrast, comparisons between adjacent environments (low vs. medium and medium vs. high) showed moderate to high correlations (CR = 0.78 and 0.79) and high percentages of individuals in common (CI = 96.8% and 98.94%). For Top 5% and 10%, CR values ranged from 0.31 (low vs. high) to 0.85 (low vs. medium), while CI values remained consistently high (> 98%).

Table 4. Rank correlations (CR, above the diagonal) and percentages of individuals in common (CI, below the diagonal) for walking ability in turkeys, comparing rankings of females with at least 50 offspring across low, medium, and high environmental conditions, based on the contemporary group for body weight at 17 – 21 weeks.

	Top 20 animals			**Top 1% (N=94)**		
	Low	**Medium**	**High**	**Low**	**Medium**	**High**
Low	**1.00**	0.88	0.18	**1.00**	0.79	0.32
Medium	90.00%	**1.00**	0.56	96.80%	**1.00**	0.78
High	90.00%	95.00%	**1.00**	100.00%	98.94%	**1.00**
	Top 5% (N=469)			**Top 10% (N=938)**		
	Low	**Medium**	**High**	**Low**	**Medium**	**High**
Low	**1.00**	0.77	0.31	**1.00**	0.85	0.49
Medium	98.93%	**1.00**	0.80	99.57%	**1.00**	0.84
High	99.57%	98.93%	**1.00**	99.89%	99.25%	**1.00**

Figures 5 show the estimated breeding values (EBVs) for the top 20 females with at least 50 offspring, plotted along the environmental gradient. Similar to the males, the ranking of the females varied across environments, which further supports the presence of G×E interaction for walking ability in this population.

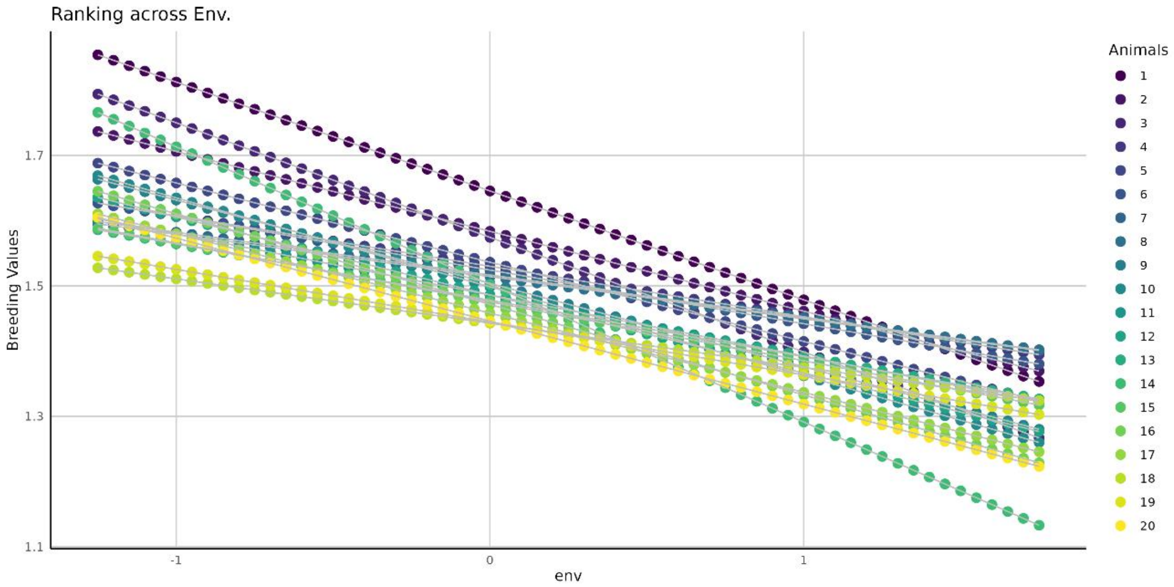

Figure 5. Breeding values for walking ability in turkeys for the top 20 females with at least 50 offspring across the environmental gradient based on the contemporary group for body weight at 17 – 21 weeks.

3.5 Discussion

The best model for evaluating genotype-by-environment interaction (GEI) for walking ability in turkeys was the one that considered three classes of heterogeneous residual variances (Model M3). This model had the lowest AIC value and was significantly better than simpler models with homogeneous residual variance (M1) or two variance classes (M2), as indicated by Likelihood Ratio Tests (LRT) with p-values < 0.001. These findings clearly demonstrate that accounting for residual variance heterogeneity improves the fit of reaction norm models under G×E conditions. Assuming constant residual variance across environments (as in M1) may oversimplify biological reality, leading to biased genetic parameter estimates and inaccurate breeding value predictions (Calus et al., 2004). Conversely, when residual variance is permitted to vary across environmental conditions (as in M3), the model can more effectively distinguish genetic effects from environmental noise, resulting in more reliable genetic evaluations.

These findings align with previous studies emphasizing the importance of modeling heteroscedasticity in GEI analyses to improve genetic evaluation accuracy. Carvalho Filho et al. (2022) showed that using heteroscedastic reaction norm models improved the evaluation of GEI in Nellore cattle for various traits, such as growth, reproductive performance, and visual scores.

Those strategies have also been applied in poultry, Silva et al. (2021) evaluated residual variance heterogeneity in meat quails and found that, although heritabilities for residual variance were low, selection for uniformity could be feasible. Likewise, Felipe et al. (2012) investigated the sensitivity of breeding values for body weight in meat quails under different crude protein levels and observed heterogeneity of additive genetic variance and residual variance across diets, indicating the presence of genotype-by-environment interaction. These studies reinforce the relevance of considering heteroscedasticity in genetic evaluations of poultry species, particularly under varying environments.

Similarly, the present study with turkeys found that accounting for residual variance heterogeneity allowed better evaluation of GEI. This finding reinforces the value of this approach in poultry breeding programs. However, the impact of modeling heteroscedasticity and GEI can vary by species and trait. Márquez et al. (2015) found that heteroscedasticity had a minimal effect on weight traits in crossbred lambs but was more relevant for ultrasonic traits, which may be more sensitive to environmental variation. These findings show that the benefits of modeling residual heterogeneity depend on the trait and context, suggesting that different models should be evaluated for reaction norms analysis.

Ignoring variability, especially in challenging environments where residual variance tends to be higher, can lead to an underestimation of genetic variance and less efficient selection (Rowiński & Rogell, 2017). Accurately partitioning phenotypic variance into genetic and environmental components is essential for making informed breeding decisions, especially when selecting animals that are robust across varying environmental conditions. Figures 1 and 2 illustrate how estimates of additive genetic variance and heritability for walking ability in turkeys change along the environmental gradient defined by contemporary group solutions for body weight. These results emphasize that the pattern of genetic variation strongly depends on the model used and how it accounts for environmental differences.

In this study, as the environment became more favorable for body weight, it became less favorable for walking ability. This inverse relationship is crucial for interpreting the results.

Under the model with homogeneous residual variance (M1), heritability estimates for walking ability was 0.708 in the worse environment. It decreased with the improvement of the environment for body weight and reached the minimum value (0.317). Lower heritability in the worse environment for walking ability can be attributed to biological limitations that reduce the expression of genetic differences when animals experience locomotor stress. This pattern is consistent with observations in livestock under GEI scenarios (Kolmodin et al., 2002).

Using the heterogeneous residual variance model (M3), which allowed residual variance to vary across three classes, produced more stable heritability estimates for walking ability across the environmental gradient, despite the gradient being challenging for the trait. The average heritability values were 0.426 in unfavorable environments for body weight (but favorable for walking ability), 0.408 in intermediate environments, and 0.407 in less challenging environments for body weight. By better accounting for residual variance, M3 avoids inflating the genetic variance component to compensate for environmental effect that M1 does not explain. The residual variances estimated for each class followed a declining trend: 0.531 for class 1, 0.416 for class 2, and 0.386 for class 3 and for M1 the estimated residual variance was 0.415. Additive variance in M3 and M1 decrease with the improvement of the environment for body weight, which indicates the existence of GEI for walking ability. Corrêa et al. (2009) and Oliveira et al. (2018) reported increases in additive genetic variance and heritability for yearling weight in cattle along environmental gradients, which supports the presence of GEI.

The genetic correlations and breeding value rankings observed across the environmental gradient provide strong evidence of genotype-by-environment interaction (GEI) for walking ability in turkeys. The environmental gradient, which was defined based on contemporary group solutions for body weight, revealed biological relationships between growth performance and locomotion capacity. Figure 3 showed that genetic correlations for walking ability decrease as environmental contrast increases, ranging from 1.00 (similar environments) to 0.30 (extreme environments). According to Robertson (1959), genetic correlations below 0.80 indicate GEI since they reflect changes in the relative performance of genotypes across environments. The drop in correlation in this study suggests that genetic expression for walking ability differs substantially depending on the production environment.

This study also revealed an inverse relationship between walking ability and body weight. As environmental conditions became more favorable for body weight, breeding values (EBVs) for walking ability decreased. In other words, animals raised under growth-optimized conditions tended to have lower breeding values for locomotion. It may be attributed to physiological or structural challenges imposed by increased body weight. This reinforces the biological trade-off between production-related and welfare-related traits, highlighting a critical consideration for selection strategies.

Ranking correlations showed in tables 3 and 4 provide additional evidence for GEI. For both males and females, the CR values dropped sharply between low and high environments, despite CI values remaining relatively high. This indicates that while the same animals may be selected across environments, their ranking changes significantly, which could affect selection intensity and long-term genetic progress. The effect was even more pronounced when focusing on the top 1% of animals, where small shifts in ranking can lead to large impacts on selection outcomes.

From a breeding perspective, the findings can be used to support that selection based only on performance in favorable environments may reduce locomotion ability, particularly in heavier animals. This could negatively impact animal welfare. Furthermore, Mulim et al. (2024) demonstrated that walking ability is moderately heritable ($h^2 = 0.23$) and negatively associated with leg defects, and mortality, emphasizing its importance as a selection trait. Their genomic findings also revealed that distinct biological processes influence walking ability, highlighting the trait's complexity and the necessity of strategies in genetic evaluations.

The results of this study emphasize the importance of modeling GEI through reaction norm models, and compute genetic correlations and ranking across environment to guide more effective selection. As demonstrated in this study and previous research (Corrêa et al., 2009; Oliveira et al., 2018; Alvarenga et al., 2022; Silva Neto et al., 2024), failing to consider GEI can result in an overestimation of genetic potential and reduced selection efficiency, particularly in the face of environmental changes. Therefore, breeding programs aiming to improve walking ability and maintain high performance must incorporate GEI into their selection decisions and consider environmental conditions' impact on trait expression.

3.6 Conclusion

This study confirmed the presence of genotype-by-environment interaction for walking ability in turkeys, evidenced by changes in genetic parameters and breeding value rankings across the environmental gradient. The model that accounted for heterogeneous residual variances (M3) provided a better fit and more stable estimates of heritability, highlighting the importance of modeling residual heterogeneity in reaction norm analyses. Additionally, the inverse relationship observed between walking ability and body weight emphasizes the need to consider welfare traits in selection strategies.

3.7 References

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