Effect of genotype and nutritional and environmental challenges on growth curve dynamics of broiler chickens

Laura Menchetti ^(@)[,*](#page-0-0) Marco Birolo (@)[,](#page-0-2)^{†[,1](http://orcid.org/0000-0002-1236-4456)} [Cecilia Mugnai](http://orcid.org/0000-0003-0172-4978) (@),[‡] [Alice Cartoni Mancinelli](http://orcid.org/0000-0001-9888-9079) (@),^{||} [Gerolamo Xiccato](http://orcid.org/0000-0003-4572-3635) \bullet [,](http://orcid.org/0000-0001-9045-6156) [Angela Trocino](http://orcid.org/0000-0001-9045-6156) \bullet , \overline{S} [and Cesare Castellini](http://orcid.org/0000-0002-6134-0901) \bullet

* School of Biosciences and Veterinary Medicine, University of Camerino, 62024 Matelica, Macerata, Italy; † Department of Agronomy, Food, Natural Resources, Animal and Environment (DAFNAE), University of Padova, 35020 Legnaro, Padova, Italy; [†] Department of Veterinary Sciences, University of Turin, 10095 Grugliasco Torino, Italy; ⁸Department of Comparative Biomedicine and Food Science (BCA), University of Padova, 35020 Legnaro, Padova, Italy; and ^{||} Department of Agricultural, Environmental and Food Science, University of Perugia, 06121 Perugia, Italy

ABSTRACT The present study aimed to compare the dynamics of growth of various chicken genotypes exposed to heat stress, low-input diets, and free-range farming by using Gompertz model to gain insights into their capabilities to face environmental and nutritional challenges. Three in vivo trials $(T1: heat stress, T2: low-input diets,$ and T3: free-range system) were conducted, involving a total of 671 animals. Five chicken genotypes were employed in each trial: 2 Italian local breeds, Bionda Piemontese (BP) and Robusta Maculata (RM), along with their crossbreeds with Sasso hens $(BP \times SA$ and $RM \times SA$), and a commercial hybrid (Ross 308). Oneday-old male chicks were individually identified, and the 5 genotypes were randomly allocated to different challenging conditions: T1 involved 2 environmental temperatures (thermoneutral vs. high temperature); T2 involved 2 diets (standard vs. low-input); T3 involved 2 rearing systems (conventional vs. free-range). The chickens were weighed once a week from their arrival until slaughtering, and the data were used to build growth curves using the

Gompertz model. Chickens from different genotypes were slaughtered at varying ages based on their maturity. In all trials, the challenging conditions significantly reduced adult body weight $(A; -31.0\%)$ and maximum growth rate $(MGR; -25.6\%)$ of Ross chickens. In contrast, in T1 and T2, no significant changes were observed in the main growth curve parameters of local breeds and crossbreeds, while under free-range conditions, there was even an increase in the A and MGR of these genotypes. The crossbreeding was effective in increasing A and MGR of BP $(+30.5\%$ in BP \times SA), as well as in improving the precocity and MGR of RM $(+19.5\%$ in RM \times SA). Our findings highlight the effectiveness of the Gompertz model as a tool for evaluating birds' adaptability and confirm the greater ability of local breeds and crossbreeds to adapt to different challenges. In conclusion, our methodological approach could be used to choose the genotype most suited to the environmental context and confirm the potential advantages of crossbreeding for enhancing resilience and sustainability.

Key words: crossbreeding, Gompertz curve, low-input diet, heat stress, free-range

INTRODUCTION

The poultry industry is a significant contributor to the global economy, providing a vital source of protein worldwide through the production of meat and eggs. Various factors, including fluctuations in the environment, significantly impact poultry health, welfare and productivity ([Quinteiro-Filho et al., 2012](#page-11-0); [Sun et al.,](#page-12-0) [2023\)](#page-12-0). High environmental temperatures result in significant economic losses (Hatfi[eld et al., 2014\)](#page-11-1) and can exacerbate agricultural losses [\(Food and Agriculture](#page-11-2) [Organization of the United Nations \(FAO\), 2023\)](#page-11-2). Climate change aggravates challenges by impacting feed quality and availability, and by negatively affecting the fertility and growth performance of animals.

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Meanwhile, selection for rapid growth and high feed efficiency has inadvertently heightened the vulnerability of broiler chickens to stress due to their elevated metabolic rates [\(Gogoi et al., 2021;](#page-11-3) [Nawaz et al., 2023\)](#page-11-4). Fastgrowing chicken genotypes require more intensive management, housing, and feeding practices compared to local breeds, which are better suited to alternative

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¹ Corresponding author: marco.birolo@unipd.it

rearing systems such as free-range and organic farming ([Cartoni Mancinelli et al., 2021\)](#page-11-5). Nevertheless, these local breeds typically demonstrate low growth rates and high feed conversion ratios. To address these challenges, various crossbreeding strategies between local breeds and more productive chicken strains have been proposed and evaluated, demonstrating positive outcomes primarily attributed to the exploitation of heterosis ([Sungkhap](#page-12-1)[reecha et al., 2022](#page-12-1); [Wang et al., 2022;](#page-12-2) [Cartoni Mancinelli](#page-11-6) [et al., 2023](#page-11-6)). Another strategy to enhance the sustainability of local breeds involves utilizing low-input diets, which are formulated with reduced nutritional inputs and derived from locally available ingredients, to partially compensate for their low feed efficiency [\(Fiorilla et](#page-11-7) [al., 2024](#page-11-7)).

In the future, animals with the ability to combine strong production potential with resilience to stressors will become increasingly crucial. This dual capability allows them to thrive across diverse environments, ensuring sustainable and adaptable agricultural systems. From this perspective, the robustness and health of animals are closely linked to their ability to respond to environmental challenges (i.e., high temperature, low-input diets, microbial pressure of environment). Therefore, achieving an appropriate balance between chicken productivity and resilience requires further study and refinement.

In recent years, there has been an increased demand for meat of chickens raised in alternative systems due to improved attributes such as texture, taste, and nutritive characteristics ([Santos et al., 2005;](#page-11-8) [Li et al., 2017](#page-11-9)). However, in organic and free-range conditions, resilience to environmental and nutritional changes is highly necessary to cope with the diverse challenges presented by the environment including suboptimal thermal conditions and dietary constraints. In free-range settings, the consumption of grass, and small stones typically dilutes the energy and protein content of the feed ([Sossidou et al., 2015\)](#page-12-3).

Some studies suggest that local chicken breeds, despite exhibiting lower productive performance, are less sensitive to "nonoptimal" diets ([Perella et al., 2009\)](#page-11-10) and more resistant to heat stress ([Huerta et al., 2023](#page-11-11); [Perini et al., 2021](#page-11-12)) compared to fast-growing commercial hybrids. Moreover, some authors postulated that the selection for fast-growing chickens might have reduced their ability to cope with the environmental stimuli related to free-range housing, thus making these genetic strains less suitable for alternative farming systems [\(Ste](#page-12-4)[fanetti et al., 2023](#page-12-4)).

The expression of genes or proteins, such as heat shock proteins, along with decreased productive performance, are regarded as significant markers of heat stress. In this context, monitoring the development of body weight at different ages is crucial for determining the growth pattern and potential of the animals, as well as for assessing the effects of challenging conditions. The growth curve during a certain period of time has primarily been studied using nonlinear models [\(Kaplan and Gurcan, 2018](#page-11-13); [Ibiapina Neto et al., 2020\)](#page-11-14), with the Gompertz model being commonly applied in chickens [\(Gonz](#page-11-15)[alez Ariza et](#page-11-15)

[al., 2021a;](#page-11-15) [Cartoni-Mancinelli et al., 2023\)](#page-11-6). Data on the growth curve could provide more explanatory insights than live weight at slaughter because analysing this function offers crucial information on animal adaptation to the environment.

Therefore, the hypothesis of the present study it that growth models can provide additional insights into the resilience of local breeds, crossbreeds, and commercial hybrids. This can aid in selecting the genotype best suited to environmental and climatic conditions, as well as the availability of resources.

The aim of this study is to investigate the response of various chicken genotypes, including local breeds, crossbreeds, and a commercial hybrid, to nutritional and environmental challenges, specifically heat stress, lowinput diets, and free-range farming. The study employs the Gompertz model as a tool to assess and differentiate the resilience of the animals.

MATERIAL AND METHODS

Ethic Statement

The experimental protocols were approved by the Ethical Committees for Animal Experimentation of the University of Padova, Italy (Prot. N. 15481, approved on $01/02/2021$) and the University of Turin, Italy (Prot. N. 251833, approved on 22/06/2020). All animals were reared and managed according to regulation 2007/ 43/EC for the protection of chickens kept for meat production and regulation 2010/63/EU for the protection of animals used for scientific purposes. Research staff involved in animal handling were animal specialists (PhD or MS in Animal Science) and veterinary practitioners.

Animals, Facilities, and Experimental Design

This study is part of the research project "Use of local chicken breeds in alternative production chain: welfare quality and sustainability − LoChAl" (Progetti di Rilevante Interesse Nazionale - PRIN, 2017). The project deals with the characterization, valorisation and conservation of Italian local chicken breeds and the development of rearing strategies to improve the global sustainability in poultry production with special emphasis on alternative farming systems.

Three in vivo trials $(T1:$ heath stress, $T2:$ low-input diets, and T3: free-range system) were performed in the poultry farm of the Department of Agronomy, Food, Natural resources, Animals and Environment − DAFNAE of the University of Padova, Italy (T1 and T2), and in the poultry facility of the Department of Veterinary Sciences of the University of Turin, Italy (T3), from October 2020 to June 2021. The challenging conditions were chosen based on ongoing and future global scenarios affecting or potentially affecting the poultry sector:

i. Heat stress is known to significantly reduce the performance, health, and welfare of broiler chickens. Due to global warming, heat stress has become a major issue, threatening poultry producers even in temperate regions.

- ii. One of the main challenges in improving the sustainability of the poultry sector is adopting low-input diets based on local crop production, which are less demanding, less impactful, and more resilient to climate change compared to conventional crops largely imported from non-EU countries at high economic and environmental costs.
- iii. There is an increasing demand for meat from less intensive farming systems (i.e., free-range). However, these systems could pose risks to the health and productivity of birds that do not exhibit sufficient resilience to the nutritional and environmental challenges that characterize extensive production systems.

Five chicken genotypes were used: 2 Italian local breeds, namely, Bionda Piemontese (BP) and Robusta Maculata (RM), and their crossbreed with Sasso T44 hens (SA; a medium growing strain, Hendrix genetics), resulting in Bionda Piemontese \times Sasso ($BP \times SA$) and Robusta Maculata \times Sasso (\mathbf{R} M \times SA), and Ross 308 (Aviagen Group, United Kingdom) used as a control, fast-growing genotype. Data presented in this study only refer to cockerels while females were employed in another experiment as laying hens.

BP and RM are native breeds of the Piedmont and Veneto regions, respectively, and are considered dualpurpose breeds [\(Ferrante et al., 2016](#page-11-16)). The breeders of BP and RM are included in the consortium for the conservation of poultry biodiversity sponsored by the Italian Ministry of Policies Agricultural, Food, and Forestry.

The SA is a meat-type chicken developed in France and commercialised by Hendrix Genetics. It is a strain created by crossing different genotypes of chickens to obtain animals that grow quickly and have a good meat yield. The SA chickens are known for their medium size, tender meat, and medium growth rate, which make them a popular choice for organic meat production. Furthermore, this chicken genotype is commonly used in crossbreeding programs with local breeds because of its recessive plumage characteristics.

The Ross 308 is the world's number 1 genotype of fastgrowing chickens reared for meat production and known for its excellent growth rate and feed efficiency [\(Aviagen,](#page-10-0) [2022\)](#page-10-0).

The eggs of BP were provided by the Avian Conservation Centre for Local Genetic Resources of the University of Turin (Italy), whereas the eggs of RM were provided by Veneto Agricoltura (Padova, Italy). The eggs of the 2 crossbreed genotypes were provided by the University of Perugia (Italy), using roosters of the 2 local breeds and SA hens. All the eggs were incubated at the same time in a local commercial incubator (Monge, Torre San Giorgio, Italy).

One-day-old male chicks were vaccinated against coccidiosis, Marek's disease, infectious bronchitis, and Newcastle disease at the hatchery, and delivered by a commercial authorized track to the experimental poultry facilities of the University of Padova and the University of Turin.

On their arrival, the chicks were individually identified by a leg mark and randomly allocated to the experimental groups (2 replicates, i.e., pens, per group in T1 and T2; 3 replicates per group in T3) according to bi-factorial designs, i.e., T1: 5 genotypes (Ross 308, BP, RM, $BP \times SA$ and $RM \times SA$) and 2 environmental temperatures (thermoneutral vs. high temperature); T2: 5 genotypes (the same used in T1) and 2 diets (standard vs. low-input); T3: 5 genotypes (the same used in T1 and T2) and 2 rearing systems (conventional vs. free-range).

In all trials, the animals were individually weighed on the day of their arrival and then, once a week until commercial slaughtering. Mortality and pen feed consumption were daily measured during the trials. All birds had free access to water and feed.

Due to significant differences in the growth rates between commercial hybrids, local breeds, and their crossbreed with SA, the birds were slaughtered at different ages (42−49 d of age for Ross 308; 82−106 d of age for the other genotypes). Previous studies reporting the growth curve of males and females Ross 308, analysed with the Gompertz model, lasted 81 d [\(Cartoni Manci](#page-11-6)[nelli et al., 2023](#page-11-6)). However, the experimental protocols applied in the present study, involving stressful conditions (i.e., high environmental temperatures, low-input diets and free-range farming) have been considered too challenging for this high productive hybrid. Therefore, Ross 308 chickens were slaughtered at ages commonly applied under commercial conditions and at a maturity rate of approximately (55% of the adult body weight). At the same time, local breeds and crossbreeds were slaughtered once they reached comparable maturity rates (approximately 55%−65% of the adult body weight).

[Table 1](#page-3-0) summarizes the numbers of birds at the beginning of the experimental trials, those that died during the trials, and those included in the final dataset.

Heath Stress—Trial 1

The poultry farm of the University of Padova had 2 identical rooms, both equipped with a cooling system, forced ventilation, radiant heating, and controlled light systems.

A total of 131 2-day-old chickens were housed in 20 pens (1.25 m wide \times 2.60 m long \times 1.20 m height; 3.25 m² total surface; 12 pens per room) with 4 pens/genotype ([Table 1](#page-3-0)). Within the genotype, half of the pens were in a room maintained under thermoneutral conditions and half in a room under high environmental temperatures. Each pen was equipped with automatic nipple drinkers and a circular feeder. The pens had a concrete floor covered with litter made of a 50-50 mixture of wood shavings and chopped wheat straw. Twenty-four h of light were provided during the first 2 d after the chickens

Table 1. Number of chickens at the beginning of the growth trials, died during the trial, and included in the final dataset.

Trial T1 Heat stress	Genotype					Challenge condition	
	Ross 308	BP	$BP \times SA$	RM	$RM \times SA$	Thermoneutral temperature (N)	High temperature (H)
On trial, n.	53	44		18		66	65
Dead, n.							
On dataset, n.	52	42		15		64	61
T ₂ Low-input diets	Ross 308	BP	$BP \times SA$	RM	$RM \times SA$	Standard diet (Std)	Low-input diet (Low)
On trial, n.	42	25	35	35	33	84	86
Dead, n.							
On dataset, n.	37	25	30	33	32	76	81
T3 Free-range	Ross 308	BP	$BP \times SA$	RM	$RM \times SA$	Conventional farming (C)	Free-range farming (F)
On trial, n.	51	75	63	75	63	228	93
Dead, n.	6						
On dataset. n.	39	74	63	74	62	224	88

 $BP =$ Bionda Piemontese; $RM =$ Robusta Maculata; $BP \times SA =$ Cross $BP \times$ Sasso; $RM \times SA =$ Cross $RM \times$ Sasso.

arrived at the poultry house. Then, the hours of light were progressively reduced until a 18L:6D light program was reached, which was maintained from the 12th d onwards. Further details regarding the housing conditions and pen equipment can be found in [Huerta et al.](#page-11-11) [\(2023\).](#page-11-11)

The environmental temperature used in the thermoneutral room was set according to recommendations for broiler chickens until the 5th wk of age ([Aviagen, 2018](#page-11-17)). In the high temperature room artificial heating was set to maintain a higher temperature of 1°C to 2°C during 1st wk and then increasing the difference until 6°C to 7° C, to provoke a moderate heat stress that can often occur in field conditions, but always in the acceptable range of temperature. Then, the average difference in the mean 24-h temperature between the 2 rooms was on average $+4.7^{\circ}$ C, as detailed in [Figure 1](#page-3-1).

Two commercial diets in crumble form, produced by a commercial feed mill (Consorzio Agrario di Treviso e Belluno, Paese, TV, Italy), were fed to the chickens during the trial ([Huerta et al., 2023\)](#page-11-11).

Commercial slaughtering took place at 42 d of age for Ross 308 and at 99 d of age for BP, $BP \times SA$, RM and RM×SA.

Low-Input Diet—Trial 2

A total of 170 chickens were housed in 20 pens (the same of T1) with 4 pens/genotype ([Table 1\)](#page-3-0). The lighting schedule was the same applied in T1.

Within genotype, half of the pens received the standard diet (ME 3,348 kcal/kg; CP 18.5%) and half the low-input diet (ME 3,084 kcal/kg; CP 16.7%) from 21 d of age until slaughtering (47 d for Ross 308 and 106 d for BP , $BP \times SA$, RM and $RM \times SA$). During the first 20 d of age all birds received a commercial starter diet ([Table 2](#page-4-0)). The starter and the standard diets were formulated to meet the energy and protein levels recommended for Ross 308 chickens kept in conventional farming systems ([Aviagen, 2022\)](#page-10-0). Low-input diet was specifically formulated with reduced soybean meal in favour of local ingredients, i.e., faba bean meal and GMO-free organic soybean meal [\(Table 2\)](#page-4-0).

All the diets were pelleted and produced by an industrial feed mill (Cortal Extrasoy S.P.A., Cittadella, Padova, Italy).

The diets were analysed for dry matter (DM, method 934.01), ash (method 942.05), crude protein (CP, method 984.13), crude fibre (CF, method 945.18), and

Figure 1. Values of temperature and relative humidity (average \pm maximum and minimum) recorded in the room maintained under thermoneutral conditions and in the room under high temperature conditions.

¹Slaughtering took place at 47 d for Ross 308 and at 105 d for other genotypes (Bionda Piemontese, Bionda Piemontese \times Sasso, Robusta Maculata and Robusta Maculata \times Sasso).

²Values estimated according to chemical composition of the diet ([Carr](#page-11-19)é et al., 2013).

 3 Values calculated through an *in vivo* digestibility trial carried out from 34 to 40 d of age.

ether extract (EE, method 2003.05) according to [AOAC](#page-10-1) [\(2000\)](#page-10-1) methods. The gross energy contents of the diets were measured using an adiabatic bomb calorimeter (IKAC200, Staufen, Germany). All analyses were performed in triplicate.

From 34 to 40 d of age samples of faces were collected from each pen, dried, and analysed for DM, CP, EE, gross energy, and acid-insoluble ash (AIA) content. AIA was used as a marker to determine the apparent digestibility coefficient (ADC) of the dietary DM, CP, EE, and to determine the apparent metabolizable energy level.

Free range—Trial 3

A total of 370, 1-day-old chicks were moved from the hatchery to the poultry facility of the University of Turin and reared until reaching 20 d of age in the brood, which was divided into 5 pens (1 per genotype).

The pens were 1 m wide and 2 m long, with net walls and a waterproof floor covered with wood shavings as litter (20 cm high). The brood was environmentally controlled, with temperature and relative humidity ranging from 32°C to 20°C and from 70% to 65%, respectively. The lighting schedule was set at 23L:1D until d 3 and thereafter the dark period was gradually increased up to reach 6 h/d . The environmental parameters were daily monitored during the whole the trial. During the first 20 d, all birds received the same starter diet of T2 [\(Table 2\)](#page-4-0).

At 21 d of age, 321 chicks were individually labelled with a wing mark, and allotted to the 2 different farming systems (conventional, until 33 kg live weight per m^2 vs. free-range, until 21 kg live weight per m^2) with 3 replicate pens per treatment for a total of 30 pens ([Table 1](#page-3-0)). To ensure similar stocking densities at slaughter, given the expected weight variations among genotypes, different numbers of chicks were allocated per pen. In the conventional farming system, 10, 18, and 15 chicks were

assigned per pen for Ross 308, local breeds (BP and RM), and crossbreeds (BP \times SA and RM \times SA), respectively. For the free-range farming system, 7, 10, and 9 chicks were allocated per pen for Ross 308, local breeds, and crossbreeds, respectively.

In the conventional system, a 18L:6D light program was maintained throughout the trial. Temperature and relative humidity in the poultry house were set according to Aviagen guidelines [\(Aviagen, 2018](#page-11-17)).

In the free-range system, the poultry house was divided into an indoor and an outdoor area: the indoor pens were equipped with wood shavings as litter and the final stocking density was set at 21 kg/m^2 ; outdoor the animals had 1 m^2 available surface per animal, according to [Commission Regulation \(EC\) No 543/2008 \(2008\)](#page-11-18). Moreover, the birds were exposed to natural temperature and photoperiod. The mean temperature in the poultry house during daylight hours was $21.0 \pm 5.0^{\circ}$ C, whereas during night it was $12.0 \pm 3.0^{\circ}$ C. The mean hours of daylight during the trial were 14 h/d. The animals were free to stay either outside or inside; the outdoor area was protected from wild birds and predators with fences.

The same batch of diets used in T2 were used in T3 ([Table 2\)](#page-4-0). Specifically, from 21 d until slaughter, birds in the conventional system received the standard diet, whereas those in the free-range system received the lowinput diet.

At 42 d of age, all Ross chickens in the conventional system were slaughtered. In the free-range system, 2 birds per replicate $(n = 6)$ were selected based on average live weight and slaughtered. All remaining birds were slaughtered at 82 d of age in both farming systems.

Statistical Procedure

The data set was checked to verify that the final body weight (BW) of birds, within each genotype and

Figure 2. Effects of the changes in Gompertz model coefficients $(A = upper$ asymptote or adult weight; $B =$ parameter of the curve describing the proportion of the asymptotic adult weight to be gained after birth; $k =$ the instantaneous relative growth rate). The direction of the grey curve indicates an improvement in the parameter from a production perspective. An increase in the value of A and k indicates an increase in adult weight and precocity, respectively, while a shift of the curve to the left is indicated by lower values of B.

treatment, did not exceed the average value ± 2.5 standard deviations. No outliers were found.

The Gompertz model, as described by [Gonz](#page-11-15)a[lez Ariza](#page-11-15) [et al. \(2021a\)](#page-11-15) and [Cartoni Mancinelli et al. \(2023\)](#page-11-6) was used to analyse the growth curve of each animal according to the following equation:

 $Y = A * EXP(-B * EXP(-k * t))$

where Y is the BW reached at age t; \bf{A} is the upper asymptote or adult weight; t is the time; \bf{B} is the parameter of the curve describing the proportion of the asymptotic adult weight to be gained after birth; and k is the instantaneous relative growth rate. High k values indicate early maturing animals. The effects of differences in the parameters of the Gompertz model are illustrated in [Figure 2.](#page-5-0)

Moreover, the following parameters were calculated: the time taken to reach 50%, 70%, and 99% of adult BW $(T_{50}, T_{70}, \text{and } T_{99}, \text{respectively}),$ the BW at the inflection point (BW_{ip}, g) obtained as $BW_{ip} = A/e$, the age at inflection point (t, d) obtained as $t_{ip} = (\ln[b])/k$, and the maximum growth rate (MGR) or growth rate at the inflection point (g/d) obtained as $MGR = BW_{\text{in}} \times k$ ([Gonz](#page-11-20)a[lez Ariza et al., 2021b](#page-11-20)).

Diagnostic charts were used to verify assumptions; A and MGR parameters were ln transformed before subsequent analyses to improve their distribution. Differences among genotypes were evaluated by Generalized Linear Models (GLM) using normal as probability distributions and Identity as link function. Curve parameters and their derivatives were included as dependent variables. A first, broad evaluation was made to estimate the effect of the genotype in the parameters of the curve regardless of the challenging condition; thus, only the genotype (5 levels: BP, BP \times SA, RM, RM \times SA, Ross 308) was included in these GLM. Then, for each challenging condition, the GLM evaluated the effect of genotype (5 levels: BP, BP \times SA, RM, RM \times SA, Ross 308), the challenging condition (2 levels: thermoneutral vs. high temperature in T1; standard diet vs. low-input diet in T2; conventional farming vs. free-range farming in T3), and their interaction. Sidak adjustment was used for pairwise comparisons.

GraphPad Prism, version 8.0 (GraphPad Software, SanDiego, CA) was used to build growth curves while SPSS Statistics version 25 (IBM, SPSS Inc., Chicago, IL) to analyze the parameters. The level of statistical significance was set at $P < 0.05$.

RESULTS

Effect of Genotype

In all trials the commercial hybrids showed the highest A (5022 g), k (0.05), BW_{ip} (1848 g), and MGR (87.9 g/d , while exhibiting the lowest T50 (40 d), T70 (54 d), T99 (130 d), and t_{ip} (34 d) values ($P < 0.05$; [Supplemen](#page-10-2)[tary Table SM1\)](#page-10-2). Between local breeds, RM chickens showed higher A $(+46.8\%)$, BW_{ip} $(+44.9\%)$ and MGR $(+21.4\%)$ values compared to BP ($P < 0.05$). However, the k parameter was lower in RM chickens (-18.8%) therefore requiring longer periods to reach the inflexion point $(+15 d)$, as well as to reach 50%, 70% and 99% of the adult body weight $(+18, +23, +50, d)$, respectively) compared to BP birds ($P < 0.05$). The crossbreeding was effective in increasing A $(+14.3\%$ on average), BW_{ip} $(+15.3\%$ on average), and MGR $(+25.8\%$ on average) of local breeds, especially when BP was crossed $(A = +30.5\%, BW_{ip} = +30.5\%, MGR = +33.5\%; P <$ 0.05). On the other hand, the crossbreeding with SA improved the precocity of RM, with $RM \times SA$ chickens showing higher k $(+7.7\%)$ and lower T50 (-11 d) , T70 (-15 d) , and T99 (-36 d) compared to RM chickens (*P* < 0.05).

Effect of Heat Stress

Under heat stress conditions, the values of A , BW_{ip} , and MGR decreased $(-15.2\%, -15.2\%, \text{ and } -13.4\%, \text{)}$ respectively; $P < 0.001$; [Supplementary Table SM2](#page-10-2)), while k increased $(+6.7\%; P < 0.01)$. A significant interaction "Genotype \times Temperature" was observed for almost all growth curve parameters ($P < 0.05$; [Supple](#page-10-2)[mentary Table SM3](#page-10-2)). Indeed, the growth parameters of the local breeds and crossbreeds did not differ under heat stress and thermoneutral conditions ([Figures 3 and](#page-6-0) [4](#page-6-0)).

In contrast, Ross 308 chickens exhibited a higher value of k with high environmental temperatures compared to standard conditions, while A and MGR

Figure 3. Effect of heat stress on the Gompertz growth curves of local breeds (BP = Bionda Piemontese; RM = Robusta Maculata; Panel A), crossbreeds $(BP \times SA = Bionda$ Piemontese \times Sasso; $RM \times SA$ = Robusta Maculata \times Sasso; Panel B), and commercial hybrids (Ross 308; Panel C).

worsened $(P < 0.001)$. In detail, the A of Ross 308 decreased by 37.3%; it did not differ from those of $BP \times SA$ and RM and it was lower than those of $RM \times SA$ ([Figure 4A](#page-6-1)). At the same time, the k value of Ross 308 increased by 20.0% while its MGR was reduced by 21.9% [\(Figures 4B](#page-6-1) and [4C](#page-6-1)). Within the genotype, the environmental temperature did not affect the B parameter while T50, T70, T99 and t_{ip} were reduced with elevated temperatures $(-8, -10, -24, \text{ and } -6 \text{ d},$ respectively) only in the Ross 308 ($P < 0.05$; [Supplemen](#page-10-2)[tary Table SM3\)](#page-10-2). At the same time, BW_{ip} decreased in commercial hybrids with increasing environmental temperature $(-37.3\%; P < 0.001)$.

Effect of Low-Input Diet

The low-input diet significantly affected all the growth curve parameters by reducing A $(-11.1\%; P \le$ 0.001), B $(-5.5\%; P < 0.001)$, k $(-14.3\%; P < 0.01)$, BWip $(-11.1\%; P < 0.001)$, and MGR $(-20.0\%; P < 0.001)$ 0.001), while increasing T50, T70, T99, and t_{in} (+7 d on average; $P < 0.05$) with respect to the standard diet ([Supplementary Table SM2](#page-10-2)). In particular, the Gompertz growth curve of Ross 308 chickens significantly

Figure 4. Effect of interaction "Genotype \times Temperature" on A (adult weight/ upper asymptote; Panel A), k (maturity of animals; Panel B) and the maximum growth rate (MGR; Panel C) of local breeds (BP = Bionda Piemontese; RM = Robusta Maculata), crossbreeds ($BP \times SA =$ Bionda Piemontese \times Sasso; RM $\times SA =$ Robusta Maculata \times Sasso), and commercial hybrids (Ross 308). Different letters over the bars denote significant differences among means ($P \leq$ 0.05).

worsened ([Figure 5;](#page-7-0) A and MGR -35.4% and -38.7% , respectively). In contrast, only minor and nonsignificant changes were observed in A and MGR of local breeds and crossbreeds [\(Figure 6](#page-7-1)).

Precocity (i.e., k parameter), as well as almost all growth curve parameters, significantly changed according to the interaction "Genotype \times Diet" ($P < 0.05$; [Sup](#page-10-2)[plementary Table SM4\)](#page-10-2). In detail, pairwise comparisons revealed that with the low-input diet, the k parameter was reduced only for BP and Ross 308 chickens $(-33.3\%$ and -25.0% , respectively); both B and BW_{ip} decreased only in the Ross $308 (-14.9\% \text{ and } -35.4\%, \text{ respectively})$ tively); T50, T70, and T99 were delayed in BP and RM 8 MENCHETTI ET AL.

Figure 5. Effect of low input diet on the Gompertz growth curve of local breeds (BP = Bionda Piemontese; RM = Robusta Maculata; Panel A), crossbreeds $(BP \times SA = Bionda$ Piemontese \times Sasso; $RM \times SA$ = Robusta Maculata \times Sasso; Panel B), and commercial hybrids (Ross 308; Panel C).

 $(+10, +14, \text{ and } +37 \text{ d}$ on average, respectively), while they did not change in the other genotypes [\(Supplemen](#page-10-2)[tary Table SM4](#page-10-2)). Accordingly, the t_{ip} increased in BP and RM chickens $(+8 d)$ on average), whereas nonsignificant variations were recorded in crossbreeds and commercial hybrids fed the low-input diet.

Effect of Free-Range System

The free-range system significantly affected all the growth curve parameters of local breeds, crossbreeds, and commercial hybrids $(P < 0.001)$, except for MGR ([Supplementary Table SM2](#page-10-2)). On average of the different genotypes, A, B and BW_{ip} increased by $24.7\%, 3.4\%,$ and 22.7%, respectively, compared to the conventional system. At the same time, in the free-range system T50, T70, T90, and t_{in} increased by 16, 22, 50, and 14 d, respectively.

A significant interaction "Genotype \times Farming system" was found for all the Gompertz growth curve parameters ($P < 0.001$; [Supplementary Table SM5](#page-10-2)). The free-range system increased the growth curve in the

Figure 6. Effect of interaction "Genotype \times Diet" on A (adult weight/ upper asymptote; Panel A), k (maturity of animals; Panel B) and the maximum growth rate (MGR; Panel C) of local breeds (BP = Bionda Piemontese; RM = Robusta Maculata), crossbreeds $(BP \times SA =$ Bionda Piemontese \times Sasso; RM $\times SA =$ Robusta Maculata \times Sasso), and commercial hybrids (Ross 308). Different letters over the bars denote significant differences among means ($P \leq$ 0.05).

local breeds and crossbreeds [\(Figures 7](#page-8-0)A and [7B](#page-8-0)), whereas it decreased the growth curve of Ross 308 birds ([Figure 7C](#page-8-0)).

Compared to the conventional system, the free-range increased A in BP $(+42.0)$, BP \times SA $(+37.9%)$, and RM (+102%) ([Figure 8A](#page-9-0); [Supplementary Table SM5\)](#page-10-2); it reduced the k parameter in BP (-33.3%) and RM (-33.3%; [Figure 8](#page-9-0)B), while increasing MGR in both local breeds (+14.4% on average) and crossbreeds $(+7.3\%$ on average; $P < 0.05$; [Figure 8](#page-9-0)C). In contrast, Ross 308 reared in free-range exhibited reduced A (-20.2%) and MGR $(-16.0\%).$

Regarding the other parameters, B and BWip increased in BP, BP \times SA, and RM genotypes (+8.6% and +56.2% on average, respectively) when reared in

Figure 7. Effect of free-range farming on the Gompertz growth curve of local breeds (BP = Bionda Piemontese; RM = Robusta Maculata; Panel A), crossbreeds (BP \times SA = Bionda Piemontese \times Sasso; $RM \times SA$ = Robusta Maculata \times Sasso; Panel B), and commercial hybrids (Ross 308; Panel C).

free-range, while they did not change in $RM \times SA$. Accordingly, BP , $BP \times SA$, and RM genotypes required longer periods to reach 50% (T50 = $+27$ d on average), 70% (T70 = +36 d on average), and 99% (T99 = +82 d) on average) of the adult body weight, as well as to reach the inflection point $(t_{ip} = +23$ d on average) when reared in the free-range compared to the conventional system ($P < 0.05$; [Supplementary Table SM5\)](#page-10-2). Conversely, the free-range system reduced B and BW_{ip} in Ross 308 (-6.9% and -20.2% , respectively; $P < 0.05$).

DISCUSSION

The present work introduces, for the first time, the use of the Gompertz model for evaluating the resilience of different chicken genotypes to environmental and nutritional challenges.

Indeed, it is well-established that regardless of the specific stressor, the physiological response to chronic stress primarily results in a reduction of fitness and compromised productive performances in all farm animals ([Etim et al., 2013\)](#page-11-21), including poultry ([Akinyemi and](#page-10-3) [Adewole, 2021\)](#page-10-3). The Gompertz model comprehensively describes the birds' entire growth pattern [\(Rizzi et al.,](#page-11-22) [2013\)](#page-11-22), providing valuable insights for assessing their adaptability across diverse rearing conditions. This includes details such as the speed and trend of growth, age at maturity, and the optimal age for slaughter ([Gonz](#page-11-15)a[lez Ariza et al. 2021a\)](#page-11-15). Our methodological approach is in accordance with the study of [Boonkum et](#page-11-23) [al. \(2021\),](#page-11-23) who found that Gompertz traits, associated with the Temperature Humidity Index, are suitable tools for comparing the effect of heat stress in native or synthetic slow-growing chicken lines and for predicting selection strategies.

Native chicken breeds typically demonstrate greater resilience compared to commercial hybrids in coping with environmental challenges. Specifically, when exposed to elevated temperatures, local breeds exhibited lower levels of physiological stress indicators [\(Soleimani](#page-12-5) [et al., 2011\)](#page-12-5), and experienced less reduction in daily weight gain and final body weight compared to commercial hybrids [\(Lu et al., 2007](#page-11-24); [Huerta et al., 2023](#page-11-11)). Several authors have also reported that the decrease in body weight in commercial hybrids subjected to heat stress was accompanied by a worsened feed efficiency ([Zhang](#page-12-6) [et al., 2017](#page-12-6); [Goo et al., 2019](#page-11-25)). The results of the current study support the resilience of local breeds and crossbreeds to environmental temperatures out of the thermoneutral range, as indicated by the absence of significant changes in their growth curve parameters. Differently, multiple comparisons indicated that elevated temperatures decreased A and MRG while increasing the precocity of Ross 308. A negative correlation between the A and k parameters was previously documented by [Mignon-Grasteau et al. \(2000\)](#page-11-26), elucidating how a swift decrease in growth rate after the inflection point resulted in a diminished asymptotic body weight.

The higher thermotolerance observed in local breeds, in contrast to commercial hybrids, can be primarily attributed to their lower body weight ([Soleimani et al.,](#page-12-5) [2011](#page-12-5)). Indeed, [Gogoi et al. \(2021\)](#page-11-3) found that, when comparing birds of the same genetic line and age, the physiological response to heat stress becomes more severe as the weight of broilers increases. Moreover, while genetic selection has led to significant advancements in the growth and muscle development of broiler chickens over the years, it has not concurrently improved the physiological function of the thermoregulatory system ([Onagbesan et al., 2023](#page-11-27)). Consequently, fast-growing chickens display increased susceptibility to heat stress compared to slow-growing lines, partly due to their elevated metabolic rates and underdeveloped cardiovascular and respiratory systems [\(Bruga](#page-11-28)[letta et al., 2022\)](#page-11-28).

The significant effect of the Genotype \times Diet interaction on all the growth curve parameters indicated that different genotypes also responded differently to nutritional challenges. Thus, while a low-input diet did not significantly influence the curve parameters of local

Figure 8. Effect of interaction "Genotype \times Farming system" on A (adult weight/ upper asymptote; Panel A), k (maturity of animals; Panel B) and the maximum growth rate (MGR; Panel C) of local breeds (BP = Bionda Piemontese; RM = Robusta Maculata), crossbreeds (BP \times SA = Bionda Piemontese \times Sasso; RM \times SA = Robusta Maculata \times Sasso), and commercial hybrids (Ross 308). Different letters over the bars denote significant differences among means ($P \leq$ 0.05).

breeds and crossbreeds, a notable worsening of growth was observed in Ross 308. The observed impairment in the main growth curve parameters and derived indexes with the low-input diet could be primarily attributed to the reduced protein and apparent metabolizable energy levels (-11% and -8%, respectively). In particular, the crude protein content of the low-input diet was approximately 15% lower than the requirements of Ross 308 broilers ([Aviagen, 2022](#page-10-0)). Therefore, the most productive genotype (Ross 308), which has the highest dietary nutrient requirements, experienced greater challenges (reduced A and MGR) with the use of the low-input diet

compared to the less productive chickens (local breeds and their crosses with SA). Previous studies report conflicting results on the effect of low-input diets, probably due to the different compositions of the feeds. When soybean was substituted with faba bean (20% dietary inclusion) and other legumes (peas and blue sweet lupins at approximately 10% and 28% dietary inclusion, respectively) in iso-protein diets, no adverse effects were observed on the final body weight and carcass traits of local breeds, high-performance genotypes, and their crosses ([Nolte et al., 2020](#page-11-29)). However, including faba beans at 16% in the diet from the starting phase until slaughtering can lead to reduced daily weight gain and carcass weight, even in slow-growing genotypes ([Dal](#page-11-30) [Bosco et al., 2013](#page-11-30)). Indeed, when high dietary levels (>20%) are utilized, the presence of glycosides and tannins, known to reduce the digestibility of protein and apparent metabolizable energy (Vilariño et al., 2009; [Woyengo and Nyachoti, 2012](#page-12-8)), along with a suboptimal amino acid profile of faba bean [\(Usayran et al., 2014](#page-12-9)), may contribute to the lower performance of broiler chickens. In a companion paper using the same experimental plan, the apparent digestibility coefficients of dry matter, crude protein, and gross energy were observed to decrease with the administration of the lowinput compared to the standard diet [\(Birolo et al.,](#page-11-31) [2023\)](#page-11-31), even though the experimental diets were used from 21 d of age onward, and the dietary inclusion of faba bean was restricted to 16%.

Finally, the analysis of the Gompertz growth curves demonstrated that free-range farming also has a different impact on the growth of the fast-growing birds and the other evaluated genotypes (as indicated by the significant effect of the interaction between the genotype and the farming system and by multiple comparisons). Free-range farming reduced the MGR Ross 308 chickens, representing a too-demanding condition for them. Conversely, the values of A and MRG even increased in the local breeds and crossbreeds, showcasing their remarkable adaptability to the alternative rearing system. The BP and RM breeds, along with their crossbreeds with SA hens, demonstrated thus a good adaptation to outdoor space and grass resources.

These findings agree with previous studies that have also compared body weight with other welfare indicators. Applying a multitraits adaptability index that considered behaviour, tonic immobility, feather condition, body lesions, and physiological indicators to different chicken genotypes (fast-growing, medium-growing, and slow-growing), [Castellini et al. \(2016\)](#page-11-32) showed that the adaptability of broilers to outdoor environments and organic farming diminishes with increasing growth rate. Indeed, daily weight gain has been identified as a moderately accurate related predictor for evaluating broilers' adaptability to organic farming, suggesting its potential use to discourage the rearing of fast-growing genotypes in alternative rearing systems ([Cartoni Mancinelli et al.,](#page-11-5) [2021\)](#page-11-5).

According to [Fiorilla et al. \(2022\)](#page-11-33), Ross 308 broilers reared in a free-range system (open environment and low-input diet) exhibit reduced productive performance, increased mortality, and compromised welfare. In contrast, local chicken breeds and crossbreeds can achieve similar final live weights and daily weight gains under both conventional and free-range farming conditions ([Fiorilla et al., 2023](#page-11-34)).

Robustness and health play pivotal roles in alternative poultry production systems, focusing on the bird's capacity to respond to environmental stressors such as temperature fluctuations, low-input diets, and freerange farming. While local breeds possess this ability, as demonstrated by the elasticity of their growth parameters measured under various challenging conditions, they also exhibit lower body weight and/or delayed precocity compared to commercial birds [\(Bilalissi et al.,](#page-11-35) [2022\)](#page-11-35). In this context, the practice of crossbreeding emerges as a valuable strategy for enhancing the productive as well as the reproductive capacities of native chicken breeds ([Taye et al., 2022](#page-12-10)). In this study, the crossbreeding of local male chickens with SA hens proved to be particularly effective in enhancing the precocity of the heaviest breed (RM) and increasing the values of A and MGR in the lightest breed (BP). Indeed, the SA breed is notable for its considerable adult body weight (around 3,000 g) and displays precocious characteristics similar to those observed in the Ross 308, making it an optimal candidate for enhancing local chicken breeds [\(Cartoni Mancinelli et al., 2023](#page-11-6)). Furthermore, various studies have consistently utilized SA chickens to produce crossbreeds with superior growth rates and carcass traits compared to pure lines [\(Alemneh et al., 2021](#page-10-4); [Bilalissi et al., 2022](#page-11-35)).

A limitation of the present study is that the growth curves of different genotypes were constructed using diverse time frames due to their varying slaughter ages (specifically, the Ross 308 were slaughtered earlier than the others). This can represent a bias in the calculation of the parameters, especially for parameter A, which represents an asymptotic value. However, if it exists, the error for the Ross 308 is systemic and does not affect the validity of the comparison between standard and challenging conditions.

CONCLUSIONS

The current study evaluated the resilience of local chicken breeds, crossbreeds, and commercial hybrids when exposed to stressors and challenges commonly encountered in alternative poultry farming systems. The findings indicate that the Gompertz model is an effective tool for assessing bird adaptability, as it offers insights into the entire growth curve. This makes the model valuable for guiding decision-making processes when choosing the suitable genotype for diverse scenarios, including high temperatures, limited availability of protein and energy raw materials, uncontrolled environmental conditions, and the need to forage in outdoor areas. The methodological approach has thus practical implications for poultry farming, helping farmers choose the genotype that promises greater productivity under different farming systems while respecting animal welfare.

The analysis of the parameters of the Gompertz model indeed showed that challenging conditions worsened the growth of Ross chickens while not influencing, or even improving (in the case of free-range), the performance of local breeds and crossbreeds. This raises concerns about the adaptability of fast-growing genotypes to alternative farming systems, emphasizing the potential advantages of local chicken breeds and their crosses, which may exhibit better thermotolerance and resilience to dietary and environmental changes. Finally, it reaffirms crossbreeding as a valuable strategy to enhance the growth potential of native breeds, preserving their resilience to various challenges and offering additional opportunities for the conservation of local chicken biodiversity.

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DISCLOSURES

The authors of the original research article entitled "Effect of genotype and nutritional and environmental challenges on growth curve dynamics of broiler chickens" Laura. Menchetti, Marco Birolo, Cecilia Mugnai, Alice Cartoni Mancinelli, Gerolamo Xiccato, Angela Trocino, Cesare Castellini have no conflicts of interest to disclose.

SUPPLEMENTARY MATERIALS

Supplementary material associated with this article can be found, in the online version, at $\frac{d}{dt}$ [doi:10.1016/j.](https://doi.org/10.1016/j.psj.2024.104095) [psj.2024.104095.](https://doi.org/10.1016/j.psj.2024.104095)

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