Robustness to chronic heat stress in laying hens: a meta-analysis.

Sandrine Grasteau, Utlwanang Moreri, Agnès Narcy, Xavière Rousseau, T. B. Rodenburg, Michèle Tixier-Boichard, Tatiana Zerjal

Robustness to chronic heat stress in laying hens: a meta-analysis

S. Mignon-Grasteau,[*,1] U. Moreri,[*,†,‡] A. Narcy,[*] X. Rousseau,[*] T. B. Rodenburg,[§] M. Tixier-Boichard,[#] and T. Zerjal[#]

[*] *UR083 Recherches Avicoles, INRA, 37380 Nouzilly, France;* [†] *WUR Animal Breeding and Genomics Group, P.O. Box 338, 6700 AH, Wageningen, the Netherlands;* [‡] *AgroParisTech UFR Génétique Elevage et Reproduction, 75231 Paris cedex 05, France;* [§] *WUR Behavioural Ecology Group, P.O. Box 338, 6700 AH, Wageningen, the Netherlands; and* [#] *UMR1313 GABI, INRA, Domaine de Vilvert, 78352 Jouy-en-Josas cedex, France*

ABSTRACT Chronic heat is a major stress factor in laying hens and many studies on the effect of heat stress have been published. It remains difficult, however, to draw general conclusions about the effect of chronic heat stress on performance and its relationship with genetic and environmental factors, as these studies have been done under varying experimental conditions and using various experimental designs. A meta-analysis enabled us to make a quantitative review of the results from 131 published papers. The relative effects of four factors (genotype, age, group size, and amplitude of temperature variation) and their interactions with temperature were analyzed for 13 traits. After pre-correcting the data for a random study effect, the best model for each trait was selected in a step-wise procedure based on its residual sum of squares. Shell strength, daily feed intake, egg mass, and hen-day egg production were found to be more sensitive to heat stress than the other traits as they dropped by 9.0 to 22.6% between thermo-neutrality (15 to 20°C) and heat stress (30 to 35°C) while yolk and albumen proportions or Haugh units showed nearly no variation with temperature (<1.2% between thermo-neutrality and heat stress). Many interactions (17) were found between temperature and one or more factors in the 13 traits studied here, which reinforces the interest of using a meta-analysis to summarize data from the literature. This study highlighted that the impact of heat stress in laying hens depends on the genotype, age, and group size, some of which have rarely been investigated.

Key words: heat stress, layer, meta-analysis, egg production, egg quality

2015 Poultry Science 00:1–15
http://dx.doi.org/10.3382/ps/pev028

INTRODUCTION

The economic losses in poultry production due to heat stress are important both for tropical areas, where mean ambient temperatures frequently exceed 30°C (Al-Saffar and Rose, 2002; Tan et al., 2010), and for temperate countries exposed to recurrent summer heat waves (COPA/COGECA report, 2003). For example, in the USA, heat has been estimated to increase layers mortality by 0.03 to 0.96% and to decrease egg production by 0.5 to 7.2%, leading to a global yearly economic loss of $98.1 million (Saint-Pierre et al., 2003). Layers are particularly vulnerable to heat stress because they have to maintain a long production cycle (50 to 70 weeks). Besides the effect on egg quantity, heat stress also decreases egg quality (Balnave and Muheereza, 1997; Al-Saffar and Rose, 2002), reproductive efficiency (Novero et al., 1991), and efficiency of the immune response (Bollengier-Lee et al., 1998; Al-Saffar and Rose, 2002).

Heat stress is caused by a combination of high environmental temperature, high humidity and low air velocity (Yahav et al., 2004; Balnave and Brake, 2005). The chicken's thermoregulatory mechanisms to avoid heat stress are normally activated above 24°C, i.e., above the 18 to 24°C thermoneutral zone (Celik et al., 2004; Etches et al., 2008), but St-Pierre et al. (2003) showed that this limit can be lower depending on air humidity. Heat stress effects become noticeable when temperatures exceed 30°C (Çiftçi et al., 2005; Seven, 2008) and are accentuated by the fact that chickens cannot dissipate heat efficiently because of the insulating property of feathers and the lack of sweat glands.

Apart from the intensity and duration of the heat stress itself, several factors affect a bird's sensitivity to high temperatures. The most frequently reported factors are the age of the bird, the cyclic variations of the temperature, and genotype. For example, egg shape is less affected by high temperature in old than in young hens (Tůmova and Gous, 2012) but laying intensity is more affected by a severe heat stress (37°C) at the end than at the beginning of the laying period (Bordas and Mérat, 1992). Cyclic variations of temperature reduce the effect of heat stress by providing

Received June 19, 2014.
Accepted October 29, 2014.
[1]Corresponding author: sandrine.grasteau@tours.inra.fr

recovery periods for birds during the cooler periods (Mashaly et al., 2004). Finally, regarding genotype effects, the most productive genotypes are more affected than the less productive ones, and the brown egg genotypes, which are heavier and more feathery, are more affected than the white egg ones (Franco-Jimenez et al., 2007; Melesse et al., 2011).

Despite the large number of studies on chicken heat stress, predictors for heat tolerance are not yet easily accessed. It is difficult to synthesize the results from multiple studies because of the heterogeneity of the experimental conditions, especially regarding age, genotype, intensity of heat stress, and cyclic variation of temperature during stress. To overcome this difficulty, we performed a meta-analysis of data collected from a large number of published studies evaluating the main effects of heat stress on laying hens to produce a quantitative synthesis of the literature results.

MATERIALS AND METHODS

Literature Search and Paper Filtering Criteria

A detailed search of studies from 1970 to 2013 was carried out in the ISI Web of Science and PubMed through key word searches and cited references of other published papers. Keywords and search strings included terms related to robustness (robustness, tolerance, and resistance), heat stress (heat stress, high ambient temperature, high environmental temperature, etc.), laying hens (laying hen, layer, chicken or poultry), genotype by environment interaction (genotype by environment interaction or genotype-environment interaction), and egg production (egg production, egg quality, laying rate or performance).

The numerous papers obtained by this search were then filtered before inclusion in the database. Papers were not eligible for inclusion if: 1) they studied other poultry species like quail or meat-type genotypes such as broiler breeder hens, 2) they were conference proceedings without details on results or on conditions, 3) they were literature reviews with no original data, 4) they did not specify the genotype, the age of the hens, the daily amplitude of temperature variation or the group size, 5) the experiment was performed under one temperature, 6) the experimental temperatures were below 15°C or above 35°C, 7) the exposure time was too short to be considered as long-term (less than one week), and 8) the parameters recorded, even if interesting, were too scarce and rarely measured (e.g., heat shock proteins).

After the filtering step, the final database included data collected from 131 papers with 99 to 1,335 data points per trait (see Appendix). Because the raw data from each study was not available, the final database was made up of the mean values for each trait per heat treatment as summarized in tables and graphs for the retained papers. Similarly, in most cases standard errors were not reported for each trait, as such, in order to account for variability in the mean estimates due to differences in sample sizes among studies, we instead estimated the number of independent statistical units (**NIS**) and included them in the database. For example, in a study presenting the results obtained during 2 weeks of egg collection, with an egg production rate of 50%, and 32 hens in each heat temperature group, reared in cages of 2 hens with a common feeder for 2 cages, we counted 32 independent samples for body weight, 16 for egg production rate and egg mass, 8 for feed intake and feed conversion ratio, and 224 for egg weight (i.e., 14 d × 32 hens × 50% egg production rate). The detailed description of the database and the complete list of references included can be found as supplementary data.

Database Recording

Parameters recorded included minimum and maximum daily temperatures, and mean daily temperature (**T**). We also recorded genotype (**GEN**) in 3 categories: commercial white birds (**CW**, 48.8% of final data), commercial colored birds (**CC**, 24.5%), and experimental lines or indigenous flocks (**EXP**, 26.7%). To maintain a sufficient number of data per category, it was not possible to define more detailed categories of genotypes. We note that EXP represented a heterogeneous group, as it included both local breeds, generally less productive under standard conditions but well adapted to local conditions, as well as experimental lines selected on a single trait (as opposed to commercial lines selected on wider number of traits) and characterized by a small effective population size. Although more homogeneous, the CC and CW groups exhibited pronounced variability as they included several different commercial lines issued from selection programs over the time span included in the database (1970 to 2014). Group size (**GS**), representing single versus collective rearing (56.9% and 43.1% of the cases, respectively) was also included in the database. For all traits recorded between X and Y weeks of age of hens, we attributed a mean age value (**A**; in weeks) corresponding to the mean of X and Y. The mean hen age in the database was 39.6 wk, with 25.8% of data coming from hens younger than 30 wk, 36.1% from 30 to 40-wk-old hens, 17.1% from 40 to 50-wk-old hens, 11.4% from 50 to 60-wk-old hens, and 9.6% from hens older than 60 wk. Finally, daily amplitude of temperature variation (Δ) was calculated as the difference between maximum and minimum temperatures. In the database, 64% of the data arose from studies with no amplitude of variation or variation less than 3°C. For the remaining studies, the daily variation of temperature averaged 9.5°C.

Traits included in the database were hen-day egg production rate per flock (**EPR**; in %), egg weight (**EW**; in g), egg mass (**EM**; in $g.d^{-1}$), daily feed intake (**FI**; in $g.d^{-1}$), feed conversion ratio (**FCR**) calcu-

Table 1. Summary statistics for each trait.

Trait[1]	Unit	N	Mean	Std	Minimum	Maximum
FI	$g.d^{-1}$	1097	99.55	19.90	23.20	162.00
EPR	%	1323	76.18	15.03	18.00	100.50
EW	g	1335	56.04	6.11	37.60	72.00
EM	$g.d^{-1}$	1281	43.07	10.39	10.40	70.38
FCR	$g.g^{-1}$	881	2.355	0.569	1.150	4.990
HU	-	254	81.49	10.84	48.72	104.53
ST	mm	307	0.3565	0.0387	0.2670	0.4900
SS	g	99	3199	704	1958	4895
YP	%	170	28.19	2.27	24.08	34.99
AP	%	138	61.82	2.56	54.61	67.01
SP	%	251	9.736	1.507	69.24	14.31
MORT	$\%.wk^{-1}$	159	0.5310	0.9198	0.00	6.78
BWc	$\%.wk^{-1}$	529	−0.3483	2.5671	−14.9511	19.7880

[1]FI: feed intake; EPR: egg production rate; EW: egg weight; EM: egg mass; FCR: feed conversion ratio; HU: Haugh units; ST: shell thickness; SS: shell strength; SP: shell proportion; YP: yolk proportion; AP: albumen proportion; MORT: mortality rate; BWc: relative weekly body weight change.

lated as the ratio of feed consumption to egg mass (in $g.g^{-1}$), Haugh units (**HU**), shell thickness (**ST**; in mm), shell strength (**SS**; in g), shell proportion (**SP**; in %), yolk proportion (**YP**; in %), albumen proportion (**AP**; in %), body weight (g), and mortality (%). Weekly mortality rate (**MORT**) was calculated by dividing the total mortality during the experiment by the length of the experiment (in weeks). We calculated a relative weekly body weight change ($\mathbf{BW_c}$) during heat exposure as:

$$BW_c = 100 \times \left(1 - \left[\frac{BW_2}{BW_1}\right]^{\left(\frac{1}{T_2 - T_1}\right)}\right),$$

where BW_1 and BW_2 were body weight at the beginning and at the end of the heat stress period, respectively, and T_1 and T_2 the hens' ages (in weeks) at the beginning and at the end of the heat stress period, respectively.

Summary statistics for these traits are presented in Table 1.

Statistical Analysis

Egg production rate, mortality, body weight change, and shell proportions were expressed in percentages, which were below 20% or above 80% and were, thus, transformed by arcsine square root, while FCR was log-transformed before analysis. The corresponding abbreviations for transformed traits are, respectively, $\mathbf{EPR_t}$, $\mathbf{MORT_t}$, $\mathbf{BWc_t}$, $\mathbf{SP_t}$, and **logFCR**.

The analysis consisted of two steps. In the first step, data were pre-corrected for a random study effect through the MIXED procedure of SAS (SAS Institute Inc., 2009). In the second step, we ran the GLMSELECT (SAS Institute Inc., 2009) procedure on the residuals of the first step to automatically select the best model for each trait. Models were allowed to include the fixed main effects of genotype (**GEN**), group size (**GS**) as fixed effects, and covariates for linear effect of temperature (**T**), nonlinear effects of temperature ($\mathbf{T^2}$, $\mathbf{T^3}$), linear effects of age and amplitude of temperature variation, and all possible two-by-two interactions. In addition, we introduced observation weights into the model to account for study-specific differences in the variability of data measured on differing numbers of independent samples (**NIS**), particularly as the NIS varied considerably among the traits and studies considered. For example, mean albumen proportions were calculated on 19 to 2421 NIS depending on the study. However, weighting observations using the raw NIS (which would correspond to giving the latter study in the previous example 127 times more weight than the former) would lead to attributing a disproportionate influence to the few large-scale experiments and largely excluding data from small to medium-sized studies, even though small studies are often more accurate in their monitoring of heat stress than large studies, so that data quality of small-scale studies is quite reliable. To avoid this, the NIS were split into 5 quantiles for each trait, from 1 (20% of studies with the smallest NIS) to 5 (the 20% of studies with the largest NIS), and these categories were used as weighting factors in the analyses of variance. A stepwise procedure was used to successively introduce new effects in the model up to the entry significance level of $P < 0.15$, which is the common standard in stepwise regressions in SAS. The best-fitting parsimonious model was then selected on the criterion of lowest predicted residual sum of squares. The final selected models for each trait are presented in Table 2.

To assess whether a trait was a good indicator of sensitivity to heat stress, we calculated an index of sensitivity (IS) in a "standard" situation (defined as single-caged birds of CW genotype, 30-weeks-old, reared under constant ambient temperature), as the relative difference between estimated performances at thermoneutrality (**TN**, 15 to 20°C) and under heat stress

Table 2. Trait mean estimated values at thermo-neutrality (TN, 15 to 20°C) and under heat stress (HS, 30 to 35°C); index of sensitivity (IS); and models fitted to each trait.

	EPR[1,2] (%)	FI (g.d^{-1})	EW (g)	EM (g.d^{-1})	FCR[2] (g.g^{-1})	HU	ST (mm)	YP (%)	AP (%)	SP[2] (%)	BWc[2] (%.wk^{-1})	MORT[2] (%.wk^{-1})	SS (g)
TN	86.91	112.82	58.09	48.48	2.313	-	0.3631	-	61.48	9.86	−0.6828	0.2610	3513
HS	77.06	87.27	53.93	44.11	2.224	-	0.3439	-	62.23	9.43	0.2028	0.2900	3009
IS	−11.3	−22.6	−7.2	−9.0	−3.9	-	−5.3	-	1.2	−4.3	−129.7	126.1	−14.3
P[3]	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	-	<0.0001	-	<0.0001	<0.0001	<0.0001	0.0010	<0.0001
Significance level of main effects[4]													
T	<0.0001				0.0002		<0.0001			0.0239	0.0610	0.0089	0.0788
T^2	<0.0001	<0.0001		<0.0001			<0.0001		0.0003	<0.0001	0.0002	<0.0001	<0.0001
T^3							0.0179			0.0129			
A	<0.0001	0.0120	<0.0001	<0.0001		0.0002	0.0039			0.1142	0.0411		0.0040
GS	<0.0001	<0.0001	<0.0001	0.0008									0.0521
GEN	<0.0001	0.0243	<0.0001	0.0018	0.0180						0.0795		
Significance level of interactions													
T×A			<0.0001										
T×GS	<0.0001	<0.0001	0.0022	0.0354									0.1360
T×GEN	<0.0001												
T^2×A		0.0112	0.0046	<0.0001									0.0972
T^2×GS													
T^2×GEN				0.0494									
A×GS	<0.0001			<0.0001									
A×GEN			<0.0001										
GS×GEN			0.0061	0.0233									
Adj R^2	0.34	0.43	0.37	0.24	0.02	0.05	0.30	0.00	0.09	0.17	0.04	0.16	0.42

[1]FI: feed intake; EPR$_t$: egg production rate on transformed scale; EW: egg weight; EM: egg mass; logFCR: feed conversion ratio on log-transformed scale; HU: Haugh units; ST: shell thickness; SS: shell strength; SP$_t$: shell proportion on transformed scale; YP: yolk proportion; AP: albumen proportion; MORT$_t$: mortality rate on transformed scale; BWc$_t$: relative weekly body weight change on transformed scale.

[2]EPR, FCR, SP, BWc and MORT were transformed before analyses.

[3]P: significance of the difference between TN and HS.

[4]T: mean temperature; T^2: squared mean temperature; T^3: cubed mean temperature; Δ: daily amplitude of temperature variation; A: hen age; GS: group size; GEN: genotype.

(**HS**, 30 to 35°C):

$$IS = 100 \times \frac{HS_S - TN_S}{TN_S},$$

where HS_S is the mean of estimated values between 15 and 20°C (6 estimated values, i.e., 1 estimated value by degree) and $\mathbf{TN_S}$ the mean of estimated values between 30 and 35°C (6 estimated values, i.e., 1 estimated value by degree).

For traits for which an interaction between temperature (T, T^2, T^3 or Δ) and one other effect was fitted in the model, we similarly calculated an index showing the differences between levels of fixed effects or pre-defined levels of covariates (30, 50, and 70 wk for age, 0, 5, and 10°C for amplitude of temperature variation). For example, for an interaction between temperature and genotype, we calculated:

$$IS_{CW} = IS$$

$$IS_{CC} = 100 \times \frac{HS_{CC} - TN_{CC}}{TN_{CC}}$$

$$IS_{EXP} = 100 \times \frac{HS_{EXP} - TN_{EXP}}{TN_{EXP}},$$

where IS_X is the index of sensitivity for the level X of genotype (X = CW, CC, EXP), HS_X and TN_X the means of estimated values under HS and at TN for the level X of genotype.

Finally, when an interaction between two effects A and B (except temperature) was fitted in the model, we calculated a similar index showing the importance of this interaction as:

$$INT_{A_{X\,X'},B_{Y\,Y'}} = 100 \times \frac{P_{A_X,B_Y} - P_{A_{X'},B_{Y'}}}{P_{A_{X'},B_{Y'}}},$$

where P_{A_X,B_Y} ($P_{A_{X'},B_{Y'}}$) were the means of estimated values between 15 and 35°C for level X (X′) of effect A and level Y (Y′) of effect B. Only combinations in which one effect was fixed (X = X′ or Y = Y′) while the other was varying were tested. For example, for an interaction between age and genotype, we calculated the difference between CC and CW birds at 30 weeks, the difference between CC birds at 30 and 50 weeks, but not the difference between CC birds at 30 weeks and CW birds at 50 weeks.

To test the significance of indices defined above, estimated data (one point by degree) were analyzed with a GLM procedure with model 1 for IS, model 2 for an interaction between temperature and another effect, and with model 3 for interactions between two effects except

temperature:

$$\text{Model 1} : EV_{ij} = \mu + TZ_i + e_{ij}$$

$$\text{Model 2} : EV_{ijk} = \mu + TZ_i + E1_j + TZ_i \times E1_j + e_{ijk}$$

$$\text{Model 3} : EV_{ijk} = \mu + E1_j + E2_k + E1_j \times E2_k + e_{ijk},$$

where $EV_{ij(k)}$ is the estimated value at j (k) degrees (j = 15 to 20 and 30 to 35°C in model 1, k = 15 to 20 and 30 to 35°C in model 2, k = 15 to 35°C in model 3), μ the general mean, $\mathbf{TZ_i}$ the fixed effect of the i^{th} temperature zone (TN, HS), $\mathbf{E1_j}$ and $\mathbf{E2_k}$ the fixed effects of level j of effect 1 and level k of effect 2 (j, k = CC, CW, EXP for genotype, 30, 50, 70 wk for age, 0, 5, 10°C for amplitude of temperature variation, Individual or Collective for group size), $TZ_i \times E1_j$ the interaction between the i^{th} temperature zone and the j^{th} level of effect 1, $E1_j \times E2_k$ the interaction between the j^{th} level of effect 1 and the k^{th} level of effect 2, and $\mathbf{e_{ij(k)}}$ the residual for estimated value at j (k) degrees.

RESULTS

The list of significant main effects and interactions for the 13 modeled traits is presented in Table 2. Amplitude of variation of temperature was found to be insignificant as both a direct effect and in interactions with other effects and will thus not be included in the Tables of results. Interactions were present 17 times in the fitted models, involving all effects. The selected fitted models can be found in Table 3. Adjusted R^2 of the models are sometimes null or close to zero (e.g., for HU or YP), which implies that these traits are not affected by heat and that factors affecting these traits are not included in our model. However, it is also important to remember that data have been pre-corrected for the random effect of the study, which generally is the largest cause of variability in a meta-analysis. Indeed in our case, the pseudo-R^2 calculated for the initial model including only the random study effect averaged at 0.65.

Index of Sensitivity and Direct Effect of Temperature

The index of sensitivity describes the intensity of response to heat stress for each trait in the reference situation between TN and HS (Table 2). Mortality and body weight change were the most affected traits and varied sharply with temperature (+126.1% and −129.7%, respectively), which is partly because their proportions were close to zero under TN conditions. Other traits such as feed intake, shell strength, egg production, and egg mass were also strongly affected by heat as their values decreased by 9.0 to 22.6% between TN and HS in the standard situation. Egg weight, feed conversion ratio, shell thickness, and percentage showed lower but non-negligible variation with temperature (−3.9 to −7.2%). In contrast, Haugh units and albumen and yolk proportions did not vary with temperature.

The temperature effect included only quadratic direct or interaction effects for AP (Table 2) or both linear and quadratic direct or interaction effects for EPR_t, FI, EW, ST, BW_{ct}, $MORT_t$, EM, SP_t, SS, and ST (Table 2). For ST and SP_t, the model also included a cubic term. A nonlinear relationship could be due to the presence of quadratic or cubic terms (FI, EM, ST, and SS) or to the nonlinear transformation of the traits before analysis (EPR_t and SP_t). For shell characteristics and egg production rate, the values initially increased at lower temperatures, reached their maximum between 20 and 22°C, and declined at higher temperatures (e.g., Figure 1A for shell proportion). Conversely, mortality decreased at low to medium temperature before increasing at high temperatures (Figure 1B). For most traits however, we observed a continuous increasing (AP) or decreasing (FI, EM, EW, FCR) trend with increasing temperature.

Genotype Effect

Genotype by temperature interaction This interaction was found for EPR_t and EM (Table 2), and was mostly due to a difference in heat sensitivity between the commercial colored group and the other genotypes (Figure 2). At TN, EPR was equivalent for the 3 genotypes (Table 4), while under HS egg production was lower for CC than for CW (−8.4%, $P = 0.001$) and tended to be lower than in EXP (−5.7%, $P = 0.08$). At TN, egg mass of CC was 5.9% higher than that of EXP ($P < 0.0001$), but under HS no difference of EM between EXP and CC was observed ($P = 0.52$). This result can be interpreted by looking at the egg weight of CC that was constantly higher compared to EXP, both at TN and HS (3.4 and 3.7%, respectively, $P < 0.0001$). The fact that CC hens have higher EW but lower EPR at HS, leads to the lack of difference in EM observed between CC and EXP.

Genotype by age interaction A genotype by age interaction was found for egg weight (Table 2), due to a difference between EXP and CW genotypes (Table 5). Egg weight was not different between commercial hens and EXP at 30 weeks (+4.3%, $P = 0.12$), but egg weight increased more with age for EXP than for commercial birds (+15.8% and +6.2%, respectively), leading to 4.6% heavier eggs in EXP birds at 70 wk ($P = 0.04$, Table 5).

Genotype by group size interaction This interaction was found for egg weight and egg mass (Table 2) and was due to a lower sensitivity to the group effect in CW birds than in CC and EXP birds (−0.4% vs −2.8

Table 3. Equations of solutions.

Trait[1]	Common Terms[2]	Terms specific to several categories[3]			
		CC	CW	G	I
EPR_t (%)	$1.011 + 2.647\ 10^{-2}\ T - 6.973\ 10^{-4}\ T^2 - 2.966\ 10^{-3}\ A$	$0.1426 - 5.769\ 10^{-3}\ T$	$3.402\ 10^{-2} - 1.674\ 10^{-4}\ T$	$-0.2769 + 4.990\ 10^{-3}\ T + 2.656\ 10^{-3}\ A$	
FI (g)	$115.1 - 2.801\ 10^{-2}\ T^2 + 0.2075\ A - 2.020\ 10^{-4}\ T^2 \times A$	2.813	2.078	$-10.39 + 1.092\ 10^{-2}\ T$	
EW (g)	$48.87 + 0.5125\ A - 1.771\ 10^{-2}\ T \times A + 2.144\ 10^{-4}\ T^2 \times A$	$4.627 - 8.818\ 10^{-2}\ A$	$6.098 - 0.1253\ A$	$-4.228 + 5.359\ 10^{-2}\ T - 0.3715$ (CC only) $+ 0.9582$ (CW only)	$-6.777\ 10^{-2}$
EM (g)	$44.35 - 3.587\ 10^{-3}\ T + 0.1336\ A - 1.650\ 10^{-4}\ T^2 \times A$	$4.410 - 4.833\ 10^{-3}\ T^2$	$1.957 - 7.960\ 10^{-4}\ T^2$	$-14.45 + 3.510\ 10^{-3}\ T + 0.1166\ A + 4.326$ (CC only) $+ 7.010$ (CW only)	
logFCR ($g.g^{-1}$)	$0.9192 - 2.625\ 10^{-3}\ T$	$-2.425\ 10^{-2}$	$-3.465\ 10^{-2}$		
HU	$83.44 - 4.906\ 10^{-2}\ A$				
ST (mm)	$0.1157 + 2.905\ 10^{-2}\ T - 1.016\ 10^{-3}\ T^2 + 1.055\ 10^{-5}\ T^3 - 1.680\ 10^{-4}\ A$				
AP (%)	$61.17 + 1.001\ 10^{-3}\ T^2$				
SP_t (%)	$0.2017 + 1.467\ 10^{-2}\ T - 5.650\ 10^{-4}\ T^2 + 6.752\ 10^{-6}\ T^3 - 5.033\ 10^{-5}\ A$				
BWc_t ($\%.wk^{-1}$)	$0.5426 - 3.752\ 10^{-3}\ T + 8.871\ 10^{-5}\ T^2 + 2.440\ 10^{-4}\ A$	$-1.880\ 10^{-3}$	$3.950\ 10^{-3}$		
$MORT_t$ ($\%.wk^{-1}$)	$0.1198 - 7.003\ 10^{-3}T + 1.740\ 10^{-4}\ T^2$				
SS (g)	$1921 + 193.5\ T - 4.844\ T^2 - 13.04\ A + 1.008\ 10^{-2}\ T^2 \times A$				$-385.7 + 0.3662\ T$

[1]FI: feed intake; EPR_t: egg production rate on transformed scale; EW: egg weight; EM: egg mass; logFCR: feed conversion ratio on log-transformed scale; HU: Haugh units; ST: shell thickness; SS: shell strength; SP_t: shell proportion on transformed scale; YP: yolk proportion; AP: albumen proportion; $MORT_t$: mortality rate on transformed scale; BWc_t: relative weekly body weight change on transformed scale.

[2]T: mean temperature; T^2: squared mean temperature; T^3: cubed mean temperature; Δ: daily amplitude of temperature variation; A: hen age.

[3]CC: commercial colored genotypes; CW: commercial white genotypes; G: group-caged birds; I: individually-caged birds.

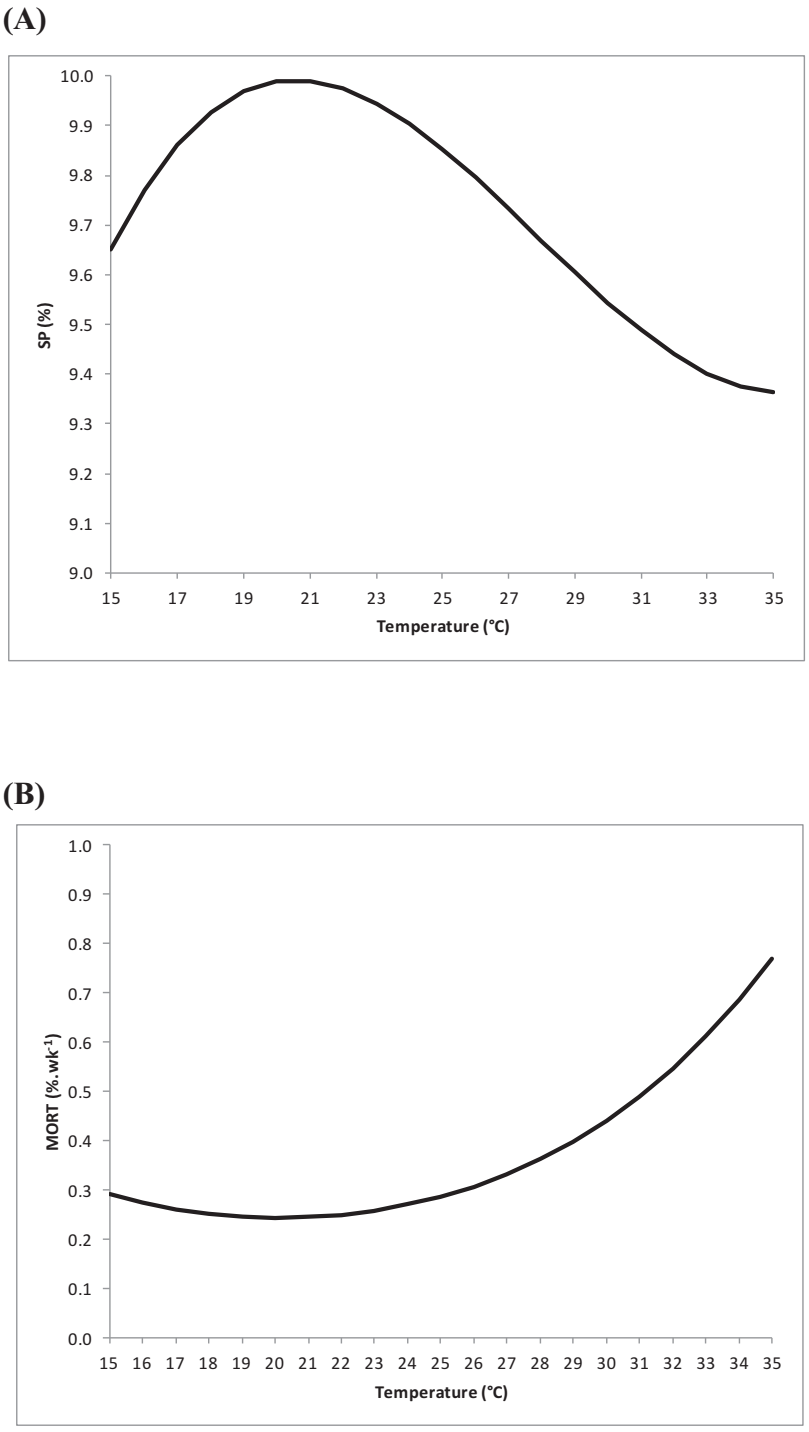

Figure 1. Fitted curves for evolution of (A) shell proportion (**SP**) and (B) weekly mortality rate (**MORT**) with temperature, in the standard situation (commercial white genotype, 30-wk-old birds, single-caged, daily amplitude of variation of temperature = 0°C).

to −7.9% for EW, −8.3% vs −14.1 to −24.1 for EM, Table 5). In both individual cages and in group housing, egg weight was higher in commercial birds than in EXP, but a difference was found between CW and CC only in collective systems. Similarly, for egg mass, no difference was found between genotypes in individual cages, but commercial birds showed better performances than EXP birds in collective systems (Table 5).

Genotype main effect Genotype differences were observed for FI and FCR ($P = 0.02$, Table 2), EXP birds eating 2.1 to 2.8 g.d^{-1} less and exhibiting a 0.06 to 0.08 higher FCR than commercial birds (Table 3). The effect of the genotypes on FI was nevertheless minor compared to the effect of the temperature itself (−34.1 g between estimated values at 20 and 35°C in the standard situation).

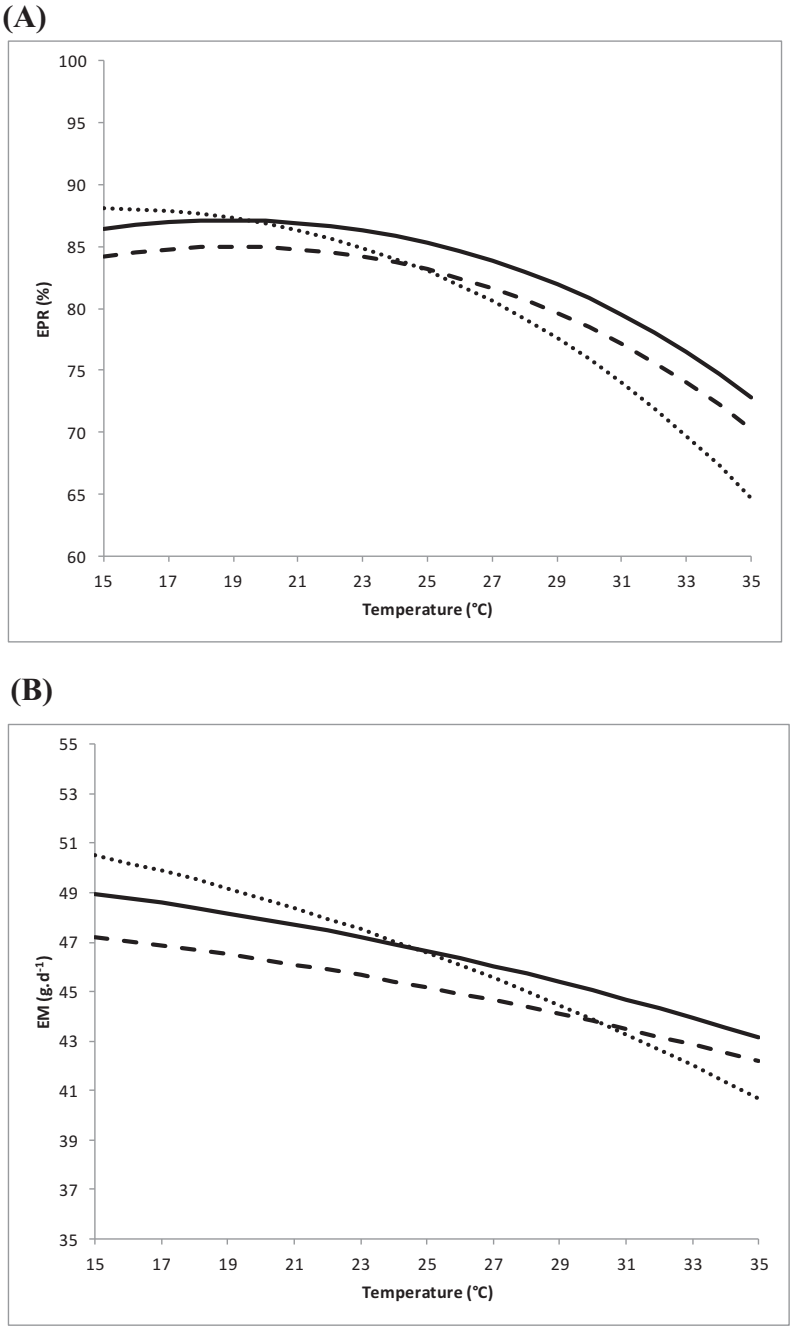

Figure 2. Fitted curves showing the interaction between genotype and temperature for (A) egg production rate (**EPR**) and (B) egg mass (**EM**). Full line stands for commercial white, dotted line for commercial colored, and dashed line for experimental and indigenous lines.

Hen Age Effect

Age by temperature interaction Age by T or T^2 interactions were included in our models for EW, FI, and EM (Figure 3 for EM and EW) and SS, even if weakly significant for the latter ($P = 0.10$, Table 2). Egg weight, egg mass, and feed intake were more affected by heat in older than in younger hens (respectively, −13.1%, −18.0% and −26.7% at 70 wk vs. −7.2%, −9.0%, and −22.7% at 30 wk between TN and HS, Table 4). On the contrary, shell strength decreased more with heat in younger than in older hens (−14.3% at 30 wk, −6.4% at 70 wk, between TN and HS, Table 4). In both cases, this pattern led to the presence of a significant or nearly significant difference between 30-wk-old hens and 70-wk-old hens under TN ($P = 0.002$ for SS, <0.0001 for EW and EM, 0.10 for FI). Under HS, the differences between young and old hens vanished for FI and SS and were largely reduced for EW and EM (Table 4).

Age by group size interaction This interaction affected EPR ($P < 0.0001$) and EM ($P < 0.0001$, Table 2). At 30 weeks, egg production rate and egg mass were, respectively, 6.7% and 8.3% lower in group-caged birds than in individual cages. At 50 and 70 weeks, these differences between group sizes disappeared (Table 5).

Table 4. Mean fitted values for each trait under thermoneutrality (TN, at temperatures between 15 and 20°C) and under heat stress (HS, between 30 and 35°C) for traits exhibiting an interaction between temperature and other effects.

Traits[1]	Mean of fitted values by effect level[2] At TN			Under HS			Difference between HS and TN (%)			Relative difference between levels of effects at TN (%)[3]			Relative difference between levels of effects at HS (%)[4]		
							Group Size[5]								
	Ind	Coll		Ind	Coll		Ind	Coll		Coll-Ind			Coll-Ind		
EPR (%)	86.91^{a}	78.66^{b}		77.06^{b,c}	74.09^{c}		−11.32	−5.81		−9.49			−3.86		
FI (g.d^{-1})	112.82^{a}	102.62^{b}		87.27^{c}	77.24^{d}		−22.65	−24.74		−9.04			−11.50		
EW (g)	58.09^{a}	56.95^{b}		53.93^{c}	54.61^{c}		−7.16	−4.11		−1.97			1.25		
EM (g.d^{-1})	48.48^{a}	44.60^{b}		44.11^{b}	40.29^{c}		−9.00	−9.67		−8.00			−8.67		
SS (g)	3513^{a}	3133^{b}		3009^{b}	2636^{c}		−14.32	−15.88		−10.80			−12.42		
							Age								
	30 wk	50 wk	70 wk	30 wk	50 wk	70 wk	30 wk	50 wk	70 wk	50–30 wk	70–50 wk	70–30 wk	50–30 wk	70–50 wk	70–30 wk
FI (g.d^{-1})	112.82^{a}	115.72^{a}	118.62^{a}	87.27^{b}	87.14^{b}	87.01^{b}	−22.65	−24.70	−26.65	2.57	5.14	2.51	−0.15	−0.30	−0.15
EW (g)	58.09^{c}	60.97^{b}	63.84^{a}	53.93^{e}	54.71^{d,e}	55.49^{d}	−7.16	−10.26	−13.08	4.95	9.89	4.71	1.44	2.88	1.42
EM (g.d^{-1})	48.48^{c}	50.13^{b}	51.78^{a}	44.11^{d}	43.29^{d,e}	42.47^{e}	−9.00	−13.64	−17.99	3.41	6.82	3.30	−1.86	−3.73	−1.90
SS (g)	3513^{a}	3314^{ab}	3116^{bc}	3009^{c}	2962^{c}	2915^{c}	−14.32	−10.62	−6.44	−5.65	−11.29	−5.99	−1.57	−3.13	−1.59
							Genotype[6]								
	CW	CC	EXP	CW	CC	EXP	CW	CC	EXP	CC-CW	EXP-CW	EXP-CC	CC-CW	EXP-CW	EXP-CC
EPR (%)	86.91^{a}	87.61^{a}	84.74^{a}	77.06^{b}	70.60^{c}	74.63^{b,c}	−11.32	−19.41	−11.93	0.81	−2.49	−3.27	−8.38	−3.16	5.70
EM (g.d^{-1})	48.48^{a}	49.68^{a}	46.76^{b}	44.11^{c}	42.29^{d}	43.00^{c,d}	−9.00	−14.88	−8.05	2.48	−3.53	−5.87	−4.13	−2.53	1.68

[1]EM: egg mass, EPR: egg production rate, FI: feed intake; EW: egg weight; SS: shell strength.

[2]Traits within line with no common superscript are significantly different ($P < 0.05$).

[3]Relative difference "A-B" is calculated as $\frac{TN_B - TN_A}{TN_A}$, where TN_A and TN_B are the means of estimated values for levels A and B of effects at thermo-neutrality.

[4]Relative difference "A-B" is calculated as $\frac{HS_B - HS_A}{HS_A}$, where HS_A and HS_B are the means of estimated values for levels A and B of effects under heat stress.

[5]Ind: Individual rearing; Coll: collective rearing.

[6]CW: commercial white birds; CC: commercial colored birds; EXP: experimental birds.

Table 5. Means of fitted values for traits exhibiting interactions between effects, except temperature.

Effect 1[1]		Means of estimated values by level of effect 2[2]			Relative difference between levels of effect 2 within effect 1(%)[3]		
			Egg Production Rate (%)				
	Group	*Ind*	*Coll*		*Coll-Ind*		
30 wk		83.3[a,4]	77.7[b]		−6.7		
50 wk		78.7[b]	77.2[b,c]		−1.9		
70 wk		73.6[c]	76.7[b,c]		−4.1		
			Egg Weight (g)				
	Age	30 wk	50 wk	70 wk	50–30 wk	70–50 wk	70–30 wk
CW		55.9[d,e]	57.6[c,d]	59.3[b,c]	3.1	3.0	6.2
CC		55.5[d,e]	57.9[b,d]	60.5[a,b]	4.5	4.3	8.9
EXP		53.5[e]	57.8[b,d]	62.0[a]	7.9	7.3	15.8
	Group	*Ind*	*Coll*		*Coll-Ind*		
CW		55.9[a]	55.6[a]		−0.4		
CC		55.5[a]	53.9[b]		−2.8		
EXP		53.5[b]	49.3[c]		−7.9		
			Egg mass ($g.d^{-1}$)				
	Group	*Ind*	*Coll*		*Coll-Ind*		
30 wk		46.4[a,b]	42.6[c]		−8.3		
50 wk		46.9[a,b]	45.4[b]		−3.2		
70 wk		47.4[a,b]	48.2[a]		1.7		
	Group	*Ind*	*Coll*		*Coll-Ind*		
CW		46.4[a]	42.6[b]		−8.3		
CC		46.2[a]	39.7[c]		−14.1		
EXP		45.0[a]	34.1[d]		−24.1		

[1]CW: commercial white birds, CC: commercial colored birds; EXP: experimental birds.

[2]Ind: individual rearing, Coll: collective rearing.

[3]Relative difference "B-A" is calculated as $\frac{P_B - P_A}{P_A}$, where PA and PB are the means over the whole temperature range (15 to 35°C) of estimated performances of levels A and B of effect 2, within effect 1.

[4]Traits within interaction with no common superscript are significantly different ($P < 0.05$).

Main age effect An age effect was fitted for BWc_t ($P = 0.04$), SP_t ($P = 0.11$), ST ($P = 0.04$), and HU ($P = 0.0002$, Table 2). These effects were very low, leading to weekly decreases of 0.03 to 0.06% for HU, ST, and SP. For BWc, this effect changed the temperature above which hens started to gain weight. At 70 wk, body weight change was positive whatever the temperature. At 50 and 30 weeks, body weight change became positive above 32°C and 28°C, respectively (Figure 4).

Group Size

Group size by temperature interaction Group size by temperature interaction was fitted to EPR_t ($P < 0.0001$), EM ($P = 0.04$), EW ($P = 0.002$), SS ($P = 0.14$), and FI ($P < 0.0001$, Table 2). For egg production and egg mass, the advantage of individual cages compared to collective ones was reduced at high temperatures (Figure 5 for EPR). Indeed, EPR and EW were 11.3% and 7.2% lower in collective than in individual cages at TN, but only 5.8% and 4.1% lower at HS (Table 4). On the contrary, the difference between group sizes slightly increased with heat for feed intake and egg mass. Mean values in collective cages were lower than those in individual cages by 22.6% for FI and by 9.0% for EM at TN, and by 24.7% and 9.7%, respectively, under HS.

DISCUSSION

In previous studies evaluating the effect of heat stress on production and feed efficiency traits, the effects of age, genotype, group size, and amplitude of temperature have been mostly studied separately and largely on CW hens reared in single battery cages. A few attempts have been made to model changes in traits with ambient temperature, but they never studied simultaneously the effects and traits analyzed in this study. For example, Marsden and Morris (1987) modeled the effects of temperature on various metabolic and production traits considering only the genotype effect, while Al-Saffar and Rose (2002) considered the genotype, group size, and temperature program but only on egg related traits. None of these reviews included the effect of hen age or interactions between traits.

One of the main results of this study is to have highlighted the existence of interactions between temperature and the different factors (age, group size, and genotype) for four main traits: 1) feed intake, 2) egg production, 3) egg weight, and 4) egg mass. This underscores that it is incorrect to apply the results

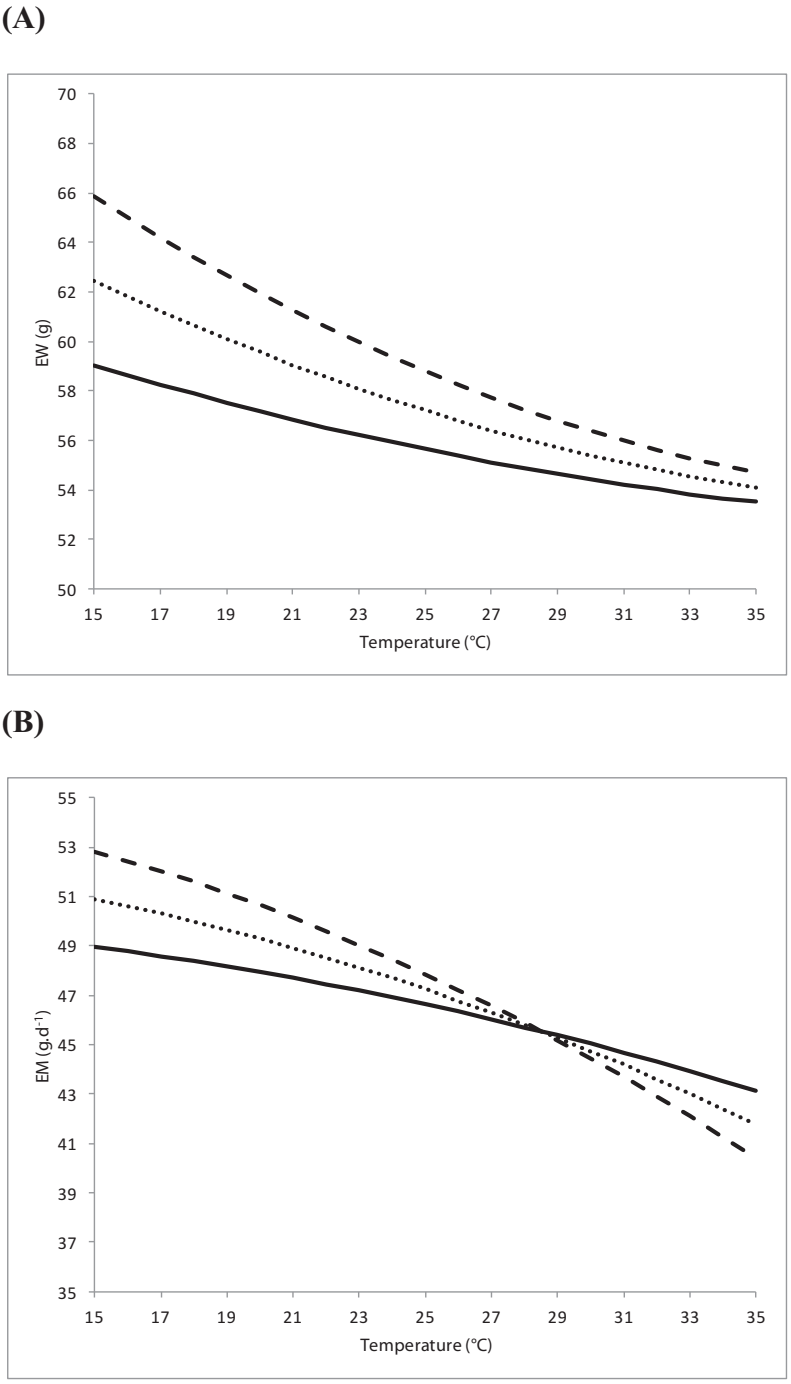

Figure 3. Fitted curves showing the interaction between age and temperature for (A) egg weight (EW) and (B) egg mass (EM). Full line stands for 30-wk-old birds, dotted line for 50-wk-old birds, and dashed line for 70-wk-old birds.

obtained from one study done under specific conditions of genotype, hen age, and group size to other conditions, unless the mechanisms leading to the aforementioned interactions are well understood. This also enhances the importance of providing the equations corresponding to fitted models, which will allow people to apply the appropriate equation for a given situation.

Joint analysis of independent studies would be facilitated by a standardization of experimental procedures. For example, mortality recording is not standardized between studies. Age or causes of mortality are usually not recorded, and it is difficult to summarize the available data on mortality although it is influenced by chronic heat stress. Similarly, some other effects such as air velocity or humidity could not be included in the models because of the paucity of records, even if they are known to influence the response to heat stress (Yahav et al., 2004). This information should be systematically included in a general effort of standardization of data recording in future experiments.

Concerning the temperature effect, our study confirmed that large negative effects of heat stress (more

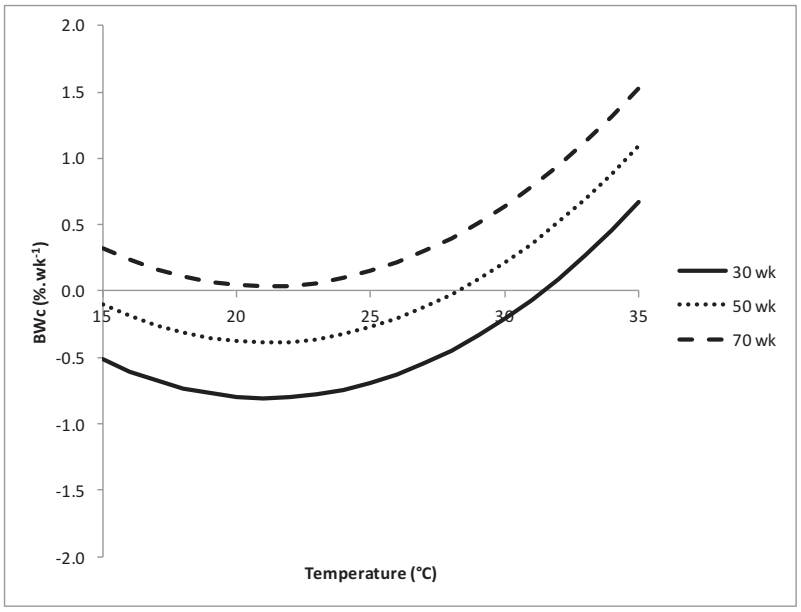

Figure 4. Fitted curve showing the effect of age on relative weekly body weight change (**BWc**). Full line stands for 30-wk-old birds, dotted line for 50-wk-old birds, and dashed line for 70-wk-old birds.

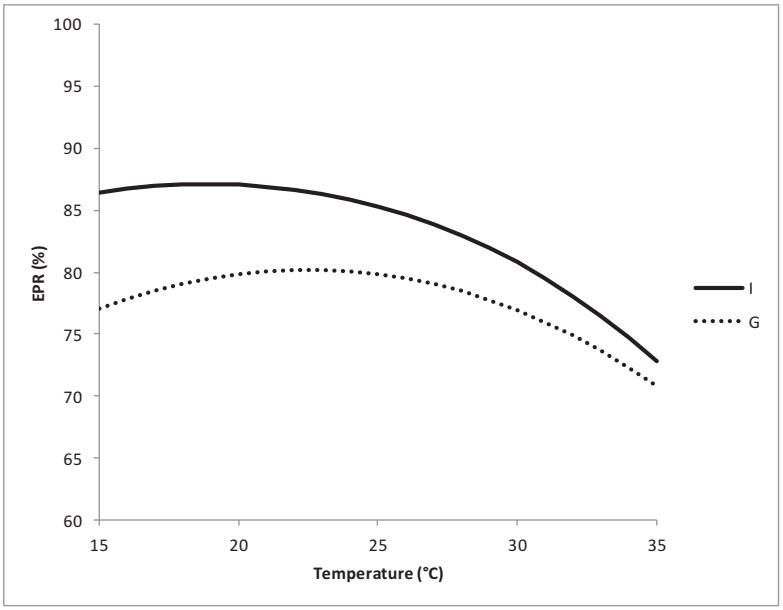

Figure 5. Fitted curve showing the interaction between group size and temperature for egg production rate (EPR). Full line stands for single-caged birds (I), dotted line for collective rearing (G).

than 10% of decrease compared to the optimal value) are observed above 30°C. However, a real impact of heat stress on some traits was observed below the threshold value of 30°C. This was the case for feed intake, with a 10% decrease observed already at 24°C, and for important production traits such as egg mass, egg production rate, egg weight, and shell strength with losses of 5% (i.e., values comparable to what is forecast as heat related losses in the USA, St-Pierre et al., 2003) at lower temperatures (24 to 29°C). This observation is relatively novel since previous studies generally considered heat stress in layers to start only above 30°C. To avoid any heat stress effect, it is recommended that ambient temperature remains below 25°C. As feed intake is the trait that is the most largely affected at the lowest temperature, we can hypothesize that this trait has a higher adaptive value than the other traits, as a lower feed intake is associated with a reduced need to dissipate heat. Moreover, there is probably a cascading effect of the reduced feed intake on the other traits, as they are all correlated.

Furthermore, the impact of using cyclic temperatures appears to be much more limited than previously thought. This underlines the severe consequences that climate change may have on egg production, considering that the Intergovernmental Panel on Climate

Change (**IPCC**) scenarios are suggesting that temperatures could rise by +2 to +4°C by the end of the century.

Genotype Effects

The high egg production rate and egg mass in the CC hens under TN temperatures followed by a stronger drop under heat stress (HS) reflect the limited ability of the CC hens to cope with high temperatures. High-performing hens have been shown to be more sensitive to environmental changes (Chen et al., 2009) but differences exist between genotypes. For example, the ability of the CW hens to tolerate heat stress better than the CC hens is well documented (Marsden and Morris, 1987; Franco-Jimenez et al., 2007; Melesse et al., 2011, 2013), and several hypotheses have been proposed. At elevated environmental temperatures, animals dissipate body heat by sensible heat loss (radiation, convection, and conduction) and evaporative heat loss (DEFRA, 2005; Mutaf et al., 2008). However, sensible heat loss is reduced in brown birds because of heavy feather coverage that limits heat dissipation (Franco-Jimenez et al., 2007). Furthermore, brown birds are heavier and, therefore, require more energy for maintenance (Franco-Jimenez et al., 2007). This was also the case in our database, where, at onset of lay (before 25 wk), CC were on average 174 g heavier than CW birds. Marsden and Morris (1987) showed that energy available for production is maximum at 23°C for brown birds and at 24°C for white birds. In our analysis birds from experimental lines (EXP) were mostly composed of White Leghorn hens and it is, therefore, not surprising that they showed a similar response to HS as the CW birds. Moreover, the EXP was a heterogeneous group. It was composed on the one hand of experimental lines selected on one unique selection criterion (residual feed intake, clutch length, etc). This might confer an advantage under HS conditions compared to commercial birds selected on multiple criteria selection indexes and that exhibit high performance for several production traits shown here to be sensitive to heat. On the other hand, the EXP group also included indigenous breeds coming mostly from hot countries and, thus, adapted to heat, and lines segregating for genes like the naked neck, sex-linked dwarf gene or frizzle genes, which have been shown to improve tolerance to heat (Chen et al., 2004, 2009; Cahaner et al., 2008; Zerjal et al., 2013).

The small difference between the commercial lines in changes in feed intake and FCR in response to increasing temperatures was expected. Reducing feed intake during heat stress is a physiological reaction to reduce diet-induced thermogenesis and the consequent metabolic energy to dissipate, thus, increasing the tolerance to heat stress. The differences in feed intake between the commercial and EXP hens can be explained considering that some EXP lines were selected for low residual feed intake, and others were carrying the sex-linked dwarf mutation, that causes a reduced appetite due to thyroid insufficiency (Guillaume, 1976; Marks, 1980). In both cases, a relatively low feed intake was expected. Small variation in BWc observed at HS in CW hen temperatures could be explained by the smaller size of the White leghorns compared to Brown hens and their higher tolerance to heat.

Age Effects

The combined effect of temperature and age can be explained by the larger drop in the level of production for age groups having the highest values in optimal conditions. Shell strength was more affected in young birds and EW was more affected in old ones. A similar scenario was reported by Tůmova and Gous (2012) who found a stronger impact of heat in highly productive laying hens compared to less productive broiler breeder hens.

When hens get older, egg weight and egg surface increase, implying an increased need for calcium to build shell matrix (Tůmova and Gous, 2012). However, the capacity to absorb calcium decreases with age (Cordts et al., 2002). This can explain why at TN, when heat stress does not affect production, shell strength is lower in old than in young hens. Under heat stress, birds hyperventilate to dissipate heat leading to an additional lack of bicarbonate ions required for shell structure (Tůmova and Gous, 2012). This should increase the disadvantage of old hens, however, old hens show a sharp decrease in egg weight under heat stress, thus limiting their needs in calcium, which may in turn explain why young and old hens have similar shell strength under HS.

Similarly, the interaction between temperature and egg weight can be linked to the trend observed with age and temperature, for egg number and feed intake. When birds get older, their needs increase for both maintenance and production as hens and eggs get heavier. Under HS, feed intake decreases sharply, and less resources are available both for maintenance and for production. As egg production does not decrease at the same rate as feed intake, there are proportionally less resources available for each egg, especially for older hens producing larger eggs, so that the age effect on egg weight is completely erased by the heat stress.

Group Size

The relationship between group size and ambient temperature has been less studied, as most of the studies on heat-stress effects in laying hens have been performed in single cages. In our database, almost 60% of the studies were done in individual cages, and only two considered both collective and individual rearing. In agreement with our results, Saki et al. (2012) showed that group-caged hens have lower egg production, egg

weight and egg mass than hens kept in single cages under neutral temperatures of 18 to 22°C. This could be due, on the one hand, to the lower feed intake of hens reared in group-cages compared to hens kept in single cages (Table 4), and, on the other hand, to the higher activity levels in group-caged hens (Guo et al., 2012), both leading to a reduction of energy resources available for production. The absence of large differences for egg production between group and individual cage systems under HS (Table 4) could reflect that above a certain temperature, HS outweighed stress represented by the collective cage. For other traits such as FI and EM the stronger effect of heat stress in collective groups than in individual cages may be due to the greater difficulty of the former to dissipate heat. Etches et al. (2008) mentioned that under heat stress, caged birds tend to increase the distance between birds and to lift their wings from the body to increase the surface of evaporative heat loss. These behavioral adaptations are probably more difficult in collective cages than in individual ones. For further studies, it will be important to include the information on surface available per bird to make a realistic estimation of this effect.

CONCLUSIONS

This analysis provides a quantification of main effects that should be taken into account to predict the expected impact of chronic heat stress in given conditions. Age and genotype are crucial factors for resilience to heat stress in laying hens as both contribute strongly to variation in egg production and quality. The genotype plays a vital role in determining egg production rate, egg weight, egg mass, and feed intake. Commercial colored birds, which generally are heavier, have more difficulties in handling heat stress. The effect of age was more prominent on egg production rate, egg weight, and feed intake, which calls for consideration of the production length of hens in rearing conditions where house temperatures are not controlled. Finally, group size (single or group-cage) is important for egg production rate, egg mass, egg weight, and feed intake. This meta-analysis points out that shell strength, feed intake, egg mass, egg production rate, and relative body weight change are good indicators of robustness in laying hens as they are more sensitive to temperature.

SUPPLEMENTARY DATA

Supplementary data is available at PSA *Journal* online.

ACKNOWLEDGMENTS

The authors would like to thank the following institutions for their support and collaboration in this project: AgroParisTech (Paris, France), Wageningen University (Wageningen, The Netherlands), and Institut National de la Recherche Agronomique (Nouzilly, France). Utlwanang Moreri benefited from a joint grant from the European Commission and "Institut de Sélection Animale", the layer breeding division of Hendrix Genetics within the framework of the Erasmus-Mundus joint doctorate "European Graduate School of Animal Breeding and Genetics." We would also like to thank Wendy Brand-Williams and Andrea Rau for the English revision of the manuscript (INRA/AgroParisTech, Jouy-en-Josas, France).

REFERENCES

Al-Saffar, A. A., and S. P. Rose. 2002. Ambient temperature and the egg laying characteristics of the laying fowl. World's Poult Sci. J. 58:317–331.

Balnave, D., and J. Brake. 2005. Nutrition and management of heat stressed pullets and laying hens. World's Poult. Sci. J. 61:399–406.

Balnave, D., and S. K. Muheereza. 1997. Improving eggshell quality at high temperatures with dietary sodium bicarbonate. Poult. Sci. 76:588–593.

Bollengier-Lee, S., M. A. Mitchell, D. B. Utomo, P. E. V. Williams, and C. C. Whitehead. 1998. Influence of high dietary vitamin E supplementation on egg production and plasma characteristics in hens subjected to heat stress. Br. Poult. Sci. 39:106–112.

Bordas, A., and P. Mérat. 1992. Performance of homozygous naked neck layers, heterozygous naked neck layers and normal plumage layers of a brown-egg dwarf line subjected to constant or fluctuating temperature [Performances de ponte de poules de génotypes NaNa (Cou nu homozygotes), Nana+ (Cou nu hétérozygote) et na+na+ (plumage normal) d'une lignée naine (dw) de type "oeuf brun" soumises à; une chaleur constante ou avec des fluctuations]. Arch. Geflugelkd. 56:22–27.

Cahaner, A., J. A. Ajuh, M. Siegmund-Schultze, Y. Azoulay, S. Druyan, and A. V. Zárate. 2008. Effects of the genetically reduced feather coverage in naked neck and featherless broilers on their performance under hot conditions. Poult. Sci. 87: 2517–2527.

Celik, L. B., A. Tekeli, and O. Oztürkcan. 2004. Effect of supplemental L-carnitine in drinking water on performance and egg quality of laying hens exposed to a high ambient temperature. J. Anim. Physiol. Anim. Nut. 88:229–233.

Chen, C. F., A. Bordas, D. Gourichon, and M. Tixier-Boichard. 2004. Effect of high ambient temperature and naked neck genotype on performance of dwarf brown-egg layers selected for improved clutch length. Br. Poult. Sci. 45:346–354.

Chen, C. F., D. Gourichon, N. Z. Huang, Y. P. Lee, A. Bordas, and M. Tixier-Boichard. 2009. Performance comparison of dwarf laying hens segregating for the naked neck gene in temperate and subtropical environments. Genet. Sel. Evol. 41:13.

Çiftçi, M., O. N. Ertas, and T. Güler. 2005. Effects of vitamin E and vitamin C dietary supplementation on egg production and egg quality of laying hens exposed to a chronic heat stress. Rev. Méd. Vét. 156:107–111.

COPA COGECA. 2003. Assessment of the impact of the heat wave and drought of the summer 2003 on agriculture and forestry. Committee of Agricultural Organisations in the European Union General Committee for Agricultural Cooperation in the European Union. Brussels: COPA-COGECA, 15 pp.

Cordts, C., M. Schmutz, and R. Preisinger, 2002. New alternatives for improving egg shell stability through breeding. Lohmann Inf. 26: 1–4.

Department for Environment, Food and Rural Affairs (DEFRA). 2005. Heat stress in poultry solving the problem. WebMD. http://www.defra.gov.uk/publications/files/pb10543-heat-stress-050330.pdf10.3382/ps/pev028.html. Accessed Mar. 2012.

Etches, R. J., T. M. John, and A. M. Verrinder Gibbins. 2008. Behavioural, physiological, neuroendocrine and molecular responses to heat stress. Pages 48–79 in Poultry Production in Hot Climates. 2nd ed. N. J. Daghir, ed. CAB International, Wallingford, UK.

Franco-Jimenez, D. J., and M. M. Beck. 2005. Intestinal calcium uptake, shell quality and reproductive hormones levels of three laying hen varieties after prolonged egg production. Int. J. Poult. Sci. 4:518–522.

Franco-Jimenez, D. J., S. E. Scheideler, R. J. Kittok, T. M. Brown-Brandl, L. R. Robeson, H. Taira, and M. M. Beck. 2007. Differential effects of heat stress in three strains of laying hens. J. Appl. Poult. Res. 16:628–634.

Guillaume, J. 1976. The dwarfing gene dw: its effect on anatomy, physiology, nutrition, management. Its application to poultry industry. World Poult. Sci. 32:285–303.

Guo, Y. Y., Q. Q. Song, H. C. Jiao, and H. Lin., 2012. The effect of group size and stocking density on the welfare and performance of the hens housed in furnished cages during summer. Anim. Welfare 21:41–49.

Marks, H. L. 1980. Growth, Feed Intake, and Feed Conversion of Dwarf and Nondwarf Broiler-Type Chickens. Poult. Sci. 59:2183–2188.

Marsden, A., and T. R. Morris. 1987. Quantitative review of the effects of environmental temperature on food intake, egg output, and energy balance in laying pullets. Br. Poult. Sci. 28:693–704.

Mashaly, M. M., G. L. Hendricks, 3rd, M. A. Kalama, A. E. Gehad, A. O. Abbas, and P. H. Patterson. 2004. Effect of heat stress on production parameters and immune response of commercial laying hens. Poult. Sci. 83:889–894.

Melesse, A., S. Maak, R. Schmidt, and G. von Lengerken. 2011. Effect of long-term heat stress on key enzyme activities and T3 levels in commercial layer hens. Int. J. Livest. Prod. 2:107–116.

Melesse, A., S. Maak, H. Pingel, and G. von Lengerken. 2013. Assessing the thermo-tolerance potentials of five commercial layer chicken genotypes under long-term heat stress environment as measured by their performance traits. J. Anim. Prod. Adv. 3:254–264.

Mutaf, S., N. S. Kahraman, and M. Z. Firat. 2008. Surface wetting and its effect on body and surface temperature of domestic laying hens at different thermal conditions. Poult. Sci. 87: 2241–2450.

Novero, R. P., M. M. Beck, E. W. Gleaves, A. L. Johnson, and J. A. Deshazer. 1991. Plasma progesterone, luteinizing hormone concentrations, and granulosa cell responsiveness in heat-stressed hens. Poult. Sci. 70:2335–2339.

Saki, A. A., P. Zamani, M. Rahmati, and H. Mahmoudi. 2012. The effect of cage density on laying hen performance, egg quality and excreta minerals. J. Appl. Poult. Res. 21:467–475.

SAS Institute Inc. 2009. SAS/STAT® 9.2. User's guide, 2nd ed. Cary, NC, SAS Institute Inc.

Seven, P. T. 2008. The effects of dietary Turkish propolis and vitamin C on performance, digestibility, egg production and egg quality in laying hens under different environmental temperatures. Asian-Aust. J. Anim. Sci. 21:1164–1170.

Saint Pierre, N. R., B. Cobanov, and G. Schnitkey. 2003. Economic losses from heat stress by US livestock industries. J. Dairy Sci. 86(E. Suppl.):E52–E77.

Tan, G. Y., L. Yang, Y. Q. Fu, J. H. Feng, and M. H. Zhang. 2010. Effects of different acute high ambient temperatures on functions of hepatic mitochondrial respiration, antioxidative enzymes, and oxidative injury in broiler chickens. Poult. Sci. 89: 115–122.

Tůmová, E., and R. M. Gous. 2012. Interaction of hen production type, age, and temperature on laying pattern and egg quality. Poult. Sci. 91:1269–1275.

Yahav, S., A. Straschnow, D. Luger, D. Shinder, J. Tanny, and S. Cohen. 2004. Ventilation, sensible heat loss, broiler energy, and water balance under harsh environmental conditions. Poult. Sci. 83:253–258.

Zerjal, T., D. Gourichon, B. Rivet, and A. Bordas. 2013. Performance comparison of laying hens segregating for the frizzle gene under thermoneutral and high ambient temperatures. Poult. Sci. 92:1474–1485.