Pre-slaughter stress responses and adrenal responsiveness in broilers of fast and slow growth rate genotypes

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Abstracts: The effects of pre-slaughter stress depend on their specificity, intensity and length, but also on their perception by the bird, depending upon its genotype and previous experience, possibly in interaction with its specific slaughter age. The objective of the study was to better characterise their impact and dynamic, by measuring changes in plasma corticosterone levels in broilers from slow (S, female) and fast (F, Ff [female] & Fm [male line]) growth rate lines. The tests were done at their respective slaughter age, i.e. 42 (F) and 84 (S) days. In order to perform the tests, broilers have been captured, placed in containers and then submitted to different treatments. The treatments were: 1) keeping birds in rooms set up at various temperatures (20, 32 or 35°C), 2) transporting birds for a period of 120 or 210min. In the meantime, their respective maximal adrenal reactivity was estimated by measuring corticosterone response following the i.m injection of a single dose of immediate (IS) or delayed (DS) Synacthen (1-24 ACTH). Blood was collected from the wing vein, just after capture (T0), at various delays after treatment onset (15, 60, 120, 180, 240, and 360min) and-or on the shackel line. Corticosterone data were subjected to a multifactorial ANOVA and Fisher test (PLSD) post hoc tests if appropriate (ANOVA: P < 0.05).
Basal corticosterone levels were significantly lower for genotype S and differences in sensitivity to stressors have been found. As an illustration, maximal corticosterone level was already reached after 15min of transportation for broilers Ff, whereas it was still at basal for genotype S. Longer transport duration and hanging on the shackel line (2min) induced comparable responses (30ng/ml). Interestingly, these responses to physical treatments were of comparable amplitudes to those resulting from the injection of a high dose of IS or DS, i.e. to maximal reactivity in both genotypes. Room temperature of 35°C only induced a significant rise in corticosterone in genotype Ff and this response was of limited amplitude (10-15ng/ml). In conclusion, these results indicate that high temperature had a limited effect in comparison to transportation or hanging on the shackel line, which were perceived as very intense stresses inducing maximal corticosterone levels. They also showed that broilers of genotype F were more rapidly affected by physical stress, which in turn suggest a higher degree of sensitivity.

Keywords: Broiler; growth rate; pre-slaughter stress; corticotrope axis.

Introduction

The conditions to which the animals are subjected during the period which immediately precedes slaughtering, such as the capture, placement in crates, transportation, hanging on the shackel line, are various sources of stress which can affect meat quality (Gregory, 1996). Moreover, works intended to
study the genetic variability of meat quality and estimating the heritability of linked characters indicated that pre-slaughter conditions had a major impact on these parameters (LeBihan-Duval et al., 2001). Besides, the consequences of stressful conditions vary depending to their characteristics (nature, intensity and duration), but also according to the intrinsic perception which the animal will have of them; perception which is going to evolve according to age, sex, previous experiences but also to the genotype (Rémignon et al., 1998, Hazard et al., 2005). The broilers used for standard productions (fast growth: F) and label (slow growth: S) not only differ by their genotype, but also by the age at slaughtering and their rearing conditions. Therefore, we cannot exclude the hypothesis that they have differences in their sensibility to stress and in such case it might be advisable to adapt the methods used for each of the different genotypes in order to minimize their effects. Thus the objectives of the present experiments were to characterize the stress sensitivity of broilers from fast and slow growth rate genotypes, by quantifying their responses in corticosterone (B), to physical treatments and pharmacological challenges.

Material and Methods

Female broilers from grand-parental stocks of fast (female line: Ff) and slow (S) growth rate (Hubbard, Chateaubourg, France), corresponding to genotypes from the standard and label type of production, respectively, were used in a first set of experiments. They were reared in a closed barn. Animals were fed ad libitum with a food, whose composition was adapted to each of the genotypes. The birds were subjected to the experimental tests at their respective age at slaughter, i.e. 42 and 84 day of age, respectively. After capture, broilers were randomly placed in crates (73x53x26cm) by group of 5, and then subjected to one of the various experimental treatments: submitted during 2 hours to a moderate (approximately 20°C) or high temperatures (32°C) and transported in a vehicle during 2 hours. Broilers from additional groups were placed in an opaque bag the first 15 minutes, then in a crate. Two blood samples (± 3ml) were collected first, immediately after initial capture in the pen (T0) and second, by the end of the experiment, in a delay of 120mn. Two additional samples were collected on half of the birds, i.e. 15 and 60min after the initial one, in order to establish the response kinetics.

In order to quantify the adrenal maximal response capacity, broilers placed in crates under moderate temperature (approximately 20°C) were submitted to a pharmacological challenge after catching. They were injected i.m. with an analogue agonist of the ACTH (1-24 ACTH, Immediate [IS: dose 25µg/bird, genotype S and Ff] or Delayed Synacthen [DS: doses 2.5 or 25µg/bird, genotype S] Novartis, 0.25mg/ml; diluted in 0.9% NaCl, injection volume = 0.5ml). Blood sampling were realized from the wing vein, on heparinized syringes, before (T0) and 120mn after injection (T120). Additional samples were taken 15 and 60min after injection on half of the birds, as well as 180, 240 and 300mn after injection of DS (2 doses, genotype S) and from control non-injected broilers. Samples were stored on ice, then plasma separated by centrifugation (10mn, 2000g, 4°C) and freezeed (-20°C) before B assay (Etches, 1976). The number of birds per experimental treatment (genotype x treatment x 2 (two or four samples)) is 10.

In a 3rd set of experiment, the high temperature was set at 35°C, instead of 32°C in the 1st one, and no pharmacological treatments were applied. The number of blood samples per bird was of 1 (time 0, 15, 60 or 120), 2 (0 and 120) or 4 (time 0, 15, 60 and 120). Birds from an additional group were transferred to the slaughterhouse immediately after capture (5-10min for transfer) and suspended by the leg, head down, on a shackles line during 2min.

In a 4th set of experiment, a 3rd genotype, corresponding to the male line of the fast growth rate genotype (Fm) was used. The Fm broilers were also slaughtered at 42 day of age. After capture, broilers from the 3 genotypes were placed in crates at random (7 per) and subjected to one of the following 3 treatments. Transfer to the slaughterhouse either, after a stay of 240min duration in a conditioned room [35°C and 70%HR (CSIT)], or immediately following capture (2-3min transportation) and suspension on a running shackles line just before stunning (C) or for a period lasting 2min (CSI). Each of these 3 pre-slaughter conditions was applied to 30 birds from each genotype. Blood samples were collected, in heparinized tubes, at bleeding, after stunning.

Experimental data were analyzed by multifactorial analysis of the variance (ANOVA). An additional test was performed using the test of Fisher (PLSD) whenever the 5% threshold was reached.
Figure 1: Changes in corticosterone levels (ng/ml plasma) in female broilers from the S and Ff genotypes after placement in a bag for 15min, then in a crate under normal temperature (20°C) or directly in a crate and transported, or placed in room under a temperature of 32 or 35°C for 120min, or left under normal temperature (20°C) and injected or not with of 1-24 ACTH (Immediate Synacthen (IS) at a dose of 25μg/bird) at time 0. (Means ± S.E.)
Results and discussion

The objectives of the present study were to characterise the impacts, of pre-slaughter stress in genotypes of broiler differing in their growth rates, on corticotrope axis sensitivity and reactivity. Broilers having fast or slow growth rate, which correspond to genotypes used for the standard and the label type of production, respectively. At their respective slaughtering age of 42 and 84 days, basal corticosterone (B) levels were low, i.e. below 5ng/ml, for both S and Ff genotypes (Figs. 1 & 2). However, significantly (P<0.05) lower basal concentrations of B were measured for the S genotype. It is not to exclude that this difference could result of a somehow difference in their sensitivity to the capture. Indeed, while placement in a bag and constraint for 15min had no further effect, placement in the crate induced a rise in B, that was measurable in a shorter delay for the Ff genotype (at 15min [Fig. 1]) than for the S one (120min [Figs. 1 & 2]). Likewise, different kinetics were observed following transportation, with maximal levels being reached at 15 or 60min for the genotype Ff and S, respectively. Furthermore, an increase in B was only observed for the genotype Ff under the
temperature of 35°C if the birds were submitted to a single sampling (Fig. 1). Although these sets of data suggest a difference in sensibility per se between the two genotypes, we cannot conclude firmly, because different factors, such as age at slaughter and metabolic state can also impact B levels. Whatever the cause for the difference in timing for the response to physical stress, it probably originates from more central mechanisms, than the pituitary and adrenal levels, which remain to be explored. Interestingly, behaviours and activities expressed (wing flapping, vocalisations) on the slaughtering shackle line were quantified (Debut et al., 2004) and differences between genotypes observed. However, these differences do not explain the results obtained in terms of change in B levels because, in contrast, broilers of the S genotype, which had the lowest B levels, were paradoxically the ones having more activity on the slaughter line.

Transportation induced large increases and comparable maximal levels were measured for both genotypes (between 20 to 30ng/ml). These last results suggest that maximal adrenal capacities are comparable for both genotypes. Indeed, the i.m. injection of 1-24 ACTH led to a large rise in B (Fig. 2) and comparable levels, ranging between 20 to 30ng/ml, were reached 15min post-injection of IS for both genotypes. Consequently, differences in adrenal capacities cannot be accounted for the differences between the genotypes in basal levels measured after capture and in the kinetics observed following transportation and/or placement under high temperature.

The kinetics established in broilers placed under warm atmosphere, 32 (Exp 1) or 35°C (Exp 2), showed that the rise in temperature lead to significant increases in B (Fig. 1). Similarly, data from the literature indicate that high temperatures rapidly lead to a rise in B levels possibly followed by a decrease if the test is lasting long enough (El Halawani et al., 1973; Edens, 1978). However, in the present study, under the single bleeding procedure, significant increases (P < 0.05) were observed at 60 and 120min for the genotype FF, under 35°C (Fig. 1) but placement under the temperature of 32°C had no significant effect upon B levels, in both genotypes. Such an absence of effect was observed previously in quails under comparable temperatures (Hazard et al., unpublished data). Consequently, we cannot exclude that the increases observed following repeated sampling, when establishing kinetics in the present study, can possibly be the consequence of a cumulative effect of theconstraint, the repeated manipulations and the temperature. In such a case, the present result will be indicative of the potential effects of cumulative stress, each one having a too weak impact to have a measurable effect. This hypothesis is further emphasised by the results from experiment 3. In any case, the observed increases in B were limited in amplitude (±10ng/ml) and of much lower amplitudes than those which were caused by transportation or hanging on the slaughtering shackle line.

The i.m. injection of 1-24 ACTH led to a rise in B (Fig. 2). Maximal and comparable levels (20 in 30ng/ml) were reached 15min post-injection of Immediate Synacthen for both genotypes. On the other hand, a dose-response relationship was observed after injection of Delayed Synacthen, with maximum levels (approximately 25ng/ml) reached at 60min post-injection with a dose of 25μg. Therefore, the nature of the 1-24 ACTH used, i.e. IS or DS, lead to comparable maximal level reached, but it did modify B kinetics and consequently the overall amount of B released. Consequently, IS would be more appropriate in mimicking the effect of acute stress, while DS, stress of longer duration. Interestingly, one of the physical treatments, i.e. transportation, led to B increases of the same amplitude than a large dose of 1-24 ACTH, inducing maximal release. Although we have no ready explanation for the underlying mechanisms involved, such similar amplitudes in the responses to physical and pharmacological challenge have not been observed in different other species of birds such as the duck (Faure et al., 2003, Guémené et al., 2006) or quail (Hazard et al., 2005), for which responses to physical treatment doesn’t exceed half of those induced by intense pharmacological challenge. In the third experiment, significant effects of the genotype and treatment factors were observed with the lowest and highest concentrations measured for the S and Fm genotypes, respectively. In the meantime, concentration increases were observed and their respective amplitudes were dependant upon the intensity of the treatment.

In conclusion, while the 3 genotypes, used in the present study, had a similar maximal response capacity, the F genotype, having a higher growth rate, is more sensitive to physical stress whatever
their nature. As a consequence, it seems more difficult to minimise the impact of stress for the F genotype at the time of slaughter, at least according to change in B levels. Concerning the S genotype, it is possible to minimise the impact of stress by limiting its intensity or duration, for example by better controlling the temperatures to which the birds will be exposed during transportation or the duration of transport and hanging on the slaughter line. In any case, in order to limit their potential side effects, it seems desirable to limit the duration of the periods of stress notably by reducing the duration of transportation and waiting, for all genotypes.

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**References**


