Effects of cage model, density, group size, genotype and their interactions upon productivity, behaviour patterns and physiological welfare indicators in laying hens

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Abstracts: The aim of the current study was to examine the differential effects of cage models, stocking density, group size, genotype and their interactions on zootechnical performances, behaviour patterns and physiological welfare indicators, in laying hens, during the first 35 weeks of the productive period. Hens were from two genotypes (GA [n=2100] & GB [n=270]) and placed in different cage models. The first one was a conventional one (CC) and the 3 others furnished ones (FC). FC differed in their surface and equipment: perches, nest lining, scratching area. Zootechnical performances collected were mortality rates, egg laying intensities, egg laying timing, egg shell qualities. Behavioural patterns observed were nesting sites, perching, presence at feeders, dust bathing, scratching. Physiological indicators were basal corticosterone (B) levels and adrenal sensitivity and maximal reactivity. Zootechnical and physiological data were subjected to repeated measure or multifactorial ANOVA and Fisher test (PLSD) post hoc tests. Behavioural data were analysed using Kruskall Wallis or Mann and Whitney, tests. Production performances measured in furnished cages are either comparable or lower than those from standard cages. Eggs laid in the nest had comparable shell quality than those from standard cages, but others had a much poorer one. Location of the nest, distance from the scratching area, floor lining, rigid or flexible sides but also the genotype are some factors that have been identified has having a significant impact on nesting. On the other hand, the surface of the available nest area had no impact neither on the percentage of eggs laid in the nest, nor on the egg laying timing. Hens present at the feeders were not evenly distributed along it, in the furnished cages Consequently, the percentage of hens present never exceeded 80%, whatever the available length was (from 9 to 16cm/hen). The perches were highly used and associated with better foot quality. Expression of scratching behaviours was observed on the Astroturf® mat and associated with claw abrasion. Dust bath expression occurred in more than 50% of the case on this surface, but occurrence was not increase in FC compared to CC. Physiological responses indicated differences in adrenal reactivity between genotypes as well as in sensitivity and basal levels, depending upon group size and stocking density and in interaction with cage models, respectively. In conclusion, some improvements can be already suggested but further investigations would have to be done in order to set up a so called “ideal” furnished cages being economically acceptable that will provide welfare improvement for the different genotypes being commercially used.

Keywords: Laying hens; welfare; furnished cages; egg production.
Introduction

The application of the directive (1999/74/CE) implies the total ban of the conventional cages (CC) in Europe from January 1st, 2012, for all flocks of more than 350 hens. One alternate rearing system is the furnished cage (FC) which minimal characteristics were defined in the directive. The manufacturers have now taken into account these new obligations to propose various cage models. They vary in the setting, material (nest, perches, scratching area, claw shortener device etc...) as well the overall cage sizes. In a concern to respect the minimal surface per hen imposed by the regulation and to rationalise space use, the manufacturers tend to propose cages of increasing size. This evolution is not without consequence on its general organisation, as well as on the living conditions imposed to the hens and their usage of the different equipments included in the cage. Using a systemic approach, the objectives of the present experiment were to compare and evaluate different furnished cage systems differing in their overall surface, group size, and/or density, as well as the nature and the position of the equipments. The impact of the genetic factor was evaluated in a limited number of experimental modalities. Zootechnical, physiological and behavioural data were collected during the first “half” of the productive period. Among the available data, we will focus on those related to the impact of the equipments in order to be able to provide some practical advises to the breeders and the cage’s manufacturers.

II - Material and Methods

Housing conditions: The barn was subdivided into 2 rooms, each including two batteries of 4 different cage models (R1: B1 and B2, R2: B3 and B4). One model of conventional cage (CCM [B1], 96 cages, 3302cm²) and three models of furnished cages (FCMP1 [B2 and B4], 60 cages, 11340cm², nest(N)=1080cm², scratching area(SA)=1320cm², perches(P)=226, 228 or 248cm; FCMP2 [B2], 6 cages, 22680cm², N=2x1080cm², SA=2640cm², P=460cm and FCMM [B3], 24 cages, 17375cm², N=4380cm², SA=1386cm², P=302cm) were used. FCMP2 model corresponded to 2 FCMP1 cages. The FCMP1 model was declined in 4 versions differing mainly in the presence and/or position of the equipment. Nest floor consisted of a plastic mat (Astroturf®, B2 and B4), a plastic netting (B2) or of both (FCMM [B3]). Nest walls were plain and solid (FCMM [B3]) or made out of striped curtains (B2 and B4). The FC models differed also for the arrangement of the perches (2 long plastic perches in parallel to the feeder (B2, B4 and B3) or one longitudinal and 2 smaller ones (plastic or metal) in perpendicular (B4)). A plastic mat (Astroturf®) was included in all furnished models as a scratching area, whereas no dust or litter was provided. Claw shortener devices were either present (B3 & B4) or absent (B2). Group size in CCM ranged from 4 to 6 while group size were of 15 (FCMP1 [B2]), 30 (FCMP2 [B2]), 23 or 31 (FCMM [B3]) and 11, 15 or 20 (FCMP1 [B4]). Therefore, depending on the density, the available nest and scratching areas, the lengths of feeder and of perches per hen differed greatly.

Animals: Hens from two brown egg genotypes (GA [n=2100] & GB [n=270]; 18 cages CCM and 12 cages FCMP1-B2) were housed in conventional or furnished cages after transfer, at the age of 18 weeks. GB hens were transferred into the barn, 4 weeks after GA ones and consequently abruptly submitted to long photoperiod. GA hens were submitted to progressive increase and final photoperiod was of 15hrs (2.00-17.00pm). Light intensity was set at 25lux minimum, at the level of the feeders. Standard food were available ad libitum and distributed twice day (9.00 and 15.00).

Experimental data collected: The present results concern the 31th(GB) or 35th(GA) first weeks of production. Mortality, egg laying, egg shell quality (5D/7), nesting site, nest, scratching or "other" area were recorded daily. Egg laying timing was recorded hourly during the 1st 8 hours then 11 and 13 hours after light on, throughout the light period, on the 40th (GB) or 44th week of production (GA). Eggs were collected in the meantime and subjected to an "egg-cracking" test for detection of micro-cracks. The same test was realised the following week on eggs originating from a unique daily collection. Perch uses were appreciated on the 19th (GA) or 15th week of production (GB) by counting the number of hens perching (resting or perching on perches) as well as resting on cage floor. On a specific day, we realised 5 series of observation (1 hour before and, 1 and 10 hours after light-on, 1 hour before and 1 hour after light-off). On the 22nd (GA) or 18th week of production (GB), the number
of hens having the head out of the cage, over the feeders, was quantified around the times of feeding (5min before and 5, 15, 30 and 60min after). On the 47th (GA) or 43rd week of production (GB), the hens expressing the “dust-bath” behaviour were counted every 30min during an 8 hour period (between 6.30am and 2.30pm) and the site where expressed identified (nest, scratching area or "other"). Body weights, plumage conditions (Method adapted from Tauson (1984) in Laywel (2006)) 5 zones [neck, breast, back, tail, wings] with note ranging from 1(poor) to 4 (good), foot conditions (Absence/presence of keratoma, injury and/or bumble foot) and claw lengths (right and left medium taws) were measured on the 27th (GA) or 23rd week of production (GB).

**Adrenal functionality:** On the 32nd (GA) or 28th week of production, adrenal sensitivity and maximal adrenal responsiveness was evaluated 10 and 60min post-injection by measuring the changes in blood corticosterone from basal level induced by the i.m. injection of 0.5 and 10µg/kg BW of 1-24 ACTH (Immediate Synacthen, Novartis, 0.25mg/ml; diluted in 0.9% NaCl, injection volume = 0.5ml; n = 16 to 24 randomly selected hens per cage model), respectively. Samples were stored on ice, then plasma was separated by centrifugation (10mn, 2000g, 4°C) and frozen (-20°C), before running B assay (Etches, 1976).

**Statistical analysis:** Zootechnical data were submitted to an ANOVA for repeated measures and those relative to egg quality to a factorial ANOVA. Whenever, the ANOVA was significant (P<0.05), post-hoc tests were realised using the Fisher PLSD test. The behavioural variables were analysed using the non-parametric tests of Kruskall Wallis and/or Mann and Whitney. The few cages having more than 2 hens dead on the day of observation were excluded from the statistical analysis for behavioural and eggshell quality data.

### III - Results and Discussion

**Laying intensity and mortality:** As the experimental period only cover the first half of the productive period, the discussion concerning mortality and laying intensity will not be evoked in detail. Briefly, after 35 (GA) or 31 (GB) weeks of production, egg laying intensities were at least comparable to those expected for the respective genotypes in all cage systems. However, results obtained in conventional cages (CC) were comparable or significantly higher than those obtained in furnished cages (FC). A result which is in agreement with others from previous studies (Guesdon and Faure, 2004; Guémené *et al.*, 2004; Guesdon *et al.*, 2006). Interestingly, while, we did not observed any significant impact of the density, ranging between 550 and 825cm²/hen, in CC, our present results suggest that a minimum surface of 750cm²/hen is necessary to enable the obtaining of optimal performances in FC cages. Therefore, much more space per hen are requested in FC, probably resulting from difficulties to cope with a more complex environment associate with larger group sizes. As an illustration, although a definite conclusion should not be drawn before than we have a more complete picture of the full productive period, significant interactions of surface with group size were already observed at this stage of the productive period. Incidentally, we also observed a delay of at least 5 days in the onset of egg laying for the GB hens housed in FC, which will have probably not have been observed if the GB hens had not been submitted to an abrupt photostimulation instead of a progressive one. This observation is suggesting that they had more difficulties to adapt to this more complex environment and/or indirectly results from the higher group size. At this stage of the productive period, the mortalities were very limited and comparable between systems, ranging between 0 and 2%. An important variability has been however observed later; the risk and variability being increased with larger group size.

**Nesting behaviour an egg laying timing:** The percentage of egg laid in the nest ranged between 70% and 95% depending upon the experimental group. This result comforts those of previous studies (EFSA, 2005) showing that laying hens are strongly motivated to lay in the nest, if it is attractive. The overall evolution of nesting during the course of the productive period is similar for both genotypes, with an initial peak on the 4-5th week of production, then a decrease with minimum rates observed between the 8-12th week of production, followed by a gradual increase. A significant difference of approximately 10% was observed between the two genotypes. It would be of interest to know if this difference is due to the attractiveness of the nest or to a lower need for isolation of one of the genotypes. For both genotypes, the percentage of egg laid in the nest was significantly higher when
nest floor was covered with a plastic mat (Astroturf®; 75 to 95%) rather than a plastic netting (60 to 80%). The remaining eggs were somehow equally laid on the scratching area and in the rest of the cage. However, the scratching area being much smaller in surface, the present observation suggests that the hens were more inclined to lay on this Astroturf® material. The percentage of nesting was significantly lower in B3 cages (73%), than in B2 (81%) and B4 ones (84%). This difference may arise from multifactorial origin (group size, density, nest characteristics (lining, size and plain walls)). In B2 cages, the percentage of egg laid in the nest was higher in the larger cages having a double group size [30 (FCMP2) (89%)/15 (FCMP1) (83%)], while identical densities and surface of nest were provided per hen. Under our specific experimental conditions, the presence of the scratching material closer to the nest in the larger cages, while located at the opposite side in the smaller ones, did not seem to lead to conflict of motivation. On the opposite, it may have improved nest attractiveness. Large differences in the available nest surface per hen (141 or 190cm² (B3) and 98, 72 or 54cm²/hen (B4)) did not impede nor the rate of nesting, neither the egg laying timing. More than 85% of the oviposition took place between 0 and 6 hours after light on (15 to 25%/hourly) with a peak occurring between 2 to 3 after light on.

**Egg shell quality:** The percentages of dirty eggs (approximately 8%) were comparable in the various systems while, the rate of crack eggs were lower in CC (1.7%) than in FC (2.8%(B2), 3.9%(B3) or 3.1%(B4)). However, shell integrity was dependent of nesting sites and the percentages of crack eggs, among those, which were laid in the nest (B2 and B4), were comparable to those obtained from CC. This result is in agreement with previously published ones (Mallet et al., 2006) and comforts the interest of increasing nest attractiveness. Interestingly, although we will have expected the reverse situation, the percentage of crack eggs was inversely correlated to group size. The rates of micro-crack eggs were high in spite of the presence of an "egg saver" device, and highly variable, ranging from 6 to 15% (hourly collection) and from 16 to 25% (daily collection). No correlation between egg laying timing and the external eggshell quality was observed. All these results indicate that egg collection frequency is of major importance in order to minimise detrimental economical consequences. As an example, egg collector speed would have to be adjusted according to battery organisation, in particular nest location, as well as the timing of the photoperiod.

**Use of the scratching area:** It is stated in the directive (1999/74/CE) that hens should have access to litter, allowing them to express behavioural priorities, such as dust-bathing as well as foraging and scratching behaviours (EFSA, 2005); a litter being defined as crisperd material. In our systems, the scratching area only consisted in Astroturf® mat and litter was not made available. However, scratching and dust-bathing behaviour expressions were both observed. Although occurrence of the first one was not quantified, it resulted, later during the productive period, in the abrasion of claws even in the absence of claw shortener device (FCMP1 (B2)). For both genotypes, the percentage of hens dust bathing averaged 20% in CC, while 10% in the different systems of FC. The fact that hens can sham dust bathing, i.e. in the absence of substrate, have been previously reported (EFSA, 2005). It is however suggested that in such case, the sequence is incomplete and the number of attempts consequently increased. This hypothesis could have been an explanation to our findings, however, due to the high variability between cages, the observed differences were not significant. Dust bathing behaviour was often, but not exclusively, observed in the scratching area (47%: B2, 67%: B3, 52%: B4). Therefore, although, we cannot conclude on the degree of satisfaction of the hens’ behavioural needs both scratching and dust-bathing behaviours were expressed, mainly on the Astroturf® mat even in the absence of litter.

**Perches’ use and foot conditions:** When they have the opportunity to perch, hens are mainly observed perching during the scotoperiod, but also during the photoperiod (EFSA, 2005). In the present study, between 20 to 60% of the hens were observed perching during the photoperiod, generally standing. Surprisingly, a similar percentage of hens were observed standing on the perches during the scotoperiod. As 30 to 50% were in the meantime resting on the perches, up to 90% were perching in total; a result, which was comparable for the 2 genotypes and in agreement with what has been previously reported (EFSA, 2005). A limited number of hens (10%) were however resting on cage floor, even if space was available on perches. Incidentally, the percentage of hens perching was slightly, but significantly higher, when perches were placed in parallel compared to a perpendicular disposition for a given cage model (FCMP1: B4). The percentage of hens perching was also higher when available perch length per hen was of 22.5cm compared to 12,4cm, while an intermediate figure
which did not differ significantly from both, was observed with 16.5cm/hen. This result is consistent with others (Wall et al., 2004) showing no advantage of a length of 15cm compared to 12cm/hen. An impact of group size and/or perch location was observed, with an higher percentage of hens observed perching with a group size of 15 (70 to 80%) compared to one of 30 (50 to 60%).

**Body weight, plumage and foot conditions:** At this stage, body weight did not differ between CC and FC, whereas group size had a significant impact (FCMP2 > FCMP1) as well as, which is puzzling, nest lining and perch positioning. Plumage conditions exceeded the note of 15 out of 25 in all cage models and overall did not differ between hens housed in CC and FC. However, it was the lowest in one model of FC at the highest density, and a significant negative impact of the density was observed in both FC and CC. However, perches had a positive effect on foot conditions, with better results obtained in FC compared to CC, although foot condition remained in all case very good. Interestingly, these results in FC also differed depending upon perch shape (FCMP vs. FCMM). Average claw lengths were in all cases shorter than 22mm and did not differ significantly nor between CC and FC, neither between FC equipped with claw shorteners or not.

**Presence at the feeders:** The percentage of hen present at the feeder before food distribution was around 20% while up to 80% at the time of feeding. Presence then decreased gradually or abruptly in the following hour, at the onset and end of the productive period, respectively. Only minor differences were observed between genotypes. Difference in the available space at the feeder (16, 12 or 9cm/hen: B4) did not affect the percentage of hen present at the feeder, except once, immediately after food distribution, with rates significantly lower for a 9cm length. On the other hand, the percentage of hen present at the feeder in conventional cages for a 12cm length was higher than in furnished cages with 12 or 16cm of feeder/hen. In fact, hen’s distribution along the feeder was less homogeneous with increasing cage length, i.e. in FC. The period during which the hens will have limited access to the feeder was however of very short duration.

**Adrenal axis functionality:** Responses to the injection of 1-24ACTH did not differ between genotypes at 10min post injection, but levels remained higher at 60min post-injection for one genotype, thus indicating its higher adrenal reactivity. Likewise, a higher sensitivity was measured in group of 30 (FCMP2) compared to 15 hens per cage (FCMP2), although they had a similar surface per hen. Stocking density in interaction with cage model (FCMP1-B4) differing only in the position of the perches) affected corticosterone basal level. In conclusion, larger stocking density or group size, as well as their respective interactions with the cage model, can affect HPA axis functionality by increasing sensitivity. Although, no difference was reported previously between hens housed in CC and FC (Guesdon et al., 2004), the present result suggests a higher sensitivity to stressful events under specific housing conditions in FC systems. Otherwise, it remains to be explored if the differences observed between genotypes are due to differential responsiveness capacity and if it results in different sensitivity to stress.

**IV - Conclusion**

Results for the entire productive period would have to be available before drawing any definite conclusion regarding the impact of housing hens in FC, as well as the more specific respective effect of group size and density. However, as previously shown (Guesdon and Faure, 2004; Guémené et al., 2004; Guesdon et al., 2006), laying performances measured in FC, for the present period, were equivalent or lower to those obtained in CC. Egg shell quality for those laid in the nest was comparable to CC ones, while lowest for the eggs laid in the other areas of the cage. It is thus of major importance to set up conditions that will make the nest more attractive. Characteristics such as location in the cage, proximity to the scratching area, nest setting (floor lining, walls), all affects its rate of use. On the other hand, the available space/hen, in our experimental conditions, had no impact nor on its use, neither on the egg laying timing. Difference in the percentage of egg laid in the nest was also observed between genotype. Concerning feeder length, 12cm per hen in conventional cage was an optimal length, while it was not the case in furnished cages, since the percentage of hens simultaneously present at the feeder never exceeded 80%. Perches were highly used, especially during the night (over 90%) and some hens were resting on cage floor. Nevertheless, a length of 15cm of perch per hen seems appropriate even if their rates of use differ according to their location and
orientation. Interestingly, comparable percentages of hens were perching standing during the scoto and the photoperiod, but we don’t know if the motivation for such a posture is the same. Presence in the cage of an appropriate surface, for example an Astroturf® mat, allowed the expression of scratching and foraging behaviour. In approximately 50% times or more, such a surface was also used for dust bathing, but its presence or absence did not affect its rate of expression. We cannot however exclude that the sequence of this behaviour was incomplete in its absence and, in such a case, that the hen’s behavioural needs would not have been fully satisfied. Differences in stress sensitivity and behavioural preferences between the two genotypes included in the present study have been observed. Therefore, there is a need for further investigations regarding specific request for different genotypes, however, from the present results, it is already possible to propose some concrete propositions in order to optimise the set up of furnished cages.

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