SSPE-CT-2004-502315

LAYWEL

Welfare implications of changes in production systems for laying hens

Specific Targeted Research Project (STReP)

Thematic Priority: Integrating and strengthening the ERA, Area 8.1.B.1.4, task 7

Deliverable 5.4

Final report for WP5

Physiology and stress indicators

Due date of deliverable: 23
Actual submission date: 24

Start date of project: 1/1/2004 Duration: 24 months

Organisation name of lead contractor for this deliverable: INRA
Revision [Final]

Project co-funded by the European Commission within the Sixth Framework Programme (2002-2006)		
Dissemination Level		
	lic	x
	tricted to other programme participants (including the Commission Services)	
	tricted to a group specified by the consortium (including the Commission Services)	
	fidential, only for members of the consortium (including the Commission Services)	

1. Introduction.

When discussing physiology and stress indicators, the nervous and endocrine systems are of primary focus (Siegel, 1971). External and internal stimuli are channelled via the nervous system to the hypothalamus. Once a stress has been perceived, two distinct pathways involving physiological reactions are evoked: the sympathetic adrenomedullary (SA) system, which lead to a very short term response, and the hypothalamic-pituitary-adrenal (HPA) axis (Fig. 1). Depending upon the intensity/frequency of their activation, these primary responses will give rise or not to secondary (glucose, blood pressure, heart rate, etc…) and tertiary ones (immunity, growth, reproduction, etc…) (Fig. 1). A first objective of the WP5 was to provide a list of physiological parameters that have been used in order to provide objective informations on the actual stress levels of laying hens in the different studies that we will refer to (Chap. 2). The second objective was to report experimental data regarding those previously defined physiological parameters. They were used for the assessment of the hens' welfare (Chap. 3) in various housing systems including standard, enriched cages and alternative housing systems, differing in the equipments, the systems and the managements as well as the hybrids or genotypes used. The major experimental factors explored were:

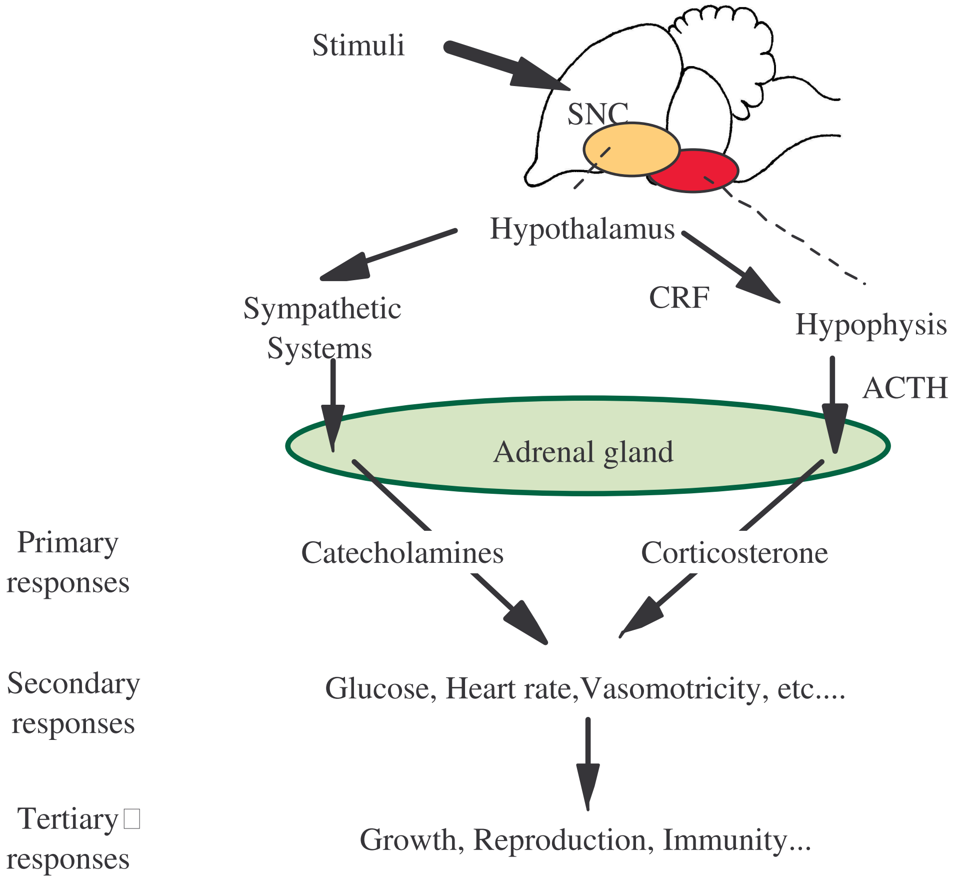
3 – 1 Comparison of housing systems during the productive period:
- Systems comparisons and effects of densities, group sizes or breeds.

3 – 2 Adaptation: interaction between rearing (growing period) and housing (laying period) conditions,

3 – 3 Feather pecking:
- Impact of beak trimming,
- Consequences of divergent selection programs for feather pecking.

In this report for the WP5, we have included a brief summary and/or the main conclusion of the different studies conducted, and, whenever available referred to a more complete communications of various types (article, manuscript to be submitted, short communications, abstract) presented in an annexe section. A specific general conclusion has been drawn thereafter from these different studies for this WP5.

Figure 1: Schematic representation of the physiological implications of stress in birds.



2. Physiological parameters identified as relevant for the assessment of acute and chronic stress state in laying hens.

Once an acute stressor has been perceived, the primary physiological responses are the activation of the sympathetic system and of the HPA axis Assessment of an activation of the sympathetic system can be done directly by catecholamine measurements in the plasma or indirectly by heart rate frequency measurements, but such measures are not easy to perform on a large scale basis, such as “commercial” conditions, especially in poultry, and for this reason can only be used in experimental approaches. It is also possible to analysed heart rate frequency variability, an analysis which provides indications about the balance between the para- and orthosympathetic systems involved in the control of the heartbeat rate. As it is accelerated by the sympathetic system and slowed down by the parasympathetic system, the analysis of the variability of the intervals between two cardiac beats makes possible to determine if the control is under the prevalence of the ortho- or parasympathetic systems and to draw hypothesis on the animal emotional state. Indeed, stresses are associated with overall increases in sympathetic tonicity whereas feed consumption or rest are associated to a parasympathetic preponderance. It is further hypothesize that parasympathetic tonicity would be associated with more passive responses and vice versa (Korte *et al.*,1999).

Once again, this approach is not easy to set up in "commercial conditions" the number of birds

Activation of the adreno-corticotrope axis of birds in response to an acute stress has been demonstrated and is reflected by an increased concentration of corticosterone in the plasma (Beuving and Vonder, 1978; Harvey *et al.,* 1980 (Fig. 1). On the other hand, various environmental conditions are also known to up or down regulate corticosterone concentrations and for example, increased concentrations in corticosteroids can also be associated with experiences that are pleasurable such as sex and food anticipation (Toates, 1995). Changes in its concentration are thus rather a preparation for an action that may be either aversive or pleasurable, that is to say associated with poor welfare and welfare enhancement in the opposite context. In brief, corticosteroids are part of the means that animals have to mobilise stores of glucose in order for actions. Extreme caution should be therefore the rule before stating firm conclusions in term of stress, after a single measurement of basal circulatory levels. On the other hand, chronic stress or repeated acute stresses induces long-term changes in the regulation of the HPA axis resulting in a hypersensitivity, then to a progressive decrease in adrenal capacity. Thus, chronic stress investigation consists in using ACTH challenges with low and high dose (Thorn et al., 1953) to address the HPA sensitivity and maximal reactivity, respectively (Landsbergis and Weiss, 1976; Koelkebeck et al., 1986; Guémené et al., 1999, 2001). With regards to variability in the responses, it is well known that they might differ depending upon the genotype (Beuving *et al*., 1989, Korte *et al*., 1997, Faure *et al*., 1996, Guémené *et al*., 2005).

Secondary physiological effects impede production of antibodies and effective cell-mediated immunity. Moreover, owing to a change in leukocyte population, an increase in the heterophil-lymphocyte ratio (H/L) is considered as a good stress indicator (Gross & Siegel, 1983). It should be also remembered that these responses could differ with regards to different genotypes. These different physiological parameters (listed above), that can provide valuable information on how an animal perceives its environment and so how suitable this environment is to the animal, have thus been considered as pertinent in the context of this work package.

The list of physiological parameters among which we will mainly refers in this report are listed below, but it should be stated that in the different studies, only appropriate ones depending upon the experimental context have been chosen

- Basal plasma corticosterone concentrations
- Corticosterone responses to physical challenges (capture or constraint for 10-15min),
- Corticosterone responses to pharmacological challenges:
    - HPA axis sensitivity: low dose of 1-24 ACTH (.5µg/kg B.W.),
    - HPA axis maximal reactivity: high dose of 1-24 ACTH (5-10µg/kg B.W.),
- Corticosterone concentrations in faeces,
- Blood cells numeration and heterophyl/lymphocyte ratio (H/L ratio),
- Humoral responses following challenge with immunogens.

Incidentally, other physiological factors such as organ weight (adrenal glands, liver, spleen), enzymes or bone strength have been used in specific appropriate experimental context.

3. Experimental data: Physiology and stress indicators.

3-1 Comparison of housing systems during the productive period: Systems comparisons and effects of densities, group sizes or breeds.

3-1-1 Humeral quality and adrenal responsiveness in laying hens reared in standard and furnished cages by GUESDON *et al.*, 2004 (INRA-SRA, Partner 5 – Annexe Manuscript 1).

In order to find out whether furnished cages contribute to improving the welfare of laying hens, humerus quality and adrenal responsiveness were evaluated in laying hens reared in standard (S) and furnished cages (F). Four cage models were used: S5, a standard cage model with 5 hens per cage; S6, a standard cage model with 6 hens per cage; F7, a furnished cage model with 7 hens per cage (with a nest, dust-bathing box, two perches, and claw-shortening) and F15, a furnished cage model with 15 hens per cage (with a nest, dust-bathing box, two perches, and claw-shortening). At 72 weeks of age, maximal adrenal responsiveness was evaluated by measuring the changes in blood corticosterone level induced by the i.m. injection of 10µg per hen of 1-24 ACTH (n = 15 hens per cage model). Hens (n = 15 to 23 hens per cage model) were slaughtered and the left and right humeri were used for measurement of weight, biomechanical characteristics in a flexion test, dry matter and ash percentage.
Basal corticosterone levels did not differ significantly while the injection of ACTH produced a significant rise in corticosterone levels ($P < 0.001$) of similar amplitude for all cage models. Humeri weights, biomechanical characteristics (elastic strain, bioyield point, stiffness and breaking strength), dry weight and percentage of dry matter were not significantly different between cage models. The humeri ash percentage was significantly ($P = 0.03$) lower in birds from the S6 cage model (57.4%) than in birds from other cage models (S5: 59.0%; F7: 58.9%; F15: 59.7%). In conclusion, adrenal responsiveness and major humeral characteristics were not significantly improved in furnished compared to standard cages in our experimental conditions.

3-1-2 Stress parameters in laying hens, beak-trimmed or not, rose in standard or furnished cages (Guémené *et al.,* 2004) (INRA-SRA, Partner 5 - Annexe Manuscript 2).

The present experiment was undertaken to compare results obtained for both production parameters and physiological stress indicators, for hens which were beak trimmed or not at 9 days of age and placed in four different models of standard (2) and furnished (2) cages. The hypothesis was that if furnished cages were going to be an appropriate alternative, they should provide better welfare conditions for the birds. ISA Brown laying hens (n = 2028) were reared on litter floor and placed in four types of cages, two standard and two furnished ones, during the productive period. Groups of 5 (S5) or 6 (S6) hens were initially housed per standard cages, whereas 15 birds were housed in each model of the furnished cages (F15M and F15P). The number of birds per cage was calculated in order to fulfil all the criteria of the directive 1999/74 EC of the European Commission (1999). Half of the birds were beak-trimmed at 9 days of age. Basal corticosterone levels, adrenal sensitivity and maximal reactivity, heterophyl/lymphocyte ratio and immune competence were assessed at the end of the productive period (70 weeks of age and over). Corticosterone measurements, heterophyl/lymphocyte ratio and antibody production in response to immune challenge show that stress levels might be higher in enriched than in standard cages and in non beak-trimmed than in beak-trimmed hens in our experimental conditions, i.e. with this specific genotype. We did not observed any systematic effect of cage type (standard vs. furnished) on the various stress indicators studied however, when significant differences are observed, they are indicating higher stress levels in furnished than in standard cages, even when used at very low densities exceeding the official requirements. It is likely that this observation does not result from a difference between cage type *per se* but of a single model effect and/or of an interaction between different factors such as cage type, group size and genotypes. Our results also indicated that, when tendencies or significant beak effects are observed, beak-trimmed hens are showing lower stress levels. This result is further emphasised by behavioural data, showing that these beak-trimmed hens also expressed lower fear reactions than intact hens (Guesdon, 2004). Taken together, these results would suggest that beak trimming results in possibly better welfare for this specific genotype.

3-1-3 The effects of cage model, density, group size and genotype by Guémené *et al.,* (unpublished data) (INRA-SRA, Partner 5 - Annexe Manuscript 3).

The aim of the current study was to examine the differential effects of cage models, stocking density, group size, genotype and their interaction on corticosterone basal levels as well as adrenal sensitivity and maximal reactivity. One model of standard cage (SC) and 3 models of furnished cages (FC) were located in two different rooms of a same barn. Group size in SC ranged of 4 to 6 on a surface of $3302cm^2$. In FC, group size were of 11, 15 or 20 in one model (FCPi A, B, C, D & E:$11340cm^2$), 30 in a second model (FCPi C: $22680cm^2$) and, 23 and 31 in a third model (FCM: $17375cm^2$). FC differed in their equipment: perches, nest, scratching area. On the 32 week of lay, adrenal sensitivity and maximal adrenal responsiveness was evaluated 10 and 60 min 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 (n = 16 to 24 hens per cage model), respectively. Responses to the injections neither did nor differ between genotypes at 10min post injection but levels remained higher after 60min for one genotype indicating a higher adrenal reactivity (Fig. 1). Likewise, a higher sensitivity was measured in group of 30 compared to 15 birds per cage, although they had a similar surface per hen (Fig. 2). Stocking density in interaction with cage model (FCPi C & D differing only in the position of the perches) affected corticosterone basal level (Fig. 3). In conclusion, larger stocking density or group size, as well as their respective interactions with the cage model, can affect HPA axis functionality, a result which suggests a higher sensitivity to stressful events under these housing conditions. On the other hand, it remains to be explored if the differences observed between genotypes are due to differential responsiveness capacity per se and if it can result in different sensitivity to stressful conditions.

3-1-4 The effects of cage model, genotype and rearing treatment by Levrino *et al.*, & unpublished) (UNIZAR, Partner 4 - Annexe Manuscript 4).

The aim of the current study was to examine the differential effects of housing system, rearing system and breeds by measuring corticosterone metabolites levels in faeces, a physiological indicator of chronic stress. Four experiments were realized. In the first one, brown hens from two different breeds were placed in three different housing systems (2 models of furnished cages and 1 conventional one; n ≥ 240 x 3 x 2). In the second one, hens from a unique breeds (n = 320) were originally reared on floor or in cages and placed in a single model of furnished cage. In the third one, hens from two different breeds (White (Leghorn) vs. Brown (Rhode Island); n = 160 x 2) were reared in cage and placed a single model of furnished cage. In experiment 4, two breeds and two housing conditions (Furnished and conventional cages) were look at in a factorial approach. Corticosterone levels were evaluated after extraction and measurement of its metabolites present in faeces, using an antibody directed against 5β–pregnane, 3-α, 11β–diol 20one. In experiment 1, higher levels were measured in conventional than in furnished cages, and within the two models of furnished cages, the lowest data were measured in the "Spanish" model (Table 1). It could have been concluded from these results that the conventional cages are a more stressful environment and that differing stress states can results from the use of furnished cages. However, although the same situation prevailed by the end of the laying period for one genotype out of two in experiment 4, the reverse situation was observed for both genotypes earlier in the laying period (Table 4). Therefore, it is not to exclude either that the present data resulted from differences in the bird metabolic states due, for example, to differing levels of physical activity and/or feed intake, two parameters that were not looked at in the course of these studies. In experiment 2, floor-rearing hens presented significantly higher corticosterone levels than cage rearing hens when placed in cages during the laying period. These results suggest that hens reared in cages will better adapt to cage systems latter than those reared in floor systems. Data from experiments 3 and 4 showed significant breed effects with white breeds having higher corticosterone levels than brown ones (Table 3) and differences between brown breeds with interaction between the age and housing system factors (Table 4). The question being whether hens from a specific breed have higher basal levels, related from example to different overall metabolites rates, or if they are more stressful/stressed in these specific experimental conditions? From the present data, it is thus not possible to conclude from the differential results obtain with different breeds, but the existence of such differences should be taken into account when evaluating and comparing adaptation of hens to different housing systems across laboratories. On the other hand, the harmonisation of the pre-laying rearing environment with the one used later appears to be a major focus to ensure a better adaptability of the hens during their productive period.

3-1-5 The effects of stocking rate and modified management on the welfare of laying hens in Non-Cage Systems by Zimmerman et al., 2005 & Nicol et al., 2005) (UNIBRIS & ADAS [funded by DEFRA project AW0233]**,** Partners 3 & 7 - Annexe Manuscript 5),

The aim of the current study was to examine the differential effects of stocking density, flock size and management on physiological indicators of welfare of laying hens under commercial conditions. This study used a broad range of physical and physiological indicators to assess the welfare of hens (113,400 beak-trimmed Shaver laying hens in total) in 36 commercial flocks originating from 7 rearing flocks and housed in commercial single-level non-cage systems (6 flocks per treatment). Three stocking rates were compared (7, 9 and 12 birds m^{-2}). At the highest stocking rate birds were kept in small (n= 2450) or large (n=4200) flocks. Additionally, at 12 birds m^{-2}, in both small and large flocks, birds were subjected to either standard (SM) or modified (MM) management. Bone strength, fracture incidence, heterophil:lympocyte (H:L) ratio, live weight, organ weights, serum creatine, serum osmolality, muscle pH and faecal corticosterone were measured on samples of birds at the end of the rearing period and at the end of lay (50 birds in each flock). During the laying period, mortality, production and integument condition were recorded at regular intervals.

The most striking finding of the post-mortem analyses was the high prevalence of old fractures in birds from all treatments. It was known from the rearing flock results that, at the end of the rearing period, no birds had sustained fractures. However, by the end of the laying period the percentage prevalence of fractures ranged from 56 to 68 but was not significantly influenced by treatment. Increased liver and decreased spleen weights tended to occur in the birds at the lower stocking densities, suggesting theses birds may have been more stressed. H/L ratio, CPK and faecal corticosterone were not affected by treatment effect, although large variability within flock or between rearing conditions were observed. In conclusion, there were no clear effects of flock size on the welfare indicators recorded in single-level non-cage systems. On the other hand, while fracture incidence was negligible and H:L ratio within a normal range at the end of the rearing period, fracture incidence was 60% and H:L ratio was high across all treatments, by the end of lay, suggesting an overall deterioration in the welfare of these birds under all conditions.

3-1-6 Welfare of laying hens kept in cages and in aviaries: physiological indicators of stress and fearfulness by Colson et al., 2005 (AFSSA & INRA-SRA, Partner 5 - Annexe Manuscript 6),

In the context of the setting of the directive 1999/74/CE, we compared welfare of laying hens kept in three housing systems: hens **F**loor-reared then kept in conventional **C**ages (**FC**), hens **F**urnished-floor reared then kept in a laying-**A**viary (**FA**) and hens **A**viary-reared then kept in a laying-**A**viary (**AA**). Welfare was evaluated by several parameters that are presented in two complementary communications. This paper deals with stress response to transfer in laying system (Heterophiles to Lymphocytes ratio [H/L] before and after transfer), stress response to housing conditions (H:L at the end of the laying period and increase of corticosterone level after injection of 1-24 ACTH, 10µg/kg) and level of fearfulness (response to a novel environment at the end of the laying period). After transfer, H/L in FC and FA hens was higher than in AA hens ($p<0.001$). At the end of the laying period, H/L was higher in FC than in AA hens ($p=0.071$). After transfer, corticosterone level after the injection of 1-24 ACTH increased less in AA than in FA hens ($p=0.053$). FC hens were the less mobile, the less active and the less explorative in the novel environment test ($p<0.001$). H/L indicates that transfer stresses more FC and FA hens, and that FC hens are more stressed at the end of the laying period. H/L results are not confirmed by corticosterone, but they are confirmed by the higher fearfulness of FC hens at the end of the laying period. In conclusion, under the present conditions, global level of stress seemed to increase between AA, FA and FC hens, and FC hens were more fearful. Both strong stress responses and fearfulness are linked to adaptation' difficulties. Therefore, the risk of impaired welfare increases between AA, FA and FC hens.

3-2 Adaptation: interaction between rearing (growing period) and housing (laying period) conditions.

3-2-1 Adrenal- and immune responsiveness in laying hens: Interactions between housing system and pre-laying rearing conditions by Moe et al., 2004 & submitted

(Norwegian School of Veterinary Science & INRA – SRA, Partners 5 & 6 - Annexe Manuscript 7)

The objectives of the present study were to study the effects of housing conditions during the laying period in interaction with pre-laying rearing conditions on various indicators of welfare, in white, not beak trimmed laying hens. Hens were kept on deep litter with no perches, or in conventional rearing cages during the pre-laying rearing period (0-16 weeks of age), and then housed in furnished cages (n = 8 hens per cage/ 12 cages) or conventional cages (n = 3 hens per cage/ 36 cages) until end of the productive period (76 weeks of age). Forty hens per cage and rearing alternative (i.e. a total of 160 hens) were included in the experiment. Adrenal reactivity was assessed by measuring corticosterone following a challenge with 1-24 ACTH at 50 weeks of age, humoral immune response by measuring antibody responses to immunization with sheep red blood cells (SRBC) and key hole limpet hemocyanin (KLH) at 60 weeks of age in the same individuals, and chronic stress by the measurement of heterophil/lymphocyte (H/L) ratio at 70 weeks of age in other individuals. Adrenal reactivity and anti-SRBC titre were unaffected whereas anti-KLH titre ($p \leq 0.001$) and H/L ratio ($p \leq 0.01$) was affected by housing system. Adrenal reactivity was unaffected, H/L ratios were close to significantly affected ($p \leq 0.06$) and anti-SRBC titres ($p \leq 0.0004$) and anti-KLH titres ($p \leq 0{,}002$) were significantly affected by pre-laying rearing conditions. In conclusion, several physiological endpoints of welfare indicators were significantly affected by the pre-laying rearing environment and to some extent by the housing environment during the laying period. Several interactions were also found. It was however not possible to make a clear statement regarding the welfare situation in furnished vs. conventional cages based on the chosen indicators. The potential risk of confounding interpretation of welfare in differential housing systems due to effects of factors in the pre-laying rearing environment is important. Last but not least, optimising the pre-laying rearing environment is one important future focus to ensure animal welfare in hens to be housed in cage alternatives during their productive period.

3-2-2 H/L ratio measurement as an assessment of welfare in laying hens: Interactions between housing system and pre-laying rearing conditions by Wall & Tauson, 2005 (SLU, Partners 6)

The objectives of the present study were to analyse H/L ratio of non beak trimmed hens from LSL and LB genotypes placed in three different housing systems (FCS - 8 birds/cage; multi-tiered aviary system and a traditional single tiered system). In the FCS birds were reared (0-16 w.) in cages or in aviaries while for the floor laying systems they were all reared on floor. Birds were bled at 17 w. and 61 w. At both ages the hens from the LB genotype showed higher H/L ratio than the LSL one. At 16 weeks (one week after collection from the rearing site) the aviary reared hens of both genotypes in the FCS had higher H/L ratio than the cage reared but the former also had the same levels as the aviary reared birds placed in the multi-tiered laying system. At 61 weeks of age there were no remaining differences between rearing systems.

In conclusion, the homogeneity of the system used during the rearing and the laying periods is of key importance in order to limit stress levels. However, it could not be excluded either that the effect of floor rearing per se and/or of the collection procedure of these birds in comparison to the rearing in cages is more important than the move from floor to a cage. It seems that apart from the reported remaining differences between genotypes at 61 weeks of age, the earlier differences after 44 weeks of being kept in the respective laying systems levels out, i.e. that some kind of adaptation occurred in the present study.

3-2-3 Other studies

Although, it was not the primary focus of those studies, specific results regarding the problem of adaptation emerging from the transfer from the rearing systems to the housing condition during the laying period were also obtained in several of the studies mentioned above (**3-1-3, 3-1-4, 3-1-6**).

The main common conclusion is that the rearing and the housing conditions should be the more comparable as possible in order to minimise acute but also chronic stress resulting from the transfer in different environments.

3-3 Feather pecking:

Feather pecking occurrence will adversely affect plumage condition, productivity (Hughes and Duncan, 1972) and indirectly induce an increase in feed consumption in order to compensate for the energy losses (Leeson and Morrison, 1978). Feather pecking can also be the primary cause of lesions that in turn will stimulate the occurrence of cannibalism and the source of mortality (Blockhuis and Wiepkema, 1998). The underlying mechanisms of feather pecking are not known although the genotype background can account for. Its multifactorial origin make it difficult to control especially after its onset in a flock and a very effective way to prevent its expression is the practice of beak trimming, but its usage is ban in some Nordic European countries while still tolerated in the others. One way, to solve this dilemma is to select genotype of birds that will not express this behaviour. In this chapter, we will report data concerning the impact of beak trimming on physiological indicators of welfare throughout a reproductive period in laying hens in the first paragraph and the consequence on the HPA axis functionality of two independent divergent selection programs for feather pecking in a second one.

3-3-1 Impact of beak trimming upon physiological indicators of stress

(Guémené *et al.,* 2004) (INRA-SRA, Partner 5 - Annexe Manuscript 2).

This was one the objectives of the study presented above in paragraph 3-1-2. In some Northern European countries, the practice of beak trimming is already banned although it is presently used in most laying operations to prevent the possible dramatic consequences of feather pecking and/or cannibalism. Such consequences were unfortunately observed in the present study and resulted in high mortality rates. Concerning the physiological indicators of stress, the results of the present study indicated that, when tendencies or significant beak effects are observed, beak-trimmed hens are showing results that could be interpreted as if they had lower levels of stress. This result is further emphasised by behavioural data, showing that these beak-trimmed hens also expressed lower fear reactions than intact hens (Guesdon, 2004). Moreover, whatever the cause is, beak trimmed hens showed higher immune responsiveness which is an indication of their higher capacity to respond in case of challenge and indirectly of an improved welfare state. Taken altogether, these results would thus suggest that beak trimming resulted in a possibly better welfare for this specific genotype under the present experimental conditions. Although the genetic factor was not included in the present study, it is known that among the multifactorial causes, large differences between breeds in the tendency to express feather pecking behaviours and cannibalism exist. The selection against its occurrence could be one effective way to solve the problem although it is not such an easy task to set it up in practice. At present, caution should be taken if beak trimming practice was to be ban for all genotypes and housing systems.

3-3-2 Consequences of divergent selection programs related to feather pecking

In this paragraph, we will refer to the results obtained by corticosterone level measurements in terms of adrenal responsiveness and basal functionality, in two independent studies aiming to divergently select against feather pecking behaviour using a direct or an indirect approach.

3-3-2-1 Plasma corticosterone levels in male chickens from two divergently selected lines of broiler for feather pecking by Guémené et al., (unpublished data) (INRA-SRA, Partners 5 - Annexe Manuscript 8)

Recently published data suggest that feather pecking trait is associated with specific adrenocorticotropic axis reactivity in egg laying lines (Korte *et al*., 1997; van Hierden *et al*., 2001). However, these results originated from comparisons between poults (van Hierden *et al*., 2001) and laying hens (Korte *et al*., 1997) of independent lines having different genetic backgrounds. Therefore, the reported differences may not be directly associated with this behavioural trait. The present study was thus undertaken to further investigate this possible functional relationship.
Male chickens originating from the 3rd generation of two divergently selected lines (FP+ & FP- : n=30x2), of a slow growth rate strain of broiler were used. Selection was realised using a slightly modified procedure of a previously reported method (Bessei, 1996). In brief, the birds are selected depending upon their tendency to peck to a fake with feathers (Picometer) at adult age and while raised in individual cages. For this study, the progenies were raised collectively in two independent rooms. Basal corticosterone levels and, responses to a physical constraint and a pharmacological challenge were compared at the age of 21 weeks. Blood samples were collected from the wing vein, before, 15 and 60 min after i.m. injection of an ACTH agonist (1-24 ACTH: immediate synacthen [IS], Novartis, 40µg/birds, vol =1ml, NaCl 0,9%) and/or physical restraint. Plasma samples were assayed for corticosterone using a specific RIA (Etches, 1976).
Basal corticosterone levels were significantly (P=.02) higher for chickens from the FP- group (4.5±.4 vs. 3.1±.3ng/ml). Increases were observed under both treatments and for both delays compared to basal levels. Alternately, genetic origin had no significant effect at 15 min. whereas much higher levels (P<.001) were measured following IS challenge (28.1±2.9 vs. 7.1±.9). After a delay of 60min., significant decreases (P<.05)

were observed but chickens from the FP- group showed higher concentrations under both treatments (9.2±.9 vs. 5.9±.6). Furthermore, a complementary genetic sub-classification of the broilers (FP++, FP+, FP- & FP--) showed a good hierarchy between this classification and corticosterone levels measured at the 60min. delay under both treatments (FP -- ≥ FP - ≥ FP + ≥ FP ++).
The present results emphasise the hypothesis that a change in the HPA axis' functionality is associated with feather pecking trait. Chickens from the higher feather pecking line showing comparable maximal reactivity but lower basal levels and different response kinetics. Further studies will be necessary in order to elucidate if these results apply to both sexes and at different physiological stages.
It should be stated here that, when raised collectively during the rearing period, these genotypes appeared to have exactly the reverse phenotypic classification in term of feather pecking; FP- birds appearing feather pecker and vice versa.

3-3-2-1 Stress reactivity in laying hens as affected by genetic selection by Kjaer & Guémené, (unpublished data) (DIAS & INRA, Partners 4 & 5, Annexe Manuscript 9)

Feather pecking in domestic fowl is a behavioural disorder that consists of pecking directed at and damaging the feathers of other birds. Feather pecking is considered a ”multi-factorial” problem because a number of causative factors have been found, environmental as well as genetic. The aim of this study was to compare adrenocortical activity and reactivity to an acute stressor (manual restraint) white Leghorn hens of the sixth generation of lines divergently selected on feather pecking behaviour (FP+ and -) and from a control line. Corticosterone levels (ng/ml plasma) were measured at 85 weeks of age, before being submitted to any treatment (basal level), after manual restraint during 10min and 10min post-injection of 1-24 ACTH at a dose of 10µg/kg B.W..
Hens from the three lines had comparable basal levels of corticosterone and overall adrenal capacity, while they differed in the reactivity to handling. Thus, FP+ hens showed levels after handling during 10min than FP- hens, while controls hens showed intermediate levels.

The results of this study indicated that a divergent selection program using a direct measure of feather pecking bouts had an effect on the HPA reactivity. This finding contradicts those of previous studies which reported higher responsiveness for FP- hens. The, FP+ hens showed higher corticosterone levels after handling during 10min than FP- hens, while controls hens showed intermediate levels.

However, results for poults (van Hierden *et al.*, 2001) and laying hens (Korte *et al.*, 1997) originated from independent lines having different genetic backgrounds. The observed effects were possibly only coincidental results. On the other hand, results from Guémené *et al.*, (manuscript 8) originated from comparisons between birds selected on their capacity to peck a fake. It was incidentally found that when raised collectively during the rearing period, the FP- will express significantly more feather pecks and vice versa. The results of the two studies in which hens from divergently selected lines for feather pecking (laying and broiler respectively) were used are therefore coherent.

4. Conclusion WP5: Physiology and stress indicators.

The data compiled in the present workpackage originated from 16 independent experiments and were provided by five LayWel's partners. As a consequence, the experiments differed first in their scientific objectives but also in numerous other aspects including rearing and housing conditions or densities, as well as the genotypes which entered the study, which could make difficult, if not impossible, to reach firm conclusions. Indeed, most of the physiological parameters chosen in a specific study have been found to be affected. However, depending upon which, it could have been possible to conclude for example that welfare was improve, comparable or decrease in enriched cages or alternative systems compared to standard cages. Alternately, this general statement should not be interpreted as if physiological indicators are not relevant to access welfare. Indeed, it illustrates the risk of misinterpretation that can result by taking into account a single or a limited number of welfare indicators of the same category and-or to conclude from a single study or by concluding using only one genotype. Moreover, some original and interesting findings come out of this work package.

First, it is of major importance to keep in mind before to conclude firmly that the responses measured for the different physiological indicators differ pending upon genotypes and/or the period of lay. Therefore, differing levels of responses should not be misinterpreted. Moreover, results originating from different independent studies have shown that there is strong interactions between the physiological responses measured during the laying period and the rearing conditions to which the poults were submitted to during the growing period. This observation clearly indicates that this initial period of life is of primary importance for a better adaptation of the hens to their future housing conditions and consequently their welfare. A second major finding is that there is no evident negative effect of the density in floor system or of the cage system (conventional vs. furnished) as such, whereas one specific cage model can be at the origin of different responses. Fourth, consequences of beak trimming can be controversial in term of welfare and care should be taken before banning this possibility to prevent feather pecking and cannibalism occurrence in some strains. Last but not least, in this context the selection against feather pecking is an interesting approach, which have been shown to be successful and such selection programs seems to be positively associated with lower HPA axis reactivity to stress in low feather pecking hens.

Acknowledgement
This report has been co-financed by the European Commission, within the 6th Framework Programme, contract No. SSPE-CT-2004-502315. The text represents the authors' views and does not necessarily represent a position of the Commission who will not be liable for the use made of such information

References: (References of the articles issued from the studies included in this report)

Colson S., Arnould C., Guémené D. & Michel V., 2005. Bien-être de poules pondeuses logées en cage ou en volière : paramètres physiologiques et comportementaux. 6èmes Journées de la Recherche Avicole, St Malo, France, March 30-31 Ed ITAVI, Paris (FRA).

Guémené D, Couty M, Guesdon V, Moe RO, Lervik S, Michel V & Faure JM. (2004) Bien-être des poules pondeuses en cages conventionnelles ou aménagées : quels sont les enseignements des essais conduits en station expérimentale ? Journée Nationale ITAVI des Professionnels de la pondeuse et de l'œuf de consommation, 9 Décembre 2004, Ploufragan, France. 11p.

Guémené D, Couty M, Guesdon V, Moe RO, Lervik S, Michel V, Colson S, Faure JM, Buil T, Chacon G, Maria G, Cepero R, Wilkins LJ, Brown SN, Zimmerman PH, Nicol CJ. 2005. Physiological indicators: contribution to the welfare assessment in various housing systems for laying hens. 7th European Symposium on Poultry Welfare, Lublin (Poland), 15-19 juin 2005 (Poster). Animal Science Papers and Reports 23(1): p269-270.

Guémené D, Couty M, Guesdon V, Moe RO, Lervik S, Michel V & Faure JM. (2005) Impact sur plusieurs indicateurs de bien-être des systemes « cage » et des pratiques d'élevage chez la poule pondeuse. 1ères Journées d'Animation Scientifique du Département PHASE (INRA), Tours, France, Mars 15-16. p223.

Guémené D, Couty, M. & Simon, E. (2005) La cage idéale : avec quels aménagements et pour quels génotypes ? Journée Nationale ITAVI des Professionnels de la pondeuse et de l'œuf de consommation, 8 Décembre 2005, Ploufragan, France. 9p.

Guémené D, Guesdon V, Moe RO, Michel V & Faure JM. (2004) Production and stress parameters in laying hens beak-trimmed or not, housed in standard or furnished cages. *In :* 22nd World's Poultry Congress; Istanbul (TUR); 2004/06/08-13, Proceedings:321(abstract), CDROM:[/1225.pdf] 4p. WPSA, turkish branch, Izmir (TUR).

Guémené D, Guesdon V, Moe RO, Michel V & Faure JM. (2005) Paramètres de production et indicateur de bien-être chez des poules pondeuses épointées ou non, élevées en cages standard et aménagées. 6 èmes Journées de la Recherches Avicoles, St Malo, France, March 30-31, 2005.

Guémené D., Guesdon, V., Moe, RO., Michel, V., Faure, JM., 2005. Paramètres de production et indicateur de bien-être chez des poules pondeuses épointées ou non, élevées en cages standard et aménagées. 6èmes Journées de la Recherche Avicole, St Malo, France, March 30-31 Ed ITAVI, Paris (FRA). Abstract p 29. Full text (CD Roms) p82-86.

Guesdon V. (2004) Etude comparative de poules pondeuses épointées ou non élevées en cage standard ou aménagées : estimation multicritères du bien-être. Thèse U. Rennes I, 220p.

Guesdon V, Leterrier C, Constantin P, Guémené D, Couty M & Faure JM. (2004) Humeral quality and adrenal responsiveness in laying hens reared in standard and furnished cages. Animal Research, 53: 235-243.

Moe RO, Guémené D, Larsen HJS, Bakken M, Lervik S, Hetland H & Tauson R. (2004) Effect of pre-laying rearing conditions in laying hens housed in standard or furnished cages on various indicators of animal welfare. *In :* 22nd World's Poultry Congress; Istanbul (TUR); 2004/06/08-13, Proceedings:329(abstract), CDROM:[\1619.pdf] 4p. WPSA, turkish branch, Izmir (TUR).

Moe RO, Guémené D, Larsen HJS, Bakken M, Lervik S, Michel V. & Tauson R. Adrenal- and immune responsiveness in laying hens: Interactions between housing system and pre-laying rearing conditions. Submitted for publication to British Poultry Science.

Nicol, C.J., Brown, S.N., Glen, E., Pope, S.J., Short, F.J.,* Warriss, P.D., Zimmerman, P.H., and Wilkins, L.J. (2005) Effects of Stocking Density, Flock Size and Management on The Welfare of Laying Hens in Single-Tier Aviaries. Accepted for publication in British Poultry Science.

Simon, E. (2005) Influence du modèle de cage, de la taille de groupe et du génotype sur des critères zootechniques et comportementaux chez la poule pondeuse élevée en cage aménagée. Mémoire ESA d'Angers. 34p.

Wall, H. & Tauson, R. 2005a. Uppfödningen har betydelse för värphöns i inredda burar. Fjäderfä 2005 (8): 24-26.

Wall, H. & Tauson, R. 2005b. Produktion, befjädring och stress hos två hybrider i olika inhysningssystem. Fjäderfä 2005(10): 58-60.

Zimmerman, P.H., Brown, S.N., Glen, E., Lindberg, C., Pope, S.J., Short, F.J., Warriss, P.D., Wilkins, L.J. & Nicol, C.J., (2005) The effects of stocking rate and modified management on the welfare of laying hens in Non-Cage System. 7th European Symposium on Poultry Welfare, Lublin (Poland), 15-19 juin 2005. Animal Science Papers and Reports 23(1): p181-188.

Final activity report: Annexes for WP 5,

Physiology and stress indicators.