FEATHER PECKING BEHAVIOUR AND STRESS RESPONSE IN LAYING HENS : A QTL-ANALYSIS

A.J. Buitenhuis[1], T.B. Rodenburg[2], Y.M. van Hierden[4], B. Ask[1], M. Siwek[1], S.J.B. Cornelissen[1], M.G.B. Nieuwland[3], H. Vos[1], P. de Groot[1], S.M. Korte[4], P. Koene[2], H. Bovenhuis[1] and J. J. van der Poel[1]

[1] Animal Breeding and Genetics Group
[2] Ethology Group
[3] Adaptation Physiology Group, Wageningen Institute of Animal Sciences, Wageningen University, P.O. Box 338, NL-6700 AH Wageningen, The Netherlands
[4]Department of Behaviour, Stress Physiology and Management, Institute for Animal Science and Health (ID-Lelystad BV), P.O. Box 65, NL- 8200 AB Lelystad, The Netherlands

INTRODUCTION

The concern for animal welfare in the west European countries results in a change in housing systems. In poultry, the free-range housing systems allow the birds to walk around freely. However, one major drawback is that feather pecking (FP) behaviour occurs. This behaviour can cause feather damage to the birds and ultimately the birds can be pecked to death. The increased feed intake due to the loss of feathers results in economic losses. To prevent the damage of FP, beak-trimming is a common used method (Gentle, 1986) as well as using a dimmed light scheme (Blokhuis and Wiepkema, 1998). However, in some countries beak-trimming is prohibited or will be prohibited in the near future. Therefore, it is important to identify the underlying factors for this behaviour.

Although environmental factors play an important role in FP behaviour, differences in FP behaviour between strains suggest a genetic basis. The heritability estimates for FP range from 0.04 (no S.E. mentioned) to 0.56±0.25, depending on age and method of recording (e.g. scoring of plumage condition, direct observations) (Cuthbertson, 1980 ; Bessei, 1995 ; Kjaer and Sørensen, 1997 ; Kjaer *et al.,* 2001 ; Rodenburg *et al.,* 2002). The possibility to select for FP behaviour seems feasible, as using the number of performed bouts as a selection criterion for FP has been succesful (Kjaer *et al.*, 2001). Craig and Muir (1993) have succesfully reduced mortality due to beak inflicted injuries based on group selection. Selection lines are useful to identify underlying genetic, immunological and physiological factors for FP behaviour. Differences in immune-response, serotonin levels and stress hormones as a consequence of selection for a reduced mortality have been reported (Cheng *et al.*, 2001a,b). Korte *et al.*, 1997 reported a difference in physiology (corticosterone level) between a high FP and a low FP line. More recently, van Hierden *et al.*, (2002) found that the dopamine and serotonin turnover was lower in a high FP strain than in a low FP strain. Suggesting that differences in the development and performance of FP between the two lines are associated with a difference in physiology and neurobiology.

The use of molecular genetics can be of great value to identify the chromosomal regions and genes involved in behaviour and stress response. The aim of this paper is to describe a QTL mapping experiment to identify chromosomal regions involved in behaviour traits in laying hens and to estimate the h^2 of the corticosterone response after manual restraint.

MATERIAL AND METHODS

Experimental population. The F2 population originates from a cross between randomly chosen birds of a high FP line (HFP) and a low FP line (LFP). Six males from the HFP line were mated to 6 females from the LFP line and 6 males from the LFP line were mated to 6 females from the HFP line to generate 120 F1 animals. From the F1 animals 4 males from the HL cross were selected and mated to 16 F1 females from the HL cross. Three males from the LH cross were mated to 12 F1 females from the LH cross. In total 650 F2 animals were produced. On average there were 92 progeny per male and 23 progeny per female bird.

Rearing and housing conditions of the birds. The F2 hens arrived at the experimental farm in 5 batches in a 2 week interval. The birds were not beak-trimmed and each individual bird had a wing-band. Each batch was divided over 2 pens, giving a total of 10 groups with an average of 63 birds per group. The floor area of the pen was 4.75 x 2 meters and covered with wood-shavings. Each pen had 2 light bulbs (2 x 40W) and a heating lamp. From week 0 to 4 there was continuous light, while in week 5 to 6 the scheme was changed to 8 hours light per day from 8:00 to 16:00 hours. Food and water was provided *ad libitum*. At 30 weeks of age the birds were housed individually.

Phenotyping of the F2 population. Feather pecking test. A social FP test (30 min) was performed at 6 weeks of age and repeated at 30 weeks of age in a square open-field of 1.25 x 1.25 meters. The FP behaviour was directly recorded from an observation room.

Manual restraint test. At the age of 32 weeks, the hens were exposed to manual restraint. The bird is placed on its side for 5 min. A blood sample (1ml) was taken from the wing veine after 5 min between 9:00 and 12:00 h. The blood samples were transferred to EDTA-coated centrifuge tubes and chilled on ice (0°C) and centriguged at 3000 rpm for 10 min at 4°C. The supernatant was stored at 4°C untill analysis. Corticosterone concentration was determined in duplo as described by de Jong *et al.* (2001). During the 5 min of the manual restraint the number of struggles (attempt to escape) were counted.

Genotyping of the F2 population. In total 180 microsatellite markers were chosen equally distributed over the chicken genome, approximately 20 centi Morgan (cM) apart. PCR reactions were performed in a total volume of 12 µl containing 10 to 60 ng genomic DNA, 1.5 mM $MgCl_2$, 50 mM KCl, 10 mM Tris-HCl pH = 8.3, 1 mM tetramethylammonium chloride (TMAC), 0.1% Triton X-100, 0.01% gelatin, 200 µM dNTP, 0.25 U Goldstar polymerase (Eurogentech), 2.3 pmole of each primer and covered with 10 µl mineral oil (Sigma). PCR programme used is: 2 min at 95°C and 35 cycles of 30 s at 95°C, 30 s (annealings temperature (45°C - 60°C), and 30 s at 72°C, and followed by an elongation step of 3 min 30 s 72°C. PCR products were pooled in a total volume of 50 µl. A mixture of 1 µl pooled PCR product with 1.6 µl loadingbuffer (containing 80% formamide and GENESCAN-350 TAMRA) was loaded on a 6% denaturing polyacrylamide gel (Sequagel 6, National Diagnostics) on an ABI 373. Fragment sizes were analyzed with GENESCAN fragment analysis software (Perkin-Elmer, Applied Biosystems). Allele calling was performed using GENOTYPER 2.0 software (Perkin-Elmer, Applied Biosystems).

Statistical analysis. The procedures PROC FREQ and PROC UNIVARIATE from the SAS software package (SAS, 1996) was used to investigate the distributions and basic statistics of the traits. ASREML (Gilmour *et al.,* 2000) was used to estimate the variance components for the response of corticosterone to manual restraint.

The model used was :
$Y_{ijkl} = \mu + PERS_i + BATCH_j + A_k + PERM_l + e_{ijkl}$
Y_{ijkl} : the individual observation ; μ : the average of the trait observed ; $PERS_i$: the fixed effect of the i' th person (i = 1,2,...,8) ; $BATCH_j$: the fixed effect of the j' th BATCH (j = 1,2,...,5) ; A_k : the random effect of the k' th animal (k= 1,2,...,706) ; $PERM_l$: permanent environment of the k' th animal (l = 1,2,...,649) ; e_{ijkl} : the residual effect

RESULTS AND DISCUSSION
The manual restraint test was performed to investigate the relation between the stress response and FP behaviour. The average corticosterone level (ng/ml) after manual restraint was 4.39±0.11. The distribution of the corticosterone data (Figure 1) seemed normal distributed. The reciprocal F2 cross presented here offered the possibility to make a contrast between the sex chromosome. Since we used female birds only in our observations, the HL group represented the Z^HW^L, Z^LW^L chromosomes and the LH group represented the Z^HW^H, Z^LW^H chromosomes. Taking the repeated measurement into account, the difference between the average of the HL and LH group was -0.32 (NS).

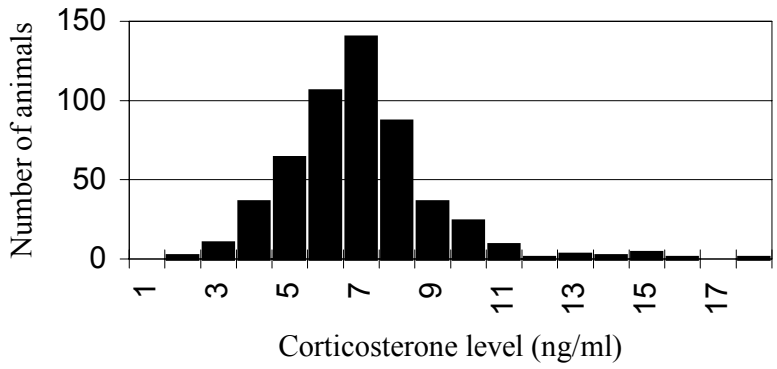

Figure 1. Frequency distribution of the average corticosterone level (ng/ml) after manual restraint, based on 2 repeated measurements

The heritability of the corticosterone level after manual restraint was low (0.06±0.05). The repeatability was found to be 0.91±0.01. The average number of struggles measured during the manual restraint was 1.75± 0.07. The number of struggles measured during manual restraint had a low heritability (0.02±0.03). The birds showing ≥8 struggles tended to have a higher corticosterone response.
Gentle FP was measured at 5 weeks and 30 weeks of age. Rodenburg *et al.* 2002, calculated the heritability for gentle FP at the age of 5 weeks. Although the levels of FP were low at 5 weeks of age, gentle FP was found to be heritable. Estimates differ between the HL and the LH group,

indicating that the sex-chromosome can be involved in the expression of the trait (Rodenburg *et al.,* 2002).

The role of glucocorticoids is important in FP behaviour (Korte *et al*., 1997) as well as in behavioural adaptation to stress (de Kloet *et al*., 1998). Recently, van Hierden *et al.*, (2002) hypothesized that a difference the mineralocorticoid receptor (MR) / glucocorticoid receptor (GR) balance in the brain of low FP and high FP birds may underly the differences in behavioural and physiological responses to environmental stimuli. We have mapped the GR (*NR3C1*) gene to GGA13, by making a comparative map between human and chicken (Buitenhuis *et al.,* 2002). We are now able to incorporate this information in the QTL analysis by adding extra microsatellite markers to GGA13 or making a marker within the gene for SNP typing to identify the role of the GR gene in FP behaviour and stress response.

To date we have finished genotyping 180 microsatellite markers and phenotyping the F2 population. By combining the genotypic and phenotypic data from this experiment we should be able to identify the chromosomal regions involved in FP behaviour and corticosterone response to manual restraint.

REFERENCES

Bessei, W. (1995) *Proc. 2nd EPBR* **73** : 9-22.

Blokhuis, H.J. and Wiepkema, P.R., (1998) *Vet. Quat.* **20** : 6-9.

Buitenhuis, A.J., Crooijmans, R.P.M.A., Bruijnesteijn van Coppenraet, E.S., Veenendaal, A., Groenen, M.A.M. and van der Poel, J.J. (2002) *Anim. Genet.* (submitted).

Cheng, H.W., Eicher, S.D., Chen, Y., Singleton, P. and Muir, W.M. (2001a) *Poult. Sci.* **80** : 1079-1086.

Cheng, H.W., Dillworth, P., Singleton, P., Chen, Y. and Muir, W.M. (2001b) *Poult. Sci.* **80** : 1278-1285.

Cuthbertson, G.J. (1980) *Br.Poult. Sci.* **21** : 444-450.

de Jong, I.C., van Voorst, A., Erfkens, J.H.F., D.A. E. and Blokhuis, H.J. (2001) *Phys. Behav.*(submitted).

de Kloet, E.R., Vreugdenhil, E., Oitzl, M.S. and Joëls, M. (1998) *Endocr. Rev.* **19** : 269-301.

Gentle, M.J. (1986) *W. Poult. Sci.* **42** : 268-275.

Gilmour, A.R., Cullis, B.R., Welham, S.J. and Thompson, R. (2000) ‹‹ASREML Reference Manual››.

Kjaer, J.B. and Sørensen, P. (1997) *Poult. Sci.* **38** : 333-341.

Kjaer, J.B., Sørensen, P. and Su, G. (2001) *Appl. Anim. Behav. Sci.* **62** : 243-254.

Korte, S.M., Beuving, G., Ruesink, W. and Blokhuis, H.J. (1997) *Phys. Behav.* **62** : 437-441.

Rodenburg, T.B., Buitenhuis, A.J., Ask, B., Koene, P., van der Poel, J.J. and Bovenhuis, H. (2002) *Proc. 36th ISAE* (submitted).

SAS (1996) *SAS/STAT® Software:Changes and enhancements through release 6.12*. SAS Inst.Inc., Cary NC.

van Hierden, Y.M., Korte, S.M., Ruesink, E.W., van Reenen, C.G., Engel, B., Korte-Bouws,G.A.H., Koolhaas, J.M. and Blokhuis, H.J. (2002) *Phys. Behav.* (accepted).