

## Feather pecking behavior in laying hens: Hypothalamic gene expression in birds performing and receiving pecks

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**ABSTRACT** Feather pecking (FP) is a welfare and economic problem in the egg production sector. Beak trimming, the current method used to reduce FP, is also criticized. The present study used gene expression to explore the biological mechanisms underlying this behavior, which could lead to a greater understanding of the cause and a tool to mitigate the problem. White Leghorn hens performing and receiving FP, as well as neutral control birds, were identified on a commercial farm. Hypothalamic RNA from 11 peckers, 10 victims, and 10 controls was hybridized onto GeneChip Chicken Genome Arrays (Affymetrix Inc., Santa Clara, CA) to compare gene expression profiles in the different groups. Eleven transcripts corresponding to 10 genes differed significantly between the 3 groups (adjusted  $P < 0.05$ ). Eight of these transcripts differed in the peck-

ers compared with the controls, 1 was upregulated in the victims compared with the controls, and 6 differed significantly in the peckers compared with the victims. Additionally, 5 transcripts showed a trend (adjusted  $P < 0.1$ ) to differ in the pecker–victim comparison. Some of the products of the differently expressed genes are involved in disorders, such as intestinal inflammation and insulin resistance, which fit well with the previously proposed hypothesis that FP is an abnormal foraging behavior. Other findings may also support the proposal that FP is linked to immune mechanisms and may serve as an animal model for obsessive compulsive disorder in humans. In conclusion, this study provides a gene list that may be useful in further research on the mechanisms behind FP.

**Key words:** gene expression, laying hen, hypothalamus, feather pecking, behavioral problem

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### INTRODUCTION

Feather pecking (FP) is a behavior in which a bird pecks at the feathers of another bird. It is considered to be an indicator of a welfare problem in the bird that develops the behavior (Weeks and Nicol, 2006). Feather pecking behavior is frequently categorized into subtypes: the gentle type in which the performer pecks at the plumage without removal of feathers and severe FP in which the pecking is more vigorous and usually feathers are pulled out (Keeling, 1995). The actual pulling of feathers is painful and can result in feather damage and wounded birds (Gentle and Hunter, 1991). Feather pecking is therefore regarded as a major welfare problem for the recipient birds.

Despite extensive research on FP, the underlying causes are not yet understood. Several studies have

shown that FP is a redirected pecking behavior related to birds' motivation to forage (e.g., Blokhuis, 1986; Huber-Eicher and Wechsler, 1998; Klein et al., 2000; Dixon et al., 2008), although others have proposed links to dustbathing behavior (Vestergaard et al., 1993). There is no doubt, however, that nutrition is an important component. Laying hens seem to adjust their eating time to the energy level of the feed and a lower energy density can consequently decrease the amount of FP (Elwinger, 1981; van Krimpen et al., 2008, 2009), whereas a low mineral content seems to increase the problem (Hughes and Duncan, 1972). Higher protein and amino acid levels, on the other hand, have been shown to have a beneficial effect on pecking behavior and plumage condition (Ambrosen and Petersen, 1997). Also, feather eating has been shown to be associated with FP (e.g., McKeegan and Savory, 1999). Harlander-Matauschek et al. (2006) showed that ingesting feathers increases the speed of feed passage and that a dietary effect of eating feathers might exist that could influence the development of FP. Feeding behavior and appetite are modulated by the hypothalamus via periph-

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eral signals about energy and nutrient status (Richards and Proszkowiec-Weglarz, 2007). The hypothalamus is therefore a potentially important part of the brain to investigate with regard to the control of FP behavior.

Regardless of strong environmental influences on the behavior, FP is performed by only a restricted number of individuals (Keeling, 1995), thus suggesting that differences on an individual level contribute to the development of the behavior. Physiological and neurobiological influences on FP have been proposed in several studies. Differences between high and low FP animals in adrenocortical reactivity and hypothalamus-pituitary-adrenal axis reactivity (Korte et al., 1997; van Hierden et al., 2002) as well as alterations in serotonergic and dopaminergic systems (Kjaer et al., 2004; van Hierden et al., 2004) are suggested to be associated with the behavior.

Strain differences in the amount of performed FP have been identified, which suggests a genetic component to the behavior (Hughes and Duncan, 1972; Klein et al., 2000; Hocking et al., 2001; Kjaer and Sorensen, 2002). This was later confirmed by selection studies in which successful selection for and against FP was performed (Kjaer and Sorensen, 2001). Heritabilities for different measures of FP have been estimated mostly as low or moderate (Kjaer and Sorensen, 1997; Kjaer et al., 2001; Su et al., 2005). Studies also show that a positive correlation exists between performing severe FP and immune response (Buitenhuis et al., 2004) as well as between pecking behavior and activity in an open-field test (Rodenburg et al., 2004).

Two studies on QTL and the performance of FP have been performed, resulting in 1 significant and 4 putative QTL (Buitenhuis et al., 2003; Jensen et al., 2005). The study by Jensen et al. (2005) also showed that FP is phenotypically linked to other behavioral traits (activity in an open-field test, novel object test, and a restraint test) as well as to nonbehavioral traits (early sexual maturation, fast growth, weak bones, and high fat accumulation in males). Biscarini et al. (2010) performed an across-line SNP association study for genetic effects on feather damage in 9 genetic lines. Fifty-seven single SNP were suggested to be associated with performing the behavior and 11 with receiving. Genes involved in the serotonergic system as well as the immune system were among those associated with performing pecks.

Knowledge about genomic differences may give a tool to reduce the problem of injurious behavior in commercial lines of birds through selection and can potentially be used to learn more about the causal mechanisms. Quantitative trait loci studies identify polymorphisms in the DNA associated with the behavior whereas gene expression studies aim to find differences in the transcriptome. The latter is much more dynamic because it is highly influenced by environmental effects. The expression of a specific gene can therefore be affected by both genetic and environmental differences. Labouriau et al. (2009) compared whole-brain gene expression in

high and moderate feather peckers from a high FP selection line. In that study, 456 genes were found to be differently expressed in birds showing high amounts of FP compared with birds performing moderate amounts of FP, but no obvious biological pathway was apparent. In a more recent study, Hughes and Buitenhuis (2010) found that several transcripts in a high FP population showed reduced variance in expression compared with control and low FP populations. No significant differences in expression were found between the populations, but 37 transcripts showed negative skewness in the control population and correlations between expression score and gentle or severe FP. The authors hypothesized that these genes are candidate genes for the major targets of selection favoring high FP. Transcripts for which the expression score was associated with severe FP, but without the negative skewness in the control population, were suggested to be genes whose expression differences are associated with severe FP but that have not been directly influenced by selection. Among these genes were several with roles in nervous system development and suppression of the immune system.

The link between the immune system and FP proposed in the studies by Biscarini et al. (2010) and Hughes and Buitenhuis (2010) are interesting because earlier results indicated that a genetic correlation exists between severe FP and primary antibody response to keyhole limpet hemocyanin (Buitenhuis et al., 2004). Moreover, Parmentier et al. (2009) found that stimulating the humoral immune response led to more feather damage. Immune mechanisms have also been suggested to be implicated in some human neuropsychiatric disorders such as obsessive compulsive disorder (OCD) and attention-deficit hyperactivity disorder syndromes (e.g., da Rocha et al., 2008; Pelsser et al., 2009). Feather pecking has been suggested to be a hyperactivity disorder (Kjaer, 2009) as well as a potential model for human OCD (van Hierden et al., 2004).

The present study focused on hypothalamic genome-wide expression in pecker, victim, and control birds. The aim was to identify genes and biological pathways involved in the mechanisms underlying FP in laying hens and thereby increase knowledge on the causation and so how to reduce this problem behavior.

## MATERIALS AND METHODS

### *Birds and Behavioral Observations*

The birds in this study were selected from a commercial poultry farm in Sweden housing Lohmann Selected Leghorns in furnished cages. The birds used in the present study were all from the same building housing 40,320 hens in 5,040 cages. Each cage contained 8 to 10 birds and was provided with a nest box, perches, and dust bath as well as ad libitum provision of feed and water. The study was performed when the birds were between 41 and 49 wk of age.

Behavioral observations were carried out to select matched batches consisting of a pecker, victim, and control bird. The cages in which the observations were performed were selected by estimating the birds' plumage condition from outside the cage. Cages housing hens with poor plumage condition were selected as potential FP cages and cages in which all birds had good plumage were selected as potential control cages. All birds in the FP cages were marked with leg rings for individual identification and the number of performed and received severe pecks (forceful pecks often involving feathers being pulled out and the victim reacting by, for example, vocalizing, turning around, or running away) were recorded for each individual. Gentle feather pecks were not recorded. In this first observation period, the birds were observed until a pecker and a victim could be identified (0.5–2.5 h). If a pecker and a victim could not be clearly identified after these observations, the cage was excluded and a new potential FP cage and corresponding control cage were selected and observed. The data collected during this observation were used only to identify the candidate peckers and victims and thus were not included in any data analysis.

Once identified, the pecker and victim as well as 2 randomly selected control birds from the neighboring control cage were marked on the head with color (red or green balanced between the different animal categories) for further individual behavioral observations. Originally, it was intended to select control birds from the FP cage. The reason for selecting the control from an adjacent cage was the difficulty in finding birds that were not involved in the pecking behavior in the FP cage. After being habituated to the observer for 30 min, the identified pecker, victim, and control birds were observed in their home cages for 30 min. This was then repeated 3 times. These 4 observations were evenly distributed over 2 d so that the birds were observed 1 h before noon on one day and 1 h in the afternoon on the other day. The observations during these 2 h were then used to estimate the frequency of severe feather pecks performed or received by the specific individuals. To be classified as a pecker and further selected for tissue sampling the bird should have been observed performing severe FP in both observations. The criteria for the victims were to receive severe FP and have a plumage with clear signs of pecking damage. Control birds that performed or received any severe FP were rejected after the last observation step. In total, 209 birds were marked and observed.

### **Feather Scoring, Tissue Sampling, and RNA Isolation**

Thirty-four birds in 11 matched batches consisting of 1 pecker and 1 victim from the pecker cage as well as 1 control from the adjacent control cage (1 batch contained 2 peckers) were selected for tissue collection. Two to 5 d after the behavioral observations, the select-

ed birds were taken from the home cage to an adjacent room where they were feather scored on the back and tail using a 4-point photographic scale where 1 is naked and 4 is fully feathered (Tauson et al., 2005). The birds were killed by injecting pentobarbitals in the wing vein followed by an injection into the abdomen when necessary (2 victims, 2 controls). To reduce the effects of handling, the order in which the birds were taken from the cages was balanced between the different animal categories within a batch and the procedures preceding the killing were standardized and performed by the same 2 persons. All birds were killed between 0845 and 1135 h and at most 2 batches of birds were killed on the same day.

The brains were removed and the hypothalamus was dissected according to Lindqvist et al. (2007) and immediately flash frozen in liquid nitrogen, transported on dry ice, and stored at  $-80^{\circ}\text{C}$  until RNA isolation. Total RNA was isolated from the frozen hypothalamus using RNeasy Lipid Tissue Mini kit (Qiagen, Valencia, CA) according to the manufacturer's instructions. Concentration and quality of the RNA was controlled measuring 260/280 and 230/260 ratios using a NanoDrop ND-1000 Spectrophotometer (NanoDrop Technologies, Wilmington, DE) and RNA integrity number using Agilent 2100 Bioanalyzer system (Agilent Technologies Inc., Palo Alto, CA).

### **Microarray Hybridization**

Because samples from 1 batch were destroyed, RNA from the hypothalamus of 31 birds (11 peckers, 10 victims, and 10 controls) in 10 batches was used. The GeneChip Expression Analysis technical manual (revision 5, Affymetrix Inc., Santa Clara, CA) was followed when preparing fragmented and biotin labeled cRNA from 2  $\mu\text{g}$  of total RNA. The cRNA was then hybridized to Affymetrix GeneChip Chicken Genome Array (representing 32,773 transcripts corresponding to more than 28,000 genes; Affymetrix Inc., Santa Clara, CA) for 16 h at  $45^{\circ}\text{C}$  and rotated at 60 rpm. The arrays were washed and stained using the Fluidics Station 450 (Affymetrix Inc.) and scanned using a GeneChip Scanner 3000 7G 8 (Affymetrix Inc.).

### **Data Analysis**

Because neither behavior nor plumage scores were normally distributed, a Mann-Whitney U-test in Minitab statistical software (Minitab Inc., State College, PA) was used for analyzing pairwise differences between bird categories. Control birds by definition did not perform or receive any severe FP and were therefore not included in the analysis of behavior.

The gene expression data were analyzed in statistical computing language R (<http://www.r-project.org>) using packages available from the Bioconductor project ([www.bioconductor.org](http://www.bioconductor.org)). The raw data were normal-

**Table 1.** Frequency of performed or received severe feather pecks (mean  $\pm$  SE) and feather score<sup>1</sup> for peckers (n = 12), victims (n = 11), and controls (n = 11) that were selected for microarrays

Group	Performed pecks (n/min)	Received pecks (n/min)	Feather score back	Feather score tail
Peckers	1.16 $\pm$ 0.24 <sup>a</sup>	0.09 $\pm$ 0.07 <sup>a</sup>	3.42 $\pm$ 0.19 <sup>a</sup>	2.75 $\pm$ 0.13 <sup>a</sup>
Victims	0.01 $\pm$ 0.003 <sup>b</sup>	0.26 $\pm$ 0.05 <sup>b</sup>	1.18 $\pm$ 0.12 <sup>b</sup>	1.55 $\pm$ 0.16 <sup>b</sup>
Controls	0	0	3.93 $\pm$ 0.09 <sup>c</sup>	3.18 $\pm$ 0.12 <sup>c</sup>

<sup>a-c</sup>Means with different superscripts within a column differ significantly ( $P < 0.05$ ).

<sup>1</sup>Four-point scale: 1 = naked, 4 = fully feathered.

ized using the robust multiarray average method, first suggested by Li and Wong (2001). An empirical Bayes moderated  $t$ -test was applied using the “linear models for microarray data” package with the default settings to search for differentially expressed genes between the groups (Smyth, 2004). Three comparisons were made: peckers versus controls, peckers versus victims, and victims versus controls. To control for the problem with multiple testing, the  $P$ -values were adjusted according to the method by Benjamini and Hochberg (1995).

## RESULTS

Among the 34 selected birds, the peckers performed more severe FP ( $W = 210.0$ , where  $W$  is the test value used by Minitab when performing a Mann-Whitney analysis;  $P < 0.001$ ) than the victims, whereas the victims received more pecks than the peckers ( $W = 93.5$ ,  $P = 0.02$ ; Table 1). The individual peckers performed between 0.57 and 3.08 severe pecks/min and the victims received between 0.03 and 0.6 pecks/min. The control birds had a better feather score on both the back and tail than peckers (back:  $W = 116.5$ ,  $P = 0.04$ ; tail:  $W = 118.5$ ,  $P = 0.03$ ) and victims (back:  $W = 66$ ,  $P < 0.001$ ; tail:  $W = 66$ ,  $P < 0.001$ ) and the peckers had a better

score than the victims (back:  $W = 209$ ,  $P < 0.01$ ; tail:  $W = 201$ ,  $P < 0.01$ ; Table 1).

All RNA samples used for the microarrays had a 260/280 ratio above 2.0 and 260/230 above 1.9. The RNA integrity number values were all 8 or greater. The hybridization of hypothalamic cRNA to Affymetrix GeneChip Chicken Genome Array identified 8 differentially expressed transcripts (adjusted  $P < 0.05$ ) in the peckers–control comparison, 1 in the victims–controls comparison, and 6 in peckers–victims comparison. Additionally, 5 transcripts tended to be differently expressed in peckers compared with victims (adjusted  $P < 0.1$ ). Because 4 of these 20 transcripts were differently expressed in 2 comparisons, the gene list consists of 16 unique transcripts ( $P < 0.05$  and  $P < 0.1$ ) listed in Table 2. Out of the 4 transcripts that were differently expressed in 2 comparisons, 3 were differently expressed in the peckers–victims and the peckers–controls comparisons. The last of these 4 transcripts differed in the victims–controls and pecker–control comparisons.

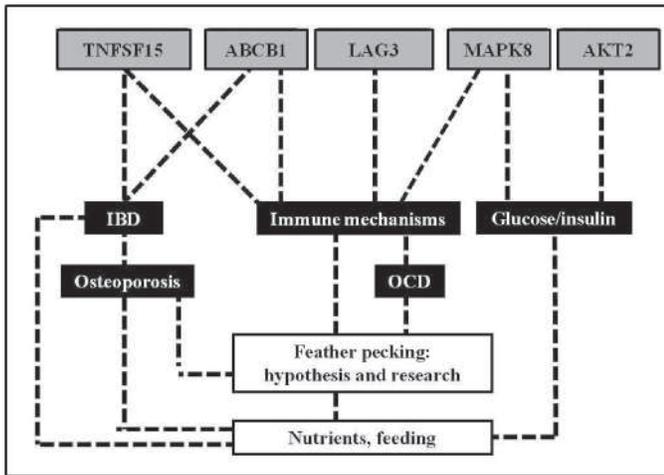
A single gene was differently expressed in victims compared with controls and the same gene was upregulated in the peckers compared with the controls. This implies that it is possible to compare pecker or victim birds with control birds despite the fact that control

**Table 2.** Gene description and log fold changes (LogFC) for the different expressed genes in the comparisons of peckers (P), victims (V), and controls (C)<sup>1</sup>

Probe set	Gene or transcript	Description	LogFC		
			P vs. C	V vs. C	P vs. V
GgaAffx.11659.1.S1_at	<i>SRI</i>	Sorcin	0.90**	0.03	0.87**
Gga.4109.1.S2_at	<i>ABCB1</i>	ATP-binding cassette, subfamily B (MDR/TAP), member 1	-1.97**	-0.12	-1.86**
Gga.4109.1.S1_at	<i>ABCB1</i>	ATP-binding cassette, subfamily B (MDR/TAP), member 1	-1.43**	-0.10	-1.32*
GgaAffx.5144.2.S1_at	<i>LOC770119</i>	Hypothetical protein LOC770119	-0.51**	-0.15	-0.36
Gga.15807.1.S1_at	Unknown	Finished cDNA, clone ChEST333m20	-0.67**	-0.15	-0.52
Gga.11554.1.S1_at	<i>LOC420998</i>	Hypothetical LOC420998	-0.31*	-0.30	-0.01
Gga.12614.2.S1_s_at	<i>TNFSF15</i>	Tumor necrosis factor (ligand) superfamily, member 15	0.42*	0.13	0.29
GgaAffx.12217.1.S1_at	<i>GTF2H5</i>	General transcription factor IIIH, polypeptide 5	0.64**	0.64**	-0.006
GgaAffx.244.1.S1_x_at	Unknown	Unknown	-0.12	0.14	-0.26*
GgaAffx.23313.1.S1_at	<i>PLD5</i>	Phospholipase D family, member 5	0.18	-0.11	0.29*
GgaAffx.25795.1.S1_s_at	<i>COMP</i>	Cartilage oligomeric matrix protein	-0.28	0.20	-0.48*
Gga.1147.1.S1_at	<i>MAPK8</i>	Mitogen-activated protein kinase 8	0.09	-0.21	0.31†
Gga.930.1.S1_at	<i>AKT2</i>	V-akt murine thymoma viral oncogene homolog 2	-0.16	0.14	-0.30†
GgaAffx.24490.2.S1_at	Unknown	Unknown	0.04	-0.18	0.21†
GgaAffx.9194.1.S1_at	<i>LAG3</i>	Lymphocyte-activation gene 3	-0.04	0.21	-0.24†
GgaAffx.21242.1.S1_at	Unknown	Finished cDNA, clone ChEST883g17	-0.11	0.07	-0.17†

<sup>1</sup>The fold change is a measure of the ratio between the phenotypes indicating the difference in expression level. A positive log fold change indicates that the transcript is upregulated in the bird category written first (e.g., upregulated in P in the P vs. C comparison) and vice versa.

\* $P < 0.05$ ; \*\* $P < 0.01$ ; † $P < 0.1$ .



**Figure 1.** Differently expressed genes (gray boxes) in peckers compared with controls or victims in the present study that have possible associations with diseases or functions (black boxes) previously implicated in hypotheses about the biological mechanisms underlying feather pecking. IBD = inflammatory bowel disease; OCD = obsessive compulsive disorder; *TNFSF15* = tumor necrosis factor (ligand) superfamily, member 15; *ABCB1* = ATP-binding cassette, subfamily B (MDR/TAP), member 1; *LAG3* = lymphocyte-activation gene 3; *MAPK8* = mitogen-activated protein kinase 8; *AKT2* = V-akt murine thymoma viral oncogene homolog 2.

birds came from adjacent cages. It further suggests that the pecker–victim comparison may be useful in identifying potential causal mechanisms behind FP in pecker birds. The results from the literature search and possible links between the genes and phenotypes are illustrated in Figure 1.

## DISCUSSION

In this study, microarrays were used to characterize hypothalamic gene expression profiles in birds performing and receiving feather pecks and birds not involved in FP. Three comparisons (peckers vs. controls, victims vs. controls, and peckers vs. victims) gave a list with 11 differently expressed transcripts and trends in 5 additional transcripts in the pecker–victim comparison.

It was originally planned to use control birds from the FP cages to avoid confounding any environmental effects when comparing pecker and victim birds with controls but, as stated earlier, too few birds in FP cages were neither performers nor receivers of FP. However, only 1 transcript (corresponding to the gene *GTF2H5*, a general transcription factor) was differently expressed in the victims–controls comparison. Because the control birds were selected from a different environment (i.e., from a cage without a feather pecker) compared with the pecker and victim, this suggests that the differential expression of this gene was probably attributable to this difference in environment. The finding that only this single gene differed between the birds with the best and worst plumage condition could be of benefit when comparing peckers and victims. It is unlikely that differences in gene expression between peckers and victims

are associated with the better plumage condition of the peckers, which otherwise might have been expected.

In the remainder of this discussion, the functions of some of the differently expressed genes are presented, illustrated by Figure 1. The functions of the differently expressed transcripts were diverse and it is difficult to interpret whether the expression of a transcript is a cause or an effect of the behavior. Therefore, only those transcripts that earlier studies clearly indicate could be important for the biological mechanisms underlying FP will be further discussed. Earlier studies by Biscarini et al. (2010), Buitenhuis et al. (2004), Hughes and Buitenhuis (2010), and Parmentier et al. (2009) suggested a link between performing FP and the immune system. It is therefore interesting that at least 3 of the transcripts differently expressed in this study are known to be related to immune defense in mammals. The protein products of tumor necrosis factor (ligand) superfamily, member 15 (*TNFSF15*) and lymphocyte-activation gene 3 (*LAG3*) are both known to have roles in modulating immune responses (Triebel, 2003; Takimoto et al., 2005). The gene *LAG3* was also the only transcript that differed both in this study and the one performed by Labouriau et al. (2009). Also, multidrug resistance protein 1 (encoded by *ABCB1*, the transcript with the largest expression difference in peckers compared with both victims and controls) has been suggested to be a possible immunomodulator. It is an efflux transporter present on the blood-brain barrier that can influence the extrusion of proinflammatory cytokines (Kooij et al., 2009). The protein product of another gene, mitogen-activated protein kinase 8 (*MAPK8*, also called *JNK* or *JNK1*), is known to play a role in immune responses because of its implications on T cell differentiation and cytokine production (Dong et al., 1998; Constant et al., 2000). The present study was performed on commercial lines in a production environment, whereas most of the other studies included birds from FP selection lines. It is therefore noteworthy that this study supports the earlier proposed link between FP and the immune system.

Two of the above-mentioned genes, *TNFSF15* and *ABCB1*, are linked to inflammatory bowel disease (IBD), which is characterized by intestinal inflammation in both humans and mice (Panwala et al., 1998; Brant et al., 2003; Thiebaut et al., 2009). Crohn's disease in humans is a variant of IBD and one in which osteoporosis is common in patients (Pigot et al., 1992). This is probably partly attributable to the poor absorption from the intestines and the reason why nutrient deficiencies of vitamin D (and, as a result, calcium), vitamin K, magnesium, protein, and trace elements are frequently reported. It is notable therefore that Jensen et al. (2005) found high FP to be correlated to low bone mineral content and weak bones (i.e., osteoporosis) in laying hens. This is interesting because deficiencies of different nutrients are believed to affect both eating behavior and the level of FP.

The second function linked to several of the differentially expressed genes in this study is glucose homeostasis. Two of the genes represented in this gene list, V-akt murine thymoma viral oncogene homolog 2 (*AKT2*) and *MAPK8*, have roles in insulin resistance (Cho et al., 2001; Velloso et al., 2008) and both tended toward being differently regulated in peckers compared with victims. A recent study showed that a selective deficiency of *MAPK8* in the nervous system of mice affects body mass, energy expenditure, and feed intake and concluded that the hypothalamic-pituitary-thyroid axis is important for the influence of *MAPK8* on metabolism (Sabio et al., 2010). All of this is relevant given that FP has been suggested to be redirected foraging behavior.

Based mainly on the compulsive characteristics of FP and the alterations in the serotonergic system, FP in poultry has been suggested to be an animal model for OCD (van Hierden et al., 2004) as well as a model of hyperactivity disorder (Kjaer, 2009). Furthermore, immune mechanisms have been implicated in the pathophysiology of some types of OCD (e.g., da Rocha et al., 2008; Pelsser et al., 2009). The prevalence of OCD as well as panic, anxiety, and major depression seems to be higher in individuals with IBD (Walker et al., 2008). Also, a possible link between disturbed glucose metabolism and depression has been discussed in several studies (for a review see Hundal, 2007). Thus, the previously presented links among immune system, IBD, the regulation of insulin, and FP behavior in poultry may be in line with links among immune mechanisms, IBD, glucose metabolism, and psychiatric disorders in humans and thus support a similar underlying mechanism for both FP and OCD.

Among the annotated genes, we only found 1 (*LAG3*) that was differently expressed in both this study and the one performed by Labouriau et al. (2009). None of the known protein coding transcripts listed in the SNP study by Biscarini et al. (2010) or the expression study by Hughes and Buitenhuis (2010) were in our gene list. Several reasons could account for the differences in the gene lists from the current study and the expression studies from Labouriau et al. (2009) and Hughes and Buitenhuis (2010). The main differences between the studies are the choice of the brain area (hypothalamus in the present study vs. whole brain in the previous studies), the criteria for the minimum number of pecks given to be classified as a pecker [0.57 in the present study vs. 0.08 in the study by Labouriau et al. (2009)], and the choice of animal material.

The advantage of using a smaller brain area, as in the present study, compared with whole brain is that the risk of diluting possible expression differences in the area of interest is decreased. On the other hand, when only a smaller part of the brain is analyzed, expression differences in other areas of the brain will be missed. Peckers in the studies differed in their rate of FP, which makes it complicated to compare the results because motivation underlying the behavior may differ

between very high frequency and occasional peckers. As discussed earlier, different underlying motivations may exist between gentle and severe FP. Finally, regarding the choice of animal material, the present study used a commercially available White Leghorn whereas both Labouriau et al. (2009) and Hughes and Buitenhuis (2010) used a high FP selection line. Studies in which selection lines are used may be misleading. Even if individuals from the same line are selected and compared, selected lines may accidentally accumulate genetic differences only indirectly or not at all related to FP. Nevertheless, despite the differences between the studies highlighted here, all these studies report links between FP and immune mechanisms, which strengthens this proposed association.

In conclusion, this study presents a list of genes with different expression patterns in FP birds than in birds not performing FP. Knowing the functions of those genes and the pathways that they are involved in could be of use when further investigating the mechanisms underlying this abnormal behavior. Identifying the cause of FP is, in turn, the most promising route toward reducing the problem. A large proportion of the differently regulated genes in this study are directly or indirectly associated with absorption of nutrients from the intestine, the regulation of glucose homeostasis, or immune mechanisms. These findings are noteworthy considering that decades of research have resulted in a general consensus that FP is linked to feeding motivation and recent studies suggest a link to the immune system.

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## REFERENCES

- Ambrosen, T., and V. E. Petersen. 1997. The influence of protein level in the diet on cannibalism and quality of plumage of layers. *Poult. Sci.* 76:559–563.
- Benjamini, Y., and Y. Hochberg. 1995. Controlling the false discovery rate: A practical and powerful approach to multiple testing. *J. R. Stat. Soc., B* 57:289–300.
- Biscarini, F., H. Bovenhuis, J. van der Poel, T. B. Rodenburg, A. P. Jungerius, and J. A. M. van Arendonk. 2010. Across-line SNP association study for direct and associative effects on feather damage in laying hens. *Behav. Genet.* 40:715–727.

- Blokhuis, H. J. 1986. Feather pecking in poultry: Its relation with ground-pecking. *Appl. Anim. Behav. Sci.* 16:63–67.
- Brant, S. R., C. I. M. Panhuysen, D. Nicolae, D. M. Reddy, D. K. Bonen, R. Karaliukas, L. Zhang, E. Swanson, L. W. Datta, T. Moran, G. Ravenhill, R. H. Duerr, J.-P. Achkar, A. S. Karban, and J. H. Cho. 2003. MDR1 Ala893 polymorphism is associated with inflammatory bowel disease. *Am. J. Hum. Genet.* 73:1282–1292.
- Buitenhuis, A. J., T. B. Rodenburg, Y. M. van Hierden, M. Siwek, S. J. B. Cornelissen, M. G. B. Nieuwland, R. P. M. A. Crooijmans, M. A. M. Groenen, P. Koene, S. M. Korte, H. Bovenhuis, and J. J. van der Poel. 2003. Mapping quantitative trait loci affecting feather pecking behavior and stress response in laying hens. *Poult. Sci.* 82:1215–1222.
- Buitenhuis, A. J., T. B. Rodenburg, P. H. Wissink, J. Visscher, P. Koene, H. Bovenhuis, B. J. Ducro, and J. J. van der Poel. 2004. Genetic and phenotypic correlations between feather pecking behavior, stress response, immune response, and egg quality traits in laying hens. *Poult. Sci.* 83:1077–1082.
- Cho, H., J. Mu, J. K. Kim, J. L. Thorvaldsen, Q. Chu, E. B. Crenshaw III, K. H. Kaestner, M. S. Bartolomei, G. I. Shulman, and M. J. Birnbaum. 2001. Insulin resistance and a diabetes mellitus-like syndrome in mice lacking the protein kinase Akt2 (PKB beta). *Science* 292:1728–1731.
- Constant, S. L., C. Dong, D. D. Yang, M. Wysk, R. J. Davis, and R. A. Flavell. 2000. JNK1 is required for T cell-mediated immunity against *Leishmania major* infection. *J. Immunol.* 165:2671–2676.
- da Rocha, F. F., H. Correa, and A. L. Teixeira. 2008. Obsessive-compulsive disorder and immunology: A review. *Prog. Neuropsychopharmacol. Biol. Psychiatry* 32:1139–1146.
- Dixon, L. M., I. J. H. Duncan, and G. Mason. 2008. What's in a peck? Using fixed action pattern morphology to identify the motivational basis of abnormal feather-pecking behaviour. *Anim. Behav.* 76:1035–1042.
- Dong, C., D. D. Yang, M. Wysk, A. J. Whitmarsh, R. J. Davis, and R. A. Flavell. 1998. Defective T cell differentiation in the absence of Jnk1. *Science* 282:2092–2095.
- Elwinger, K. 1981. Different energy levels and restricted feeding to three strains of SCWL hybrids. 1. Effects on egg production. *Swed. J. Agric. Res.* 11:149–157.
- Gentle, M. J., and L. N. Hunter. 1991. Physiological and behavioural responses associated with feather removal in *Gallus gallus* var *domesticus*. *Res. Vet. Sci.* 50:95–101.
- Harlander-Matauschek, A., H. P. Piepho, and W. Bessei. 2006. The effect of feather eating on feed passage in laying hens. *Poult. Sci.* 85:21–25.
- Hocking, P. M., C. E. Channing, D. Waddington, and R. B. Jones. 2001. Age-related changes in fear, sociality and pecking behaviours in two strains of laying hen. *Br. Poult. Sci.* 42:414–423.
- Huber-Eicher, B., and B. Wechsler. 1998. The effect of quality and availability of foraging materials on feather pecking in laying hen chicks. *Anim. Behav.* 55:861–873.
- Hughes, A. L., and A. J. Buitenhuis. 2010. Reduced variance of gene expression at numerous loci in a population of chickens selected for high feather pecking. *Poult. Sci.* 89:1858–1869.
- Hughes, B. O., and I. J. Duncan. 1972. The influence of strain and environmental factors upon feather pecking and cannibalism in fowls. *Br. Poult. Sci.* 13:525–547.
- Hundal, Ø. 2007. Major depressive disorder viewed as a dysfunction in astroglial bioenergetics. *Med. Hypotheses* 68:370–377.
- Jensen, P., L. Keeling, K. Schutz, L. Andersson, P. Mormede, H. Brändström, B. Forkman, S. Kerje, R. Fredriksson, C. Ohlsson, S. Larsson, H. Mallmin, and A. Kindmark. 2005. Feather pecking in chickens is genetically related to behavioural and developmental traits. *Physiol. Behav.* 86:52–60.
- Keeling, L. J. 1995. Feather pecking and cannibalism in layers. *Poult. Int.* 6:46–50.
- Kjaer, J. B. 2009. Feather pecking in domestic fowl is genetically related to locomotor activity levels: Implications for a hyperactivity disorder model of feather pecking. *Behav. Genet.* 39:564–570.
- Kjaer, J. B., B. M. Hjarvard, K. H. Jensen, J. Hansen-Møller, and O. Naesby Larsen. 2004. Effects of haloperidol, a dopamine D2 receptor antagonist, on feather pecking behaviour in laying hens. *Appl. Anim. Behav. Sci.* 86:77–91.
- Kjaer, J. B., and J. P. Sorensen. 1997. Feather pecking behaviour in White Leghorns, a genetic study. *Br. Poult. Sci.* 38:333–341.
- Kjaer, J. B., and P. Sorensen. 2002. Feather pecking and cannibalism in free-range laying hens as affected by genotype, dietary level of methionine + cysteine, light intensity during rearing and age at first access to the range area. *Appl. Anim. Behav. Sci.* 76:21–39.
- Kjaer, J. B., P. Sorensen, and G. Su. 2001. Divergent selection on feather pecking behaviour in laying hens (*Gallus gallus domesticus*). *Appl. Anim. Behav. Sci.* 71:229–239.
- Klein, T., E. Zeltner, and B. Huber-Eicher. 2000. Are genetic differences in foraging behaviour of laying hen chicks paralleled by hybrid-specific differences in feather pecking? *Appl. Anim. Behav. Sci.* 70:143–155.
- Kooij, G., R. Backer, J. J. Koning, A. Reijerkerk, J. van Horssen, S. M. A. van der Pol, J. Drexhage, A. Schinkel, C. D. Dijkstra, J. M. M. den Haan, T. B. H. Geijtenbeek, and H. E. de Vries. 2009. P-glycoprotein acts as an immunomodulator during neuroinflammation. *PLoS ONE* 4:e8212.
- Korte, S. M., G. Beuving, W. Ruesink, and H. J. Blokhuis. 1997. Plasma catecholamine and corticosterone levels during manual restraint in chicks from a high and low feather pecking line of laying hens. *Physiol. Behav.* 62:437–441.
- Labouriau, R., J. B. Kjaer, G. C. Abreu, J. Hedegaard, and A. J. Buitenhuis. 2009. Analysis of severe feather pecking behavior in a high feather pecking selection line. *Poult. Sci.* 88:2052–2062.
- Li, C., and W. H. Wong. 2001. Model-based analysis of oligonucleotide arrays: Expression index computation and outlier detection. *Proc. Natl. Acad. Sci. USA* 98:31–36.
- Lindqvist, C., A. M. Janczak, D. Nätt, I. Baranowska, N. Lindqvist, A. Wichman, J. Lundeberg, J. Lindberg, P. A. Torjesen, and P. Jensen. 2007. Transmission of stress-induced learning impairment and associated brain gene expression from parents to offspring in chickens. *PLoS ONE* 2:e364.
- McKeegan, D. E. F., and C. J. Savory. 1999. Feather eating in layer pullets and its possible role in the aetiology of feather pecking damage. *Appl. Anim. Behav. Sci.* 65:73–85.
- Panwala, C. M., J. C. Jones, and J. L. Viney. 1998. A novel model of inflammatory bowel disease: Mice deficient for the multiple drug resistance gene, *mdr1a*, spontaneously develop colitis. *J. Immunol.* 161:5733–5744.
- Parmentier, H. K., T. B. Rodenburg, G. de Vries Reilingh, B. Beerda, and B. Kemp. 2009. Does enhancement of specific immune responses predispose laying hens for feather pecking? *Poult. Sci.* 88:536–542.
- Pelsser, L. M. J., J. K. Buitelaar, and H. F. J. Savelkoul. 2009. ADHD as a (non) allergic hypersensitivity disorder: A hypothesis. *Pediatr. Allergy Immunol.* 20:107–112.
- Pigot, F., C. Roux, S. Chaussade, D. Hardelin, O. Pelleter, T. Du Fuy Montbrun, V. Listrat, M. Dougados, D. Couturier, and B. Amor. 1992. Low bone mineral density in patients with inflammatory bowel disease. *Dig. Dis. Sci.* 37:1396–1403.
- Richards, M. P., and M. Proszkowiec-Weglarz. 2007. Mechanisms regulating feed intake, energy expenditure, and body weight in poultry. *Poult. Sci.* 86:1478–1490.
- Rodenburg, T. B., A. J. Buitenhuis, B. Ask, K. A. Uitdehaag, P. Koene, J. J. van der Poel, J. A. van Arendonk, and H. Bovenhuis. 2004. Genetic and phenotypic correlations between feather pecking and open field response in laying hens at two different ages. *Behav. Genet.* 34:407–415.
- Sabio, G., J. Cavanagh-Kyros, T. Barrett, D. Y. Jung, H. J. Ko, H. Ong, C. Morel, A. Mora, J. Reilly, J. K. Kim, and R. J. Davis. 2010. Role of the hypothalamic-pituitary-thyroid axis in metabolic regulation by JNK1. *Genes Dev.* 24:256–264.
- Smyth, G. K. 2004. Linear models and empirical Bayes methods for assessing differential expression in microarray experiments. *Stat. Appl. Genet. Mol. Biol.* 3: Article 3.
- Su, G., J. B. Kjaer, and P. Sorensen. 2005. Variance components and selection response for feather-pecking behavior in laying hens. *Poult. Sci.* 84:14–21.
- Takimoto, T., K. Takahashi, K. Sato, and Y. Akiba. 2005. Molecular cloning and functional characterizations of chicken TL1A. *Dev. Comp. Immunol.* 29:895–905.

- Tauson, R., J. Kjaer, G. A. Maria, R. Cepero, and K. E. Holm. 2005. Applied scoring of integument and health in laying hens. *Anim. Sci. Pap. Rep.* 23:153–159.
- Thiébaud, R., S. Kotti, C. Jung, F. Merlin, J. F. Colombel, M. Lemann, S. Almer, C. Tysk, M. O'Morain, M. Gassull, V. Binder, Y. Finkel, L. Pascoe, and J.-P. Hugot. 2009. TNFSF15 polymorphisms are associated with susceptibility to inflammatory bowel disease in a new European cohort. *Am. J. Gastroenterol.* 104:384–391.
- Triebel, F. 2003. LAG-3: A regulator of T-cell and DC responses and its use in therapeutic vaccination. *Trends Immunol.* 24:619–622.
- van Hierden, Y. M., S. F. de Boer, J. M. Koolhaas, and S. M. Korte. 2004. The control of feather pecking by serotonin. *Behav. Neurosci.* 118:575–583.
- van Hierden, Y. M., S. M. Korte, E. W. Ruesink, C. G. van Reenen, B. Engel, G. A. Korte-Bouws, J. M. Koolhaas, and H. J. Blokhuis. 2002. Adrenocortical reactivity and central serotonin and dopamine turnover in young chicks from a high and low feather-pecking line of laying hens. *Physiol. Behav.* 75:653–659.
- van Krimpen, M. M., R. P. Kwakkel, C. M. C. van der Peet-Schwering, L. A. den Hartog, and M. W. A. Verstegen. 2008. Low dietary energy concentration, high nonstarch polysaccharide concentration, and coarse particle sizes of nonstarch polysaccharides affect the behavior of feather-pecking-prone laying hens. *Poult. Sci.* 87:485–496.
- Van Krimpen, M. M., R. P. Kwakkel, C. M. C. van der Peet-Schwering, L. A. den Hartog, and M. W. A. Verstegen. 2009. Effects of nutrient dilution and nonstarch polysaccharide concentration in rearing and laying diets on eating behavior and feather damage of rearing and laying hens. *Poult. Sci.* 88:759–773.
- Velloso, L. A., E. P. Araújo, and C. T. de Souza. 2008. Diet-induced inflammation of the hypothalamus in obesity. *Neuroimmunomodulation* 15:189–193.
- Vestergaard, K. S., J. P. Kruijt, and J. A. Hogan. 1993. Feather pecking and chronic fear in groups of red junglefowl: Their relations to dustbathing, rearing environment and social status. *Anim. Behav.* 45:1127–1140.
- Walker, J. R., J. P. Ediger, L. A. Graff, J. M. Greenfeld, I. Clara, L. Lix, P. Rawsthorne, N. Miller, L. Rogala, C. McPhail, and C. Bernstein. 2008. The Manitoba IBD cohort study: A population-based study of the prevalence of lifetime and 12-month anxiety and mood disorders. *Am. J. Gastroenterol.* 103:1989–1997.
- Weeks, C. A., and C. J. Nicol. 2006. Behavioural needs, priorities and preferences of laying hens. *World's Poult. Sci. J.* 62:296–307.