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Original Studies

Comparison of Selected Diagnostic Parameters in African Grey Parrots (*Psittacus erithacus*) With Normal Plumage and Those Exhibiting Feather Damaging Behavior

Susan L. Clubb, DVM, Dipl. ABVP (Avian), Carolyn Cray, PhD, Kristopher L. Arheart, EdD, and Michelle Goodman

Abstract: Feather damaging behavior is a common problem in African grey parrots (*Psittacus erithacus*). To determine if differences in clinical test results could be detected in parrots with feather damaging behavior (FDB), we studied 51 parrots, including 24 with FDB, with a variety of diagnostic tests. A predictable stress hemogram was found after administration of thyroid-stimulating hormone in both normal birds and birds with FDB. Birds with FDB had significantly lower lymphocyte counts and higher heterophil : lymphocyte ratios after thyroid-stimulating hormone injection than did normal birds. Although resting thyroxine (T_4) levels were not different between the 2 groups, the magnitude of the post–thyroid-stimulating-hormone increase in T_4 was significantly less in the FDB group. Alpha-1 and alpha-2 globulin fractions were significantly higher and gamma globulin fractions were significantly lower in birds with FDB. Birds with FDB also had higher *Aspergillus* antibody titers. No significant differences were found in *Aspergillus* antigen levels, *Candida* antibody titers, or blood concentrations of histamine, serotonin, or corticosterone. The clinical significance of these differences is not known.

Key words: feather damaging behavior, feather plucking, thyroxine, hypothyroidism, thyroid-stimulating hormone stimulation, *Aspergillus,* avian, African grey parrot, *Psittacus erithacus*

Introduction

African grey parrots (*Psittacus erithacus*) are recognized for their beauty, intelligence, and ability to mimic human language. They are also known for feather damaging behavior (FDB). Self-destructive behaviors, including feather picking, feather damage, and soft-tissue mutilation, are common in psittacine birds. A multifactorial etiology that involves behavior, parasitism, bacterial or fungal infection, nutritional deficiencies, allergy, or unrelated medical conditions has often been proposed.[1,5]

From the Rain Forest Clinic for Birds, 3319 E Rd, Loxahatchee, FL 33470, USA (Clubb); Division of Comparative Pathology, Miller School of Medicine, University of Miami, PO Box 016960 (R-46), Miami, FL 33101, USA (Clubb, Cray); Department of Epidemiology and Public Health, Miller School of Medicine, University of Miami, PO Box 016960 R-669, Miami, FL 33101, USA (Arheart); and Kaytee Products, Inc, 521 Clay St, PO Box 230, Chilton, WI 53014, USA (Goodman).

A recent report detailed that higher levels of *Malassezia* organisms were not found in birds with FDB relative to those in normal birds.[6] The role of hypothyroidism often has been mentioned in association with feather damaging behavior. Clubb et al[7,8] demonstrated low resting thyroxine (T_4) levels in birds with FDB. In addition, 1 case of hypothyroidism was reported in a scarlet macaw (*Ara macao*) with severe feather loss.[9] Allergy or hypersensitivity has also been widely associated anecdotally with FDB, and attempts have been made to document this by skin testing.[10] Related therapies, including dietary restrictions, antihistamines, and antioxidants, have reportedly been used with good results.[11]

In the current study, results from diagnostic tests in African grey parrots with FDB and in those with normal plumage were compared. The goal of the study was to investigate the roles of stress, infection, allergy, and thyroid function in birds with FDB.

Materials and Methods

Fifty-one African grey parrots, 27 with normal plumage and 24 with evidence of FDB, were used in the study. Most birds came from 4 breeding facilities (1 indoor collection in Wisconsin and 3 outdoor collections in Florida) where populations had been stable for several years. The group with normal plumage also included 4 pet birds. All but 4 birds were wild caught, and, with the exception of the 4 captive-bred birds, all were 10 years of age or older. The normal plumage group was composed of 13 females and 14 males, whereas the FDB group had 16 females and 8 males. All birds tested negative by polymerase chain reaction for circovirus I, the causative agent of psittacine beak and feather disease. Five of 24 birds were positive for avian polyomavirus antibody, although there were no known cases of polyomavirus in the tested collection during the preceding 5 years. All birds appeared clinically normal, with the exception of feather lesions in birds exhibiting FDB.

All sampling occurred in autumn and during the same period of the day (9:00–10:00 AM). Each bird was captured and restrained manually, and a blood sample was collected from the jugular vein within 30 seconds after capture. Thyroid-stimulating hormone (TSH) from bovine pituitary tissue (0.5 IU; Sigma, St. Louis, MO, USA) was administered in the pectoral muscle, as previously described.[12] A second blood sample was collected 6 hours after the TSH injection. Blood samples were placed in lithium heparin plasma separator tubes (VWR, West Chester, PA, USA), and plasma was separated by centrifugation within 30 minutes of collection. Two peripheral blood smears were prepared immediately on full-length glass slides. All plasma samples were placed on cold packs and shipped overnight to the clinical laboratory. Initial blood samples were submitted for complete blood count (CBC), plasma protein electrophoresis, T_4 level, corticosterone, serotonin, and *Aspergillus* antigen and antibody serologic testing, all of which were completed within 24 hours. The samples collected 6 hours after TSH stimulation were submitted for a CBC and T_4, corticosterone, and serotonin levels. The remaining plasma was stored at −20°C for later testing of histamine levels, as well as *Candida* antibody titers. All samples were analyzed at the University of Miami Avian and Wildlife Laboratory (Miami, FL, USA), with the exception of CBCs from birds housed in Wisconsin, where analyses were performed at Marshfield Laboratory (Marshfield, WI, USA).

The total white blood cell (WBC) count was determined by the Unopette method (Becton Dickinson, Franklin Lakes, NJ, USA) within 24 hours of sample collection. The WBC differential was performed manually after smears were stained with a modified Wright stain (Diff Quik, Southeast Vet Lab, Miami, FL, USA). Plasma protein electrophoresis was completed as previously described.[13] Serologic testing of *Aspergillus* antibody and antigen was performed by enzyme-linked immunosorbent assay (ELISA), as previously described.[14] *Candida* serologic testing was performed by using an adaptation of a commercial kit (Candida IgG, ALPCO Diagnostics, Windham, NH, USA). The kit procedure was followed, and anti-chicken immunoglobulin G (Sigma, St. Louis, MO, USA), including confirmed avian positive and negative control samples, was substituted at the conjugate stage. Concentrations of T_4 and corticosterone were determined by radioimmunoassay (Diagnostic Products Corporation, Los Angeles, CA, USA) as previously described.[12] Serotonin and histamine levels were quantitated by ELISA assays (ALPCO Diagnostics). All samples were analyzed in duplicate.

Data were analyzed as percentages and absolute numbers. Log transformation was used on the data and data were analyzed by analysis of variance. One-way analysis of variance was used for data measured once. For variables measured before and after treatment, a repeated measures analysis of variance with planned comparisons was used to test the significance of before and after differences within groups. The $P = .05$ level was used to determine statistical significance. The Statistical Analysis System (SAS, Inc, Cary, NC, USA) was used for data management and analysis.

Results

Birds in both the normal and FDB groups experienced an approximate 2-fold increase in the WBC count after TSH injection. This was a significant increase within each group ($P < .001$), but there was no significant difference between the groups. The packed cell volume decreased and heterophil counts increased slightly by the second sampling time. Notably, the percentage and absolute lymphocyte count decreased after TSH injection in both the normal and FDB groups. The percentage of lymphocytes after TSH stimulation in the FDB group was significantly lower than that in the normal group ($P = .021$), and a similar

Table 1. Results (mean ± SE) of hematologic tests at rest and 6 hours after TSH administration in normal African grey parrots and those with FDB.[a]

Analyte	Normal birds, mean ± SE	Birds with FDB, mean ± SE
WBC, $\times 10^3/\mu l$		
Resting	10.6 ± 0.9[b]	10.6 ± 0.8[b]
After TSH	20.6 ± 1.3[b]	23.0 ± 1.6[b]
PCV		
Resting	53.3 ± 1.3[c]	51.5 ± 1.3[b]
After TSH	51.8 ± 1.3[c]	49.0 ± 1.1[b]
Heterophils, %		
Resting	63.2 ± 3.5[b]	64.6 ± 2.6[b]
After TSH	80.6 ± 2.3[b]	86.1 ± 1.6[b]
Lymphocytes, %		
Resting	35.7 ± 3.5[b]	31.1 ± 2.5[b]
After TSH	20.3 ± 2.7[b,d]	12.0 ± 1.7[b,d]
Het : lymph ratio		
Resting	2.8 ± 0.5[b]	2.5 ± 0.3[b]
After TSH	7.5 ± 1.4[b,d]	12.2 ± 2.2[b,d]

[a] SE indicates standard error; FDB, feather damaging behavior; TSH, thyroid-stimulating hormone; PCV, packed cell volume; Het, heterophils; and lymph, lymphocytes.

[b] Significant difference between resting and after TSH values ($P < .001$).

[c] Significant difference between resting and after TSH values ($P < .05$).

[d] Significant difference between normal birds and birds with FDB ($P < .05$).

difference was also apparent in the heterophil : lymphocyte ratio after TSH stimulation ($P = .02$). The decrease in lymphocytes after TSH stimulation was significantly different between the normal and FDB groups ($P = .04$; Table 1).

No significant difference was seen between the normal and FDB groups in both corticosterone and serotonin levels before and after TSH injection (Table 2). Similarly, resting T_4 levels were not significantly different between the normal birds and the birds with FDB (Table 2). Both groups showed a significant increase in T_4 and serotonin levels after TSH stimulation ($P < .001$). Before and after values between the 2 groups were also significantly different ($P = .046$).

Plasma protein fractions were analyzed by electrophoresis. Birds in the FDB group demonstrated significantly higher levels of alpha-1 globulins ($P < .001$) and alpha-2 globulins ($P = .017$) and lower levels of gamma globulins ($P = .006$) (Table 3) than those in the normal group. No significant differences were present between the groups in the albumen : globulin (A : G) ratio or percentages of pre-albumin, albumin, or beta globulins.

Table 2. Corticosterone, serotonin, and thyroxine (T_4) levels (mean ± SE) at rest and 6 hours after TSH administration in normal and African grey parrots and those with FDB.[a]

Analyte	Normal birds, mean ± SE	Birds with FDB, mean ± SE
Corticosterone, ng/ml		
Resting	20.1 ± 3.4	18.6 ± 3.2
After TSH	14.8 ± 2.7	21.9 ± 3.5
Serotonin, ng/ml		
Resting	434 ± 134[b]	492 ± 162[b]
After TSH	624 ± 113[b]	678 ± 186[b]
T_4		
Resting	0.27 ± 0.05[b]	0.30 ± 0.04[b]
After TSH	0.91 ± 0.06[b]	0.82 ± 0.09[b]

[a] SE indicates standard error; TSH, thyroid-stimulating hormone; FDB, feather damaging behavior.

[b] Significant difference between resting and after TSH values ($P < .001$).

The index of reactivity in the *Aspergillus* antibody assay was significantly higher in the FDB group ($P = .018$; Table 4). No significant differences were seen between the 2 groups in either *Aspergillus* antigen or *Candida* antibody reactivity. Similarly, no significant differences were appreciated between the groups for resting histamine levels (Table 4).

Discussion

Our results support the association of FDB and altered thyroid function. In addition, stress-related differences, as well as differences in antibody levels to *Aspergillus* were documented in parrots

Table 3. Plasma protein fractions (mean ± SE) measured by electrophoresis on resting blood samples in normal African grey parrots and those with FDB.[a]

Analyte	Reference range	Normal birds, mean ± SE	Birds with FDB, mean ± SE
A : G ratio	1.4–3.5	1.7 ± 0.1	1.7 ± 0.9
Pre-albumin, %	0–19	1.8 ± 0.6	1.0 ± 0.6
Albumin, %	42–69	60.3 ± 1.1	61.0 ± 1.4
Alpha-1 globulins, %	2–7	2.7 ± 0.1[b]	3.7 ± 0.2[b]
Alpha-2 globulins, %	2–6	4.3 ± 0.2[c]	5.6 ± 0.6[c]
Beta globulins, %	12–26	21.4 ± 0.6	21.2 ± 0.7
Gamma globulins, %	4–12	9.5 ± 0.5[c]	7.6 ± 0.5[c]

[a] SE indicates standard error; A, albumen; G, globulin; and FDB, feather damaging behavior.

[b] Significant difference between normal birds and birds with FDB ($P < .001$).

[c] Significant difference between normal birds and birds with FDB ($P < .05$).

Table 4. Serologic reactivity and histamine levels (mean ± SE) in normal African grey parrots and those with FDB.[a]

Test	Normal birds, mean ± SE	Birds with FDB, mean ± SE
Aspergillus antibody[b]	1.24 ± 0.06[c]	1.45 ± 0.07[c]
Aspergillus antigen[b]	1.25 ± 0.05	1.24 ± 0.04
Candida antibody[b]	1.18 ± 0.06	1.24 ± 0.07
Histamine, nM	6.9 ± 1.5	5.0 ± 1.4

[a] SE indicates standard error; FDB, feather damaging behavior.

[b] Value is an index of the absorbance of the test sample divided by the absorbance of a pool of negative samples.

[c] Significant difference between normal birds and birds with FDB ($P < .05$).

with FDB. The clinical relevance of these findings in African grey parrots is unknown.

Routine hematologic testing showed normal total WBC and differential counts in the birds with FDB. Increases in WBC counts occurred in both groups after handling and TSH injection. Stress-related hemograms were previously described in avian species. Whereas decreases in the WBC count were observed in pigeons after handling, travel was found to increase the total WBC count in macaws.[15,16] In addition, avian species dominated by heterophils were reported to show increases in both WBC and heterophil counts after handling and transport.[17] Birds with FDB demonstrated a lower lymphocyte count and an increased heterophil : lymphocyte ratio after TSH. Because the change in leukocyte differential has been proposed to be an indicator of stress in other avian species, the significant difference between the groups in this study may show that FDB birds have a higher stress response to handling and injections than birds with normal plumage.[18]

To further investigate other possible measures of stress, blood corticosterone and serotonin levels from normal and birds with FDB were compared. Both were previously quantitated in avian species by using commercial kits for mammalian use.[12,19,20] No difference in resting corticosterone levels was appreciated between the groups. Higher levels of corticosterone were measured in birds with FDB after TSH administration, but levels were not significantly different ($P = .10$). This may be related to the time interval of sampling (6 hours after TSH injection), because corticosterone levels were shown to peak for 3 hours then decrease markedly by 4 hours after injection of adrenocorticotropic hormone.[19] Interestingly, the authors of that report found no significant differences when adrenocorticotropic hormone injections were replaced by saline solution. This result is similar to that reported in sandhill cranes (*Grus canadensis*).[20] However, handling resulted in increased corticosterone levels in American kestrels (*Falco sparrerius*).[21]

In the current study, no significant differences were seen between resting and post–TSH-injection levels of serotonin. Although serotonin has not been widely studied in avian species, it is believed to play a role in the physiology of depression and anxiety in mammals. Chickens genetically selected for high production and longevity were reported to have higher blood serotonin concentrations than those with lower levels of production and longevity.[22] In the current study, no significant difference was seen in serotonin levels between normal birds and birds with FDB. Both groups demonstrated an increase in serotonin levels by 6 hours after TSH administration. This may reflect a normal diurnal change as reported in other species.[23] The lack of corroboration of the observed stress-related hematologic changes with either corticosterone or serotonin levels suggests that these hormones may not follow the same sampling time course or that hematologic evaluation may be a more sensitive indicator of stress and may be mediated by other factors in avian species. Assessment of neurotransmitters and stress indicators in birds with FDB warrants further studies.

In limited publications, but certainly anecdotally, hypothyroidism has been linked to FDB.[1,7-9] Our previous report found that resting levels of T_4 were lower in birds with FDB than in birds with normal plumage.[8] The current study reflected a doubling of the sample size and shows that resting levels of T_4 between the groups were not significantly different. However, the magnitude of change between the before and after TSH levels of T_4 was significantly different in the normal and FDB groups ($P = .046$). It has been reported that a single resting level of T_4 does not have clinical diagnostic importance and that the TSH stimulation test is superior for the diagnosis of thyroid diseases.[12,24] In the description of hypothyroidism in a macaw, resting levels of T_4 only increased by 50% after injection with TSH.[9] In the current study, birds with FDB showed more than a 2.5-fold increase, indicating that they were not hypothyroid. However, our results supported an association between suboptimal thyroid function and FDB.

Changes in plasma protein fractions were described as indicators of acute inflammation and ongoing immune responses.[13] Although both normal birds and birds with FDB had plasma levels of alpha-1 and alpha-2 globulins within reference ranges, levels were significantly higher

in parrots with FDB than in normal birds. This difference may reflect underlying inflammatory processes associated with FDB or the effects of FDB. Interestingly, although levels were also within reference ranges, birds with FDB demonstrated a significantly lower gamma globulin level than normal birds. Lower gamma globulin levels in mammals were associated with immune suppression or immune deficiency.[25] Perhaps the long-term destructive behavior of the birds with FDB resulted in humoral anergy or suppression.

Yeast infection was examined as a potential cause of inflammatory skin disease and was positively and negatively associated with FDB in various studies.[6,26,27] Our results demonstrated no difference in antibody titers to *Candida* species between normal birds and birds with FDB. In humans, both *Aspergillus* and *Candida* have been well implicated in allergic responses. *Aspergillus* was similarly proposed as a possible allergen or infectious agent associated with FDB in birds.[8,11,26,28] In the current study, a significantly higher level of antibody to *Aspergillus* was detected in birds with FDB ($P = .018$), but no infection or allergy was documented. The presence of increased antibody titers may reflect sensitivity to infection or exposure to *Aspergillus* secondary to the effects of FDB. Future studies should characterize the complete response to *Aspergillus*, both at the antibody and lymphocyte levels.

Histamine levels were not significantly different between normal birds and birds with FDB. Macwhirter and Mueller[28] reported that only some psittacine birds responded to intradermal injections of histamine and proposed that there may be other mediators of allergic responses in avian species. African grey parrots in the current study had exhibited long-term FDB, and only 1 blood sample was submitted for histamine quantitation. Histamine-mediated allergic reactions possibly form an initial or recurrent cause of FDB but do not represent a constant process. It would be interesting to compare histamine levels in birds with acute onset FDB with those that demonstrate the chronic behavior.

There is a body of literature regarding the variability in general chemistry and hormone levels in some avian and mammalian species. Circadian and seasonal variability in general biochemical determinations, as well as the influences of sex and subspecies, was demonstrated in budgerigars (*Melopsittacus undulatus*).[29] Although T_4 levels were not addressed in the budgerigar study, changes in T_4 levels with season, time of day, and sex have been documented in nonpsittacine bird species. This was inclusive of rising levels of T_4 in molting penguins (*Spheniscus humboldi*), the importance of T_4 in controlling seasonal reproduction in tree sparrows (*Spinzella arborea*), and diurnal variation of T_4 in chickens.[29-32] In a study of T_4 levels in pigeons (*Columba livia*) housed indoors and outdoors during the summer and winter, diurnal variation of T_4 did not vary significantly between indoor and outdoor birds, but higher overall T_4 levels were found in the summer than in winter.[33]

In the current study, 2 populations were assessed. The 26 birds from an indoor facility in Wisconsin received artificial light on a timer to simulate a natural photoperiod. Notably, the analysis of this population revealed results of hematologic testing, TSH response, plasma protein electrophoresis, and *Aspergillus* antibody similar to those described in a previous study of this population.[8] Interestingly, the Wisconsin FDB group had significantly lower resting T_4 levels than birds with normal plumage.[8] In an effort to increase the sample size, additional data were collected from birds housed outdoors in Florida. Inclusion of this outdoor population added differences in environment, housing, and food. With the addition of samples from the outdoor group, resting levels of T_4 were no longer significantly different between groups, as was found in the previous study.[8] In the present study, all sampling was done during the nonbreeding season and at the same time of the day to minimize sample variation. Although the normal plumage group had a nearly equal sex ratio, hens predominated the FDB group. Future studies should investigate possible differences in the risk of FDB in male and female birds.

An examination of test results from African grey parrots with normal plumage and with FDB revealed several differences and similarities. It is not clear if the differences were clinically significant or if they constituted a cause or consequence of FDB. The results do suggest a basis for future studies and support the premise that FDB likely has a multifactorial etiology.

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