Fecal corticosterone excretion in captive healthy and feather picking African grey parrots (*Psittacus erithacus*)

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Feather picking (FP) is a common problem in companion parrots, especially in African grey parrots *(Psittacus erithacus).* Many hypothesis have been made about this self-mutilating behavior, and serious psychological conflicts can play a pivotal role in the initiation of this self-defeating and self-punishing behaviour. Even though ethological distress is difficult to study, the monitoring of fecal corticoids is a useful non-invasive tool that can be used to assess stress in animals. The purpose of the present study was to compare the fecal corticosterone levels of companion African grey parrots kept as wild-type parrots (WT), healthy pet parrots (HP) and feather picking pet parrots (FPP) during the breeding season and out of the breeding season. An African grey parrot group, composed of 82 individuals, has been studied; these birds were divided into three sub-groups: 1) WT, parrots that were reared by their biological parents, kept in couples and with a regular reproduction activity; 2) HP, parrots that were hand-reared, but did not show any FP symptoms; 3) FPP, parrots that were hand-reared and that showed signs of FP. Fecal samples were collected in the morning on alternate days, during the breeding seasons and out of the breeding season. The fecal corticosterone and immunoreactive corticosterone metabolites were determined using a pan-specific corticosterone enzyme immunoassay kit (K014; Arbor Assays®, Ann Arbor, MI) validated for dried fecal extracts. Analysis of Variance was used to examine any variations due to the breeding season and among the groups of parrots. WT and HP parrots showed lower corticosterone levels than FPP parrots. The fecal corticosterone concentration showed an average (sum of the two seasons) of 74.10 ± 16.89 ng/g in the WT parrots; 79.56 ± 21.23 ng/g in the HP parrots and 185.53 ± 34.83 in the FPP parrots. A statistical trend between fecal corticosterone concentration in the different seasons for the WT parrots ($P=0.085$) was observed, but not for the other groups. The results suggest that 1) the highest levels of fecal corticosterone are excreted by birds affected by FP; 2) the observed highest level of corticosterone in FPP parrot suggests a role of stress in this self-injuring stereotypic behavior.

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Short title: Corticosterone and feather picking

Abstract

Background. Feather picking (FP) is a common problem in companion parrots, especially in African grey parrots *(Psittacus erithacus)*. Many hypothesis have been made about this self-mutilating behavior, and serious psychological conflicts can play a pivotal role in the initiation of this self-defeating and self-punishing behaviour. Even though ethological distress is difficult to study, the monitoring of fecal corticoids is a useful non-invasive tool that can be used to assess stress in animals. The purpose of the present study was to compare the fecal corticosterone levels of companion African grey parrots kept as wild-type parrots (WT), healthy pet parrots (HP) and feather picking pet parrots (FPP) during the breeding season and out of the breeding season.

Methods. An African grey parrot group, composed of 82 individuals, has been studied; these birds were divided into three sub-groups: 1) WT, parrots that were reared by their biological parents, kept in couples and with a regular reproduction activity; 2) HP, parrots that were hand-reared, but did not show any FP symptoms; 3) FPP, parrots that were hand-reared and that showed signs of FP. Fecal samples were collected in the morning on alternate days, during the breeding seasons and out of the breeding season. The fecal corticosterone and immunoreactive corticosterone metabolites were determined using a pan-specific corticosterone enzyme immunoassay kit (K014; Arbor Assays®, Ann Arbor, MI) validated for dried fecal extracts. Analysis of Variance was used to examine any variations due to the breeding season and among the groups of parrots.

Results. WT and HP parrots showed lower corticosterone levels than FPP parrots. The fecal corticosterone concentration showed an average (sum of the two seasons) of 74.10 ± 16.89 ng/g in the WT parrots; 79.56 ± 21.23 ng/g in the HP parrots and 185.53 ± 34.83 in the FPP parrots. A statistical trend between fecal corticosterone concentration in the different seasons for the WT parrots (P=0.085) was observed, but not for the other groups.

Discussion. The results suggest that 1) the highest levels of fecal corticosterone are excreted by birds affected by FP; 2) the observed highest level of corticosterone in FPP parrot suggests a role of stress in this self-injuring stereotypic behavior.

Keywords: African grey parrot, stress; corticosterone; bird ethology; sexual behavior.

Introduction

Feather picking (FP) is a behavioral disorder that is frequently encountered in captive parrots; it includes plucking, chewing, fraying and/or biting, and it results in the loss of or damage to feathers (van Zeeland et al., 2009; van Zeeland et al., 2013). FP is common in many species of parrots, and it has been observed in African grey parrots and cockatoos (Jayson SL, Williams DL &Wood, 2014; Jen-Lung Peng S, Hessey J, Tsay T & Chang-Young Fei A., 2014), Amazon parrots (Garner et al., 2006), *Ara spp*., *Agapornis spp*. (Costa et al., 2015), Crimson bellied Conure (van Hoek & King, 1997) and in other Psittacine species. FP is typical of captive parrots, but has rarely been observed in the wild and often occurs when birds reach sexual maturity (Wedel, 1999). Grindlinger (1991) estimated that approximately 10.0% of the captive parrot population suffered from FP, while other authors have declared a prevalence of 15.8% (Kinkaid et al., 2013).

Bordnick, Thyer & Ritchie (1994) compared FP to the psychological mechanisms that generate the appearance of trichotillomania in humans, and pointed out how serious conflicts can generate self-defeating and self-punishing behavior. However, the study of behavioral suffering in animals is difficult, but some kinds of ethological distress are manifested physically, such as stress lesions, self mutilation and other outward displays of abnormal or stereotypic behavior (Engebretson, 2006).

The methodologies frequently used to try to quantify pain and stress in animals include quantification of the circulating stress hormones (e.g. cortisol, β-endorphin and catecholamines) (Ayala et al., 2012; Livingston, 2010; Schmidt et al., 2010). The monitoring of glucocorticoid concentrations is increasingly being applied to zoo animals thorough the sampling of urine, feces and saliva, and this methodology can provide a very useful longitudinal measure of the adrenal output to detect potentially indicative patterns of chronic stress, even in birds (Shepherdson et al., 2004), and associated with behavioral and physiological changes in energy demand (Harvey et al. 1984). Furthermore, the levels of circulating corticosterone are considered a reliable indicator of stress levels in birds (Dehnhard et al., 2003; Hartup et al., 2004), although blood sampling can be inappropriate because its high invasiveness can cause stress responses (Nemeth et al., 2016), thus compromising the stress assessment (Hamilton & Weeks, 1985; Le Maho et al., 1992). The non-invasive glucocorticoid measurement, especially when used in conjunction with other parameters, such as behavior, can give an accurate and important insight into the welfare status of an individual or a group of animals (Lane, 2006). Fecal corticoids can be used as a useful non-invasive tool for the assessment of stress in birds and other animals (Shepherdson, Carlstead & Wielebnowski, 2004), as this method does not require restraint of the animals .

FP is the most common problem in African grey pet parrots (*Psittacus erithacus*) (Clubb *et al.*, 2007), and it has been demonstrated that the fecal corticosterone levels in African grey parrots are linked to stress and FP damage (Owen & Lane, 2011). Since all companion parrots are born in captivity, they can be defined as " pet parrots" or "wild type parrots" according to the rearing system. The "pet parrots" include any bird that has been hand-reared at the neonatal age (Schmid, Doherr & Steiger, 2006); these birds develop an imprinting toward humans. The "wild-type parrots" have been reared by their biological parents and can maintain natural or normal behavior that is more in line with the natural pattern (Glendell, 2003); these birds do not have a close contact with humans. Our group previously conducted a study, considering this classification, which showed a notable difference in FP prevalence in the two different populations of parrots. The wild-type population was composed of 1488 birds and showed a plumage disorder prevalence of 1.3%, while the pet parrot population showed a feather picking prevalence of 17.5% (Costa, et al., 2015).

The purpose of the present study was to compare the fecal corticosterone level in companion African grey parrots kept as wild-type parrots (WT), in pet parrots with feather picking (FPP) and in healthy pet parrots (HP) during the breeding season and in the out of breeding season.

Material & Methods

Animal and selection criteria

The study was based on a web questionnaire of a previous study (Costa et al., 2015), addressed to the owners of all species of pet parrots. The questionnaire was distributed throughout Italy through on-line parrot association sites, social networks and e-mails, in collaboration with the Italian Psitacine Club (named "Club degli Psittacidi" http://psittacidi.webservice-4u.com/) and the Italian Association of Parrots Breeders (named "Associazione Italina Allevatori Pappagalli", http://www.assopappagalli.it/).

Only the owners of African grey parrots (*Psittacus erithacus*) were considered for the present study, since they had resulted to be the most represented breed among the Italian respondents and also because this breed is considered very sensitive to FP (Jayson et al., 2014).

A total of 82 African grey parrots, kept in captivity, were included in the study. In order to be included in the study the birds had to be at least thirty-six months old, so that only birds that had a fully formed character and sexual behavioral patterns were considered. The animals were classified according to the typology of breeding. The WT group (Figure 1a) was composed of birds reared by their biological parents and had natural or normal behavior in line with the natural pattern (e.g they lived with a conspecific partner and had not developed a relationship with humans) (n=30 couples). The animals included in this group were kept in at least standard minimum cage (1 m^3) produced by specialized factory for birds and were exposed to natural photoperiod. All these birds were healthy and never showed the FP or any other plumage disorders. These birds have a regular reproduction activity.

The other groups included pet parrots that had been hand-reared from a neonatal age, and had thus developed an imprinting toward humans; these birds lived mostly outside a cage, without any other parrots and with no possibility of reproduction. These pet parrots were divided into two groups: healthy parrots (HP, n=11), which did not showed any sign of FP (Figure 1b) and feather picking parrots (FPP, n=11) (Figure 1c). The diagnosis of FP was conducted by an expert veterinary surgeon who took into consideration all the possible differential diagnoses according to van Zeeland et al., 2009. In this way, it was possible to rule out any clinical problems. The HP birds were age (± 2 years) and sex matched with the FPP birds. All the birds had free access to water and to a standard commercial diet formulated specifically for parrots.

The owners of all the parrots included in the study compiled a questionnaire; however, only data about care management and the main body regions affected by FP have been dealt with in this work.

Fecal sampling

Fecal samples were collected throughout October – December 2014 (out of the breeding season) and throughout April – July 2015 (breeding season). Although most African grey parrots start copulating in October, with egg laying starting in November and chicks hatching in December, Italian breeders prefer to start the breeding season in spring, thus reversing the natural habit. In other words, the WT African grey parrots did not have nests during the autumn sample collection period. Fecal samples were collected in the morning (9:00 – 11:00 AM) on alternate days to obtain at least 30 g material. The samples were collected directly from the cleaned bottom of the bird's habitual cage, where the parrot lived. The fecal samples were stored in 50 ml plastic tubes and immediately frozen at -20°C until analysis. A total of 30, 11 and 11 samples were collected for each sampling time for the WT, HP and FPP, respectively.

Fecal sample analyses

The feces were lyophilized and weighed, completely crushed, and two aliquots of pulverized feces (0.25 g each) were put into extraction tubes, which were then sealed with a Teflon cap. In order to extract steroids, the feces were subjected to an organic phase extraction using ethanol; the use of ethanol is recommended as a way of completely solubilizing the dried steroid, because certain steroids have limited aqueous solubility. An aliquot of 1 mL of ethanol (Sigma Aldrich, St. Louis, MO) was added to each tube for each 0.1 g of solid, and the mixture was shaken vigorously for 30 min. The samples were centrifuged at 3,300 × g for 15 min, and the supernatant was recovered in a clean tube for evaporation to dryness in a SpeedVac (Thermo Fisher Scientific, *Waltham, MA).* Extracts were stored at –80°C for the subsequent analysis.

The extracted samples were dissolved in 100 µL ethanol, and 400 µL of kit Assay Buffer (Arbor Assays, AnnArbor, MI) was added. The mixture was then vortexed and left to rest for 5 min twice to ensure complete steroid solubility. The fecal corticosterone and immunoreactive corticosterone metabolites were determined using a pan-specific corticosterone enzyme immunoassay kit (K014; Arbor Assays®, Ann Arbor, MI) validated for dried fecal extracts.

All the analyses were repeated twice. The inter- and intra-assay coefficients of variation were less than 10%. The sensitivity of the test was determined by measuring the smallest amount of hormone standard consistently distinguishable from the zero concentration standard, and it was calculated to be 17.3 ng/mL^{-1}. All the fecal samples were analyzed at multiple dilutions (1:4, 1:8, 1:16 and 1:32) and were found to be parallel to the standard curve ($p < 0.05$). The mean recovery rate of corticosterone added to dried excreta was 96.7%. According to the manufacturer, the corticosterone kit presents the following cross reactivity: 100% with corticosterone, 12.3 % with desoxycorticosterone, 0.62% with aldosterone, 0.38% with cortisol and 0.24% with progesterone.

Data analysis

The fecal corticosterone amount of the WT, HP and FPP parrots were compared. Before testing for group differences, normality of data distribution and homogeneity of variance were assessed using the Shapiro-Wilk test and the Levene test, respectively. Analysis of Variance was used to examine any variations due to the breeding season and among the groups of parrots.

Correlation between the corticosterone levels and age was also investigated in the HP and FPP. Statistical significance was set at 0.05, and a statistical trend was considered for *P* values below 0.1. Statistical analyses were performed using SPSS, version11.5.1, for Windows (SPSS Inc., Chicago, IL, USA).

Results

The average of age of the parrots was 125 months. The average volume of the aviary cages in which the birds belonging to the WT group were kept was 4.85 m^3. The average volume of the cage for the pet parrots was 1.70 m^3, although they were kept outside the cage on a daily basis, for at least five hours, thus living in close contact with their owners.

The main region affected by FP in the FPP birds was the chest (90.9%) (Figure 2a), and this was followed by the wings (18.2%) (Figure 2b), the shoulders and the rump (9.1%) (Figure 2c),. No sign of FP was observed on the head. Different quantities of fecal corticosterone were found for the three groups of African grey parrots ($P<0.001$). The mean corticosterone value was 74.10 ± 16.89 ng/g in the WT parrots; 79.56 ± 21.23 ng/g in the HP and 185.53 ± 34.83 ng/g in the FPP ones (Table 1).

Fecal corticosterone was not influenced by the sampling time (out-of breeding season vs breeding season) , but a statistical trend was observer for the WT parrots ($P=0.085$) (Table 1). No correlation between corticosterone and age was observed in either the HP or FPP.

Discussion

Feather picking is a concern for the welfare of parrots. The mechanisms and causes of this phenomenon are not completely clear, although FP is frequently encountered in captive

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parrots (van Zeeland et al., 2009). FP is considered an abnormal type of behavior that develops or persists in the absence of clinical causes, and it may be associated with a number of management factors, such as inadequate diet, social isolation and lack of environmental stimulation (Mertens, 1997). The present study has shown that the most affected area is the chest (90.9%), and this is followed by the wings (18.2%), shoulders and rump (9.1%). The head was not affected by FP and this data thus supports the hypothesis that FP is self-inflicted. In fact, the presence of feathers in good condition in areas of the body not directly reachable by the birds is one of the criteria that has been used to make a distinction between FP and other diseases (Galvin, 1983; Harisson, 1986; Westerhof & Lumeij, 1987).

Emotional stress and self-injury have also been observed in captive primates, and some authors have compared FP behavior in parrots to obsessive, compulsive and impulsive human disorders (Bordnick, Thyer & Ritchie, 1994; Jenkis, 2001; Garner, Meehan & Mench, 2003). Self-injuring behavior has also been observed in rhesus macaques (Novak, 2003). Self-injuring is considered a strategy to alleviate an acute negative affect or affective arousal (Favazza, 1992; Gratz, 2003; Haines, Williams & Brain, 1995).

The quantification of fecal corticoids is a useful non-invasive tool for the assessment of stress in birds (Shepherdson, Carlstead & Wielebnowski, 2004). The predominant glucocorticoid in birds is corticosterone, a hormone released in response to a potential threat to homeostasis, which is used to regulate behaviour and suppress body processes that are nonessential to survival (Wingfield, 1994), and the levels of circulating glucocorticoids are considered a reliable indicator of stress levels in birds (Dehnhard et al., 2003; Hartup et al., 2004). Like cortisol in mammals, corticosterone levels can be interpreted as an adaptive response to stressful events. Owen & Lane (2011) measured fecal corticosterone in African grey parrots, and they observed that the corticosterone level in the feces of feather picking parrots was higher than that of healthy pet parrots (261 ng/g and 75·1 ng/g, respectively). However, the authors did not consider the breeding season and they did not study the amount of fecal corticosterone in WT parrots. Our samples have shown a similar trend (74.10 ng/g in the WT; 79.56 ng/g in the HP and 185.53 in the FPP). A statistical trend for the corticosterone level of WT parrots was observed for the two sampled seasons, thus suggesting a probable influence of season and reproductive activity on fecal corticosterone excretion. According to Cherel et al. (1988), changes in circulating hormones are linked to the breeding season, and an increase in corticosterone concentration is a characteristic phase of a bird's reproduction.

The higher fecal corticosterone value in the FPP group, than in the HP and WT groups, confirms that these values consistently show elevations in adrenal activity after periods of stress, as suggested by Wasser et al. (2000). The link between FP and fecal corticosterone levels has also been observed by Jen-Lung Peng et al. (2014) in two cases of FP sulphur-crested cockatoo (*Cacatua galerita*): the authors found a decrease in corticosterone levels after bird's treatments, which consisted of socialization, a training program, medication and feeding enrichments. The authors suggested that high corticosterone levels can be associated to FP, and the present study confirms this hypothesis.

In this study, we did not investigate the relationship between the environmental captivity condition, and the inclusion criteria were related to the rearing method. WT are not hand-reared and can have behavior that is more in line with the natural pattern, that is, living with conspecific partner, without developing a relationship with humans. In a previous work (Costa et al., 2015), it was found that WT did not usually show signs of FP. On the other hand, pet parrots that have been hand-reared from a neonatal age, develop an imprinting toward humans, and live without

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any other parrots, with no possibility of reproduction. According to Fox (2006), an abnormal sexual imprinting and a strong social preference for humans may cause behavioral problems in pet parrots, which are probably more prone to inappropriately direct sexual behavior toward their owners. In the absence of a conspecific during the growing period; parrots probably develop sexual imprinting toward humans during the hand-rearing or weaning period. Furthermore, high levels of corticosterone are associated with depressive-like behaviour in animal models and a sex-dependent manner (Kott et al., 2016). Schmid, Doherr & Steiger (2006) suggested that the imprinting of parrots during the first two weeks of life is essential for the maturation of normal sexual behavior and fundamental for their later sexual activity. In fact, an aberration of this ethological process, for example, the absence of a conspecific, can cause a sexual deprivation in parrots, and hand-rearing consequently has the potential of producing physical as well as behavioral problems in parrots (Harcourt-Brown 2003). It has been noted that FP often develops after the onset of sexual maturity (Wedel, 1999), and that FP is connected to sexual frustration in parrots (Lantermann, 1989; Scott, 1948), which is manifested by behavior disorders, such as masturbation and food regurgitation.

It can be assumed that HP parrots can cope with stress, because they have similar corticosterone values to WT parrots, which rarely show signs of FP. However, it could be interesting to monitor the stress levels in those birds to establish whether they can change in time. Moreover, it could be interesting to study the corticosterone levels in birds that live naturally and are never kept in captivity. The present study has shown that fecal corticosterone can be used as a marker of animal welfare and that it should be interpreted in conjunction with other parameters (such as behavioral observations) in order to better assess a stress condition.

Conclusions

Corticoid excretion can be monitored by quantifying the corticosterone levels in fecal samples by using a non-invasive sampling method, which does not require animal restraining. The present study has investigated the level of corticosterone in companion parrots with different ethological constructs. A statistical difference has been observed between the FPP and HP parrots. The HP parrots that did not show signs of self-injuries had similar corticosteroid levels to the WT parrots, thus showing that they can cope with their environment.

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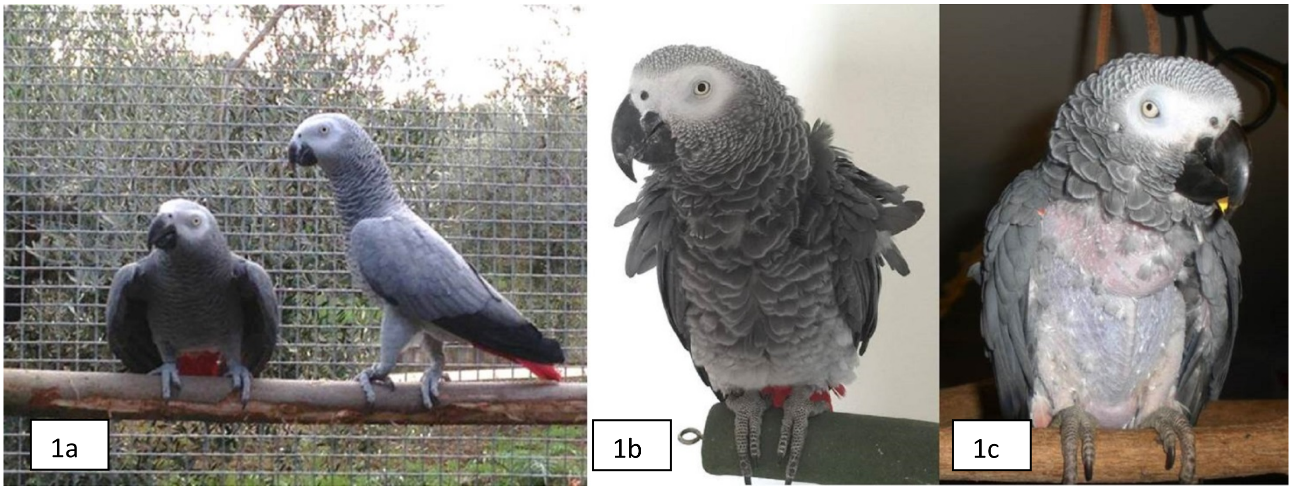
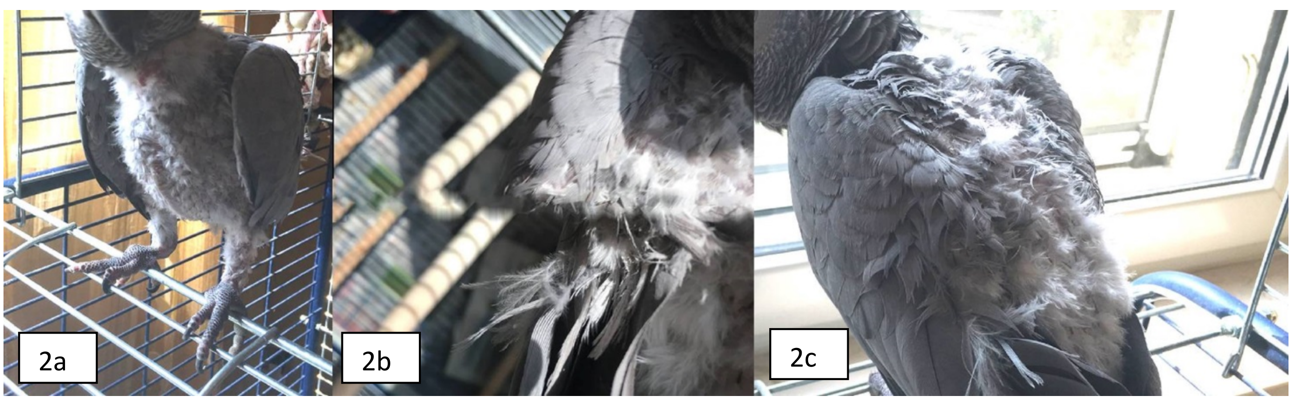
Figure 1a, 1b, 1c. Type of African grey parrots (*Psittacus erithacus*) observed in the present study



a) WT: wild type couple; b) HP: healty pet parrots and c) FPP: feather picking parrots.

Figure 2a, 2b, 2c. Deplumation area in feather picking African grey parrots



a) chest area; b) wings; c) shoulders and rump

Table 1: Fecal corticosterone excretion in captive healthy and feather picking African grey parrots (*Psittacus erithacus*) (ng/g) (mean ±s.d.)

	WT[1]	**HP[2]**	**FPP[3]**	***P***
	n = 30	n = 11	n = 11	
Out of breeding season	$77.85^{B} \pm 17.40$	$83.77^{B} \pm 20.84$	$183.67^{A} \pm 29.78$	< 0.001
In breading season	$70.35^{B} \pm 15.77$	$75.35^{B} \pm 21.75$	$187.19^{A} \pm 40.66$	< 0.001
P	0.085	0.365	0.809	
Total	n = 60	n = 22	n = 22	
Total mean	$74.10^{B} \pm 16.89$	$79.56^{B} \pm 21.23$	$185.53^{A} \pm 34.83$	< 0.001

A,B: P < 0.001

[1]WT: Wild-type parrots

[2]HP: Healthy pet parrots

[3]FPP: Feather picking pet parrots