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FEATHER DAMAGING BEHAVIOUR IN PETS PARROTS: SURVEY ON AN ITALIAN POPULATION AND FECAL CORTICOIDS EXCRETION IN AFRICAN GREY PARROTS (*Psittacus Erithacus*)

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PREFACE

The most popular birds in human environment

Parrots are one of the birds that many people like to keep as pets. This has been a long practice among many people for a very long time. In Italy, this phenomenon appeared especially in the last years of the Twentieth Century and increased in the New Millennium. Euromonitor (2014), e.g., claim that in Italy there are 13.000.000 birds in the country, but this data is certainly underestimated. In other states of the world, parrots are kept in household environment more than Italy: in North America about 10 million parrots are kept as household pets alone (Tweti, 2008). The reasons of these attentions are many, but certainly because the parrots are the one of the most distinctive groups within the avian kingdom. Their appearance is generally unmistakable, in spite of the wide diversity in size and coloration which exist within the seventy-seven genera which make up the order, Psittaciformes. Perhaps their most distinctive feature is the shape of the bill, with the upper portion being curved and fitting over the extended lower part on the beak (Alderton, 1992). More than any other group of birds, parrots have become closely associated with people, usually as companions although on occasions, larger species such as the macaws have been, and still are, shot for the pot. A fascination with parrots extends right back to the dawn history. The earliest settlers in South America probably kept parrots around their villages in the same way that their descendants do today. In some areas, there is also evidence to show that such birds were kept and bred on professional basis.

Goal of the study

The differences in morphology, eating habits and behavior cause in household environment many difficulties in management of these birds: often, many biological requirements are not satisfied and parrots can show a behavior disorder. Feather damaging behavior (FDB) in parrots has been studied for many years and it is the most dangerous abnormal behavior for parrots in household conditions. Despite the numerous studies conducted, it cannot still rely on a definition that would clarify unequivocally the mechanisms underlying this pathological condition, as well as the interpretation: the scientific community is yet to agree due to differences in opinions of scientists on what the factors that determine the appearance of this manifestation of discomfort and what the mechanisms modulate the expression. The primary aims of this work is to analyze literature and theories about this abnormal behavior to give the basis to study FDB in some Italian parrots populations.

The project started with the analysis of the Italian parrots population to understand how many and what species of parrots are kept as pet, what is the prevalence of FDB in our country and which species showed this phenomenon. Another objective was to compare the prevalence of FDB in hand-reared pet parrots (living alone) compared to parent-raised parrots (living in couple).

In order to understand the relationship between ethological evidence and physiological consequences, another purpose is provided. The analysis of glucocorticoid in birds can provide many information about their welfare conditions. Our study also evaluated the stress in FDB parrot through quantification of corticosterone levels in fecal samples: fecal samples collection is a not invasive method for evaluation of stress in birds. This insights were used to compare ethological evidence, management condition and individual characteristic of birds, to understand the link and possible explanation of this abnormal behavior. Levels of fecal corticosterone in hand-reared parrots compared to parent-raised birds was considered. Lastly, we considered the influence of environmental enrichments on the FDB evolution, examining the effect of commercial toys for pet birds on the corticosterone excretion.

GENERAL SECTION

1 BIOLOGY AND MANAGEMENT OF CAPTIVE PARROTS

1.1 Scientific classification

Parrots, also known as psittacines, are birds of the roughly 393 species in 92 genera that compose the order Psittaciformes, found in most tropical and subtropical regions.

The parrots are one of the most marvelously diverse groups of birds in the world. They range size from tiny pygmy parrots weighing just over 10 grams to giant macaws (Picture 2.1), weighing over kilogram. They consume a wide variety of foods, including fruits, seeds, nectar, insects, and in a few cases, flesh. They produce large repertoires of sounds, ranging from grating squawks to cheery whistles to, more rarely, long melodious songs. They live in a broad array of habitats, from lowland tropical rainforest to high-altitude tundra to desert scrubland to urban jungle (Toft & Wrigth, 2015).

Parrots are members of *Psittaciformes* order (Table 2.1): this order includes all parrots, parakeets, lories, lorikeets, conures, macaws, budgerigars and cockatoos. In particular, the order of *Psittaciformes* includes two family: *Cacatuidae* and *Psittacidae* (De Kloet, 2005).

The *Cacatuidae* include families belonging to *Probosciger* genus, *Calyptorhynchus*, *Callocephalon*, *Eolophus*, cockatoos and *Nymphicus*. This is the group numerically much small and includes, according to the classifications, from eighteen to twenty-one species, with several subspecies.

The tribe of *Cacatuini* includes the biggest parrots of the Indo-Australian: their size varies from thirty to eighty centimeters and are characterized mainly by a showy tuft of erectile feathers on the head, which sometimes have a different color from that of the plumage of the body (e.g. *Cacatua galerita*).

These birds are distributed in Australia, but some species also live in New Guinea, Philippines and in the Moluccas regions. Parrots of the *Cacatuidae* family does not show bright liveries; they are much less striking than the South Americans, but they are well spread in captivity. The most represented in captivity is certainly *Nymphicus hollandicus*, commonly called Cockatiel; from bred and selected this little cockatoo showed different color mutations.

Picture 2.1 Green-winged macaw (*Ara chloropterus*). Photo by “El Lorito” farm (© 2014 Pierluca Costa).

The massive spread of this species in household environment and the state of wild populations has allowed the exclusion from list of the Washington Convention. All cockatoos live mainly in the arboreal zone, preferring zones crossed by rivers in tropical and equatorial forests.

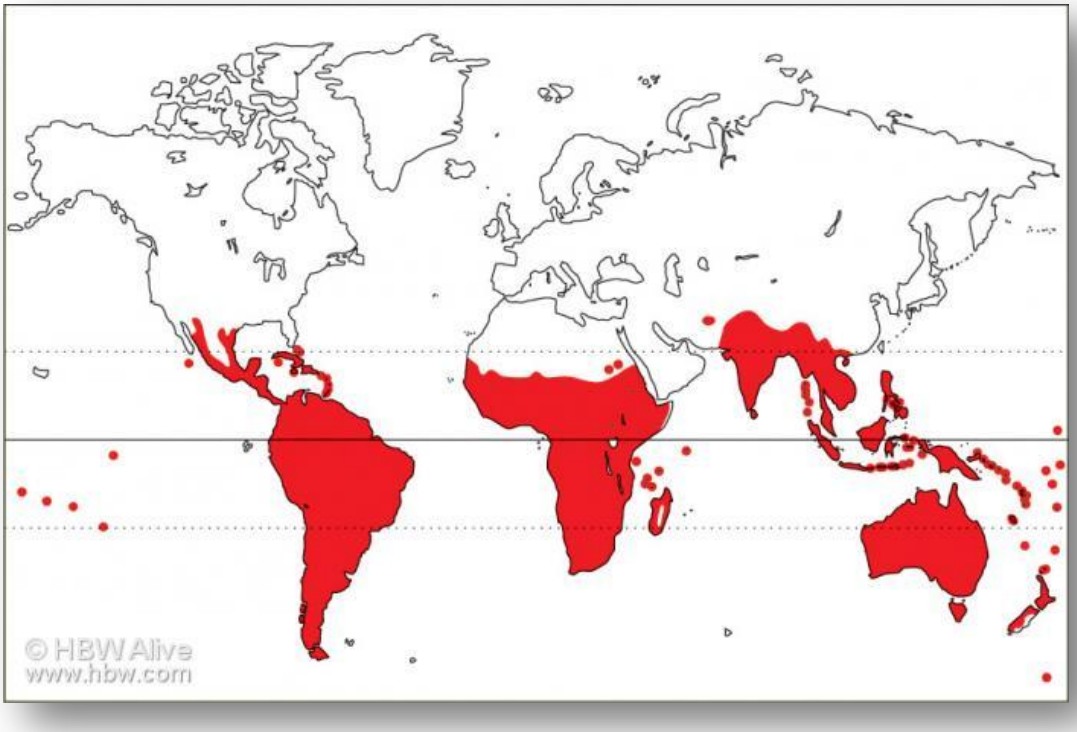
The family of *Psittacidae* includes all the parrots that are not included in the previous family. It is the most numerous family and includes very heterogeneous species compared to form, color and diffusion in captivity: large macaws, amazons, parrots, African greys *etc*. and the diffusion concerns almost all three continents (Costa, 2014). The size and general appearance of the species in this family vary widely: for example, it is possible to compare the small size of *Forpus passerinus* (8 cm) to Macaws, that can reach one meter in length. About feed preference, *Psittacidae* family can be granivorous/frugivorous/florivorous/onnivorous, or combination of these groups; also, behavioral habits are very different: it is possible to find animals that live in small groups and animals that live in a huge flocks (Khaleghizadeh, 2004).

The group of macaws include the most large, coloured, long tailed parrots of the *Psittacidae* family, come to South America. There are twenty-four species and sub-species: the most large parrots of this group is *Anodorhynchus hyacinthinus*, called Hyacinth Macaw. This bird's length is about 91 cm; eyes medium gold and they have a black beak. General color deep rich cobalt blue with the wings showing the darkest shadings. Eye rings and skin around the base of beak are orange yellow. Feet and legs are black. No dimorphism is present between male and female, however some authors consider the female smaller than male (Rogers, 1981). Among macaws, Hyacinth Macaw is consider the most affectionate and best birds to make a good pet.

Table 2.1 Parrots classification.

Kingdom	***Animalia***
Subkingdom	***Eumetazoa***
Superphylum	***Deuterostomia***
Phylum	***Chordata***
Subphylum	***Vertebrata***
Superclass	***Tetrapoda***
Class	***Aves***
Subclass	***Neornithes***
Order	***Psittaciformes***

Family	***Cacatuidae***
Subfamily	*Cacatuini*
Family	***Psittacidae***
Subfamily	*Arini*
Subfamily	*Loriinae*
Subfamily	*Psittaculini*
Subfamily	*Platycercini*
Subfamily	*Psittacini*
Subfamily	*Micropsittini*
Subfamily	*Cyclopsittacini*
Subfamily	*Nestorini*
Subfamily	*Strigopini*
Subfamily	*Psittrichacini*



Picture 2.2 Parrots distribution in world (http://www.hbw.com/family/parrots-psittacidae).

1.2 African grey parrots

The most common parrot in captivity is the African grey parrot (*Psittacus erithacus*), and at the same time it is the most popular parrot that shows a behavioral problem: in fact, FDB is most common in African grey parrots (Clubb et al., 2007, Chitty 2003; Costa et al., 2016a; Garner et al. 2008; Rosenthal et al. 2004; van Zeeland 2009a, 2013b). The African grey parrot (*Psittacus erithacus*) is an old world parrot in the family Psittacidae; it is a medium-sized bird, predominantly grey (Picture 2.3), black-billed parrot which weighs around 400 g, with a length of 33 cm and an average wingspan of 46–52 cm (Holman, 2008). The African grey parrot is native to Equatorial Africa, including Angola, Cameroon, Congo, Côte d'Ivoire, Ghana, Kenya and Uganda. The species is found from Kenya to the eastern part of the Ivory Coast (Picture 2.4). Between 120,100 and 259,000 Timneh African grey parrots remain worldwide. Current estimates for the global population of Congo African grey parrots are uncertain and range from 0.63 to 13 million birds. Populations are decreasing worldwide. The species seems to favor dense forests, but can also be found at forest edges and in more open vegetation types (gallery and savanna forests; Bellamy *et al*., 2016).

There are two subspecies of African grey parrots – *Psittacus erithacus erithacus*, also known as the Congo African Grey, and *Psittacus erithacus timneh*, or Timneh African Grey. African grey parrots do not show sexual dimorphism (Juniper & Parr, 1998) and may live for 40–60 years in captivity, although their mean lifespan in the wild appears to be shorter at about 23 years.

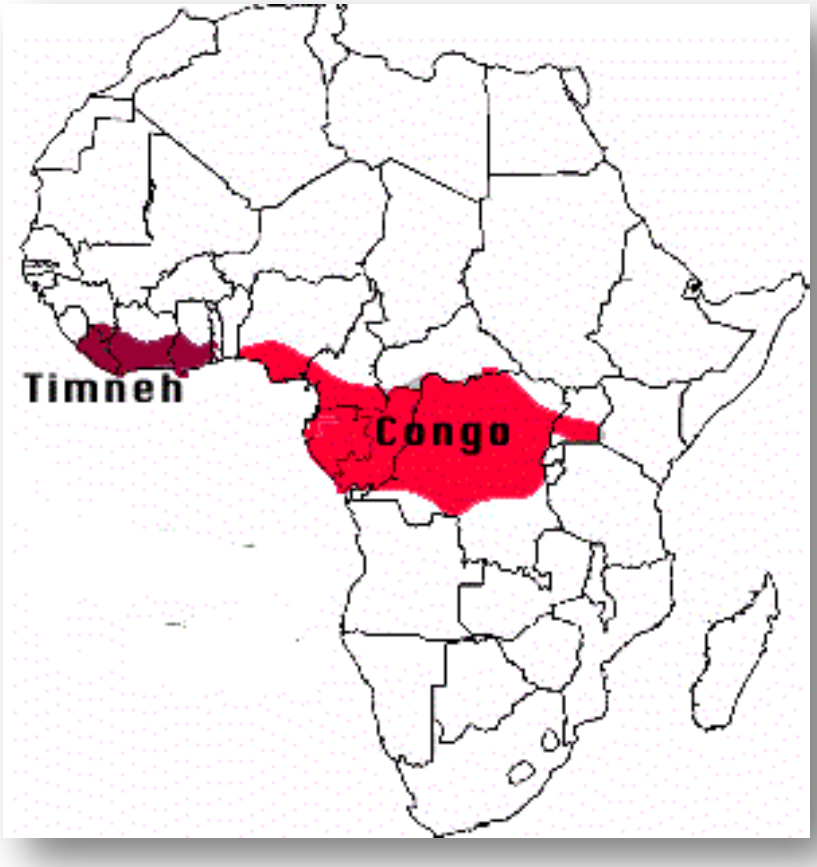
African grey parrots are monogamous breeders and nest in tree cavities. Each couple of parrots needs its own tree to nest. The hen lays three to five eggs, which incubates for 30 days while

being fed by her mate. The adults defend their nesting sites. Both parents help take care of the chicks until they became independent. African grey parrot chicks require feeding and care from their parents in the nest. The parents take care of them until four or five weeks after they are fledged (Griffin, 2012). The young birds leave the nest at the age of 12 weeks. Little is known about the courtship behavior of this species in the wild; they weigh between 12 and 14 g at hatching.

They are mostly frugivorous; most of their diet consists of fruit, nuts, and seeds. The species prefers oil palm fruit and also eat flowers and tree bark, as well as insects and snails. In the wild, the African grey is partly a ground feeder. In captivity, it can eat sunflower seeds, bird pellets, a variety of fruits (such as pears, orange, pomegranate, apple, and banana, and vegetables such as carrots, cooked sweet potato, celery, fresh kale, peas, and green beans), and in particular they also need a source of calcium. Lumeji (1990), showed that they also need a source of calcium because this parrots are more prone to develop hypocalcaemia that rarely developes in the other psittacine species.

A study published in 2015 stated that the species disappeared in Ghana, probably due mainly to pet trade (Annorbah *et al.*, 2016). Populations are thought to be stable in Cameroon, and in the Congo an estimated 15,000 are taken every year for the pet trade, from the eastern part of the country. The annual quota is 5,000.

Picture 2.3 A companion African grey parrot (© 2016 Pierluca Costa).



Picture 2.4 In general the African Grey Parrot comes from a broad area of Central Africa (http://thegreyroost.com/congogrey.html)

1.3 Feeding behavior

Formulating or selecting appropriate diets for captive birds for the order Psittaciformes requires knowledge of the birds' wild feeding strategy, digestive physiology and specific knowledge of nutrient requirements in that species. However, little data concerning psittacine nutrient requirements have been published. Clearly, much work remains to be done before a complete picture of the nutrient requirements of psittacine birds is elucidated (Koutsos et al., 2001). The selection or formulation of appropriate diets that meet the nutrient requirements of psittacine birds is based upon several factors. Firstly, the wildtype foraging habits and behaviors of a particular parrot species provide important information regarding the animal's evolutionary adaptations to feeds and feeding. Secondly, the understanding of bird's digestive physiology, which often reflects its wild-type feeding habits, assists nutritionists in determining the nutrient and food requirements of that species (Matson & Koutsos, 2006).

The findings on eating habits show that the parrots eat in the wild environment various feeds: seeds, fruits, nectar, flowers, plant parts etc.. Parrots are very flexible animals in eating habits, that are modulate based on the seasonality of the environments in which they live. In fact, it was observed that the consumption of flowers, for example, becomes very important quantitatively when the production of fruits decreases, especially during the dry season in the natural environments.

Observations conducted in the canopy forest of the Pantanal show that both small and large species of parrots eat fruits: parakeets most of the pulp, while large species generally the pulp and seeds (Ragusa-Netto et al., 2006).

A further classification of the birds, based on eating preferences includes the *granivorous* (diet of seeds and grains), the *frugivorous* (fruit-based diet and flowers) and *nectarivorous* (a nectar-based diet). These three sub-categories do not consider the numerous species of birds that can be classified in more than one or even all three categories. In fact, it is often considered the *granivorous-frugivorous* association for many species such as the macaw or parakeet black tail (Costa, 2014).

Even species considered exclusively nectarivourus, as the Rainbow Lorikeet (*Trichoglossus h. haemathodus*) or Henderson Lorikeet (*Vini stepheni*), often also feed on nectar, fruits, seeds and insects. Many species of parrots are considered omnivorous, as some cockatoos and parakeets. Cannon (1984), in a study on the diet of *Platycercus eximius* and *Platycercus adscitus*, have shown that these birds eat grass, mainly flowers, shrubs and plants. In addition, significant intake of insects was observed, especially in July *Psyllidae* are found on *Eucalyptus* leaves; this even makes up about 50% of the *Platycercus eximius* diet. Since parrots in nature demonstrate a very heterogeneous set of food habits, in domestic environment is very difficult to organize a standard diet, and the knowledge of biology to each family is fundamental before study the diet. In any case, experimental and clinical evidence demonstrates that diets based on unsupplemented domestic foods are nutritionally incomplete and must be fortified with a variety of amino acids, vitamins, and minerals (Koutsos et al., 2001).

Table 2.2 Reported food intake of captive adult birds in the order Psittaciformes (Koutsos et al., 2001).

Species	**Environment**	**Diet (ME)[a]**	**Intake[b] (% BW/d)**
Budgerigar (*Melopsittacus undulatus*)	colony cage	corn/soy meal (3.3 kcal/g, 14.0 MJ/kg)	25%
Budgerigar	colony cage	millet, canary grass, oat groats	26%
Budgerigar	Temperature-controlled room small cage	Japanese millet,	19.6%
		millet (2.9 Kcal/g, 12.1 MJ/kg	14%
Rainbow lorikeet	metabolism cage	sucrose/dextrin/egg white (4.0 kcal/g, 16.73 MJ/kg)	8.4%

(*Trichoglossus haematodus*)			
Rainbow lorikeet	small cage	bread, honey, dried milk (4.4 kcal/g, 18.3 MJ/kg)	10.4%
African grey parrot (*Psittacus erithacus*)	small enclosure	extruded granules[c] (2.7 kcal/g, 11.5 MJ/kg)	9.8%
Amazon parrots (*Amazon species*)	small enclosure	extruded granules[c] (2.7 kcal/g, 11.5 MJ/kg)	6.2%
Lesser crested cockatoo (*Cacatua sulfurea sulfurea*)	small enclosure	extruded granules[c] (2.7 kcal/g, 11.5 MJ/kg)	9.1%
Blue and gold macaw (*Ara ararauna*)	small enclosure	extruded granules[c] (2.7 kcal/g, 11.5 MJ/kg)	7.8%

[a] Metabolizable Energy (ME) content is based on values for chickens except when ME content of mixed seeds diets cannot be estimated because the proportion of seed consumption was not reported; [b] Dry matter intake, excluding hulls; BW indicates body weight; [c] Commercially available extruded granules, unknow composition.

Table 2.3 Levels of protein and amino acid shown to be adequate for birds in the order Psittaciformes at a given physiological state (Koutsos et al., 2001)

Species	**Physiological state**	**Protein source (ME level)[a]**	**Protein level**
Budgerigar	Maintenance	purified amino acids (3.4 kcal/g, 14.4 MJ/kg)	6.8%
Budgerigar	Maintenance	corn (+ lysine, 3.4 kcal/g, 14.2 MJ/kg	12%
Cockatiel	Maintenance	soybeans (+ methionine 3.5 kcal/g, 14.6 MJ/kg)	11%
African grey parrot	Maintenance	corn and soybeans; NR	10-15%
Rainbow lorikeet	Maintenance	Egg white (4.0 kcal/g, 16.6 MJ/kg)	2.9%
Bugerigar	Reproduction	corn and soybeans (+ lysine and methionine, 3.2 Kcal/g, 13.4 MJ/kg)	13.2%

Cockatiel	Growth	soybeans (+ methionine 3.5 kcal/g, 14.6 MJ/kg)	20%
Cockatiel	Growth	Purified aminoacids (4.0 kcal/g, 16.7 MJ/kg)	0.8% lysine

a ME indicates metabolizable energy; + indicates additive; NR, energy concentration not reported

Picture 2.5 *Cacatua galerita Eleonora* (© 2016 Pierluca Costa).

1.4 Legal detention

Parrots are protected animals and keeping them in captivity required to observe specific government laws and regulations. In 1973 the Convention on international trade in animal and plant in danger of extinction was signed in Washington; the Washington Convention is today one of the most important international regulatory instruments to make trade sustainable by preserving the biodiversity of our planet. The priority task for each state is to adequately monitor and regulate the national and international trade of specimens and products derived from animal and plant species in order to avoid extinction in a salient economic situation which is adversely affected by the adverse effects caused by the multiple human activities and climate change that are destroying the natural habitats of many species (www.corpoforestale.it). This convention (signed today by 160 countries including Italy since 1980) was born from the need to safeguard and protect the wildlife, regulating catches and marketing. Countries that have signed the convention can regulate independently the animal breeding and possession within its borders.

Picture 2.6 In captive household, it is necessary that parrots are ringed at neonatal age (© 2016 Pierluca Costa).

In Italy the implementation of the Washington Convention is entrusted to various ministries: Environment, Finance and Foreign Trade, but the most important part was held by the Ministry of Agriculture, as required by the Convention on International Trade in Endangered Species (CITES) Service coordinated by the National Department of Forestry Preservation, which handles the administrative management for the purpose of certification and specialized technical control for the respect of the Convention. The CITES Service of the National Department of Forestry Preservation is structured in a General Inspectorate at the Coordinating Centre in Rome and in forty branch offices.

The Washington Convention provides that each plant and animal species, including parrots, is comprise in the appendix I, II and III (in Europe there are annexes A, B and C, that match to appendix), based on their conservation status. In Italy the detention of parrots is regulated by the Legislative Decree of 22 February 2001.

1.5 Household environment and cage

Every animal not genetically modified by humans, or not object of artificial selection pushed and protracted over time, is an identical ethological copy of a conspecific who live in nature. Parrots, also bred for many years, are still particularly genetically intact; this inevitably leads to some reflections before addressing the chapter on the housings for these birds.

An animal that has not undergone extensive changes following artificial selection retains all the morphological and behavioral characteristics of its conspecific free natural environment: the genetic code of each animal provides for morphological, physiological and ethological adaptations molded to the environment which it has evolved. Therefore, it is useful to consider that parrots also retain all the evolutionary characteristics of their species: even if they sometimes amaze themselves for their particular availability to domesticity they still need to receive as much stimulation as possible in accordance with their adaptation. Parrots, as other birds, are very active animals and the fly capacity constrain the owners to dedicate their large spaces and to use many environment enrichment to ensure welfare.

1.6 Hand-rearing of baby parrots

Hand-rearing is an important tool in the breeding of endangered species, being the most effective way of rapidly increasing the numbers of birds which would be normally produce by only one clutch per year. When eggs are removed for artificial incubation or chicks at an early age for hand-rearing, the results are usually that the female lays again. Hand-rearing requires that parrot chicks are separated from their parents at birth or at a very early life-stage. It has long been appreciated that even short-term separation is stressful, and that disruptions in parental care can disrupt normal behavioral and physiological development (Fox, 2006). There are a number of different situations which will result in the need to hand-rear parrot chicks hatched in cages aviaries. Rosemary Low, an important ornithologist and aviculturist, showed in her book "*Hand-rearing parrots and other birds*" (1991), the following main rules to hand-rear:

1) The death or illness of the parent (s). This rarely occurs.
2) Chicks which hatch during cold weather and which the parents cease to brood before they have acquired enough down of feathers to survive without being brooded. This is a very common cause of death in many species, including cockatiels, lories, australian parakeets and others.

3) Failure to feed chicks at all (rare) or inadequate feeding (fairly common). This may be due to inexperience and in subsequent nests the parents may rear their young successfully. On the other hand they may prove to be inadequate parents on all future occasions.
4) Serious plucking of young by one or both parents (common). If left in the nest they may be so badly denuded that they could die from chilling on leaving the nest and/or be unable to fly. It is advisable to remove the chicks well before they are due to leave the nest as the older they are the more difficult they are to feed initially.
5) To supply birds as pet. This is very widely practiced in the USA where many breeders remove all young parrots for hand-rearing. It should be pointed out that a young parrot removed from its parent as soon as it is independent and kept in a cage in dwelling house will usually become tame quite soon, provided that it constantly exposed to human care. It is therefore not essential to hand-rear chicks if a time bird required. However, hand-reared birds are automatically tame and this fact appeals to those who breed parrots to supply the pet trade. Such birds command higher prices because the task of taming them unnecessary. The consequences of selling all hand-reared parrots as pets should be considered by those who practice this. Breeding pairs are not immortal. The wise breeder will retain one of his young birds and exchange another for an unrelated youngster to make up a second pair which will be ready to breed in a few years' time. This is most important with the rarer species, with large parrots where breeding age females are usually in short supply and with species which are not frequently bred in captivity. Secondly, the wise breeder will sell some hand-reared youngster for breeding purpose, making up unrelated pairs where possible. If, as happens in USA, hand-reared parrots of the larger species are almost invariably sold as pets, there could be an acute shortage of breeding pairs in a few years' time, when importations of wild-caught parrots are drastically reduced or have ceased entirely.

The only redeeming factor in selling hand-reared birds as pets is that some pet owners eventually become breeders. When their pet matures they understand its need for a mate or they become absorbed in the quite different sphere of bird breeding rather than pet keeping. Former pets are usually excellent as breeding birds.

1.6.1 Hand-rearing techniques

There are many methods to deal with the practice of artificial administration of feed to nestlings of parrot and with different instruments: spoon, syringe and syringe with a tube.

The spoon methodology foresees that the feed is administered through a spoon, that allows the liquid to slip in the beak of the small parrots, and then to be ingested: this technique the substance mimics fairly well the food administration carried by biological parents, that regurgitating the predigested food and they let slip varying amounts in the beak of the chicks. The spoons that are used for this purpose are special and have the slightly raised edges, to allow sliding of food. With this methodology in a short time the chicks learn to swallow the liquid and, therefore, to obtain supplies of

food offered by the breeder. This technique requires some dexterity because often before animals learn to receive food with the spontaneity with which respond to their natural parents, they face significant risks: the most important is to be victims of *ab-ingestis* pneumonia (Costa, 2014).

Disposable plastic syringes (without needles) are often used to hand-feed chicks. Syringe feeding is a rather quick and easy method to use. A disadvantage of syringe feeding can also be the danger of the parrots injuring their beaks against the plastic while eating (Wagner, 1999). Besides, you need to be careful that the chicks can choke on the food when it is injected too fast into the birds' beaks (Schmidt & Lightfoot, 2006).

The description, the modalities and the consequences of the above spoon technique are also valid for the method with the syringe, which provides that the liquid food is provided through the aid of a syringe without needle. Instead, place the tip of the spoon on the semi-open beak of *pullus*, waiting for the food to slide toward the mouthpiece opening, it will support the tip of the syringe and the sliding of the food will not be gravitational, but for the manual action of the breeder on the plunger of the syringe.

This latter differentiation allows to prefer the use of the syringe to the spoon since the liquid food disintegration is mechanical, so it is easier to control and rationalize. The main concern for this type of administration is always to use new syringes and no more than a dozen doses. The plastic material making up the plunger in contact with hot food quickly loses its softness and lubrication: friction between the plunger and the body of the syringe can cause sudden leakage of food (Costa, 2014).

As for the syringe with a tube, this one is placed on the end of a syringe and goes directly into the crop of the parrot. There are two different sorts of tubes that can be fixed onto syringes. The tubes are either made of soft rubber (catheter) or of metal with a small round ball at the end (called gavage needles) to prevent injuries of the crop. The main advantage of using tubes is that parrots that refuse to eat can rapidly be force-fed (Voren & Jordan, 1992).

The main concern for this type of administration is always to use new syringes and no more than a dozen doses. The plastic material that composes the plunger in contact with the hot food quickly loses its softness and lubrication: a friction between the plunger and the syringe body can determine sudden leakage of food. Holding the head of the animal with thumb and index inserts into the oral cavity the catheter gently tread the path along the esophageal tract until it reaches the lumen of the ingluvies (Picture 2.9): the esophageal tract is placed laterally on the trachea on the right side of the bird. Once arrived at the destination, that is inside the ingluvies, very gently will push on the plunger of the syringe to bring the contents into the crop. In time small parrots begin to associate this method to satiety resulting from filling of the crop and will themselves stand in a favorable position for the operation (Costa, 2014).

This technique has the advantage of speed. The quantity of food can also be measured very precisely (Reinschmidt, 2000). However, this hand-feeding procedure has many disadvantages. The main disadvantage is the danger of killing a chick if food enters the windpipe (Low, 1987;

Reinschmidt, 2000). Besides, tube-feeding can injure chicks when not done properly. The tubes made of soft rubber can harden after repeated use and may harm the birds and lacerate their mouths or crops. The metal tubes can be very dangerous as it takes very little pressure to force them through the oesophageal wall of the parrots (Voren & Jordan, 1992). Another drawback is when an oversized tube is pulled out of the crop. A vacuum is then made pulling the inner wall of the crop into the hole at the tip of the tube and perforating the tissue (Wagner, 1999). Tubes are also very difficult to maintain in an irreproachable hygienic condition, as they are very difficult to clean. Last but not least, this technique is the most unnatural one for the birds, as it does not allow chicks to swallow the food (Reinschmidt, 2000).

Picture 2.7 Instruments for hand rearing (© 2016 Pierluca Costa).

Picture 2.8 Food administration to small African grey parrots through the use of syringe without catheter

(©2014 "El Lorito" farm).

Picture 2.9 Section of baby parrots during the administration of food through the use of syringe with catheter ((© 2012 Pierluca Costa).

2 WELFARE IN CAPTIVE BIRDS

2.1 Welfare and abnormal behavior in birds

Parrots have a very complex organization of behavior and show to be more sensible to stress than many other birds. When a parrot is stressed, it may become ill or resort to abnormal behaviors like excessive aggression or display of feather picking. Parrots and other birds whose environmental and behavioral needs are not met in captivity may engage in a variety of distressing abnormal behaviors (Mehaan et al., 2006): lack of environment and social stimuli can cause a parrot to become stressed. Parrots also have a strong learning behavior, and traumatic events (such as hand-rearing, phobias, frights etc.) can cause ongoing stress, long after the event has occurred. It is very difficult to organize a correct management for these birds, because the species are highly different in biological features and requirements: for example, the behavioral requirements of an small *Forpus passerinus* can are very different than an *Anodorhynchus hyacinthinus*, and these not only in relation to dimensions, but also for eat habits, reproduction habits, behavior etc.

Since the environment is a composite of interacting stressors, a bird's success in coping with it depends on the severity of the stressor(s) and the bird's physiological ability to respond properly (Siegel, 1980). Parrots and other birds in household environment are subjected to many stimulatory conditions (external like temperature, light, etc., or internal, like disease organisms, parasites etc.) that cause a different responses. Another ensemble of stimuli are those ethological, like social, sexual and communicative behavior incitement. In any way the parrots are stimulated, they respond with a significant changes in regulatory processes to attempt to reestablish or maintain the homeostatic state. There are two general types of regulatory processes: specific and nonspecific. A particular condition will elicit a specific response. For example, when the external environment causes a bird's body temperature to rise, surface blood vessels dilate to permit greater blood flow to the skin for more rapid heat dissipation, and feathers are rearranged to reduce insulation (Siegel, 1980). However, stress occurs when an organism experiences allostatic load (McEwen, 2000) which refers to the physiological cost of maintaining physiological stability (homeostasis) in changing environments. Secretion of glucocorticoids increases with allostatic load and in the short run, they are essential for adaptation and maintenance of homeostasis, but if they are elevated over a longer period they exert a cost (allostatic overload) that can lead to adverse health conditions (Wingfield, 2005; Korte et al., 2005).

Regards to behavior, it is more difficult to see an unequivocal responsible stimuli that cause a specific abnormal behavior, and vice versa. In fact, the mechanisms that control the pathological behavior in animals are not very clear.

Many parrots in captive conditions show stereotype behavior. In ethological terms, stereotype refers to invariant and repeated chains of behavior or speech. Animals kept in cages often develop a stereotypic behavior, hence they are often called cage stereotypies. These behaviors are “abnormal” in the most neutral sense of not being normally observed in wild, free-ranging animals. That is, they

differ quantitatively or qualitatively and can be statistically distinguished from behaviors routinely performed by wild animals. Also, they are abnormal in the sense that they often have no readily identifiable function even in the captive environment (Toft at al., 2015).

Between the abnormal behaviors in captive animals, self-injuring is rather widespread and it is often linked with removal or damage to parts of the body (air pulling, feather damage and pulling, skin pulling etc.); it is observed in human, mice, dog and cats, birds and non-human primates (Reinhardt, 2005).

2.2 Glucocorticoids and welfare in birds

Methodologies frequently used to try to quantify pain and stress in animals include quantification of circulating 'stress' hormones (e.g. cortisol, β-endorphin, or catecholamines; Ayala et al., 2012, Livingston, 2010 and Schmidt et al., 2010). Cortisol concentrations, in particular, are useful for quantifying stress responses to a variety of diverse types of stressors, including handling and transport methods, alterations in social structure, experimental physiological manipulations, disease states, surgical procedures, or therapeutic interventions (Hart, 2012).

In birds, the predominant glucocorticoid (GC) is corticosterone (Holmes and Phillips 1976), a hormone released in response to a potential threat to homeostasis that is used to regulate behavior and suppress bodily processes that are nonessential to survival (Wingfield, 1994), and levels of circulating GC are considered a reliable indicator of stress levels in birds (Dehnhard et al., 2003; Hartup et al., 2004). Such as cortisol in mammals, corticosterone levels can be interpreted as an adaptive response to stressful events.

Selection can act on patterns of hormone secretion as shown by the fact that individuals in populations that are frequently exposed to environmental stresses tend to have lower corticosterone production in response to a standard stressor than individuals in populations that are infrequently exposed to such stresses (Wingfield, 2005). In birds corticosterone is associated with behavioral and physiological changes in energy demand (Harvey et al. 1984). Many authors showed that this corticoid is linked to sensitivity of the hypothalamic-pituitary-adrenal axis and how can be useful as indicator of physiological condition and habitat quality for birds (Marra & Holberton, 1998). Moreover, high levels of corticosterone are associated with depressive-like behavior in animal models and sex-dependent manner (Kott et al., 2016). Changes in circulating hormones are linked to breeding season and the increase in corticosterone concentration is a characteristic phase of bird's reproduction, when body mass loss and protein utilization increase (Cherel et al., 1988). An acute elevation of corticosterone levels tends to switch on what is called the emergency life history stage and tends to increase foraging behavior in many birds (Wingfield, 2003; 2002). It is chronic elevation of corticosterone that tends to suppress foraging and reproductive behavior (Wingfield, 2003).

3 THE FEATHER DAMAGING BEHAVIOR IN PARROTS

3.1 Feather damaging behavior (FDB) in parrots

Feather damaging behavior (FDB) (also referred to feather damaging behavior or feather plucking) is a behavioral disorder that is frequently encountered in captive parrots (van Zeeland at al., 2009). Various degrees of feather destructive behavior, from over-preening to feather plucking and self-mutilation, are commonly encountered in avian practice. Based on skin biopsies, many of these cases have an underlying lesion that would account for pruritus and self-trauma. In some birds there is no evidence of skin or systemic disease or condition and these case have been ruled out. Since self-trauma can lead to lesions, histologic changes must be carefully assessed before a diagnosis of behavioral FDB is made (Schmidt & Lightfoot, 2006). Despite the numerous studies conducted, it cannot still rely on a definition that would clarify unequivocally the mechanisms underlying this pathological condition, as well as the interpretation. As claimed by Rubinstein and Lightfoot (2012), initial problem lies in the relative scarcity of controlled studies related to the underlying causes of feather loss in companion avian species and the paucity of current veterinary medical knowledge regarding feather loss and feather destructive behavior.

3.2 Body areas affected by FDB

FDB is a condition that manifests with plucking, chewing, fraying and/or biting, resulting in loss of or damage to the feather (Van Zeeland et al., 2013). One of the criteria that drive researchers to make a distinction between FDB and other diseases is the presence of feathers in good condition in areas of the body that animals do not to directly reach; from this basic assumption it was suggested to consider FDB as a form of self-injury (Galvin, 1983; Harisson, 1986; Westerhof et al., 1987).

Picture 4.1 Some species of *Psittacidae* affected from FDB (© 2016 Pierluca Costa).

3.3 FDB prevalence and sensible species

Many authors consider this disorder as stereotypic behavioral or obsessive compulsive disorders (Jenkis, 2001) and literature show studies of prevalence of FDB in different parrots populations. Grindlinger (1991) estimated that approximately 10% of the captive parrot population suffers from FDB; in a sample of 538 parrots McDonald Kinkaid et al. (2013) found an incidence of 15.8%. This impact on captive parrots populations drive authors to consider this problem such as the more common and frustrating reasons that avian patients are presented to veterinary hospitals (Rubinstein et al., 2014). FDB has been suggested to vary across different captive species in both prevalence and severity (Rosenthal, 1993; Rosskopf and Woerpel, 1996; Briscoe et al., 2001; Chitty, 2003a; Chitty, 2003b; Seibert, 2006). FDB is most common in African grey parrots (*Psittacus erithacus*) (Clubb et al., 2007) and this species proved is particularly sensitive (Chitty 2003; Costa et al., 2016a; Garner et al., 2008; Rosenthal et al., 2004; Van Zeeland 2009a, 2013b) and in Cockatoos it was observed that the abnormal behavior evolution can be characterized also by a subsequent involvement of the epidermis, that is injured through chewing and traction, in particular in the Moluccan cockatoo (*Cacatua moluccensis*) and sunrise in cockatoos (*Cacatua alba*) (Rosenthal,

1993). In any case, generally, the most sensitive genus are *Psittacus, Cacatua, Ara, Myiopsitta, Eclectus* and any species of Conure.

3.4 FDB Aetiology

3.4.1 Physiological data

Methodologies regularly used to try to quantify pain and stress in animals include quantification of circulating 'stress' hormones (e.g. cortisol, β-endorphin, or catecholamines) (Ayala et al., 2012, Livingston, 2010 and Schmidt et al., 2010). In birds, principal glucocorticoid (GC) is corticosterone, a hormone released in response to a potential threat to homeostasis that is used to regulate behavior and suppress bodily processes that are nonessential to survival (Wingfield, 1994) and levels of circulating GC are considered a reliable indicator of stress levels in birds (Dehnhard et al., 2003; Hartup et al., 2004). In the matter of to FDB in parrots, Owen (2011) showed that compared to healthy parrots, FDB parrots showed a levels of faecal corticosterone of 261 [83] ng/g, than in the control parrots (75·1 [15·6] ng/g).

Picture 4.2 White cockatoo (*Cacatua alba*) with serious feather and skin damage from feather damaging behavior (© 2011 Pierluca Costa).

3.4.2 Clinical factors linked to feather damaging behavior

Clubb et al. (2007) support the association between FDB and altered thyroid function. Additionally, their work showed a significant difference in lymphocyte count and they linked this to an higher stress response. Feather follicles that are itchy or irritated attract the bird's attention. In its attempt to make itself comfortable, the bird may chew or pick at the feather (Picture 4.3). Possible causes include an infection associated with mites, bacteria, fungi or a virus. Some species of mite live deep in the feather follicle, wedged between the outer wall of the feather and the lining of the feather follicle. A simple in-aviary test that might indicate the presence of mites is to gently roll a damaged feather out of its follicle. If mites are present, there may be a collar of dry dandruff-like material around the feather. A vet can scrape this material onto a drop of oil on a microscope slide and examine it. Examination can reveal adult mites, nymphs and also eggs. A feather with a healthy follicle is often harder to remove and the section of feather below the skin is clean and shiny (Clubb et al., 2007).

3.4.3 Bacteria, fungi, parasites and virus factors

Disturbance of skin irritants may be due to bacteria, viruses, fungi, parasites such as mites, fleas, flies, lice and mosquitoes. Feather encrustations (scabs) and skin debris should be examined by standard laboratory procedures for parasitic, bacterial and fungal disease. Many times these conditions cause such skin irritation that the parrot starts to pick its own feathers (Burr, 1982).

Infection associated with bacteria such as Staphylococcus sp. can be intensely itchy, while fungi (such as *Mucor sp*. and *Rhizopus sp.*) have been associated with itchiness in pigeons as well as parrots. Veterinarians can take skin scrapings and squash feather contents onto slides for microscopic examination. Sometimes, special stains can aid in diagnosis. Feather and skin samples can also be cultivated for bacteria and fungus. Both *Polyoma virus* (associated amongst other things with 'French Moult' in budgies) and *Circo virus* (the agent of some feather disorders in cockatoos and other birds) can inflame the feather follicle, leading to the growth of abnormal feathers and variable degrees of irritation. These viruses are tested in blood and feather samples where either evidence of the virus itself or antibody to the virus is detected. Interestingly, itchy birds that may appear quite normal sometimes test positive for *Circo virus*, which means that even though no obvious feather damage is visible, the virus should not be discounted.

Picture 4.3 Feather damaging in shoulders regions in *Ara ararauna* male (© 2016 Pierluca Costa).

3.4.4 FDB ethological factors

Regardless of the numerous studies conducted, it still cannot rely on a definition that would clarify unambiguously the mechanisms underlying this pathological condition, as well as the interpretation. The interpretation of the authors in recent decades, give a very big jointly of interpretation and theories about this abnormal behavior: e.g., Galvin (1979) states that feather problems may start after a new pet has joined a family unit, a family member has passed away or a person has spent less than the customary amount of time with the bird. Lumeij et al., (2007) considers that FDB may result from a redirected foraging behavior and through its studies support this hypothesis through the positive results that are obtained by increasing environmental enrichment and promoting foraging activity. However, this interpretation does not take into account that if a foraging behavior actually take place in order to resolve any conflicts of behavior, then, in theory, any behavior could play an equally any other function from the original one and it would be sufficient to change the environmental stimuli to cause a shift of the functional behavioral repertoire of animals.

Nevertheless, in many cases there was also a loss of plumage in areas unreachable by birds which is uncertain attribute organic causes. It is widely thought that the FDB is usually self-inflicted, but, when housed in groups, it can sometimes be directed to cage mates or nestling. In these instances, the primary target area appears to be the head and face (Wedel, 1999; Fox and Millam, 2004; Lightfoot and Nacewicz, 2006). This interpretation, however, creates a problem: if so, the victim of the parrot feather pecking caused by other parties should not own sensitivity to pain and even

superficial behavioral mechanisms of intraspecific aggression, aimed at modulating the aggressive behavior of conspecifics, during social relations. Instead, parrots bred in captivity there is intraspecific aggression, that in many cases the causes for aggression between birds are the same as aggression toward humans (Welle et al., 2006).

Interpret the expression of the disorder of the plumage as different expression of a state of generalized stress is necessary.

This does not account for the manifestations of sudden loss of plumage, even in areas unreachable by the beak, which involve birds reared in social isolation. Many individuals, in fact, seem to show that plumage loss as a result of sudden changes in environmental and social issues, such as the change of environment and/or the change of partners and are not always in close relationship with a conspecific partner or with groups of individuals.

According to Harrison (1994), the origin of FDB can be related to an a aberrant imprinting. In fact, birds hand-reared will imprint as people, not birds. As they mature, their natural instincts to choose a mate may cause objectionable behaviors (e.g. FDB, screaming). An imprinted bird will spend all of its time attempting to drive unwanted individuals, other pets or object out of its territory, while trying to find one chosen person with whom mate. FDB may be caused by pathologic and psychologic condition, and the first step in solving the problem is a through physical examination. Once medical cause of FDB have been ruled-out, psychologic causes should be explored. The two most common primary causes of FDB in the author's experience are frustrated mating instincts and lack of proper training. Sexual frustration is common in birds, especially in cockatoos and many domestically bred birds. Programmed in the wild to be constantly with mate, a birds becomes distraught when its "person mate" is gone much of the day. It may also become jealous of other family members or maladjusted following a change in environment (e.g. change of enclosure location, a new dog or child). Even the client's emotional state can affect the bird's behavior.

3.4.5 FDB and nutritional deficiency

Nutritional aspect are potentially implicated in FDB, although malnutrition has been associated with FDB, research in the potential nutritional mechanisms is lacking (Rubinstein et al., 2014). Authors suggested many hypothesis but the nutritional deficiencies do not seem to affect a FDB appearance. When amino acid deficiencies were induce, parrots did not develop FDB (Koutsos et al., 2001).

3.5 Ethological interpretations of FDB

The abnormal behavior differ from somatic pathology because its substrate is not visible: animals show abnormal behavior with different actions that cause a maladaptative state when the welfare of animals is compromise. Many birds in household environment show abnormal behavior with stereotypies, self-damaging behavior, aggressive behavior, abnormal or absent parent care and abnormal sexual behavior. There is sufficient evidence that the pecking behavior, chewing and traction of feathers are actually indicators of forms of stress and distress behavior: it should be

emphasized that any cause or nature of clinical etiological factor has not been demonstrated and any observations of coexistence of other diseases could be random (van Zeeland et al, 2009).

FDB has multifactorial origin. On the other hand, the FDB has many considerations from the ethological point of view, being considered now as a behavioral pathology.

Several theoretical explanations are aimed at understanding the causes and mechanisms of pathological behavioral disorders. One of these consider the stereotyped behaviors (at the base of FDB) as a process of replacement of dominant behaviors with normal pathological ones, through a modification of the behavior "control center", however, the abnormal behaviors as responses to environmental stimuli quantitatively excessive abnormal (van Zeeland at al, 2006; Garner, 2003). However, pathological behaviors such as stereotypes can also be interpreted as new behaviors, that is not covered by the behavioral repertoire of the species taken into consideration and therefore, characterized by a new modulation, consisting of behavioral patterns of different origin and function: as indeed are the more obviously self-injurious behaviors. Interpreting in this way the pathological behaviors at the base of the FDB (hence the behavioral stereotypes and behaviors aimed at the removal and damage of plumes and feathers) open a different way of ethological analysis: it aims to break down these behaviors in their various modulations and understand the origin biologically correct. For example, if the basis for the removal of a feather could be considered as a trigger for the behavior an excess of aggression (probably due to a deep sense of frustration over the lack environmental and social stimulation) then, theoretically, it is possible to assume that the pain generated by the removal of the follicle from its seat may have the same functions they perform in humans, which have an affect-regulation (to alleviate acute negative affect or aversive affective arousal) and sensation-seeking effect (to generate exhilaration or excitement) (Klonsky & Muehlenkamp, 2007).

The removal of feathers may serve to assuage the feelings of anxiety and frustration by the animal, responding to psychological mechanisms similar to what happens to the human species.

Beyond the theoretical ethological interpretations that can be given to this event pathological behavior, what is particularly striking is that the FDB presents a set of symptoms (often progressive character) common (for modulations and behavioral consequences) in all affected individuals, and this motivates us to propose a classification of symptoms on the basis of field observations.

Picture 4.4 Feather picking African grey parrot (http://bestfriends.org/resources/feather-plucking).

EXPERIMENTAL SECTION

Introduction

Understanding mechanisms and causes of abnormal behavior is very difficult, because it is often necessary to know the ontogenetic history of each animal that the researcher intends to study (Costa, 2016); in order to these considerations, plan an research program must consider any possible aspect.

The relationship between emotional stress and self-injury (such as FDB), is also seen in captive non-human primates: e. g., self-injurious rhesus macaques display greater emotional responsiveness than their not injurious counterparts do (Novak, 2003), and the stress of relocation to novel housing produced long-lasting increases in self-biting behavior in these animals (Davenport et al., 2008). Leaving aside the possible involvement of organic nature phenomena, for which there is not objectively possible to impute a partnership in observable behavioral modification, it is possible to ascertain that there are different environmental factors that can predispose animals to the onset of self-injurious behaviors. In particular, a scientific research has shown that foraging plays a can have a role in the prevention of these behaviors and that environmental enrichment in the home environment may have a positive role (Lumeij, 2008) in preventing the onset of FDB.

Our program of research about FDB had considered different approach: i) a survey about pet parrots population in Italy, ii) a quantification of faecal Corticosterone in African grey parrot and iii) the effects of use of environmental enrichment in feather picking parrots.

According to Zammuner (1998), the most common types of research, where a questionnaire or structured interview is used is the survey (sample, statistic, market, opinion, social) and research (descriptive, experimental, longitudinal, sampling, social, statistical, market, evaluative). The researches vary based on: search goals; population considered and sampling method; type of objects; degree of generality; degree of standardization. According these considerations, we had organized an a questionnaire addressed to pet parrots owners, in order to collect data about sensible species, risk factor and ethological evidence on FDB parrots.

In order to understand which risk factor can promote FDB it was necessary find a physiologically parameters to monitor together the behavior and body symptoms of FDB parrots. The literature suggests that some behavioral problems in animals start after stress conditions and that faecal steroid hormone metabolites are becoming increasingly popular as parameters for reproductive functions and stress (Palme et al., 2013). In second study of this work, were observed the quantity of corticosterone metabolites in fecal sample obtained from two types of African grey parrots: a group were composed to African grey parrot not hand-rearing and that lived in pairs, with possibility of reproduce and a group composed to African grey parrots as pet, hand-rearing and that do not lived with conspecific partners.

Last study was projected to get information about the effects of environmental enrichment (as a parrots toys), on FDB and general status of birds, quantifying the response through quantity of fecal corticosterone response, in according to Luescher et al. (in Luescher, 2006), who support that toys are a valuable means of encouraging development in pet birds. The activities involved in toy play can

promote learning, relieve stress, and occupy idle time, and in according to Lumeji et al. (2008), who find that foraging plays can have a decisive role in the prevention of these behaviors and that environmental enrichment in the home environment may have a positive role in preventing the onset of FDB.

The experimental section consist of three investigation that which will be described further, on the following topics:

- *Experiment 1*: investigation on evidence about feather damaging behavior in Italian pet parrots population;
- *Experiment 2*: quantification of faecal corticosterone in African grey parrots, compared to hand-rearing and not hand-rearing birds in house-hold condition;
- *Experiment 3*: the influence of reinforcement foraging toys in the welfare in two feather picking African grey parrots (*Psittacus erithacus*).

Two of this investigation (Experiment 1 and 2, reported in the appendix), were published in ISI journals (Costa et al., 2016a and Costa et al.2016b).

4. MATERIALS AND METHODS

4.1 Experiment 1: Investigation on evidence about feather damaging behavior in Italian pet parrots population

A web questionnaire, addressed to the owners of all species of pet parrots, was distributed throughout Italy through on-line parrot association sites, social networks and e-mails. The web questionnaire was drawn up through a Google Drive application. This questionnaire was on line from June 2014 to October 2014 and a total of 31 questions were created (Table 5.1). The title of the questionnaire was 'Questionnaire for companion parrots owners' and the participants discovered that there was a feather picking part only during the compilation. The questionnaire was divided into two parts: one addressed to all parrot owners and the second to the owners of feather plucking parrots. The first part of the questionnaire was divided into two section. First sections was on general information (name of owner, contact details, parrot species, age, etc.), while the second section was dedicated to information about the history and management of the parrot. The second part of the questionnaire, on the feather picking information, featured appropriate questions about feather picking parrots, such as behavior, stereotypic behavior, feather picking regions, etc. All the owners who filled in the questionnaires about their feather plucking parrots were followed by the owners' clinician veterinary who had diagnosed the problem and excluded other possible pathologies. In order to confirm their diagnoses, all the veterinarians were contacted to obtain verbal confirmation of the data. The questions were based on hypothetic risk factors for feather picking and were formulated on data and hypotheses published on this subject. Any incomplete questionnaires (e.g. the absence of the owner's name or contact details,
the species of the parrot, etc.) were excluded from the analysis. The questionnaire contained closed questions. The owners, after choosing among the possible closed answers, could provide a descriptions about its observation and this data were analyzed for evaluated objectively the answers. The questions about behavior observation were only in FP section and were created for to be simples, not interpreted by owners and directly linked to data that we wanted to get. The choice of terms and words to describe the abnormal behavior were based on the description of the movements that abnormal behavior show, on the basis of the descriptions currently available in the literature. The question about aggressiveness were created in according to actually knowledge on aggressiveness in companion parrots. The possible choices were linked to only aggressiveness type: dominance aggressions over the owner (specifying to which human family member) and dominant behavior towards conspecifics (Schmid et al. 2006), that the participants could further describe in 'other' section. Also sexual behavior questions were created with this methods. In Table 1 the overview about the FP questions. In order to have a control sample and verify any differences between hand-raised and parent-raised parrots (those kept in pairs and not hand-reared), a second questionnaire, addressed to parent-raised type parrot breeders, was created. The aim was to have a comparison parrot group of birds that had not been hand-reared and which were living in more suitable ethological

conditions (they were living with a conspecific partner and had the opportunity to reproduce). Nine questions were asked about the number of parrots present on the farm, their species, and on the number of animals showing signs of feather loss. All the data acquired from the questionnaires were registered in a database and analyzed. Genera prevalence, feather picking region prevalence, and any associations between the feather picking behavior and categorical risk factors were evaluated using the chi square test, and were considered to be significant when $p<0.05$. A statistical trend was considered for p values below 10%. The data were processed through the use of SASVR (Statistical Analysis Software). The results were presented with their p value and a 95% confidence interval (C.I.).

Table 4.1. Overview of 31 questions of questionnaire for pet companion parrots.

First part – addressed to all pet parrots
General data
Name, address, email address and phone
Parrots curriculum
Age, sex, ringed, feather picking or not
Social life
Hand-rearing method
Weaning with other chicks or not
Live with other parrots
hand-rearing history
Management
Management method (lives caged when owners absent; lives always free; lives always caged, live always in home or live too in garden)
Placement of the cage (indoor; outdoor; indoor in winter and outdoor in summer; parrot management climate depending)
Perches materials (plastic; wood; natural branches; metal and other)
Use of environment enrichments (parrot's toys, natural items or either; nothing)
Possibility of washing (every day; at least two time/week)
Diet
Diet (mainly dry seed, mainly fresh food or either in equal parts)
Eat extruded food (always; many times in weeks; don't administered; it don't eat them)
Eat human food (always; sometimes in week; rarely and never)
Second part – addressed to feather picking pet parrots
Knowledge
What is the feather picking for participants (virus disease; bacterial disease; fungi disease; abnormal behavior; don't now)
Beginning of feather picking
Events linked F.P. before feather loss (owner change; other animals attack; change in number of human family; added or disappeared other parrots; no events; parrots adopted F.P. already)
Feather loss (owners seen feather traction; owners seen body's regions without feather; owners seen increase in time of preening; increase aggressiveness; other)
Signal of feather picking: presence and evolution
Body's areas (chest; rump; wings; tail; under wing regions; paws; head)
Feather picking evolution (start to biting and damaging feather; immediately start to traction feather; immediately compared body's regions naked; increase aggressiveness behavior it-

self, such as peck it wings or other parts)

Sexual behavior

Sexual behavior showed (It is displayed on perches or objects in the birds' surroundings or parts of human body; in owners presence parrot regurgitates; owners don’t seen sexual behaviors; other)

Aggressiveness

Presence of aggressiveness (bites owners hands; the parrots assumes an attack posture and tries to come in against to bite; don’t presence of aggressiveness; other)

Victim of aggression (everybody; only owners; toward everybody except owners; toward only person; other)

Self-injuries behavior

Presence of self-injuries behavior (slaps it-self; bite it wings; it traction feather in nervous expression when the owners or any human approaches; bite it feet; other)

4.2 Experiment 2: Quantification of faecal Corticosterone in African grey parrots, compared to hand-rearing and not hand-rearing birds in house-hold condition

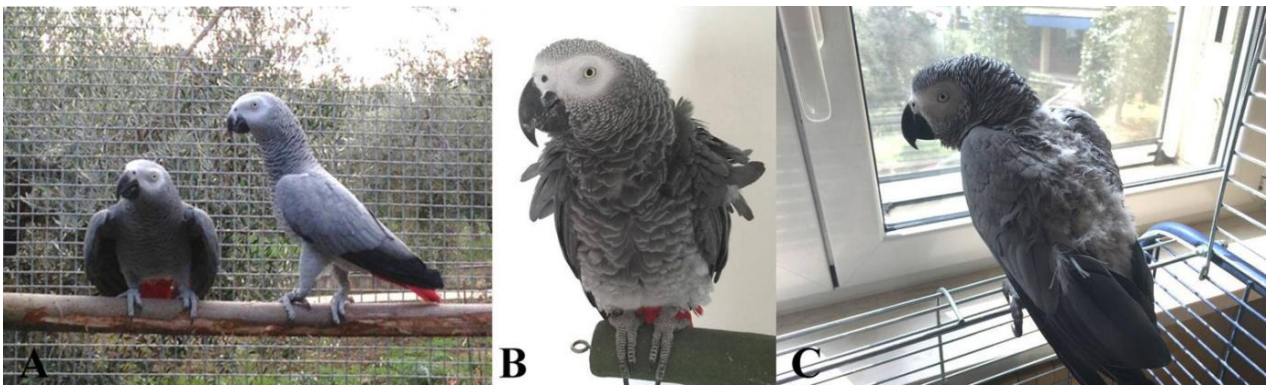
Animal and selection criteria

The study was based on a web questionnaire used in a previous study (Costa et al., 2016a) that was addressed to the owners of all species of pet parrots. The questionnaire was distributed throughout Italy through online parrot association sites, social networks and e-mails in collaboration with the Italian Psittacine Club (known as the "Club degli Psittacidi" http://psittacidi.webservice-4 µ.com/) and the Italian Association of Parrot Breeders (known as the "Associazione Italiana Allevatori Pappagalli", http://www.assopappagalli.it/). In the present study, we only considered African grey parrots because these were the most represented species among the Italian respondents (Costa et al., 2016a) and because this species is considered to be very sensitive to FDB (Jayson at al., 2014; Schmid et al., 2006).

All birds considered in our study were born in captivity, and no wild-caught birds were used. A total of 82 African grey parrots (*Psittacus e. erithacus*) was considered. 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. Based on the different methodologies of rearing at the neonatal stage, hand-reared and parent-reared parrots were considered. Among the hand-reared parrots, a further distinction was made between parrots displaying FDB and parrots not displaying FDB. According to these criteria, three samples of birds were defined: 1. Parent-reared (PR) parrots; 2. Healthy hand-reared parrots (H-HR); 3. FDB hand-reared parrots (FDB-HR) (Picture 5.1).

1. The parent-reared (PR) parrots (Figure 1a) included birds kept in pairs (n=30 pairs) with a conspecific partner of the opposite sex, since they were specifically reared for reproduction. These birds were reared by their biological parents, and contact with humans was minimal and related only to their care and daily management. The PR parrots were permanently housed in a standard parrot cage with a minimum volume of 1 m3 and exposed to natural light variation. All of these birds were healthy and never showed signs of FDB. All of the birds included in this sample were housed in the same facility. We included this sample that we considered a valid control for stress coping since (usually considered well-balanced birds that have learnt all of the specific behavioral patterns of their species). We included this sample since parent-reared captive parrots are usually considered well-balanced birds that have learnt all of the specific behavioral patterns of their species (Schimd et al., 2006).

2. The healthy hand-reared parrots (H-HR) (Figure 1b) included pet parrots that were hand-reared. These birds did not display any sign of FDB. This sample was composed of 11 birds (7 males and 4 females). Each bird was individually kept by a owner.



Picture 4.1. African grey parrots (*Psittacus e. erithacus*) observed in the present study. A) a parent-reared pair; B) healthy hand-reared parrot and C) hand-reared parrots that display feather damaging behavior (Costa et al., 2016b).

3. The FDB hand-reared parrots (FDB-HR) (Figure 1c) included pet parrots that were hand-reared. These birds displayed FDB (Picture 5.2). This sample was composed of 11 birds (7 males and 4 females). Each bird was individually kept by a owner. The diagnosis of FDB was made by a veterinary expert in exotic birds who took into consideration all of the possible differential diagnoses according to van Zeeland et al. (2009). In this way, it was possible to rule out any clinical problems.

The H-HR were age (± 2 years) and sex matched with the FDB-HR. Both H-HR and FDB-HR parrots lived mostly outside a cage without any other parrots and had a close relationship with humans. All of the birds were privately owned and had free access to water and to commercial diets formulated specifically for parrots that were supplemented with fruit and vegetables. The owners of all of the parrots included in the study completed a questionnaire about the care and management of the parrots and, only for FDB-HR parrots, the main body regions affected by FDB.

Droppings sampling and analysis

Droppings were collected throughout autumn 2014 and spring 2015 in the middle of each season. The droppings were collected in the morning (9:00 – 11:00 AM) for three days on alternating days. This time frame was chosen with the intention to reduce the effect of daily patterns in CM excretion. The samples were collected directly from the cleaned bottom of the bird's habitual cage where the parrot lived. For PR parrots, the dropping samples represent a pool of the excreta from the parrot pairs, whereas the droppings were individually collected for the H-HR and FDB-HR parrots. The 3-day samples were pooled, stored in 50-mL plastic tubes and immediately frozen at -20 °C until analysis. A total of 30, 11 and 11 samples were collected at each sampling time for the PR, H-HR and FDB-HR parrots, respectively.

To extract steroids, we used the methanol-based procedure described by Palme et al. (2013) with slight modifications. Briefly, the droppings were lyophilized, weighed, and completely crushed, and two aliquots of the samples (0.25 g each) were placed into extraction tubes, which were then

sealed with a teflon cap and stored at -20 °C. Each aliquot was thoroughly mixed for 30 min using a multivortex with one mL of 80% methanol (Sigma Aldrich, St. Louis, MO). The suspension was then centrifuged at 500 g for 20 min and the supernatant was recovered. An aliquot (0.5 mL) of the supernatant was transferred into a new vial and evaporated at 50 °C for 14 h. After evaporation, the dried extracts were stored at room temperature in dark boxes for 15 days and then kept at -80 °C until they were assayed. One day before the CM analyses, the dried extracts were re-diluted in 0.5 mL of 80% methanol. An aliquot of the extract was diluted to 1:10 in the assay buffer (Arbor Assays®, Ann Arbor, MI). The mixture was then vortexed and left to rest for 5 min twice to ensure complete steroid solubility. The CM were determined using a multi-species corticosterone enzyme immunoassay kit (K014; Arbor Assays®, Ann Arbor, MI). All of the analyses were repeated twice. The inter- and intra-assay coefficients of variation were less than 10% (6% and 8%, respectively). The sensitivity of the assay was 11.2 ng/g droppings. All of the droppings samples were analyzed at multiple dilutions (1:4, 1:8, 1:16 and 1:32), and all regression slopes were parallel to the standard curve (r2 = 0.983). The mean recovery rate of corticosterone added to dried excreta was 95.8%. 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. The concentration of CM was expressed as ng/g of droppings dry matter.

Picture 4.2. Deplumation area in feather damaging behavior African grey parrots. A) chest area; B) wings; C) shoulders and rump (Costa et al., 2016b).

Data analysis

The CM of the PR, HP and FDB-P parrots were compared. Before testing for group differences, the normality of the data distribution and the homogeneity of variance were assessed using the Shapiro-Wilk test and Levene's test, respectively. Split-plot repeated-measure ANOVA was used to examine any differences using one within-subject variable (season) and one between-subject variable (the three samples of birds) and considering the interaction between these main effects. When the main effect was significant, a Tukey's post hoc test was performed to analyze the differences between groups. To explore the effects of sex and age on CM within the H-HR and FDB-HR groups, a t-test and a correlation analysis (Pearson's r) were performed, respectively. The data

are presented as the mean and the pooled standard error of the mean (SEM). Statistical significance was set at 0.05, and a trend of significance was considered at $p<0.1$. All statistical analyses were performed using SPSS version 15.0 for Windows (SPSS Inc., Chicago, IL, USA).

4.3 Experiment 3: The influence of reinforcement foraging toys in the welfare in two feather picking African grey parrots (Psittacus erithacus)

Animal and selection criteria

The study was based on the data obtained from a web questionnaire used in a previous study (Costa et al., 2016a) that was addressed to the owners of all species of pet parrots. The questionnaire was distributed throughout Italy through online parrot association sites, social networks and e-mails in collaboration with Italian parrots associations. From this previous questionnaire we obtained the parrots data about age, sex and life story information.

The four birds considered in our study belonged to *Psittacus erithacus* species, were born in captivity and was hand-reared by human in neonatal age. These birds have been studied in a previous work (Costa et al., 2016a), that observed the comparison between parrots that were breeding by biological parents and by human, with and without FDB symptoms in regards to CM value, in two season.

Previous research allowed us to compose two groups of parrots: FDBP parrots (FDBP – feather damaging parrot) and HHP-C parrots (healthy hand-reared parrot – HHP-C). FDBP parrots group was composed of 2 birds (1 males and 1 females), that showed clearly FDB symptoms. HHP-C parrot (1 males and 1 females) do not showed FDB symptoms; these birds were considered in order to have a control. Each bird was individually kept by a private owner. Of this birds, it is present a set of information (age, sex, levels of CM in past years, behavioral trend, FDB body regions, etc.).

The diagnosis of FDB was made by a veterinary expert in exotic birds who took into consideration all of the possible differential diagnoses according to van Zeeland et al. (2009).

Questionnaire for African grey's owners

A questionnaire about behavior characteristic of birds before the reinforcement foraging toys and the behavior modification after toy use was compiled by each owners (Table 1). Ten questions regarding state of plumage, vocalizations, aggressive behavior and relation with human were considered.

Droppings sampling and analysis

The parrots of this study were studied in a previous works (Costa et al., 2016a). The CM values observed in parrots that was used in this study are in Table 3. Droppings were collected before the reinforcement foraging toys use and after a period of three days. The droppings were collected in the morning (9:00 – 11:00 AM) for three days on alternating days. This time frame was chosen with the intention to reduce the effect of daily patterns in CM excretion. The samples were collected directly from the cleaned bottom of the bird's habitual cage where the parrot lived. The 3-day samples were pooled, stored in 50-mL plastic tubes and immediately frozen at -20 °C until analysis.

To extract steroids, we used the methanol-based procedure described by Palme et al. (2013) with slight modifications. Briefly, the droppings were lyophilized, weighed, and completely crushed, and two aliquots of the samples (0.25 g each) were placed into extraction tubes, which were then sealed with a Teflon cap and stored at -20 °C. Each aliquot was thoroughly mixed for 30 min using a multivortex with one mL of 80% methanol (Sigma Aldrich, St. Louis, MO).

The suspension was then centrifuged at 500 g for 20 min and the supernatant was recovered. An aliquot (0.5 mL) of the supernatant was transferred into a new vial and evaporated at 50 °C for 14 h. After evaporation, the dried extracts were stored at room temperature in dark boxes for 15 days and then kept at -80 °C until they were assayed. One day before the CM analyses, the dried extracts were re-diluted in 0.5 mL of 80% methanol. An aliquot of the extract was diluted to 1:10 in the assay buffer (Arbor Assays®, Ann Arbor, MI). The mixture was then vortexed and left to rest for 5 min twice to ensure complete steroid solubility. The CM were determined using a multi-species corticosterone enzyme immunoassay kit (K014; Arbor Assays®, Ann Arbor, MI). All of the analyses were repeated twice. The inter- and intra-assay coefficients of variation were less than 10% (6% and 8%, respectively). The sensitivity of the assay was 11.2 ng/g droppings. All of the droppings samples were analyzed at multiple dilutions (1:4, 1:8, 1:16 and 1:32), and all regression slopes were parallel to the standard curve (r^2 = 0.983). The mean recovery rate of corticosterone added to dried excreta was 95.8%. 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. The concentration of CM was expressed as ng/g of dropping dry matter.

Reinforcement foraging toys

A reinforcement foraging toys (Picture 5.3), was used for this study. This toy had a rotating top with two 1/2" by 1" slots through which birds may retrieve their favorite snacks. The bird can learn to rotate the toy so that the openings are over the material they wish to retrieve. Reinforcement foraging toys are made of polycarbonate with stainless steel. The toy is product by Northern Parrots© (1995 – 2016, Unit 21 Cuba Estate, Ramsbottom, Bury, BL0 0NE, United Kingdom).

Picture 4.3 Reinforcement foraging toys. Reinforcement foraging toy had a rotating top with two 1/2" by 1" slots through which birds may retrieve their favorite snacks.

Data analysis

The CM of the FDB parrots were quantified. The data are presented as the individual value for each bird.

5 **RESULTS**

5.1 Experiment 1

General data returned

A total of 335 surveys was obtained, of which 292 (87.2%) were useful for the statistical analysis and all regions of the country were represented. The acquired data referred to 20 different genera of parrots, kept as pets distributed throughout all the regions in Italy, genera representing less than 5% prevalence were not presented in the table. The most popular species kept as pets are Psittacus erithacus (24.3%), Agapornis spp. (19.5%), Nymphicus hollandicus (18.0%) and Amazona spp. (9.0%). In the sample, 40.2% of the parrots were female and 59.7% male (Table 5.1).

In regards to breeding, 80.2% of the parrots have been hand-reared; in particular, 41.8% were fed at the neonatal stage, by the breeder and weaned by the buyer; 38.4% were fed at the neonatal stage and weaned by the breeder and was then sold when weaning had been completed (Table 5.2). It emerged that 84.3% of the parrots were caged when the owners were absent and left free when they were present and that 66.4% of the pet parrots were left free from 1 to 6 hours/day; while lives always caged 4.1%. Most of the roosts were made of wood (43.1%), 22.7% were natural branches and 21.6% plastic perches. It was also found that 90.4% of the parrots were able to wash their plumage every week, because the owners nebulized their bodies or put special bowls with water into their cages or near their roosts.

As far as eating habits are concerned, 40.4% of the birds eat dry seeds and fresh vegetables in equal amounts; 23.5% eat more dry seeds (4 times a week) than vegetables. As for extruded feed consumption, 37.9% of the birds were not fed this type of food, because the owner does not provide it or the parrots do not like it. Finally, 71.2% of the birds were occasionally or regularly fed human food (e. g. cookies, bread, yogurt, meat, etc.) (Table 5.2).

Feather picking general data, prevalence and symptoms

This study has shown a FP prevalence rate of 17.5% (Table 5.2). A statistical trend showed that FP has been found to mostly affect males (70% of the FP parrots, $p=0.10$) (Table 5.2). It has emerged that 52.9% of the owners stated that the birds had not suffered from episodes that could have caused FP, such as trauma, fear or changes in family. A significant difference in the feather picking site was detected ($p<0.001$). The most affected body region was the chest (58.8%), followed by the rump (41.2%) and under wing regions (25.5%). It has emerged that 98.0% of the FP parrot's owners consider feather picking to be a behavioral disorder. This study has not shown any link between type of diet and abnormal behavior.

Sensible species

A statistical trend (p=0.09) was observed in differences among FP prevalence in parrot's genera, the highest prevalence was reported for Agapornis spp. (26.3% of the FP) and Psittacus spp. (22.5%) (Table 5.1).

Imprinting, neonatal age and grow-up

The 82.0% of FP parrots and 42.3% of non-FP parrot (p<0.001) were fed and weaned by the breeder after birth and were then sold after weaning had been completed. By contrast the 18.2% of FP parrots and 57.7% of non-FP parrot (p<0.001) were fed after birth by the breeder and weaned by the owner (Table 5.3).

Age

The average age in all FP parrots were 82 months; in particular, in sensible species, were Agapornis spp. 60.5 months, in Psittacus spp. 75.4 and in Amazona spp. 78 months. In one case the age was not known (Agapornis roseicollis); all other cases were sexual mature.

Aggressiveness, abnormal and sexual behavior

In regard of abnormal behavior, 64.6% of the parrot owners claimed they had seen their parrots pulling at their feathers; 51.0% of the owners observed abnormal behavior before the birds attempted to remove their feathers (e.g. feather chewing and biting, increase aggressive behavior and self-injuries), but others reported a sudden appearance of feather removal behavior (48.9%).

Of the feather picking parrots, 50.0% showed behavior stereotypes and most sexual behavior disorders observed was food regurgitation in front of the owner (30.5%); aggressiveness was observed in 60.7% of case.

Other self-injuring behavior (such as biting their legs, hitting their face, etc.) was observed in 39.2% of the feather picking parrots. In general, FP frequency has been shown to vary according to the parrot species.

Environments and human relationship

The 62.7% of FP parrots and 42.7% of non-FP parrot (p<0.01) live with another parrot (Table 6.3). Moreover, 2.9% of non-FP parrots and 10.0% of FP parrots lived always caged (Table 4), and this difference show a statistic trend (p=0.06).

Parent-raised type parrots data

At the same time, we conducted another study to better understand some information from the companion parrots through a comparison with the parent-raised type parrots (genera: Nymphicus, Agapornis spp., Amazona spp., Ara spp., Psittacus spp.). With the help of the second questionnaire, 18 parrot breeders were found with 1488 parrots in the most suitable ethological conditions (not hand-

reared, not alone and with the possibility of reproduction). Of these 1488 parrots, only 19 birds showed feather loss (1.3% of the parent-raised type population).

Table 5.1 - Parrot genera represented in our studied population (n=292 parrots) and percentage of feather picking parrots in each genus.

Genus	**Prevalence of population (%)**	**Feather picking (%)**	**(95% CI[2])**
Psittacus spp.	24.3	22.5	12.8 - 32.2
Agapornis spp.	19.5	26.3	14.9 - 37.7
Nymphicus spp.	18.0	7.6	0.4 - 14.7
Amazona spp.	9.0	15.4	1.5 - 29.2
Ara spp.	6.5	15.8	0.0 - 32.2
Other genera[1]	22.7	-	-
P	**-**	**0.09**	**-**

[1]Other genera each represented below 5% prevalence: Aratinga, Cacatua, Cyanoramphus, Diopsittaca, Eclectus, Eolophus, Eos, Melopsittacus, Myiopsitta, Nandayus, Pionus, Psephotus, Psittacula, Poicephalus, Trichoglossus.

[2]confidence interval.

Table 5.2 – Variables investigated in the sampled Italian pet parrots (n=292) population and in Italian feather picking parrots (n=51) of the sample.

Variable	**% Prevalence (95% CI[1])**
First part (n=292) – addressed to all pet parrots	
Social Life	
Lives with others parrots	46.2 (40.5 - 51.9)
Lives alone	52.74 (47.0 - 58.5)
Grew up alone	21.6 (16.9 – 26.3)
Grew up with other chicks	71.0 (47.4 – 58.9)
Hand-rearing and weaned from breeders	38.4 (32.8 - 43.9)
Hand-rearing from breeders and weaned from owners	41.78 (36.1 – 47.4)
Management	
• Lives caged when owners absent	84.3 (78.6 - 87.2)

• Lives always free	11.7 (8.0 - 15.3)
• Lives always caged	4.0 (1.9 - 6.4)
• Lives always in owners home	83.0 (78.6 - 87.2)
• Lives outdoor	4.8 (2.3 - 7.2)
Cage enrichments	
Materials of roosts:	
• Wood	43.1 (37.4 - 48.8)
• Plastic	21.6 (18.9 - 27.0)
• Natural branches	22.7 (18.0 - 27.4)
• Other materials	11.3 (7.7 - 15.0)
Other information:	
• Presence of toys and natural object	89.7 (86.2 - 93.2)
• Possibility of bathing water	90.4 (87.0 - 93.7)
Environment	
• Lives in home and outdoor	83.0 (78.6 - 87.2)
• Lives always in closed	25.3 (20.3 - 30.3)
Diet	
• Eat human food	71.9 (66.7 - 77.0)
• Eat seeds and vegetables in equal quantities	40.4 (34.8 - 46.0)
• Eat extruded feed	60.6 (55.0 - 66.22
Feather picking	
• Prevalence[2]	17.5 (13.1 - 21.8)
• Male	70.0 (55.8 - 84.2)
• Female	30.0 (15.8 - 44.2)
• No episode linked feather picking	52.9 (39.2 - 66.6)
• Immediate removal of plumage without damage results first	33.3 (20.4 - 46.3)
• Sexual behavior	45.1 (31.4 - 58.7)
• Aggressive behavior	60.7 (47.4 - 74.2)
• Self-injury behavior showed	39.2 (25.8 - 52.6)
• Owners claim that feather picking is linked to behavioral disorder	98.0 (94.2 - 100.0)

[1]Confidence interval; [2]Calculated on the whole sampled Italian pet parrots population (n=292).

Table 5.3 - Risk factors associated with the feather picking behavior in the sampled Italian pet parrots population (n=292).

Risk factor	**Healthy pet parrots (%) (n= 241)**	**Feather picking pet parrots (%) (n= 51)**	**P**
Living with other parrots	42.7	62.7	<0.01
Hand-rearing and weaned from breeders	42.3	82.0	<0.001
Hand-rearing from breeders and weaned from owners	57.7	18.2	<0.001
Lives always caged	2.9	10.0	0.06

5.2 Experiment 2

The average age of the birds was 8.1±1.7, 7.9±5.4, 7.8±5.4 years for PR, H-HR and FDB-HR parrots, respectively. The average volume of the aviary cages in which the birds belonging to the PR group were kept was 4.85 m3. The average volume of the cages of each H-HR and FDB-HR parrot was 1.70 m3, 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 FDB in the FDB-HR birds was the chest (90.9%; Picture 4.2a), and this was followed by the wings (18.2%; Picture 5.2b), the shoulders and the rump (9.1%; Picture 4.2c). No sign of FDB was observed on the head.

Different quantities of CM in droppings were found for the three samples of African grey parrots. The mean CM value was 587 ng/g in the PR parrots, 494 ng/g in the H-HR parrots and 1744 ng/g in the FDB-HR parrots, irrespective of the season (Table 5.4). The excretion of CM in FDB-HR parrots was higher than in PR and H-HR parrots ($p<0.001$). CM in droppings were not influenced by the season (autumn vs spring); furthermore, the interaction between parrot groups and the sampling season was not significant (Table 5.4).

To explore the effect of sex on CM excretion in the H-HR and FDB-HR samples, a t-test was performed, considering the mean CM amount (autumn and spring) for each bird, given the non-significance of the within-subject effect (sampling season); moreover, in these samples, a correlation analysis (Pearson's r) using the same response variable was conducted to assess the effect of age on CM excretion. The results showed that there was a trend in the difference in the mean CM excreted by male and female birds, with the levels of males being higher than those presented in females: HP, mean of males=554, mean of females=388 ($t=1.851$, $p=0.097$); FDB-HR, mean of males=1852, mean of females=1556 ($t=1.906$, $p=0.089$). When the effect of age was considered (in the two separate populations), there was a statistically significant positive correlation only for H-HR ($r=0.609$ $p=0.047$); in contrast, no correlation was found for FDB-HR ($r=0.398$, $p=0.225$).

Table 5.4. Corticosterone metabolite (ng/g dry matter) excretion in the droppings of healthy and FDB1 African grey parrots (Psittacus erithacus) (mean and pooled SEM)

				95% Confidence Interval	
Group	***Season***	***Mean***	***SEM***	***Lower Bound***	***Upper Bound***
PR[2] parrots	autumn	617	25	558	676
	spring	558	31	467	649
	mean	587	20		
H-HR[3] parrots	autumn	519	58	421	616
	spring	469	45	318	620

	mean	494	36		
FDB-HR[4] parrots	autumn	1749	55	1652	1847
	spring	1739	133	1589	1890
	mean	1744	70		
Main effects:					
Group:		F = 194.477	p < 0.0001		
Season:		F = 1.305	p = 0.259		
Interaction (group × season):		F = 0.191	p = 0.826		
Group contrasts (LSD test):					
PR vs. H-HR parrots:		p = 0.140			
PR vs. FDB-HR parrots:		p < 0.0001			
HR vs. FDB-HR parrots:		p < 0.0001			

[1]FDB: feather damaging behavior [2]PR: parent-reared; [3]H-HR: healthy hand-reared; 4FDB-HR: feather damaging behavior hand-reared.

5.3 Experiment 3

The average age of the birds was 5.4 years. Each animal was kept in a commercial cage from different dimensions. The parrots had the opportunity to exit from the cage for a minimum of 4 hours a day; two of five of this parrots lived with the cage ever open (Table 5.5.a and 5.5.b). All of the birds were privately owned and had free access to water and to commercial diets formulated specifically for parrots that were supplemented with fruits and vegetables. The mean CM value was 587 ng/g in the two FDBP parrots and 494 ng/g in the two H-HRP parrots (Table 1). The excretion of CM in FDB-HR parrots was higher than in FDBP, and H-HR parrots (p<0:001). CM in droppings were not influenced by the season (autumn vs. spring); furthermore, the interaction between parrot groups and the sampling season was not significant (Table 5.7).

Table 5.5.a Information about feather damaging behavior (FDB) parrots.

Parrot FDB1 (ScNap)			
Age	4	**Sex**	Male
CM levels ng/g 2014-2015		1416,8 - 1045,13	
Behavioral set			
Neonatal period history			
• Hand reared with syringe by breeder			
• Weaned from breeder from 3 to 5 months			
• Kept and weaned with other chicks			
• After weaned live isolations from other parrots			
Management			
• Parrot can outside from cage daily			
• The parrot have a relation with only family member			
• The parrot is most active in the afternoon			
• The parrot vocalize most in the afternoon			
• No toys in the cage			
Sexual behavior: Regurgitation			
Aggression: Ever with other humans are not the owner			
FDB set			
• Start at the age of 1,5 years			
• Preceded by increasing in aggression and shouting			
• Period of increasing FDB is evening			
• Skin lesions			

- Body regions interested are chest and abdomen

Parrot FDB2 (CnAn)

Age	15	**Sex**	Female
CM levels ng/g 2014-2015	1924,92 - 1937,00		

Behavioral set

Neonatal period history

- Hand reared with syringe by breeder
- Weaned from breeder
- Kept and weaned without other chicks
- After weaned live isolations from other parrots

Management

- Live ever outside from cage
- The parrot have a relation with only family members
- The parrot is most active in the afternoon
- The parrot vocalize most in the morning and in the afternoon
- Toys are present in the cage

Sexual behavior: Regurgitation

Aggression: Sometimes with other humans who are not the owner

FDB set

- Start at the age of 12 years
- Preceded by increasing in aggression, shouting and sexual behavior
- Period of FDB is all day
- No other symptoms
- Body regions interest are chest and abdomen

Table 5.5.b Information about healthy parrots (HHP).

Parrot HHP-C1 (VgTor)			
Age	6	**Sex**	Male
CM levels ng/g 2014-2015	637,84 **-** 569,28		

Behavioral set

Neonatal period history

- Hand reared with syringe by breeder
- Weaned from breeder from 5 to 8 months
- Kept and weaned without other chicks
- Live actually with other parrots (*Amazona aestiva*)

Management

- Parrot can outside from cage daily
- The parrot have a relation with only family member
- The parrot is most active in the morning
- The parrot vocalize most in the morning
- No toys in the cage

Sexual behavior: Copulation with body part of owner

Aggression: No aggression with other humans

Parrot HHP-C2 (PPTor)			
Age	4	**Sex**	Female
CM levels ng/g 2014-2015	358,92 - 515,75		

Behavioral set

Neonatal period history

- Hand reared with spoon by breeder
- Weaned from breeder
- Kept and weaned with other chicks
- Live actually with other parrots (*Eos bornea*)

Management

- Parrot can outside from cage daily
- The parrot have a relation with only family member
- The parrot is most active in the afternoon
- The parrot vocalize most in the morning
- No toys in the cage

Sexual behavior: Regurgitation

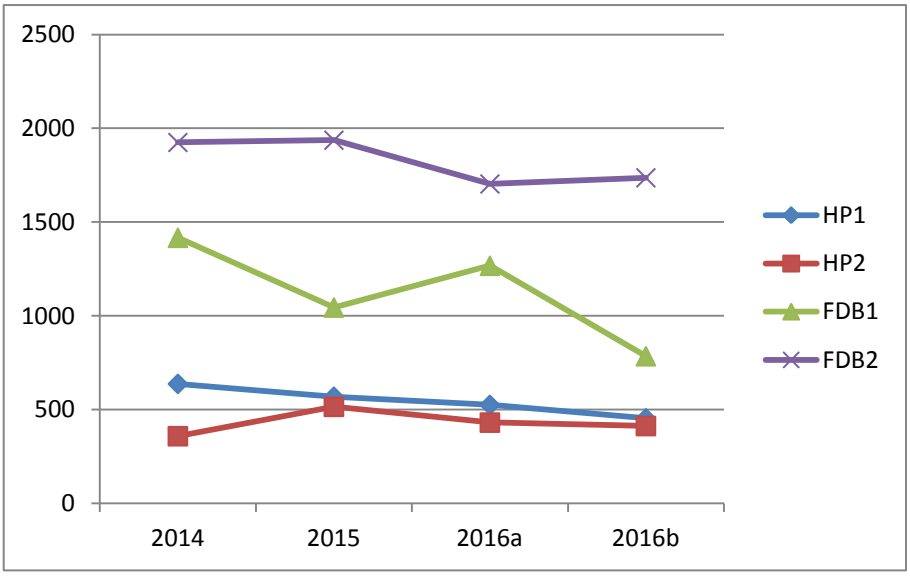
Aggression: Ever with other humans are not the owner

Table 5.6. Questionnaire for FDB African grey parrots owners.

Questions	Answer
How do you judge the plumage of your parrot after a month of use of the toy?	Very improved Improved It is the same of the time before use of the toy Worsened
It is declined the aggressive behaviors (bite, aggression etc.) after a month of use of the toy?	They are definitely diminished Are somewhat diminished They are the same of the time before use of the toy Are increased
How are the vocalizations after a month of use of the toy?	They are definitely diminished Are somewhat diminished They are the same of the time before use of the toy Are increased
How is the human relation after a month of use of the toy?	The parrot still want more contact The parrot seems to want a little more contact Is the same of the time before use of the toy The parrot want less contacts
The stereotypes behavior (if present) are increased or decreased?	They are very increase They are a little increased They are the same of the time before use of the toy They are significantly diminished
What stereotypes behavior specifically has increased/decreased? How often you filled the wheel-toy with food? How much time the parrot has had to take for "understand" the operation of the wheel-toy?	Daily Every two or three days Weekly Less than a week Over a week About a month
The Reinforcement foraging toy was used	With any food

with normal daily food or with particular foods, such as tidbit?	Only with tidbit food

Figure 5.7 Corticosterone metabolites levels.



6 DISCUSSION

6.1 Experiment 1: Investigation on evidence about feather damaging behavior in Italian pet parrots population

The use of web questionnaires to collect data in surveys can be a limit since they suffer response bias (Dohoo et al., 2003). However, our questionnaire attained a good response rate, enabling a valid data collection from a wide study population.

Given the nature of the conduct underlying the FP brought to light by the present data, it is possible to consider the damage and the removal of the plumage as a behavioral pathology, in which factors of different origin (aberrant imprinting, hand-rearing techniques, environment isolation, sexual frustration) can cause behavioral changes that give rise to the manifestation of quite similar abnormal behavior to that of the common forms of self-injury. On the other hand, the relationship between emotional stress and self-injury is also seen in captive primates and Bordnick et al. (1994) compared feather-picking behavior in parrots to compulsive and impulsive human disorders, such as human trichotillomania. In other animals it has been observed that. Self-injuring rhesus macaques display greater emotional responsiveness than their non-injurious counterparts do (Novak 2003), and the stress of relocation to novel housing has been shown to produce long-lasting increases in self-biting behavior in these animals (Davenport et al., 2008).

Feather picking general data and sensible species

FP is a behavioral disorder that is frequently encountered in captive parrots (van Zeeland et al. 2009). Many authors have shown relatively low FP rates (10% Grindlinger et al. 1991; 13% Gaskins et al. 2011), but the present results are similar to those found by McDonald Kinkaid et al. (2013) who determined a FP prevalence rate of 15.8%. The present survey suggests that the prevalence of FP in Italian parrots is 17.5%. This prevalence is much higher than in parrots bred in more ethologically suitable conditions (1.3%), i.e. those bred from biological parents, were living in a couple and without direct human contact. Such a finding could suggest that the hand-rearing of parrots may be a risk factor for the development of FP, together with other living conditions of pet parrots.

The present results, in agreement with these authors, show FP in 22.5% of Psittacus spp. and in the sample of Cacatua genus the prevalence of FP was high (40%), but the sample was small (2% of the parrot population; data not shown in the table). The present results show, that the Agapornis genus is also sensitive to FP (26.3% prevalence). Agapornis spp. has become a very popular pet parrot in Italy in the last few years. This data suggest that the species may be a risk factor for FP onset.

Post natal period and sexual behavior

The mean age of the FP parrots examined in our study was 82 months; this observation is supported by van Hoek et al. (1998), who reported that most of the birds showed significant

behavioral distortions upon reaching sexual maturity. Many authors agree that FP is more common in African grey parrots (*Psittacus spp.*) and in the *Cacatua genus* (Chitty 2003; Rosenthal et al. 2004; Garner et al. 2008; van Zeeland 2009a, 2013b).

This difference between FP parrots and non-FP parrots could be determined by the imprinting process that evolved during the hand-rearing period. In particular, the sexual imprinting (which develops later than parental imprinting) may play a role in the onset of FP. Sexual imprinting refers to the process by which animals learn the characteristics of appropriate mates by learning the characteristics of their parents or siblings (Fox, in Luescher 2006). FP often develops after the onset of sexual maturity (Wedel 1999) and the data recorded in this study confirmed this thesis. In fact, on the average, the age of the two sensible species was 60.5 in *Agapornis spp*. and 75.4 in Psittacus. Sexual maturity is reached earlier in budgerigars, at 5 months (Kavanau 1987), while many of the large-bodied species of cockatoos, e.g., do not reach sexual maturity until approximately four to five years of age (Forshaw 1981). These data could be also confirmed from the observation of the behavior of the birds by the owners: those who had observed sexual behavior directed toward humans claim that the most frequent habit was food regurgitation (30.5%). In fact, food regurgitation is typical sexual behavior in parrot couples (courtship feeding or allofeeding, Spoon, in Luescher 2006) and this fact leads to the hypothesis that FP parrots show redirected sexual behavior due to a lack of partner, in according to Lantermann (1989) who claim that this behavior is the expression of the parrots' sexual frustration. This hypothesis is based on the fact that parrots probably develop sexual imprinting toward humans during the hand-rearing or weaning period. According to Fox (2006), these results confirm that an abnormal sexual imprinting and a strong social preference for humans may cause behavioral problems in hand-raised parrots, which are probably more likely to inappropriately direct sexual behavior toward their owners and the presence of other parrots don't change this preference. In addition, our results showed that the parrots that are more in contact with humans showed higher prevalence of FP than the parrots that live always in cages. In addition, it seems that FP mostly affects males (70% of the FP parrots). Jayson et al. (2014) considered that the sex of the bird was a significant factor in the occurrence of FP. Fox (2006) suggested that hand-rearing apparently influences sexual imprinting in males more strongly than in females.

Although popular literature suggests that hand-raised parrots make better pets than parent-raised parrots (Blanchard 1999), hand-rearing has the potential of producing physical as well as behavioral problems in parrots (Harcourt-Brown 2004). Different methods of breeding can affect the onset of behavioral problems, such as FP, to a great extent (Lightfoot 2002). This indicates that various hand-rearing techniques could influence the prevalence of FP and that is reduced if the sexual imprinting is directed to the final owner (this is possible if the final owner is responsible of the last phase of hand-rearing). In particular, it is probably the imprinting mechanism that causes the bird to deviate incorrectly. In the course of the classical sensitive phase in early development of zebra finches, the birds establish a social bond with their parents. Under normal circumstances, this

narrowing of social preference to the parent species guides young male zebra finches in their first courtship attempts when they are sexually mature (Öetting et al., 1995).

Environment

As already observed by Jen-Lung Peng et al. (2014), inadequate socialization may be a factor of feather picking in some birds. They claims that using conspecific rather than human exposure do not have an effect on FP reduction. Our results showed that the presence of other parrots in the same environment was more high in FP parrots (62.7%), compared to non-FP parrots (42.7%). Preiss & Frack (1974) and Van Zeeland (2009), claimed that these abnormalities in behavior resulted from the isolation of hand-raised birds during the fledgling period, which fail to become integrated into a conspecific social group later on. If parrots construct their sexual imprinting to humans (in absence of other parrots during the weaning period) they can develop an adaptation to human that does not find the same stimulatory satisfaction.

6.2 Experiment 2- Quantification of faecal Corticosterone in African grey parrots, compared to hand-rearing and not hand-rearing birds in house-hold condition

In our study, we observed increased excretion of CM in FDB-HR parrots, which was approximately three times higher than that of PR and H-HR parrots, irrespective of the season of sampling. Moreover, no differences were found in CM excretion between H-HR and PR parrots, the latter of which were kept in pairs for reproduction and so they can maintain sexual and social activity.

Our results confirm the findings of Owen & Lane (2006), who showed higher CM in the droppings of FDB-HR parrots than in control parrots. To the best of our knowledge, the paper of Owen & Lane (2006) is the only study comparing the CM excretion in droppings of FDB and non-FDB African grey parrots (261 ng/g and 75 ng/g, respectively). Our results confirm these observations in terms of significant differences between FDB-HR and H-HR parrots, but the magnitude of the values measured in our study was more than 6 times higher than those observed by Owen & Lane (2006). In the study of Owen & Lane (2006), the control group was composed of ten birds that were kept all together in a large aviary, so they presumably maintained their social and sexual activity. In contrast, in our study, we considered two samples of parrots that did not display FDB: PR and H-HR, which both display similar levels of CM excretion. Parent reared parrots are usually considered well balanced birds since parent rearing methods let them to learn all the specific behavior pattern, which is a great benefit for their welfare (Schmid et al., 2006). The link between FDB and the corticosterone levels of excreta has also been observed by Peng et al. (2014) in two cases of FDB in sulphur-crested cockatoos (*Cacatua galerita*); the authors found a decrease in corticosterone levels after treatments that consisted of socialization, a training program, medication and feeding enrichments. Even though we did not measure the environmental or enrichment management and the activity of parrots included in our study, it has been previously demonstrated that parrots with FDB display higher activity compared to parrots without FDB in a number of behavioral tests, suggesting that FDB is a proactive

stress response pattern; under chronic stress conditions, proactive birds seemed to be more prone to develop behavioral disorders (van Zeeeland et al., 2013).

FDB can lead to, or result from, underlying skin pathologies that itch or irritate (Garner et al., 2008). FDB may also cause health problems related to tissue damage, hemorrhage, infection, or hypothermia (Meehan et al., 2003; Van Zeeland et al., 2009).

In the present study, the body area most affected by FDB was the chest, and the head was not affected by FDB. The presence of feathers in good condition in areas of the body that are not directly reachable (i.e., the head) by the birds is one of the criteria that has been used to make a distinction between FDB and other skin or plumage diseases (Galvin, 1983; Harison, 1986; Westerhof & Lumeij, 1987).

The higher CM excretion in the FDB-HR parrots than in the H-HR and PR parrots, suggest an increase in adrenal cortical activity (Möstl & Palme, 2002). The adrenal glands have a key role in the hormonal response to short-term and chronic stress, which result in an increase in glucocorticoid secretion (Möstl et al., 2002). The measurement of CM in bird droppings has been proposed to assess the welfare status of birds (Meehan, Garner & Mench, 2004; Van Zeeland et al., 2009; Cussen & Mench, 2015; Young & Hallford, 2013; Ferreira et al., 2015; Shepherdson et al., 2004), the results of such analyses are hard to interpret because the biological perspective suggests only an increase in adrenocortical activity. For these reasons, the importance of these data could lead to misinterpretation because they are a result of a complex interaction between a wide range of physiological, endocrine and behavioral variables that occur simultaneously (Gaskins & Bergman, 2011; Cussen & Mench, 2015; Van Zeeland et al., 2009).

In our sample of hand-reared parrots, a trend in the significance of the difference in the mean CM excreted between male and female birds was found for both H-HR and FDB-HR parrots, with the levels excreted by males being higher than those excreted by females. In contrast, Ferreira et al. (2015) did not find any gender effect in the CM excretion of blue-fronted parrots (*Amazona aestiva*). Furthermore, a positive correlation between age and CM excretion was found for H-HR parrots. However, these results should be considered with caution in both studies due to the small sample sizes and the different species considered. The demographic features of FDB (i.e., sexual maturation) and gender predisposition (female > male) have been reviewed by van Zeeland et al. (2009), who state that the literature on this topic is related to field studies of small group of animals and that consequently larger surveys are thus necessary to confirm these results.

FDB is observed mainly in hand-reared parrots, occurring in from 10 to 17.5% of individuals (Grindlinger 1991; Kinkaid et al., 2013; Costa et al., 2016a), while in parent-reared parrots, FDB does not occur or occurs rarely (approximately 1%) (Costa et al., 2016a). Hand-rearing has been considered to be a risk factor in the incidence of FDB (Costa et al., 2016a; Schmid, Doherr & Steiger, 2006). Furthermore, social isolation and sexual behavior frustration can have important roles in the development of abnormal behavior (Lantermann, 1989; Harrison, 1994; van Hoek et al., 1998; Wedel, 1999; Fox, 2006; Jayson et al., 2014). According to Fox (2006), abnormal sexual imprinting and a

strong social preference for humans may cause behavioral problems in pet parrots, which are most likely more prone to inappropriately direct sexual behavior toward their owners. Since both H-HR and FDB-HR were in social and reproductive isolation in our study, this suggests that there is something different about their management that could be linked to environmental enrichment or breeding methods; thus, from an animal welfare perspective, it is fundamental to deeply research the risk factors that are involved in the incidence of FDB.

6.3 Experiment 3 - The influence of reinforcement foraging toys in the welfare in two feather picking African grey parrots (Psittacus erithacus)

Environmental enrichment describes improved living conditions. It is the process of enhancing the pet's environment using its behavioral biology and natural environment characteristics. It is providing opportunities for birds to hide, socialize, exercise, and occupy time. Environmental enrichment increases the bird's behavior choices, draws out their species-appropriate behaviors, and enhances their mental welfare (Rupley & Simone-Freilicher, 2015). Concerns about abnormal and stereotypic behavior displayed in captive animals have resulted in the development of environmental enrichment to allow animals to express more natural behavior (Swaisgood and Shepherdson, 2005) and Rodríguez-López (2016) in his study giving enrichment to parrots, has been shown to have an effect on their activities.

Our Results showed an decrease in CM levels in FDB1 parrot after a month of use reinforcement foraging toys in cage, while in FDB2 parrot this decrease is was not possible evaluated. On the other hand, the CM levels in healthy pet parrot (HHP-C1 and 2) do not shown an appreciable change after use reinforcement foraging toys. In particular, FDB1 showed a change in feather condition in body regions interested (chest and abdomen). In fact, the visual analysis prove an sight increase in accretion down-feather and plumule, and the damage of feather structure was diminished. Van Hoek & King (1997) concluded after they study on a Crimson-Bellied Conure (*Pyrrhura perlata perlata*) that the introduction of environmental enrichments led to a decrease in over-preening and feather-picking behaviors, while Jen-Lung Peng et al. (2014), after our study on a plucking Sulphur-Crested Cockatoos (*Cacatua galerita*) consider the most important factor of FDB start can be a lack in mental stimulation.

It is possible speculate about the role of play and mental stimulation in order to understand how parrots can modulate their motivation state, made up of all those internal and external factors that have a causal effect upon behavior (McFarland, 2006).

7 CONCLUSIONS

7.1 Possible ethological causes of feather damaging behavior

Regardless of the numerous studies conducted, it still cannot rely on a definition that would clarify unambiguously the mechanisms underlying this pathological condition, as well as the interpretation. Since it seems there is no conformity yet opinions in the scientific community to what are the factors that determine the appearance of this manifestation of discomfort and what are the mechanisms that modulate the expression. The loss of the feathers and/or its removal from the subjects affected appear as self-mutilation: in fact, one of the criteria that drive researchers to make a distinction between FDB and other diseases is the presence of feathers in good condition in areas of the body, not directly reachable from the animals from this basic assumption is suggested to consider it as a form of self-injurious (Galvin, 1983; Harisson, 1986; Lumeij and Westerhof, 1987).

Nevertheless, in many cases there was also a loss of plumage in areas unreachable by birds which is uncertain attribute organic causes. It is widely thought that the FDB is usually self-inflicted, but, when housed in groups, it can sometimes be directed to cage mates or nestling. In these instances, the primary target area appears to be the head and face (Wedel, 1999; Fox and Millam, 2004; Lightfoot and Nacewicz, 2006). This interpretation, however, creates a problem: if so, the victim of the parrot feather pecking caused by other parties should not own sensitivity to pain and even superficial behavioral mechanisms of intraspecific aggression, aimed at modulating the aggressive behavior of conspecifics, during social relations. Instead, parrots bred in captivity there is intraspecific aggression, that in many cases the causes for aggression between birds are the same as aggression toward humans (Welle and Luescher, 2006).

Interpreting the expression of the disorder of the plumage as different expression of a state of generalized stress, it is therefore necessary to begin to consider the possibility that there are two forms of FDB: the first, commonly defined false moult, where we are witnessing a loss of feathers coverts in certain areas of the body parrots (chest, abdomen, face and neck), and the other is self-defeating form itself, where the subjects get caught and/or excise directly and voluntarily in the feathers of its body, damaging and removing them.

The latter can be characterized also by a subsequent involvement of the epidermis, that is injured through chewing and traction, especially in various species of cockatoos, in particular in the Moluccan cockatoo (*Cacatua moluccensis*) and sunrise in cockatoos (*Cacatua alba*) (Rosenthal, 1993).

Therefore considering also forms of sudden loss of plumage not caused by direct self-injurious behaviors, it has been observed that sometimes some subjects placed in groups of more individuals may undergo the removal of the feathers and pecking by other entities in the group (Wedel, 1999; Fox and Millam, 2004; Lightfoot & Nacewicz, 2006). This does not account for the manifestations of sudden loss of plumage, even in areas unreachable by the beak, which involve birds reared in social isolation. Many individuals, in fact, seem to show that plumage loss as a result of sudden changes in

environmental and social issues, such as the change of environment and / or the change of partners and are not always in close relationship with a conspecific partner or with groups of individuals.

According to these observations, therefore it is necessary to consider a form of FDB not characterized by self-injurious behaviors directly, but rather as a result of various forms of acute and chronic stress that are expressed in the subject through the psychophysiological manifestation of diseases affecting the skin and appendages: psychophysiological diseases are those disorders in which the course of the skin disease is influenced by the patient's psychological state (Yadav et al., 2013).

Moreover, it is not yet clear whether the appearance and the evolution of FDB may correlate directly or indirectly factors such as inflammation of the skin, diseases from malnutrition, bacterial and viral infections. Considerations relating the possibility that this form of disease can be determined by brain dysfunction or incorrect development of the brain have not been confirmed (van Zeeland at al., 2009), while completing the broad framework of investigation about this phenomenon.

Regarding the sharing (causal or consequential) of disorders of the epidermis, a preliminary study conducted on a small sample of animals seems to have shown that there is no correlation between the inflammatory processes of the delicate skin of the parrots and the appearance of conduct related to FDB (Rosenthal et al, 2004); on the contrary, a study decidedly wider, conducted on a sample of about 400 subjects suffering from FDB (Garner et al., 2008) presented contrasting results compared to the study of 2004, determining a relationship between inflammation of the epidermis and the presence of FDB.

The most important survey in regards to FDB in parrots can be the data investigations about hormones and their metabolites. In our study of Corticosterone excretion in African grey parrots (Costa et al., 2016b), we analyzed the differences in CM excretion between African grey parrots characterized by 1. different rearing histories (parent rearing vs. hand rearing) and 2. the presence or absence of FDB in hand-reared parrots. The highest amount of CM excretion was found in FDB-HR parrots, and a positive correlation between age and CM excretion was found in H-HR.

In regard to the explanation about the effect of environmental enrichment, Lumeij et al., (2007) consider that the behavior of FDB may result from a redirected foraging behavior and through its studies support this hypothesis through the positive results that are obtained by increasing environmental enrichment and promoting foraging activity. However, this interpretation does not take into account that if a foraging behavior actually take place in order to resolve any conflicts of behavior, then, in theory, any behavior could play an equally any other function from the original one and it would be sufficient to change the environmental stimuli to cause a shift of the functional behavioral repertoire of animals. In our study, the use of reinforcement foraging toys in FDB parrots cage has shown a significant decrease of CM in one parrot of two, but we have not observed variations of this hormone in HHP-C. The abnormal behavior under FDB may be modulated through use of toys and environmental enrichment that can help the birds to redirected their strong motivation for some behavior (e.g. sexual behavior) in other object, but serve many other studies and whit more animals

for understand the most appropriate operations for resolve FDB in parrots. Taking into account the small number of birds in our study, it is difficult establish if the use of reinforcement foraging toys can be really useful, without other operations in feeding, environment and social setting.

7.2 Can be feather damaging behavior a form of neurosis?

There is sufficient evidence that the pecking behavior, chewing and traction of feathers are actually indicators of forms of stress and distress behavior: it should be emphasized that any cause or nature of clinical etiological factor has not been demonstrated and any observations of coexistence of other diseases could be random (van Zeeland et al., 2009).

The causes which determine the FDB can be many and there are a lot hypotheses. Several authors have devoted themselves to research in this sense, that is, further investigation aimed to exclude the less likely sources, but now the data are still few, fragmentary and conflicting with each other and the main reason for this uncertainty is that the majority of research was conducted on a relatively small sample of subjects (van Zeeland, 2009). On the other hand, the FDB has many considerations from the ethological point of view, being considered now as a behavioral pathology.

Several theoretical explanations are aimed at understanding the causes and mechanisms of pathological behavioral disorders. In fact, you can highlight two schools of thought most shared: one that considers the stereotyped behaviors (at the base of FDB) as a process of replacement of dominant behaviors with normal pathological ones, through a modification of the behavior "control center", however, the abnormal behaviors as responses to environmental stimuli quantitatively excessive abnormal (van Zeeland at al, 2006 and Garner, 2003). The study of Italian pet parrots populations has shown that FDB can be caused by the imprinting process during the fledgling period and we could hypothesize that sexual frustration plays an important role in developing FDB. As FDB is a distinctly pathological condition related to captive-kept parrots, as shown by our study, it is quite likely that defective environmental stimuli, aberrant imprinting and a lack of sexual satisfaction and reproduction could lead to a higher incidence of the onset of the disease. In fact, it is possible to ascertain that the onset of this condition is particularly high in hand-raised birds, namely those subtracted from the biological parents and hand fed by breeders, while in the parent-raised type parrots is almost nothing.

However, pathological behaviors such as stereotypies can also be interpreted as new behaviors, that is not covered by the behavioral repertoire of the species taken into consideration and therefore, characterized by a new modulation, consisting of behavioral patterns of different origin and function: as indeed are the more obviously self-injurious behaviors.

Interpreting in this way the pathological behaviors at the base of the FDB (hence the behavioral stereotypes and behaviors aimed at the removal and damage of pens and feathers) opens a different way of ethological analysis: it aims to break down these behaviors in their various modulations and understand the origin biologically correct. For example, if the basis for the removal of a feather could be considered as a trigger for the behavior an excess of aggression (probably due to a

deep sense of frustration over the lack environmental and social stimulation) then, theoretically, it is possible to assume that the pain generated by the removal of the follicle from its seat may have the same functions they perform in humans, which have an affect-regulation (to alleviate acute negative affect or aversive affective arousal) and sensation-seeking effect (to generate exhilaration or excitement; Klonsky, 2007).

The removal of feathers may serve to assuage the feelings of anxiety and frustration by the animal, responding to psychological mechanisms similar to what happens to the human species.
Beyond the theoretical ethological interpretations that can be given to this event pathological behavior, what is particularly interesting is that the FDB presents a set of symptoms (often progressive character) common (for modulations and behavioral consequences) in all affected individuals, and this motivates us to propose a classification of symptoms on the basis of field observations.

Given the nature of the conduct underlying the FDB brought to light by various authors and in regards to the data obtained after this work, is possible to consider the damage and the removal of the plumage as a behavioral pathology, in which factors of different origin (imprinting, social isolation, etc.) cause behavioral changes that give rise to pathological manifestations quite similar to the common forms of self-injurious. For these reasons, it is proposed to identify the disease's behavior under the term of Self-Damaging Neuroses.

7..3 General conclusion

The relationship between emotional stress and self-injury is also seen in captive non-human primates. Self-injurious rhesus macaques display greater emotional responsiveness than their not injurious counterparts do (Novak, 2003), and the stress of relocation to novel housing produced long-lasting increases in self-biting behavior in these animals (Davenport et al., 2008). These considerations lead us to suggest new approaches to this behavior pathology in the future.

Leaving aside the possible involvement of organic nature phenomena, for which there is not objectively possible to impute a partnership in observable behavioral modification, it is possible to ascertain that there are different environmental factors that can predispose animals to the onset of self-injurious behaviors. In particular, a scientific work has shown that foraging plays a decisive role in the prevention of these behaviors and that environmental enrichment in the home environment may have a positive role (Lumeij, 2008) in preventing the onset of FDB.

Being the FDB a distinctly pathological condition related to domesticated parrots, it is quite likely that defective environmental stimuli, aberrant imprinting and lack of species-specific stimuli could lead to a higher incidence in the onset of the disease. In fact, it is possible to ascertain that the onset of this condition is particularly high in artificially reared parrots, or those subtracted from the biological parents and hand fed by breeders.

These individuals develop all probability forms of imprinting in childhood to the human species, with the result of developing high motivations for certain behaviors that occur in adulthood with zero or

low frequencies. For example, an unsatisfied sexual behavior may be the main cause of the widespread of self-destructive phenomena, on the other hand it is hypothesized hormonal control because this behavior pathological form usually occurs after sexual maturity (Wedel, 1999), but also the frustration felt by the animals for the lack of development of social relationships within the family unit, which is predominantly human and can not respond completely to the ethological needs evolved from species belonging.

At the moment there are still no reliable data on effective measures to solve this disease. Injuries that cause the animals seem to resist any drug treatment and behaviors that cause does not seem to be definitively eliminated with the use of psychotropic drugs. Leaving aside the preventive precautions, such as environmental enrichment and care that can be implemented during the relationship with the domesticated animal, there are currently no therapeutic protocols experimentally demonstrated and actual therapeutic success.

Store parrots affected in better condition, such as inclusion in a social group and / or provide them with a sexual partner, may be interventions that improve the overall status of the individual, even if this may not be decisive in cases of aberrant imprinting or severe cases of self-mutilation: it is known as a modification of behavior in childhood could lead to behavioral tendencies and attitudes individually constant throughout the life of the individual.

It might be useful in the future to study possible surgical and / or pharmacological feasible to physiologically decrease the sexual disposition and, consequently, to appease the sense of frustration that may result from dissatisfaction behavior in captivity. For example, it may be interesting to determine how the sexual sphere can participate in the onset of the disease: in this sense a temporary castration of those affected could be a feasible method to verify this partnership, as has already been tried by Mans et al. (2014), to resolve disorders of reproduction in birds, parrots in particular.

It is essential in order to conduct a study of the phenomenon also in-depth analysis of the role of certain chemical mediators, such as hormones, as it already been tried (Owen et al., 2006), to determine which physiological processes can be a contributory cause.

Another particularly interesting aspect might be the study of the nature of these behavioral disorders, since the expression and modulation of these suggested mechanisms similar to those known to the human species. Bordnick et al. (1994), overlap to FDB the psychological mechanisms that generate the trichotillomania in humans, highlighting how serious conflicts can generate self-defeating and self-punishing needs. On the other hand other authors (Garner, 2003) investigate the prospect of an overlap with serious human diseases such as autism and schizophrenia.

In other fields of research, some authors depart from the assumption that a pathological behaviors regarding excessive grooming activity in humans (and more generally in all those activities related to exacerbated attention to specific areas of the body), can be explained through the use of animal models, and that these may suggest a lot of information about the treatments, however, and

pointing out that any obsessive behavior towards one's own body is derived from distorted grooming rituals (Feusner et al., 2009).

An interdisciplinary inquiry in this regard can not only increase understanding of the mechanisms underlying the FDB, but can open up new frontiers in understanding the biological nature of behavioral disorders in humans.

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APPENDIX

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Feather picking in pet parrots: sensitive species, risk factor and ethological evidence

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PAPER

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Feather picking in pet parrots: sensitive species, risk factor and ethological evidence

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ABSTRACT

The goals of this study were to estimate the prevalence of feather picking (FP) in Italian pet parrots and evaluate the risk factors and possible ethological correlation. A web survey was created and addressed to owners of all species of companion parrots, distributed through on line parrots association websites, social networks and by mail. The survey was available for compilation from June to October 2014. In 292 compiled surveys, 20 different parrots genera were indicated; *Psittacus* spp. (24.3%), *Agapornis* spp. (19.5%), *Nymphicus hollandicus* (18.0%) and *Amazona* spp. (9.0%) were the most represented species. Our study showed a FP prevalence of 17.5%, with the highest prevalence in *Agapornis* spp. (26.3%) and *Psittacus* spp. (22.5%). Living with other parrots was positively associated to FP ($p < 0.05$), such as being fed in neonatal age and weaned by the breeder and not by the final owner ($p < 0.001$). Moreover, 2.9% of non-FP parrots and 10.0% of FP parrots lived always caged ($p = 0.06$). A significant difference in the feather picking site was detected ($p < 0.001$). The most affected body region was the chest (58.8%), followed by the rump (41.2%) and under wing regions (25.4%). At the same time, we conducted another study to better understand some information from the hand-raised parrots through a comparison with the parent-raised parrots. We recorded 1488 parent-raised parrots, and only 1.3% were affected by feather loss. This finding confirm that FP is be considered a multi-factorial behavioral pathology in which factors of different origin may cause behavioral disorders associated to self-injuries.

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Introduction

Feather picking (FP), also referred to as feather damaging behavior or feather plucking, is a behavioral disorder that is frequently encountered in captive parrots (van Zeeland et al. 2009). FP includes plucking, chewing, fraying and/or biting, and it results in the loss of or damage to feathers (van Zeeland et al. 2013). Many authors consider it as stereotypic behavior or an obsessive compulsive disorder (Jenkins 2001), and has been observed in a variety of captive species with differing levels of prevalence and severity (Chitty 2003, 2005; Seibert 2006). Grindlinger (1991) estimated that approximately 10% of the captive parrot population suffered from FP, but other works have reported contradictory data. McDonald Kinkaid et al. (2013), in a sample of 538 parrots, found a prevalence of 15.8%. As claimed by Rubinstein and Lightfoot (2012), the problem about understanding FP mechanisms is related to the relative scarcity of controlled studies on the underlying causes of feather loss in pet avian species and the paucity of current veterinary medical knowledge regarding feather loss and feather destructive behavior. In fact, despite the numerous studies that have been conducted, there is not yet agreement among the scientific community on what the factors are that determine the appearance of this manifestation of discomfort and what the mechanisms are that modulate its expression. Lumeij and Hommers (2008) considered that FP may be the result of redirected foraging behavior. In his studies, the author showed a decrease in FP when the environment was enriched and foraging activity was promoted. However, the removal of feather is regarded as self-mutilation. In fact, one of the criteria that have allowed researchers to make a distinction between FP and other diseases, is the presence of feathers in good condition in areas of the body not directly reachable by the birds (Galvin 1983;

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Harrison et al. 1986; Westerhof & Lumeij 1987). Nevertheless, in many cases, there is also a loss of plumage in areas that cannot be reached by the birds, which has uncertainly been attributed to clinical causes. It is widely thought that FP is usually self-inflicted, but, when birds are housed in groups, in some cases it can be directed to cage mates or nestlings. In these circumstances also the head and face are affected (Wedel 1999; Fox & Millam 2004; Lightfoot & Nacewicz 2006) and this behaviors appear more similar to hair pulling, whiskers eating and barbering observed in mice (Garner et al. 2008).

The aim of this study was to estimate the prevalence of FP self-mutilation expression in the Italian population of pet parrots, but also of determining the most sensitive species and evaluating the risk factors. Another objective was to compare the prevalence of FP in pet parrots with the prevalence of FP in parent raised type parrots held in captivity, which had not been hand-raised and which lived in couples with the possibility of reproducing. We used a web questionnaire as data-gathering tool, in order to collect data from a wide study population across Italy.

Materials and methods

A web questionnaire, addressed to the owners of all species of pet parrots, was distributed throughout Italy through on-line parrot association sites, social networks and e-mails. The web questionnaire was drawn up through a Google Drive application. This questionnaire was on line from June 2014 to October 2014 and a total of 31 questions were created. The title of the questionnaire was 'Questionnaire for companion parrots owners' and the participants discovered that there was a feather picking part only during the compilation. The questionnaire was divided into two parts: one addressed to all parrot owners and the second to the owners of feather plucking parrots. The first part of the questionnaire was divided into two section. First sections was on general information (name of owner, contact details, parrot species, age, etc.), while the second section was dedicated to information about the history and management of the parrot. The second part of the questionnaire, on the feather picking information, featured appropriate questions about feather picking parrots, such as behavior, stereotypic behavior, feather picking regions, etc. All the owners who filled in the questionnaires about their feather plucking parrots were followed by the owners' clinician veterinary who had diagnosed the problem and excluded other possible pathologies. In order to confirm their diagnoses, all the veterinarians were contacted to obtain verbal confirmation of the data. The questions were based on hypothetic risk factors for feather picking and were formulated on data and hypotheses published on this subject. Any incomplete questionnaires (e.g. the absence of the owner's name or contact details, the species of the parrot, etc.) were excluded from the analysis. The questionnaire contained closed questions. The owners, after choosing among the possible closed answers, could provide a descriptions about its observation and this data were analyzed for evaluated objectively the answers. The questions about behavior observation were only in FP section and were created for to be simples, not interpreted by owners and directly linked to data that we wanted to get. The choice of terms and words to describe the abnormal behavior were based on the description of the movements that abnormal behavior show, on the basis of the descriptions currently available in the literature. The question about aggressiveness were created in according to actually knowledge on aggressiveness in companion parrots. The possible choices were linked to only aggressiveness type: dominance aggressions over the owner (specifying to which human family member) and dominant behaviour towards conspecifics (Schmid et al. 2006), that the participants could further describe in 'other' section. Also sexual behavior questions were created with this methods. In Table 1 the overview about the FP questions.

In order to have a control sample and verify any differences between hand-raised and parent-raised parrots (those kept in pairs and not hand-reared), a second questionnaire, addressed to parent-raised type parrot breeders, was created. The aim was to have a comparison parrot group of birds that had not been hand-reared and which were living in more suitable ethological conditions (they were living with a conspecific partner and had the opportunity to reproduce). Nine questions were asked about the number of parrots present on the farm, their species, and on the number of animals showing signs of feather loss.

All the data acquired from the questionnaires were registered in a database and analyzed. Genera prevalence, feather picking region prevalence, and any associations between the feather picking behavior and categorical risk factors were evaluated using the chi-square test, and were considered to be significant when $p < 0.05$. A statistical trend was considered for p values below 10%. The data were processed through the use of SAS® (Statistical Analysis Software). The results were presented with their p value and a 95% confidence interval (C.I.).

Table 1. Overview of 31 questions of questionnaire for pet companion parrots.

First part – addressed to all pet parrots

General data
Name, address, email address and phone

Parrots curriculum
Age, sex, ringed, feather picking or not

Social life
Hand-rearing method
Weaning with other chicks or not
Live with other parrots
Hand-rearing history

Management
Management method (lives caged when owners absent; lives always free; lives always caged, live always in home or live too in garden)
Placement of the cage (indoor; outdoor; indoor in winter and outdoor in summer; parrot management climate depending)
Perches materials (plastic; wood; natural branches; metal and other)
Use of environment enrichments (parrot's toys, natural items or either; nothing)
Possibility of washing (every day; at least two time/week)

Diet
Diet (mainly dry seed, mainly fresh feed or either in equal parts)
Eat extruded feed (always; many times in weeks; don't administered; it don't eat them)
Eat human food (always; sometimes in week; rarely and never)

Second part – addressed to feather picking pet parrots

Knowledge
What is the feather picking for participants (virus disease; bacterial disease; fungi disease; abnormal behavior; don't now)

Beginning of feather picking
Events linked FP before feather loss (owner change; other animals attack; change in number of human family; added or disappeared other parrots; no events; parrots adopted FP already)
Feather loss (owners seen feather traction; owners seen body's regions without feather; owners seen increase in time of preening; increase aggressiveness; other)

Signal of feather picking: presence and evolution
Body's areas (chest; rump; wings; tail; under wing regions; paws; head)
Feather picking evolution (start to biting and damaging feather; immediately start to traction feather; immediately compared body's regions naked; increase aggressiveness behavior it-self, such as peck it wings or other parts)

Sexual behavior
Sexual behavior showed (It is displayed on perches or objects in the birds' surroundings or parts of human body; in owners presence parrot regurgitates; owners don't seen sexual behaviors; other)

Aggressiveness
Presence of aggressiveness (bites owners hands; the parrots assumes an attack posture and tries to come in against to bite; don't presence of aggressiveness; other)
Victim of aggression (everybody; only owners; toward everybody except owners; toward only person; other)

Self-injuries behavior
Presence of self-injuries behavior (slaps it-self; bite it wings; it traction feather in nervous expression when the owners or any human approaches; bite it feet; other)

Results

General data returned

A total of 335 surveys was obtained, of which 292 (87.2%) were useful for the statistical analysis and all regions of the country were represented. The acquired data referred to 20 different genera of parrots (Table 2) kept as pets distributed throughout all the regions in Italy, genera representing less than 5% prevalence were not presented in the table. The most popular species kept as pets are *Psittacus erithacus* (24.3%), *Agapornis* spp. (19.5%), *Nymphicus hollandicus* (18.0%) and *Amazona* spp. (9.0%). In the sample, 40.2% of the parrots were female and 59.7% male.

Table 2. Parrot genera represented in our studied population ($n = 292$ parrots) and percentage of feather picking parrots in each genus.

Genus	Prevalence of population, %	Feather picking, %	(95% CI)
Psittacus spp.	24.3	22.5	12.8–32.2
Agapornis spp.	19.5	26.3	14.9–37.7
Nymphicus spp.	18.0	7.6	0.4–14.7
Amazona spp.	9.0	15.4	1.5–29.2
Ara spp.	6.5	15.8	0.0–32.2
Other genera[a]	22.7	–	–
p	–	0.09	–

[a]Other genera each represented below 5% prevalence: Aratinga, Cacatua, Cyanoramphus, Diopsittaca, Eclectus, Eolophus, Eos, Melopsittacus, Myiopsitta, Nandayus, Pionus, Psephotus, Psittacula, Poicephalus, Trichoglossus.

In regards to breeding, 80.2% of the parrots have been hand-reared; in particular, 41.8% were fed at the neonatal stage, by the breeder and weaned by the buyer; 38.4% were fed at the neonatal stage and weaned by the breeder and was then sold when weaning had been completed (Table 3). It emerged that 84.3% of the parrots were caged when the owners were absent and left free when they were present and that 66.4% of the pet parrots were left free from 1 to 6 hours/day; while lives always caged 4.1%. Most of the roosts were made of wood (43.1%), 22.7% were natural branches and 21.6% plastic perches. It was also found that 90.4% of the parrots were able to wash their plumage every week, because the owners nebulized their bodies or put special bowls with water into their cages or near their roosts. As far as eating habits are concerned, 40.4% of the birds eat dry seeds and fresh vegetables in equal amounts; 23.5% eat more dry seeds (4 times a week) than vegetables. As for extruded feed consumption, 37.9% of the birds were not fed this type of feed, because the owner does not provide it or the parrots do not like it. Finally, 71.2% of the birds were occasionally or regularly fed human food (e.g. cookies, bread, yogurt, meat, etc.) (Table 3).

Feather picking general data, prevalence and symptoms

This study has shown a FP prevalence rate of 17.5% (Table 3). A statistical trend showed that FP has been found to mostly affect males (70% of the FP parrots, $p = 0.10$) (Table 3). It has emerged that 52.9% of the owners stated that the birds had not suffered from

Table 3. Variables investigated in the sampled Italian pet parrots ($n = 292$) population and in Italian feather picking parrots ($n = 51$) of the sample.

Variable	% Prevalence (95% CI)
First part ($n = 292$) – addressed to all pet parrots	
Social Life	
Lives with others parrots	46.2 (40.5–51.9)
Lives alone	52.74 (47.0–58.5)
Grew up alone	21.6 (16.9–26.3)
Grew up with other chicks	71.0 (47.4–58.9)
Hand-rearing and weaned from breeders	38.4 (32.8–43.9)
Hand-rearing from breeders and weaned from owners	41.78 (36.1–47.4)
Management	
• Lives caged when owners absent	84.3 (78.6–87.2)
• Lives always free	11.7 (8.0–15.3)
• Lives always caged	4.0 (1.9–6.4)
• Lives always in owners home	83.0 (78.6–87.2)
• Lives outdoor	4.8 (2.3–7.2)
Cage enrichments	
Materials of roosts:	
• Wood	43.1 (37.4–48.8)
• Plastic	21.6 (18.9–27.0)
• Natural branches	22.7 (18.0–27.4)
• Other materials	11.3 (7.7–15.0)
Other information:	
• Presence of toys and natural object	89.7 (86.2–93.2)
• Possibility of bathing water	90.4 (87.0–93.7)
Environment	
• Lives in home and outdoor	83.0 (78.6–87.2)
• Lives always in closed	25.3 (20.3–30.3)
Diet	
• Eat human food	71.9 (66.7–77.0)
• Eat dry seed and vegetables in equal quantities	40.4 (34.8–46.0)
• Eat extruded feed	60.6 (55.0–66.22)
Second part ($n = 51$) – addressed to feather picking pet parrots	
Feather picking	
• Prevalence[a]	17.5 (13.1–21.8)
• Male	70.0 (55.8–84.2)
• Female	30.0 (15.8–44.2)
• No episode linked feather picking	52.9 (39.2–66.6)
• Removal of plumage without feather damage	33.3 (20.4–46.3)
• Sexual behaviour	45.1 (31.4–58.7)
• Aggressive behaviour	60.7 (47.4–74.2)
• Self-injury behaviour showed	39.2 (25.8–52.6)
• Owners claim that feather picking is linked to behavioral disorder	98.0 (94.2–100.0)

[a]Calculated on the whole sampled Italian pet parrots population ($n = 292$).

episodes that could have caused FP, such as trauma, fear or changes in family. A significant difference in the feather picking site was detected ($p < 0.001$). The most affected body region was the chest (58.8%), followed by the rump (41.2%) and under wing regions (25.5%) (Table 5). It has emerged that 98.0% of the FP parrot's owners consider feather picking to be a behavioural disorder. This study has not shown any link between type of diet and abnormal behavior.

Sensible species

A statistical trend ($p = 0.09$) was observed in differences among FP prevalence in parrot's genera, the highest prevalence was reported for Agapornis spp. (26.3% of the FP) and Psittacus spp. (22.5%) (Table 2).

Table 4. Risk factors associated with the feather picking behavior in the sampled Italian pet parrots population ($n = 292$).

Risk factor	Healthy pet parrots, % ($n = 241$)	Feather picking pet parrots, % ($n = 51$)	p
Living with other parrots	42.7	62.7	<0.01
Hand-rearing and weaned from breeders	42.3	82.0	<0.001
Hand-rearing from breeders and weaned from owners	57.7	18.2	<0.001
Lives always caged	2.9	10.0	0.06

Table 5. Areas of the body that are mainly pecked or plucked in feather picking pet parrots ($n = 51$).

Regions	Prevalence, %	CI
Chest	58.8 a	(45.3–72.3)
Rump	41.2 ac	(27.7–54.7)
Under wing regions	25.4 bc	(13.5–37.4)
Wings	21.6 bcd	(10.3–32.9)
Feet	21.6 bcd	(10.3–32.9)
Tail	19.6 bd	(8.7–30.5)
Head	7.8 d	(0.5–15.2)
p	<0.001	–

a–d: $p < 0.05$.

Imprinting, neonatal age and grow-up

The 82.0% of FP parrots and 42.3% of non-FP parrot ($p < 0.001$) were fed and weaned by the breeder after birth and were then sold after weaning had been completed. By contrast the 18.2% of FP parrots and 57.7% of non-FP parrot ($p < 0.001$) were fed after birth by the breeder and weaned by the owner (Table 4).

Age

The average age in all FP parrots were 82 months; in particular, in sensible species, were *Agapornis* spp. 60.5 months, in *Psittacus* spp. 75.4 months and in *Amazona* spp. 78 months. In one case the age was not known (*Agapornis roseicollis*); all other cases were sexual mature.

Aggressiveness, abnormal and sexual behavior

In regard of abnormal behavior, 64.6% of the parrot owners claimed they had seen their parrots pulling at their feathers; 51.0% of the owners observed abnormal behavior before the birds attempted to remove their feathers (e.g. feather chewing and biting, increase aggressive behavior and self-injuries), but others reported a sudden appearance of feather removal behavior (48.9%). Of the feather picking parrots, 50.0%

showed behavior stereotypes and most sexual behavior disorders observed was feed regurgitation in front of the owner (30.5%); aggressiveness was observed in 60.7% of case. Other self-injuring behavior (such as biting their legs, hitting their face, etc.) was observed in 39.2% of the feather picking parrots. In general, FP frequency has been shown to vary according to the parrot species.

Environments and human relationship

The 62.7% of FP parrots and 42.7% of non-FP parrot ($p < 0.01$) live with another parrot (Table 4). Moreover, 2.9% of non-FP parrots and 10.0% of FP parrots lived always caged (Table 4), and this difference show a statistic trend ($p = 0.06$).

Parent-raised type parrots data

At the same time, we conducted another study to better understand some information from the companion parrots through a comparison with the parent-raised type parrots (genera: *Nymphicus*, *Agapornis* spp., *Amazona* spp., *Ara* spp., *Psittacus* spp.). With the help of the second questionnaire, 18 parrot breeders were found with 1488 parrots in the most suitable ethological conditions (not hand-reared, not alone and with the possibility of reproduction). Of these 1488 parrots, only 19 birds showed feather loss (1.3% of the parent-raised type population).

Discussion

The use of web questionnaires to collect data in surveys can be a limit since they suffer response bias (Dohoo et al. 2003). However, our questionnaire attained a good response rate, enabling a valid data collection from a wide study population.

Given the nature of the conduct underlying the FP brought to light by the present data, it is possible to consider the damage and the removal of the plumage as a behavioral pathology, in which factors of different origin (aberrant imprinting, hand-rearing techniques, environment isolation, sexual frustration) can cause behavioral changes that give rise to the manifestation of quite similar abnormal behavior to that of the common forms of self-injury. On the other hand, the relationship between emotional stress and self-injury is also seen in captive non-human primates and Bordnick et al. (1994) compared feather-picking behavior in parrots to compulsive and impulsive human disorders, such as human trichotillomania. In other animals it has been observed that self-injuring rhesus macaques display greater emotional responsiveness than their non-injurious counterparts do (Novak 2003), and the stress of relocation to novel housing has been shown to produce long-lasting increases in self-biting behavior in these animals (Davenport et al. 2008).

Feather picking general data and sensible species

FP is a behavioral disorder that is frequently encountered in captive parrots (van Zeeland et al. 2009). Many authors have shown relatively low FP rates (10% Grindlinger 1991; 13% Gaskins & Bergman 2011), but the present results are similar to those found by McDonald Kinkaid et al. (2013) who determined a FP prevalence rate of 15.8%. The present survey suggests that the prevalence of FP in Italian parrots is 17.5%. This prevalence was much higher than in parrots bred in more ethologically suitable conditions (1.3%), i.e. those bred from biological parents, were living in a couple and without direct human contact. Such a finding could suggest that the hand-rearing of parrots may be a risk factor for the development of FP, together with other living conditions of pet parrots.

The present results, in agreement with these authors, show FP in 22.5% of *Psittacus* spp; in the sample of *Cacatua* genus the prevalence of FP was high (40%), but the sample was small (2% of the parrot population; data not shown in the table). Our results show that the *Agapornis* genus is also sensitive to FP (26.3% prevalence). *Agapornis* spp. has become a very popular pet parrot in Italy in the last few years. This data suggest that the species may be a risk factor for FP onset.

Post natal period and sexual behaviour

The mean age of the FP parrots examined in our study was 82 months; this observation is supported by van Hoek & Ten Cate (1998), who reported that most of the birds showed significant behavioral distortions upon reaching sexual maturity. Many authors agree that FP is more common in African grey parrots (*Psittacus* spp.) and in the *Cacatua* genus (Chitty 2003; Rosenthal et al. 2004; Garner et al. 2008; van Zeeland et al. 2009, 2013).

This difference between FP parrots and non-FP parrots could be determined by the imprinting process that evolved during the hand-rearing period. In particular, the sexual imprinting (which develops later than parental imprinting) may play a role in the onset of FP. Sexual imprinting refers to the process by which animals learn the characteristics of appropriate mates by learning the characteristics of their parents or

siblings (Fox 2006). FP often develops after the onset of sexual maturity (Wedel 1999) and the data recorded in this study confirmed this thesis. In fact, on the average, the age of the two sensible species was 60.5 months in *Agapornis* spp. and 75.4 months in *Psittacus*. Sexual maturity is reached earlier in budgerigars, at 5 months (Kavanau 1987), while many of the large-bodied species of cockatoos, do not reach sexual maturity until approximately four to five years of age (Forshaw 1981). These data could be also confirmed from the observation of the behavior of the birds by the owners: those who had observed sexual behavior directed toward humans claim that the most frequent habit was food regurgitation (30.5%). In fact, food regurgitation is typical sexual behavior in parrot couples (courtship feeding or allofeeding, Spoon 2006) and this fact leads to the hypothesis that FP parrots show redirected sexual behavior due to a lack of partner, in according to Lantermann (1989) that claim that this behaviour is the expression of the parrots' sexual frustration. This hypothesis is based on the fact that parrots probably develop sexual imprinting toward humans during the hand-rearing or weaning period. According to Fox (2006), these results confirm that an abnormal sexual imprinting and a strong social preference for humans may cause behavioral problems in hand-raised parrots, which are probably more likely to inappropriately direct sexual behavior toward their owners and the presence of other parrots don't change this preference. In addition, our results showed that the parrots that are more in contact with humans showed higher prevalence of FP than the parrots that live always in cages. In addition, it seems that FP mostly affects males (70% of the FP parrots). Jayson et al. (2014) considered that the sex of the bird was a significant factor in the occurrence of FP. Fox (2006) suggested that hand-rearing apparently influences sexual imprinting in males more strongly than in females.

Although popular literature suggests that hand-raised parrots make better pets than parent-raised parrots (Blanchard 1999), hand-rearing has the potential of producing physical as well as behavioral problems in parrots (Harcourt-Brown 2004). Different methods of breeding can affect the onset of behavioral problems, such as FP, to a great extent (Lightfoot 2002). This indicates that various hand-rearing techniques could influence the prevalence of FP and that is reduced if the sexual imprinting is directed to the final owner (this is possible if the final owner is responsible of the last phase of hand-rearing). In particular, it is probably the imprinting mechanism that causes the bird to deviate incorrectly. In the course of the classical sensitive phase in early development of zebra finches, the birds establish a social bond with their parents. Under normal circumstances, this narrowing of social preference to the parent species guides young male zebra finches in their first courtship attempts when they are sexually mature (Oetting et al. 1995).

Environment and human relationship

As already observed by Jen-Lung Peng et al. (2014), inadequate socialization may be a factor of feather picking in some birds. They claim that using conspecific rather than human exposure do not have effect on FP reduction. Our results showed that the presence of other parrots in the same environment was more high in FP parrots (62.7%), compared to non-FP parrots (42.7%). Preiss and Frack (1974) and van Zeeland et al. (2009), claimed that these abnormalities in behavior resulted from the isolation of hand-raised birds during the fledgling period, which fail to become integrated into a conspecific social group later on. If parrots construct their sexual imprinting to humans (in absence of other parrots during the weaning period) they can develop an adaptation to human that does not find the same stimulatory satisfaction.

Conclusions

This study has shown that there is a significant prevalence of FP in Italian pet parrots. Many species are sensitive to this abnormal behavior and, in particular, the most common genus sensitive to FP is *Agapornis*. The collected data suggest that FP can be caused by the imprinting process during the fledgling period and we could hypothesize that sexual frustration plays an important role in developing FP. For these reasons, it is proposed that FP should be termed Parrot's Self-Damaging Neuroses. As FP is a distinctly pathological condition related to captive-kept parrots, as shown by our study, it is quite likely that defective environmental stimuli, aberrant imprinting and a lack of sexual satisfaction and reproduction could lead to a higher incidence of the onset of the disease. In fact, it is possible to ascertain that the onset of this condition is particularly high in hand-raised birds, namely those subtracted from the biological parents and hand fed by breeders, while in the parent-raised type is almost absent. Further studies should be performed to further analyze the present results. Further studies could help in clarifying the complex of FP behavior. The research of physiological parameters is necessary for assess many thesis published from authors in the last years. For our study and the hypothesis of the sexual

behavior role, e.g. could be worthwhile study the consequence and/or influence on stress condition.

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An association between feather damaging behavior and corticosterone metabolite excretion in captive African grey parrots (*Psittacus erithacus*)

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ABSTRACT

Background. African grey parrots (*Psittacus erithacus*) are kept as pets and are frequently hand-reared. It has been observed that hand-reared African grey parrots may develop behavioral disorders such as feather damaging behavior (FDB). It is well known that chronic stress is involved in behavioral disorders in captive parrots. The main glucocorticoid in birds is corticosterone; its quantification provides information about adrenocortical activity and is considered to be a reliable indicator of stress levels in birds. We analyzed the differences in the excretion of corticosterone metabolites (CM) in the droppings of African grey parrots characterized by: 1. different rearing histories (parent rearing vs. hand rearing); and 2. the presence or absence of FDB in hand-reared parrots.

Methods. A total of 82 African grey parrots that were kept in captivity were considered. According to breeding methods, three groups of birds were defined: 1. The parent-reared (PR) parrots included birds kept in pairs ($n = 30$ pairs) with a conspecific partner of the opposite sex. All of these birds were healthy and never showed FDB signs; 2. The healthy hand-reared parrots (H-HR) included pet parrots individually kept, that were hand-reared and did not display any sign of FDB ($n = 11$, 7 males and 4 females); 3. The FDB hand-reared parrot (FDB-HR) included pet parrots individually kept, that were hand-reared and displayed FDB ($n = 11$, 7 males and 4 females). Droppings were collected in the morning over three alternating days in autumn 2014 and spring 2015. The CM were determined using a multi-species corticosterone enzyme immunoassay kit. Split-plot repeated-measure ANOVA was used to examine any differences using group, season and group × season as the main factors.

Results. Different quantities of CM in droppings were found for the three groups. The mean CM value was 587 ng/g in the PR parrots, 494 ng/g in the H-HR parrots and 1,744 ng/g in the FDB-HR parrots, irrespective of the season. The excretion of CM in FDB-HR was significantly higher than in PR or H-HR parrots. CM in droppings were not influenced by the season (autumn vs. spring); furthermore, the interaction between group and sampling season was not significant. Limited to the H-HR and FDB-HR groups, a trend in the significance of the difference in the mean CM excreted by male and female birds was observed, with the levels excreted by males being higher than

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those excreted by females. When the effect of age was considered (in the two separate groups), there was a statistically significant positive correlation only for H-HR. **Conclusions**. The highest amount of CM excretion was found in FDB-HR parrots, and a positive correlation between age and CM excretion was found in H-HR. Given that the CM excretion of both PR and H-HR parrots was similar in our study, future research is recommended to investigate the specific aspects of hand-rearing to improve parrot welfare.

Subjects Animal Behavior, Veterinary Medicine
Keywords African grey parrot, Stress, Corticosterone, Feather picking, Feather plucking

INTRODUCTION

African grey parrots (*Psittacus erithacus*) are kept as pets in private households because of their sociability and also for their ability to imitate human speech.

African grey parrots may be hand-reared, and this practice has been increasingly carried out over the last 30 years. Based on the hand-rearing method used, hand-reared parrots can be divided into different groups according to the incubation system (natural vs. artificial) and the age of removal from the nest (at hatch, less than approximately five weeks or more than approximately five weeks) (*Schmid, Doherr & Steiger, 2006*). In contrast to parent-reared parrots, which imprint toward conspecifics (*Glendell, 2003*), hand-reared parrots imprint on humans and seem to be socially dependent on them. The exact consequences of the different hand-rearing methods on the development of behavior in adult birds are still not clear. However, it has been observed that hand-reared grey parrots may develop behavioral disorders, such as aggressiveness, feather picking, stereotypies or abnormal sexual behaviors, and thus it is expected that they are prone to develop such behavioral disorders (*Schmid, Doherr & Steiger, 2006*). Moreover, is has been observed that hand-reared chicks that were less than 5 weeks old when removed from the nest developed stereotypies more often than chicks that stayed longer with their parents (*Schmid, Doherr & Steiger, 2006*).

Feather damaging behavior (FDB) 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*). FDB in parrots is usually self-inflicted and generally includes all mutilation of the feathers accessible to the bird's beak (*Harrison, 1986*). *Grindlinger (1991)* estimated that approximately 10% of the captive bird population suffered from FDB. *Kinkaid et al. (2013)*, in a sample of 538 parrots, found an FDB prevalence of 15.8%. Our group previously conducted a study considering this classification, which showed a notable difference in the FDB prevalence in the two different populations of parrots. The parent-raised population ($n = 1{,}488$) showed an FDB prevalence of 1.3%, while the pet parrot population ($n = 292$) showed an FDB prevalence of 17.5% (*Costa et al., 2016*). FDB has rarely been observed in the wild and usually occurs in captive birds when they reach sexual maturity (*Wedel, 1999*), even though some authors have reported the onset of FDB prior to the occurrence of sexual maturity (*Jayson, Williams & Wood, 2014*). FDB occurs in many species of parrots, and it has been observed in African grey parrots (*Psittacus erithacus*) and

cockatoos (*Cacatua* spp.) (*Clubb et al., 2007*; *Jayson, Williams & Wood, 2014*; *Peng et al., 2014*), *Amazona* spp. parrots (*Garner et al., 2006*), *Ara* spp. and *Agapornis* spp. (*Costa et al., 2016*), crimson-bellied conures (*Pyrrhura perlata*, *Van Hoek & Ten Cate, 1998*) and other psittacine species. It has been suggested that FDB could be a coping strategy for negative affective states (e.g., stress and boredom) and/or living in a suboptimal environment (*Rosskopf Jr & Woerpel, 1996*; *Levine & Practice, 2003*). In many cases, these patterns may represent an exaggeration or expansion of normal behavior, resulting from inadequate environmental stimuli and/or early weaning and/or social isolation (*Garner et al., 2006*; *Schmid, Doherr & Steiger, 2006*; *Van Zeeland et al., 2013*).

It is well known that chronic stress is involved in behavioral disorders in captive parrots (*Ferreira et al., 2015*; *Owen & Lane, 2006*). In vertebrates, the front-line hormones for overcoming stressful situations are β-endorphin, glucocorticoids and catecholamines (*Ayala et al., 2012*; *Johnstone, Reina & Lill, 2012*; *Livingston, 2010*; *Möstl, Rettenbacher & Palme, 2005*; *Schmidt et al., 2010*). The main glucocorticoid in birds is corticosterone; its quantification provides information about adrenocortical activity (*Ferreira et al., 2015*) and is considered to be a reliable indicator of stress levels in birds (*Dehnhard et al., 2003*; *Hartup et al., 2004*; *Young & Hallford, 2013*), giving important insight into the welfare status of an individual or a group of animals (*Lane, 2006*), especially when used in conjunction with other parameters such as behavior. The analysis of fecal corticosterone is preferred over blood sampling because is less invasive and can cause fewer stress responses (*Nemeth et al., 2016*) without compromising the welfare assessment (*Hamilton & Weeks Jr, 1985*; *Le Maho et al., 1992*). Several authors have reported a correlation between the concentrations of plasma glucocorticoids and their metabolites in the feces of mammals (*Möstl et al., 1999*; *Palme et al., 1999*; *Stead, Meltzer & Palme, 2000*, *Palme et al., 2005*) or in the droppings of birds (*Dehnhard et al., 2003*).

Owen & Lane (2006) measured corticosterone in the droppings of African grey parrots, and they observed that the corticosterone level in the excreta of FDB parrots was higher than that of healthy pet parrots. However, these authors did not consider the sex and age of the parrots or the season in which the samples were taken. The purpose of the present study was to compare the excretion of corticosterone metabolites (CM) in the droppings of hand-reared (with or without FDB) and in parent-reared African grey parrots (kept in pairs for reproduction) during autumn and spring. For the hand-reared parrots, the influence of sex and age on the amount of corticosterone in droppings was also considered. An increase in CM in hand-reared parrots with FDB was expected. Furthermore, we aimed to determine if healthy hand-reared parrots and parent-reared parrots display similar patterns in CM excretion.

MATERIALS & METHODS

Animal and selection criteria

The study was based on a web questionnaire used in a previous study (*Costa et al., 2016*) that was addressed to the owners of all species of pet parrots. The questionnaire was distributed throughout Italy through online parrot association sites, social networks and e-mails in collaboration with the Italian Psittacine Club (known as the "Club degli Psittacidi"

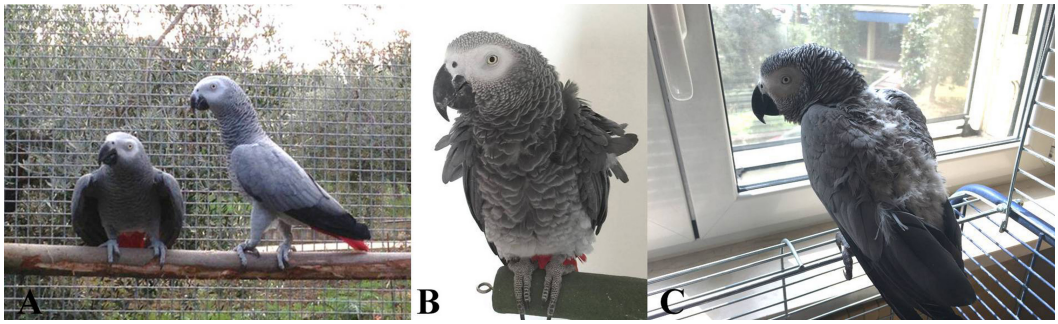

Figure 1 African grey parrots (*Psittacus erithacus*) observed in the present study. (A) A parent-reared pair; (B) healthy hand-reared parrot and (C) hand-reared parrots that display feather damaging behavior.

http://psittacidi.webservice-4u.com/) and the Italian Association of Parrot Breeders (known as the "Associazione Italiana Allevatori Pappagalli," http://www.assopappagalli.it/). In the present study, we only considered African grey parrots because this was the most represented species among the Italian respondents (*Costa et al., 2016*) and because this species is considered to be very sensitive to FDB (*Jayson, Williams & Wood, 2014*; *Schmid, Doherr & Steiger, 2006*).

All birds considered in our study were born in captivity, and no wild-caught birds were used. A total of 82 African grey parrots were considered. 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. Based on the different methodologies of rearing at the neonatal stage, hand-reared and parent-reared parrots were considered. Among the hand-reared parrots, a further distinction was made between parrots displaying FDB and parrots not displaying FDB. According to these criteria, three samples of birds were defined: 1. Parent-reared (PR) parrots; 2. Healthy hand-reared parrots (H-HR); 3. FDB hand-reared parrots (FDB-HR).

1. The parent-reared (PR) parrots (Fig. 1A) included birds kept in pairs ($n = 30$ pairs) with a conspecific partner of the opposite sex, since they were specifically reared for reproduction. These birds were reared by their biological parents, and contact with humans was minimal and related only to their care and daily management. The PR parrots were permanently housed in a standard parrot cage with a minimum volume of 1 m^3 and exposed to natural light variation. All of these birds were healthy and never showed signs of FDB. All of the birds included in this sample were housed in the same facility. We included this sample that we considered a valid control for stress coping since (usually considered well-balanced birds that have learnt all of the specific behavioral patterns of their species). We included this sample since parent-reared captive parrots are usually considered well-balanced birds that have learnt all of the specific behavioral patterns of their species (*Schmid, Doherr & Steiger, 2006*).

2. The healthy hand-reared parrots (H-HR) (Fig. 1B) included pet parrots that were hand-reared. These birds did not display any sign of FDB. This sample was composed of 11 birds (7 males and 4 females). Each bird was individually kept by a owner.

3. The FDB hand-reared parrots (FDB-HR) (Fig. 1C) included pet parrots that were hand-reared. These birds displayed FDB. This sample was composed of 11 birds (7 males and 4 females). Each bird was individually kept by a owner. The diagnosis of FDB was made by a veterinary expert in exotic birds who took into consideration all of the possible differential diagnoses according to *Van Zeeland et al. (2009)*. In this way, it was possible to rule out any clinical problems.

The H-HR were age (±2 years) and sex matched with the FDB-HR. Both H-HR and FDB-HR parrots lived mostly outside a cage without any other parrots and had a close relationship with humans. All of the birds were privately owned and had free access to water and to commercial diets formulated specifically for parrots that were supplemented with fruit and vegetables. The owners of all of the parrots included in the study completed a questionnaire about the care and management of the parrots and, only for FDB-HR parrots, the main body regions affected by FDB.

Droppings sampling and analysis

Droppings were collected throughout autumn 2014 and spring 2015 in the middle of each season. The droppings were collected in the morning (9:00–11:00 AM) for three days on alternating days. This time frame was chosen with the intention to reduce the effect of daily patterns in CM excretion. The samples were collected directly from the cleaned bottom of the bird's habitual cage where the parrot lived. For PR parrots, the dropping samples represent a pool of the excreta from the parrot pairs, whereas the droppings were individually collected for the H-HR and FDB-HR parrots. The 3-day samples were pooled, stored in 50-mL plastic tubes and immediately frozen at −20 °C until analysis. A total of 30, 11 and 11 samples were collected at each sampling time for the PR, H-HR and FDB-HR parrots, respectively.

To extract steroids, we used the methanol-based procedure described by *Palme et al. (2013)* with slight modifications. Briefly, the droppings were lyophilized, weighed, and completely crushed, and two aliquots of the samples (0.25 g each) were placed into extraction tubes, which were then sealed with a Teflon cap and stored at −20 °C. Each aliquot was thoroughly mixed for 30 min using a multivortex with one mL of 80% methanol (Sigma Aldrich, St. Louis, MO, USA). The suspension was then centrifuged at 500 g for 20 min and the supernatant was recovered. An aliquot (0.5 mL) of the supernatant was transferred into a new vial and evaporated at 50 °C for 14 h. After evaporation, the dried extracts were stored at room temperature in dark boxes for 15 days and then kept at −80 °C until they were assayed. One day before the CM analyses, the dried extracts were re-diluted in 0.5 mL of 80% methanol. An aliquot of the extract was diluted to 1:10 in the assay buffer (Arbor Assays®, Ann Arbor, MI, USA). The mixture was then vortexed and left to rest for 5 min twice to ensure complete steroid solubility. The CM were determined using a multi-species corticosterone enzyme immunoassay kit (K014; Arbor Assays®, Ann Arbor, MI, USA). All of the analyses were repeated twice. The inter- and intra-assay coefficients of variation were less than 10% (6% and 8%, respectively). The sensitivity of the assay was 11.2 ng/g droppings. All of the droppings samples were analyzed at multiple dilutions (1:4, 1:8, 1:16 and 1:32), and all regression slopes were parallel to the standard curve ($r^2 = 0.983$).

Figure 2 Deplumation area in feather damaging behavior African grey parrots. (A) Chest area; (B) wings; (C) shoulders and rump.

The mean recovery rate of corticosterone added to dried excreta was 95.8%. 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. The concentration of CM was expressed as ng/g of droppings dry matter.

Data analysis

The CM of the PR, HP and FDB-P parrots were compared. Before testing for group differences, the normality of the data distribution and the homogeneity of variance were assessed using the Shapiro–Wilk test and Levene's test, respectively. Split-plot repeated-measure ANOVA was used to examine any differences using one within-subject variable (season) and one between-subject variable (the three samples of birds) and considering the interaction between these main effects. When the main effect was significant, a Tukey's post hoc test was performed to analyze the differences between groups. To explore the effects of sex and age on CM within the H-HR and FDB-HR groups, a t-test and a correlation analysis (Pearson's r) were performed, respectively. The data are presented as the mean and the pooled standard error of the mean (SEM). Statistical significance was set at 0.05, and a trend of significance was considered at $p < 0.1$. All statistical analyses were performed using SPSS version 15.0 for Windows (SPSS Inc., Chicago, IL, USA).

RESULTS

The average age of the birds was 8.1 ± 1.7, 7.9 ± 5.4, 7.8 ± 5.4 years for PR, H-HR and FDB-HR parrots respectively. The average volume of the aviary cages in which the birds belonging to the PR group were kept was 4.85 m^3. The average volume of the cages of each H-HR and FDB-HR parrot 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 FDB in the FDB-HR birds was the chest (90.9%) (Fig. 2A), and this was followed by the wings (18.2%) (Fig. 2B), the shoulders and the rump (9.1%) (Fig. 2C). No sign of FDB was observed on the head.

Different quantities of CM in droppings were found for the three samples of African grey parrots. The mean CM value was 587 ng/g in the PR parrots, 494 ng/g in the H-HR

Table 1 Corticosterone metabolite (ng/g dry matter) excretion in the droppings of healthy and FDB[a] African grey parrots (*Psittacus erithacus*) (mean and pooled SEM).

Group	Season	Mean	SEM	95% confidence interval	
				Lower bound	Upper bound
PR[b] parrots	Autumn	617	25	558	676
	Spring	558	31	467	649
	Mean	587	20		
H-HR[c] parrots	Autumn	519	58	421	616
	Spring	469	45	318	620
	Mean	494	36		
FDB-HR[d] parrots	Autumn	1,749	55	1,652	1,847
	Spring	1,739	133	1,589	1,890
	Mean	1,744	70		
Main effects:					
Group:		$F = 194.477$	$p < 0.0001$		
Season:		$F = 1.305$	$p = 0.259$		
Interaction (group × season):		$F = 0.191$	$p = 0.826$		
Group contrasts (LSD test):					
PR vs. H-HR parrots:		$p = 0.140$			
PR vs. FDB-HR parrots:		$p < 0.0001$			
HR vs. FDB-HR parrots:		$p < 0.0001$			

Notes.
[a]FDB, feather damaging behavior.
[b]PR, parent-reared.
[c]H-HR, healthy hand-reared.
[d]FDB-HR, feather damaging behavior hand-reared.

parrots and 1,744 ng/g in the FDB-HR parrots, irrespective of the season (Table 1). The excretion of CM in FDB-HR parrots was higher than in PR and H-HR parrots ($p < 0.001$). CM in droppings were not influenced by the season (autumn vs. spring); furthermore, the interaction between parrot groups and the sampling season was not significant (Table 1).

To explore the effect of sex on CM excretion in the H-HR and FDB-HR samples, a *t*-test was performed, considering the mean CM amount (autumn and spring) for each bird, given the non-significance of the within-subject effect (sampling season); moreover, in these samples, a correlation analysis (Pearson's *r*) using the same response variable was conducted to assess the effect of age on CM excretion. The results showed that there was a trend in the difference in the mean CM excreted by male and female birds, with the levels of males being higher than those presented in females: HP, mean of males = 554, mean of females = 388 ($t = 1.851$, $p = 0.097$); FDB-HR, mean of males = 1,852, mean of females = 1,556 ($t = 1.906$, $p = 0.089$). When the effect of age was considered (in the two separate populations), there was a statistically significant positive correlation only for H-HR ($r = 0.609$, $p = 0.047$); in contrast, no correlation was found for FDB-HR ($r = 0.398$, $p = 0.225$).

DISCUSSION

In our study, we observed increased excretion of CM in FDB-HR parrots, which was approximately three times higher than that of PR and H-HR parrots, irrespective of the season of sampling. Moreover, no differences were found in CM excretion between H-HR and PR parrots, the latter of which were kept in pairs for reproduction and so they can maintain sexual and social activity.

Our results confirm the findings of *Owen & Lane (2006)*, which showed higher CM in the droppings of FDB-HR parrots than in control parrots. To the best of our knowledge, the paper of *Owen & Lane (2006)* is the only study comparing the CM excretion in droppings of FDB and non-FDB African grey parrots (261 ng/g and 75 ng/g, respectively). Our results confirm these observations in terms of significant differences between FDB-HR and H-HR parrots, but the magnitude of the values measured in our study was more than six times higher than those observed by *Owen & Lane (2006)*. In the study of *Owen & Lane (2006)*, the control group was composed of ten birds that were kept all together in a large aviary, so they presumably maintained their social and sexual activity. In contrast, in our study, we considered two samples of parrots that did not display FDB: PR and H-HR, which both display similar levels of CM excretion. Parent reared parrots are usually considered well balanced birds since parent rearing methods let them to learn all the specific behavior pattern, which is a great benefit for their welfare (*Schmid, Doherr & Steiger, 2006*). The link between FDB and the corticosterone levels of excreta has also been observed by *Peng et al. (2014)* in two cases of FDB in sulphur-crested cockatoos (*Cacatua galerita*); the authors found a decrease in corticosterone levels after treatments that consisted of socialization, a training program, medication and feeding enrichments. Even though we did not measure the environmental or enrichment management and the activity of parrots included in our study, it has been previously demonstrated that parrots with FDB display higher activity compared to parrots without FDB in a number of behavioral tests, suggesting that FDB is a proactive stress response pattern; under chronic stress conditions, proactive birds seemed to be more prone to develop behavioral disorders (*Van Zeeland et al., 2013*). FDB can lead to, or result from, underlying skin pathologies that itch or irritate (*Garner et al., 2008*). FDB may also cause health problems related to tissue damage, hemorrhage, infection, or hypothermia (*Meehan, Millam & Mench, 2003*; *Van Zeeland et al., 2009*). In the present study, the body area most affected by FDB was the chest, and the head was not affected by FDB. The presence of feathers in good condition in areas of the body that are not directly reachable (i.e., the head) by the birds is one of the criteria that has been used to make a distinction between FDB and other skin or plumage diseases (*Galvin, 1983*; *Harrison, 1986*; *Westerhof & Lumeij, 1987*).

The higher CM excretion in the FDB-HR parrots than in the H-HR and PR parrots, suggest an increase in adrenal cortical activity (*Möstl & Palme, 2002*). The adrenal glands have a key role in the hormonal response to short-term and chronic stress, which result in an increase in glucocorticoid secretion (*Möstl & Palme, 2002*). The measurement of CM in bird droppings has been proposed to assess the welfare status of birds (*Meehan, Garner & Mench, 2004*; *Van Zeeland et al., 2009*; *Cussen & Mench, 2015*; *Young & Hallford,*

2013; Ferreira et al., 2015; Shepherdson, Carlstead & Wielebnowski, 2004), the results of such analyses are hard to interpret because the biological perspective suggests only an increase in adrenocortical activity. For these reasons, the importance of these data could lead to misinterpretation because they are a result of a complex interaction between a wide range of physiological, endocrine and behavioral variables that occur simultaneously (Gaskins & Bergman, 2011; Cussen & Mench, 2015; Van Zeeland et al., 2009).

In our sample of hand-reared parrots, a trend in the significance of the difference in the mean CM excreted between male and female birds was found for both H-HR and FDB-HR parrots, with the levels excreted by males being higher than those excreted by females. In contrast, Ferreira et al. (2015) did not find any gender effect in the CM excretion of blue-fronted parrots (*Amazona aestiva*). Furthermore, a positive correlation between age and CM excretion was found for H-HR parrots. However, these results should be considered with caution in both studies due to the small sample sizes and the different species considered. The demographic features of FDB (i.e., sexual maturation) and gender predisposition (female > male) have been reviewed by Van Zeeland et al. (2009), who state that the literature on this topic is related to field studies of small group of animals and that consequently larger surveys are thus necessary to confirm these results.

FDB is observed mainly in hand-reared parrots, occurring in from 10 to 17.5% of individuals (Grindlinger, 1991; Kinkaid et al., 2013; Costa et al., 2016), while in parent-reared parrots, FDB does not occur or occurs rarely (approximately 1%) (Costa et al., 2016). Hand-rearing has been considered to be a risk factor in the incidence of FDB (Costa et al., 2016; Schmid, Doherr & Steiger, 2006). Furthermore, social isolation and sexual behavior frustration can have important roles in the development of abnormal behavior (Lantermann, 1989; Harrison, 1994; Van Hoek & Ten Cate, 1998; Wedel, 1999; Fox, 2006; Jayson, Williams & Wood, 2014). According to Fox (2006), abnormal sexual imprinting and a strong social preference for humans may cause behavioral problems in pet parrots, which are most likely more prone to inappropriately direct sexual behavior toward their owners. Since both H-HR and FDB-HR were in social and reproductive isolation in our study, this suggests that there is something different about their management that could be linked to environmental enrichment or breeding methods; thus, from an animal welfare perspective, it is fundamental to deeply research the risk factors that are involved in the incidence of FDB.

CONCLUSIONS

In the present study, we analyzed the differences in CM excretion between African grey parrots characterized by: 1. different rearing histories (parent rearing vs. hand rearing); and 2. the presence or absence of FDB in hand-reared parrots.

The highest amount of CM excretion was found in FDB-HR parrots, and a positive correlation between age and CM excretion was found in H-HR.

Given that the CM excretion of both PR and H-HR parrots was similar in our study, future research is recommended to focus on the specific aspects of hand-rearing needed to improve the welfare of parrots.

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The authors declare there are no competing interests.

Author Contributions

- Pierluca Costa conceived and designed the experiments, performed the experiments, wrote the paper, prepared figures and/or tables.
- Elisabetta Macchi conceived and designed the experiments, performed the experiments, wrote the paper.
- Emanuela Valle analyzed the data, wrote the paper, prepared figures and/or tables.
- Michele De Marco prepared figures and/or tables, reviewed drafts of the paper.
- Daniele M. Nucera analyzed the data.
- Laura Gasco contributed reagents/materials/analysis tools, reviewed drafts of the paper.
- Achille Schiavone conceived and designed the experiments, analyzed the data, contributed reagents/materials/analysis tools, wrote the paper, prepared figures and/or tables.

Data Availability

The following information was supplied regarding data availability:

The raw data has been supplied as Data S1.

Supplemental Information

Supplemental information for this article can be found online at http://dx.doi.org/10.7717/peerj.2462#supplemental-information.

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