

**EFFECTS OF LITHIUM CHLORIDE ON FEATHER DAMAGING  
BEHAVIOR IN PARROTS**

An Undergraduate Research Scholars Thesis

by

ELIZABETH CRUSER

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Dr. J. Jill Heatley

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## **ABSTRACT**

### Effects Of Lithium Chloride On Feather Damaging Behavior In Parrots

Elizabeth Cruser  
Department of Biology  
Texas A&M University

Research Advisor: Dr. J. Jill Heatley  
Department of Small Animal Clinical Services  
Texas A&M University

Feather damaging behavior, the intentional removal of feathers without the biological need to do so (i.e. lining a nest, breeding display, preening), is a complex syndrome frequently seen in captive parrots. Feather damaging behavior may be caused by systemic health or psychological problems and can result in self-mutilation, infection, and death. Many cases of feather damaging behavior are never successfully resolved. Lithium has been used to treat depression in humans for over 100 years and may also be a needed dietary micronutrient for avian species. Birds showing damaging behaviors or self-mutilation could have a form of depression or a dietary deficiency. I investigated lithium for potential to reduce the presence of feather damaging behavior in 5 Quaker Parrots who had feather damage. I offered the birds free choice access to a Lithium Chloride solution while tracking lithium intake, feather damage prevalence, electrolytes, body weight, body condition score, and skin cytology. Results include that when bird's intake lithium at an average of 0.054 mg/day when provided a low sodium diet, do not develop detectable plasma lithium levels but do have other plasma electrolyte changes. While lithium supplementation did not apparently affect feather regrowth based on this study, we

found that supplementation also caused no apparent harm to avian health and some skin cytology parameters normalized after supplementation.

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## NOMENCLATURE

FDB	Feather Damaging Behavior
ppm	Parts Per Million
BCS	Body condition score
PCR	Polymerase Chain Reaction
INas	Intranasal
NFG	New feather growth
NCA	Near complete alopecia
PA	Partial alopecia

# CHAPTER I

## INTRODUCTION

Feather preening is a normal social and grooming behavior seen in parrots and other birds, but intense preening can result from chronic stress from their environment and be a form of “excessive self-comforting.”<sup>1</sup> This excessive self-preening is a form of feather damaging behavior (FDB). Plucking and picking are some of the most common types of FDB reported by avian veterinarians and parrot sanctuaries. Potential causes of FDB include social causes such as socialization, inability to preen, bathing, or cage position; behavioral/psychological causes such as separation anxiety; and medical causes such as feather mites or lice.<sup>2,3</sup> FDB “cannot be classed as a single disease” because it is almost never triggered by one single factor, but each factor may contribute to the birds stress level.<sup>3</sup> Since FDB are thought to result from complex behavioral and social issues, many cases are never successfully resolved.<sup>4</sup> Most often, however, treatment is simply not attempted.

FDB can be detrimental to the relationship between the parrot and the owner because it can be frustrating, time consuming, and expensive to treat. Initially, it may be hard for the owner to distinguish between normal and excessive preening.<sup>5</sup> Other times, owners may blame themselves and feel guilty that their bird has begun to feather pluck. Owners may have unrealistic expectations of the treatment options and believe that this is a simple disorder.<sup>1</sup> All of these factors combined can strain the owner pet relationship, which can result in relinquishment of the bird. More research on FDB can help repair the owner-pet relationship and guide owners through treatment of FDB, and hopefully find a cure.

Moreover, it is estimated that the captive parrot population could reach 100 million by the year 2020 in the USA alone. As said in the article “Welfare and Suitability of Parrots as Companion Animals: A Review,” many significant aspects of parrot behavior in the wild are denied in varying degrees when parrots are kept as companions.<sup>6</sup> Many of these aspects were highlighted previously as potential factors that could trigger FDB. As the number of captive parrots increases, it is likely that the number of FDB cases is going to increase, as well, thus highlighting the lack of treatment options and emphasizing the need for further research into the issue.

FDB has been noted to have similarities to the human disorder trichotillomania, the compulsive removal of hair, and other self-mutilation neuroses.<sup>1,7</sup> Through close examination of the two disorders, they have been found to be analogous with similar treatments attempted including physical restraints.<sup>7</sup> Despite this close relationship between FDB and trichotillomania, information regarding treatment options is often limited in avian species.<sup>1</sup> These limitations only emphasize the importance of research on this topic. Non-human animals have been used as models in biomedical research since the “dawn of time.”<sup>8</sup> Despite the use of animals in medical research being controversial, the use of FDB in avian species as a non-human model for trichotillomania research allows for advances for both humans and avian species.<sup>7,8</sup> If one model of research on type of neurosis begins to make more rapid progress than the other, it is possible that the developments made could be related to and evaluated within the other model.<sup>8</sup>

Our interest in lithium as a treatment option was developed from two concepts: 1) evidence of lithium’s ability to enhance mood<sup>9</sup> and 2) preference of free-living birds to eat soil with significantly higher amounts of lithium.<sup>10</sup> Lithium, in the form of inorganic lithium, has been used to treat depression in humans for over 100 years.<sup>9</sup> Lithium enhances the transport of



folate and vitamin B<sub>12</sub>, both of which have the ability to effect mood.<sup>9</sup> Birds that have the neurotic feather plucking behavior could have a form of depression and therefore we believe treatment with lithium has potential to reduce the presence of feather damaging behavior.<sup>1</sup>

Lithium certainly seems to play an important role in brain health. From 1970-1990, low-lithium levels in rats and goats were studied. These animals showed a marked increase in numerous health issues, including those related to reproduction and mortality.<sup>9</sup> Furthermore, populations whose water is naturally higher in lithium reportedly live longer, healthier lives.<sup>9</sup> Despite the apparent correlation between lithium and many health factors, the lithium requirements for various animal species is still unknown.<sup>9</sup> This shows the importance of lithium in the diet across vastly different species.

Through this research, I will be testing whether or not lithium, provided in an aqueous solution as lithium chloride, can reduce or eliminate neurotic FDB in Quaker Parrots. With this research, I also hope to further expand on a causal relationship between a reduction in FDB and diet, possibly signifying that lithium is an essential micronutrient.

## CHAPTER II

### MATERIALS AND METHODS

#### Setup

At Schubot Exotic Health Center Aviaries, 5 adult Quaker Parrots (*Myopsitta monachus*), 1 male and 4 females, weighing from 100 g to 140 g, were chosen from a large colony based on observed feather damage. These birds were housed individually in galvanized wire caging (1 cm x 1 cm), with hardware cloth, dowel perches, and similar enrichment. The birds had a 12-hour light cycle, with natural light. With use of air conditioning, the birds were maintained at a temperature of about 21°C (70°F). Each cage had two water bottles made of PVC and a controllable valve attached to each cage wall (Bird Butler™ Birds Etc. Liberty Hill, Texas) (Figure 1). Figure 2 illustrates the cage setup with the two water bottles attached.



Figure 1. One of the water bottles used in this study.



Figure 2. An example of one of the cages fully setup to house the birds.

The water bottles attached to each cage wall were labeled either ‘A’ or ‘B’ with the corresponding cage number. The bottles contained either A) Deionized water or B) an electrolyte solution. Birds had *ad libitum* access to these water bottles. Fresh deionized water and electrolyte solution was provided every other day and the water bottles were washed with hot, soapy tap water once weekly.

Each bird was provided 60 g of a low sodium, 280-parts per million (ppm) pelleted diet, daily (See Appendix 1). The regular recommendation for sodium concentration in a Quaker parrot's diet is 1500-ppm. The reduction of sodium in the food resulted in salt seeking behavior in the form of geophagy. The geophagy supplement was provided in the form of an aqueous 500-ppm sodium chloride solution in the 'B' bottles.

### **Preliminary Phase**

A preliminary health examination was conducted to test for disease-based causes for FDB. A choanal/cloacal swab and a feather sample from each bird were tested at a commercial laboratory via polymerase chain reaction (PCR). These samples were tested for Psittacine Beak and Feather Disease, Avian Polyoma Virus, *Chlamydophila* Genus, *Giardia*, *Malassezia* spp. yeast, *Dermanyssus* spp. and *Ornithonyssus* spp. mites, Ringworm (*Microsporium canis*, *Microsporium gypseum*, or *Trichophyton mentagrophytes*), and *Staphylococcus aureus*. Any diseases detected were treated for and eliminated. During treatment of any detected infectious diseases, the birds were provided with a 500-ppm sodium chloride solution in the 'B' bottles. A choanal/cloacal swab and feather sample was taken from each bird again at the end of the study.

### **Treatment Phase**

After treatment for any infectious disease which could contribute to FDB, birds were treated with lithium chloride (Sigma-Aldrich). To begin, an initial assessment was conducted. Prior to this assessment, each bird was sedated with 1 mg/kg intranasal (INas) Midazolam (midazolam hydrochloride, 5 mg/mL, Alvogen, Bengaluru, India) and 1 mg/kg INas Butorphanol (butorphanol tartrate, 10 mg/mL, VETone Vetorphic™ CIV, Boise, ID). The assessment included recording: weight, body condition score (BCS), a dermatological assessment checking the cytology and skin health of each, and recording the prevalence of missing feathers via images

of each bird's ventral and dorsal sides. This was repeated three more subsequent times over the course of 17 weeks. At the initial and final assessments, blood was also drawn to check electrolytes using an i-STAT CHEM8+ handheld blood analyzer (Abbot Point of Care Inc.) and clinical pathology testing using VITROS® 4600 (Ortho Clinical Diagnostics).

The birds were provided with a 500-ppm sodium chloride and 50-ppm lithium chloride provided in the 'B' bottle on each cage (See Appendix 2 Lithium Chloride Solution). Lithium added to the geophagy supplement allowed the birds to self-medicate the lithium. The free-choice access of the lithium reduced stress on the birds and allow them to have *ad libitum* lithium access.

The bird's lithium intake was then tracked over a three-day period each week throughout treatment. Every Tuesday, 50 mL of the sodium lithium solution and 150 mL deionized water was provided at a given time and then the remaining volume was measured on the following Wednesday and Thursday while recording the time. I was thus able to track lithium intake while the birds had constant free-choice access. The birds were never provided with more the 50mL of the sodium lithium solution to eliminate the risk of lithium toxicity.

## **Analysis**

To analyze the effect of lithium, skin cytology of an affected area, weight, body condition score, areas affected, area affected, and overall appearance recorded at each progress check. These were compared throughout the study.

Once lithium chloride supplementation began, we photographed each bird's ventral and dorsal sides to record feather loss at each assessment. Figure 6 illustrates image gridding methodology used to map alopecia on each bird. The area affected was analyzed via a gridding system to calculate the area of alopecia. During each of the assessments conducted, the birds

were spread out on top of a FISKARS® cutting mat (12" x 12") (Figure 3) and pictures were taken of their ventral and dorsal sides using an Olympus Tough TG-5 12-megapixel camera. Figures were coded so that assessment could be done blindly, the gridded was not aware of bird ID or date. On the blind images, the lines on the grid below the bird were extended across each of the images to calculate the area of alopecia. And areas without feathers were counted and added to create a score for each image. These values were compared throughout the study for ventral and dorsal sides of each of the birds.

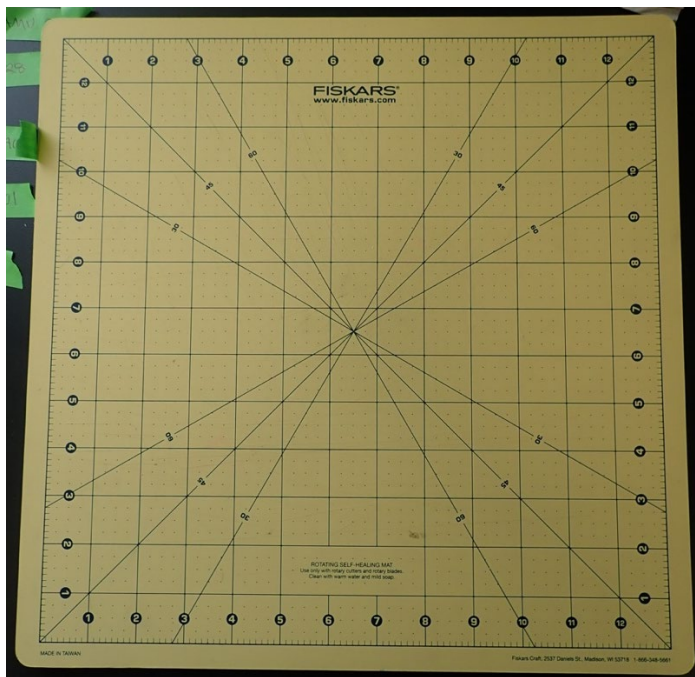


Figure 3. FISKARS® cutting map used for imaging.

Cytology was compared using a grading scale (1-4) to determine the number of organisms and inflamed cells presents. These numbers were compared throughout treatment. The areas affected were determined before and after treatment based on apparent feather damage to 5 different body sections: legs, back, ventrum, wings, and neck. A score of 1 was given to body sections with feather damage and a score of 0 was given to body sections without damage. These scores were totaled and compared from before and after treatment. All plasma analytes,

including lithium were evaluated before and after treatment based on 2 tailed Student's T test, alpha  $\leq$  0.05 for significant differences.

## CHAPTER III

### RESULTS

Prior to treatment with lithium chloride, birds were tested for infectious diseases that could contribute to FDB. All birds tested negative for Psittacine Beak and Feather Disease, Avian Polyoma Virus, *Chlamydophila* Genus, Giardia, *Ornithonyssus* spp. Mites, and Ringworm (*Microsporum canis*, *Microsporum gypseum*, or *Trichophyton mentagrophytes*). Some birds tested positive for *Malassezia* spp. yeast, *Dermanyssus* spp. mites, and *Staphylococcus aureus*. These results can be seen below in Table 1 with indication as to which test (choanal/coacal swab or feather sample) was positive.

Table 1. Results of infectious disease testing (PCR) for Quaker parrots (*Myopsitta monachus*) with feather damaging behaviours. Negative (-), Positive (+) method of positive test.

CAGE	BIRD ID	Test Date	<i>Malassezia</i> spp.	<i>Dermanyssus</i> spp.	<i>Staphylococcus aureus</i>
17	TAMU 219	22-Aug-18	-	-	+ swab
		28-Aug 18	-	-	-
		22-Feb-19	-	+ blood, swab	-
18	TAMU 428	22-Aug-18	-	+ feather	+ swab
		28-Aug-18	-	+ feather	-
		22-Feb-19	-	-	-
19	TAMU 401	22-Aug-18	+ swab	-	-
		28-Aug-18	-	+ feather	-
		22-Feb-19	-	+ blood, swab	-
31	TAMU 467	22-Aug-18	-	-	+ swab
		2-Oct-18	-	-	+ swab
		22-Feb 19	-	-	-
32	TAMU 327	22-Aug-18	+ swab	-	-
		28-Aug-18	-	-	-
		22-Feb-19	-	-	-



These diseases were treated before beginning treatment. This test was repeated after treatment. All birds tested negative for Psittacine Beak and Feather Disease, Avian Polyoma Virus, *Chlamydophila* Genus, Giardia, *Malassezia* spp. yeast, *Ornithonyssus* spp. mites, Ringworm (*Microsporum canis*, *Microsporum gypseum*, or *Trichophyton mentagrophytes*), and *Staphylococcus aureus*. Two of the birds tested positive for *Dermanyssus* spp. Mites.

Once treatment started, we began using pictures of each bird's ventral and dorsal sides to record feather loss prevalence at each assessment. In Figures 4 and 5 below, an example of the initial images taken for one of the birds, TAMU 401, can be seen. In Figures 6 and 7, the final images taken for TAMU 401 can be seen. Using the gridding method (Figure 8) of determining amount of alopecia on each bird, it was found that little overall feather regrowth occurred on all of the birds except TAMU 401. With consumption of 0.054 mg/day of lithium, four of the birds appeared to have increased feather loss on their ventral sides. All but one of the birds had decreased feather loss by the last assessment. Figure 9 illustrates the amount of feather loss present on each of the birds throughout the study. The birds with much more severe feather loss (Figure 10) presented these results. However, when inspecting each bird based on individual areas affected by feather loss, each bird showed feather regrowth in at least one of the areas initially reported.

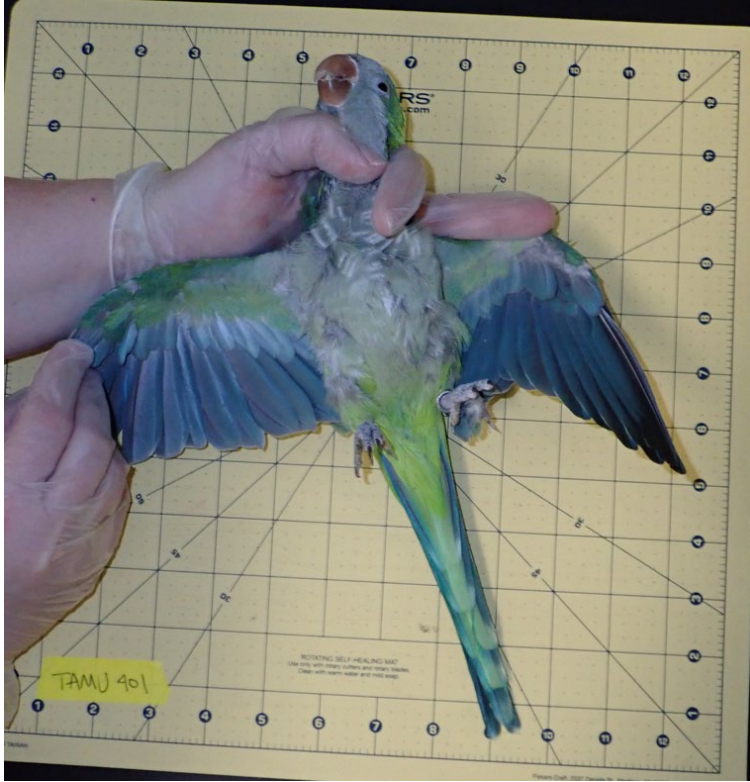


Figure 4. Ventral Side of TAMU 401 on October 19<sup>th</sup>, 2018.

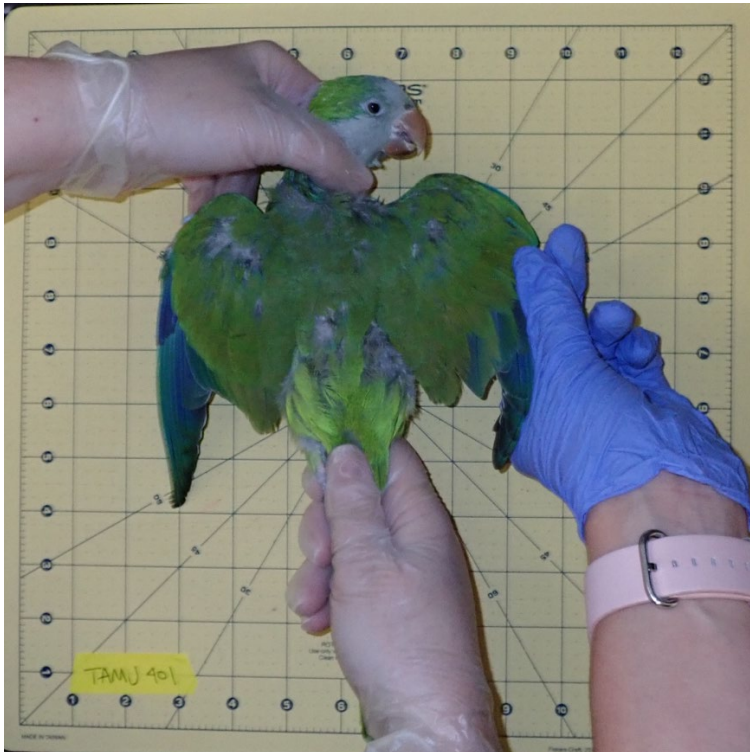


Figure 5. Dorsal Side of TAMU 401 on October 19<sup>th</sup>, 2018.

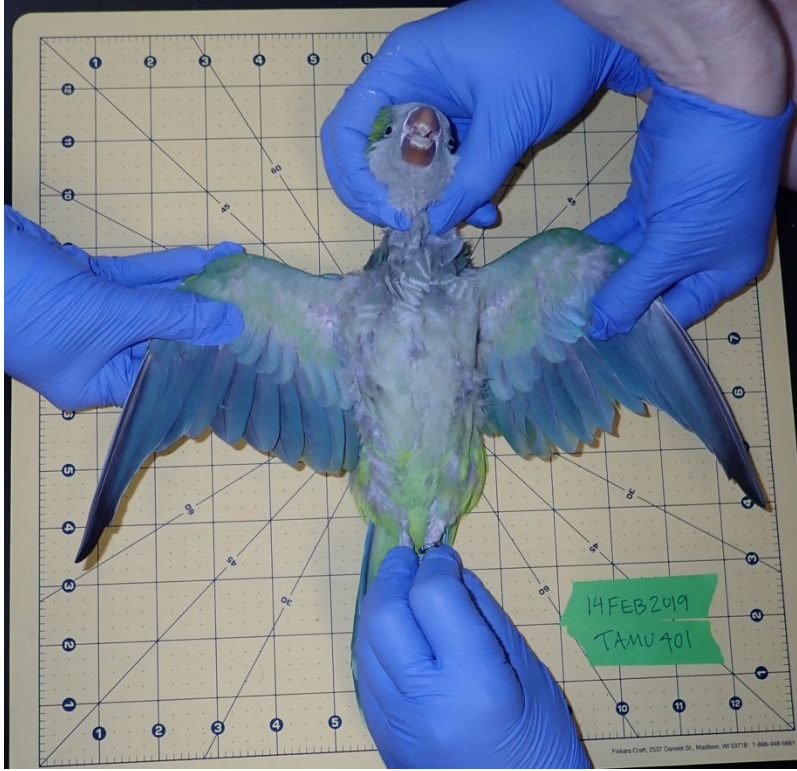


Figure 6. Ventral Side of TAMU 401 on February 14<sup>th</sup>, 2019.

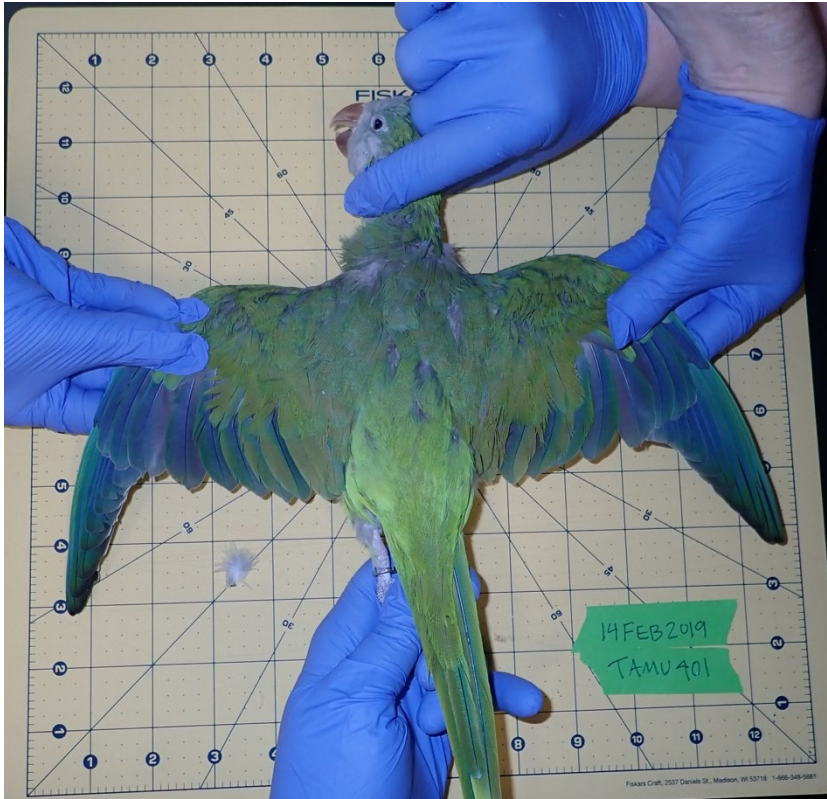


Figure 7. Dorsal Side of TAMU 401 on February 14<sup>th</sup>, 2019.

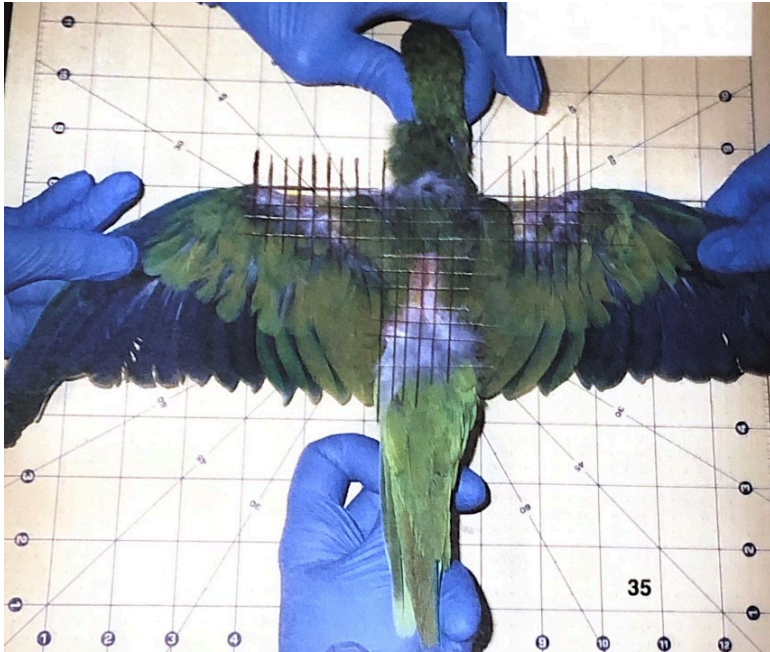


Figure 8. Blind image 35 gridded over area of alopecia visible.

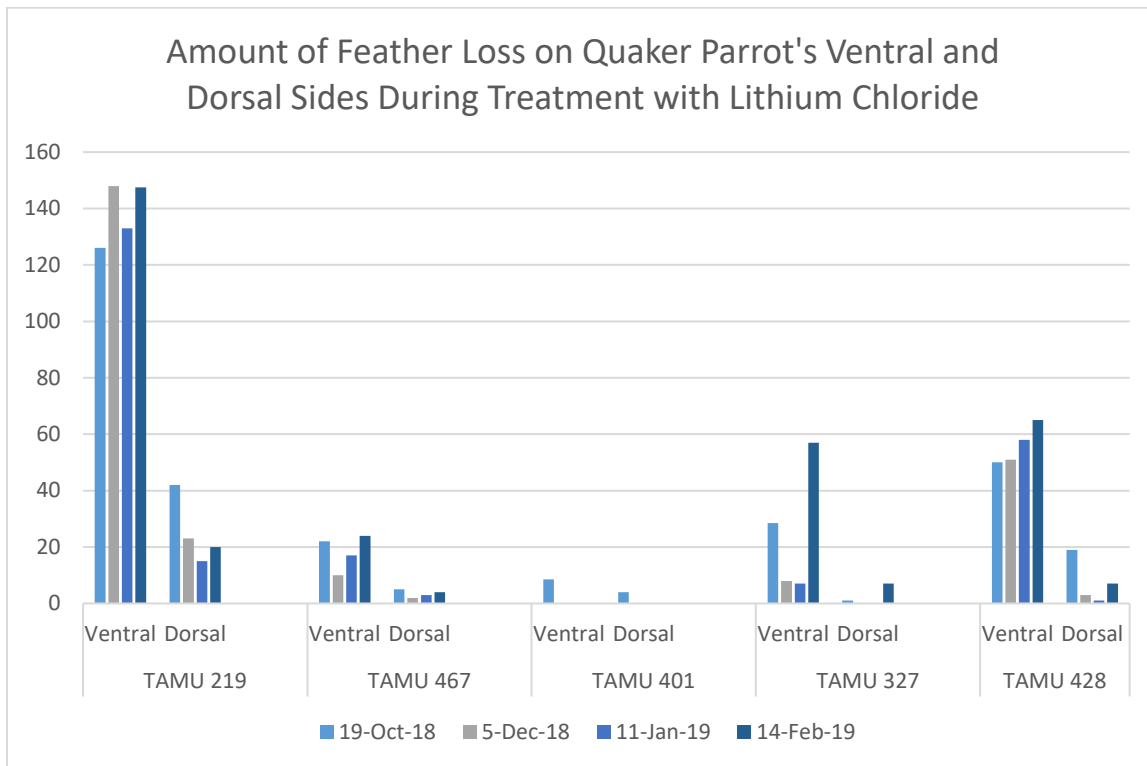


Figure 9. Amount of feather loss present on each Quaker Parrot's Ventral and Dorsal sides.

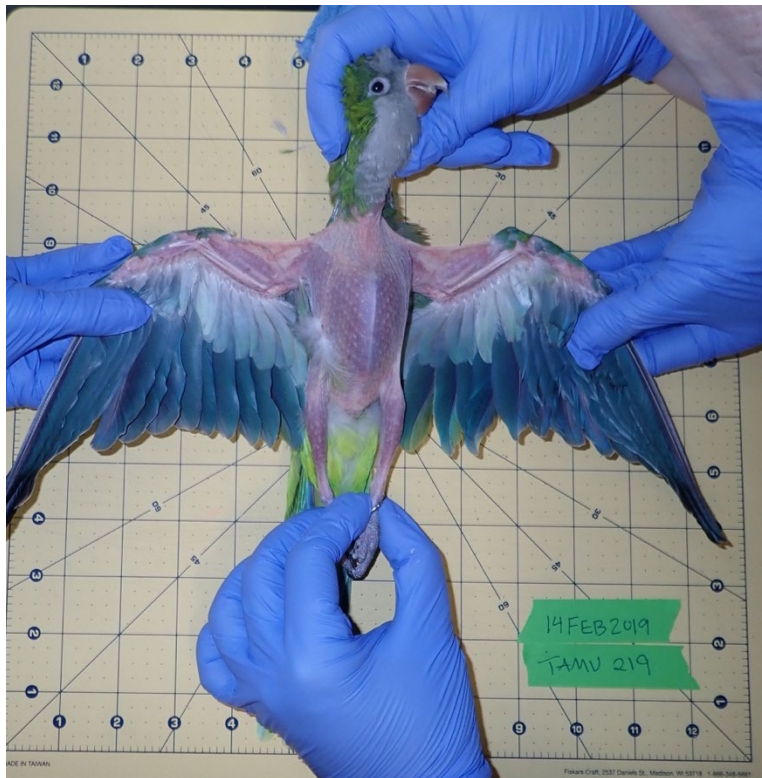


Figure 10. Sever feather loss: ventral side TAMU 219 on February 14<sup>th</sup>, 2019

All values for plasma lithium concentrations and blood urea nitrogen were below detectable levels. Sodium decreased based on the plasma analyzed by clinical pathology laboratory at a 5% significance level ( $p$ -value = .0018) but was not significantly different based on i-STAT CHEM8+ blood analysis at 5% significance level. Figure 11 illustrates the decrease of sodium concentrations based on clinical pathology laboratory testing at a 5% significance level. Potassium was increased in birds post treatment in plasma analyzed by the clinical pathology laboratory at a 5% significant level ( $p$ -value = 0.0003) but not for values determined by the iSTAT ( $p < 0.05$ ). Figure 12 illustrates the change in potassium based on clinical pathology laboratory testing. All other blood and plasma values were not significantly different.

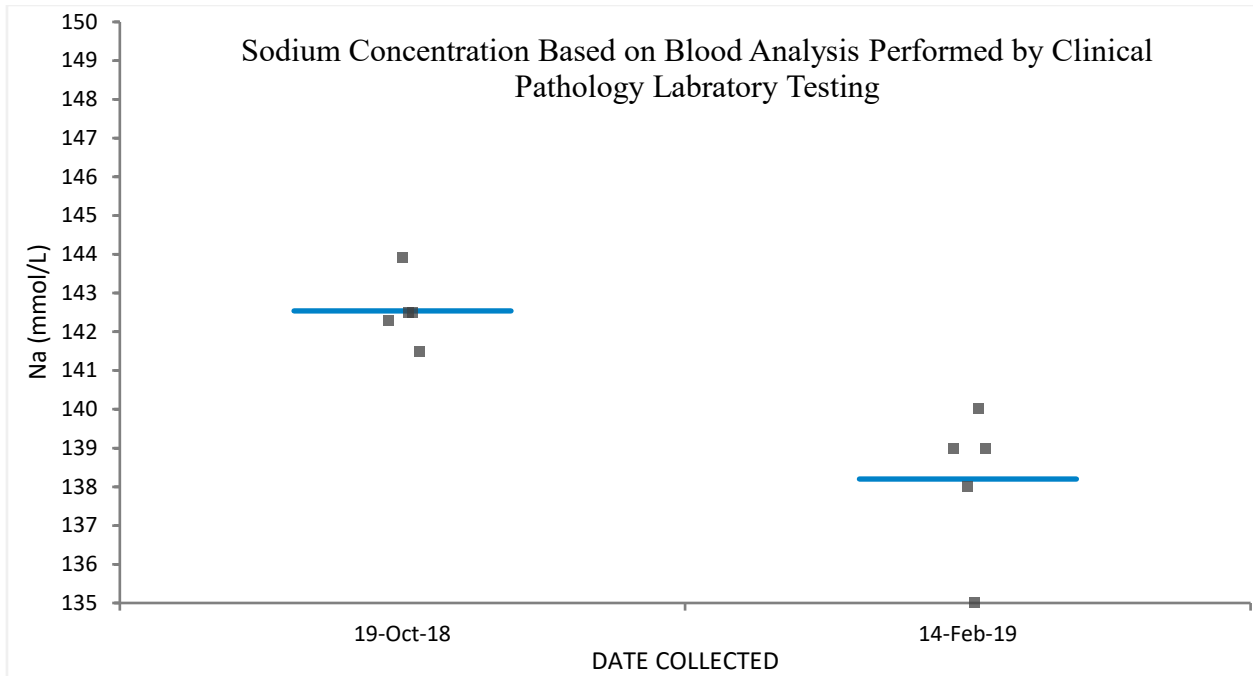


Figure 11. Sodium concentration based on clinical pathology laboratory testing before and after treatment with lithium chloride.

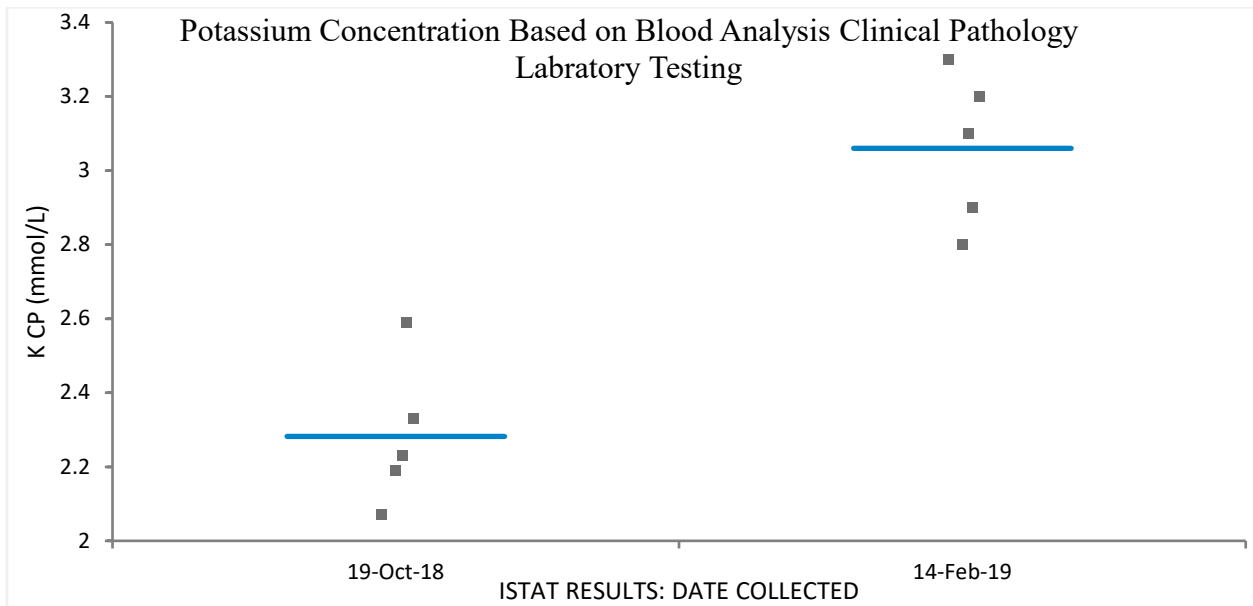


Figure 12. Potassium concentration based on clinical pathology laboratory testing before and after treatment with lithium chloride.

Weights and body condition scores were similar throughout the study for each bird. None of the birds had dramatic weight loss or weight gain. The dermatological exam revealed many birds had a reduction in organisms on their skin, and all but one of the birds had no organisms or inflammatory cells observed by the last assessment (Table 2).

Table 2. Dermatology Exam Results for 5 Quaker parrots (*Myopsitta monachus*) during lithium supplementation.

Bird	Result Type	Exam 1	Exam 2	Exam 3	Exam 4
327	Observation	PA of medial legs and keel. More complete alopecia of ventral neck. Skin of ventral neck is thickened. Mild PA of wrists. Dorsum Feathers disheveled but present.	PA of ventral neck and cranial keel, NFG. Skin of neck subjectively less thick. NFG of dorsal wrists. Dorsal Feathers are relatively smooth, less disheveled.	NCA of ventral neck, some evidence of NFG. PA of medial wings and legs. A large amount of NFG observed along the ventrum. NFG also on dorsum. No evidence of inflamed skin is observed	PA along the ventral neck and proximal keel with evidence of NFG. Partial to complete alopecia remains along the medial legs with NFG. The dorsum is fully feathered with no areas of alopecia. No evidence of inflammation is observed.
	Cytology Tape, ventral neck	rare heterophils, no organisms seen	single area on slide shows 2+ cocci, 4+ rods. No inflammatory cells are observed (contamination?)	rare nuclear streaming (inflammatory debris)	rare heterophils
401	Observation	mild PA along the left axillary region. PA is noted along the medial legs bilaterally. Feathers are disheveled but present along the ventrum and dorsal neck.	Evidence of NFG is present within the left axillary region. Mild PA remains along the right medial wing with evidence of NFG. Feathers along the dorsum are relatively	The bird is nearly fully feathered at this time. NFG is appreciated along all body surfaces. No alopecia is observed.	The bird remains nearly fully feathered. NFG is appreciated along all body surfaces. No alopecia observed.

			smooth and less disheveled in appearance.		
	Cytology: Tape, Left axilla	rare yeast, rare heterophil	no organisms or inflammatory cells observed	no organisms or inflammatory cells observed	no organisms or inflammatory cells observed
219	Observation	NCA of entire ventrum, ventral neck, medial-ventral aspects of both wings, and circumferentially around both legs. NCA on the caudal dorsum more lateralized to the left. Alopecia on the dorsal wrist of both wings.	NCA of entire ventrum, ventral neck, medial-ventral aspects of both wings, and circumferentially around both legs. Tuft of feathers present in the right inguinal fold. Evidence of NFG present along the caudal dorsum. Alopecia is noted on the dorsal wrist of both wings.	NCA of entire ventrum extending from the neck, along the medial wings, and along the legs. NFG of the right side of the chest. Partial to complete alopecia of dorsal wings and caudal dorsum with some NFG.	NCA of entire ventrum extending from the neck along the medial wings, keel and legs. Previously noted NFG of right chest present. Partial to complete alopecia remains of dorsal wings and caudal dorsum with evidence of NFG. Mild inflammation noted of left dorsal wing.
	Cytology Tape, ventrum	2+ yeast, rare heterophils	single yeast organism observed	rare nuclear streaming (inflammatory debris), no organisms	no organisms or inflammatory cells observed
467	Observation	PA along the ventral neck, medial wings, and medial legs bilaterally. Mild PA is present along the ventrum with disheveled feathers. Feathers along the dorsal neck are also mildly disheveled.	PA along the ventral neck, medial wings, and medial legs bilaterally. Evidence of NFG is present along the neck and wings. NFG is present along the dorsal wings bilaterally.	PA of ventral neck, wings, and inguinal folds (right > left). NFG appreciated within described body regions. Dorsum fully feathered. No evidence of inflammation observed.	PA of ventral neck, medial wings, and legs; NFG is appreciated in described body regions. Dorsum appears fully feathered. No evidence of inflammation observed.
	Cytology Tape, ventral neck, wing	No organisms or inflammatory cells	no organisms or inflammatory cells observed	no organisms or inflammatory cells observed	no organisms or inflammatory cells observed



428	Observation	<p>NCA of ventral neck. The medial wings bilaterally have mild hemorrhage at wrists extending to the metacarpals. PA along the keel lateralized to the left.</p> <p>Circumferential alopecia of legs bilaterally. PA of dorsal right wrist and cranial dorsum.</p> <p>Alopecia with hemorrhage of dorsal left wrist</p>	<p>PA of ventral neck and cranial keel with NFG. NCA medial aspects of both wings with NFG. PA circumferentially around the legs with NFG. NFG of cranial dorsum and dorsal wings.</p> <p>Previously noted hemorrhage of wrists resolved.</p>	<p>NCA of ventral neck and medial wings. Mild inflammation observed along medial wings bilaterally. NFG is of ventral neck and entire chest. PA circumferentially along the legs, dorsal wings, and majority of the dorsum.</p>	<p>NCA of ventral neck and medial wings. PA of right lateral chest. NFG is along the neck, wings, and chest. Wings are not inflamed. Legs remain near completely alopecic circumferentially with some evidence of NFG. A large amount of NFG is appreciated along the majority of the dorsum. PA remains along the dorsal wings and lower on the dorsal midline.</p>
	Cytology	<p>Tape (ventral neck, wings, chest): 1+ cocci (bacteria), rare heterophil</p>	<p>Tape (ventral neck, chest, wings): rare heterophil</p>	<p>Tape (ventral neck, chest, wings): periodic cluster of heterophils observed</p>	<p>Tape (ventral neck, chest, wings): rare clusters of heterophils, no organisms observed</p>

Table Key: NFG - New feather growth, NCA - Near complete alopecia, PA - Partial alopecia.

## CHAPTER IV

### CONCLUSION

Birds were treated with lithium for 17 weeks and did not develop any ill needs or problems based on numerous measurements such as BCS, weight, and hematology. A lack of detectable levels of lithium suggest that the dosage determined in this study appears safe for long term treatment of Quaker Parrots. Furthermore, the fact that the birds did not lose weight and all other electrolytes remained within healthy levels suggest that treatment with lithium chloride did no harm. Further research will be required to expand on the safety of lithium chloride in avian species.

The apparent increase in feather loss after treatment on the bird's dorsal side could be due to variability between pictures. The initial assessment was the first time attempting to take pictures of the birds using this method. It is possible that if the birds were not spread the exact same way, calculating the amount of feather loss could be skewed. In future research, the variability between images will need to be addressed. Potential ways to address this is to use a tripod or computer software. Moreover, the fact that feathers did not grow on some of the bird's ventral sides could be due to follicle damage. If the follicle is damaged, the bird cannot physically regrow feathers. In that case, future research would be needed to determine if lithium affected skin picking prevalence. Skin cytology indicated that skin health changed for the better and could suggest that lithium had a role in this. *Dermanyssus* spp. mites could have been passed to the birds in the study from surrounding birds, and may reinfest the environment and this may have reinfected the treated birds of this study.

This experiment would need to be repeated several times, with a larger group of birds, as a blind study to further expand on the causal relationship between lithium chloride treatment and feather damaging behavior. Furthermore, this study suggests additional research is needed for this species regarding micronutrient requirements. Most information about avian nutrition is derived from knowledge of chickens. This study could lead to research on micronutrients in avian species, to determine if lithium is a micronutrient necessary for feather and skin health.

Despite not getting the exact results expected, this study provides evidence as a potential modality for treatment and amelioration of Quaker mutilation syndrome, or other FDBs based on some positive results and lack of negative impact on avian health

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# APPENDIX 1

## RECIPE AND INSTRUCTIONS FOR MAKING LOW SODIUM 280 PPM

### PELLETED QUAKER PARROT FEED

----- Used Ingredients -----										----- Nutrient Solution -----					
Ingr Code	Ingredient Name	Unrounded Lbs	Pct	Owning \$/100Lb	-- Range --		-- Restriction --			Nutr Nb	Nutrient	(Q ass t)			
					Low	High	Mn Pct	Max Pct	Rcost			Minimum	Actual	Maximum	
1	CORN YELLOW #	275.93	68.982	6.000		12.300	40.0000			I	2 DRY MATTER		89.89		
15	MCLASSES, DEHY	5.20	1.300	0.000			1.3000	1.3000		I	3 MO STUFF		10.11		
20	SOYBEAN ML48%	68.01	17.002	16.600	10.650	50.830	13.0000			I	4 PROTEIN	16.00	16.00		
40	ALFALFA- Na	36.00	9.000	8.000			9.0000	9.0000		I	5 CRUDE FAT		3.03		
50	DL-MET98	0.925	0.231	135.000	15.690	690.330		0.3200		I	6 CRUDE FIBER		4.44		
53	LYSINE HCL	0.262	0.066	100.000	25.530	272.730				I	7 CALCIUM	1.00	1.00	1.00	
70	LIMESTONE	5.29	1.323	3.000		22.650				I	8 PHOSPHORUS		0.7071		
73	BI OFOS 16/21P	7.18	1.796	12.000	3.510	889.380	1.0000			I	9 AV PHOSPHATE	0.50	0.5000		
79	TAMU TRACE M N	0.200	0.050	100.000			0.0500	0.0500		I	13 ME POULTRY kca	2850.00	2880.46		
80	TAMU VITAMINS	1.00	0.250	150.000			0.2500	0.2500		I	14 ME POULTRY kca		1309.30		
										I	16 XANTHOPYLL m		31.53		
Total Bat ch:		400.00 Lbs at		174.79 \$/Ton		8.739 \$/100Lb		0.0874 \$/Lb		I	21 AV-MET		0.4602		
										I	22 AV-TSAA		0.6730		
										I	23 AV-LYS		0.6937		
----- Binding Nutrients -----															
Nutr Nb	Nutrient Name	Unit of Measure	Nutr Cost	Increment Change							Nutr Nb	Nutrient	Minimum	Actual	Maximum
4	PROTEIN	PCT	3.2524	1.00 PCT							I	32 METHIONINE	0.35	0.4863	
7	CALCIUM	PCT	-0.0460	0.10 PCT							I	33 TSAA	0.75	0.7500	
9	AV PHOSPHATE	PCT	0.7985	0.10 PCT							I	34 LYSINE	0.80	0.8000	
33	TSAA	PCT	2.4802	0.10 PCT							I	35 TRYPTOPHAN		0.1879	
34	LYSINE	PCT	2.0501	0.10 PCT							I	36 THREONINE		0.5801	
											I	37 ARGININE		0.9159	
											I	43 GLYCINE		0.6500	
											I	59 LI NOLEIC ACID	1.00	1.63	
											I	61 ELECTROLYTES		204.86	300.00
----- Unused Ingredients -----															
Ingr Code	Ingredient Name	Current \$/100Lb	At \$/100Lb	Would Use	Minimum Pct	Maximum Pct	Restriction Pct	Restriction Rcost		Nutr Nb	Nutrient	Minimum	Actual	Maximum	
67	SOYBEAN OIL	20.000	3.870				1.5000	3.231		I	62 SODIUM		0.0277		
75	SALT	Suppressed	2.500	3.870				-0.27		I	63 POTASSIUM		0.8550		
										I	64 CHLORIDE		0.0918		
										I	94 18:2 n6		1.31		
										I	95 18:3 n3		0.0957		
----- Nutrient Ratio Requirements -----															
Nutr Nb	Nutrient Name	Units	Requirement Mn	Requirement Max	Per	Nutr No	Nutrient Name	Units	Ratio	Formula Nutr Amount	Restr Cost	At Limit			
94	18:2 n6	PCT			1.00	95	18:3 n3	PCT	13.68	1.31					

## **APPENDIX 2**

# **RECIPE AND INSTRUCTIONS FOR MAKING 500-PPM SODIUM CHLORIDE AND 50-PPM LITHIUM CHLORIDE ELECTROLYTE SOLUTION**

### **Materials**

3.045 grams of lithium chloride

12.75 grams of sodium chloride

10 liters of deionized water

### **Methods**

Using an analytical balance, measure out sodium and lithium chloride. Using a deionized water dispenser, dispense 10 liters of deionized water. Add sodium and lithium chloride and stir until fully dissolved. Be sure to stir the solution before future use.