EVALUATION AND ASSESSMENT OF HYPOTHYROIDISM IN PSITTACINE BIRDS

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INTRODUCTION

Hypothyroidism has long been suspected as a reasonably common disorder, but its diagnosis continues to present a challenge in psittacine birds. Thyroid hormones influence, or are influenced by, multiple physiological, environmental and nutritional parameters (McNabb, 1998). There are no specific patterns as yet identified for this endocrine disorder although low levels of thyroid hormone have been associated with obesity, reduced gonadal function, generalized feather loss and abnormal feather growth and development (Harrison and Lightfoot, 2006). Modifications in plumage maturity including fringed and elongated feathers, structural defects in contour feathers, loss of colour and delayed molt have been attributed to hypothyroidism (Rae, 1995). Current options for diagnostic testing appear unreliable and limited information is available on treatment protocols and efficacy. This presentation describes results of physical examination, blood testing, biopsy and thyroid hormone supplementation in a small number of birds presenting at a private veterinary practice.

MATERIALS AND METHODS

Individuals were selected for this investigation based on a pattern of feather change that the authors have observed in galahs (*Eolophus roseicapilla*), cockatiels (*Nymphicus hollandicus*) and budgerigars (*Melopsittacus undulatus*). These changes included elongation of powder down feathers, narrowing and elongation of outer primary feathers and generalized feather colour changes. Birds in this investigation were brought to the practice for varying reasons including self mutilation, owner concern about abnormal plumage, owner concern about reduced activity levels and 'well bird' exams. Owner preferences at times dictated the range of tests performed on each bird.

Three cockatiels exhibiting feather dystrophy were selected for baseline thyroid testing. To approximate standard plasma thyroid hormone concentrations, blood was collected from two clinically healthy cockatiels with normal plumage (Table 1). Previously published mean resting thyroxine concentrations in cockatiels (n=3) reported by Lothrop et al. (1985) were 11.83 ± 6.76 ng/ml. These concentrations were determined with a solid phase radioimmunoassay specific for T_4 and validation of the assay was completed for T_4 quantification in psittacine birds (Lothrop et al.,

1985). Five galahs exhibiting feather dystrophy were selected for baseline thyroid testing. To approximate standard plasma thyroid hormone concentrations, blood was collected from eight clinically healthy galahs with normal plumage (Table 2). Budgerigars were not tested because sufficient blood could not be obtained safely. All samples were placed in lithium heparin plasma separator tubes, spun and immediately stored on ice. Plasma thyroid hormone concentrations were analyzed at VETPATH laboratory services (Perth, Western Australia) using solid-phase competitive chemiluminescent enzyme immunoassay specific for T_4. All samples were evaluated using a human product kit to determine Total T_4 concentrations and canine product kit to determine canine T_4 concentrations. These assays have not been validated for use in birds.

One galah had both baseline T_4 and six hour post TSH (bovine 0.5 IU; Sigma) T_4 measured.

Various tissue samples were collected from four affected galahs, two 'normal' galahs and one affected cockatiel. Skin samples, including feather follicles, were taken from the flank. Liver biopsies were taken via a midline laparotomy incision just caudal to the sternum. Thyroid biopsies were performed with birds in dorsal recumbency with the head and neck dorso-flexed. A 2.7mm endoscope was introduced through the thoracic inlet and into the clavicular air sac. It was easier to see the thyroid glands (typically on the medial aspect of the internal carotid arteries midway between the thoracic inlet and the syrinx) on the left side because the oesophagus was present on the right-hand side. In overweight birds fat deposits in the thoracic inlet hampered visualization of the glands. Biopsy samples were collected with 9 Fr rigid cup biopsy forceps. Complication associated with the biopsy included collecting all or part of the parathyroid and portions of nerve ganglia. This did not appear to create significant post-operative problems. The close adherence of the thyroids to the carotid vessel means great care must be taken not to inadvertently include any portion of this vessel in the biopsy forceps. The cockatiel samples were collected post-mortem.

Some of the birds presenting with feather changes suspicious of hypothyroidism were trialed on oral thyroxine. Supplementary L-thyroxine (Oroxine® 200 µg tablet – Sigma Pharmaceuticals (Australia) Pty Ltd) was administered by either dissolving one quarter of a 200 µg tablet in 15 ml of water and dosed at 0.5 ml/kg orally once daily or a dosage of 333 µg/L drinking water.

RESULTS

The baseline thyroid testing was unreliable in both species tested. All cockatiels (normal or abnormal plumage) returned low (< 13 nmol/L or <6.4 nmol/L) T_4 plasma values (Table 1). All four galahs with abnormal plumage also had T_4 levels below analyser range, but so did 3/10 galahs with normal plumage. The remaining seven normal galahs returned T_4 levels between 15.4 nmol/L and 26.9 nmol/L (Table 2). Interestingly, one of the galahs with abnormal feathering that had been receiving daily oral thyroxine for over twelve months was reported to have a total T_4 concentration of 26 nmol/L and a canine T_4 concentration of < 6.4nmol/L using the same lithium heparin plasma sample. The tests were repeated on the same day, with the same blood sample resulting in similar values. The cause of the discrepancy between the total T_4 and canine T_4 concentration results is uncertain.

In the galah receiving TSH the pre- and post-TSH plasma T_4 values were < 13 nmol/L (Table 2).

Biopsy samples from two of the four galahs with suspect hypothyroidism had similar dermatological and thyroid appearance. Skin histology revealed diffuse orthokeratotic, hyperkeratotic dermatosis. The thyroid tissue revealed epithelial hyperplasia and hypertrophy along with a deficit of colloidal material and undersized follicles consistent with thyroid hyperplasia (Figure 1). The remaining two

suspect hypothyroid galahs had predominantly normal histological appearance of both skin and the thyroid (Figure 2). All four liver biopsies obtained from the suspect hypothyroid galahs demonstrated mild to moderate hepatic lipidosis (Figure 3). Liver biopsies taken from two clinically healthy galahs with normal plumage, fed and housed identically to two of the 'hypothyroid' birds had the same pattern of mild to moderate hepatic lipidosis as the suspect hypothyroid birds. The thyroid from the abnormally feathered cockatiel was relatively normal with only mild follicular size variation.

All birds treated with supplemental thyroxine showed visible improvements in feather morphology. Elongated powder down feathers in particular were quickly lost and replaced with normal powder down. Primary feathers that moulted and were replaced during treatment were also more normal in appearance (shorter and broader) and some discoloured contour feathers were replaced with normally coloured feathering. No birds however regained totally normal plumage.

DISCUSSION

Reasons for the unreliability of T_4 testing may include the insensitivity of the thyroxine assays used to determine the relatively low thyroxine concentrations in birds (Greenacre, 2009). The lowest concentration of T_4 values within linear range for the canine test is < 6.4nmol/L and the human test is < 13nmol/L. In addition, the enzyme immunoassays used in this report were not validated in birds. Seven galahs considered clinically healthy with normal plumage had measurable T_4 values considerably higher (>13 nmol/L) than baseline values obtained for the hypothyroid suspect galahs (<13 nmol/L). Three of the galahs with normal plumage had thyroxine values of < 13 nmol/L. Liver biopsies were performed on two of these galahs revealing histological evidence of hepatic lipidosis. These birds may have been euthyroid.

T_4 values alone do not reflect the function of the thyroid glands. Many factors including environmental conditions and food intake can influence results. The best method considered to accurately assess thyroid function in birds is through the administration of TSH followed by measurement of the serum T_4 concentration after a particular time (Schmidt, 2002, Greenacre, 2009). However, only one confirmed diagnosis of hypothyroidism in a scarlet macaw [*Ara macao*] has been published by using bovine TSH as described by Lothrop et al. (1985) (Oglesbee, 1992).

Avian, bovine and porcine pituitary tissue have been used for avian TSH testing, although logic would dictate that avian-derived TSH would be the most effective. Avian TSH is a large complex glycoprotein that is either isolated or prepared using recombinant methods and unable to be chemically synthesized. Avian TSH could not be obtained for this clinical trial. Bovine and porcine pituitary tissue is also expensive and difficult to acquire. In unpublished data described by Danylyk et al. (2009) porcine pituitary tissue was used to test the thyroid function in six adult galahs. Moderate T_4 concentration increases were observed in five clinically healthy galahs with normal plumage. In comparison, one galah with abnormal plumage (and histological evidence of thyroid disease) failed to respond. In the current study a galah with abnormal feathering also failed to respond to TSH administration. However, in this case, skin and thyroid biopsy results were not supportive of hypothyroidism (normal tissue).

Two of four galahs with feather changes suspected to be related to hypothyroidism had histologically identifiable thyroid changes. All four hypothyroid suspect galahs had histological lesions consistent with hepatic lipidosis, as did two birds with normal plumage. Interestingly, both these birds had low baseline T_4 values. TSH stimulation testing was not performed but it is possible that these birds may have been euthyroid.

FATTY LIVER SYNDROME (HEPATIC LIPIDOSIS) AND THYROID HYPERPLASIA; IS THERE A CORRELATION?

Fatty liver syndrome is commonly reported in species such as Amazon parrots, cockatiels, budgerigars, macaws and galahs (Schmidt et al., 2003). Similar species of birds are commonly implicated in thyroid disorders. The cause of lipidosis in birds is typically attributed to a dietary excess of fatty food (Schmidt et al., 2003). However, experimental thyroidectomy has been reported to result in glycogen accumulation in the liver, as well alterations in feather structure (fringed, elongated feathers) and reduced gonadal function (Rae, 1995). Thyroid hormones are also known to augment the process of protein synthesis and lipogenesis in the avian liver (Harrison and Lightfoot, 2006). In a variety of mammals, endocrine disorders, such as diabetes and hypothyroidism, can produce hepatocellular lipidosis (McGavin and Zachary, 2007). The histological evidence of hepatic lipidosis in two of the four hypothyroid suspect galahs may be a manifestation of abnormal metabolism due to thyroid hyperplasia.

All birds administered supplemental thyroid hormone showed some normalization of abnormal feather morphology. However no birds developed completely normal feathers. Reasons for this may include insufficient duration of treatment, insufficient/inaccurate dosage, or a response to thyroxine independent of thyroid dysfunction. It has been well documented that an increase in thyroid hormone leads to moulting, possibly by stimulating new feather growth (Pant et al., 1993).

With unreliable baseline T_4 values, difficulties in obtaining pituitary tissue for TSH testing and non-specific clinical signs, it is evident that diagnosis of avian hypothyroidism is clinically challenging. Thyroid gland biopsy may offer an alternative and specific diagnostic option for birds suspected of thyroid dysfunction. While the technique requires some practice it appears relatively safe, at least in larger species. In addition, appropriate samples (thyroid, skin and liver) can be obtained post-mortem from birds demonstrating this characteristic pattern of feather change. Much more data is required to further understand whether this clinical appearance can be linked with thyroid gland and/or hepatic dysfunction.

Table 1- T_4 Results for Cockatiels

Name	Date of testing	Species	History	Clinical Appearance	T_4 Result nmol/L
Hypothyroid Suspect Cockatiel 1	16/03/2010	Cockatiel	6 year old probable female outdoor aviary mixed seed diet only	Narrow primaries Elongated powder down Green/yellow feather colour Elongated maxillary rhamphotheca	< 6.4
Hypothyroid Suspect Cockatiel 2	20/03/2010	Cockatiel	7 year old probable male indoor/outdoor pet mixed seed diet	Narrow primaries Elongated powder down Green/yellow feather colour	< 6.4
Hypothyroid Suspect Cockatiel 3	22/03/2010	Lutino cockatiel	8 year old indoor pet mixed seed diet	Narrow primaries Elongated powder down Bleached feather colour Elongated maxillary rhamphotheca	No initial T_4 testing,
	18/05/2010		Trial Oroxine @ 333ug/l drinking water	Beak no longer elongated Feather colour returning to normal appearance	< 13
Normal plumage Cockatiel 1	31/05/2010	Cockatiel	>2 year old indoor pet seed diet	Normal plumage	< 6.4
Normal plumage Cockatiel 2	03/06/2010	Lutino cockatiel	4 year old indoor pet seed diet, celery, apple	Considered Overweight at 117 grams Normal plumage	< 6.4

Table 2 - T_4 Results for Galahs

Name	Date of Testing	Species	History	Clinical Appearance	T_4 Result nmol/L
Hypothyroid Suspect Galah 4	01/04/2010 09/06/2010	Galah	>10 year old Male aviary bird Mixed seed diet	Narrow primaries. Elongated powder down. Red feather replacement of normally grey feathers	< 6.4 < 13
Hypothyroid suspect Galah 2	40362	Dilute Galah	>10 year old aviary bird, mixed seed diet	Narrow primaries Elongated powder down Red feather replacement of Normally grey feathers	<6.4
Hypothyroid Suspect Galah 5	17/05/2010	Galah	>10 year old indoor pet. Seed and vegetables	Narrow primaries Elongated powder down Red feather replacement of Normally grey feathers	<13
Hypothyroid Suspect Galah 3	23/06/2010	Galah	> 10 year old female aviary bird. Mainly seed, some native browse	Narrow primaries Elongated powder down Red feather replacement of Normally grey feathers	<13 Pre TSH <13 Post TSH <13
Hypothyroid suspect Galah 1	26/03/2008 07/05/2008 19/01/2010 **3/06/2010**	Galah	> 10 year old Male aviary bird. Mixed seed diet	Narrow primaries Elongated powder down Red feather replacement of Normally grey feathers Not on Thyroxine treatment **Improvement to plumage post Thyroxine treatment**	<13 Pre TSH 14.3 Post TSH 13.2 16.3 **Total T4 26 Canine T4 <6.4**
Galah 1	26/03/2008	Galah	Unknown age aviary bird. Murdoch University Mixed seed diet	Normal plumage	16.9
Galah 2	26/03/2008	Galah	Unknown age aviary bird Murdoch University Mixed seed diet	Normal plumage	15.4
Galah 3	26/03/2008	Galah	Unknown age Aviary bird Murdoch University Mixed seed diet	Normal plumage	18.3
Galah 4	26/03/2008	Galah	Unknown age Aviary bird Murdoch University Mixed seed diet	Normal plumage	17.4
Galah 5	26/03/2008	Galah	Unknown age Aviary bird Murdoch University Mixed seed diet	Normal plumage	25.5
Galah 6	40394	Galah	Unknown age (<10) Aviary bird Male Mixed seed diet	Normal plumage	18.3
Galah 7	40394	Galah	Unknown age (<10) Female Aviary bird Mixed seed diet	Normal plumage	26.9
Galah 8	18/05/2010	Galah	>2 years Indoor pet Mixed seed/vegetables	Normal plumage	<13

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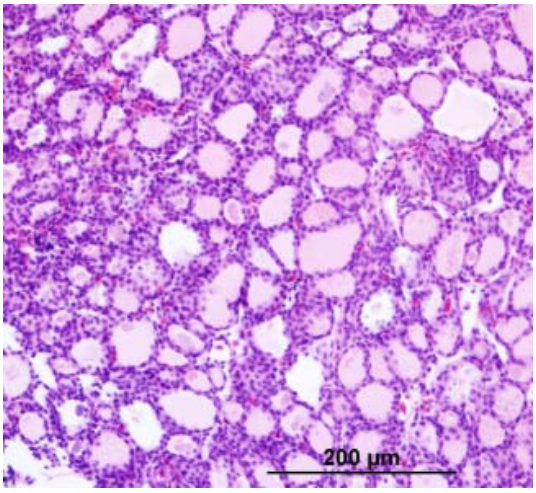

Figure 1: Thyroid tissue epithelial hyperplasia and hypertrophy along with a deficit of colloidal material and undersized follicles

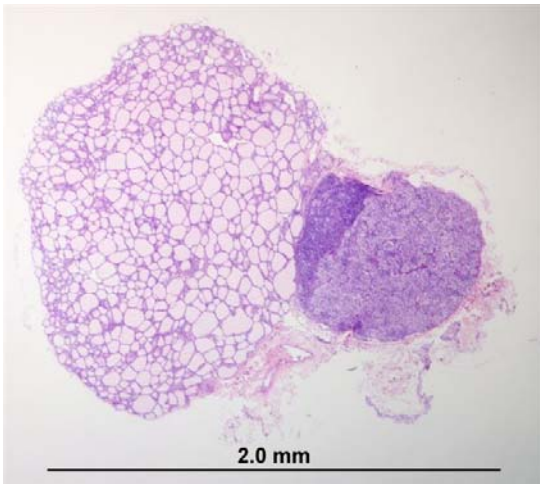

Figure 2: Normal thyroid, parathyroid and ultimobranchial gland of a wild galah

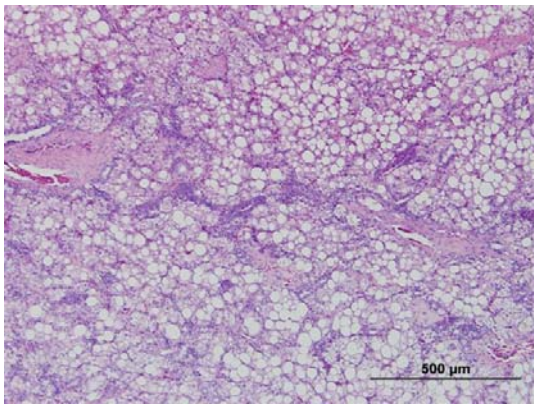

Figure 3: Moderate hepatic lipidosis