J. Raptor Res. 39(1):84–88

The Role of Thyroxine on the Production of Plumage in the American Kestrel (*Falco Sparverius*)

Michael J. Quinn, Jr.[1]
Department of Animal and Avian Sciences, University of Maryland, College Park, MD 20742 U.S.A.

John B. French, Jr.
U.S. Geological Survey, Patuxent Wildlife Research Center, 11510 American Holly Drive, Laurel, MD 20708 U.S.A.

F.M. Anne McNabb
Department of Biology, Virginia Tech, Blacksburg, VA 24061 U.S.A.

Mary Ann Ottinger
Department of Animal and Avian Sciences, University of Maryland, College Park, MD 20742 U.S.A.

Key Words: *American Kestrel*; Falco sparverius; *thyroxine*; *plumage*; *feather*; *molt.*

In a prior study (Quinn et al. 2002), we examined the effects of Aroclor 1242 (a mixture of polychlorinated biphenyls [PCB]) on feather production in the American Kestrel (*Falco sparverius*). The development of plumage is heavily influenced by the action and timing of thyroid hormones (Owens and Short 1995, Kuenzel 2003). Although Aroclor 1242 was shown to cause decreases in plasma thyroxine (Quinn et al. 2002), no significant differences were observed in feather production or appearance between PCB and control treatments. The data presented here illustrate the relative roles of thyroxine on molt and plumage pigmentation in the American Kestrel.

Although it is well known that molt and feather regrowth are dependent on thyroid hormones, the underlying mechanisms remain unclear. Experimentally-administered thyroid hormones typically induce molt, and normal schedules of molt and feather regrowth are associated with changes in thyroid hormones (Kuenzel 2003). Sequence of molt also is modulated by thyroid hormones (Payne 1972). Feather loss and regrowth occurs in a regular sequence along each feather tract. Patterns in thyroxine-induced molts may depart from the orderly sequence of feather replacement observed in natural molts. The melanin content and structure of barbules in feathers, that would affect feather color and patterns, also can be altered by high levels of circulating thyroid hormones (Payne 1972).

The timing and quality of feather loss and regrowth can affect many aspects of a bird's life. Thermoregulation and flight can be significantly affected by changes in plumage production (Dawson et al. 2000). Feathers also communicate mate quality and age in a number of species. The plumage colors and patterns measured in this study are important secondary sexual characters for the American Kestrel (Wiehn 1997a, 1997b). The objectives of this study were to: (1) investigate the roles of thyroid hormones in molt in American Kestrels, and (2) determine the relative roles of thyroxine in plumage pigmentation.

Methods

American Kestrels used in this study were 1-yr-old offspring from a captive colony at the U.S. Geological Survey Patuxent Wildlife Research Center (Laurel, MD). Pairs were randomly assigned to flight cages measuring ca. 24 × 4 × 6 m. Seven pairs of kestrels per treatment received food that was treated with either 10 ppm thyroxine (3,3′,5,5′ tetradiodo-L-thyroxine free acid) or 2000 ppm propylthiouracil (6-n-propyl-2-thiouracil), a thyroid hormone blocker (Sigma Chemical Co., St. Louis, MO U.S.A.). Treatment levels were chosen to be nonlethal, but above estimated no-observed-effects levels (May 1980, Leung et al. 1985, Lien et al. 1987, Kai et al 1988, Siopes 1997, al-Afaleq and Homeida 1998). Chemicals were mixed with rice flour, and the resulting mixture was added to the kestrels' standard diet of horsemeat (Nebraska Brand Bird-of-Prey Diet, Central Nebraska Packing, North Platte, NE U.S.A.). Rice flour constituted 1% of the final mixture; therefore, six pairs of kestrels received the control diet of horsemeat with flour and without chemicals at 1%. Treated food was given ca. 1 wk before egg laying began (second week of June) and ended when the birds finished molting in the fall (second week of October). Each kestrel was fed two 2.0 g treated horsemeat balls daily from Monday through Friday and two untreated mice on Saturday. They were fasted on Sunday to help ensure hunger for the treated food.

We collected the outermost secondary-flight feathers from the right wing and the second rectrix from the middle on the right side of the tail from each male and female after they had regrown under treatment. Feather position was numbered according to Willoughby (1966) The width of the male black subterminal band on the

[1] Email address: mquinn@umd.edu

second rectrix was measured at the rachis to the nearest 0.1 mm using slide calipers.

We assessed color in two ways: by comparing the colors of the feathers to color chips in a Munsell soil color chart (Macbeth 1994), and by measuring feather reflectance from 230–800 nm with a reflectance spectrophotometer (PerkinElmer UV/VIS/NIR Spectrophotometer, Lambda 19, Norwalk, CT U.S.A.). For visual analysis, the colors were ranked from 1 (light) to 4 (dark) according to four soil color chips per color that best matched the brown or gray colors analyzed (Quinn et al. 2002). When we ranked females' rectrices, we measured the first brown band above the black subterminal band on the wider side of the vane. For females' secondaries, we ranked the area in the first complete band from the tip. Color was ranked in the first available 3 × 10 mm area of brown right above the subterminal band on males' rectrices and in a similar sized area of charcoal gray on the males' secondaries. An overall score was determined by taking the mean of the tail and wing ranks, and this score was used in subsequent analyses.

The same areas of color on retrices that were scored with the soil color chart were analyzed for reflectance using the reflectance spectrophotometer. Reflectance measurements were recorded from 230–800 nm at 1 nm intervals. A 3 × 10 mm diameter spot on each area of color was presented flat against the aperture of the spectrophotometer, with the vane perpendicular to the beam of light. Data consisted of reflectance calculated as a percentage of light reflected from a standard white block of vitrolite, a near-flawless diffusing surface. Reflectance measurements were conducted at the National Institute of Standards and Technology, Gaithersburg, MD.

The progress of molt for primaries and retrices was recorded weekly by capturing each bird and recording feathers lost and the length of growing feathers. Timing of molt was measured by date of onset and total duration of molt. Date of molt onset is the date when the first primary feather dropped. The duration of molt was measured as the interval between the onset of molt and the complete regrowth of the last rectrix. The sequence of molt was averaged within treatment groups for each primary and rectrix and compared to sequences recorded by Willoughby (1966).

Approximately 1.0 ml of blood was collected weekly from the jugular vein using a syringe rinsed with a sodium heparin solution. Each sample was centrifuged, and the plasma was collected and stored at −70°C. Plasma thyroxine was measured in duplicate samples with a double antibody radioimmunoassay (RIA; Wilson and McNabb 1997). We validated the RIA for hormone measurements on kestrel plasma by demonstrating parallelism of the standard curve and diluted and hormone-spiked samples of kestrel plasma. Sensitivity was 12.5 μl and precision, as coefficient of variation, was 3.1%.

Data on reflectance were averaged for every 100 nm from 800–400 nm (visual range) and analyzed by multivariate analysis of variance (MANOVA). Data from 399–230 nm (UV range) were analyzed separately by two-way analysis of variance (ANOVA). Plasma-thyroxine levels were analyzed by analysis of covariance with week of sample collection as the covariate to account for possible time differences. All other data were analyzed by two-way ANOVA. Data were analyzed separately by sex for sexually-dimorphic characters and when sex-by-treatment interactions were significant. Tukey tests were used for *post hoc* pairwise comparisons. Statistical computations were performed using SPSS for Windows, Version 8.0 (SPSS 1998).

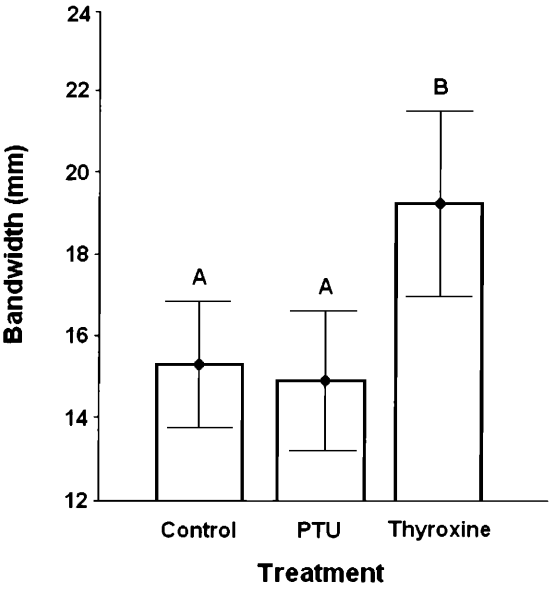

Figure 1. Width (mm) of subterminal bands on the retrices of male American Kestrels treated with 10 ppm thyroxine or 2000 ppm propylthiouracil. Error bars are standard errors of the mean. $N = 7$ for the thyroxine and propylthiouracil treatments and 6 for the control treatment. Means labeled as "A" are significantly different than those labeled as "B."

RESULTS

There was no overall effect of treatments on plumage color as measured by the Munsell color scores. However, treatments did have a significant effect on the width of the subterminal bands on male retrices (Fig. 1; $P = 0.02$). Subterminal bands from thyroxine-treated birds were ca. 4 mm wider than those from control ($P = 0.038$) and PTU ($P = 0.008$) treated birds.

Reflectance of feather colors did not differ significantly among males of different treatments in the visual range, and only marginal differences existed for males in the ultraviolet range ($P = 0.07$). However, there were significant treatment differences between reflectance for females (Fig. 2) in the visual ($P = 0.004$) and the ultraviolet ranges ($P = 0.019$). Feathers from thyroxine-treated birds were significantly more reflective of light of wavelengths from 400–700 nm (400–499 nm: $P < 0.05$; 500–599 nm: $P < 0.05$; 600–699 nm: $P < 0.05$) and only marginally more reflective from 700–800 nm ($P = 0.057$).

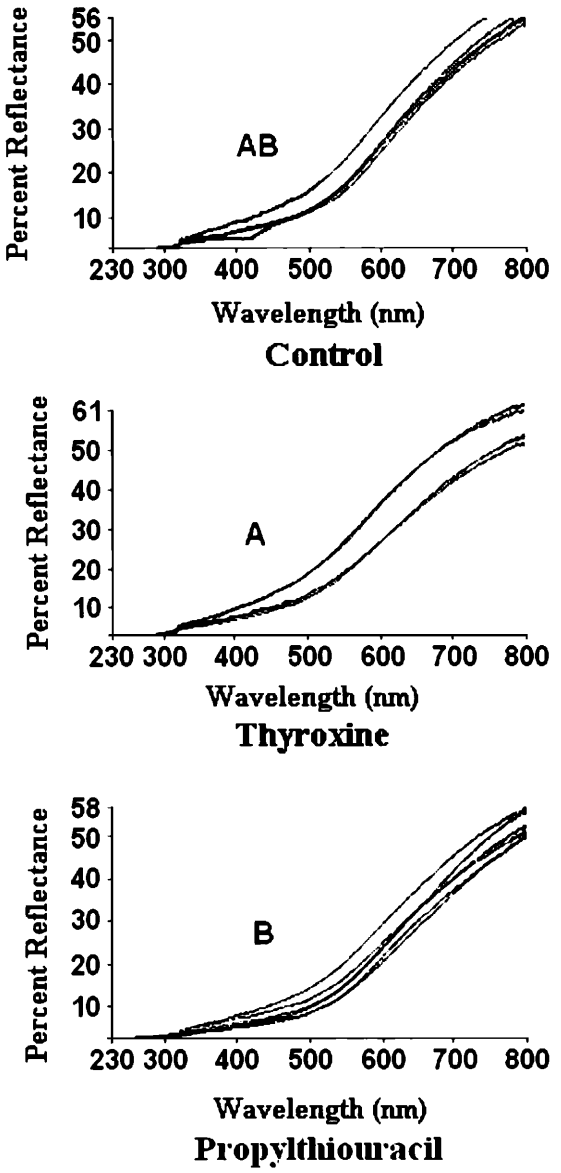

Figure 2. Reflectance of feathers in the visual (400–800 nm) and ultraviolet (230–399 nm) ranges from female American Kestrels treated with 10 ppm thyroxine or 2000 ppm propylthiouracil. $N = 4$ for the thyroxine treatment, 6 for the propylthiouracil treatment, and 5 for the control treatment. Mean reflectance from graph labeled as "A" was significantly different than graph labeled as "B."

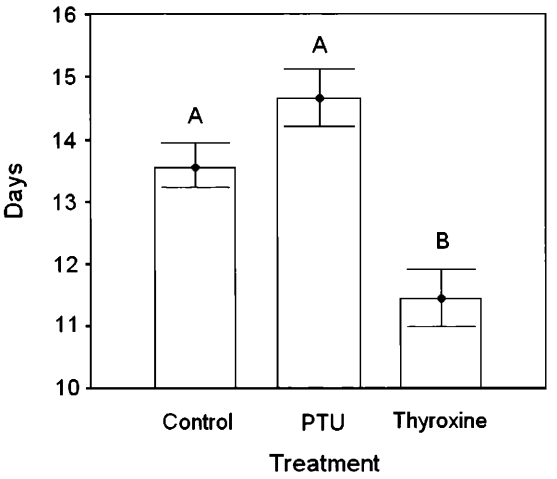

Figure 3. Mean (±SE) number of days in molt period of American Kestrels treated with 10 ppm thyroxine or 2000 ppm propylthiouracil. Error bars are standard errors of the mean. $N = 7$ for the thyroxine and propylthiouracil treatments and 6 for the control treatment Means labeled as "A" are significantly different than that labeled as "B."

The duration of molt (Fig. 3) differed significantly among the treatment groups in both males and females ($P < 0.001$). Birds that received thyroxine-treated food required fewer days to complete their molt than birds from control ($P < 0.005$) and PTU ($P < 0.001$) treatments. The sequence of molt from the thyroxine-dosed kestrels differed from the rest of the treatment groups only in the order of loss and replacement of the first and tenth primaries. The primaries from the thyroxine group molted in the same order as wild American Kestrels observed by Willoughby (1966). Although the sequence of molt for the retrices did not differ among treatments, slight differences were observed between all our treatment groups and Willoughby's birds in the sequence of molt of the last three retrices. However, the variations in our observed molt sequences did not deviate from the natural pattern of variability originally described by Willoughby.

Hormone assays confirmed clearly the effects of the thyroxine treatment on plasma thyroxine concentrations in both males and females from the second to the fifth week of treatment ($P < 0.0001$; Fig. 4). Plasma thyroxine concentrations from PTU-treated kestrels were consistently lower than controls; however, these differences were not significant.

DISCUSSION

The goals of this research were to determine the relative roles of thyroxine in plumage pigmentation and molt in American Kestrels. The observed changes in

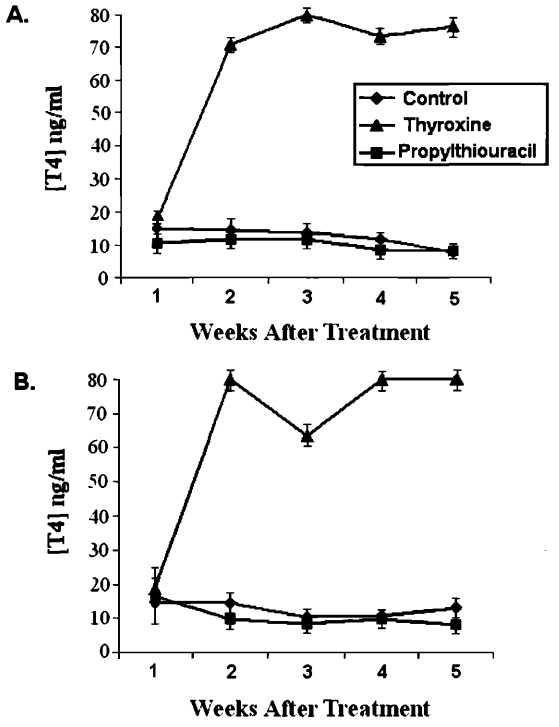

Figure 4. Mean (±SE) plasma thyroxine concentrations (ng/ml) in male (A) and female (B) American Kestrels treated with 10 ppm thyroxine or 2000 ppm propylthiouracil. Error bars are standard errors of mean. $N = 7$ for the thyroxine and propylthiouracil treatments and 6 for the control treatment.

feather pattern and reflectance in thyroxine-treated birds suggest an influence of thyroxine on feather pigmentation. No such alterations were observed in the PTU-treated birds, suggesting that although thyroid hormones modulate certain aspects of molt and feather production, other hormones may also play a role. Estradiol, testosterone, and prolactin also have strong effects on feather loss and regrowth (Vevers 1962, Nolan et al. 1992, Dawson and Sharp 1998). Clearly more studies are needed to better understand the hormonal interactions in this complex process.

Our data suggest that thyroxine may not play as significant a role in the American Kestrel for initiation of molt as in other species. Although the onset of molt was unaffected by treatments, thyroxine decreased the amount of time required to complete a molt. Dawson et al. (2000) state that in most birds, onset of molt is most heavily influenced by termination of breeding and that rate of molt is more affected by seasonal changes in day length. Longer day lengths combined with increased levels of thyroxine play a stronger role in implementing refractoriness to reproduction (Wilson and Reinert 1996, Siopes 1997). During this time, birds are reproductively unresponsive to light and, therefore, are able to devote more energy and resources to molting and plumage production. A possible explanation of our results is that the onset of molt may only require a threshold level of thyroxine that may have been exceeded in all of the treatments. If this was true, we may have seen an effect in the PTU-treated birds if that treatment had significantly lowered thyroxine levels.

In regard to the slight differences observed in molt sequence, Payne (1972) suggested that the pattern of feather loss and regrowth may be altered in thyroxine-induced molts. However, there is little evidence in the current literature or in our study to support this. Sequence of molt may be modulated more by genetic factors than hormonal ones.

Feather loss and regrowth involve an intricate interplay of environmental, nutritional, behavioral, and hormonal factors that varies among species (Berry 2003, Kuenzel 2003). Much of what is known about feather production comes from studies of poultry and songbirds. This was the first study to the authors' knowledge that begins to examine the role of the endocrine system on molt and plumage pigmentation in a raptor. We found that thyroxine modulated plumage production in American Kestrels; however, it appeared that other unidentified factors may have played stronger roles in this process. More studies are needed to explore the individual roles and interactions of the myriad of signals that are involved with feather loss and regrowth.

PAPEL DE LA TIROSINA EN LA PRODUCCIÓN DEL PLUMAJE EN *FALCO SPARVERIUS*

RESUMEN.—Aunque es sabido que el desarrollo del plumaje es modulado en parte por la acción de las hormonas tiroideas, se sabe poco sobre el grado al cual estas hormonas controlan la pérdida y reemplazo de las plumas y la pigmentación en las aves rapaces. En este estudio, halcones de la especie *Falco sparverius* recibieron alimento tratado con 10 ppm de tirosina o con 2000 ppm de propiltiouracilo (PTU, un bloqueador de la tiroides) diariamente desde una semana antes de que comenzara la puesta de huevos hasta el final de la muda. La duración de la muda de las plumas de vuelo fue significativamente más corta en halcones que recibieron tirosina La aparición de las plumas fue evaluada midiendo el color (utilizando una carta de colores de referencia), la reflectancia de 230–800 nm y el ancho de la banda subterminal de la cola del macho. Los halcones tratados con tirosina tuvieron bandas terminales significativamente más anchas. Las plumas de hembras tratadas con tirosina presentaron valores de reflectancia significativamente mayores, tanto dentro del rango visual como del ultravioleta, que las hembras que recibieron PTU.

[Traducción del equipo editorial]

ACKNOWLEDGMENTS

We are thankful for the guidance of Yvonne Barnes at the National Institute of Standards and Technology with the reflectance measurements. We are also grateful for the help and guidance of Greg Paulson and Tim Maret at Shippensburg University and the helpful suggestions of the *Journal of Raptor Research* referees. This work was funded in part by EPA Star Grant No. R-827400-01-0.

LITERATURE CITED

AL-AFALEQ, A.I. AND A.M. HOMEIDA. 1998. Effects of low-doses of oestradiol, testosterone, and dihydrotestosterone on the immune response of broiler chicks. *Immunopharmacol. Immunotoxicol.* 20:315–327.

BERRY, W.D. 2003. The physiology of induced molting. *Poult. Sci.* 82:971–980.

DAWSON, A., S.A. HINSLEY, P.N. FERNS, R.H.C. BONSER, AND L. ECCLESTON. 2000. Rate of molt affects feather quality: a mechanism linking current reproductive effort to future survival. *Proc. R. Soc. Lond.* 267:2093–2098.

——— AND P.J. SHARP. 1998. The role of prolactin in the development of reproductive photorefractoriness and postnuptial molt in the European Starling (*Sturnus vulgaris*). *Endocrinology* 139:485–490.

KAI, O., H. NAGASE, N. ISHIKAWA, M. SUZUKI, T. KAKEGAWA, AND K. SATO. 1988. Effects of propylthiouracil (PTU) on the immunological status of the chicken. *Dev. Comp. Immunol.* 12:145–156.

KUENZEL, W.J. 2003. Neurobiology of molt in avian species. *Poult. Sci.* 82:981–991.

LEUNG, F.C., J.E. TAYLOR, AND A. VAN IDERSTINE. 1985. Effects of dietary thyroid hormones on growth, plasma T3, and T4, and growth hormone in normal and hypothyroid chickens. *Gen. Comp. Endocrinol.* 59:91–99.

LIEN, R.J., J.R. CAIN, AND S.L. BEASOM. 1987. Effects of dietary and parental estrogens on bobwhite reproduction. *Poult. Sci.* 66:154–161.

MACBETH. 1994. Munsell soil color chart. Macbeth Division of Kollmorgen Instrument Corporation, New Windsor, NY U.S.A.

MAY, J.D. 1980. Effect of dietary thyroid hormone on growth and feed efficiency of broilers. *Poult. Sci.* 59: 888–892.

NOLAN V., JR., E.D. KETTERSON, C. ZIEGENFUS, D.P. CULLEN, AND C.R. CHANDLER. 1992. Testosterone and avian life histories: effects of experimentally elevated testosterone on prebasic molt and survival in male Dark-eyed Juncos. *Condor* 94:364–370.

OWENS, I.P.F. AND R.V. SHORT. 1995. Hormonal basis of sexual dimorphism in birds: implications for new theories of sexual selection. *Trends Ecol. Evol.* 10:44–47.

PAYNE, R.B. 1972. Mechanisms and control of molt. Pages 103–155 *in* D.S. Farner and J.R. King [EDS.], Avian biology. Academic Press, New York, NY U.S.A.

QUINN, M.J., JR., J.B. FRENCH, JR., F.M.A. MCNABB, AND M.A. OTTINGER. 2002. The effects of polychlorinated biphenyls (Aroclor 1242) on thyroxine, estradiol, molt, and plumage characteristics in the American Kestrel (*Falco sparverius*). *Environ. Toxicol. Chem.* 21· 1417–1422.

SIOPES, T. 1997. Transient hypothyroidism reinitiates egg laying in turkey breeder hens: termination of photorefractoriness by propylthiouracil. *Poult. Sci.* 76:1176–1782.

SPSS Inc. 1998. SPSS base 8.0 for Windows user's guide SPSS Inc., Chicago, IL U.S.A.

VEVERS, H.G. 1962. The influence of the ovaries on secondary sexual characters. Pages 263–289 *in* S. Zuckerman [ED.], The ovary. Academic Press, New York, NY U.S.A.

WIEHN, J. 1997a. Plumage characteristics as an indicator of male parental quality in the American Kestrel. *J. Avian Biol.* 28:47–55.

———. 1997b. Mate choice and reproductive success in the American Kestrel: a role for blood parasites? *Ethology* 103:304–317.

WILLOUGHBY, E.J. 1966. Wing and tail molt of the sparrow hawk. *Auk* 83:201–206.

WILSON, C. AND F.M.A. MCNABB. 1997. Maternal thyroid hormones in Japanese Quail eggs and their influence on embryonic development. *Gen. Comp. Endocrinol.* 107:153–165.

WILSON, F.E. AND B.D. REINERT. 1996. The timing of thyroid-dependent programming in seasonally breeding male American Tree Sparrows (*Spizella arborea*). *Gen Comp. Endocrinol.* 103:82–92.

Received 27 June 2003; accepted 6 October 2004