Seasonal Changes in Supraorbital Comb Characteristics and Fecal Testosterone Levels in Captive Male Svalbard Rock Ptarmigans (Lagopus muta hyperboreus)

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[Received 14 November 2015; accepted 8 April 2016]

ABSTRACT

The comb is a good indicator of physical condition in male birds. We monitored the seasonal changes in supraorbital comb characteristics with visual assessments and fecal testosterone concentrations by enzyme immunoassay in three captive male Svalbard rock ptarmigans (Lagopus muta hyperboreus). Although there were interindividual variations, the size and redness increased from February to August; i.e., before and during breeding season. The fecal testosterone levels increased markedly between May and June, and declined sharply from late June. The color simultaneously changed from deep to pale red but size was kept until testosterone concentrations fell to baseline. We suggest that changes occurring in supraorbital comb, especially its redness, reflect testicular activity and may be useful indicators for captive breeding of this species.

Key words: Supraorbital comb, Svalbard rock ptarmigan, Testosterone

Tetraonidae birds, belonging to order Galliformes, have a supraorbital comb, which acts as a secondary sexual trait, and the characteristics of the comb are strongly related to reproductive success. Sexual ornamentation (the color, length, and size of the comb in males) and the role of testosterone (T) have been studied in male red jungle fowl (Gallus gallus) and domestic fowl (G. domesticus) species [1-3]. In birds, comb is usually more conspicuous in males than in females [4]. Accumulating evidence suggests that fleshy comb plays a prominent role as a sexual signal in male red jungle fowl, but Parker and Ligon [5] indicated in their recent review that the influence of T on comb growth is dependent on bird’s physical condition (such as health and immune function). In addition, comb acts as an important signal during male-male competition and impacts on mate choice by females [1]. A relationship between T levels and comb has been detected in Tetraonidae birds. T treatment induced comb growth in willow ptarmigans (Lagopus lagopus lagopus) even though the birds had been kept in short day conditions [6]. Stokkan [7] reported that comb growth is directly dependent on T levels and indirectly related to day length in willow ptarmigans. Studies of male willow ptarmigans have indicated that plasma T levels control comb expression [8] and that mating male willow ptarmigans have larger combs than unmating territorial males; however, the comb sizes of polygynous and monogamous males did not differ [9]. In addition, T might also regulate immune function, as suggested by the immunocompetence handicap hypothesis [10], which proposes that males who can afford to reduce the quality of their immune systems are able to express exaggerated sexual traits. In a previous study, T treatment had positive effects on the reproduction of red grouse (L. lagopus scoticus), e.g., it increased the birds’ comb size and competitive ability, whereas their weight decreased and they were carrying more parasites [11]. Female birds

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prefer males with outstanding sexual traits because they infer that such males have genes that enable their chick to resist parasite infections [12]. Thus, the combs of male birds might be indicative of their reproductive ability and immune status. To extrapolate, reproductive hormonal status based on the appearance of external structures, such as comb or wattle [4], may help captive breeding, especially during the pairing of male with female in preparation for the reproductive season.

The purpose of our study was to evaluate the relationships between supraorbital comb characteristics and T concentrations in male Svalbard rock ptarmigans (L. lagopus hyperboreus). We used fecal samples for the hormonal assays, which enabled us to perform longitudinal monitoring in a non-invasive manner.

Three male ptarmigans (studbook No. S53, S65, and S85; the birds were 2.5, 1.4, and 1.4 years of age at the start of the experiment) used in this study were kept at Toyama Municipal Family Park. Each bird was kept in a wire cage under controlled lighting conditions, as described in our previous study [13]. Maximum continuous lighting (0 h dark: 24 h light) was employed from mid-May to July, and minimal lighting (15.5 h dark: 8.5 h light) was provided from December to February. During the intervening periods, light exposure was increased or decreased at approximately 2-week intervals. The birds had ad libitum access to rabbit food pellets, Japanese mustard spinach (Brassica rapa var. perviridis), and water. Morphological changes in the supraorbital comb were visually assessed in all three males by the same zookeeper twice a month (usually on the 1st and 15th days of each month). Supraorbital comb size was visually evaluated using the following scoring system: 1 = small, 2 = medium, and 3 = large (Fig. 1). Supraorbital comb redness was classified as pale or deep. Excreted fecal samples were collected from two males (No. S53 and S65) on 1-4 days/week. The sampling period lasted from January 3 to November 13, 2012, for No. S53 and from December 20, 2011, to June 25, 2012, for No. S65. One ptarmigan (No. S65) died unexpectedly on June 28, 2012. The samples were stored at -20°C until hormone analysis. Each fecal sample was dried, and T was extracted using the methanol extraction method.

**Figure 1** Heads of male Svalbard rock ptarmigan showing supraorbital combs. The size of comb was visually evaluated using the following scoring system: 1 = small, 2 = medium, and 3 = large. Redness of combs was classified as either pale or deep.
Supraorbital comb and testosterone in male ptarmigan

Fecal T concentrations were assayed by an enzyme immunoassay, as reported previously [14]. The T values for No. S53 and S65 represent the mean concentrations for the period from day 1 to day 15 (the first half of the month) or from day 16 to the end of the month (the second half of the month).

Figure 2 shows the changes in the supraorbital comb status of all three males (No. S53, S65, and S85) studied and the changes in fecal testosterone concentrations for two males (No. S53 and S65). In No. S53 bird, marked changes in the size and redness of the supraorbital comb were noted on February 15 and March 15, respectively. The comb size score for No. S53 changed from 1 to 2 and then increased further to 3, and the color of its comb also changed from pale to deep red. The fecal T concentrations of No. S53 ranged from 24.23 ng/g in second half of February (n=1) to 388.03 ng/g in second half of May (n=8) (Fig. 2). This bird’s fecal T levels started to increase in April and peaked in late May. Changes in the color of No. S53’s supraorbital comb occurred simultaneously with the increase in its fecal T concentration. The fecal T concentrations of No. S53 fell markedly in late June, and its comb color became paler. However, No. S53 continued to exhibit a comb score of 3 until July 15. In this bird, the supraorbital comb started to increase in size and change to deep red in the lead up to the reproductive period, and a simultaneous rise in fecal T levels was also detected. The bird’s comb color switched to pale red as its fecal T concentrations fell, producing an annual cycle.

Thus, we infer that changes in comb color are a sensitive marker of circulating T levels, which in turn are indicative of fluctuations in testis activity. In No. S85 bird, the comb became bigger and its color became more intense later than in No. S53 (Fig. 2). Specifically, No. S85 first demonstrated a comb size score of 3 on May 15 (compared with May 1 in No. S53), and the color of the comb changed from pale to deep red on May 1 (compared with March 15 in No. S53). These differences might have been related to the males’ social ranks because it has been reported that supraorbital comb height is correlated with social rank in willow ptarmigans [15]. However, it is unclear whether social hierarchy among individuals do exist, as these birds were kept in cages individually. Moreover, existence in the difference of fecundity related age cannot be ignored. In red jungle fowl, used for male-male staged fights, older males (2-year old and above) had significantly longer spurs than 1-year old males [1]. Svalbard rock ptarmigans can breed from yearling age. The birds used in this study were 2.5 and 1.4 years old at the start of the examination and we monitored their third and second breeding challenges. Difference in reproductive success between ages (1 to 4-year old) have been reported in white-tailed ptarmigan (L. leucurus) and willow ptarmigan (L. l. perdix), where older males had higher reproductive success.
ptarmigan as well, with older females exhibiting high fecundity than 1-year old females [16]. These variations may be related the breeding condition too. Thus, results obtained in this study also may reflect the age difference; further research is necessary to confirm this inference.

In No. S65 bird, changes in supraorbital comb size/redness and fecal T concentrations were not as marked (Fig. 2) as those seen in No. S53 and S85. No. S65’s fecal T concentrations ranged from 30.54 ng/g in second half of February (n=3) to 172.26 ng/g in second half of May (n=8). As No. S65 died unexpectedly during the experimental period, we postulate that the factors that caused the bird’s death might have had a negative influence on the supraorbital comb changes.

Carotenoids also play a role in comb expression. For example, they are considered to affect coloration and self-maintenance (they buffer T-mediated immunosuppression), and the allocation of carotenoids is influenced by physiological constraints [17]. Based on the variations seen in No. S65, we consider that carotenoids are preferentially used for immunohomeostasis rather than comb exaggeration. Thus, the condition of the comb seems to provide information about immune status and general conditioning.

The condition and size of the supraorbital comb and T levels have been studied as characteristics associated with sexual selection and signaling in birds belonging to the Tetraonidae family [18-20]. Castrated male willow ptarmigans failed to show supraorbital comb growth even during photostimulation [7]; however, supraorbital comb growth was induced in male red grouses that were implanted with T under the skin of their breasts [19]. In copulating male black grouses (Tetrao tetrix), a significant positive relationship was detected between supraorbital comb size and copulatory success, and supraorbital comb size was significantly correlated with the T level [18]. Thus, in Tetraonidae birds supraorbital comb expression is regulated by testicular hormones.

In previous studies of Tetraonidae birds [8, 17, 18, 20], T assays were carried out by collecting blood samples. In contrast, we assessed the utility of the supraorbital comb as a non-invasive external index of testicular activity by visually assessing the supraorbital comb and the changes in fecal hormonal concentrations. The use of feces as an alternative to blood is non-invasive and makes repetitive collection feasible.

As such, we suggest that the appearance of the supraorbital comb can be used as an indicator of testicular status in male Svalbard rock ptarmigans had in captivity, and the color of the comb offers a good indication of testicular regression. These findings may aid decision-making regarding the timing of male and female pairing and assessments of the fertility potential and general conditions of male ptarmigans kept in captivity.

This study was supported by the Tokyo Zoological Park Society Wildlife Conservation Fund.

We acknowledge the assistance of Dr. Sachi Sri Kantha in improving the manuscript text.

REFERENCES


研究短報 繁殖学

飼育下スバールバルライチョウ（*Lagopus muta hyperboreus*）における眼窩上肉冠とテストステロン値の季節変化

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[2015年11月14日受領，2016年4月8日採択]

要 約

飼育下の雄スバールバルライチョウにおける眼窩上肉冠のサイズと赤さを目視により段階評価し、糞中テストステロン（T）含量と比較した。夏の繁殖期に向けて肉冠のサイズと色の増強がみられ、同時にテストステロン含量は5月から6月に著しく増加した。6月末にT含量の低下がみられ、肉冠は退色した。肉冠の変化は精巣活動を反映していることが示唆され、飼育下繁殖時の個体管理に役立つ指標になると考えられた。

キーワード：スバールバルライチョウ，テストステロン，肉冠

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