



An appraisal of endocrine activity in strutting sage grouse  
by Roger Cecil Thorvilson

A thesis submitted to the Graduate Faculty in partial fulfillment of the requirements for the degree of  
MASTER OF SCIENCE in Zoology  
Montana State University  
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**Abstract:**

An effort was made to appraise differential endocrine activity in reproductively active adult and subadult male sage grouse. Adult sage grouse showed significantly heavier testes than subadults. Pituitaries and thyroids were significantly heavier in subadults. Protein bound iodine (thyroxin) level was significantly higher in the serum of adult birds. There were no significant differences in the weights or histological appearance of adrenal glands in adults and sub adults. Testes weights were shown to be negatively correlated with pituitary, thyroid, and adrenal weights in subadults. Adult grouse showed a positive correlation between testes weights and adrenal weights, but thyroids and pituitaries negatively correlated, with testes and adrenals.

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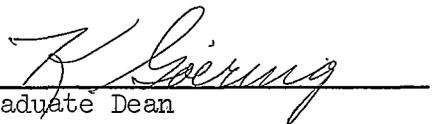
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Approved:

  
Head, Major Department

  
Chairman, Examining Committee

  
Graduate Dean

MONTANA STATE UNIVERSITY  
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## ABSTRACT

An effort was made to appraise differential endocrine activity in reproductively active adult and subadult male sage grouse. Adult sage grouse showed significantly heavier testes than subadults. Pituitaries and thyroids were significantly heavier in subadults. Protein bound iodine (thyroxin) level was significantly higher in the serum of adult birds. There were no significant differences in the weights or histological appearance of adrenal glands in adults and subadults. Testes weights were shown to be negatively correlated with pituitary, thyroid, and adrenal weights in subadults. Adult grouse showed a positive correlation between testes weights and adrenal weights, but thyroids and pituitaries negatively correlated with testes and adrenals.

## INTRODUCTION

In recent years there has been considerable study of the inter-relations between social behavior and endocrine functions in the control of animal reproduction. Christian (1959) and Christian and Davis (1964) have discussed the involvement of the pituitary-adrenocortical axis in an intrinsic control of population size in mammals. They propose that increased social interactions in dense animal populations trigger ACTH release by the pituitary, increased adrenocortical output, and the associated stress responses, which result in reduced reproduction. Highly dominant animals in the social hierarchy are least affected by crowding of populations.

Position in the social dominance order has been related to the number of successful matings, eggs fertilized, and viable offspring in chickens (Guhl and Warren, 1946). Siegel (1960) found heavier adrenals and pituitaries in cockerel chickens caged at high density levels. Heavier thyroids and adrenals, adrenal lipid depletion, and slow weight gain of testes, with accompanying degenerative changes have been found in socially subordinate male chickens caged with females in groups of six, as opposed to chickens caged as male-female pairs (Flickinger, 1961). Neave and Wright (1968) correlated the adrenal weights of ruffed grouse with population density on drumming sites.

The complex social structure present during the breeding season of the sage grouse has been investigated in considerable detail (Patterson, 1952). However, little work has been done to relate this behavior to

hormonal activity. Patterson (1952) and Eng (1963) have compared testis development of the adult males with that of subadult (yearling) males, but they did not study the other endocrine organs.

Sage grouse behavior during the breeding season has been described by Patterson (1952) and Lumsden (1968). Social dominance is strongly evident throughout the strutting season of the sage grouse. Among the adult males there is a social hierarchy, with a master cock and a few other dominant cocks performing most of the mating. However, all the adults act in repelling subadults from the main strutting ground. Any subadult that ventures onto the ground from the periphery is immediately chased off by an adult. No fighting occurs between adults and subadults, the subadults retreating without observable resistance. Although the subadults are physiologically capable of breeding, the adults effectively keep them from doing so.

It was the purpose of this study to collect both adult and subadult males from wild populations and to look for possible physiological differences between these two groups. No attempt was made to determine dominance patterns within either of the groups. The weights of adrenals, thyroids, pituitaries, and testes were recorded, histological appearances of the adrenals were compared, and an attempt was made to index blood levels of thyroid hormone.

## METHODS

### Collection and Handling

Thirteen sage grouse were collected from two strutting grounds in Fergus and Petroleum Counties, Montana, from March 30 to May 12, 1968. After a period of observation to distinguish between adult and yearling males, birds were collected with a 22 caliber rifle. Blood was collected from the major vessels and heart. If necessary, clotted blood from the body cavity was also taken. The testes and adrenal glands were excised and placed in separate marked containers. The glands and the blood were then placed on ice. The heads were removed and also put on ice in order to cool the pituitary body. Age determination was confirmed by examination of the outer two primary feathers for characteristic differences as described by Patterson (1952).

The pituitary body and the thyroid glands were excised in the laboratory. The adrenals, thyroids, testes, and pituitary were dissected free of surrounding tissue and weighed to the nearest 0.1 mg on a Mettler precision balance. The adrenal glands were preserved in 10% formalin and the other glands placed in absolute alcohol. Eight to ten hours elapsed from shooting until completion of weighing. The body weight was measured to the nearest 10 gm with a twin pan balance.

The means of gland weights and gland weight/body weight ratios were calculated for the adult and subadult groups, and the differences between means were tested for significance by analysis of variance. Group variances were compared with the F test.

7-13-68  
5-1-68  
2-1-68

### Protein Bound Iodine Determination

Blood which was collected from the birds in the field was allowed to stand approximately twenty four hours. The serum was then drawn off and placed in separate tubes. Initially the serum was stored under refrigeration until used. However, the serum from birds collected April 21 and later was frozen until use. Protein bound iodine determinations were started within two hours after thawing.

The Hycel method (Anon., 1967) of protein bound iodine determination was used to estimate the level of thyroxin in the blood. In this method the protein bound iodine in the serum acts as a catalyst in the decolorization of yellow ceric ammonium sulfate. Protein bound iodine is measured by the rate of change of ceric ion to colorless cerous ion. Transmittance at 435 mu was determined with a Bausch and Lomb Spectronic 20 colorimeter. From each serum sample, three one-ml portions were analyzed for protein bound iodine, and the mean of the transmittance values was recorded.

The preliminary step of removal of inorganic iodine with anionic exchange resin was not employed. Protein bound iodine was determined in several separate aliquots of the serum from a single animal. The exchange resin was used with half of these aliquots; this step was eliminated in determinations with the remaining half. No significant difference in results was found. Since results were comparable and use of the resin introduced a possible source of variability, this step was not used in the following analyses of bird sera.

An analysis of variance was used to compare the transmittance means and variances.

Adrenal Histology

Portions of adrenal glands were sectioned at eight microns and stained with hematoxylin and eosin. Interrenal (cortical) cells were compared as to cell size, separation of cells, degree of vacuolization, and size of nuclei. An interrenal cell/medullary cell ratio was derived from photomicrographs of the adrenal tissue.

## RESULTS

The means of body weights, gland weights, gland weight/body weight ratios, and PBI transmittance values are shown in Tables I and II. The adult birds' mean testes weight was significantly greater ( $p < 0.01$ ) than that of subadults. The testes weights relative to body weight were also significantly greater ( $p < 0.01$ ) in the adult group. The adrenal weight/body weight ratios were somewhat greater in the subadult group; however, this difference was not significant. Pituitary weight/body weight ratios were significantly higher ( $p < 0.05$ ) in the subadult grouse. Thyroid weights relative to body weight were also significantly greater ( $p < 0.01$ ) in subadults. The protein bound iodine level in the blood as shown by percent transmittance (percent transmittance is directly proportional to thyroxin concentration) was higher in the adult birds. This difference

Table I. The means, ranges and standard errors of body weights, gland weights, and gland weight/body weight ratios.

	Body Wt. (Kg)	Testes Wt. (Gm) **	Testes(Gm)/ Body(Kg) **	Adrenal Wt. (Mg)	Adrenal(Mg)/ Body(Kg)
<u>Adults</u>					
Mean	2.90	4.272	1.47	163.5	55.4
Range	2.67-3.06	3.17-5.06	1.12-1.80	123.1-234.6	43.6-76.7
S $\bar{x}$	---	0.24	0.09	---	5.7
N	7	7	7	5	5
<u>Subadults</u>					
Mean	2.40	2.165	0.89	135.7	56.7
Range	2.12-2.70	1.26-3.26	0.56-1.29	127.9-167.6	40.9-62.1
S $\bar{x}$	---	0.31	0.11	---	3.3
N	6	6	6	6	6

\*\* Difference between means is highly significant ( $p < 0.01$ ).

Table II. The means, ranges, and standard errors of gland weights, gland weight/body weight ratios, and PBI transmittance values.

	Pituitary Wt. (Mg)	Pit. (Mg)/ Body(Kg) *	Thyroid Wt. (Mg)	Thyroid(Mg)/ Body(Kg) **	PBI (% Trans.) *
<u>Adults</u>					
Mean	9.80	3.36	105.0	35.9	14.7(17.3) <sup>t</sup>
Range	7.50-11.7	2.52-4.15	87.1-143.9	29.2-50.8	6.6-18.0
S	---	0.34	---	4.1	2.0
$\bar{x}$					
N	5	5	5	5	5
<u>Subadults</u>					
Mean	9.90	4.13	112.2	62.5	8.9(15.3)
Range	7.40-12.1	2.92-4.84	96.9-205.6	38.3-97.0	5.7-17.5
S	---	0.29	---	9.2	2.0
$\bar{x}$					
N	6	6	5	5	6

\* Difference between means is significant ( $p < 0.05$ ).

\*\* Difference between means is highly significant ( $p < 0.01$ ).

<sup>t</sup> Values in parentheses are means of uncorrected transmittance readings.

was significant ( $p < 0.05$ ) when corrected transmittance values were compared.

This correction factor is discussed in the next section.

Variances for the adult and subadult groups did not differ significantly for any of the gland weights or the PBI levels. Thyroid weight relative to body weight varied much more in the subadults than in the adults; the F value of 5.88 approached significance at the 5% level.

An attempt was made to bioassay pituitary activity of both adults and subadults using frogs. Although standard procedures were followed, no activity could be demonstrated. Perhaps this was due to low pituitary activity, a low response of amphibians to bird gonadotropins, or to procedural deficiencies.

Adrenal cortical activity, as indicated by cell size and vacuolization of interrenal cells, did not appear to differ between adult and subadult males. There was considerable variation in the degree of separation of interrenal cells. This separation did not appear to correlate with age, however. As much variation was found within adult and subadult groups as was shown between them. Nor did histological appearance seem to correlate with time or with the adrenal weights. A grid was placed over photographs of adrenal sections, and the proportion of intersects touching interrenal cells was counted. The interrenal-medullary ratios did not differ significantly for adult (mean 2.19; range 1.24-3.33) and subadult (mean 2.31; range 1.85-3.70) birds.















