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Comparative Biochemistry and Physiology Part C 122 (1999) 173–179

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The effect of ACTH on the GnRH-induced release of LH and testosterone in male white-tailed deer

George A. Bubenik [a,*], Robert D. Brown [b], Dieter Schams [c], Ludek Bartos [d]

[a] *Department of Zoology, University of Guelph, Guelph, Ont. N1G 2W1, Canada*
[b] *Department of Wildlife and Fisheries, Texas A&M University, College Station, TX 77843, USA*
[c] *Department of Physiology, Technical University of Munchen-Weihenstephan, D-8050 Freising, Germany*
[d] *Research Institute of Animal Production, Ethology Group, CZ-104 00 Praha-10, Uhrineves, Czech Republic*

Received 17 December 1997; received in revised form 4 August 1998; accepted 13 August 1998

Abstract

In order to investigate the possible link between stress and the impairment of the reproductive system, 12 yearling white-tailed bucks, born to mothers captured wild in southern Texas, were immobilized every 6 weeks over the period of 1 year. In half of experiments deer were injected i.m. with 20 IU of ACTH; in the second half, we used saline only. Simultaneously, in each experiment we also injected all deer i.m. with 100 μg of GnRH. Three blood samples were taken before and seven after treatment and plasma levels of cortisol, LH and testosterone (T) were later measured by RIA. Half of our yearlings were born to mothers which were fed high-protein–high-energy (HP-HE) diet during their pregnancy; the other half was fed high-protein–low-energy diet (HP-LE). ACTH increased cortisol levels in both nutritional regimes. Cortisol levels in controls decreased with time but a more pronounced reduction was observed in HP-HE bucks as compared to HP-LE deer. GnRH significantly increased LH and T levels. However, only in summer, LH levels were higher in HP-LE fed deer than those fed HP-HE; in other seasons they were equal. Conversely, only in winter T levels were elevated in HP-HE fed deer as compared with HP-LE deer. We concluded that the pronounced suppression of reproductive hormones by ACTH or cortisol reported previously in domestic ungulates does not occur in white-tailed deer yearlings. Conversely, the low level of energy provided in food to mothers during their pregnancies significantly reduced peak levels of testosterone in their male offspring. This study further proved that white-tailed deer is a highly adaptable cervid species resistant to environmental stress.

Keywords: White-tailed deer; Reproduction; Nutrition; Stress; ACTH; GnRH; Cortisol; Luteinizing Hormone; Testosterone

1. Introduction

An overwhelming evidence link stress to the impairment of the reproductive system [11,12]. Research studies indicate, that the response to stress is mediated through the hypothalamo–pituitary axis. It was assumed that stress induces the release of ACTH, which then initiates secretion of glucocorticoids. These events can interfere with the hypothalamo–pituitary–gonadal axis [19–21]; however, the exact mechanism of this action is not yet known. Both, ACTH and cortisol lower plasma concentration of LH and testosterone in bulls [15] and decrease levels of testosterone in plasma of boars [18]. In addition, exogenous administration of ACTH, reduced GnRH-induced secretion of LH in heifers [19,21]. Finally, results of a similar study performed in rams [13] indicate that the impairment of LH secretion after stress is probably a direct effect of ACTH alone and not a result of a subsequent release of cortisol. Conversely, in bulls an increase in cortisol secretion induced by an intense sexual stimulation did not alter testosterone levels [27]. Deer species vary substantially in their tolerance to social stress. Some

* Corresponding author. Tel.: +1 519 8244120, ext. 8786; fax: +1 519 7671656; e-mail: gbubenik@uoguelph.ca

0742-8413/99/$ - see front matter
PII S0742-8413(98)10101-9

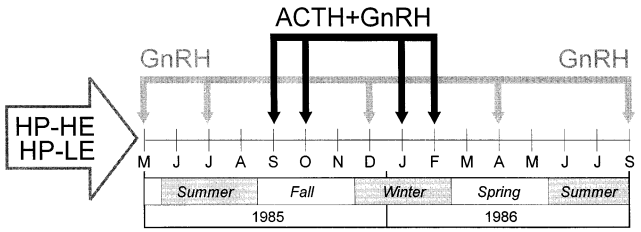

Fig. 1. Schematic diagram of the experimental design. HP-HE, high-protein–high-energy diet; HP-LE, high-protein–low-energy diet. ACTH, 20 IU per deer; GnRH, 100 μg per deer.

species, such a white-tailed deer (*Odocoileus virginianus*) can tolerate an extremely high population density without exhibiting signs of stress [23]. Conversely, in other species, such as red deer (*Cervus elaphus*), stress might alter secretion of adrenal steroids as was manifested in the impairment of their antler cycle [28].

In order to test the hypothesis that submature white-tailed deer exposed to stress might have an impaired reproductive response, GnRH-induced secretion of LH and testosterone was determined in plasma of yearling white-tailed bucks, in which stress was simulated by simultaneous administration of ACTH. In addition, as male offsprings of mothers who were stressed during their pregnancies were exhibiting impaired testosterone response [30], we decided to test a secondary hypothesis postulating that in white-tailed deer the levels of energy in diet of mothers would influence response of reproductive hormones to ACTH in their sons.

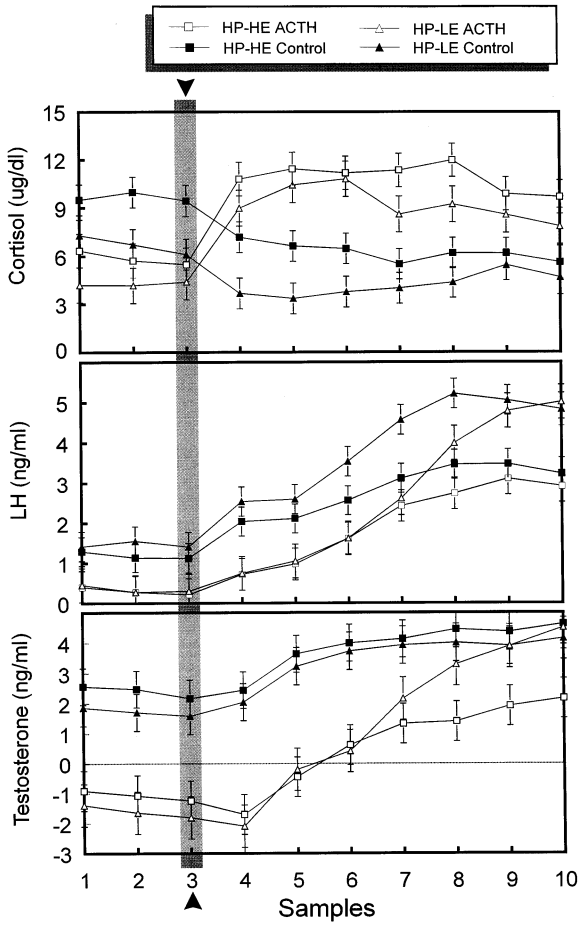

Fig. 2. LSMEANs ($\pm$ SE) of cortisol (μg dl^{-1}) (top), LH (ng ml^{-1}) (middle), and testosterone (ng ml^{-1}) (bottom) during the sampling. Vertical bars indicate application of GnRH (100 μg per deer) and ACTH (20 IU per deer) (or saline).

2. Material and methods

2.1. Animals and experimental design

Twelve yearling white-tailed bucks, born to mothers captured wild in southern Texas, were kept in a 0.5-ha enclosure at the Caesar Kleberg Wildlife Research Institute of the Texas A&I University in Kingsville, Texas. During the pregnancy, the mothers of our yearlings were fed two types of diet: (a) high-protein–high-energy (HP-HE); and (b) high-protein–low-energy (HP-LE). The composition of these diets were described in the previous paper [10]. The yearlings were fed year round a standard pelleted deer ration ad libitum which, except for winter months (December–February), was supplemented by a browse of deciduous trees and a fresh sorghum. During a 16-month period (from May 1985 to September 1986) all bucks were immobilized every 6 weeks by a 1:1 mixture of Rompun and Ke-

Table 1
Results of GLM procedure for cortisol, LH, and testosterone

Class	df	Cortisol		LH		Testosterone	
		F	*P*<	*F*	*P*<	*F*	*P*<
Model	51 827	8.53	0.001	16.19	0.001	16.57	0.001
Sample (nutrition/ACTH application/Category)	37 827	5.78	0.001	8.68	0.001	7.72	0.001
Category	1827	10.19	0.001	305.52	0.001	109.87	0.001
Nutrition	1827	5.61	0.01	5.20	0.05	0.00	NS
Deer	9827	16.66	0.001	7.05	0.001	4.83	0.001
Season	3827	14.93	0.001	50.18	0.001	173.67	0.001

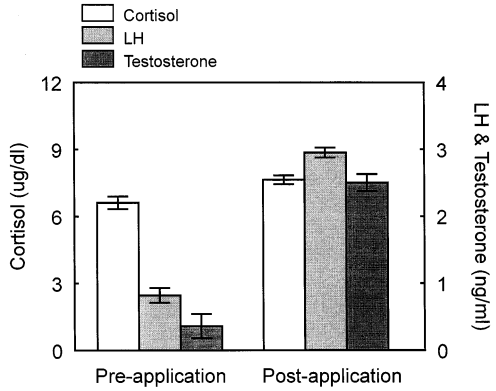

Fig. 3. LSMEANs (± SE) of cortisol, LH and testosterone according to Category.

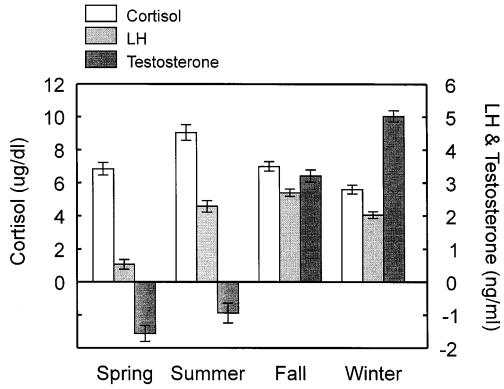

Fig. 5. LSMEANs (± SE) of cortisol, LH and testosterone according to season.

tamine, delivered by a Telinject dart pistol. The average dose of combined anesthetic was around 3 mg kg^{-1} body weight.

After immobilization (10–15 min after injection) a polypropylene cannula (Cathlon, Mississauga, Ont., Canada) was inserted into a jugular vein and secured in place by a suture. Three blood samples (5 ml each) were then drawn in 10-min intervals. After that, 100 μg of gonadotropin-releasing hormone (GnRH) (Hoechst, Frankfurt, Germany) was administered i.m. and seven more blood samples were taken in 30-min intervals. After completion of sampling the deer were given antibiotics i.m. and an antidote, yohimbine (0.125 mg kg^{-1}), was injected i.v. in order to speed up recovery. All samples were kept on ice and centrifuged within 2 h of their collection. Plasma was then frozen at -20°C for later radioimmunoassay (RIA).

In May, July, and December of 1985 and April and September 1986[1] only GnRH was administered. In September and October 1985, and January and February of 1986, GnRH was preceded by an i.m. administration of 20 IU of porcine adrenocorticotropic hormone (ACTH) (ACTHAR Gel H.P., Armour Pharmaceutical Co., Kankakee, IL, USA). The schematic diagram of the experimental design is depicted in Fig. 1.

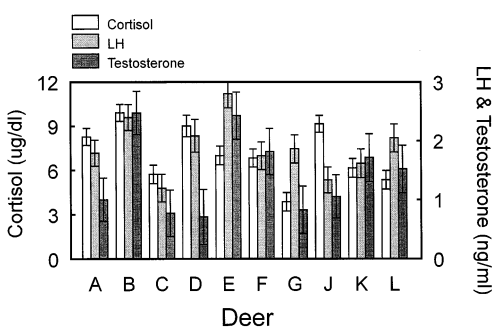

Fig. 4. LSMEANs (± SE) of cortisol, LH and testosterone according to individual deer.

[1] September of 1986 was added to the basic 1-year sampling period (May–April) in order to elucidate whether a 1-year difference in age would influence the pre-treatment levels.

2.2. Determination of hormones

Details of RIA for testosterone, LH and cortisol were described in detail in previous publications [1,4,16,22,25]. Testosterone assay was validated for white-tailed deer plasma by recovery experiment. There was a negligible crossreactivity of the testosterone antisera with other steroid hormones. Sensitivity of testosterone assay was 50 pg ml^{-1}. The interassay coefficient of variation (CV) was 7.5–12%. Inhibition curves using white-tailed deer pituitary extracts and deer plasma samples showed parallelism with the bovine LH standard. The sensitivity of LH assay was 0.05 ng per tube. The interassay CV averaged 8.6%; the interassay CV was 11.5–19%. Cortisol was measured using commercially available RIA kit (Joldan Bioclinical, Scarborough, Ont., Canada). Cortisol assay was validated for deer by recovery experiment. The sensitivity of assay was found to be < 0.14 μg dl^{-1}. Inter-assay CV was $\sim 7.5\%$; intra-assay CV was 20.8%.

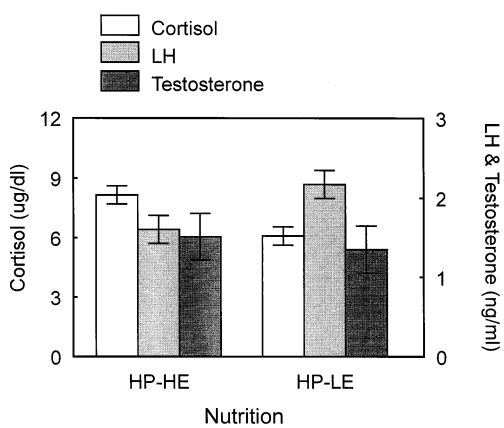

Fig. 6. LSMEANs (± SE) of cortisol, LH and testosterone according to nutrition.

Table 2
Spearman residual correlations between cortisol, LH and testosterone

	Pre-application			Post-application		
	n	r_s	*P*<	*n*	r_s	*P*<
ACTH application						
Cortisol vs LH		−0.14	NS		−0.10	NS
Cortisol vs testosterone	113	0.32	0.001	266	0.33	0.001
LH vs testosterone		−0.19	0.05		0.25	0.001
No ACTH application						
Cortisol vs LH		0.26	0.01		−0.05	NS
Cortisol vs testosterone	137	0.25	0.01	312	0.16	0.01
LH vs testosterone		0.67	0.001		0.23	0.001

2.3. Statistics

The data were subjected to the General Linear Models Procedure (GLM) for Unbalanced ANOVA (SAS). Classes were 'deer' (individual males marked A, B, C, D, E, F, G, J, K and L), 'nutrition' (HP-HE and HP-LE), 'samples' (1–10), 'ACTH application' (yes and no), 'category' (preapplication and postapplication), and 'season' (spring, summer, fall, and winter). The class 'sample' was nested within classes in interaction 'nutrition', 'ACTH application', and 'category'. Hormonal levels of animals treated with ACTH are referred to as 'ACTH treated', while others are called 'controls'. Least-square means (LSMEANs) were computed for each class and differences between classes were tested by *t*-test. LSMEANS and Spearman residual correlation coefficients were computed according to specific aspects described bellow.

3. Results

Models for all three hormones showed highly significant variation (Table 1). Sample values of interacting classes 'nutrition', 'ACTH application', and 'category' (Fig. 2), 'category' (Fig. 3), 'deer' (Fig. 4), and 'season' (Fig. 5) demonstrated significant influence on at least the $P < 0.01$ level (Table 1). The only exception was 'nutrition' that showed no difference between HP-HE and HP-LE groups in concentrations of testosterone, while cortisol levels of HP-HE were higher than those in HP-LE animals. In the case of LH the results were just the opposite (Fig. 6).

ACTH-treated deer showed significantly lower preapplication levels of cortisol (and LH and testosterone as well) than control animals (ACTH-treated versus controls, $P < 0.02$, for both, HP-HE and HP-LE groups). Nevertheless, no post-treatment change was detected in controls. Conversely, ACTH-treated animals demonstrated a significant increase in cortisol levels after ACTH application (Fig. 2, top). All post-application levels were higher than those before the treatment (at least $P < 0.01$). In contrast, control levels tended to decrease with time. In HP-HE animals, all levels after saline application were significantly lower (at least $P < 0.05$) than the levels before the application, except in sample 4 ($P = 0.06$). In HP-LE animals the decrease of cortisol levels was less pronounced. After a temporary decrease in samples 4–6 (at least $P < 0.05$ versus preapplication levels). Then the levels gradually increased again reaching the preapplication amplitude in some cases.

No simple consequence of ACTH application was apparent for either LH (Fig. 2, middle) or testosterone levels (Fig. 2, bottom). Overall, LH tended to increase (Fig. 2, middle). ACTH-treated, HP-LE animals exhibited a temporary, significantly lower increase than the HP-HE controls (in samples 4–6, at least $P < 0.05$). Similarly, the HP-LE ACTH-treated deer had lower LH levels between samples 4 and 8 (at least $P < 0.05$). The initial, preapplication testosterone levels were significantly higher in controls than in ACTH-treated bucks. Therefore, we were unable to distinguish the effect of ACTH in most post-treatment samples and the suppression of testosterone levels in response to GnRH was detected only in sample 4.

In all three hormones, postapplication levels were higher than those before the application (Fig. 3). The individual deer have shown significant variation in either hormone (Fig. 4). There were meaningful seasonal differences in all hormones (Fig. 5). The highest cortisol levels were found in summer ($P < 0.001$ against all other parts of the season). Cortisol concentrations in spring and fall were higher than those in winter ($P < 0.05$ and $P < 0.001$, respectively). The lowest seasonal LH levels were detected in spring ($P < 0.01$ against summer, fall and winter). Summer LH levels differed neither from fall nor winter amplitudes ($P < 0.05$). Winter LH levels were lower than those in fall ($P < 0.01$). Testosterone concentrations were lowest in the spring

Table 3
Results of GLM procedure for cortisol, LH, and testosterone according to season and nutritional status

Class	df	Cortisol		LH		Testosterone	
		F	*P*<	*F*	*P*<	*F*	*P*<
Model	25 448	10.78	0.001	20.09	0.001	54.13	0.001
Season (nutrition)	7448	15.91	0.001	27.32	0.001	167.95	0.001
Sample	9448	6.66	0.001	27.17	0.001	13.49	0.001
Deer	9448	7.33	0.001	3.44	0.001	3.93	0.001

and highest in winter. All seasons differed from each other ($P < 0.001$) except spring and summer ($P = 0.06$).

In order to clarify the relationship between cortisol and LH and testosterone, Spearman residual correlations were calculated for the Pre- and postapplication periods (Table 2). These correlations were calculated after the treatment of the data by the GLM model shown in Table 1.

To solve the above hypothesis that deer fed a low-energy diet will exhibit an impaired activation of the hypothalamo–pituitary–gonadal function, only the data on saline-treated deer were included, in order to eliminate a possible influence of ACTH treatment. Classes in the GLM model were 'season' nested to 'nutrition', 'sample', and 'deer'. GLM analyses demonstrated a significant variation for all three hormones and all classes involved (Table 3). No difference between groups of deer based on nutritional status were shown in cortisol levels across seasons (Fig. 7). LH levels were higher in HP-LE fed deer than those fed HP-HE in summer ($P < 0.01$), while in other seasons they were equal (Fig. 8). On the other hand, testosterone levels were remarkable elevated in HP-HE fed deer as compared to HP-LE only in winter (Fig. 9).

Residual correlation coefficients between cortisol, LH and testosterone according to season and nutritional status are shown in Table 4. These Spearman correlations were computed after treatment of the data by the GLM model presented in Table 3. No relationship was found between the seasonal increase of body weight, final size of antlers and the hormonal parameters.

4. Discussion

Stimulation of the pituitary–gonadal axis by GnRH has become a standard technique for the evaluation of the reproductive system of domesticated as well as wild animals [13,31]. GnRH has been also used to study seasonal variation of pituitary and gonadal activity in male cervids. Several species of deer were investigated, such as white-tailed deer [3,8], red deer [17,26], rusa deer [29], and axis deer [9].

In wild animals the sensitivity of the pituitary and the testes to a standard dose of GnRH varies considerably throughout the annual cycle. In addition, a substantial seasonal variation of the LH/T ratio was detected in cervids [1,3,17,26]. As the changes of LH/T ratio appears to be dependent on the social rank of the individual male, it was proposed to use the GnRH test for an assessment of the reproductive potential of male wild ungulate [3,14].

Study of stress in deer presents technical difficulties, the results of which would endanger the animals. In addition it is difficult to apply a uniform level of stress to all individuals investigated. Therefore, a simulated stress has been used in various cervids, using synthetic ACTH [6,7,9,23,25]. These tests revealed differences in sensitivity in various cervid species to a simulated stress. In the present experiment ACTH suppressed temporarily GnRH-induced LH increase in HP-HE as well as HP-LE animals. This was more pronounced in HP-LE deer. A similar trend was also apparent for testosterone levels. However, because the initial, preapplication testosterone levels were significantly higher in controls than in ACTH-treated animals, it was difficult to ascertain the effect of ACTH treatment. Therefore the suppression of testosterone in response to GnRH was observed only in sample 4. The general pattern of the hormone increased slightly in controls, while it further decreased in ACTH-treated deer.

The highest cortisol levels detected in summer may indicate a thermal stress to which our deer were exposed during the summer months. During a 6-week period in June and July, daily maxima of ambient temperature in Kingsville were rarely below 40°C.

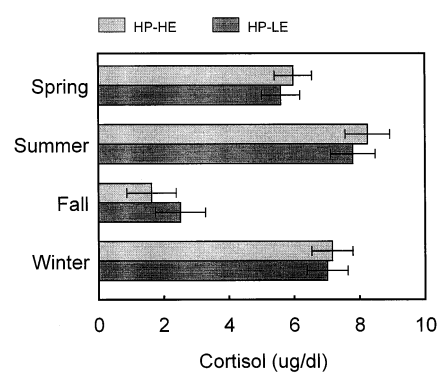

Fig. 7. LSMEANs (±SE) of cortisol according to season and nutritional status.

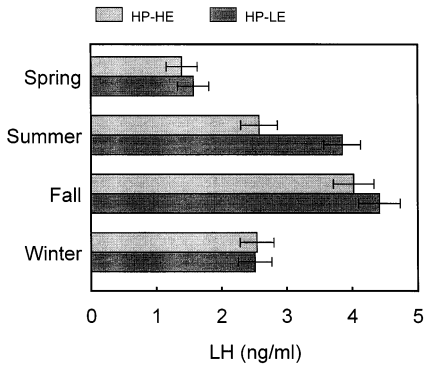

Fig. 8. LSMEANs (± SE) of LH according to season and nutritional status.

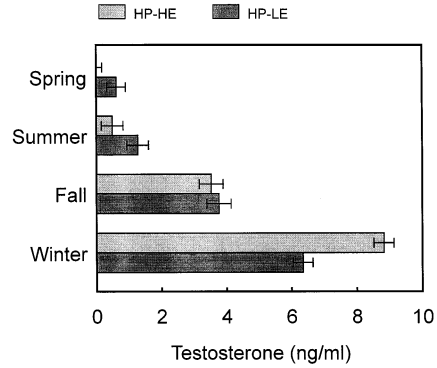

Fig. 9. LSMEANs (± SE) of testosterone according to season and nutritional status.

In most deer species seasonal levels of LH reach peak several months before testosterone exhibits its maximum [1,9,24,26]. Similar pattern was found in the Texas white-tailed deer yearlings where peak LH levels were detected in the summer and fall. Testosterone levels reflect obvious seasonal pattern, well established in boreal cervids [2,24,29]. However, unlike in more northern locations, in white-tailed yearlings of southern Texas rutting season appears to culminate in winter and not in the fall. This confirms a slight shift in the seasonality of reproductive hormones of white-tailed deer in Texas, as reported in our previous study [5].

An unexpected differences in pre-treatment levels were detected between controls and treated deer. As the same individual deer were treated either with ACTH or with saline (albeit in the different months), it can be hypothesized that the differences between pre-administration levels of hormones were purely coincidental, resulting mostly from the circannual variation of LH and testosterone. Because of the differences in the pre-treatment levels our results do not equivocally support the hypothesis that stress (as simulated by an ACTH-induced elevation of cortisol) substantially suppresses the GnRH-induced secretion of LH and testosterone. However, the secondary hypothesis postulating the effect of fetal nourishment on the postnatal responses of yearlings to a simulated stress, appears to be at least partly confirmed. Although during the summer, HP-LE bucks exhibited significantly higher levels of LH than HP-HE deer, peak levels of T observed in winter months (rut), were significantly higher in the HP-HE yearlings.

We have concluded that the lack of any long-term impairment of LH or testosterone secretion after stress (as simulated by ACTH administration) indicate that white-tailed deer is a species highly resistant to environmental stress. In addition the higher testosterone levels observed in the rut in bucks whose mothers were fed a

Table 4
Spearman residual correlations between cortisol, LH and testosterone according to season and nutritional status (ACTH treated date removed)

	HP-HE			HP-LE		
	n	r_s	*P*<	*n*	r_s	*P*<
Spring						
Cortisol vs LH	94	0.24	0.05	85	0.44	0.001
Cortisol vs testosterone		0.02	NS		0.07	NS
LH vs Testosterone		0.83	0.001		0.45	0.001
Summer						
Cortisol vs LH	50	−0.22	NS	50	−0.10	NS
Cortisol vs testosterone		0.16	NS		0.10	NS
LH vs testosterone		0.14	NS		−0.01	NS
Fall						
Cortisol vs LH	40	0.34	0.05	30	0.31	NS
Cortisol vs testosterone		−0.29	0.07		0.11	NS
LH vs testosterone		0.16	NS		0.57	0.001
Winter						
Cortisol vs LH	50	0.18	NS	50	−0.41	0.01
Cortisol vs testosterone		0.36	0.01		−0.04	NS
LH vs testosterone		0.15	NS		0.48	0.001

high-energy diet indicate the importance of a balanced fetal nutrition for the proper pre- and postnatal gonadal development.

Acknowledgements

We thank Dr Tom Dailey and the staff of Caesar Kleberg Wild. Res. Institute in Kingsville, Texas, for their excellent help in maintenance and sampling of deer and Yoko Imai for the preparation of the manuscript.

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