

Influence of restraint and isolation stress on plasma cortisol in male karakul sheep

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ABSTRACT

The investigation was carried out to study the effect of Restraint and Isolation Stress (RIS) on plasma cortisol hormonal profile in male Karakul sheep. To carry out the objectives of this study six apparently healthy male adult animals were subjected to restraint and isolation stress. The blood samples were collected and analysed at control or pre-stress condition and on 1st, 2nd and 3rd day of RIS, respectively. A highly significant ($P \leq 0.01$) effect of RIS on plasma cortisol with increasing trend was observed.

Key words: plasma, karakul sheep, cortisol, stress

Introduction

Among domestic animals the sheep is one of the most sensitive to emotional factors (STEPHENS, 1980) and seems to be a very good model in stress experiment. In animal husbandry there are some management stresses, which cannot be eliminated from the daily work operations. Stress could be physiological, physical, environmental, chemical and emotional. Restraint is one of the most common practices in domestic animal husbandry. Exposure of livestock to stress has been associated with activation of the hypothalamic-pituitary-adrenal axis (HPA) (MINTON, 1994).

The susceptibility of the endocrine system to stress has been established (McKENZIE, 1979). The response of the thyroid and adrenal gland to stress appears to depend on the severity and type of stress and the species.

The stress reactions are characterized by enhanced pituitary-adrenal reactivity, which facilitates the return of the body to homeostatic conditions. Sheep, being gregarious

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animals, are very sensitive to the stress of becoming separated from the flock. Earlier studies have reported that subjecting sheep to Restraint and Isolation Stress (RIS) caused a robust increase in plasma concentrations of ACTH and cortisol (MINTON and BLECHA, 1990; COPPINGER et al., 1991). Stress of any kind will affect the rate of cortisol production. Cortisol is the major glucocorticoid produced and secreted by the adrenal cortex which affects the metabolism of protein, fat and carbohydrates, maintains muscle and myocardial integrity and suppresses inflammatory and allergic activities. The glucocorticoids have slight mineralocorticoid activity.

Therefore keeping this in view, the present study was undertaken with the objectives to estimate the normal cortisol hormonal level in male Karakul sheep and to study the effect of Restraint and Isolation Stress (RIS) on cortisol hormonal levels in Karakul sheep.

Materials and methods

The present study was planned in two phases:

1st phase of experiment. Ten apparently healthy adult male animals procured from the project scheme in the department of Gynecology and Obstetrics, College of Veterinary and Animal Sciences, Bikaner (Rajasthan), India were included in this study. These animals were housed in a flock undisturbed during the experimental period for ten days and were treated as the control group.

2nd phase of experiment. Each animal of the control group was subjected to Restraint and Isolation Stress (RIS) for three consecutive days. The stress treatment comprised the removal of the animal from the flock and transferring them to separate trial cages away from visual and tactile contact with other animals. Animals were fed with dry roughage with approximately 250 Gms of concentrate and some quantity of salt and mineral mixture, as per the standard conditions of feeding and management. Drinking water was provided ad libitum.

Collection, processing and preservation of samples. The samples were collected at 9.30 am each day for control and 1st, 2nd and 3rd consecutive days of RIS, respectively. The samples were collected under aseptic conditions directly into the hot air oven sterile tubes. 10 mL of blood was taken in a sterile heparinized tube (Heparin was added 30 IU/mL of blood). Plasma for each sample was separated out on the same day. For separation of plasma the test tubes were centrifuged at 3000 rpm for 20 minutes, plasma was pipetted out into clear dry plasma tubes, only non-haemolysed plasma samples were used. After collection, plasma was stored at -20 °C.

Hormonal assay (cortisol). Plasma cortisol hormonal level was estimated by Radio Immuno Assay (RIA) technique using coat-A-count kit supplied by Diagnostic Production

Corporation, Los Angeles, USA. The RIA protocol, specificity of antisera and critical evaluation of assays have been reported (RASTOGI and AGARWAL, 1990).

Statistical analysis. The results were presented as Mean \pm SE. The mean values were determined according to RIS and sex groups. The data was subjected to analysis of variance (ANOVA) (SNEDECOR and COCHRAN, 1967). The critical differences among various means were worked out by “Least Significance Difference (LSD)” method.

Results

The mean \pm SE concentrations of plasma cortisol are shown in Table 1, according to the effect of RIS. In the present study, the statistical analysis (ANOVA) reflected the highly significant effect ($P < 0.01$) of RIS on plasma cortisol concentration in male karakul sheep. The highest plasma cortisol concentration was observed during the 1st day of RIS and the lowest in the control group. Initially on the 1st day of RIS an increase of 110.924%, plasma cortisol concentration was noted and thereafter the level of cortisol declined during the 2nd day and 3rd day of RIS, respectively. However as a result of the decline the overall concentrations of cortisol during the 2nd and 3rd day of RIS were comparatively higher than that of control group. The plasma cortisol concentration increased significantly during the course of RIS.

Table 1. Mean \pm SE concentrations of cortisol hormone according to the effect of RIS and sex including interaction between RIS and sex in Karakul sheep

Main effects	Cortisol hormone (nmol/L)			
	Sex 1 (6 males)	Sex 2 (6 females)	Overall mean \pm SE (12 animals)	% increase/ decrease
Control or pre-RIS condition (T_1)	27.038 \pm 1.742	27.681 \pm 1.574	27.360 ^a \pm 1.123	-
On 1 st day of RIS (T_2)	57.663 \pm 0.488	57.755 \pm 0.470	57.709 ^c \pm 0.323	110.92
On 2 nd day of RIS (T_3)	32.464 \pm 0.502	33.245 \pm 0.842	32.855 ^b \pm 0.482	20.08
On 3 rd day of RIS (T_4)	31.268 \pm 0.454	32.004 \pm 0.565	31.636 ^b \pm 0.363	15.63
Overall mean \pm SE	37.108 ^a \pm 2.550	37.671 ^a \pm 2.496	-	-

Mean comparison have been made within main effects. Mean superscripted by different letters differ significantly ($P \leq 0.05$) from each other.

Discussion

Reported values of mean plasma cortisol concentration in this present investigation were more or less in agreement with the findings of NIEZGODA et al. (1993). The values reported in present investigation were below the normal values as given by BOBEK et al. (1986) (46.27 ± 4.23 nmol/L) and KANEKO et al. (1997) in sheep. Possibly this may be due to the breed difference and different metabolic status of animals and it is also important to mention here that the cortisol level in the blood stream differs with each individual, because some animals secrete more stress hormones than others.

The analysis of variance revealed that the mean plasma cortisol level increased significantly ($P > 0.01$) during the course of RIS and this pattern was well in accordance with the previous research which has demonstrated increase secretion of cortisol in reference to RIS in various breeds of sheep (APPLE et al., 1993; BOBEK et al., 1986; NIEZGODA et al., 1993). Sheep, being gregarious animals, are very sensitive to the stress of becoming separated from the flock. MINTON and BLECHA (1990) and COPPINGER et al. (1991) have shown that subjecting sheep to restraint and isolation stress (RIS) caused a robust increase in plasma concentrations of ACTH and cortisol (APPLE et al., 1993). MINTON and BLECHA (1990) in lambs reported elevated cortisol level in response to RIS and suggested that acute exposure to elevated ambient temperature or restraint plus isolation of lambs activated pituitary-adrenal-axis and this is one of the important contributing factors for elevated cortisol level due to the effect of RIS. Further, the pattern of the present findings is also similar to the findings of BOBEK et al. (1986).

ADRICHEM and VOGT (1993) also observed a similar response in respect to isolation stress in sheep and concluded that isolation acts as a stress syndrome to the animal which triggers a non-specific endocrine response, characterized by the activation of the adrenocortical axis which can be demonstrated by an increase in circulating ACTH and cortisol level (MORMEDE, 1988) and they further reported that in sheep total isolation has been shown to be a potent stimulus for cortisol release (PARROTT et al., 1987). In our present study the highest cortisol level was observed during the first day of RIS and thereafter a decline was observed in the cortisol level. These findings are more or less in accordance with the findings of PIERZCHALA et al. (1999), BOBEK et al. (1986) in Polish-Mountain sheep, DOUBEK et al. (2003) in Merinolandschaf and Romney Marsh breeds of sheep, in which all they reported an overall increase in blood/plasma cortisol concentration in response to stress. A similar pattern has also reported by several workers in other species, in calves (COOPER et al., 1995) and in gilts (ANTOINE et al., 1995).

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SAŽETAK

Istražen je učinak stresa zbog sputavanja i odvajanja na razinu kortizola u plazmi karakulskih ovnova. U pokus je uzeto šest klinički zdravih odraslih ovnova koji su bili podvrgnuti stresu zbog sputavanja i izolacije. Uzorci krvi bili su pretraženi prije podvrgavanja stresu te prvi, drugi i treći dan stresa. Ustanovljen je značajno velik ($P \leq 0,01$) učinak stresa na razinu kortizola u plazmi s trendom povećanja.

Cljučne riječi: plazma, karakulska ovca, kortizol, stres
