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Effects of Level of Feeding and Season on Thermoregulation and Semen Characteristics in Desert Rams (*Ovis aries*)

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Abstract: The purpose of this study was to evaluate the effects of level of feeding lucerne hay (high, medium, low) and seasonal change in thermal environment (winter vs summer) on the mean body weight (BW), rectal temperature (Tr), serum albumin (Alb) concentration, scrotal circumference (SC) and semen characteristics in Desert rams. Feeding low level of lucerne hay significantly decreased BW and Tr in both seasons compared to medium and high levels. The afternoon values of Tr were significantly higher during summer with all levels of feeding. In both seasons, serum Alb value and the ejaculate volume (EV) were significantly lower with the low level of feeding as compared to the other feeding levels. The sperm mass motility (SMM) was significantly lower with the medium and low levels of feeding as compared to the higher level during summer. The percentage of sperm individual motility (SIM) was significantly lower with the medium and low levels of feeding during summer and winter. During summer, the live sperm percent (LSP) was significantly lower with medium level of feeding compared to the other groups. The sperm cell concentration (SCC) was significantly higher during winter and summer with medium and low levels of feeding, respectively as compared to the higher level of feeding. The abnormal sperm percent (ASP) was significantly higher with the low level of feeding during summer as compared to the other groups. With all feeding levels, there were significantly lower values of serum Alb, EV, SMM, SIM and significantly higher ASP values during summer as compared to winter. It was concluded that thermoregulation and seminal traits of Desert rams were influenced by feed restriction and seasonal change in thermal environment.

Key words: Rams · Level of feeding · Season · Thermoregulation · Semen characteristics

INTRODUCTION

The nutritional status in rams has great impact on their physiological responses and reproductive performance. Feed restriction of ram lambs prevented them attaining puberty in their first potential breeding season [1]. The sexual activity and successful fertilization necessitate consideration of nutritional management of rams [2, 3]. Previous studies have demonstrated the close relationship between energy intake and the performance of adult rams [4- 6]. Also, it has been reported that protein deficiency was associated with reduced sexual activity and semen quality in rams [7,8]. Hot climate is usually associated with declined food intake and utilization [9] and a rise in body temperature and respiration rate [10,11] which increases energy requirements for maintenance [12]. In temperate regions, the most important cue for ram reproductive seasonal variation is the annual cycle of photoperiod and the magnitude of change in daylength depends on the latitude [13]. In tropical areas, semen quality was superior during the rainy season, which indicates that seasonality of semen quality is influenced by nutrition in areas wheres changes in daylength and responses to photoperiod are minimal [14,15]. It has been suggested that nutrition influences reproductive activity in rams in both GnRH- dependent and GnRH-independent way [16]. It has been indicated [17] that there are direct relationships between plane of nutrition and gonadotropin secretion in male ruminants. Higher plane of nutrition results in increased LH pulse frequency, whereas submaintenance level of feeding leads to rapid decrease in LH pulse frequency in rams [18,19].

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The sheep population in Sudan is estimated at about 49 million head [20], constituting about 36% of livestock in this country. Desert sheep comprise about 60% of the sheep population; they are reared within the low rainfall savannah and semi-arid zones under extensive systems where, the availability of food is highly dependent on season. During the long dry season, sheep thrive with low quality range grasses, water scarcity and high ambient temperature [21]. The high variability in amount and distribution of rainfall affects the vegetation, water availability and general husbandry of sheep. Accordingly, the nutritional status, welfare and potentials for high production of sheep are adversely influenced during the hot season. As desert sheep have considerable contribution to national economy, investigations into the factors that influence their productive and reproductive potentials are recommended. Virtually no information is available on the effect of nutrition on the reproductive performance of Desert rams.

The present study was undertaken to assess the effects of plane of nutrition during tropical summer and winter conditions on the body weight, thermoregulation, scrotal circumference and seminal traits of Desert rams.

MATERIALS AND METHODS

Climate and Location: Studies were performed at the Department of Physiology at Shambat located at 15° 36' N, 32° 35' E and an altitude of 390 m. The climatic conditions prevailing during the experimental period are depicted in Table 1.

Animals: Nine adult Desert rams aged 2.5 -3 years and weighing 39.0-46.5 kg were used in these studies. Rams were screened with regard to the health of external genitalia and fertility. Animals were individually housed in shaded well ventilated pens under natural photoperiod. Pens were provided with appropriate facilities for feeding and watering. Initially animals were offered lucerne hay (*Medicago sativa*) (CP: 17.5 %; ME: 8.48MJ / kg) and clean tap water *ad libitum*. During the experimental period, a general management protocol including deworming, clinical examination, claws trimming and sanitary measures was adopted.

Experimental Procedure: Rams were allowed an adaptive period of 2 weeks, followed by an experimental period of 12 weeks. The average value for *ad libitum* level of dry matter intake (100%) was considered as the high plane of nutrition (1200 g/day); accordingly the medium level, 66% (800g/ day) a nd the low level, 33% (400g/ day) were

Table 1: The mean values of ambient temperature (Ta) (°C) and relative humidity, (RH) (%) during the experimental period

	Ta (°C)			
Season	Max.	Min.	Mean	RH (%) (Mean)
Winter	31.58±1.51	15.83±1.66	23.71±7.88	28.17±4.57
Summer	38.23±2.61	25.59±1.18	31.91±6.32	42.09±11.49

calculated. The maintenance requirements (Mm) for sheep kept indoors, was calculated as Mm/k g = 1.2 + 0.1 BW [22]. The initial average (BW) of rams was 43.22 kg; accordingly the maintenance energy allowance was 6.82 MJ/day. The metabolizable energy (ME) of lucerne hay in ruminants was calculated as 8.80 MJ/kg [23]. The amount of lucerne hay needed for maintenance requirement was computed (775 g). The medium level of feeding used in this experiment provided approximately the maintenance requirements, and the low level imposed was below maintenance. A similar protocol of feeding regimen was adopted in both summer and winter.

Measurements of Tr were performed twice daily, in the morning (7:00 a.m.) and in the afternoon (2:00 p.m.), during summer and winter for 12 weeks. The scrotal circumference was weekly measured. In each season, blood samples were collected weekly for 12 weeks and semen samples were collected for 10 weeks. The samples were collected at 9.00 - 10.00 a.m.

Measurement of Rectal Temperature (T_r), Body Weight (BW) and Scrotal Circumference (SC): The rectal temperature (Tr) was measured with certified mercury-in-glass clinical thermometer with an accuracy of ±0.1°C. The body weight (BW) of rams was measured by a spring balance (Salter No.235-Trade No.2892- England). The scrotal circumference (SC) was measured using a tape at the widest scrotal diameter.

Blood Analysis: Blood samples were collected by jugular venipuncture using plastic disposable syringes. Haemolysis - free serum samples were used for determination of albumin concentraton [24].

Semen Collection and Evaluation: Semen samples were collected using a transistorized rectal probe with two ring electrodes (The Ruakura MK 1V Ram ejaculator; Alfred Cox, Surrey, England). The probe was inserted so that the ring electrodes penetrated to a depth of approximately 11 cm. Semen samples were evaluated using standard methods [25,26]. The ejaculate volume (EV) was measured in a graduated tube. The sperm mass motility (SMM) was

evaluated by transferring a drop of undiluted semen to a warm slide, placing a cover slip and observing under a microscope (x 40). The (SMM) assessment was performed on a scale of 0 (immotile) and 5 (vigorous motility). The Sperm individual motility (SIM) was estimated using a scale of 1-10 representing increments of 10%. Sperm cell concentration (SCC) was determined after diluting semen with a 0.05% formaldehyde saline solution (1:400) and examining under the microscope (x 400). The live sperm percent (LSP) and dead spermatozoa were determined using nigrosin- eosin staining technique by counting 100 spermatozoa under oil immersion objective (x 1000) using random fields. The abnormal sperm percent (ASP) was determined by examining a total of 200 spermatozoa from nigrosin -eosin stained smear.

Statistical Analysis: The data obtained are presented as (Means ± SE). The statistical analysis was performed according to standard methods [27]. The analysis of variance (ANOVA) test was used to evaluate the effects of plane of nutrition and season on the parameters investigated.

RESULTS AND DISCUSSION

The climatic data depicted in Table 1 indicate that the rams experienced marked seasonal change in ambient temperature. During both seasons, the rams exhibited significantly lower values of rectal temperature (Tr) with the low level of feeding in the morning ($P< 0.05$) (Table 2) and the afternoon ($P< 0.01$) (Table 3), as compared to respective values obtained with the other feeding levels. The lower Tr with low plane of nutrition is attributed to decrease in metabolic heat production in response to feed restriction [28-31]. This response in body temperature could also be associated with alteration in thyroid activity which influences metabolic rate. Following feed restriction or food deprivation, the plasma concentrations of thyroid hormones were reduced in sheep [32-34].The present findings are in agreement with results previously reported for desert rams [10]. The significantly ($P<0.01$) higher Tr values obtained in the afternoon with all feeding levels during summer compared to winter is clearly associated with the increase in thermal load with rise in ambient temperature.

Table 4 shows that in both seasons, feed restriction significantly ($P<0.01$) reduced the scrotal circumference (SC). Also rams maintained on high level of feeding had significantly ($P<0.01$) lower values of (SC) during summer as compared to winter values. The reduction in (SC) with

Table 2: Effects of level of feeding lucerne hay and season on rectal temperature, (Tr) (°C) of desert rams at 7:00 a.m (n = 252, mean ± S.E.)

	Season		
Level of feeding	Winter	Summer	SL
High	$^{A}37.98^{a}\pm0.04$	$^{A}38.12^{a}\pm0.02$	NS
Medium	$^{A}37.74^{a}\pm0.05$	$^{B}37.89^{a}\pm0.04$	NS
Low	$^{B}37.40^{a}\pm0.06$	$^{B}37.54^{a}\pm0.10$	NS
SL	*	*	

Mean values within the same row bearing similar superscripts (small) are not significantly different. Mean values within the same column bearing different superscripts (capital) are significantly different

SL: Significance level.; *: Significant at P<0.05. N; S: Not significant

Table 3: Effects of level of feeding lucerne hay and season on rectal temperature, (Tr) (°C) of desert rams at 2:00 p.m. (n = 252, mean ± S.E.)

	Season		
Level of feeding	Winter	Summer	SL
High	$^{A}38.72^{b}\pm0.03$	$^{A}39.10^{a}\pm0.05$	**
Medium	$^{A}38.64^{b}\pm0.04$	$^{A}38.99^{a}\pm0.06$	**
Low	$^{B}38.39^{b}\pm0.04$	$^{B}38.76^{a}\pm0.05$	**
SL	**	**	

Mean values within the same row bearing different superscripts (small) are significantly different

Mean values within the same column bearing different superscripts (capital) are significantly different

SL: Significance level. **: Significant at P< 0.01; NS: Not significant

Table 4: Effects of level of feeding lucerne hay and season on scrotal circumference (SC) (cm) in desert rams. (n = 30,mean±S.E.)

	Season		
Level of feeding	Winter	Summer	SL
High	$^{A}32.52^{b}\pm0.40$	$^{A}30.75^{a}\pm0.19$	**
Medium	$^{B}30.55^{a}\pm0.35$	$^{AB}29.72^{a}\pm0.42$	NS
Low	$^{B}27.77^{a}\pm0.51$	$^{B}28.77^{a}\pm0.45$	NS
SL	**	**	

Mean values within the same row bearing different superscripts (small) are significantly different

Mean values within the same column bearing different superscripts (capital) are significantly different

SL: Significance level. **: Significant at P<0.01; NS: Not significant

Table 5: Effects of level of feeding lucerne hay and season on the mean body weight (BW) (kg) of desert rams. (n = 36,mean±S.E.M.)

	Season		
Level of feeding	Winter	Summer	SL
High	$^{A}44.16^{a}\pm0.33$	$^{A}42.16^{b}\pm0.40$	***
Medium	$^{B}39.57\pm0.40^{a}$	$^{B}38.40^{b}\pm0.37$	***
Low	$^{B}35.70^{a}\pm0.39$	$^{B}34.45^{b}\pm0.60$	***
SL	**	***	

Mean values within the same row bearing different superscripts (small) are significantly different

Mean values within the same column bearing different superscripts (capital) are significantly different

SL: Significance level. **: Significant at P< 0.01; ***: Significant at P< 0.001

Table 6: Effects of level of feeding lucerne hay and season on ejaculate volume (EV) (mL) in desert rams. (n = 30, mean±S.E.)

	Season		
Level of feeding	Winter	Summer	SL
High	$^{A}1.96^{a}\pm0.09$	$^{A}1.66^{b}\pm0.09$	**
Medium	$^{A}1.98^{a}\pm0.10$	$^{A}1.58^{b}\pm0.09$	**
Low	$^{B}1.58^{a}\pm0.08$	$^{B}1.33^{b}\pm0.07$	*
SL	**	**	

Mean values within the same row bearing different superscripts (small) are significantly different

Mean values within the same column bearing different superscripts (capital) are significantly different

SL: Significance level. *: Significant at P< 0.05.; **: Significant at P< 0.01

Table 7: Effects of level of feeding lucerne hay and season on sperm mass motility, (S M M) (0 – 5) in Desert rams. (n = 30, mean±S.E.)

	Season		
Level of feeding	Winter	Summer	SL
High	$^{A}4.65^{a}\pm0.08$	$^{A}3.66^{b}\pm1.41$	***
Medium	$^{A}4.42^{a}\pm0.08$	$^{B}2.86^{b}\pm1.51$	***
Low	$^{A}4.32^{a}\pm0.10$	$^{B}3.02^{b}\pm1.34$	***
SL	NS	*	

Mean values within the same row bearing different superscripts (small) are significantly different

Mean values within the same column bearing different superscripts (capital) are significantly different

SL: Significance level. *: Significant at P<0.05; ***: Significant at P< 0.001; NS: Not significant

feed restriction is associated with the general loss in the mean (BW) (Table 5) and loss of subcutaneous fat in the scrotal sac.Testicular mass and seminiferous tubule length and diameter were reported to be lower in Poll Merino rams fed a sub-mainbtenance diet [35]. The current results are also in agreement with previously reported findings [6, 36-38]. The high (SC) value with the high feeding level in both seasons lends support to the hypothesis that the growth of testis can be affected when the animals are fed above their maintenance requirement [39]. The significantly (P<0.01) higher SC recorded for high level of feeding during winter compared to summer values (Table 6) is presumably related to an increase in the number of seminiferous tubules and spermatogenic activity. Similarly, Al Sayed [40] reported relatively higher values of SC during tropical winter conditions in Desert rams. In temperate zone, higher values of SC were reported for Finnish Landrace and Tasmanian rams during the cold season [41]. Exposure of rams to a hot environment for 14 days caused a reduction in testis weight to about 70 % of control values [42].

The ejaculate volumes (EV) obtained in the present study for Desert rams (Table 6) are relatively large compared to values previously reported in literature. This could be related to the recovery of semen by electrical stimulation. Other studies reported that electroejaculation resulted in larger ejaculate volume [43-45]. The response to electrical stimulation resulted in large semen volume presumably due to a high secretion of accessory glands [43]. The EV in Desert rams was significantly (P<0.01) lower with low level of feeding in both seasons (Table 6). This response is related to decrease in the concentration of luteinizing hormone (LH) and reduction of testosterone secretion in response to feed restriction [8]. The low (EV) obtained could be attributed to the decreased function of pituitary gland and testis due to decrease in their sizes in feed restricted rams [36]. Consequently, the secretion of androgen dependent organs (epididymis, testis and accessory glands) are expected to decrease resulting in low (EV). It has been indicated [39] that the EV tended to be higher with improvement of nutrition in Bakhtiary rams. The significantly (P<0.05) higher EV observed during winter, with all levels of feeding, as compared to summer values (Table 6), could be related to the effects of endocrine activation on exposure of rams to cold and stimulatory effects of testosterone on accessory genital glands. The current results are in agreement with the findings reported previously for Desert rams under tropical conditions [40,46].

Sperm mass motility (SMM) was significantly (P<0.05) lower with feed restriction during summer (Table 7). This is clearly related to decline in the nutritional status of rams. The low food intake during summer could have induced low fructose level in seminal plasma and consequently decreased SMM as these parameters are positively related [47]. Moreover, Galil and Galil [48] reported a low level of fructose in seminal plasma of Desert rams during summer and they attributed this result to high ambient temperature and reduced secretion of testosterone. The reduction of SMM during summer has been attributed to low fructose level and sperm metabolic rate [49]. The reduction of (SMM) could be partially associated with low serum albumin (Alb) concentration reported in the present study in feed restricted rams (Table 8). Miyamoto and Chang [50] suggested that serum Alb is the best medium for increasing sperm motility *in vitro*. Serum Alb is considered as very sensitive and early nutritional indicator of protein status in animals because its turnover is only 16 days [51]. The significantly (P<.001) lower

Table 8: Effects of level of feeding lucerne hay and season on serum albumin (Alb) concentration (g/dL) in Desert rams. (n = 36, mean ± S.E.)

	Season		
Level of feeding	Winter	Summer	SL
High	$^{A}3.57^{a}\pm0.04$	$^{A}3.45^{b}\pm0.04$	*
Medium	$^{B}3.50\pm0.04^{a}$	$^{B}3.37^{b}\pm0.05$	*
Low	$^{AB}3.54^{a}\pm0.04$	$^{B}3.38^{b}\pm0.05$	*
SL	*	*	

Mean values within the same row bearing different superscripts (small) are significantly different

Mean values within the same column bearing different superscripts (capital) are significantly different

SL: Significance level. *: Significant at P<0.05

Table 9: Effects of level of feeding lucerne hay and season on sperm individual motility (S IM) (%) in desert rams. (n = 30, mean ± S.E.)

	Season		
Level of feeding	Winter	Summer	SL
High	$^{A}76.67^{a}\pm2.24$	$^{A}59.14^{b}\pm4.26$	**
Medium	$^{B}68.50^{a}\pm2.90$	$^{B}30.71^{b}\pm4.71$	**
Low	$^{B}63.33^{a}\pm2.84$	$^{B}35.15^{b}\pm4.69$	**
SL	**	*	

Mean values within the same row bearing different superscripts (small) are significantly different

Mean values within the same column bearing different superscripts (capital) are significantly different

SL: Significance level. *: Significant at P< 0.05; **: Significant at P<0.01

SMM observed with all levels of feeding during summer compared to winter values (Table 7) might be attributed to the high ambient temperature and increase in body temperature of rams reported during summer. These results are in general conformity with previous findings [46].

Table 9 shows that the percentage of sperm individual motility (SIM) was significantly (P<0.01) lower during winter (P<0.01) and summer (P<0.05) with medium and low levels of feeding. This might be related mainly to the low plane of nutrition and protein intake resulting in a relatively low concentration of seminal plasma metabolites. Setchell *et al.* [52] indicated that the decrease in SIM in feed restricted rams was attributed to low seminal plasma fructose concentration and depressed activity of the pituitary gland. However, low SIM has been related to decline in sex hormones binding globulins [53]. Irrespective of level of feeding, SIM was significantly (P<0.001) higher during winter as compared to summer values (Table 11). This could be attributed to the relative efficient testicular thermoregulation mechanism in the cool environment. High values of SIM were reported in Desert rams during winter and autumn compared to summer values [46].

Table 10: Effects of level of feeding lucerne hay and season on sperm cell concentration (SCC) ($\times10^{9}$/mL) in desert rams. (n = 30, mean ± S.E.)

	Season		
Level of feeding	Winter	Summer	SL
High	$^{B}2.07^{a}\pm0.133$	$^{AB}1.64^{a}\pm0.21$	NS
Medium	$^{A}2.37^{a}\pm0.15$	$^{B}1.24^{b}\pm0.13$	***
Low	$^{B}2.15^{a}\pm0.21$	$^{A}1.80^{b}\pm0.21$	NS
SL	*	*	

Mean values within the same row bearing different superscripts (small) are significantly different

Mean values within the same column bearing different superscripts (capital) are significantly different

SL: Significance level. *: Significant at P<0.05; ***: Significant at P< 0.001; NS: Not significant

Rams maintained on medium level of feeding showed significantly (P<0.05) higher sperm cell concentration (SCC) during winter (Table 10). This response could be associated with the low EV obtained with feed restriction and the fact that EV depends primarily upon the secretion of seminal plasma rather than SCC [25]. The significantly (P<0.05) lower SCC reported during summer with the medium level of feeding could be associated with the increase in ambient temperature. These findings are consistent with previous reports [36,54,55]. The SCC was lower with all levels of feeding during summer compared to winter; the decrease was significant (P<0.001) in rams maintained on medium level of feeding (Table 10). The findings are attributed to decrease in spermatogenic activity and epididymal reserve during the hot season. Setchell [56] indicated that the most obvious cell to be the primary site of heat is the Sertoli cell which provides nutrients and controls the development of germ cells. Similarly, a lower SCC was reported in Desert rams during summer compared to winter [46].

The current study revealed significantly (P<0.05) lower live sperm percent LSP values during summer compared to winter in rams maintained on medium level of feeding (Table 11). This response could be related to the increase in body temperature resulting in increase in metabolic activity of testicular cells leading to testicular hypoxia which probably plays a role in heat-induced spermatogenic disturbance [49]. However, it has been indicated that damage may be caused not so much by hypoxia directly, as by generation of reactive oxygen species during recovery [56]. Galil and Galil [46] reported lower LSP in Desert rams during summer compared to winter values. The reduction in LSP in association with feed restriction could be attributed to the decrease in number of seminiferous tubules and increase in dead

Table 11: Effects of level of feeding lucerne hay and season on the live sperm percent (LSP) (%) in desert rams. (n = 30, mean±S.E.)

	Season		
Level of feeding	Winter	Summer	SL
High	$^{A}94.80^{a}\pm1.37$	$^{A}93.24^{a}\pm3.40$	NS
Medium	$^{A}94.97^{a}\pm0.82$	$^{B}87.60^{b}\pm3.37$	*
Low	$^{A}94.55^{a}\pm1.00$	$^{A}91.28^{a}\pm2.14$	NS
SL	NS	*	

Mean values within the same row bearing different superscripts (small) are significantly different

Mean values within the same column bearing different superscripts (capital) are not significantly different

SL: Significance level. *: Significant at P<0.05. NS: Not significant

Table 12: Effects of level of feeding lucerne hay and season on abnormal sperm percent (ASP) (%) in desert rams. (n = 30, mean±S.E.)

	Season		
Level of feeding	Winter	Summer	SL
High	$^{A}3.18^{b}\pm0.60$	$^{B}13.07^{a}\pm4.07$	*
Medium	$^{A}5.45^{b}\pm1.22$	$^{B}13.36^{a}\pm3.29$	*
Low	$^{A}3.86^{b}\pm1.11$	$^{A}21.18^{a}\pm4.03$	***
SL	NS	*	

Mean values within the same row bearing different superscripts (small) are significantly different

Mean values within the same column bearing different superscripts (capital) are significantly different

SL: Significance level. *: Significant at P< 0.05; ***: Significant at P< 0.001.; NS: Not significant

sperm percent due to increase in degenerative processes. Recently, it has been reported that the LSP tended to be higher in supplemented Zebu bulls [57].

Feed restriction significantly (P<0.05) increased the incidence of abnormal sperm percent (ASP) during summer (Table 12). This response might be due to the decrease in availability and supply of essential nutrients required for sperm production in the testis and epididymal maturation associated with thermal stress during summer [52,58]. Similar findings were reported by other workers [1,8,59]. The ASP was higher during summer as compared to winter with all levels of feeding (Table 12). The responses were significant with the high and medium (P<0.05) and low (P<0.001) levels of feeding. This could be accounted for by the high body temperature reported during summer and consequently testicular hyperthermia which results in disturbed spermatogenesis [60]. Other studies have reported that increased testicular temperature provokes testicular degeneration [61-64].

In conclusion, this study indicates that the plane of nutrition and thermal environment influence the physiological performance and reproductive potentials of desert rams. The responses to level of feeding were modulated by thermal environment. Accordingly, these factors should be considered adequately in order to improve the productivity of sheep under tropical conditions. Future studies should investigate the endocrine responses and alterations in the composition of semen plasma in relation to nutrition and thermal environment. Also the genetic component should be explored by comparative studies involving different indigenous breeds and ecotypes of sheep.

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