

ORIGINAL ARTICLE

Evaluating fertility and growth rate potential of indigenous sheep breeds submitted to heat stress under different management systems

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ABSTRACT

Objective: Sheep farming is an important source of livelihood in the southern Khyber Pakhtun Khwa province of Pakistan where they are reared under extensive system (ES). This system has limitations of improper feeding, rearing, and management which negatively affect their productivity. The present study compares different rearing systems and their stresses on fertility, behavior, and growth rate potential of two sheep breeds of Pakistan.

Materials and Methods: Eighty ewes were selected; forty each from Damani and Balkhi breeds reared in semi-intensive system (SIS) and ES at Paharpur district, Dera Ismail Khan. Blood samples were collected in triplicate on days 0, 14, and 28 of May, and the molecular and behavioral stress, growth, and fertility rates were recorded.

Results: The highest growth and fertility rate were observed in both the breeds in SIS than in ES ($p < 0.01$). The values of stress indicators (cortisol, heat shock protein (HSP-70)) and behavioral stress parameters were found lower in Damani breed as compared to Balkhi breed in both rearing systems ($p < 0.01$). All the molecular stress and behavioral stress parameters were negatively associated with metabolic hormones (T3 and T4) ($p < 0.01$). A positive correlation was observed among all the molecular stress and behavioral stress parameters ($p < 0.01$). Similarly, T3 and T4 were positively interlinked to one another ($p < 0.01$).

Conclusion: It was concluded that both Damani and Balkhi sheep showed lower stress levels and better fertility and growth parameter in SIS than ES system of rearing. Local Damani breed exhibited well tolerant ability and genetic adaptation to the local environment as compared to Balkhi breed.

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Introduction

Among other factors, heat stress is one of the crucial factors which negatively affect the livestock health, productivity, and fertility [1]. The annual economic loss resulting from environmental stress ranges from 0.1% to 4% of total farm earnings [2]. The estimated range of temperature tolerance for adult sheep is between 12°C and 32°C. Any fluctuation in temperature below and above that range will deteriorate ewe's health and eventually the production and fertility [3]. Including productivity, other physiological

and reproductive parameters are also affected due to heat stress in sheep and goats [4].

Different management systems could impose different types of stresses which significantly affect physiological parameters, weaning weight, growth rate, and percentage mortality in small animals [1,5]. Sheep is usually reared in semi-intensive system (SIS) and ES. ES of rearing imposed greater stress on Comisana ewes, Italy, which negatively affected their physiological parameters and reduced their productivity [6]. The production and fertility were badly

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affected in ewes reared in an existing system in Bangladesh which may be due to the provision of insufficient class feed resources [4].

During heat stress, various members of heat shock proteins (HSPs) are induced intracellularly, which protect cellular proteins from the damage of heat stress [7]. HSP-70 production protects from cell injury and also takes part in the acclimatization mechanism with a temperate environment [8]. The farmers of Dera Ismail Khan usually kept Damani and Balkhi sheep under ES in the rural areas. The animals are allowed to graze for 8–10 hours with occasional feeding of concentrates. This system reduces the growth and fertility performance which eventually causes economic losses.

The present study was conducted in Dera Ismail Khan district (Pakistan) to explore various factors affecting stress markers and behavioral stress indicators of two different sheep breeds under various management systems and their subsequent implications on the growth rate potential and fertility during stress.

Materials and Methods

Ethical approval

An experimental study was approved by the Department of Livestock Management and ABG, The University of Agriculture Peshawar LSEC no: 1115-112.

Selection and management of animals

About 80 healthy Damani and Balkhi ewes of age 3–4 years, average body weight of 36 kg \pm 2.3 kg and 43 kg \pm 1.2 kg, respectively, and body condition score of 3.5 \pm 0.1 were selected. They were divided into two groups, i.e., extensive system (ES; temperature and humidity index (THI), 90.68 Tdb, 42°C, Rh, 38%), 20 each from Damani and Balkhi ewes, and similarly in SIS (THI, 88.10 Tdb, 40°C, Rh, 42%). The ES was comprised of a management system of grazing throughout the year on rangeland and other available plants, especially *Sesbania aculeata* (dhaincha/jantar) plants in summer and *Acacia nilotica* (kiker) in winter. Under ES, no proper vaccination, deworming, housing, feeding, and medication facilities were practiced during an experimental trial. SIS was comprised of proper management, vaccination, and well-constructed sheds with adjacent sufficient open grazing area. Green forages were provided *ad libitum* and concentrated at the rate of 500 gm per day. The SIS was selected from Livestock Research and Development Center, Paharpur district of Dera Ismail Khan, and the ES was selected from the adjacent areas of Paharpur district.

Measurement of THI

The temperature and humidity were measured three times a day: morning (08:00), noon (12:00 AM), and afternoon (04:00) using the formula described previously [9].

THI: $(1.8 \times T \pm 32) - (0.0055 \times RH) \times (1.8 \times T - 26)$, where T is the temperature (°C) and RH is the relative humidity (%). The average THI during the experimental period was 88.10 in SIS and 90.68 in ES.

Fertility parameters

Data regarding fertility parameters were obtained through information obtained from the owner during May 2013–July 2014. The parameters recorded were conception rate (%), live birth (%), stillbirth (%), and abortion (%).

Growth rate parameters

Data on the following growth rate parameters were recorded from the information obtained from owners (May 2013–July 2014). The growth rate parameters recorded were birth weight (kg in two digits), weaning weight (kg in two digits), daily weight gain (kg in two digits), and adult weight (kg in two digits).

Behavioral stress indicators

Rate of respiration/minute, pulse rate/minute, and rectal temperature were noted at each time of sampling.

Blood sampling and analysis

The jugular vein was used to collect blood samples from each ewe and put into Ethylenediamine Tetraacetic Acid (EDTA) and non EDTA containing vacutainer tubes for plasma and serum separation. Blood samples in EDTA tubes were centrifuged at 3,000 rpm for 20 min to obtain plasma, whereas serum was obtained from non EDTA tubes and stored at –20°C until further analysis. Blood samples were collected in triplicate at an interval of 14 days, i.e., on days 0, 14, and 28. Tests were carried out for the estimation of HSP-70 from plasma using Enzyme-linked Immunosorbent Assay (ELISA) kit by Cusabio Biotech (Wuhan, China). Hormonal profile including cortisol and metabolic hormone, such as tri-iodothyronine (T3) and thyroxine (T4), was assayed in serum using commercially available kits by Calbiotech (El Cajon, CA) and enzyme immunoassay kit by BioCheck-323 (Vintage Park, Foster City, CA), respectively.

Statistical analysis

The data were analyzed using SPSS software. The difference between the means of different groups was analyzed for significance using analysis of variance. DMR test was applied for

ranking of means of various stress markers and reproductive and metabolic hormones. Correlation of different factors was studied using Pearson's correlation procedure [9].

Results

Behavioral changes, fertility, and growth rate

Values for behavioral stress parameters were higher for both breeds in ES comparatively (Table 1, $p < 0.05$). A significant variation in values of growth rate parameters (birth weight, daily weight gain, weaning weight, and adult weight) and fertility parameters (conception rate (%), live birth (%), stillbirth (%), abortion (%), and twinning (%)) was observed among breeds and systems of rearing. In SIS, the values of growth parameters were higher for birth weight (14.5%), daily weight gain (17.7%), weaning weight (13.2%), and adult weight (13.8%) in Balkhi sheep as compared to ES (Table 1, $p < 0.05$). Similarly, increased values for growth parameters such as birth weight (23.7%), daily weight gain (17.9%), weaning weight (16.8%), and adult weight (18.8%) were recorded in local Damani sheep as compared to ES (Table 1, $p < 0.05$).

In the same way, in SIS, improved values of fertility parameters such as conception rate (4.4%) and live birth (6.2%) were higher, whereas stillbirth (50%) and abortion (50%) were lower, and twinning (0%) was observed in Balkhi sheep as compared to ES. Similarly, for local Damani breed, the improved values of fertility parameters such as conception (10.4%), live birth (6.2%), and twinning (100%) were higher, whereas stillbirth (50%) and abortion (50%) were lower than that of ES. The improved values of all growth and fertility parameters of Damani breed were higher as compared to Balkhi breed because Damani breed is the local breed of this area and more resistant to the local environments (Table 2, $p < 0.05$).

Effect of breed and management systems on stress markers

The level of HSP-70 was significantly higher in Balkhi sheep as compared to Damani sheep (Table 3, $p < 0.01$). It reflects that Balkhi sheep breed is more prone to thermal stress. The level of HSP-70 of both breeds was higher in ES as compared to SIS (Table 4, $p < 0.01$). The difference in HSP-70 concentrations was not significant at different days of sampling (Table 4, $p < 0.23$).

Cortisol level was significantly higher in Balkhi sheep than in Damani sheep (Table 4, $p < 0.01$) reflecting a lower level of stress on the later breed, probably because of the higher level of tolerance to the local harsh environment. Cortisol level was higher in both breeds reared in ES as compared to SIS (Table 4, $p < 0.01$). The difference in blood cortisol level during different sampling days was not significant (Table 4).

MDA level of Balkhi sheep was higher than Damani sheep probably because Damani sheep is the local breed and is well resistant to the local harsh environment (Table 4, $p < 0.01$). Similarly, on different days of sampling, the level of MDA in various breeds of sheep also varied significantly (Table 4, $p < 0.01$). The level of MDA in both breeds was also higher in ES as compared to SIS. It shows that ewes were more exposed to heat stress in ES.

Effect of breed and system on metabolic hormones

The mean values of T3 and T4 were significantly higher in Damani sheep than in Balkhi sheep (Table 4, $p < 0.01$). Similarly, the values of the two hormones were considerably ($p < 0.01$) higher in SIS system than in ES. Both the metabolic hormones (T3 and T4) were found negatively correlated with HSP-70 ($r = 0.811$, Table 4; $p < 0.01$), whereas they were positively correlated in each other ($r = 0.882$, Table 4; $p < 0.01$).

Table 1. Changes in growth rate parameters in Balkhi and Damani sheep breeds with respect to different grazing systems.

Sheep breeds	Extensive grazing system				Semi-intensive grazing system			
	B.WT	D.WT.G	W.WT	AD.WT	B.WT	D.WT.G	W.WT	AD.WT
Balkhi	2.58 ± 0.19	0.13 ± 0.003	17.70 ± 1.03	33.50 ± 2.51	3.02 ± 0.17	0.16 ± 0.01	20.40 ± 1.71	38.91 ± 1.14
Damani	1.54 ± 0.20	0.10 ± 0.01	14.8 ± 0.57	25.8 ± 0.57	2.02 ± 0.35	0.12 ± 0.006	17.81 ± 1.15	31.82 ± 1.78

B.WT = Body weight, D.WT.G = Daily weight gain, W.WT = Weaning weight, AD.WT = Adult weight.

Table 2. Changes in fertility parameters in Balkhi and Damani sheep breed with respect to different grazing systems.

Sheep breeds	Extensive grazing system					Semi-intensive system				
	CR%	LB%	SB%	AB%	TWIN%	CR%	LB%	SB%	AB%	TWIN%
Balkhi	86	90	4	4	0	90	96	2	2	0
Damani	86	96	3	3	10	96	100	1	1	20

CR = Conception rate, LB = Live birth, SB = Still birth, AB = Abortion.

Table 3. Behavioral stress parameters in two different indigenous breeds of sheep under different rearing systems.

Independent Variable		Respiration rate (RR/min)	Rectal temp RT (°F)	Pulse rate (PR/min)
Breed	Balkhi	56.40 ± 1.92	103.50 ± 0.17	74.56 ± 1.09
	Damani	49.43 ± 1.66	103.00 ± 0.01	70.53 ± 0.69
	<i>p</i> -value	<0.01	<0.01	<0.01
System	SIS THI, 88.10	46.53 ± 1.63	102.92 ± 0.13	69.40 ± 0.76
	ES THI, 90.68	59.30 ± 1.35	103.58 ± 0.16	75.70 ± 0.82
	<i>p</i> -value	<0.01	<0.01	<0.01
Day	Day 0 THI, 85.95	52.70 ^a ± 1.59	103.30 ^a ± 0.20	72.25 ^a ± 1.59
	Day 14 THI, 88.10	53.45 ^a ± 2.69	103.23 ^a ± 0.17	73.60 ^a ± 1.22
	Day 28 THI, 89.74	52.60 ^a ± 2.63	103.22 ^a ± 0.21	71.80 ^a ± 2.63
	<i>p</i> -value	NS	NS	NS

^{a, b, c} Means with different superscripts within the same column are different significantly.

Table 4. Data showing the level of different thermal and hormonal stress parameters in two different indigenous breeds of sheep under different systems of rearing.

Independent Variable		HSP-70 (ng/ml)	Cortisol (nmol/l)	T3 (nmol/l)	T4 (nmol/l)	MDA (nmol/l)
Breed	Balkhi	55.26 ± 0.48	2.64 ± 0.02	55.26 ± 0.48	1.08 ± 0.02	6.38 ± 0.13
	Damani	47.54 ± 0.87	2.34 ± 0.01	47.54 ± 0.87	1.42 ± 0.02	5.18 ± 0.15
	<i>p</i> -value	<0.01	<0.01	<0.01	<0.01	<0.01
System	SIS THI, 88.10	48.14 ± 0.97	2.40 ± 0.02	48.14 ± 0.97	1.38 ± 0.03	5.04 ± 0.12
	ES THI, 90.68	54.66 ± 0.58	2.59 ± 0.03	54.66 ± 0.58	1.12 ± 0.03	6.53 ± 0.11
	<i>p</i> -value	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01
Day	Day 0 THI, 85.95	50.89 ^a ± 1.18	2.47 ^a ± 0.04	50.89 ^a ± 1.18	1.24 ^a ± 0.05	5.69 ^a ± 0.23
	Day 14 THI, 88.10	51.91 ^a ± 1.22	2.50 ^a ± 0.04	51.91 ^a ± 1.22	1.26 ^a ± 0.04	5.76 ^{ab} ± 0.22
	Day 28 THI, 89.74	51.40 ^a ± 1.30	2.51 ^a ± 0.04	51.40 ^a ± 1.30	1.25 ^a ± 0.05	5.89 ^b ± 0.22
	<i>p</i> -value	NS	NS	NS	NS	< 0.01

^{a, b, c} Means with different superscripts within the same column are different significantly.

Relationship between stress markers and physiological parameters

Cortisol was found positively correlated with all physiological parameters such as Pulse rate (PR), Respiration rate (RR), and Rectal temperature (RT) ($r = 0.622$, $r = 0.613$, and $r = 0.366$; $p < 0.01$), respectively, but negatively correlated with both metabolic hormones, i.e., T3 and T4 ($r = 0.860$, $p < 0.01$). All behavioral stress parameters were found positively correlated with HSP-70 ($r = 0.528$; $p < 0.01$).

Discussion

Sheep production constitutes an important part of agriculture in developing countries. The production is at risk if subjected to high ambient temperature during the summer season [10]. Alteration in various physiological parameters could act as an indicator of heat stress. Physiological adaptation may be related to normal RR, pulsation rate, and RT during stress [11]. In the present study, higher values of behavioral stress parameters (PR, RR, and RT)

in Balkhi sheep indicate higher sensitivity of this breed toward heat stress, whereas Damani sheep breed is more resistant and well adapted to the local heat stress environment. Similarly, the elevated behavioral stress values observed in the ES may be associated with poor management, feeding, and so on. The results are in agreement with Pennisi et al. [6] who reported similar effects of different housing systems on behavioral stress parameters during the hottest period of the year in the Mediterranean area. Similar observations were found in Ouled Djellal breed of ewe in Algeria. Fertility and growth rate parameters were severely affected by thermal, nutritional, and walking stress resulted in decrease productive and reproductive performance [12]. In this study, a significant variation in values of growth rate parameters and fertility parameters was observed among breeds and systems of rearing. The findings of this study are in accordance with Munir et al. [13] who reported the average weaning weight of lambs in increasing order from extensive, semi-intensive, and intensive management system. Appreciably, lambs with higher weaning weights were observed in intensive group in comparison to other groups. The highest mortality was observed in lambs of ES rearing, whereas no mortality was observed in SIS and intensive rearing system. In another study, a constant increase in live weight of sheep under intensive system of rearing was observed from the 1st day of trial up to the end of that week compared to ES [14]. It strongly supports the findings. We observed the highest weaning weight and higher live birth % age in SIS system of rearing comparatively. It might be due to concentrate feeding (500 gm), proper housing, and management as compared to ES.

Different management systems for ewes were compared in Bangladesh. The reproductive efficiency was improved in ewes kept in SIS than in intensive system, but lamb survivability was improved in intensive system. Increased lamb survivability was due to proper management practices, healthcare at the time of birth, and weaning stage [15]. As shown in the SIS system of this study, the conception rate and live birth rate were higher in both Damani and Balkhi sheep kept in SIS.

Air movement is an important factor in relieving heat stress; therefore, proper ventilation facility may be provided in management practices [10]. It supports this study because in the SIS system of rearing, proper ventilation, floor space requirements, and sheds were the probable reasons for reduced level of PR, RR, and RT. Farmers may consider proper ventilation facility during farm building construction. Fans may be installed in the farm building to ensure optimum airflow. It may be concluded that SIS is a better system of rearing as compared to ES of rearing for both breeds of sheep regarding physiological adaptation during thermal stress.

Cortisol is a stress hormone which has a crucial role in many physiological processes, particularly in the thermal regulation of the body [16]. Cortisol is secreted by the adrenal glands, which allows better patience to the thermal stress in dairy cows [17]. In various studies, an increased level of cortisol was reported in sheep under heat stress [18]. In this study, the cortisol level in two different breeds of sheep was, therefore, determined with an objective to determine which breed performs well in high ambient temperature. A higher concentration of cortisol in ES ewes indicates that ewes bear more stress in ES than SIS. Comparing breeds, Balkhi breed showed higher cortisol level than local Damani breed in both SIS and ES, which indicates increase susceptibility of Balkhi breed to heat stress.

HSP-70 is produced in the body in response to different kinds of stresses. Its expression increases in heat stress [19] and plays an important role in protection from cell injury [20]. HSP-70 constitutes a part of mechanism responsible for heat dissipation in hairy sheep in tropical and subtropical climates [21]. In the present study, the concentration of HSP-70 was higher in Balkhi sheep and ES of rearing. It revealed that HSP-70 level is increased with an increase in thermal stress. These findings are compatible with the previous findings. Heat stress significantly amplified HSP-70 in bovine Angus and Hereford bovine breeds. However, it was unaltered in bovine Brahman breeds such as Senepol and Romosinuano under heat stress. The reason might be the well acclimatization and tolerance of Brahman bovine breeds [22]. A similar pattern of HSP-70 was noted in goats acclimatized to the barren region compared with less adapted goats [23]. In this study, the interaction of breed and system also showed a significant effect on HSP-70 level which is supported by the findings of Romero et al. [22]. The results are also in agreement with the metabolic parameters of goats submitted to heat stress in Egypt [24]. It may be concluded from this study that Balkhi ewes showed a higher level of HSP-70 in both rearing systems, indicating that this breed is more susceptible to stress than local Damani breed of ewes because of its low adoptability to the local hot environment.

We found that both T3 and T4 levels were higher in Damani sheep in both SIS and ES which revealed that it is more stress tolerant under local conditions as compared to Balkhi sheep. A strong positive relationship was observed between T3 and T4, which is in line with the previous studies [25]. Sejian et al. [26] reported the highest T3 level in Malpura ewes of India kept at 21°C, whereas an increase in the temperature showed a negative impact on the expression of T3. T3 and T4 levels were also found lower in goats during May–July [27] and ewes [28]. We found a similar pattern of T3 and T4 ewes. In contrast to the above results, camels showed a higher level of serum T3 and T4 during the

summer season as compared to winter. It is related to sufficient water intake. If water intake is reduced, the thyroid function is reduced with eventual reduction in fertility [29].

Conclusion

Comparing Balkhi and Damani sheep breed, Damani breed is more resistant to heat stress, and it could be preferred to rear in warm geographical area without a danger of losing productivity due to heat stress. Comparing two management systems SIS and ES, SIS of rearing is more desirable for sheep management because it poses little danger to animal's health.

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Conflict of interest

The authors declare no conflict of interest.

Author's contribution

S designed and conducted the study; Imdad Ullah Khan, Alam Zeb Khan, Shahid Zaman, Abdul Majid, Attiq ur Rehman, and Mumtaz Ali Khan helped in data collection, sampling, sample processing, and data analysis; Hamza Maris and Rahman Ullah helped in manuscript writing setting and data analysis; Subhan Qureshi provided the idea and supervised the experiment as a whole.

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