



Clinical, behavioral and biochemical alterations due to shearing stress in Ossimi sheep

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ABSTRACT. The effects of shearing on behavioral patterns, antioxidants, and inflammatory and stress biomarkers was investigated in Ossimi sheep. Clinical parameters and behavioral patterns were recorded, and serum samples were collected pre-shearing and 1, 5, 10 and 15 days post-shearing from 60 Ossimi sheep. The results revealed that grooming and standing idle frequencies were significantly ($P<0.01$) increased post-shearing. There were significant ($P<0.01$) increases in IL-6, cortisol, and MDA detected from the 1st to the 10th day post-shearing compared to pre-shearing values. IL-2 and TNF- α significantly ($P<0.01$) increased from the 1st until the 5th day post-shearing compared to pre-shearing values, while significant ($P<0.01$) decreases in the values of catalase from the 1st until the 5th day post-shearing compared to pre-shearing values were recorded. Regarding to glutathione reductase, there was a significant ($P<0.01$) decrease from the 1st until the 10th day post-shearing compared to pre-shearing values. Shearing leads to significant changes in antioxidants, inflammatory, stress biomarkers, and some behaviors in sheep.

KEY WORDS: behavior, oxidative stress, physiology, shearing

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Routine management procedures in sheep breeding, such as shearing, isolation and transportation induce some biological changes known as stress [7, 29]. The reaction to such stressful conditions is mainly centered in the activation of the sympathetic system and the hypothalamic- hypophysis- adrenal axis through catecholamine and glucocorticoid production [36]. Shearing usually results in an enhancement of energy exchange between the animal and its surroundings, and this may affect the productivity and welfare of the sheep [3].

Domestic animals react to stress by alerting their behavioral and physiological responses, which result from their individual emotional reactivity [21]. The physiological response to stress includes activation of the central nervous system and the endocrine and immune systems, and it can affect the animal's production performance. In sheep, different studies have demonstrated the worsening of production performance in response to stressful management procedures [16, 45].

Wool shearing is one of the management practices that can influence the success of sheep growing. Wool shearing is usually applied by farmers to minimize external parasites that could cause health problems. In addition, farmers believe that shearing sheep would improve growth performance and could make them more adaptable to the environment [40].

Shearing is considered to be stressful to sheep, capable of causing some behavioral changes, including comfort behaviors that change immediately after shearing [4, 9] and results in thermal stress [17]. Stressful conditions lead to excessive production of free radicals, which results in oxidative stress. This is commonly defined as an imbalance between oxidants and antioxidants in favor of oxidants at the cellular level [39]. Oxidative stress causes cellular injuries in cells and tissues in the form of lipid peroxidation which includes malondialdehyde (MDA) [42] and cytokines [19]. Determination of MDA [20], catalase and glutathione reductase [18] allows the detection of the degree of oxidative stress in ruminants [1].

The aim of this work was to investigate the effect of shearing on some welfare parameters, antioxidants, and inflammatory and stress biomarkers in Ossimi sheep.

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Table 1. Behavioral ethogram of sheep

Behavior	Description
Grooming	Sheep scratch the accessible parts of the body with their teeth or claws
Rumination	Regurgitation of food from the rumen; re-chewing and re-swallowing it
Grazing	Sheep collect vegetation from the ground
Walking	Move from one point to another
Standing idle	Pointless positioning of all four feet

MATERIALS AND METHODS

The current study procedures were carried out according to the guidelines of the faculty of veterinary medicine Benha University for the use of animals under ethics approval no (fvtm.bu/01).

This study was carried out in September 2017 with a flock of 60 clinically healthy Ossimi rams (average body weight 30 ± 2.3 kg, aged between 1–2 years). Sheep health status was evaluated daily, based on behavior, rectal temperature, heart rate, respiration, cough, nasal and eye discharges, appetite, ruminal movements, and the physical characteristics of the fecal matter.

The environmental temperature and humidity levels were recorded during the experimental period. The average environmental temperature was 31°C , and the average relative humidity was 75%. During the day, the animals grazed on natural pasture, and they were penned at night, where they received a concentrate ration of 250 g per head (crude protein 18%), with free access to hay and water *ad libitum*.

Experimental procedures

Blood sampling. Blood samples were collected from the animals just before shearing, and then at 1, 5, 10 and 15 days after shearing. Serum was used to measure Catalase (CAT), Glutathione reductase (GSH-R), Malondaldehyde (MDA), Interleukin 6 (IL6), Interleukin 2 (IL2), Tumor necrosis factor alpha (TNF- α) and cortisol.

Behavioral observation. Behavioral patterns were recorded by means of live observations. Each animal was observed for 10 min once per week through continuous focal sampling [5]. The behavioral patterns of each animal were recorded pre-shearing, and on the 1st, 5th, 10th, and 15th day post-shearing. The behaviors recorded during the study are presented in Table 1.

Biochemical analysis. Serum cortisol concentrations were determined using an ELISA kit (Eucardio Laboratory, Inc., Encinitas, CA., U.S.A.). CAT levels were determined using the methods described by Goth [27]. TNF- α , IL-6 and IL-2 levels were determined with ELISA according to the methods described by Fan *et al.* [24]. MDA levels were determined using the methods described by Draper and Hardley [22]. GSH-R levels were determined according to Bulfield and Moore [11].

Statistical analysis. The obtained results from the experiments were expressed as means \pm SEM and were analyzed by analysis of variance (ANOVA) for repeated measures using SPSS Statistics for Windows, version 23.0 (IBM Corp: Armonk, NY, U.S.A.). Differences were declared significant when ($P < 0.05$).

RESULTS

Clinical and behavioral observations

Rectal temperature, pulse, and respiratory rates increased until the 5th day post-shearing. Rumination, grazing, and walking were not significantly affected by shearing, while there was a significant ($P < 0.01$) increase in frequency of grooming post-shearing, especially on the 10th and 15th day. Shearing also significantly increased the frequency of standing ($P < 0.05$), (Table 2, Fig. 1).

Biochemical analysis

There were significant ($P < 0.01$) increases in IL-6, cortisol, and MDA from the 1st to the 10th day post-shearing compared to pre-shearing values, while at the 15th day there were no significant differences from pre-shearing values. IL-2 and TNF- α significantly ($P < 0.01$) increased from the 1st to the 5th day post-shearing compared to pre-shearing values, while there were no significant differences from the 10th day. Significant ($P < 0.01$) decreases in the values of CAT from the 1st to the 5th day post-shearing compared to pre-shearing values were recorded. Regarding to GSH-R, there was a significant ($P < 0.01$) decrease from the 1st until the 10th days post-shearing compared to pre-shearing values, while there was no significant difference at the 15th day from the pre-shearing values (Table 3, Fig. 2).

DISCUSSION

Evaluation of the effect of shearing on some welfare parameters, antioxidants, and inflammatory and stress biomarkers in Ossimi sheep revealed significant alterations in some clinical, behavioral and biochemical parameters regarding to oxidative stress.

Clinical and behavioral observations

The physical stress of shearing produced a transient increase in rectal temperature in terms of stress-induced hyperthermia

Table 2. Frequency of sheep behavioral patterns pre and post-shearing

Behavior	Pre-shearing	Post -shearing			
		1st day	5th day	10th day	15th day
Grooming	7.5 ± 0.68 ^{b)}	8 ± 0.68 ^{b)}	9.66 ± 0.68 ^{b)}	14 ± 0.68 ^{a)}	15 ± 0.83 ^{a)}
Rumination	8 ± 0.67	8.16 ± 0.67	8.33 ± 0.67	7 ± 0.67	5 ± 0.82
Grazing	9 ± 0.62	10.66 ± 0.62	10 ± 0.62	9.83 ± 0.62	9.86 ± 0.76
Walking	6.73 ± 0.86	7.33 ± 0.86	8.33 ± 0.86	9.66 ± 0.86	6.50 ± 1.06
Standing idle	1.30 ± 0.68 ^{b)}	6 ± 0.68 ^{a)}	4 ± 0.68 ^{a)}	3.66 ± 0.68 ^{a)}	3 ± 0.83 ^{a)}

Means (± SE) in the same row with different superscript letters are significantly different when ($P < 0.05$).

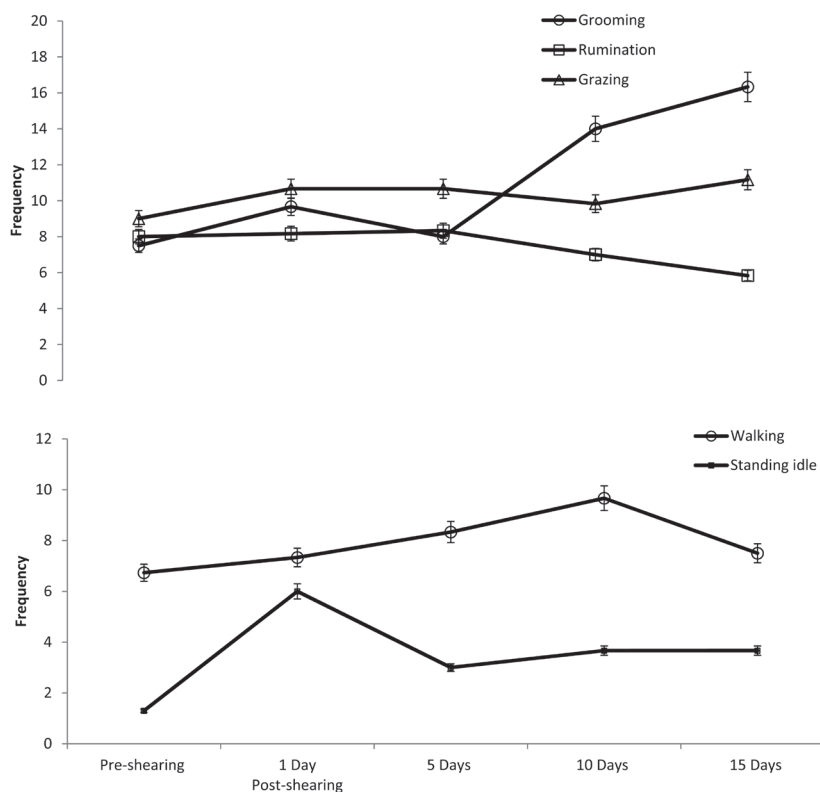


Fig. 1. Frequency of sheep behavioral patterns pre-shearing and at 1,5,10, and 15 days post-shearing.

[17, 48], which coincided with the current study. The increased respiratory rate probably occurred because it tends to closely follow heat loss by evaporation [38]. Heart rate showed a similar trend in respect to respiratory rate due to the direct effect of heat stress inducing hemodynamic adaptations, because the animals produced high quantities of heat dispersed by an enlarged peripheral vascular system [40].

The shearing process significantly affects grooming behaviors, as the frequency of grooming among shorn sheep was higher compared to that of unshorn sheep. The current result agrees with Naglaa *et al.* [37] who found that there were no significant differences in the behaviors of shorn and unshorn ewes, except in grooming behaviors which were significantly greater among the shorn ewes. The increase in grooming behaviors post-shearing may be attributed to the stimulus effect of shearing in the skin of sheep, which initiates grooming. The current results also agree with Benjamin and Patricia [9] and Hart and Pryor [30], who studied the effect of the shearing process on grooming and reported that immediately after shearing the skin shows different bouts of cutaneous stimuli leading to an increase in grooming behaviors.

Although frequency of rumination decreased at the 10th and 15th day post-shearing, the effect of shearing was not significant. Additionally, the results showed that shearing had no significant effect on grazing. This may be attributable to the fact that the sheep received the same type and amount of food, and they grazed at the same pasture both before and after shearing. Previous studies [8, 37] reported no change in food intake and feeding behaviors in shorn and fleeced sheep. Badawy *et al.* [6] stated that shorn sheep showed a decline in rumination frequency compared with unshorn sheep.

The current results showed variations in walking frequencies of sheep. Higher levels of walking were recorded at the 1st, 5th, and 10th day post-shearing. This increase in walking activities may be attributable to the painful effect or fear reaction resulting

Table 3. Biochemical parameters of sheep pre and post-shearing

Parameters	Pre-shearing	Post-shearing			
		1st day	5th day	10th day	15th day
IL6 ($\mu\text{g/ml}$)	6.54 \pm 0.65 ^b	13.94 \pm 1.3 ^a	15.53 \pm 2.1 ^a	12.42 \pm 0.90 ^a	7.46 \pm 0.47 ^b
IL2 ($\mu\text{g/ml}$)	0.37 \pm 0.07 ^b	0.85 \pm 0.08 ^a	1.15 \pm 0.56 ^a	0.55 \pm 0.02 ^b	0.48 \pm 0.05 ^b
Cortisol ($\mu\text{g/dl}$)	6.96 \pm 0.82 ^b	20.64 \pm 2.5 ^a	23.79 \pm 1.9 ^a	11.58 \pm 0.84 ^a	9.55 \pm 0.49 ^b
TNF- α (U/ml)	30.25 \pm 1.9 ^b	63.73 \pm 6.2 ^a	52.88 \pm 2.3 ^a	39.72 \pm 1.6 ^b	30.95 \pm 1.4 ^b
CAT (kU/l)	43.65 \pm 3.9 ^a	19.46 \pm 2.03 ^b	18.68 \pm 2.4 ^b	35.39 \pm 3.02 ^a	41.72 \pm 3.3 ^a
GSH-R (kU/l)	7.7 \pm 0.5 ^a	3.6 \pm 0.2 ^b	3.3 \pm 0.7 ^b	6.1 \pm 0.4 ^b	7.8 \pm 0.3 ^a
MDA (mmol/l)	0.66 \pm 0.6 ^b	1.2 \pm 0.5 ^a	1.3 \pm 0.7 ^a	0.97 \pm 0.4 ^a	0.67 \pm 0.5 ^b

Means (\pm SE) in the same row with different superscript letters are significantly different when ($P < 0.05$).

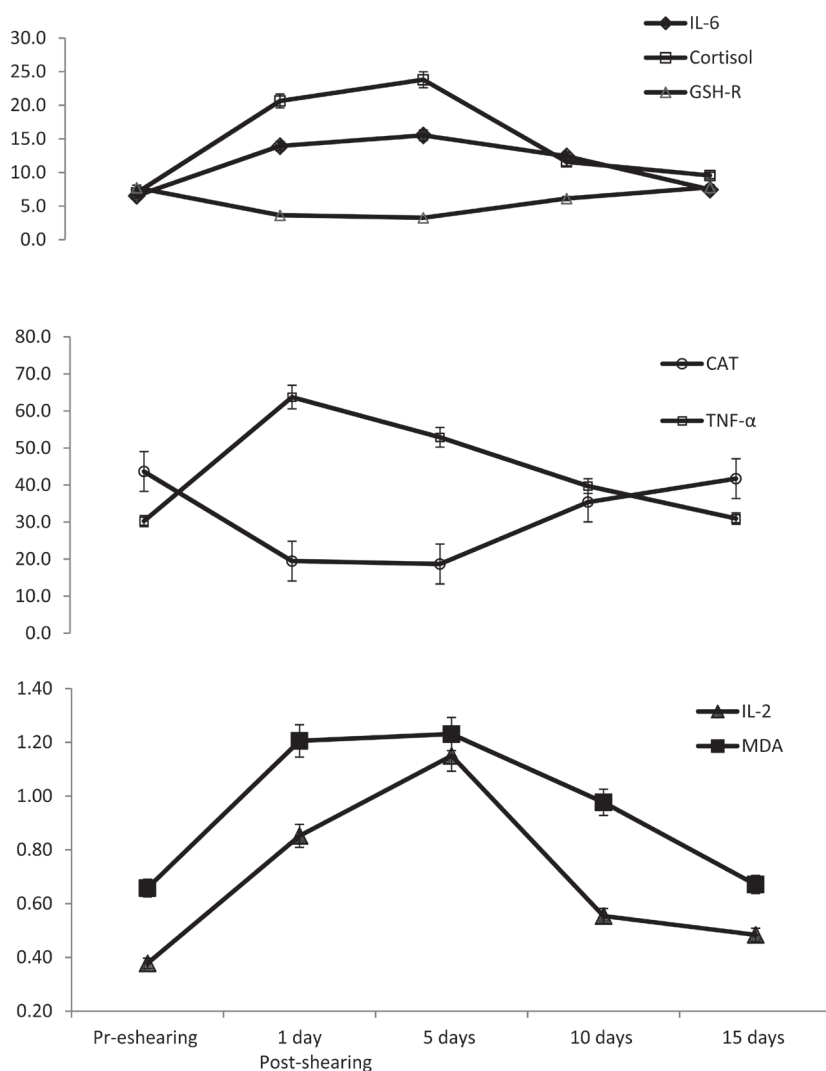


Fig. 2. Biochemical parameters of sheep pre-shearing and at 1, 5, 10, and 15 days post-shearing.

from the shearing process, as reported by Badawy *et al.* [6]. On the 15th day after shearing, walking frequency decreased, which may be attributable to the sheep's adaptation to post-shearing conditions.

The current results revealed higher frequencies of standing post-shearing compared to pre-shearing as a sign of stress resulting from shearing [44]. Teresa *et al.* [47] reported that unshorn sheep stood less than the shorn sheep ($P < 0.05$). This was dissimilar to the results of Naglaa *et al.* [37] who found no effect of shearing on standing idle among shorn and unshorn sheep.

Biochemical analysis

Stress has been defined as the cumulative response of an animal resulting from interactions with its environment via receptors [26]. Blood cortisol levels were higher post-shearing than in pre-shearing; the high levels were maintained at the same rate between the 1st and 10th day post-shearing. The rise in blood cortisol levels that was observed post-shearing coincided with previous reports [13, 28]. Several studies have demonstrated that plasma cortisol concentrations increase in sheep in response to stressors, like the shearing process [25, 28, 34, 41]. In agreement with this finding, the present study recorded, marked elevations in plasma cortisol concentrations post-shearing, suggesting that shearing by itself would be a stressful event.

In the present study, the shearing procedure induced a significant increase in blood MDA concentrations, indicating the occurrence of oxidative stress. Increase in MDA was interpreted as a marker of excess free radical production [1] in sheep post-shearing. Lipids are the most susceptible substrates to free radical damage. MDA is the last product of lipid peroxidation, and is a key marker of oxidative stress [18]. Various studies have reported that lipid peroxidation products, such as MDA, increase in diseased and some managemental conditions as transportation [2]. On the other hand, the antioxidant defense systems include small molecular antioxidants and antioxidant enzymes like CAT and GSH-R [25].

Increased production of free radicals as hydrogen peroxide under stressful conditions leads to augmented oxidative stress, resulting in enhanced lipid peroxidation and MDA production [43] as well as reduction in CAT [32, 35] and GSH-R [23]. This agrees with the results of the current study, which can be induced under some conditions by exposure to oxidative stress during some management procedures as is the case with sheep during shearing. Therefore, GSH-R can be considered a special system of antioxidant enzymes controls the concentrations and fluxes of superoxide radicals, hydrogen peroxide, and the reactive products [12, 33]. A stressful condition leads to the excessive production of free radicals, which results in the oxidative stress associated with an imbalance between oxidants and antioxidants in favor of oxidants at the cellular level [31, 39, 46]. This result could be explained by the occurrence of free radicals due to the stress factors present during the shearing process. Stressors can determine the secretion of pro-inflammatory cytokines IL-2, IL-6, and TNF- α . In turn, cytokine production and actions are controlled by cortisol [10, 14]. The direct relationship found between the plasma cortisol and cytokine concentration [15, 19] results from this study demonstrated that, significant increases in cortisol, IL-2, IL-6, and TNF- α levels post-shearing were considered to be stressors.

In conclusion, the shearing procedure may be considered a stressful condition, as evidenced by the significant increase in cortisol, MDA, IL-6, IL-2, and TNF- α concentrations associated with decreased GSH-R and CAT concentrations, as well as clinical and behavioral changes. The increases in plasma cortisol and MDA concentrations in response to shearing could be additional factors responsible for oxidative stress. The determination of oxidative stress parameters could be a new approach for evaluating stress in sheep, and antioxidant treatment is recommended for decreasing stressful condition that resulted from shearing.

CONFLICT OF INTEREST. The authors declare no conflict of interest.

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