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Impact of saccharomyces enriched with selenium on growth performance and metabolic status of sheep exposed to heat stress.

Haidy E. Mohamed [*] and Hala N. Ibrahim [**]

[*]Biochemistry Department, Agriculture Research Center (ARC), Animal Health Research Institute (AHRI), Ismailia Branch 41511, Egypt.
[**]Clinical pathology Department, Agriculture Research Center (ARC), Animal Health Research Institute (AHRI), Ismailia Branch 41511, Egypt.

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ABSTRACT:

The objective of this research was to determine the effect saccharomyces enriched with selenium yeast (SeY) on Rahmani sheep and to evaluate the influence of the oral supplementation of saccharomyces enriched selenium on growth performance, Haematobiochemical, and antioxidant parameters in such sheep. Twenty Rahmani sheep were allocated into 4 groups , each group have five sheep, which were control group : received basal diet and not exposed to heat stress, Heat stress group (HS): received basal diet and exposed to heat stress, heat stressed with Saccharomyces enriched selenium(HS+SeY):received basal diet containing Saccharomyces enriched selenium and exposed to heat stress, non-heat stressed group with Saccharomyces enriched selenium (NHS+SeY):received basal diet with Saccharomyces enriched selenium and not exposed to heat stress. Temperature and humidity index were calculated. Experiment undergoes for two months (July and August). The results showed that Saccharomyces enriched selenium causes significant improvement in feed efficiency in sheep under heat stress. Saccharomyces enriched selenium supplementation to Hs sheep showed improvement of Haematological indicator and tendency toward normal values indicating positive effect of Saccharomyces enriched selenium. Furthermore, the findings of the study demonstrated that selenium enriched saccharomyces diet have positive impact on various blood metabolites, such as glucose, total protein, albumin, globulin, liver and kidney enzymes, and triglyceride levels in heat stressed sheep. Feeding Saccharomyces enriched selenium under heat stress to sheep not only cause significant decrease in serum malonaldehyde (MDA) concentration as indicator of lipid peroxidation, but also cause significant increase in glutathione peroxidase (GPx), catalyse (CAT) and superoxide dismutase (SOD) activity. In summary, inclusion of Saccharomyces enriched selenium to sheep diet during summer condition, is advantageous to growth performance, haematobiochemical and antioxidant parameters of Rahmani sheep.

*Corresponding author: Haidy Elsayed Mohamed, biochemistry Department, Agriculture Research Center (ARC), Animal Health Research Institute (AHRI), Ismailia Branch. Egypt
E-mail: haidyvet_2000@yahoo.com
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INTRODUCTION

Sheep live in complex environments where they are constantly confronted with short- and long-term environmental changes including nutrition, geographical areas, temperature, and photoperiod. Livestock production is the primary component which adversely affect by detrimental effects of extreme climatic conditions around the world **(Keshri et al. 2022)**. Heat stress is the most significant stressor influencing sheep development, growth and reproduction **(van Wettere et al. 2022).** Heat stress, combined with increased solar radiation, will have an impact on livestock health **(Serrano et al. 2022)**, HS have a direct impact on animals by reducing feed intake, changing production traits, negatively affecting reproduction, decreasing disease resistance, and thus affecting the animal's overall efficiency and health. While sheep are more heat tolerant than other ruminants, the negative impact of heat stress on sheep productivity is well documented **(DiGiacomo et al. 2021)**.

Heat stress not only affects the physical condition but also affects reproductive performance in sheep. Climatic variables can trigger physiological, biochemical, hematological and hormonal alterations **(De et al. 2017; Haire et al. 2022; McManus et al. 2020)**, lowering serum trace mineral concentrations involved in antioxidant defense in the body **(Khan et al. 2016; Kumbhar et al. 2018),** blood electrolyte imbalance, and imbalance of the gut microbiota and barrier function, which all can affect animal performance, health, and welfare and cause economic losses **(Patra and Kar, 2021; Podder et al. 2022).** exposure of sheep to heat stress evokes a series of drastic changes in the biological functions, which include a decrease in feed intake efficiency and utilization, disturbances in water, protein, energy and mineral balances, enzymatic reactions, hormonal secretions and blood metabolites **(Marai et al. 2007),** impairs liver function, causes oxidative stress, jeopardizes the immune response and decreases reproductive performance **(McManus et al. 2020).**

HS moreover, it affects immune response and increased reactive oxygen species(ROS) production and/or deficiency of serum antioxidants micronutrient **(Alhidary et al. 2012)** as selenium, zinc, and vitamin E during heat stress leading to an imbalance between oxidant and antioxidants and resultant oxidative stress **(Chauhan et al. 2021),** ROS stimulation suggested to be result of HS **(Slimen et al. 2019)**. Most studies with ROS in sheep look at the role of antioxidants in modulating or mitigating HS **(Chauhan et al. 2014; Proietti et al. 2018)**.

Nutritional strategies have been investigated as a promising approach to mitigating the negative effects of heat stress. Se plays critical metabolic role, optimizing its level in the diet for improving immunity and antioxidant status would aid in the amelioration of the negative effects of HS in animals **(Aderao et al. 2023; Alhidary et al. 2012)**.

Se exists primarily in two forms as a food additive: inorganic Se and organic Se **(Naiel et al. 2021).** Organic Se possesses antioxidant properties and has higher bioavailability and rates of product accumulation as well as lower toxicities as compared to inorganic form **(Liu et al. 2021; Lu et al. 2018; Pan et al. 2007; Payne et al. 2005; Thiry et al. 2012).** selecting selenium additives such as organic selenium to produce selenium-enriched animal products is a relatively easy and achievable method **(Bai et al. 2022).** Organic forms like selenium-enriched yeast are utilized in many countries as high-quality source of organic selenium with excellent absorption rate, minimal toxicity, and wide safety margin **(Kieliszek et al. 2015)**, it has the ability to improve meat quality as well as production of Se-enriched meat **(Wu et al. 2011).** Addition of enriched yeast with selenium resulted in a higher proportion of lymphocytes in the blood, and a diet with high selenium content did not negatively impact various blood metabolites such as total protein, albumin, globulin, liver enzymes, glucose, and triglyceride levels. Taken together, these results indicate that enriched yeast with selenium is a safe source of selenium for sheep, and its dietary supplementation beyond the nutritional requirements enhances feed efficiency in growing lambs **(Mousaie, 2021a).**

Including selenium yeast (SeY) in the diet can plays a critical role in preserving intracellular redox balance by effectively neutralizing harmful reactive radicals. As a result, it helps mitigate cellular oxidative damage **(Guo et al. 2015; Li et al. 2023; Liu et al. 2021; Liu et al. 2020a; Luo et al. 2018) by** mitigating the adverse effects of heat stress on the immune system, physiological status **(Abbas et al. 2022),** growth performance by increasing antioxidant capacity, immune function, and suppressing inflammatory response **(Liu et al. 2021).**

Selenium yeast has the potential to enhance retention of selenium and absorption in ruminant animals. Previous research has demonstrated that incorporating selenium into diet can enhance the activity of key antioxidant enzymes such as glutathione peroxidase (GPx) and superoxide dismutase (SOD). Furthermore, it reduces the levels of malondialdehyde (MDA) in sheep serum, thereby improving the overall antioxidant capacity **(Shi et al. 2017; Sobeková et al. 2006).**

Furthermore, the yeast enriched with selenium can work properly as a clinical health product or drug for a variety of diseases such as Alzheimer's **(Zhang et al. 2018).** The advantageous effects of Saccharomyces enriched selenium supplementation on intestinal health and growth in the upon oxidative stress revealed that it could be used as a therapeutic antioxidant factor **(Liu et al. 2020b).** As a result, Se protective action, which is primarily focused on boosting internal antioxidant defense in metabolic diseases, has received a lot of attention **(Huang et al. 2022).** Selenium and other antioxidants have the ability to remove harmful reactive oxygen species (ROS) and help maintain the balance of redox reactions. They safeguard the integrity of cell membranes, regulate the immune response, reduce damage to the intestinal barrier, and enhance the production of heat shock proteins (HSPs) in animals experiencing stress **(Chauhan et al. 2016)**

Accordingly, the present research was carried out to observe whether supplementation with Saccharomyces enriched selenium in the diet of sheep could affect the hematobiochemical and antioxidant parameter during heat stress.

MATERIAL and METHODS

Ethical approval:

The animal-related procedures carried out in this study in accordance with the guidelines set by the Ethics of Animal Use Research Committee at (ARC-IACUC) at agricultural research center, protocol number ARC- AHRI- 39-23.

MATERIAL

Yeasel® has been received from Angel yeast Co., LTD., Egypt.

Yeasel® is a well-known source of organic selenium, which was obtained through a process of submerged fermentation. *Saccharomyces cerevisiae* fermented in a selenium-enriched medium to produce this valuable source of selenium

Dose: Yeasel 2000ppm, 300gm/ton.

Animals' management and experimental design

Twenty Rahmani sheep (with average weight 32.56±23 kg) and aged 5-6 months were used in this experiment, which provided farm vaccination routine protocols. Investigated Sheep were fed on concentrated feed mixture (CFM) (**Table 1**) twice a day, basal diet for growing sheep were set using guidelines established by the **(NRC, 2007)**. After 14 days of adaptation to the basal diet and experimental condition, the sheep were randomly allocated to one of the dietary treatments, five animals were placed in each of the four experimental treatments, which were assigned as following: **(control**): negative control, non -heat stressed and basal diet without Yeasel , (**HS**): positive control heat stressed, basal diet without Yeasel, (**HS+SeY**): sheep were heat stressed and receive basal diet with Yeasel and (**NHS+SeY**): sheep were non heat stressed and received basal diet with Yeasel. The experimental period lasted for 8 weeks, each group was individually penned (2.5 × 2.5 m pens) with free access to fresh water, the heat stress exposures were performed from 12:00 to 3:00 pm. The leftover feed from the previous day was collected and weighed for each group before the morning meal. According to the following equations, average total weight gain and feed conversion efficiency were calculated: Average total weight gain (kg) = final body weight - initial body weight. The body weight (BW) of sheep was recorded every week during the experiment after an overnight fast

Table 1. the ingredient and chemical composition of experimental basal diet:

Component	Ingredient Kg/ Ton	Ingredient %
Corn	500	50%
Soya bean meal	150	15%
Wheat bran	328	32.8%
Salt (Sodium chloride)	7	0.7%
Limestone (Calcium carbonate)	10	1%
Premix® (Vitamin- mineral)	5	0.5%
Total	1000	100%

The premix contained the following amounts of nutrients per kilogram: 12,000,000 IU of vitamin A, 1,000 mg of vitamin E, 2,000 mg of vitamin K3, 1,000 mg of vitamin B1, 4,000 mg of vitamin B2, 10 mg of vitamin B12, 3.33 g of pantothenic acid, 33 mg of biotin, 0.83 g of folic acid, 200 g of choline chloride, 5 g of manganese (Mn), 12.5 g of iron (Fe), 0.5 g of copper (Cu), 133.3 mg of iodine (I), 16.6 mg of selenium (Se), and 66.7 g of magnesium (Mg).

Blood collection for hematological and biochemical examination

At the end of experiment period (60 days), from each sheep two blood samples were collected from jugular vein in heparinized tubes for hematological examination included estimation of RBCs count, Hb concentration, PCV, MCV, MCHC, MCH, total and differential leukocytic counts according to the method that was adopted by **Bain et al. (2006).** Another blood sample taken on non-heparinized tube for biochemical tests, the blood was stored at 4 ºC for 12 h and allowed to coagulate to produce sera and then the blood samples were centrifuged at 3,000 rpm for 15 min, and then sera were collected. The plasma sera were kept at –20◦C until analysis. Individual serum samples were analyzed for estimation of serum total protein and albumin were estimated according to **Doumas et al. (1981)** and **Drupt (1974)** respectively, aspartate aminotransferase (AST, U/L) and alanine aminotransferase (ALT, U/L) activities according to **Reitman and Frankel (1957)** and uric acid (UA, mole/L) according to **Barham and Trinder (1972)** and creatinine (μmol/L) levels according to **Schirmeister et al. (1964),** glucose according to **Barham and Trinder (1972) and** triglycerides according to**Fossati and Prencipe (1982)** . Also, serum glutathione peroxidase (GPx) according to **Paglia and Valentine (1967),** catalase (CAT) according to **Aebi (1984),** superoxide dismutase (SOD) activities were determined according to **Nishikimi et al. (1972),** and serum malondialdehyde (MDA) level were determined according to **Kei (1978)**, using assay kits purchased from Bio-diagnostic Co., Giza, Egypt All assays were performed according to the manufacturer's instructions without any modification.

Temperature and humidity index measurements

Throughout the duration of the study, the weather information was gathered daily by taken the air temperature and relative humidity data in the pens were collected daily using recording thermohygrometers, these measurements were used to calculate the daily variation of the temperature humidity index following the methodology outlined by **(Marai et al. 2001).**

temperature-humidity index **(THI) = db ◦C − [(0.31 − 0.31 RH) (db ◦C − 14.4)]**

where db represents the ambient temperature in Celsius and RH is the relative humidity percentage divided by 100. Based on this formula, THI values equal to or below 27.8 is indicate an absence of heat stress, whereas values above 28.9 are indicative of severe heat stress.

Statical analysis

Statistical analysis was performed on the data using one-way analysis of variance (ANOVA), as described in a previous study **(Bailey, 2008)**, with the aid of SPSS 16 software. The purpose was to determine the significance of the differences between the control groups and the stressed group after selenium yeast supplementation. The results were presented as means ± standard error (SE), and statistical significance was considered when the p -value was less than 0.05 (P<0.05).

RESULTS

The calculated temperature humidity index (THI) values were 25 (indicate no heat stress) in groups, control and NHS+SeY and 36.8 (sever heat stress) in groups HS and HS+SeY during experiment.

Table 2. Effect of *saccharomyces cervices* enriched selenium on growth performance.

	Control	HS	HS+ SeY	NHS+SeY
Initial body weight (Kg)	32.14±1.32	32.72±1.40	32.26±1.21	32.56±1.41
Final body weight (Kg)	$43.63±1.24^{b}$	$37.56±1.58^{c}$	$40.19±1.33^{c}$	$45.9±0.32^{a}$
Average total weight gain (gm)	$11490±1.72^{b}$	$4870±0.56^{d}$	$7930±0.93^{c}$	$13340±1.23^{a}$
Daily gain	$191.5±7.02^{b}$	$81.17±6.12^{d}$	$132.17.5±7.10^{c}$	$222.3±6.31^{a}$
Feed intake (gm/day)	$1121±3.21^{a}$	$844±2.45^{c}$	$986±3.42^{b}$	$1162±3.60^{a}$
FCR (kg/kg gain)	$5.85±0.19^{c}$	$10.39±0.18^{a}$	$7.46±0.18^{b}$	$5.23±0.17^{c}$

Data are presented as mean ± SE. Values in the same column with the different superscripts are significantly different at P<0.05.

Table 3. Effect of *saccharomyces cervices* enriched selenium on hematological profile.

Groups Parameters	control	HS	HS+SeY	NHS+SeY
RBCs ($\times 10^{6}$ /µl)	$7.62±0.17^{b}$	$6.94±0.04^{b}$	$7.33±0.27^{b}$	$8.44±0.26^{a}$
Hb (g/dl)	$8.43±0.26^{b}$	$7.23±0.12^{c}$	$7.41±0.27^{c}$	$9.15±0.10^{a}$
PCV (%)	$24.40±0.47^{b}$	$21.30±0.49^{c}$	$23.03±0.44^{b}$	$27.90±0.74^{a}$
MCV (fl)	$32.02±0.68^{b}$	$29.25±0.55^{c}$	$31.42±0.67^{b}$	$33.06±0.43^{a}$
MCH (pg)	$11.06±0.39^{a}$	$10.41±0.12^{a}$	$10.11±0.46^{a}$	$10.84±0.15^{a}$
MCHC (%)	$34.54±0.98^{a}$	$33.94±0.72^{a}$	$33.18±1.38^{a}$	$32.97±0.15^{a}$
WBCs ($\times 10^{3}$/µl)	$7.37±0.22^{b}$	$10.15±0.33^{a}$	$7.74±0.12^{b}$	$6.45±0.25^{c}$
Neutrophils($\times 10^{3}$/µl)	$2.25±0.08^{a}$	$2.19±0.11^{a}$	$2.22±0.04^{a}$	$1.86±0.01^{a}$
Lymphocytes($\times 10^{3}$/µl)	$4.58±0.15^{bc}$	$6.62±0.10^{a}$	$4.97±0.33^{b}$	$4.09±0.21^{c}$
Monocytes($\times 10^{3}$/µl)	$0.43±0.03^{b}$	$1.19±0.16^{a}$	$0.48±0.08^{b}$	$0.44±0.04^{b}$
Eosinophils($\times 10^{3}$/µl)	$0.07±0.003^{b}$	$0.12±0.006^{a}$	$0.06±0.006^{b}$	$0.04±0.01^{b}$
Basophils($\times 10^{3}$/µl)	$0.04±0.0018^{a}$	$0.038±0.004^{a}$	$0.015±0.001^{c}$	$0.028±0.002^{b}$

Data are presented as mean ± SE. Values in the same column with the different superscripts are significantly different at P<0.05

Table 4. Effect of *saccharomyces cervices* enriched selenium on serum biochemical profile.

	control	HS	HS+SeY	NHS+SeY
Total protein(g/dl)	6.98±0.44[a]	4.12±0.35[c]	5.71±0.41[b]	6.84±0.39[a]
Albumin (g/dl)	3.46±0.31[a]	2.39±0.47[c]	3.01±0.41[b]	3.22±0.39[a]
Globulin (g/dl)	3.52±0.11[a]	1.73±0.12[c]	2.7±0.22[b]	3.62±0.03[a]
Glucose (mg/dl)	54.24±1.52[c]	71.62±1.01[a]	60.83±1.3[b]	53.36±0.53[c]
Triglyceride (mg/dl)	15.23±0.20[c]	23.71±0.51[a]	20.82±0.42[b]	14.51±0.21[c]
ALT (U/l)	18.05±1.31[c]	29.47±1.56[a]	21.37±1.51[b]	17.35±1.42[a]
AST(U/l)	93.16± 1.24[c]	145.54± 1.34[a]	121.35±1.31[b]	91.2±1.21[c]
Uric acid(mg/dl)	41.14±2.72[c]	72.68±2.36[a]	53.16±3.14[b]	38.70±1.30[c]
Creatinine(mg/dl)	1.23±0.06[c]	2.95±0.11[a]	1.77±0.11[b]	1.19±0.09[c]
MDA (nmol/ml)	7.93±0.17[c]	18.46±0.61[a]	12.62±0.36[b]	6.74±0.27[d]
SOD(U/ml)	79.32±2.12[a]	41.26±1.52[b]	61.15±2.10[b]	77.42±2.43[a]
CAT(U/ml)	421.9±13.0[b]	235.6±12.2[c]	353.2±14.1[b]	485.2±13.6[a]
GPx(µmol/ml)	356.21±3.01[a]	211.11± 2.41[c]	301.2±2.01[b]	361.20±3.11[a]

Data are presented as mean±SE. Values in the same column with the different superscripts are significantly different at P<0.05.

DISCUSSION

In current study, THI data show that control and NHS+ SeY groups were not exposed to heat stress, but HS and HS+ SeY groups raised under severe heat stress throughout the hot summer months. Considering the results, the average THI was 25 in non-heat stressed groups and 36 in heat stressed groups. THI index has been widely recognized and embraced as a reliable measure of the thermal comfort of domestic animals over an extended period **(Wijffels et al. 2021)**. **(Marai et al. 1995)** classified sheep THI in sheep as following: below 27.8 is signify an absence of heat stress, while a value is increased above 28.9 is assessed sever heat stress. Therefore, with reference to the above mentioned classification, the value of 36 for THI in HS and HS+SeY groups may indicate that lambs in the current study experienced a sever heat stress.

Heat stress lower the animal's production efficiency, which costs world animal agriculture billions of dollars every year **(Bernabucci et al. 2009; Rhoads et al. 2013)**. Farm animals reared in areas and during certain seasons when effective temperature conditions are outside of their zone of thermal comfort for part or all of the time result in economic losses for the livestock industries **(Sejian et al. 2018; St-Pierre et al. 2003)** Animal production is negatively impacted by high ambient temperatures and humidity **(Fuquay, 1981; Gaughan et al. 2018; Sejian et al. 2018)** ADG(average daily gain) and ADFI (average daily feed intake) are reduced in sheep vulnerable to high temperatures **(Marai et al. 2007)**. According to **Fuquay (1981)**, HS happens when sheep are vulnerable to temperatures greater than 30 °C. The optimum critical temperature for sheep is between 25 and 30 °C. When the THI value exceeds 25.6, sheep are said to be experiencing extreme severe HS, according to **Marai et al. (2007)**. In the current investigation, the mean THI value at the peak of heat treatment in the afternoon was 36, which is like an extremely severe HS. Animals acquire specific defense mechanisms to decrease body heat production in response to persistently high ambient temperatures, such as reduced ADFI and metabolic heat generation **(Fuquay, 1981)**.

Feed intake was decreased in sheep exposed to high ambient temperature along with decrease ADG **(Mahjoubi et al. 2015; Rhoads**

et al. 2013; Zhao et al. 2019), is attributed to heat damage to the epithelial cells of intestine **(Kim et al. 2016; Yu et al. 2010)**. In the current experiment SeY supplementation to group under HS was able to maintain feed intake. SeY has important implications for industry of sheep as it can be used to lower or alleviate the consequences of thermal stress, including body weight loss and the raised rectal temperature **(Alhidary et al. 2012)**. The result stands in line with the findings of earlier studies, which indicated that using selenium had positive effect on feed intake, weight gain of growing lambs **(Kumar et al. 2009; Mariezcurrena-Berasain et al. 2022; Mousaie, 2021b; Wilkins et al. 1982)**, goat **(Shi et al. 2011)** and beef cattle **(Hefnawy and Tórtora-Pérez, 2010)**. Both organic and inorganic Se supplementation to diet of lambs improve growth rate. But organic Se was more efficient than inorganic Se at accelerating lambs' growth rates **(Kumar et al. 2009)**. This observed SeY-induced increase in feed efficiency can be mostly due to Se effect on reducing oxidative stress and its Positive impacts on nutrition utilization and digestion in the gastrointestinal tract **(Shi et al. 2011; Wang et al. 2009; Wang et al. 2019)**. The animals' daily dry matter intake and average daily gain increased with the addition of se ($P < 0.01$).

Selenium appears to boost the effectiveness of energy utilization for growth because it improves immunity, which is supported by the current meta-analysis. On the other hand, Se has been linked to thyroid activity, and more specifically, with thyroid deiodinases, selenoenzymes that accelerate the activation of T3 from T4, which explains Se's positive impact on growth **(Matics et al. 2017)**. In contrast to our findings, **(Domínguez-Vara et al. 2009; Hernandez-Calva et al. 2013; Kumar et al. 2022; Mahan and Parrett, 1996; Vignola et al. 2009)** found Se dietary supplements from inorganic, organic, and nano sources have no significant effect on growth performance, feed intake, feed to gain ratio digestibility of nutrients in lambs. This could result from the presence of selenium in the basic diet, which might be adequate to fulfill the nutritional needs of sheep (**Kumar et al. 2022**).

Present work showed a significant ($P \leq 0.05$) reduction in Hb (g/dl) and PCV% were detected in sheep under high THI. There are conflicting findings on the changes in Hb and PCV under heat stress. According to several studies, there is a decrease in RBC counts, which affects PCV and Hb values **(Kumar et al. 2010; Maurya et al. 2007; Sivakumar et al. 2010; Temizel et al. 2009)** during heat stress. It's possible that the elevated THI during the sweltering summer months led to an increase in respiratory rate, which in turn led to an increase in oxygen intake **(Kamal et al. 1984)** It caused the blood's partial pressure of oxygen to rise while plasma protein and certain trace elements decreased like cobalt, iron, and copper that lower the hemoglobin ratio. These metals are crucial for the synthesis of hemoglobin, Consequently, erythropoiesis was affected, which decreased the quantity of RBCs and, consequently, the PCV and Hb levels in the sheep breeds **(Singh et al. 2016)**, This is consistent with the findings of **Abozed (2014)**, who found that the connection between Hb concentration and ambient temperature (AT) was substantially and significantly negative. These findings are explained by the hemodilution effect of water, which is a result of animals drinking a lot of water to reduce heat load. This increased blood and plasma volume changes in cells causes a decrease in the concentration of circulating erythrocyte counts, which in turn causes a decrease in Hb concentration and PCV value.

However, **Sejian et al. (2013a)** observed that short-term heat stress in Malpura sheep led to a significant rise in the Hb and PCV% values ($P < 0.05$). Similar outcomes were attained by **Al-Haidary (2004)** with Naimey sheep and **Srikandakumar et al. (2003)** with Omani and Australian Merino sheep. According to **Alam et al. (2011)**, RBC, PCV%, Hb%, TLC, and DLC amounts in goats increased significantly. This discrepancy in the results may be the result of different experimental designs, which led to the animals being exposed for longer periods of time (in the current study under natural conditions) as opposed to shorter periods of time (either in a climate chamber for 6 hours as was done by **Sejian et al. (2013b)** or for shorter periods of time (6–8 hours) as a result of heat stress by **Alam et al. (2011)**. In the heat -stressed Saccharomyces enriched selenium yeast supplementation group the current investigation revealed an increase in RBCs, Hb, and PCV ($P \leq 0.05$), but a decrease in white blood cell count ($P < 0.05$). The results are consistent with those of **Faixova et al. (2007)**, who found that lambs fed a basal diet supplemented with SeY had higher RBC counts than lambs fed a diet without any supplementation. This is because Se increases RBC number by either stimulating erythropoiesis (RBC production) or by extending the lifespan of the animal through protection. Although the precise process is still unknown, SeY decreases erythrocyte membrane fragility by increasing osmotic resistance. This implies that SeY preserves and extends the life of red blood cells. The antioxidant properties of selenium in blood cells may be linked to the defense of cell or organelle membranes **(Surai, 2006a)**.

According to a number of publications (**Tras et al. 2000; Abbas, 2002; Mohri et al. 2005; Faixova et al. 2007**), selenium has a positive effect on hematological indicators. Additionally, for some reason, blood RBC count responded to selenium administration. Conversely, lambs fed 1 mg of Se from Se nanoparticles (**Sadeghian et al. 2012**) and lambs given varying doses of SeY supplement (**Alimohamady et al. 2013; Shi et al. 2018**) did not exhibit any changes in RBC count. It was also in line with the findings of earlier studies (**Shi et al. 2018**), which showed that SY had no appreciable impact on hematocrit or blood Hb concentration. Values of WBC raised in sheep **(Sadeghian et al. 2012)**, calves **(Mohri et al. 2005)** when selenium supplemented in diet with no appreciable impact on PCV and RBC values. When Se was supplemented into the diet of sheep using sodium selenite and organically bound Se in algae, **Pisek et al. (2008)** found no significant alterations in the white blood cell profile.

There are divergent views in the literature regarding how Se influences hematological markers. One of the reasons is that the effects of selenium and/or vitamin E and ascorbic acid were studied concurrently, which complicated interpretation. Our findings, which are displayed above, demonstrated that SeY had a favorable impact on PCV, Hb, and RBC count.

The results value showed tendency to normal indicating improvement and thus the diet containing SY reverted all alterations in the hematological parameters of the heat stressed sheep to near the control values **(Faixova et al. 2007; Mohri et al. 2005; Mousaie, 2021b)**.

Throughout heat stress, plasma proteins play a crucial role in facilitating the transfer of heat from the internal body to the outer surface of the skin for dissipation through non-evaporative processes. This is due to the substantial water content held within the intravascular fluids of these proteins, which helps maintain the viscosity of the blood **(Kamal et al. 1962)**.

Our result showed significant decrease ($p \leq 0.5$) in total protein, albumin and globulin levels in HS group without medication. This results in coordinate with **Sejian et al. (2013b); (Singh et al. 2016)** Who documented a significant ($P < 0.05$) reduction in total protein and albumin in sheep under heat stress. This reduction in total plasma protein in the heat stressed groups is believed to facilitate gluconeogenesis, ensuring the availability of energy for thermoregulatory processes **(Sejian et al. 2010)**.

Rateb and Hmdon (2015) attributed the notable reduction in blood plasma protein levels observed in Rahmani sheep exposed to heat stress to several factors. These factors include the dilution of plasma proteins due to an increase in body water content, a decrease in protein synthesis caused by a decrease in the secretion of anabolic hormones **(El-Masry and Habeeb, 1989)**, an elevation in catabolic hormones like glucocorticoids and catecholamines **(Alvarez and Johnson, 1973)**, and a decrease in the intake of feed nitrogen and minerals. Due to its high osmotic sensitivity and comparatively smaller molecular mass and size than other protein fractions, albumin may be filtered and redistributed into the extravascular spaces during heat stress, causing a decrease in the circulating amount **(Kerr, 2008)**.

Our findings reveal that increase in total protein as well as albumin in HS sheep supplemented with SeY, accumulating evidence suggests that the supplementation of selenium (Se) can have an impact on serum globulin, total protein, and cholesterol levels **(Ashouri et al. 2015; El-Demerdash and Nasr, 2014; Mahmoud et al. 2013; South et al. 2000)** in contrast **Mousaie (2021b)** found that total protein, albumin, and globulin concentrations had no significant difference after administration of SeY.

High THI increased water consumption, lowered feed intake, enhanced free radical formation, and impacted endocrine processes, which may disrupt particularly the lipid and glucose metabolism in animals **(Belhadj Slimen et al. 2016)**. Our data showed significant rise in glucose and triglyceride in serum. The observed increase of serum glucose level during hot summer conditions may be attributed to activation of cortisol secretion induced by stress. This, in turn, stimulate gluconeogenesis while inhibiting cellular glucose uptake and utilization **(Marai et al. 2007)**. The high plasma glucose level which determined in stressed sheep might be due to the increasing requirement for glucose as an energy source to sustain the effort of physiological processes for thermoregulation **(Sejian et al. 2013b)**.

Several studies pointed out that sheep subjected to heat stress had significantly higher blood serum glucose concentrations **(Ellamie, 2013; Rashid et al. 2013; Sejian et al. 2013b).** On other hand some research reported decrease in blood glucose of sheep during hot conditions of summer **(Indu et al. 2014; Kochewad et al. 2018; Ramana et al. 2013)** this drop in blood glucose in sheep was most likely caused by a decrease in food intake.

dietary Se can enhance the metabolism of cholesterol and glucose **(Shi et al. 2018)**. **Domínguez-Vara et al. (2009)** observed that lambs treated with Se had significantly lower triglyceride levels. **Novoselec et al. (2018)** showed that selenium had a significant role in decreasing the level of triglycerides in animals supplemented with it. Organic Se supplementation (SeY) has shown a considerable reduction in triglyceride content compared to inorganic and non-supplemented treatment **(Muhammad et al. 2022)**. **Ibrahim et al. (2012)** demonstrated that yeast enriched with selenium could improve blood lipid profile of mice and that the beneficial effects of Saccharomyces enriched selenium are more robust than that of probiotic or Selenium selenite used alone.

The presence of plasma levels of liver marker enzymes such as AST, ALT, ALP, and LDH are considered to be a sensitive mark of liver impairment **(Goorden et al. 2013; Wang et al. 2015)**.

Sheep under high THI showed significant elevation in AST and ALT. This is in line with **Marai et al. (1995)** who explained that reason of increasing activity of liver enzymes (ALT and AST), when sheep exposed to heat stress, is the increased stimulation of gluconeogenesis by corticoids (increase in cortisol, cortisone or adrenocorticotrophic hormone) or liver function may be adversely affected by the deleterious consequences of heat stress **(Banerjee et al. 2015; Wojtas et al. 2014)**. In addition, when the liver body has some sort of injury, the ALT, AST, and LDH activities in the plasma are released into the blood stream **(Khan et al. 2013)**. Furthermore, studies on animals have demonstrated that the activities of these liver marker enzymes, such as AST, ALP, and ALT, elevated in heat-stressed animals **(Ismail et al. 2013; Mokondjimobe et al. 2012)**. Additionally, Exposing the liver to thermal conditions can result in oxidative stress-induced damage. This damage is predominantly associated with elevated levels of ALT and AST, heightened hepatic MDA contents, and reduced activity of liver SOD, CAT, and GSH **(Li et al. 2014; Wang et al. 2018)**.

Treatment with SeY in HS sheep showed decrease level of liver enzymes which is in accordance with **Halawa et al. (2023)** who observed that AST and ALT levels lowered by using Selenium nanoparticles.

Animals increase blood flow to their skin and decrease blood flow to their internal organs **(Srikandakumar et al. 2003)** to cool down and prevent heatstroke caused by high environmental temperatures. However, this decreases in blood flow to the internal organs can damage or impair their function, such as the liver, kidneys, and intestines **(Kour et al. 2014)**. The rise in serum urea and creatinine levels in sheep as a result of heat stress may suggest that their kidneys undergo diminished blood flow under these conditions **(Čukić et al. 2023).**

Oxidative stress is a result of an imbalance between the body's generation of oxides and its antioxidant defense mechanism **(Antonenkov and Hiltunen, 2012).** Increased antioxidant capability may contribute to animal health **(Li et al. 2018).** Previous research has demonstrated that environmental HS cause farm animals to be subject to oxidative stress by activating ROS or reducing antioxidant capacity of animals **(Di Trana et al. 2006).** Higher HS can cause systemic inflammation, release proinflammatory cytokines, and increase the production of reactive oxygen species (ROS**) (Constable et al. 2016**), or impairment of the antioxidant defense system, which markedly decreases blood concentrations of markers of antioxidant capacity **(Alhidary et al. 2015)**.

Free radical generation will rise because of the biochemical and physiological responses to heat stress (**Mujahid et al. 2009; Azad et al. 2010**). Superoxide ion (O2), hydroxyl radical (HO), and hydrogen peroxide (H_2O_2) are forms of ROS that are highly reactive molecules and easily overproduce when animal under HS lead to dysfunction of antioxidant defense and oxidative damage of biological molecules including DNA/RNA, proteins and lipids **(Sevi et al., 2001; Trout et al. 1998; Yang et al. 2010)**.

Antioxidants, which are compounds that delay or prevent oxidative damage to a target molecule, are said to be substances that scavenge free radicals, comprising non-enzymatic and enzymatic systems. The non-enzymatic system comprises vitamin E, vitamin C, cysteine, glutathione (GSH), copper (Cu), iron (Fe), zinc (Zn), and selenium (Se). On the other hand, the enzymatic system includes superoxide dismutase (SOD), glutathione peroxidase (GPx), catalase (CAT), and various other antioxidant enzymes **(Ha et al. 2019; Halliwell and Gutteridge, 2015; Roman et al. 2014).**

According to **Ellamie et al. (2020)**, oxidative stress generated by ambient HS increases tissue damage markers in the form of elevated lipid peroxidation observed by the higher MDA in the plasma from Barki sheep. decreased plasmatic SOD, CAT and GPX in HS sheep reveal over production of ROS.

In the present study, the serum antioxidant status was evaluated by measuring serum CAT, GPx, and SOD. Serum MDA, as a lipid peroxidation marker. The results revealed rise in MDA level and decrease in CAT, GPx and SOD activity in HS sheep.

In our study the lambs exposed to HS without any additives showed the highest serum MDA content than other groups, this is due to the length and intensity of a lamb's exposure to thermal stress may affect how much lipid peroxidation is brought on by hyperthermia. A similar finding was reported previously by **Shi et al. (2020)** in lambs under HS. Additionally, serum SOD activity was significantly decreased in HS compared to other groups, comparable to the findings of the buffalo-cow experiment, which showed that SOD activity in serum of buffalo cows (Bubalus bubalis) was considerably lower in the summer (May to August) compared with the winter (December to February) **(Megahed et al. 2008)**. The decrease in the activities of blood enzymic antioxidants (SOD and CAT) in the HS sheep may be explained by the utilization of these enzymes to detoxify the free radicals produced by the HS, and to preserve the redox steady state **(Ellamie et al. 2020)**.

Our results revealed the addition of Saccharomyces enriched selenium to diet of HS sheep lead to significant decrease in serum MDA concentration, as well as increase serum CAT, SOD, and GPx activities. The results of the current study support earlier observations about the antioxidant impact of selenium-enriched probiotics **(Gan et al. 2014; Le and Fotedar, 2014); Liu et al. (2015),** who declare the Selenium probiotic supplementation further increased GSH, CAT and SOD activities and GPx content and reduced MDA content**.** And increased animal antioxidant capacity **(Gan et al. 2014; Surai and Dvorska, 2002).** Selenium enriched yeast can effectively increase antioxidant capacity system of sheep **(Mousaie, 2021b; Wang et al. 2019)**, SeY used has more potent positive effects in enhancing the antioxidant status of animals. Protective effect of Selenium involves all 3 categories of antioxidant defense systems in cells of animals, prevention of radical formation, inhibiting and limiting of chain formation and propagation, and excision and repair of damaged parts of molecules **(Surai, 2006b)**. The antioxidant characteristics of selenium are linked to its integration as a biocatalytic and functional element within selenoproteins (SeP), particularly the glutathione peroxidase family (GPXs), and thioredoxin reductase (TRx). The GPXs are responsible for neutralizing various peroxides, including hydrogen peroxide (H2O2), phospholipid hydroperoxide, fatty acid hydroperoxides, and hydroperoxyl groups of thymine **(Rayman, 2000)**. Saccharomyces enriched selenium dietary supplements reduced heat stress by the exertion of opposing effects, and created a preventative impact by maintaining the antioxidant system in sheep and cow **(Alhidary et al., 2015)**. The consumption of selenium, particularly from a highly bioavailable source, has the potential to enhance the activity of GPx (**Alimohamady et al. 2013; Čobanova et al. 2017**). These markers serve as indirect yet dependable indicators of the impact of selenium supplementation on the antioxidant capacity of lamb blood. Consequently, an elevation in blood GPX activity and a decrease in MDA concentration in lambs fed selenium may indicate the need for higher selenium levels to enhance the blood antioxidant status of lambs raised in the warm conditions of summer.

CONCLUSION

Continuous increase in environmental temperature has deleterious effect on sheep productivity, bring about various potential measures to enhance sheep nutrition to increase productivity and reproductive efficiency during raising of sheep. Here in our study, we have concluded that supplementing heat-stressed sheep with selenium-enriched yeast (Saccharomyces enriched selenium) can mitigate certain negative consequences associated with heat stress. This can be achieved by alleviating oxidative stress and improving certain physiological responses in sheep.

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