Veterinary and Animal Science 10 (2020) 100133

Contents lists available at ScienceDirect

Veterinary and Animal Science

journal homepage: www.elsevier.com/locate/vas

Effects of fossil shell flour supplementation on heat tolerance of dohne merino rams

Lwazi Mwanda[a], Olusegun O Ikusika[a,b,*], Conference T Mpendulo[a], Anthony I Okoh[b,c]

[a] Department of Livestock and Pasture Science, Faculty of Science and Agriculture, University of Fort Hare, Alice, South Africa
[b] SAMRC Microbial Water Quality Monitoring Centre, University of Fort Hare, Alice, South Africa
[c] Applied and Environmental Microbiology Research Group (AEMREG), Department of Biochemistry and Microbiology, University of Fort Hare, Alice, South Africa

ARTICLE INFO

Keywords:
Heat tolerance
Blood metabolites
Fossil shell flour
Dohne merino rams

ABSTRACT

This study evaluated the effects of fossil shell flour (FSF) supplementation on heat tolerance of Dohne Merino sheep. Twenty-four ($n = 24$) Dohne Merino rams of about 4 months old, weighing 22.3 ± 0.43 kg were confined in individual pens using complete randomized design for a period of 100 days. The rams were subjected to four varying inclusion levels of fossil shell flour (0 g/kg, 20 g/kg, 40 g/kg and 60 g/kg. Water and feed intake were recorded throughout the period of successive feeding. On days 0, 45 and 90, blood samples were collected for analyses. The parameters monitored included the average daily water intake (ADWI), average feed intake (ADFI), skin temperature (ST), respiration rate (RR), pulse rate (PR), rectal temperature (RT), total plasma protein (TPP), blood glucose (GLU), red blood cell (RBC),white blood cell (WBC), Heamaglobin (Hb), mean corpuscular hemoglobin concentration (MCHC), Packed cell volume (PCV), and mean corpuscular volume (MCV). The inclusion levels of FSF affected ADWI and ADFI, revealing high water intake and feed intake in rams subjected to 40 g/kg of FSF followed by 60 g FSF/kg, 20 g FSF/kg and 0 g FSF/kg ($P < 0.01$) respectively. All the physiological parameters (ST, RT, RR and PR) decreased with increase in inclusion levels of FSF ($P < 0.01$). The TPP and GLU increased as the levels of FSF increases ($P < 0.01$). The RBC and WBC were higher in rams subjected to FSF supplemented diets compared to the control ($p < 0.05$). We conclude that water and feed intake increase with increasing levels of the FSF while the physiological parameters decline as levels of FSF increases. Hence, fossil shell flour could be used as a supplement in Dohne-Merino rams' diet to mitigate heat stress and promote overall productivity of the sheep.

1. Introduction

Sheep are important species of livestock, providing meat and wool. They play a vital role in the economy of millions of people who earn their livelihood either by direct rearing sheep or indirectly using the products from sheep (Gupta, Kumar, Dangi, & Jangir, 2013). Today, sheep farming is practiced in almost all the inhabited continents of the world, being easily adaptable and instrument to several civilizations (Skapetas & Kalaitzidou, 2017). According to (Al-Haidary, Aljumaah, Alshaikh, Abdoun*, & Alfuraiji, 2012), adaptability of sheep under various geographical and environmental conditions and, even in extreme and harsh climates are easily possible. It was also reported that sheep performed better under harsh weather than other farm animals, though goats are more heat tolerant than sheep. However, despite sheep ability to tolerate wide climatic condition, the productivity of sheep often diminishes as a result of heat stress (Banerjee et al., 2014). Among many types of stressors that animals undergo, heat stress is the most challenging one with the present climatic changes been experienced globally (Silanikove & Koluman, 2015). One of the most significant stressors in semi-arid region is heat stress (Al-Dawood, 2015), and in arid (Silanikove, 2000). Gupta, Kumar, Dangi, & Jangir, 2013) defined heat stress as an alleged discomfort and physiological strain associated with an exposure to an extreme and hot environment. (Srikandakumar et al., 2003; Caroprese et al., 2012; Al-Dawood, 2015) reported temperature tolerance range for adult sheep as −12 °C to 32 °C. Kiyatkin (2005) reported that in order to afford hyperthermia and other related stress in farm animals EU legislated law minimum temperature for each species for the purpose of animal welfare. Nevertheless, the degree of tolerance depends majorly on such factors as physiological condition of the animal as well as the blood anionic-

* Corresponding author
E-mail addresses: 201307068@ufh.ac.za (L. Mwanda), 201818619@ufh.ac.za (O.O. Ikusika), tmpendulo@ufh.ac.za (C.T. Mpendulo), aokoh@ufh.ac.za (A.I. Okoh).

https://doi.org/10.1016/j.vas.2020.100133
Received 17 March 2020; Received in revised form 25 June 2020; Accepted 25 June 2020
Available online 27 June 2020
2451-943X/ © 2020 The Authors. Published by Elsevier Ltd. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/BY-NC-ND/4.0/).

cationic balance (Srikandakumar et al., 2003; Cwynar et al., 2014). (Kawas, Andrade-Montemayor, & Lu, 2010; Al-Dawood, 2017) reported needs to strongly control heat stress syndromes in animals during a high environmental temperature. An animal's body temperature, respiration rate, and rectal temperature should, therefore, be measured, as a vital and basic indicator of thermal homeostasis of the body. (Rensis De and Scaramuzzi, 2003; Cwynar, Kolacz, & Czerski, 2014) reported and recommended that rectal temperature and respiratory rate parameters are excellent indicator of thermal balance of the body and as such should be used as a research method. In the report of (Srikandakumar, Johnso, & Mahgoub, 2003; Cwynar et al., 2014) a correlation between the environmental temperature and blood parameters was confirmed.

Several strategies have been used to mitigate heat stress in livestock production. Some of these include provision of shade, adequate provision of water, sprinklers, fans, and air conditioner. However, some of these strategies are expensive and cannot be afforded by communal and small-scale farmers while some are not practicable in certain regions of the world where good water is a scarce commodity. Thus, there is a need to explore the use of other alternative management practices such as the use of feed additives that could reduce massive heat load without affecting production and reproduction performance.

This feed additive could be fossil shell flour (FSF). Recently, there is a growing interest in the use of FSF as feed additive in livestock (Ikusika et al., 2019). FSF are diatoms that occurs naturally and very rich in silicon. It is a type of hard-shelled plant algae originally deposited millions of years ago in the earth from dried up seas and lakes (Ikusika et al., 2019a; Koster, 2013). Recent studies have shown that FSF can be used as growth enhancer in livestock, natural anthelmintic, and adjuvant for vaccine in animal production (Ikusika et al., 2019; Koster, 2013). FSF is rich in minerals, cheap, available in abundance and has nontoxic characteristics (Ikusika et al., 2019). Though the knowledge on the animal response towards heat stress is common, nutritional manipulation through the use of feed additive such as FSF remains unexplored. Therefore, this study investigated the effect of fossil shell flour on heat stress indicators in Dohne-merino rams. We hypothesize that supplementation of Dohne-Merino rams' diets with FSF will be a viable way to reduce the effect of heat stress in Dohne-merino rams.

2. Material and methods

2.1. Ethical consideration

The experiment was conducted in compliance with the standards required by the Animal Ethics Committee of the University of Fort Hare (Ethical No: MPE011SMWA01/19/A). All trial procedures were conducted in accordance with standards of experimentation given by the committee of ethics on animal use of the Society for the Prevention of Cruelty to Animals (SPCA) (Constitutional Court of South Africa, 2013).

2.2. Study site

The study was conducted at the University of Fort Hare, Teaching and Research Farms Honeydale, Eastern Cape Province, South Africa. The farm is located at a latitude of 32˚46′ S and longitude 26˚50′ E, at an altitude of 535 m above sea level, and is characterized by warm temperate climate with an average annual rainfall of about 575 mm, received mainly during the summer months of November to March. The maximum temperature is 42.6 ˚C, minimum temperature is 17.1 ˚C, and average temperature is 27.8 ˚C during summer. The vegetation is dominated by the *Themeda triandra* and *Cympogon plurinodis* with woody plants such as *Acacia Karroo* and shrubs (Mucina & Rutherford, 2006).

2.3. Management of rams, feeding and experimental design

A total of twenty-four ($n = 24$) Dohne Merino rams aged 4 months, ranging from 20 to 24 kg, averaging (22.3 ± 0.43 kg) were selected from a commercial farm in Mitford village, Tarkastad, Eastern Cape of South Africa. The Dohne Merino represents one of the most prevalent sheep breeds in South Africa, and according to Cloete, Hoffman, & Cloete (2012) is one of the most genetic-stable sheep races, which was the main argument to use it in the study. Rams were confined in individual pens at the same facility with similar temperature (39.29 ˚C), relative humidity (76.75%), dry bulb temperature (36.82 ˚C), wet bulb temperature (33.15 ˚C) and the mean temperature-humidity index calculated (THI = 90.97).

THI was calculated as $0.72 \times (Td + Tw) + 40.6$ as described by NRC (1971). Where Td is the dry bulb temperature and Tw is the wet bulb temperature while 0.72 and 40.6 are constant,

Each of twenty-four rams was drenched with 2 mL of Maxicare to control gastrointestinal nematodes using a drenching gun. The experiment lasted for 100 days excluding 10 days of adaptation period. The study was carried out in summer (from December 2018 till March 2019). The rams were randomly subjected to four varying inclusion levels of fossil shell flour (0 g/kg, 20/kg, 40 g/kg and 60 g/kg) in a Completely Randomized Design with six rams per treatment. The experimental diet consisted of concentrate and hay at ratio 40:60. The concentrate was made up of, maize 8%, sun flower oil cake 10%, molasse 5%, wheat bran 15%, limestone 1.5%, Salt 0.2% and sheep premix 0.3% while the hay consist of 30% teff and 30% leucine (Table 1). All these was thoroughly milled and mixed evenly together to form basal diet. The feed was formulated to meet ram energy and protein requirement according to NRC standard (National Research Council (NRC), 2007). The dietary groups were, basal diet, basal diet + 20 g/kg FSF, basal diet + 40 g/kg FSF and basal diet + 60 g/kg FSF. The Lucerne and grass hay were purchased from Border Seed Distributors CC (Queenstown, South Africa) while the ingredients for the concentrate were purchased from Monti-feed (pty) Ltd, East London, South Africa. Rams were fed daily at 08h00 and 16h00. Fresh and clean water was made available to each ram using calibrated 10 liter buckets.

2.4. Blood sampling and laboratory analysis

Seven millilitres (ml) of blood was collected from jugular vein using a graduated syringe on day 0, 45 and 90 at 900 h. The blood samples used in heamatological analysis, was collected into EDTA vacuum tubes (Sarstedt Monovette®, Germany) while sample for biochemistry was collected into clotting tubes (Sarstedt Monovette®, Germany). Blood was centrifuged at 4000 rpm for 8 min. Samples were placed immediately on ice, and then transferred to the laboratory within two hours of collection. Blood samples were analyzed for red blood cell

Table 1
Mineral composition of fossil shell flour FSF.

Items	Quantity (% or ppm)
DM%	9.3
Ca%	0.22
Mg%	0.11
K%	0.08
Cu (mg/kg)	30
Na (mg/kg)	923
Zn(mg/kg)	118
Fe(mg/kg)	7944
Mn(mg/kg)	69
P%	0.00
Sulfate Sulfur (S)%	0.062
Aluminum (Al)%	0.065
Vanadium (V)%	0.00438
Boron (B)%	0.0023

(RBC), white blood cell (WBC), packed cell volume (PCV), hemoglobin (Hb), blood glucose (GLU), total plasma protein (TPP), mean corpuscular hemoglobin concentration (MCHC) and mean corpuscular volume (MCV). Blood tests were performed at heamatobiochemical laboratory of National Health Insurance Scheme (NHIS), East London, South Africa, certified by South Africa Medical Research Council (SAMRC).

2.5. Measurements

2.5.1. Physiological parameters

The skin temperature (ST), respiratory rate (RR), pulse rate (PR) and rectal (RT) were measured on day 0, 45, 90. In the current study ST was measured from four body parts (abdomen, scrotum, face and from animal's back) using infrared thermometer (ADTEMP™) using methods described by Veerasamy Sejian, Kumar, Gaughan and Naqvi (2017). The RR was recorded by counting flank movements per minute using a stopwatch from a distance of 2 m without disturbing the rams. The HR was measured using a stethoscope (3M™ Littmann® Master Classic II™) method described by Verschuren, Marjolijn, Tim, Paul, & Jan Willem (2008). The RT was measured using a digital thermometer (Uniontech). The thermometer was inserted about 2 to 3 cm in the rectum until a stable automated reading was obtained.

2.5.2. Data analyses

The data for feed intake, water intake, physiological and blood biochemical response were analyzed using the repeated measures procedure of SAS/JMP (2018) and the treatment and day (TRT × DAY) interaction of the physiological and blood biochemical response were analyzed. Significant treatment mean values were compared using the probability of difference (PDIFF) option of the same package. The level of statistical significance was set at $P < 0.05$.

3. Results

3.1. Feed and water intake

Results on the effects of fossil shell flour on ADWI and the ADFI are shown in Table 3. The results showed that as FSF inclusion levels increased both the ADFI and ADWI increased and peaked at 40 g/kg FSF, before declining. The ADFI and ADWI were both higher in the rams subjected to 40 g FSF/kg inclusion level ($P < 0.01$).

3.2. Physiological response

Results on the effects of fossil shell flour on skin temperature (ST), rectal temperature (RT), respiration rate (RR) and pulse rate (PR) are shown in Table 4. It was observed that as FSF inclusion levels increased, ST, RT, RR and RP values decreased, and was significant for all treatments and the sampling days ($p < 0.01$). The ST, RT, RR, and PR were high in rams fed non-supplemented FSF diet and low in rams fed 60 g/kg FSF ($P < 0.01$). The effects of the days were significant across all physiological parameters tested ($P < 0.01$). The RT and PR were high on day 0 of the experiment ($P < 0.05$), whilst the ST was high on day 0 and 45 ($P < 0.01$). The RR was smallest on day 90 ($P < 0.01$). The interaction of the effects of TRT x DAY on RT and RR were significant ($P < 0.01$).

3.3. Blood hematobiochemical response

The effects of fossil shell flour on TPP, GLU, Hb, RBC, WBC, MCV and MCHC are shown in Table 5. The concentration of glucose in ram fed with 20 g/kg, 40 g/kg and 60 g/kg FSF were higher than in those fed with 0 g/kg FSF diet. Rams fed on diet with 40 g/kg FSF had the highest concentration of glucose at day 45 and 90, and was significantly different from rams fed on diets with 0 g/kg, 20 g/kg and 60 g/kg FSF at the said days of sampling ($P < 0.05$). Also, glucose concentration of rams on 40 g/kg and 60 g/kg FSF diets were significantly different from rams on 0 g/kg FSF diet at the last 2 days of the sampling period ($P < 0.05$). The concentration of total protein was lesser in rams with 0 g/kg FSF compared to rams with 20 g/kg, 40 g/kg and 60 g/kg FSH at all the sampling days. The WBC increased as the FSF inclusion levels increases. The white blood cell (WBC) counts of rams fed diet containing 40 g/kg and 60 g/kg FSF were greater ($P < 0.01$) than rams fed on diets with 0 g/kg and 20 g/kg FSF at all the sampling days. Rams fed on diet with 60 g/kg FSF had highest red blood cell (RBC) counts compared to rams on 0 g/kg, 20 g/kg and 40 g/kg FSF diets at day 45 and 90. Rams on 40 g/kg FSF diet were not statistically different from rams on 20 g/kg and 0 g/kg FSF diet but varied significantly from rams on 60 g/kg FSF diets at the same sampling days ($P < 0.01$). The MCV was high in rams subjected to 0 g FSF/kg and 60 g FSF/kg on day 0($P < 0.01$) while the MCV was high in rams subjected to 20 g FSF/kg and 40 FSF/kg on day 45 and 90 ($P < 0.01$).

4. Discussion

The current study focused on assessing the extent at which fossil shell flour inclusion levels can reduce heat stress in Dohne Merino rams by measuring ADWI, ADWFI, ST, RR, RT, GLU, TPP, WBC, RBC, MCV and MCHC. The results revealed that FSF has an impact on average daily feed intake, average daily water intake, physiological responses and blood biochemical in Dohne merino rams.

4.1. Effects of fsf on feed and water intake

Feed and water intake are vital attributes determining adaptive ability of livestock (Minka, 2009; Sejian, Maurya, Naqvi, Kumar, & Joshi, 2010). When animals are experiencing heat stress, they tend to reduce feed intake and increase their water consumption. The increase in feed intake as the temperature reduces due to an increase in FSF inclusion levels could be because FSF increases digesta passage rate by reducing gut fill for longer and depresses rate because of the mineral content of FSF. It could also be because FSF increases blood flow to the rumen, thereby causing an increase in both ruminal motility and rumination. The presence of sulfate in FSF has been reported to stimulate microbial growth and activities in the rumen, thereby increasing feed intake (Golder et al., 2014). One of the most essential nutrients in livestock production is water. It is important for the regulation of body temperature, digestion of diets, exchange of nutrients, transportation of nutrients, excretion of waste products and heat balance (Al-Dawood, 2017). Cwynar et al., (2014) reported that sheep tend to drink more water during hot weather and when their body temperature tends to be above normal range. The result of this current study showed that water consumption decreases as the temperature decrease due to an increase in FSF inclusion levels. This is contrary to report of Cwynar, Kolacz, & Czerski (2014). This could be because FSF increase the feed intake and since the rams are not heat stressed, water consumption will proportionally be related to feed intake. The results of the current study on ADWI and ADFI justified that mineral constituents found in FSF improve insulin sensitivity because good insulin response is a major factor to successful adaption of heat load. Therefore, supplementation of diet with FSF could be an effective strategy to improve the likelihood of rams to survive heat load.

4.2. Physiological response

Physiological responses are regarded as first parameters to check whether the animal is coping with any adverse environmental temperatures (Ikusika, Mpendulo, Zindove, & Okoh, 2019; Cwynar, Kolacz, & Czerski, 2014). Respiration rate and RT have been used as a reliable heat stress indicators in sheep (Daramola & Adeloye, 2009; Marai, Habeeb, & Gad, 2002; Sejian et al., 2010). The outsets of thermal stress in animals are best indicated by increase in rectal temperature and respiration rate (Al-Haidary, 2004; Marai et al., 2007). The positive relationship between the RR and RT indicates the importance of respiratory mechanism for thermolysis and maintenance of homeothermia in the animals, to avoid an increase in body temperature (Gomes & Starling, 2003). The decrease in RT and PR as inclusion levels of FSF increased in the present study indicates that the heat stress was mitigated by the effect of fossil shell flour in this study. With 60 g FSF/kg inclusion levels, FSF was able to reduce the RT by 2.10 °C. Likewise, addition of FSF was able to bring the RT of rams fed with FSF supplemented diets (especially treatment 3 and 4) to normal RT range of 38.3 °C to 39.9 °C for sheep as reported by Heath and Olusanya (1985), and Okoruwa (2015). This effect of FSF could be attributed to its richness in Magnesium and Potassium which helps to balance body electrolytes and reduce body heat (Titto et al., 2016). Also, Wojtas, Cwynar, Kolacz, & Kupczynski, 2013) reported that heat stress leads to change in acid- base balance in Polish Merino sheep, hence, the addition of FSF could help to maintain acid-base balance since FSF in rich in minerals, thereby preventing heat stress.

Another typical measurement of the physiological state in sheep is skin temperature (ST) (Talwar & Fahim, 1998; Marai et al., 2007; Cwynar et, al.,2014; Al-Dawood, 2017). In small ruminant, assessment of ST helps to determine the rate of sensitivity of the animal to heat stress because, exchange of heat between the animal body and the environment are been carried out by the skin surface. Marai et al. (2007) reported that head, legs and abdomen are acceptable indicators of heat transfer via skin in sheep. The temperature measurements on the skin, in this current study were made at the head, back, scrotum and abdomen. The average for the four measurements were recorded as ST. It was found that the decrease in ST as the FSF inclusion levels increased in Dohne Merino rams were the direct effect of FSF during the experiment. The result from this study aligned with the report of (Krishman et al., 2017) that observed that, dietary supplementation of trace elements to animal feeds can ameliorate adverse effect of heat stress. Since, FSF is abundantly rich in about 14 trace elements, the addition of FSF to Dohne Merino rams' diet can be concluded to reduced ST in FSF supplemented diet compared to FSF non-supplemented diet.

4.3. Blood parameters

Heamatobiochemical profile of sheep is a vital indicator of physiological responses to heat stress because they are sensitive to changes in environmental temperature (Al-Dawood, 2017). Hence, the degree of heat stress could be determined by considering the blood parameters of the animal. Sivakumar, Singh, & Varshney, 2010) and Okoruwa (2014) reported that lower PCV, Hb, and WBC values were noticed in animals under heat stress compared to when the animals were in comfort zone. The heamatological results obtained in our study agreed with the reports by Winnicka (2008). The increased that occurred in parameters such as WBC, PCV and RBC as the temperature decreases as a result of inclusion of FSF could be because of the activities of minerals elements in FSF. The Potassium, Sodium and Chloride in the control diet were lost through sweating in thermal stress rams but reverse is the case in FSF supplemented diets because addition of FSF supplies these minerals that helps to maintain serum electrolytes balance. Also, during thermal stress, instead of blood, there is transportations of more water in the circulatory system for evaporative cooling. Hence, PCV and RBC in thermal stress animals are lower than non-thermal stress animals. This results also aligned with the statement of Altan, Altan, & Cabuk, 2000), who observed that when sheep are heat stressed the values of blood constituents decreases due to hemodilution effect where more water is transported by the circulatory system to help in evaporative cooling. It could therefore be suggested that FSF has opposing effect on hemodilution because of its anti-oxidant content.

Likewise, Dangi et al., 2012 reported an increase in TPP as heat stress in sheep decreases. This may be due to an increase in feed intake as the heat stress decrease. Though there are inconsistent reports of the effect of heat stress on glucose, (Ocak, Darcan, Çankaya, & İnal, 2009; Mohamad, 2012) observed blood glucose level increases under a comfort zone but decreases when the animals are under heat condition. This could be due to a decrease in the availability of nutrients and a lower rate of propionate production or as a result of the increase in plasma glucose utilization due to an increased respiration rate during heat stress. The results from this current study align with the reports of Ocak, Darcan, Çankaya, & İnal, 2009; Mohamad, 2012). The higher values of glucose and total plasma protein observed at 40 g/kg FSF and 60 g/kg FSF is because the rams were able to eat more feeds in a comfort zone thereby made more nutrients available compared to heat stress zone of high temperature.

Table 2
Composition and the proximate analysis the of experimental Diets.

Items	Percentage (%)
Maize	8
Sunflower oil cake	10
Molasses	5
Wheat bran	15
Limestone	1.6
Sheep premix	0.2
Salt	0.3
Grinded leucine hay (alfalfa)	30
Grinded teff hay	30
Chemical composition	
Dry matter (% as fed)	95.5
Organic matter	85.22
Energy ME	24.67
Crude Protein	14.56
Ash	10.33
Ether extract	1.7
Crude fibre	22.60

Table 3
Effect of fossil shell flour on feed and water intake in Dohne merino rams.

Parameter	Treatment				SEM	P-value
	T1	T2	T3	T4		
Feed intake (g/day)	593	572	694	648	39.9	0.023
Average daily weight gain (g)	84.69[c]	92.86[bc]	121.42[a]	105.35[b]	0.483	0.004
Feed efficiency ratio	0.14	0.18	0.19	0.17	0.01	0.011
Water intake (L/day)	1.33[c]	1.84[b]	2.33[a]	1.94[ab]	0.14	0.01

nonsignT1; 0 g FSF/kg, T2; 20 g FSF/kg T3; 40 g FSF/kg, T4; 60 g FSF/kg. SEM, standard error means.

5. Conclusion and recommendations

The findings from the current study have shown that FSF supplementation affected the physiological and heamatobiochemical response of Dohne merino rams, thereby reducing heat load. Also, the results from this study shows that inclusion of FSF increased feed intake thereby making more nutrients available for the rams' growth through tissue development. Therefore, FSF can be used as a supplement to boost growth performance and mitigate heat stress in Dohne Merino rams especially at inclusion rate of 40 g FSF/kg.(Table 2)

Ethical statement

The authors wish to state that the experiment and all procedures were conducted in accordance with standards of experimentation given by the committee of ethics on animal use of the Society for the Prevention of Cruelty to Animals (SPCA) (Constitutional Court of South Africa, 2013) as approved by University of Fort Hare (Ethical No: MPE011SMWA01/19/A)

Table 4
Effect of fossil shell flour on skin temperature, respiration rate, rectal temperature and pulse rate.

Parameter	Day	Treatments				SEM	P-value		
		T1	T2	T3	T4		Trt	Day	Trt*Day
ST(°C)	0	36.67	36.62	36.65	36.68	0.20	0.040	0.000	0.374
	45	36.95	35.29	35.45	35.31				
	90	36..55	35.31	35.20	35.17				
RT (°C)	0	40.08	40.10	40.12	40.06	0.23	0.001	0.011	0.000
	45	39.98	38.46	38.60	38.45				
	90	40.01	38.44	38.36	38.30				
RR (Breath/minutes)	0	53.92	53.81	54.01	53.98	0.93	0.000	0.004	0.000
	45	49.19	41.00	37.56	30.24				
	90	51.20	28.31	27.31	26.25				
PR (beats/minutes)	0	84.88	86.63	86.35	85.98	0.69	0.000	0.000	0.225
	45	86.83	76.56	72.67	70.08				
	90	86.35	72.81	70.18	70.00				

T1; 0 g FSF/kg, T2; 20 g FSF/kg T3; 40 g FSF/kg, T4; 60 g FSF/kg, ST; skin temperature, RT; rectal temperature, RR; respiration rate, PR; pulse rate, NS, non-significant; TRT, treatment; SEM, standard error means; TRT x DAY, treatment and interaction.
***$P < 0.0001$.
**$P < 0.01$.
*$P < 0.05$.

Table 5
Effect of fossil shell flour on some blood heamatobiochemical parameters.

Parameter	Day	Treatments				SEM	P-value		
		T1	T2	T3	T4		Trt	Day	Trt*Day
Hb (g/100Ml)	0	9.25	9.20	9.30	9.28	0.25	0.048	0.0001	0.0001
	45	10.13	8.80	8.38	7.83				
	90	9.74	8.20	8.20	7.85				
TPP (g/100 mL)	0	56.41	57.67	57.24	56.70		0.002	0.320	0.001
	45	56.45	58.56	58.80	58.82	1.02			
	90	56.42	58.80	59.18	59.95				
GLU (mmol/L)	0	2.35	2.43	2.38	2.40	0.12	0.003	0.001	0.001
	45	2.30	2.53	2.55	2.60				
	90	2.31	2.70	2.74	2.78				
PCV (%)	0	0.27	0.26	0.27	0.29	0.03	0.12	0.11	0.23
	45	0.26	0.25	0.25	0.26				
	90	0.27	0.23	0.23	0.24				
WBC ($x10^3/mm^3$)	0	3.60	3.58	3.63	3.50	0.33	0.224	0.045	0.325
	45	3.55	3.65	3.72	3.69				
	90	3.56	3.72	3.79	4.19				
MCV (fl)	0	39.55	39.54	39.55	40.01	0.59	0.002	0.001	0.000
	45	39.54	40.63	41.55	41.77				
	90	39.54	41.63	41.98	42.22				
MCHC (%)	0	46.03	46.52	46.11	46.34	1.81	0.001	0.054	0.003
	45	46.00	42.41	47.86	47.92				
	90	47.78	43.91	52.89	47.98				
RBC ($x10^6/mm^3$)	0	4.33	4.30	4.35	4.27	0.31	0.001	0.003	0.122
	45	4.23	4.33	4.82	4.86				
	90	4.20	4.78	5.30	5.37				

T1; 0 g FSF/kg, T2; 20 g FSF/kg T3; 40 g FSF/kg, T4; 60 g FSF/kg, HB; hemoglobin, TPP; total plasma protein.
GLU; Blood glucose, PCV; packed volume cell, WBC; white blood cells, MCV; mean corpuscular volume.
MCHC; mean corpuscular hemoglobin concentration, RBC red blood cell, NS, nonsignificant; TRT, treatment.
SEM, standard error means; TRT x DAY, treatment and interaction.

Declaration of Competing Interest

The authors wish to declare that there is no conflict of interest regarding the publication of this manuscript.

Acknowledgements

This study was financially supported by the South African Medical Research Council. Grant number SAMRC/UFH/P790

References

Al-Dawood (2017). Towards heat stress management in small ruminants – A review. *Ann. Anim. Sci. Vol. 17*(1), 59–88.
Al-Haidary (2004). Physiological responses of Naimey sheep to heat stress challenge under semi-arid environments. *International Journal of Agriculture and Biology, 2*(July), 307–309.
Al-Haidary,, A, Aljumaah, R, Alshaikh, M, Abdoun*, K, Alfuraiji, M, et al. (2012). Thermoregulatory and physiological responses of Najdi sheep ex- posed to environmental heat load prevailing in Saudi Arabia. *Pak. Vet. J. 32*, 515–519.
Banerjee, Upadhyay, C, Chaudhary, U, Kumar, R, Singh, S, Sachinandan, D, et al. (2014). Seasonal variation in expression pat- tern of genes under HSP70 family in heat- and cold-adapted goats (Capra hircus). *Cell Stress Chap. 19*, 401–408.
Caroprese, Albenzio, M, Bruno, A, Annicchiarico, G, Marino, R, Sevi, A, et al. (2012).

Effects of shade and flaxseed supplementation on the welfare of lactating ewes under high ambient temperatures. *Small ruminant research : the journal of the International Goat Association, 102*, 177–185.
Cloete, J. J. E, Hoffman, L. C, Cloete, S. W. P, et al. (2012). A comparison between slaughter traits and meat quality of various sheep breeds: Wool, dual-purpose and mutton. *Meat Science, 91*, 318–324.
Cwynar, P, Kolacz, R, & Czerski, A (2014). Effect of heat stress on physiological parameters and blood composition in Polish Merino rams. *Berl Münch Tierärztl Wochenschr, 127*, 177–182.
Wojtas, Krzysztof, Cwynar, Przemyslaw, Kolacz, Roman, & Kupczynski, Robert (2013). Effect of heat stress on acid-base balance in Polish Merino sheep. *Archiv Tierzucht, 56*(92), 917–923.
Daramola and Adeloye, (2009). Physiological adaptation to the humid tropics with special reference to the West African Dwarf (WAD) goat, 1005–1016. 10.1007/s11250-008-9267-6.
Gomes & Starling, (2003). Evaporação Cutânea e Respiratória em Ovinos sob Altas Temperaturas Ambientes Cutaneous and Respiratory Evaporation Rates of Sheep in Hot Environments, 2003, 1956–1961.
Dangi, S. S, Gupta, M., Maurya, D., Yadav, V. P, Panda, R. P., Sarkar, M., et al. (2012). Expression profile of HSP genes during different seasons in goats (Capra hircus). *Trop. Anim. Health Prod. 44*, 1905–1912.
Altan, O, Altan, A, Cabuk, M, et al. (2000). Effects of heat stress on some blood parameters in broilers. *Turkish J Vet Anim Sci, 24*(2), 145–148.
Heath and Olusanya. (1985). *Anatomy and physiology of tropical livestock* (3rd ed). Longman: Intern. Tropical Agric. Series138.
Gupta, M, Kumar, S., Dangi, S. S., & Jangir, Babu Lal (2013). Physiological, biochemical and molecular responses to thermal stress in goats. *Intern. J. Livest. Res. 3*, 27–38.
Ikusika, Olusegun. O., Mpendulo, C. T, Zindove, T.j, & Okoh, A. I (2019). Fossil shell flour in livestock production: A review. *Animals, 9*(3), 1–20. https://doi.org/10.3390/ani9030070.
Kawas, J. R., Andrade-Montemayor, H., & Lu, C. D (2010). Strategic nutrient supplementation of free ranging goats. *Small Rumin Res, 89*(2–3), 234–243.
Kiyatkin (2005). Brain hyperthermia as physiological and pathological phenomena. *Brain research reviews, 50*, 27–56.
Koster.(2013). Diatomite in Animal Feeds. In agrisilica.co.za/pdf/eng/Diatoms%20in%20Animal%20Feeds%20HH%20Koster.pdf.
Krishman, G, Bragath, M., Pragna, P., Vidya, M.k, Aleena, Joy, Bhatta, R., et al. (2017). Mitigation of the Heat Stress Impact in Livestock Reproduction. Chapter 4. *Intech Open Science,* 63–86.
Marai, et al. (2007). Physiological traits as affected by heat stress in sheep-A review. *Small Ruminant Research, 71*(1–3), 1–12. https://doi.org/10.1016/j.smallrumres.2006.10.003.
Marai, I. M. F, Habeeb, A. A. M., & Gad, A. E. (2002). Rabbits productive, reproductive and physiological traits as affected by heat stress a review. *Livestock Production Science, 78*(2), 71–90.
Minka, Ayo (2009). Physiological responses of food animals to road transportation stress. *African Journal of Biotechnology, 8*(25), 7415–7427.
Mohamad (2012). Effect of level of feeding and season on rectal temperature and blood metabolites in desert rams. *Acad. J. Nutr. 1*, 14–18.
National Research Council – NRC. (1971). *A guide to environmental research on animals.* Washington, DC: National Academy of Sciences374p.
Mucina, L., & Rutherford, M. C. (2006). The vegetation of South Africa, Lesotho and Swaziland. *South African National Biodiversity Institute, 19*, 748–756 ISBN-13:978-1-919976-21-1.
Ocak, S., Darcan, N., Çankaya, S., & İnal, T. (2009). Physiological and biochemical responses in German fawn kids subjected to cooling treatments under Mediterranean climatic conditions. *Turk. J. Vet. Anim. Sci. 33*, 455–461.
Okoruwa (2015). Effect of coat characteristics on physiological traits and heat tolerance of West African Dwarf sheep in Southern Nigeria. *Open J. Anim. Sci. 5*, 351–357.
Rensis De, & Scaramuzzi (2003). Heat stress and seasonal effects on reproduction in the dairy cow – a review. *Theriogenology, 60*, 1139–1151.
Sejian, et al. (2017). Effect of multiple environmental stressors on the adaptive capability of Malpura rams based on physiological responses in a semi-arid tropical environment. *Journal of Veterinary Behavior, 17*(December), 6–13. https://doi.org/10.1016/j.jveb.2016.10.009.
Silanikove, & Koluman (2015). Impact of climate change on the dairy industry in temperate zones: Predications on the overall negative impact and on the positive role of dairy goats in adaptation to earth warming. *Small Rumin Res, 123*, 27–34.
Sejian, V, Maurya, V. P., Naqvi, S. M. K., Kumar, D., & Joshi, A. (2010). Effect of induced body condition score differences on physiological response, productive and reproductive performance of Malpura ewes kept in a hot, semi-arid environment. *Journal of Animal Physiology and Animal Nutrition, 94*(2), 154–161. https://doi.org/10.1111/j.1439-0396.2008.00896.x.
Silanikove (2000). The physiological basis of adaptation in goats to harsh environments. *Small Rumin. Res, 35*, 181–193.
Sivakumar, A. V. N., Singh,, G., & Varshney, V. P. (2010). Antioxidants supplementation on acid base balance during heat stress in goats. *Asian-Aust. J. Anim. Sci. 23*, 1462–1468.
Skapetas, & Kalaitzzidou (2017). Current Status and Perspectives of Sheep Sector in the World. *Livestock Research for Rural Development. 29*(2), 17–19.
Srikandakumar, A., Johnso, E. H., & Mahgoub, O. (2003). Effect of heat stress on respiratory rate, rectal temperature and blood chemistry in Omani and Australian Merino sheep. *Small Rumin Res, 49*(2), 193–198.
Talwar, & Fahim (1998). Circulatory response to hyperthermia during acute normovolaemic haemodilution. *International journal of biometeorology, 41*, 169–174.
Titto, Cristiane Gonçalves, Veríssimo, Cecília José, Pereira, Alfredo Manuel Franco, Geraldo, Ana de Mira, Katiki, Luciana Morita, & Titto, Evaldo Antonio Lencioni (2016). Thermoregulatory response in hair sheep and shorn wool sheep. *Animal science journal = Nihon chikusan Gakkaiho, 82*, 591–600.
Verschuren, Olaf, Marjolijn, Ketelaar, Tim, Takken, Paul, Helders, & Jan Willem, Gorter (2008). Excercise programs for children with cerebral palsey: A systematic review of the literature. *American Journal of Physics Medicine and Rehabilitation, 87*(5), 404–417.
Winnicka, A. (2008). *Reference values of basic laboratory tests in veterinary medicine, fourth ed. (in polish).* War- saw, Poland: SGGW Press.