

RESEARCH ARTICLE

Effect of Selenium Supplementation on Secretion of Reproductive Hormones and Immune Response to *Trichostrongylidae* Nematodes Infestation in Zulu Sheep Ewes During Oestrous Cycle

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Abstract: The growing resistance of nematodes to anthelmintics has prompted research into alternative control strategies, including nutritional supplementation with micronutrients such as Selenium (Se). Research focusing on the use of mineral supplementation would not only contribute to improvement of animal performance but also to acquaint stakeholders with alternative immune boosting strategies in livestock. Thus, this research assessed how immune response and secretion of reproductive hormones of Zulu ewes were affected by Se supplementation during three oestrous cycles. Due to scarcity of the Zulu sheep breed in the study area, a total of 24 clinically healthy Zulu ewes aged 2-3 years were used and distributed into Se-supplemented (n = 12) and non-supplemented (control) groups (n = 12). Blood sampling was done from 12 ewes (6 Se-supplemented and 6 control) for determining the circulating concentrations of oestrogen (E2), progesterone (P4), Luteinizing Hormone (LH), glutathione peroxidase (GSH-Px), modulation of immunoglobulin G (IgG) and neutrophils at the beginning of the experiment (day 0), during oestrus (day 1), dioestrus (day 12), luteolysis (day 15) and ovulation (day 3) of oestrous cycle. Egg Count Per Gram of faeces (EPG), anaemic levels, Body Condition Score (BCS), Rectal Temperature (RT) & nematode species compositions were recorded from 24 experimental ewes at time points described for blood samples collection and subjected to logarithmic transformation (log10) before analysis using the repeated measures Linear Mixed Model (PROC MIXED) of SAS version 9.4. Results revealed a significantly (p<0.05) increased overall E2, LH, P4, GSH-Px, IgG, neutrophils circulating concentrations and transformed overall BCS in Se-supplemented ewes. Transformed overall values of Anaemia, EPG, burden of *Haemonchus*, *Ostertagia* and *Trichostrongylus* were significantly reduced (p<0.05) in Se-supplemented Zulu ewes. It is therefore, concluded that, selenium supplementation improved secretion of reproductive hormones by minimising oxidative stress through high GSH-Px activity during different stages of the oestrous cycle. Furthermore, selenium supplementation reduced the burden of *Trichostrongylidae*, enhanced immune response by increasing secretion of IgG and neutrophils in Zulu ewes during oestrous cycle.

Keywords: Micronutrients, Reproductive Hormones, Nematodes, Immunity, Zulu Sheep

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Introduction

Several factors are acknowledged to influence sheep reproduction, including the physical environment, nutrition, genetic merit, and management practices [1-3]. Among these factors, nutrition is the most critical determinant of reproductive success, as it directly affects gametogenesis, steroidogenesis, follicular development, ovulation, and Corpus Luteum (CL) function [4, 5]. Micronutrients such as Selenium (Se), Zinc (Zn), and Copper (Cu) play key roles in stimulating hormonal production and supporting optimal reproductive processes during the oestrous cycle in ruminants [6]. However, deficiencies of these essential micronutrients can impair the normal functioning of the reproductive and immune systems in small ruminants [7].

The South African province, KwaZulu-Natal (KZN), is one of the regions characterized by high annual rainfall and has been identified as Se-deficient due to nutrients leaching from the soil [8, 9]. The normal Se concentration in ruminants' body is said to be 0.38 ppm [10], however, the Se level in the blood of Zulu ewes of the present study was discovered to be 0.03 ppm before the study commenced, indicating deficiency in Zulu sheep. This deficiency is particularly concerning for Zulu sheep, a locally adapted breed reared primarily in pasture-based systems. Selenium deficiency is associated with reproductive challenges such as silent oestrus, cystic ovaries, and the birth of lambs with compromised immunity, as well as increased susceptibility to diseases and gastrointestinal nematode (GIN) infestations [11]. Gastrointestinal nematodes infestations, especially by *Trichostrongylidae* nematodes (e.g., *Haemonchus*, *Ostertagia*, and *Trichostrongylus*), are common in subtropical regions like KZN [12-14] and further exacerbate the challenges faced by small ruminants. These parasites not only suppress the immune system but also disrupt mineral metabolism, leading to worsened micronutrient deficiencies and reduced reproductive performance [15, 16].

The growing resistance of nematodes to anthelmintics [17] has prompted research into alternative control strategies, including nutritional supplementation with minerals such as Se to boost the immune system [18, 19]. Apart from the developing nematode resistance to anthelmintics, these treatments are said to be expensive [20, 21] in comparison to SE oral supplements as these supplements are used in smaller proportions while enhancing the functioning of multiple systems in animal body to increase productivity. Selenium, as an integral component of glutathione peroxidase (GSH-Px), plays a vital role in immune function, phagocyte activity, and humoral immune responses, helping to mitigate oxidative stress during nematode infections [19, 22-24]. It also inhibits problems related to reproduction, such as reduced fertility, abortion, and placenta retention in ruminants [25]. Despite these known benefits, there is limited understanding of how long-term Se supplementation impacts both reproductive hormone secretion and immune responses in areas with high nematode-infested regions like KZN.

This study addresses the dual challenge of reduced reproduction and nematode infestation in Zulu sheep, with the main goal of assessing Se supplementation on the secretion of reproductive hormones and immune response during oestrous cycle. The findings will provide novel insights into the potential of Se as a sustainable intervention to improve reproductive efficiency and resilience in sheep infested with nematode and reared in selenium-deficient environments like KZN. By integrating nutritional and parasitological perspectives, this study offers a unique approach to addressing a critical matter in small ruminant production systems.

Methods

Ethics Clearance

Ethical clearance for this research was permitted by the University of Zululand (Unizulu) Research Ethics Committee (Registration number: UZREC 171110-030 PGM 2022/19).

Area of the Study

This study was carried out at the Unizulu research farm, KwaDlangezwa, Empangeni, situated at 28°44' South latitude and 31°53' East longitude in the KwaZulu-Natal province of South Africa [26].

Experimental Animal Management and Feeding

Due to scarcity of the Zulu sheep breed in the study area, a total of 24 clinically healthy Zulu ewes aged 2-3 years were used. Water availability was *ad libitum* and ewes grazed on natural pastures daily for 3 consecutive oestrous cycles. The natural pastures grass species composition included *Themeda triandra*, *Allelotopsis semialata*, *Trisraccia leucothrix* and

Microcoloa caffra. The study was conducted during rainy season, which is characterised by high infestation of *Trichostrongylidae* nematodes. Members of *Trichostrongylidae* characterised in the present study include *Haemonchus contortus*, *Ostertagia circumcincta* and *Trichostrongylus colubriformis*. Experimental ewes were kept separate from rams and were not dewormed. The pastures' soil Se content was analysed and the results showed the level of Se in the soil to be less than 0.001 ppm. The soil was also discovered to be acidic with a pH of 4.30, it has been reported that acidity significantly reduce the levels of Se in the soil [28]. The grass samples were also collected from the grazing camps for Se analysis and the results revealed no detectable amounts of Se in the grass samples.

Experimental Design

A completely randomised design was used, where ewes were randomly distributed into Se (sodium selenate (Vet Chem, India)) supplemented (n = 12) and non-supplemented (control) (n = 12) groups (Table 1). The animals were balanced according to body weight, with average body weight of 29.79±2.15 and 30.03±2.24 kg on the Se-supplemented and control groups, respectively. The supplemented group were dosed with sodium selenate 0.34 mg /kg at 2 weeks intervals according to [27, 28] throughout the experimental period of 3 oestrous cycles for 3 months.

Table 1: Experimental design

Treatment	Selenium		Total no. of ewes
	Se-supplemented	Non-supplemented (control)	
Number of animals	12	12	24

Se: Selenium

Data Collection and Analysis

Oestrous Detection

All 24 experimental ewes were synchronised on day 19 of the experiment using Control Internal Drug Release (CIDR) inserted for 11 days intravaginally prior oestrous detection. Oestrous detection was done at CIDR removal, 30 days after the first selenium supplementation on all 24 ewes, by introducing 4 teaser rams wearing aprons to the ewes at 12 hours interval for 3 consecutive days. Ewes that stood on heat (wagging tail, stood when mounted, restless etc) were recorded, bled for hormonal concentration and immune response analysis, Rectal Temperature (RT) and faecal samples for Egg Count Per Gram of faeces (EPG) were also collected for 3 oestrous cycles.

Blood Sampling and Analysis

For blood collection, 12 ewes (6 from Se-supplemented and 6 from control) were bled, this was done to reduce costs of ELISA kits as they were expensive. The ewes were randomly allocated to each blood collection group and balanced according to body weight and age.

Blood was collected from the jugular vein of 12 ewes using 21G needles connected to vacutainer clot activator tubes and delivered to the laboratory for hormonal concentration analysis. Blood was collected at the beginning of the experiment (day 0) and subsequently when the ewes first showed standing heat, oestrus (day 1), ovulation (day 3), metoestrus (day 6), dioestrus (day 12) and luteolysis (day 15) after showing signs of oestrus for 3 oestrous cycles.

Hormonal concentrations were analysed using enzyme-linked immunosorbent assay (ELISA) kits (Bioassay Technology Laboratory, China) according to the manufactures' protocols, in brief, blood serum were harvested by means of centrifugation at speed of 3000 rpm for 20 minutes. To conduct the ELISA assays, microplates were first pre-coated with antibodies specific to either oestrogen (E2), Luteinizing Hormone (LH), Progesterone (P4), glutathione peroxidase (GSH-Px), immunoglobulin G (IgG) or neutrophils. The sheep neutrophils ELISA kit indirectly quantifies neutrophils as it uses sheep-specific antibodies and enzyme-linked detection systems to produce measurable signals, typically colorimetric, which link with the concentration of the target protein (Neutrophil Gelatinase-Associated Lipocalin (NGAL)), providing an indirect quantity of neutrophil activity or concentration (Bioassay Technology Laboratory, China). For all hormonal and immunity analysis, the antibodies in serum samples competed for the binding sites on the microplates pre-coated with the antibodies supplied with the ELISA kit, which were specific to either E2, P4, GSH-Px, neutrophils, IgG or LH. After the reaction was complete, the microplate wells were washed to remove the unbound antibodies and the substrates were added. Subsequently, a stop solution was added and there was a colour change from blue to yellow. Absorbances of E2, LH, P4, GSH-Px, quantitative levels of IgG and neutrophils

were then determined [29, 30] using Thermo Scientific Multiskan FC microplate photometer (Thermo Fisher Scientific Inc., Waltham, USA) set at 450 nm.

Faecal Analysis (Egg Counts)

Faecal samples were collected from 24 ewes (Se-supplemented and control groups) from the rectum at the beginning of the experiment (day 0) and subsequently when ewes showed standing heat for the first time, during oestrus (day1), ovulation (day 3), metoestrus (day 6), dioestrus (day 12) and luteolysis (day 15) after showing first signs of oestrus from each animal over 3 oestrous cycles. About 3g of the faecal sample were mixed with 42 mL of distilled water in a 50 mL glass beaker to create a homogenised faecal suspension. Thereafter, a stirring rod (Inqaba Biotechnical Industries [Pty] Ltd, South Africa) was used to stir thoroughly with the aim of breaking the faecal pellets. After being stirred, each sample's faecal suspension was filtered into a clean glass beaker using a sieve (Reflecta Laboratory Supplies, South Africa) and cloth (C.C. Imelmann [Pty] Ltd, South Africa). The filtered faecal suspension was then homogenised by pouring. Following that, a homogenised faecal suspension subsample was then poured into a 50 mL centrifuge conical test tube (Thermo Fisher Scientific Inc., USA) and spun with a table top centrifuge (Rotina 380R, Andreas Hettich GmbH, Germany) at 1000 rpm for five minutes and supernatant was discarded afterwards. The sediment left in the 50 mL conical test tube (Thermo Fisher Scientific Inc., USA) was then mixed with 15 mL of flotation fluid (sugar solution (1L of water and 500g sugar) designed to concentrate and isolate parasite eggs from most faecal debris) and formed a solution. A total of 1000 µl of the solution was pipetted into 2 chambered McMaster slide (Labotec Company, South Africa) and placed for 1 minute on the microscope according to [32] (allowing the eggs to float to the top). Eggs were counted and classified according to different species at 100X magnification of Leica compound microscope (Leica Microsystems, Danaher Corp., Germany). The Egg Count Per Gram of faeces (EPG) was determined after microscopic analysis using Eq. 1. below: [31]:

$$\text{"epg=" "A}\times\text{"B" / "C}\times\text{D}\times\text{"E" "}$$

Where:

- A. Is the total number of eggs counted
- B. Is the total volume of faecal suspension (60 mL)
- C. Is the number of chambers counted
- D. Is the grams of faeces (g)
- E. Is the standard volume of chamber (0.15 mL)

Anaemic Level

Anaemic levels of 24 ewes during the 3 oestrous cycles were assessed by individuals that received prior training from the veterinarian that works with the University of Zululand department of agriculture to mitigate the observer biasness, using the FAMACHA® guide [32] by comparing the conjunctiva's colour with the eye's colour and scored as follows:

- 1. = Red, Most favourable, Deworming not needed
- 2. = Red Pink, Satisfactory, Deworming not needed
- 3. = Pink, Marginal, Deworming may be needed
- 4. = Pink White, Threatening, Deworming is needed
- 5. = White, Fatal, Deworming is needed

Body Condition Score

The Body Condition Scoring (BCS) was done throughout the experimental period at 2 week intervals on 24 ewes by individuals that received prior training from the veterinarian to mitigate the observer biasness, since the blinding protocol was not practiced for both FAMACHA and body condition scoring. In order to assess the BCS, the loin was pressed halfway between the last rib and the hipbone to feel the pelvic bone and the results were given using a range between 1 and 5 as described below according to [33]:

- 1. Prominent spine, sharp to touch transverse processes with no detectable fat cover (Very thin)

2. Transverse processes touchable with thumb but rounded with a thin fat cover (Thin)
3. The thumb had to be applied firmly in order to feel individual transverse processes (Satisfactory)
4. Even with firm pressure, transverse processes could not be detected (Fat)
5. The transverse processes were clearly covered in a very thick layer of fat and were completely undetectable (Obese)

Statistical Analysis

Data on faecal egg counts, EPG, anaemic level, RT and BCS of the animals were subjected to logarithmic transformation ($\log_{10} x + 1.5$) to ensure normal distribution of the values according to [34]. The effect of selenium supplementation on faecal egg count, EPG, anaemic level, RT, BCS, E2, LH, P4, GSH-Px, IgG and neutrophils concentrations were analysed using repeated measures Linear Mixed Model (PROC MIXED) implemented in Statistical Analysis Software version 9.4 using the below model. Ewes ID were defined as random effect and oestrous stages as fixed effects. The post-hoc statistical power analysis that relates observed p-value to observed power was used on this study to demonstrate the validity of its statistical inferences, using Eq. 2. below. Student Newman Keuls (SNK) test was used for separation and comparison of least square means (LSMeans) and $p \leq 0.05$ was considered significant. All log transformed LSMs were reported as transformed LSMs Linear Mixed Model:

$$Y_{ijkl} = \mu + T_i + W_j + (TW)_{ij} + A_k(T_i) + \varepsilon_{ijkl}$$

Where:

Y_{ijkl} = observed value of the dependent variable (faecal egg count, EPG, anaemic level, RT, BCS, E2, LH, P4, GSH-Px, IgG, or neutrophil concentration) for the k th ewe in treatment i at time j :

μ = Overall population mean

T_i = Fixed effect of the i th selenium supplementation treatment

W_j = Fixed effect of sampling time/repeated measurement period

$(TW)_{ij}$ = Interaction effect between selenium treatment and sampling time

$A_k(T_i)$ = Random effect of the k th animal nested within treatment

ε_{ijkl} = Residual error Eq. 2: [35]:

$$\text{Observed power} = 1 - \Phi(Z_\alpha - Z_p)$$

Where:

Φ is the standard normal cumulative distribution function

Z_α is the z-score corresponding to the critical value for significance level (0.05 in a two-sided z-test)

Z_p is the z-score corresponding to the observed P-value

Results

Impact of Selenium Supplementation on Production of Reproductive Hormones and Enzyme Activity in Zulu Sheep Ewes Throughout Oestrous Cycle

Overall circulating concentration levels of E2 of Zulu ewes at different stages of the oestrous cycle are shown in Fig. 1. The baseline of E2 levels (day 0) were 371.01 ± 75.30 pg/mL vs 377.91 ± 75.30 pg/mL prior the supplementation of Se for Se-supplemented and control groups. Oestrogen circulating concentration increased ($p < 0.05$) in ewes from Se-supplemented compared to the control group during oestrus (day 1) (558.23 ± 69.04 pg/mL vs 276.49 ± 69.04 pg/mL) and ovulation (day 3) (491.50 ± 77.41 pg/mL vs 274.03 ± 77.41 pg/mL) after showing signs of oestrus. The circulating concentrations of E2 had no significant difference ($p > 0.05$) during dioestrus (day 12) and luteolysis (day 15) after showing signs of oestrus.

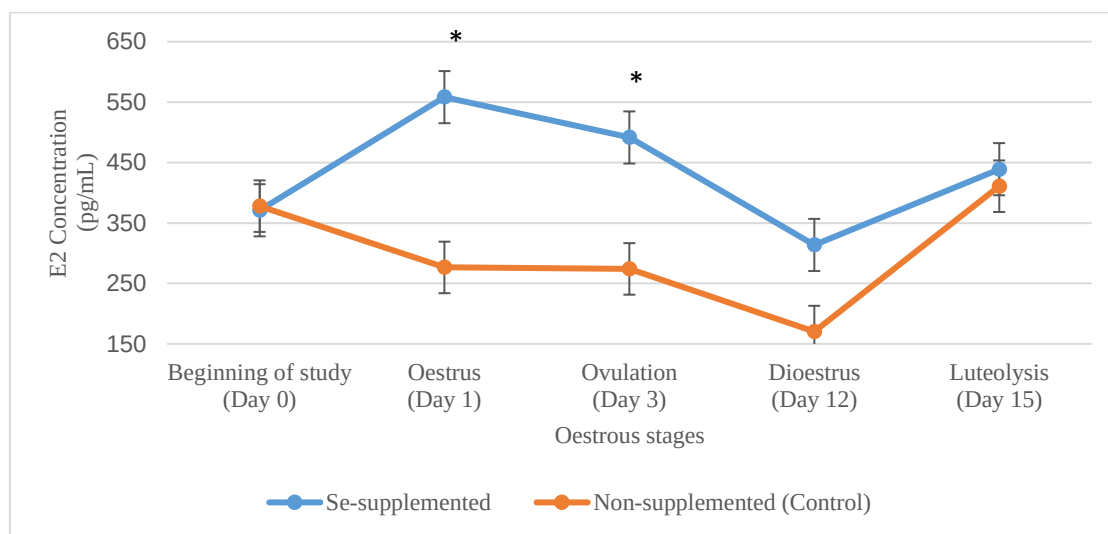


Fig. 1: Oestrogen (E2) overall circulating concentration (LSMean $\pm$ SEM) (pg/mL) over 3 oestrous cycles of Se-supplemented Zulu sheep ewes (n = 6 Se-supplemented ewes & 6 non-supplemented ewes); *- indicates significant ($p \leq 0.05$) difference

The overall levels of LH circulating concentrations of Zulu sheep ewes at different stages of oestrous cycle are shown in Fig. 2. Baseline values (day 0) of LH levels were 58.26 ± 22.50 mIU/mL vs 58.71 ± 22.50 mIU/mL before Se supplementation on supplemented and control ewes. During ovulation (day 3), LH circulating concentration was higher ($p < 0.05$) in ewes that were supplemented (59.28 ± 22.21 mIU/mL) with Se than ewes that were not supplemented (11.51 ± 22.21 mIU/mL) with Se. However, there was no significant difference ($p > 0.05$) on LH circulating concentrations when ewes were on standing heat, oestrus (day 1), during metoestrus (day 6), dioestrus (day 12) and luteolysis (day 15) after showing signs of oestrus.

Progesterone overall circulating concentrations of Zulu sheep during different stages of oestrous are shown in Fig. 3. The baseline of P4 levels were 3.46 ± 0.13 ng/mL vs 3.36 ± 0.13 ng/mL prior Se supplementation at the beginning of the experiment (day 0) for Se-supplemented and control groups. Progesterone circulating concentrations significantly increased ($p < 0.05$) in Se-supplemented ewes than the control ewes during dioestrus (day 12) (7.48 ± 1.18 ng/mL vs 3.23 ± 1.18 ng/mL) and luteolysis (day 15) (4.64 ± 0.87 ng/mL vs 2.69 ± 0.87 ng/mL) after the onset of oestrus.

The P4 circulating concentration in Se-supplemented ewes and control ewes did not differ significantly ($p > 0.05$) during oestrus (day 1), ovulation (day 3) and metoestrus (day 6).

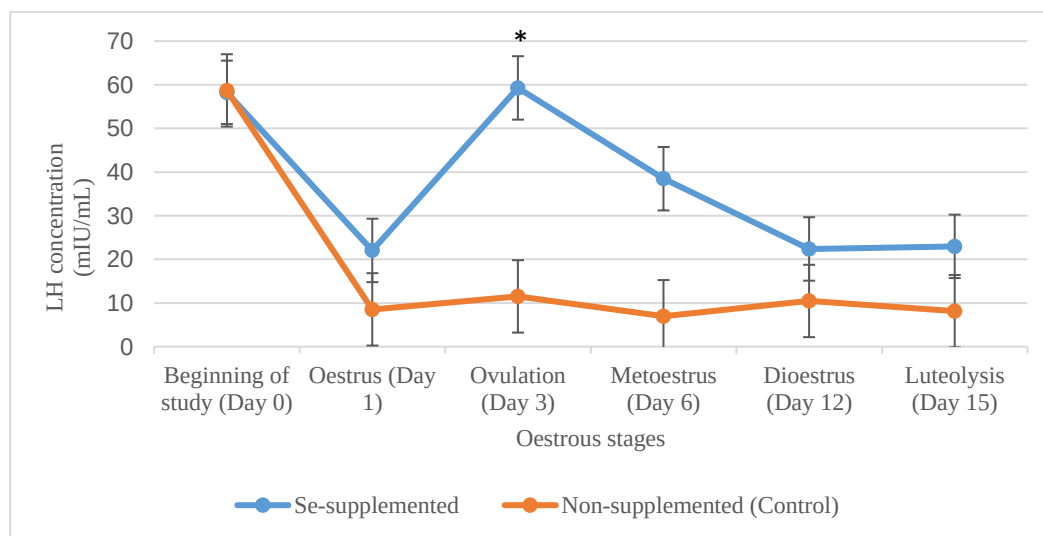


Fig. 2: Luteinizing Hormone (LH) overall circulating concentration (LSMean $\pm$ SEM) (mIU/mL) during 3 oestrous cycles of Se-supplemented Zulu sheep ewes (n = 6 Se-supplemented ewes & 6 non-supplemented ewes); *- indicates significant ($p \leq 0.05$) difference

The overall levels of GSH-Px circulating concentration of Zulu sheep at different stages of oestrous cycle are shown in Fig. 4. Baseline GSH-Px values (day 0) were 25.62 ± 17.81 ng/mL and 26.90 ± 17.81 ng/mL before Se supplementation for the supplemented ewes and non-supplemented. Ewes that were supplemented with Se had higher ($p < 0.05$) circulating concentration of GSH-Px at different stages of oestrous, during oestrus (day 1) (16.28 ± 6.56 ng/mL vs 4.88 ± 6.56 ng/mL), ovulation (day 3) (29.96 ± 8.36 ng/mL vs 3.34 ± 8.36 ng/mL), dioestrus (day 12) (35.32 ± 11.58 ng/mL vs 14.68 ± 11.58 ng/mL) and luteolysis (day 15) (35.81 ± 11.36 vs 9.32 ± 11.36 ng/mL) compared to ewes in the control group.

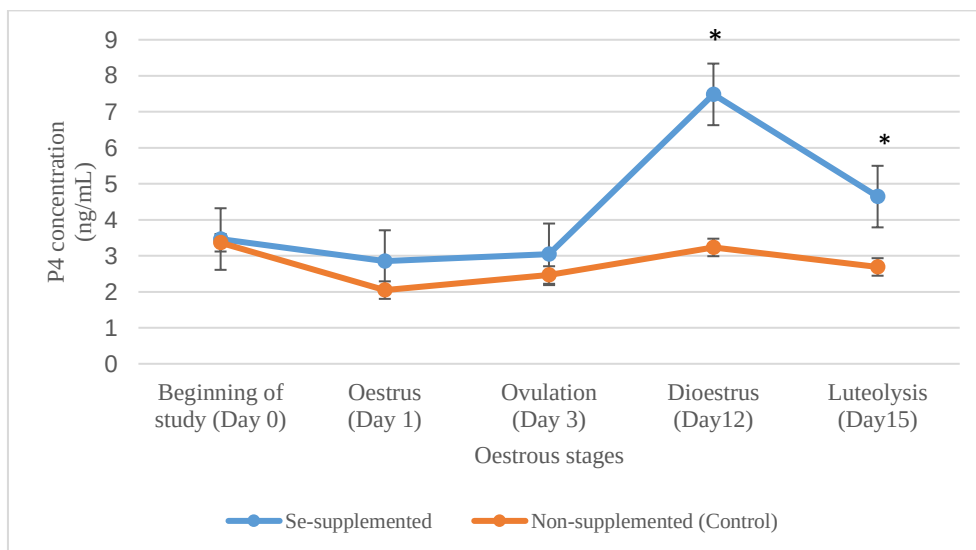


Fig. 3: Progesterone (P4) overall circulating concentration (LSMean $\pm$ SEM) (ng/mL) during 3 oestrous cycles of Se-supplemented Zulu sheep ewes (n = 6 Se-supplemented ewes & 6 non-supplemented ewes); *- indicates significant ($p \leq 0.05$) difference

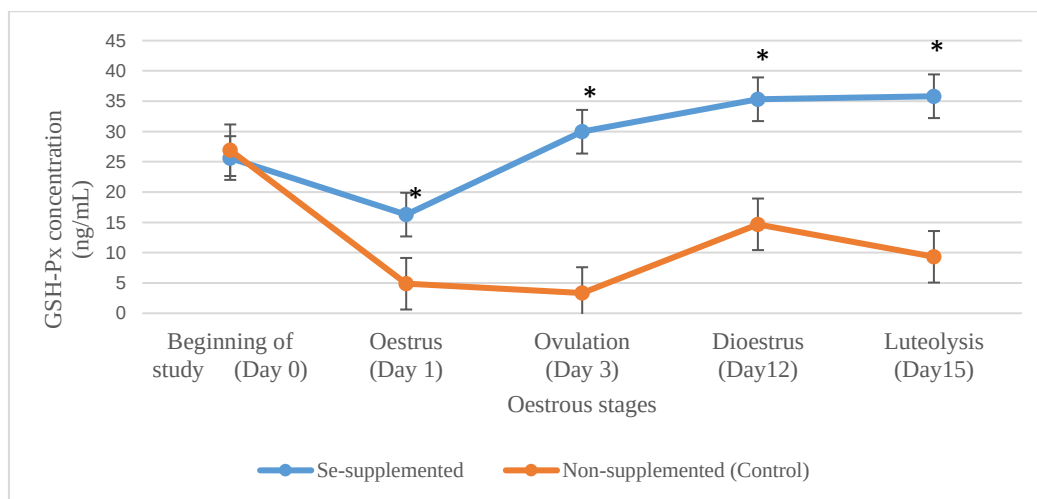


Fig. 4: Glutathione peroxidase (GSH-Px) overall concentration (LSMean $\pm$ SEM) (ng/mL) during 3 oestrous cycles of Se-supplemented Zulu sheep ewes (n = 6 Se-supplemented ewes & 6 non-supplemented ewes); *- indicates significant ($p \leq 0.05$) difference

Impact of Selenium Supplementation on the Morphological and Physiological Traits of Zulu Ewes Throughout the Oestrous Cycle

Table 2 shows overall values of morphological and physiological characteristics of Zulu sheep ewes during 3 oestrous cycles. Overall transformed anaemic levels and Egg Count Per Gram of faeces (EPG) significantly decreased ($p < 0.05$) in ewes that were supplemented (0.39 ± 0.02) and (2.76 ± 0.05) compared to the control (0.41 ± 0.02) and (3.21 ± 0.05) ewes, respectively. The overall BCS increased ($p < 0.05$) for Se-supplemented (0.39 ± 0.02) ewes compared to the control (0.35 ± 0.02)

group. However, there was no significant difference ($p>0.05$) on the overall rectal temperature values between the ewes that were supplemented (1.58 ± 0.01) and control (1.58 ± 0.01) ewes for 3 oestrous cycles.

Table 2: Anaemic level, egg count per gram of faeces, body condition score and rectal temperature (LSMean $\pm$ SEM) during 3 oestrous cycles of Se- supplemented Zulu sheep ewes (n = 12 Se-supplemented ewes & 12 non-supplemented ewes)

Treatment	Selenium		P-value
	Se-supplemented	Non-supplemented (control)	
AL	0.39 ± 0.02^b	0.41 ± 0.02^a	0.05
EPG	2.76 ± 0.05^b	3.21 ± 0.05^a	0.01
BCS	0.39 ± 0.02^a	0.35 ± 0.02^b	0.03
RT	1.58 ± 0.01	1.58 ± 0.01	0.99

Se: Selenium, AL: Anaemic Level, EPG: Egg Count Per Gram of faeces, BCS: Body Condition Score, RT: Rectal Temperature, ^{a, b} means with different superscripts within the same column differ significantly ($p\leq0.05$)

The Impact of Selenium Supplementation on *Trichostrongylidae* Nematode Burden and the Immune Response in Zulu Ewes Throughout Oestrous Cycle

Table 3 shows overall values of *Trichostrongylidae* nematode burden in 3 oestrous cycles in Zulu sheep. Selenium supplemented ewes had lower ($p<0.05$) parasite burden of the identified *Trichostrongylidae* (*Haemonchus* 0.67 ± 0.18 , *Ostertagia* 0.47 ± 0.09 and *Trichostrongylus* 0.31 ± 0.17) compared to ewes from the control (*Haemonchus* 1.03 ± 0.09 , *Ostertagia* 0.93 ± 0.17 and *Trichostrongylus* 0.65 ± 0.10) group.

Table 3: Burden of *Trichostrongylidae* in Zulu sheep ewes (n = 12 Se-supplemented ewes & 12 non-supplemented ewes) supplemented with selenium (LSMean $\pm$ SEM)

Treatment	Selenium		P-value
	Se-supplemented	Non-supplemented (control)	
<i>Haemonchus contortus</i>	0.67 ± 0.18^b	1.03 ± 0.09^a	0.01
<i>Ostertagia circumcincta</i>	0.47 ± 0.09^b	0.93 ± 0.17^a	0.04
<i>Trichostrongylus colubriformis</i>	0.31 ± 0.17^b	0.65 ± 0.10^a	0.03

Se: Selenium, ^{a, b} means with different superscripts within the same column differ significantly ($p\leq0.05$)

Table 4 shows overall immunological response of Zulu sheep ewes during 3 oestrous cycles. Immunoglobulin G (IgG) and neutrophil modulations were significantly ($p<0.05$) higher in ewes with selenium supplementation (8.66 ± 1.80 ng/mL) and (276.14 ± 52.79 pg/mL) compared to ewes from the control (4.81 ± 1.80 ng/mL) and (162.14 ± 52.79 pg/mL), respectively.

Table 4: Immune response (LSMean $\pm$ SEM) of Zulu sheep ewes (n = 6 Se-supplemented ewes & 6 non-supplemented ewes) supplemented with selenium

Treatment	Selenium		P-value
	Se-supplemented	Non-supplemented (control)	
IgG (ng/mL)	8.66 ± 1.80^a	4.81 ± 1.80^b	0.01
Neutrophils (pg/mL)	276.14 ± 52.79^a	162.14 ± 52.79^b	0.03

Se: Selenium, IgG: immunoglobulin G, ^{a, b} means with different superscripts within the same column differ significantly ($p\leq0.05$)

Discussion

The limitations of this study are that it used a very small sample size (n = 24) as these were the available ewes on the study area due to scarcity of the breed. The high costs of ELISA kits contributed to even smaller sample size (n = 12) of ewes that were bled for hormonal, enzymatic and immune response analysis. Also it focused on one breed (Zulu sheep), at a single location. The study solely focused on selenium content as it did not assess the mineral profile of other micronutrients on the grazing pastures' soil and grass. Another limitation that was noted from this research is that it was conducted for a short period of time, 3 months. These limitations have been taken into consideration for future studies purposes.

Impact of Supplementing Selenium on Secretion of Reproductive Hormones and Enzyme Activity in Zulu Ewes During the Oestrous Cycle

Selenium (Se) supplementation increased oestrogen (E2) secretion during oestrus and ovulation which may have improved oestrus behaviour and ovulation rate in Zulu sheep ewes. This observation could contribute to high oestrous response and ovulation rate in supplemented ewes. Selenium supplementation during oestrous cycle has been reported to stimulate E2 production through enhancing follicle maturation, to trigger early ovulation in ruminants [36] reported that Se and vitamin E increased E2 production in supplemented Ghezel ewes during oestrus. Selenium supplementation is also known to increase neutrophils concentration in animals during oestrous cycle to protect gonadotropin-releasing hormone receptors (GnRHR) from oxidants with the aim of promoting E2 production during oestrus [6, 37]. This study showed an increased neutrophils concentration in supplemented Zulu ewes, indicating that Se may have improved E2 production through increase in neutrophils which has previously been reported to protect GnRHR [6].

Luteinizing hormone (LH) circulating concentration was increased in selenium supplemented Zulu ewes during ovulation in the present study. Luteinizing hormone is known for stimulating follicles to ovulate [38, 39]. [36] also reported high LH concentrations in Se-supplemented dairy cows during ovulation to promote the release of an ovum. According to previous studies, Se stimulates high levels of LH secretion in animals by controlling the Hypothalamic-Pituitary-Gonadal (HPG) axis, which elevates GnRH pulses and raises LH secretion during ovulation stage [6, 40].

Selenium supplementation improved Progesterone (P4) circulating concentration in Zulu sheep ewes during dioestrus and luteolysis in this study. The current finding is in agreement with the submission by [41] who discovered that Se supplementation increases P4 secretion during the luteal phase in beef cows. This could mean that supplementation of Se may enhance the Corpus Luteum (CL) function and formation in Zulu sheep ewes which could have led to high P4 secretion during the luteal phase. The results suggest that during P4 biosynthesis in the CL, Se supplementation may have eliminated Reactive Oxygen Species (ROS) accumulation by promoting glutathione peroxidase activity during dioestrus to increase P4 secretion in supplemented ewes [36].

Selenium supplementation increased glutathione peroxidase (GSH-Px) activity of Zulu sheep ewes during oestrus, ovulation, dioestrus and luteolysis which could have led to reduced oxidative stress during oestrous cycle. The present results are in agreement with the findings of [42] who reported a high activity of GSH-Px in ewes that were supplemented with Se during the oestrous cycle. Glutathione peroxidase is one of oxidative stress indicators and serves the main role of reducing ROS including organic and hydrogen peroxides produced in animals during an oestrous cycle. It has been previously shown that supplementing animals with Se activate the activity of the glutathione peroxidase enzyme, with the aim of reducing or eliminating oxidative stress [24, 43]. Active glutathione peroxidase enzyme reduces ROS into less toxic hydrogen peroxide during oestrous cycle in animals [44, 45].

Impact of Selenium Supplementation on the Morphological and Physiological Traits of Zulu Ewes Throughout the Oestrous Cycle

The anaemic level (FAMACHA) and Body Condition Scores (BCS) had limitations on this study, as the blinding protocols were not implemented on these subjective assessments. However, anaemic level and Egg Count Per Gram of faeces (EPG) were reduced in Se-supplemented Zulu ewes in the present study. Similar findings were reported by [16] in lambs supplemented with Se. The anaemic level and EPG are normally reduced by the suppression of eggs excreted by parasite worms. Eggs suppression usually occurs after anthelmintic or nutraceuticals treatment in ruminants [16]. Therefore, selenium supplementation can be an alternative control strategy of reducing egg excretion of parasite worms apart from the anthelmintics and nutraceuticals. Lower anaemic levels observed in the supplemented group was supported by lower prevalence of nematodes in this study. Low EPG in supplemented ewes is also an indication of low prevalence of parasites as it is a diagnostic method of determining the number of parasite eggs present in animal faeces [46] to provide the intensity of parasite infection within an animal [47].

Selenium supplementation increased Body Condition Score (BCS) of Zulu ewes in this study. This aligns with [48] who reported a higher BCS in ewes after Se supplementation. This might be that Se stimulate appetite in animals causing an increase in feed consumption, body weight gain and higher BCS in sheep as observed by [49] in Se-supplemented sheep. Also, the lower EPG of *Trichostrongylidae* observed in Se supplemented ewes in this study may have contributed to higher BCS. The Rectal Temperature (RT) of Zulu ewes was not affected by Se supplementation. This is in agreement with [50] who reported no significant effect on the RT of lambs supplemented with vitamin E and Se. Rectal temperature as used for this

study for body temperature is dependent on metabolism as waste energy in the form of heat [51]. Therefore, the present findings indicate that Se has no effect on metabolism in sheep.

The Impact of Selenium Supplementation on *Trichostrongylidae* Nematode Burden and the Immune Response in Zulu Ewes Throughout Oestrous Cycle

Selenium supplementation reduced the burden of *Trichostrongylidae* nematodes (*Haemonchus contortus*, *Ostertagia circumcincta* and *Trichostrongylus colubriformis*) in Zulu ewes during oestrous cycle in the present study. This agrees with [34] who observed lower parasite load of Gastrointestinal Nematodes (GIN) following Se and Copper (Cu) supplementation in lambs. This observation is also supported by lower anaemic level in Se-supplemented ewes found in this study. Coincidentally, *Trichostrongylidae* nematodes have been shown to cause anaemia, and diarrhoea in small ruminants [52-55]. Infection of such nematodes lead to oxidative stress in sheep [19], however, selenium supplementation lowered their burden in supplemented ewes and minimised the oxidative stress as supported by high glutathione peroxidase enzyme activity of supplemented ewes on the current study [16, 19, 56].

According to [24], Se plays an essential role in modulating inflammation and immune responses. In the present study, Se supplementation increased immunoglobulin G (IgG) reflect modulation of Zulu ewes that were infested by *Trichostrongylidae* during oestrous cycle. In agreement with recent findings, [57] reported a high IgG reflect modulation in ewes following Se and vitamin E supplementation which improved their humoral immune response against GIN infestation. Multiple studies have previously indicated that Se supplementation increase IgG reflect modulation during parasitic infection to lower parasite burden in ruminants [24, 58-60]. Immunoglobulin G has been reported to reduce nematode eggs excreted with faeces in animals [61] and studies have associated high IgG with resistance towards *Trichostrongylidae* [62, 63]. The results of this study whereby lower EPG was found in Se-supplemented ewes may promote humoral immune response of supplemented Zulu ewes against *Trichostrongylidae* infestation by increasing the IgG reflect modulation and, thereby, reducing the parasitic load.

Selenium supplementation also improved cellular component of the immune system through increasing neutrophils modulation of Zulu ewes during oestrous cycle in this study. Similarly, [64] reported high neutrophils reflect modulation in Se-supplemented sheep, which enhanced cellular immune response against nematode infestation. Neutrophils are responsible for assessing signs of microbial infections in animals through rapid migrate to the site of infection and phagocytose microbes [65]. Selenium has previously been reported to improve the capability of phagocytic neutrophils in animals by increasing GSH-Px activity, which strengthens the immune function [24, 66, 67]. As observed in this study, Se supplementation increased the GSH-Px activity which could have led to high neutrophil modulation in Se-supplemented Zulu ewes [24, 65]. The results of this study suggests that Se supplementation has a potential of reducing *Trichostrongylidae* load through increase in cellular immune response during oestrous cycle in Zulu sheep ewes as indicated by high neutrophils modulation in supplemented ewes.

Conclusion

Selenium supplementation increased the secretion of circulating E2, LH, P4 and GSH-Px activity during different stages of the oestrous cycle which could enhance reproductive performance and oxidative stress biomarker enzyme activity in the Zulu ewes. Selenium supplementation reduced *Trichostrongylidae* nematodes infestation in Zulu ewes which could have led to reduced anaemic levels, EPG and high BCS, and promote immune response of Zulu ewes. It is therefore, suggested that Se can be an alternative control strategy for KwaZulu Natal communal farmers to improve reproductive performance and boost immune system of Zulu ewes in the KZN region.

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Author's Contributions

Noxolo W Nkunzi: Conceptualization and design, acquisition of data, analysis and interpretation of data, drafting the article, reviewing it critically for significant intellectual content and editora.

Fhulufhelo V Ramukhithi, Ismaila Muritala, Fabian N Fon: Analysis and interpretation of data, reviewing manuscript critically for significant intellectual content and editor.

Khoboso C Lehloeny: Conceptualization and design, analysis and interpretation of data, reviewing manuscript critically for significant intellectual content and editor, gave final approval of the version to be.

Ethics

This research adhered to the University of Zululand's ethical guidelines and standards, and experiments were carried out in compliance with the ethics committee's requirements. Animal discomfort was avoided as according to the Animal Protection Act 71 of 1962 during data collection.

Conflict of Interest

The authors declare no conflict of interests.

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