Mirror of Research in Veterinary Sciences and Animals
MRVSA/ Open Access DOAJ

OPEN ACCESS

ARTICLE INFO
Received: 17.07. 2024
Revised: 27.07. 2024
Accepted: 28.07.2024
Publish online: 30.07. 2024

Reviewed by
Assistance professor Abdulkarim Jafar Karim Tropical Biological Research Unit, College of Science, University of Baghdad

***Corresponding author:**

Olusegun Oyebade Ikusika (E-mail: Ikusika(Oikusika@ufh.ac.za)
Address: Department of Livestock and Pasture Sciences, Faculty of Science and Agriculture, University of Fort Hare, Alice, South Africa.
https://orcid.org/0000-00016646 8341

CITATION

Olusegun Oyebade Ikusika. (2024). Effect of Fossil Shell Flour Supplementation on Hematobiochemical Profiles and Parasitic Loads in Dohne Merino Wethers. MRVSA. 13 (1): 21-37.

Doi: http://dx.doi.org/10.22428/mrvsa-2024-00131-03

TYPE Research Article
PUPLISHED 30.07. 2024
Doi: http://dx.doi.org/10.22428/mrvsa-2024-00131-03

Effect of Fossil Shell Flour Supplementation on Hematobiochemical Profiles and Parasitic Loads in Dohne Merino Wethers

Olusegun Oyebade Ikusika [1*]

[1] Department of Livestock and Pasture Sciences, Faculty of Science and Agriculture, University of Fort Hare, Alice, South Africa
Corresponding author: Olusegun Ikusika(Oikusika@ufh.ac.za
Tel : +27736535941
https://orcid.org/0000-0001-6646-8341

ABSTRACT

In recent livestock production, Fossil Shell Flour (FSF), a naturally fossilized sediment, has garnered attention as a feed additive. This study investigated the impact of varying FSF levels on hematobiochemical profiles and gastrointestinal parasite loads in Dohne Merino wethers. Twenty-four wethers were randomly assigned to different treatments: a basal diet (0% FSF) and diets supplemented with 2%, 4%, or 6% FSF. Blood and fecal samples were collected at intervals during the feeding trial. Results revealed that wethers fed FSF-supplemented diets exhibited significant increases in red and white blood cell counts from day 25 to 100 compared to the control group ($P < 0.05$). Additionally, wethers on a 4% FSF diet had significantly lower blood urea and serum creatinine levels ($P < 0.05$). Overall, FSF supplementation improved hematobiochemical parameters and notably reduced gastrointestinal parasite burdens, particularly at the 4% inclusion level.

Keywords: fossil shell flour, blood haematology, serum biochemistry, faecal egg count, Dohne-Merino sheep

Doi: http://dx.doi.org/10.22428/mrvsa-2024-00131-03

Introduction

Sheep's productivity and health are closely intertwined, making the assessment of their health crucial for optimizing efficiency (Gwaze *et al.,* 2012). Traditionally, researchers have relied on methods like body condition scoring to assess health and growth rates in small ruminants (Malekkhahi *et al.,* 2015). However, these methods fall short in providing an immediate and comprehensive evaluation of blood profiles and fecal egg counts, as pointed out by Schröder and Staufenbiel, (2010). Various factors influence blood constituents, including age, sex, breed, feed intake and quality, the presence of anti-nutritional factors, and the types of supplements or feed additives used (Etim *et al.,* 2014; Babeker and Bdalbagi,

2015). Gastrointestinal parasites pose a significant challenge in sheep production (Catorci *et al.,* 2012), especially because sheep graze close to the ground, exposing them to a variety of parasites and diseases. Parasitic infections can accelerate metabolic processes, leading to hematobiochemical imbalances and reduced body weight (Odoi *et al.,* 2007). Recently, interest has grown in feed additives for ruminants, particularly as growth promoters and anthelmintics (Anassori *et al.,* 2015). Research indicates that additives, including organic supplements, can influence animal blood profiles (Wang *et al.,* 2016). These additives may also exhibit antimicrobial properties, control rumen microbial activity, and reduce greenhouse gas production, among other benefits. Wang and Wang, (2016) observed positive immunological, antioxidative, digestive, and metabolic effects in goats with the addition of Chinese herbal medicine (plant extracts) to their diets. Common additives for ruminants include prebiotics, herbs, probiotics, substances derived from plants, antibiotics, and organic acids. Nevertheless, pharmaceutical-based additives have been linked to antibiotic resistance and the potential transmission of resistant bacteria to both animals and humans (Wang and Wang, 2016). Additionally, these additives can be costly and inaccessible to communal farmers. Therefore, there is a need for affordable and readily available alternatives that promote healthy livestock production without compromising efficiency (Ruiz-Garcia and Lunadei, 2011). Fossil shell flour (FSF) emerges as a potential substitute.

Fossil shell flour, primarily composed of silicon from diatoms, is a natural sedimentary rock that is accessible, cost-effective, and nutritious for sheep. Its production is sustainable and environmentally friendly compared to synthetic compound concentrates (Koster, 2013; Ikusika *et al.,* 2019a). Emeruwa, (2016) demonstrated that diets supplemented with FSF improved serum chemistry parameters in West African Dwarf sheep, including total protein, alanine aminotransferase, cholesterol, and urea. Sheep supplemented with FSF showed higher albumin and glucose levels, suggesting enhanced energy and protein availability for productive and reproductive performance ($P < 0.05$). FSF has also proven effective in naturally controlling internal parasites in livestock (Koster, 2013). Bennett *et al.,* (2011) found that incorporating FSF at 2% in diets for laying birds increased weight gain, feed efficiency, egg production, and reduced parasite loads in commercial egg-laying breeds. As natural products gain popularity over synthetic formulations, especially for managing parasitic infestations in ruminants like FSF, there is growing interest in understanding their effects on blood parameters and nematode prevalence. This study aimed to investigate the effects of varying concentrations of FSF on blood attributes and gastrointestinal parasite burdens in Dohne Merino wethers to enhance productivity. Based on existing literature, the hypothesis was formulated that FSF inclusion in the diets of Dohne Merino wethers would improve beneficial blood changes and reduce gastrointestinal parasite loads.

Materials and Methods

Ethical approval

The handling and the use of the animals were approved by the University of Fort Hare, Animal Ethics and Use Committee (Approval number: MPE041IKU01).

Study site description

The study was carried out at the small ruminant unit of the University of Fort Hare teaching and research farm located in Alice, Eastern Cape Province, South Africa. The farm

is situated at a longitude of 26°50'E and a latitude of 32°46'S, with an annual rainfall ranging between 480-490 mm. The temperature typically varies between 11.1°C and 24.6°C, averaging 17.8°C. The farm is situated at an elevation of 535 meters above sea level.

Animal, experimental design, and management

Twenty-four five-month-old Dohne-Merino wether in 20 ± 1. 5 kg on average were selected and purchased from a commercial farm at Mitford village in Tarkastad region, Eastern Cape province, South Africa. The wethers were randomly allotted into four treatments (n = 6). They were individually housed (1.5 m × 1.5 m) in a well-ventilated roofed animal building with a concrete floor and exposed to the same environmental conditions. The experiment lasted 105 days, excluding 14 days of the adaptation period. The wethers had access to sufficiently clean and freshwater ad libitum daily. Each wether was ear-tagged and labeled for identification on a diet basis.

Experimental Diets

The diets fed to the wethers consisted of a blend of concentrate and hay in a ratio of 40:60. The basal diet included maize (8%), sunflower oil cake (10%), molasses (5%), wheat offal (15%), limestone (1.5%), salt (0.3%), sheep mineral-vitamin premix (0.2%), 30% teff, and 30% Lucerne. All ingredients were purchased from Monti Feeds (Pty) Ltd, East London, South Africa, and were finely milled and thoroughly mixed. The diet was formulated to meet the nutritional requirements (energy and protein) of the sheep used, as per NRC (2007) guidelines. The experimental diets comprised four groups: basal diet (0% FSF), basal diet + 2% FSF, basal diet + 4% FSF, and basal diet + 6% FSF. Food-grade Fossil shell flour (FSF) was obtained from Eco-Earth (Pty) Ltd, Port Elizabeth, South Africa, and produced under license by the Department of Agriculture, Forestry, and Fisheries of South Africa. Wethers were fed twice daily at 8:00h and 15:00h, based on 4% of their body weight on a dry matter basis.

Proximate analysis of the experimental diets

The dry matter, crude protein, crude fiber, ether extract, and total ash content of the samples were analyzed in triplicate using the standard procedures outlined in AOAC (2012). The proximate composition of the experimental diet is summarized in Table 1.

Table 1: Ingredients and proximate analysis of experimental diet

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

Mineral analyses

The mineral composition of the FSF used in the diet is detailed in Table 2. To determine the mineral content of FSF, 5.0 g of the sample was weighed in triplicate and incinerated at 550°C in a muffle furnace for 5.5 hours. The resulting residues were cooled in a desiccator before being dissolved in 100 mL of deionized water. Standard solutions of suitable salts for each element were prepared. These standard mineral solutions were then analyzed using an atomic absorption spectrophotometer (Jenway, FPSP 210 model 6305, United Kingdom) to determine the concentrations of Mg, Zn, Fe, Cd, Ca, Al, Mn, and B in the unknown feed sample. Sodium (Na) and potassium (K) concentrations were determined using a flame photometer (Jenway Models PFP7 and PFP7/C, Cole-Parmer, United Kingdom).

Table 2: Mineral composition of Fossil shell flour (FSF).

Items	**Quantity**
DM %	93
Ca	0.40
% CaO (calculated from %Ca)	0.55
Mg	0.21
%MgO (calculated from %Mg)	0.34
K%	0.16
Cu (mg/kg)	30
Na (mg/kg)	923
Zn(mg/kg)	118
Fe(mg/kg)	7944
Mn(mg/kg)	69
P (as P2O5)	0.037
Sulfate Sulfur (S)%	0.062

Aluminium (Al) %	0.065
Vanadium (V) %	0.00438
Boron (B) %	0.0023

Blood sample collection and analyses

At 0800 hours before feeding, blood samples were collected from five wethers per treatment on days 0, 25, 50, 75, and 100. Blood was drawn from the jugular vein into three labeled sterile bijou bottles per wether. Approximately 10 mL of blood was collected, with 5 mL deposited into a bottle containing ethylenediaminetetraacetic acid (EDTA) solution for hematological analyses. For serum biochemical studies, 3 mL of blood was placed in a second sterile bottle without anticoagulant, and 2 mL of blood was collected into sodium oxalate fluoride bottles for glucose concentration determination. Each tube was labeled with the wether's identification number, placed in a cooler box with ice packs, and transported to the laboratory within two hours. Hematological analyses were conducted on the same day as blood sample collection. Total red blood cell (RBC) counts were determined using a microscopic method with a counting chamber after dilution with Eayemis solution. Total white blood cell (WBC) counts were assessed using the improved Neubauer hemocytometer chamber with 2% acetic acid as the diluent, following John's method (2012). Blood platelet counts were obtained by diluting a small blood smear with 1% ammonium oxalate and counting in a hemocytometer under light microscopy, as described by Douglas and Harold, (2012). Packed cell volume (PCV) was measured using the microhematocrit method, and hemoglobin (Hb) estimation was conducted using the alkali-hematin method, also according to John (2012). Mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), and mean corpuscular volume (MCV) were calculated from RBC, Hb, and hematocrit (HCT) values, following John's protocols (2012). Blood samples in anticoagulant-free bottles were allowed to clot at room temperature before centrifugation at 3500 rpm for 15 minutes at 10°C (Model 5403 centrifuge, Gatenbay Eppendorf GmbH, Engelsdorp, Germany). This process facilitated the separation of serum from the cellular components of blood. The serum samples were then transferred to 1.8 mL cryovials and frozen at -20°C until blood metabolites were analyzed. Serum samples were assessed for total protein (T.P.), albumin, and creatinine using methods described by Tietz, (1995). Serum urea concentration was determined following Tietz's method (1995). Globulin levels were calculated as the difference between total protein and albumin concentrations. Glucose analysis was conducted according to the procedure outlined by Gochman and Schmitz, (1972)

Determination of faecal egg counts

Faecal samples were collected from five wethers per treatment directly from the rectum using a latex glove lubricated with glycerine on the middle finger. One hundred grams of faeces per wether were collected on days 0, 25, 50, 75, and 100. Samples were promptly placed in labeled polyethylene bags, stored in a cooler box at 4°C, and transported to the laboratory on the same day. Sodium chloride served as the flotation medium, and a modified McMaster technique was employed to determine faecal egg counts. Each faecal sample (4 g) was thoroughly mixed with a saturated NaCl solution (56 mL). The number of nematode eggs per gram (g) of faeces was calculated by multiplying the total count observed in two squares of the McMaster slide by the dilution factor of 50 (Whitlock, 1948). This modified McMaster technique detects 50 or more eggs per gram of faeces and is widely recognized

for its speed, effectiveness in removing debris before counting, and overall accuracy (Levecke *et al.,* 2012). Identification of nematode egg types and protozoa utilized keys developed by Uhlinger (1991) and Foreyt, (2013), employing a sedimentation method described by Soulby, (1982).

Statistical analyses

Blood differentials, serum chemistry, and FEC were analyzed as repeated measures using the general linear model procedure in SAS (2011). The model included treatments (0%, 2%, 4%, and 6%), days of sampling (0, 25, 50, 75, and 100), and their interaction. FEC was transformed using log10 (FEC + 1) to normalize the data. Differences between means were assessed using Tukey's studentized range test when the F-test indicated significance at $P < 0.05$.

Results

Serum biochemistry

The glucose concentrations in wethers fed FSF-supplemented diets were significantly higher than those in wethers fed a 0% FSF diet ($P < 0.05$). Wethers fed a diet containing 2% FSF exhibited the highest glucose concentrations on days 75 and 100, which were significantly higher compared to wethers fed diets with 0%, 4%, and 6% FSF on those sampling days ($P < 0.05$). Glucose concentrations increased with continued feeding days. Total protein concentrations were lower in wethers fed a 0% FSF diet compared to those fed FSF-supplemented diets on all sampling days except day 50 ($P < 0.05$). There were no significant differences observed from day 50 to day 100, except among wethers fed a diet containing 6% FSF. For urea concentration, values in wethers fed diets containing 2%, 4%, and 6% FSF were lower than those in wethers fed a 0% FSF diet across all sampling days ($P < 0.05$). However, urea concentrations did not significantly differ between wethers fed 0% and 2% FSF diets but were significantly different from those on 4% and 6% FSF diets ($P < 0.05$). There was minimal variation observed among wethers on different mineral diets (Na and K), especially on day 100. Regarding ALT concentration, wethers fed diets containing 2%, 4%, and 6% FSF had significantly higher values ($P < 0.05$) than those fed a 0% FSF diet on days 50, 75, and 100 (Table 3). A negative correlation was found between serum minerals (Na+ = -0.184 and K+ = -0.236) and total protein (Table 4). Conversely, serum urea and total protein showed a positive correlation ($P = 0.365$). Similarly, bilirubin exhibited a positive correlation with total protein.

Table 3: Blood biochemistry of sheep fed varying amounts of FSF

Parameter	Day	Treatment	SEM	P-value					
		1	2	3	4		Trt	Day	Trt*Day
Na (mmol/l)	0	144.0^{bc}	146.33^{ab}	151.0^{a}	146.0^{ab}	0.579	0.048	0.000	0.065
	25		144.0^{a}	147.66^{a}					
	50	143.0^{a}	145.0^{a}	142.0^{a}	143.0^{a}				
	75	144.0^{a}	142.66^{a}	144.33^{a}					
	100	143.0^{a}	141.66^{a}	143.33^{a}	142.33^{a}				

K (mmol/l)	0 25 50 75 100	4.96^{bc} 5.10a 5.60^{ab} 5.00^{ab} 5.00^{ab}	5.00b 4.90^{a} 5.66^{a} 5.033^{ab} 5.033^{ab}	7.03a 4.56^{b} 5.0^{bc} 4.86^{ab} 4.86^{ab}	4.833^{bc} 5.2^{a} 4.96^{bc} 4.63^{c} 4.63^{c}	0.0141	0.125	0.011	0.001
Urea (mmol/L	0 25 50 75 100	6.66^{a} 6.06^{a} 6.40^{a} 6.10^{a} 6.10^{a}	6.60^{a} 6.05^{a} 6.3^{5a} 6.06^{a} 6.08^{a}	4.93^{bc} 6.08^{a} 5.73^{c} 5.79^{a} 5.83^{a}	5.23^{ab} 6.16^{a} 6.20^{b} 5.10^{b} 4.80^{b}	0.176	0.000	0.004	0.158
Triglycer (mmol/L)	0 25 50 75 100	0.11^{b} 0.20^{c} 0.20^{b} 0.17^{c} 0.15^{d}	0.10^{b} 0.19^{d} 0.22^{a} 0.24^{b} 0.26^{b}	0.18^{a} 0.38^{a} 0.19^{c} 0.41^{a} 0.43^{a}	0.18^{a} 0.33^{b} 0.12^{d} 0.15^{d} 0.18^{c}	0.0172	0.000	0.0001	0.0001
Cholest. (mmol/L)	0 25 50 75 100	1.45^{ab} 1.37^{a} 0.98^{a} 1.01^{a} 1.13^{a}	1.42^{ab} 1.27^{a} 1.06^{a} 1.09^{a} 1.10^{a}	2.14^{a} 1.34a 1.13^{a} 1.31^{a} 1.33^{a}	1.56^{ab} 1.24^{a} 1.21^{a} 1.27^{a} 1.26^{a}	0.0711	0.012	0.000	0.444
ALP (U/L)	0 25 50 75 100	52.66b 141.33b 163.0^{b} 129.66^{b} 125.66^{b}	58.33b 91.66c 126.0^{c} 146.0^{b} 145.0^{b}	145.66a 210.33a 211.33^{a} 223.33^{a} 220.3^{a}	54.33b 84.33c 73.66^{d} 72.66^{c} 70.66^{c}	11.5	0.000	0.001	0.684
Bilirubin (µmol/L)	0 25 50 75 100	6.66^{a} 7.66^{a} 6.0^{a} 5.66^{a} 5.66^{a}	7.0^{a} 6.0^{c} 5.12^{b} 4.0^{c} 4.0^{c}	5.0^{b} 6.66^{b} 4.42^{c} 3.33^{d} 3.33^{d}	6.66^{a} 4.66^{d} 3.66^{d} 5.0^{b} 4.66^{b}	0.298	0.001	0.000	0.000
Albumin (g/L)	0 25 50 75 100	12.66^{a} 13.0^{a} 12.66^{a} 13.0a 12.50^{a}	13.0^{a} 12.0^{b} 12.0^{b} 12.66^{a} 12.16^{a}	12.66^{a} 13.33^{a} 12.33^{a} 13.0a 12.85^{a}	12.33a 12.0^{b} 12.33^{a} 13.0^{a} 12.90^{a}	0.171	0.137	0.103	0.501
Glucose(mmol/L)	0 25 50 75 100	2.53^{b} 2.76^{a} 2.60^{a} 2.25^{c} 2.20^{c}	2.30^{bc} 2.65a 2.76^{a} 3.66^{a} 3.97^{a}	2.63^{b} 2.60a 2.53^{a} 2.96^{b} 3.02^{b}	4.06^{a} 2.50^{a} 2.52^{a} 2.73^{b} 2.76^{b}	0.151	0.224	0.210	0.008
Protein (g/L)	0 25 50 75 100	61.0^{b} 57.66^{ab} 59.0^{a} 57.66^{b} 60.66^{b}	54.33^{c} 61.0^{a} 53.66^{b} 58.33^{b} 63.33^{b}	54.0^{c} 57.33^{ab} 58.2^{a} 58.50^{b} 61.0^{b}	68.66^{a} 56.0^{bc} 62.0^{a} 65.33^{a} 68.33^{a}	1.98	0.001	0.738	0.094
creatinine (µmol/L)	0 25 50 75 100	105.33^{a} 79.33^{a} 61.33^{a} 61.0^{b} 60.80^{b}	91.66^{b} 71.66^{b} 54.66^{b} 69.33^{a} 69.93^{a}	78.33^{c} 60.66^{c} 51.33^{b} 53.33^{c} 52.12^{c}	64.33^{d} 55.0^{d} 48.33^{b} 63.33^{b} 65.10^{ab}	3.29	0.001	0.000	0.194

abc mean values with different superscripts across the row are significantly different (P< 0.05). T1 = 0 % FSF diet, T2 = 2 % FSF diet, T3 = 4 % FSF diet and T4= 6 % FSH diet.

Table 4: Correlation between the blood biochemical parameters

Parameters	Creatine	protein	Albumin	Total Bilirubin	ALT	ALP	Cholest.	Triglycer	Urea	k	Na
Protein	0,201										
Albumin	0,282*	0.084*									
Total Bilirubin	0,335**	0,169	0,106								
ALT	0,187	0,093	-0,012	-0.302*							
ALP	-0,169	-0.364***	0.387***	0,073	-0,152						
Cholest.	0.342**	0,065	0.263*	0,029	0.510***	-0,052					
Triglycer	-0.337**	-0.526***	0,171	-0,230	0,064	0.602***	0,011				
Urea	0.308*	0.342***	-0,131	0.274*	-0.396***	-0,219	-0.254*	0.394***			
K	0,014	-0,236	-0.365***	0,060	0,225	-0,001	0,242	-0,133	0,066		
Na	0,175	-0,184	0,233	-0,005	0.364***	0,078	0.684***	0,181	-0,093	0.393***	

The level of significance was set at $^{*}P < 0.05$; $^{**}P < 0.01$; $^{***}P < 0.001$

Haematological parameters

White blood cell (WBC) counts showed a tendency to decrease from day 30 to 100 in control and 2% FSF-fed lambs, while increasing during the same period in wethers supplemented with 4% and 6% FSF ($P < 0.05$). Significant statistical differences ($P=0.0003$) were observed among wethers fed diets containing 2%, 4%, and 6% FSF, as well as those on a 0% FSF diet (Table 4). Red blood cell (RBC) counts increased ($P < 0.03$), whereas mean corpuscular hemoglobin (MCH) decreased ($P < 0.0001$) with increasing feeding days in wethers fed 4% FSF compared to control and other FSF-supplemented wethers (Table 4). Significant variations were also noted in hematocrit, platelet count, red cell distribution width (RDW), mean corpuscular hemoglobin concentration (MCHC), and hemoglobin (Hgb) levels between wethers on a 0% FSF diet and those fed FSF ($P < 0.05$; Table 5)

Table 5: haematological parameters of sheep fed varying amounts of FSF

Parameter	Day	Treatment				SEM	P-value		
		1	2	3	4		Day	Treat	Day*
MCHC (((g/d(g/L)	0	48.96^{b}	41.80^{c}	66.80^{a}	37.06^{c}	3.479	0.031	0.0001	0.0001
	25	33.53^{b}	53.00^{a}	51.17^{a}	37.83^{b}				
	50	57.10^{a}	51.87^{a}	56.26^{a}	42.13^{b}				
	75	55.60^{a}	56.07^{a}	42.43^{b}	45.36^{b}				
	100	56.46^{a}	56.93^{a}	41.57^{b}	46.23^{b}				
RDW (fL)	0	34.17^{b}	36.63^{a}	32.97^{bc}	38.66^{a}	1.078	0.004	0.0004	0.0063
	25	33.07^{b}	34.93^{ab}	35.67^{a}	30.86^{c}				
	50	29.77^{c}	35.60^{a}	33.93^{b}	33.93^{b}				
	75	32.90^{c}	35.40^{ab}	37.20^{a}	35.53^{ab}				
	100	31.90^{c}	36.00^{ab}	37.80^{a}	36.53^{ab}				
MPV (fL. µm3.)	0	6.83^{a}	5.77^{c}	6.27^{b}	6.06^{bc}	0.249	0.116	0.0012	0.0001
	25	7.27^{a}	5.70^{c}	6.53^{b}	6.33^{b}				
	50	6.50^{b}	7.73^{a}	6.00^{c}	6.50^{b}				
	75	5.63^{c}	7.73^{a}	6.47^{b}	6.53^{b}				
	100	4.63^{c}	8.73^{a}	7.47a	7.53^{d}				

Platelet (×103/µl)	0 25 50 75 100	53.6^{bcd} 30.6^{cd} 25^{d} 27^{d} 23^{d}	55.5^{bc} 85.3^{b} 79.6^{bc} 74.3^{c} 71.3^{c}	59.3^{b} 61.6^{c} 93.6^{b} 103.6^{b} 108.6^{b}	95.6^{a} 108^{a} 146^{a} 183.6^{a} 198.6^{a}	8	0.000	0.0001	0.0001
WBC (cells/L	0 25 50 75 100	59.3^{a} 60.2^{a} 54.0^{a} 53.6^{a} 50.6^{a}	30.8^{b} 30.6^{b} 25.6^{b} 13.9^{cd} 15.9^{cd}	19.2^{bcd} 18.3^{bc} 18.8^{bcd} 22.2^{bc} 25.2^{bc}	25.2^{bc} 29.8^{b} 22^{bc} 34.4^{b} 38.4^{b}	6.7	0.278	0.0003	0.5400
Haemoglo. (mmol/L)	0 25 50 75 100	10.36^{ab} 9.90^{a} 8.43^{a} 8.60^{a} 8.80^{a}	10.56^{a} 8.43^{b} 7.93^{bc} 7.80^{a} 7.70^{b}	9.50^{b} 8.5^{b} 7.23^{bcd} 8.10^{a} 8.40^{ab}	10.63^{a} 8.33^{bc} 8.17^{b} 8.37^{a} 8.45^{ab}	0.502	0.001	0.045	0.842
Heamotocri. (L/L)	0 25 50 75 100	0.21^{bc} 0.25^{a} 0.15^{cd} 0.14^{c} 0.13^{dc}	0.24^{ab} 0.20^{ab} 0.18^{abc} 0.20^{ab} 0.22^{ab}	0.16^{d} 0.19^{bc} 0.20^{ab} 0.23^{a} 0.25^{a}	0.27^{a} 0.23^{ab} 0.22^{a} 0.20^{ab} 0.19^{bc}	0.017	0.002	0.003	0.0003
MCV (L/L)	0 25 50 75 100	43.57^{a} 35.77^{c} 45.43^{a} 45.33^{a} 45.13^{a}	41.57^{bc} 45.17^{a} 43.10^{b} 44.00^{a} 45.00^{a}	40.20^{c} 43.73^{a} 44.60^{ab} 41.60^{bc} 40.70^{c}	41.80^{b} 38.80^{b} 41.66^{c} 42.67^{b} 42.87^{b}	0.750	0.000	0.002	0.0001
MCH (g/L)	0 25 50 75 100	21.83^{b} 12.03^{c} 25.73^{a} 25.37^{a} 25.60^{a}	18.03^{c} 24.03^{a} 22.40^{b} 21.66^{b} 20.067^{b}	27.00^{a} 22.13^{a} 22.80^{ab} 17.17^{cd} 15.43^{c}	15.93^{d} 15.63^{b} 18.00^{c} 19.43^{bc} 20.37^{b}	1.469	0.000	0.0001	0.0001
RBC (10^9/L)	0 25 50 75 100	5.05^{ab} 6.72^{a} 4.41^{bc} 3.53^{bc} 3.5^{bc}	5.99^{a} 5.46^{bc} 4.19^{b} 4.37^{b} 4.37^{b}	3.8^{bc} 3.86^{bc} 6.02^{a} 6.1^{a} 6.13^{a}	6.6^{a} 5.6^{b} 6.06^{a} 5.44^{ab} 5.44^{ab}	0.594	0.399	0.030	0.000

^{abc} mean values with different superscripts across the row are significantly different ($P<0.05$). T1 = 0% FSF diet, T2 = 2% FSF diet, T3 = 4% FSF diet and T4= 6% FSH diet.

Faecal egg count

The inclusion of FSF in wethers' diets at rates of 4% and 6% effectively eradicated ($P < 0.05$) coccidia protozoa by day 50. In contrast, a 2% FSF inclusion reduced coccidia protozoa to lower numbers but did not completely eliminate the parasite (Fig. 1). At 2% FSF inclusion, wireworm levels dropped to zero by day 50, whereas no reduction was observed with 0% FSF supplementation during the same period (Fig. 2). The impact of FSF on conical and liver fluke worms is depicted in Figures 3 and 5. Adding 2% FSF eradicated conical and liver fluke worms by 100 days. However, at this inclusion level, long-necked worms were not eliminated, except with higher inclusion rates of 4% and 6% (Fig. 4).

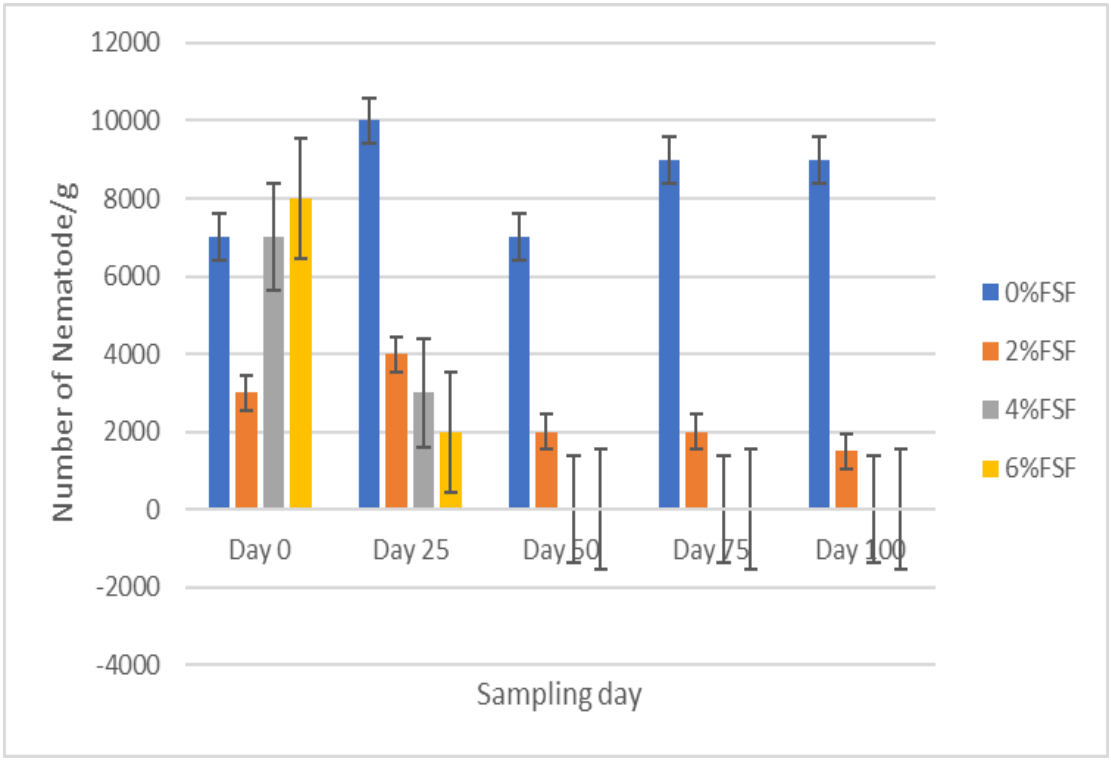

Figure 1: Mean faecal egg counts per gram of coccidia were measured in Dohne-Merino wethers fed different levels of FSF for 0, 25, 50, 75, and 100 days, presented as means ± standard errors

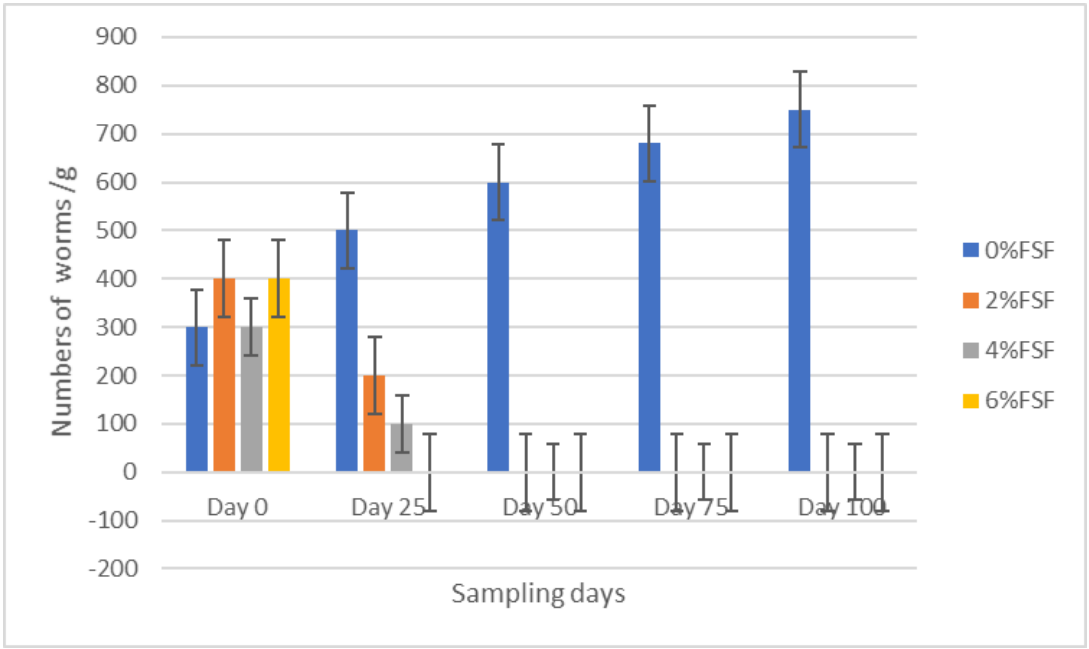

Figure 2: The mean ± standard error of fecal egg counts for wireworms in Dohne Merino wethers fed different levels of FSF over 0, 25, 50, 75, and 100 days is presented.

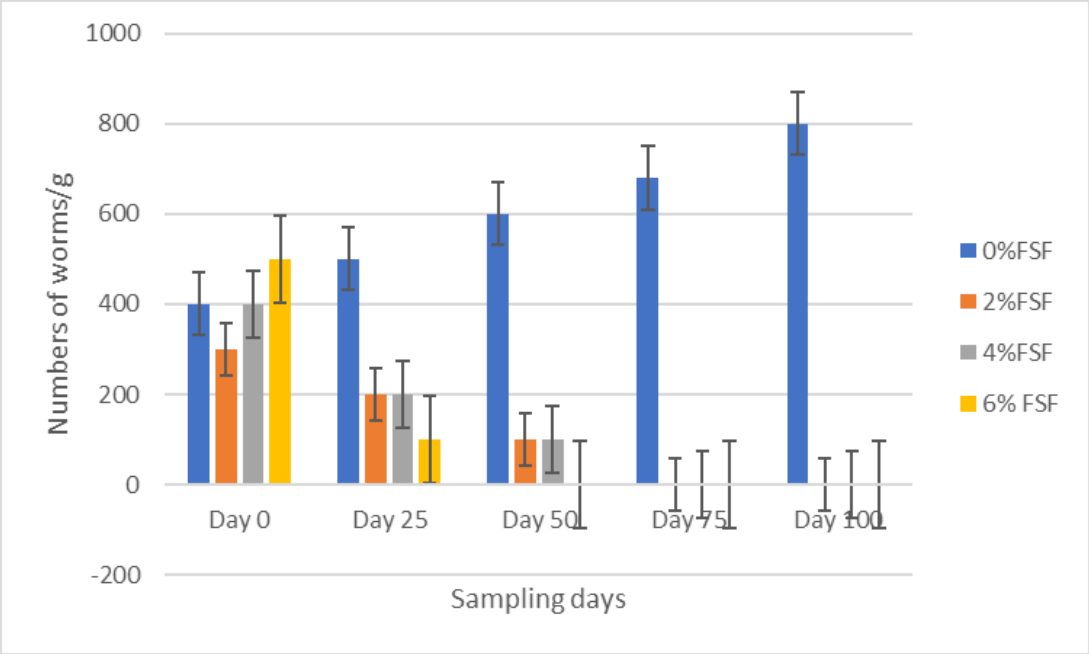

Figure 3: Fecal egg counts of conical fluke worms in Dohne Merino wethers fed various levels of FSF over 0, 25, 50, 75, and 100 days, presented as means ± standard errors.

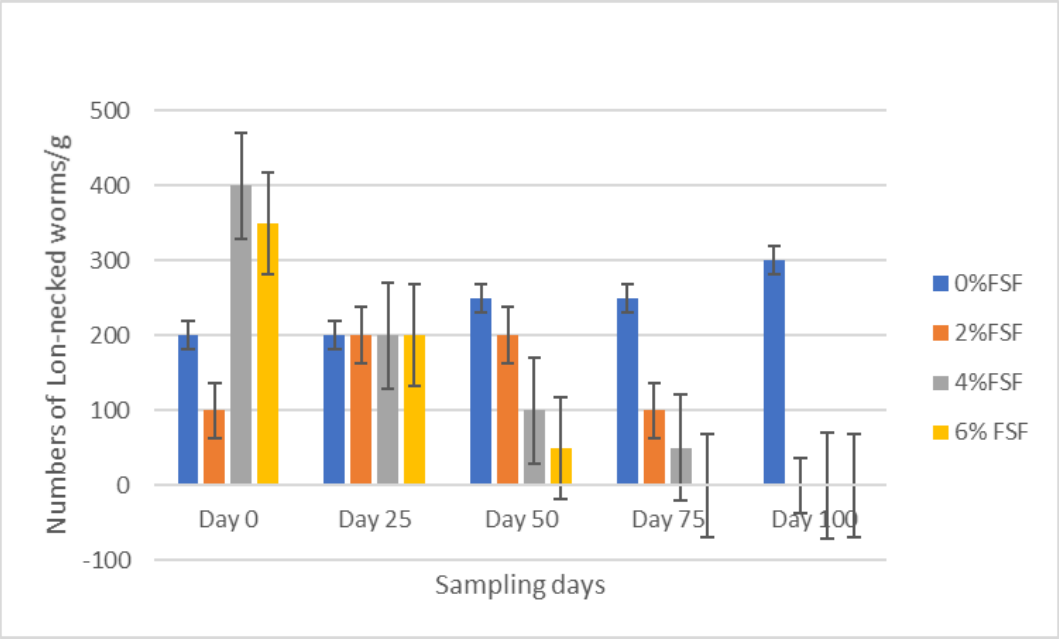

Figure 4: Fecal egg counts of long-necked worms in Dohne Merino wethers fed different levels of FSF over 0, 25, 50, 75, and 100 days, presented as means ± standard errors

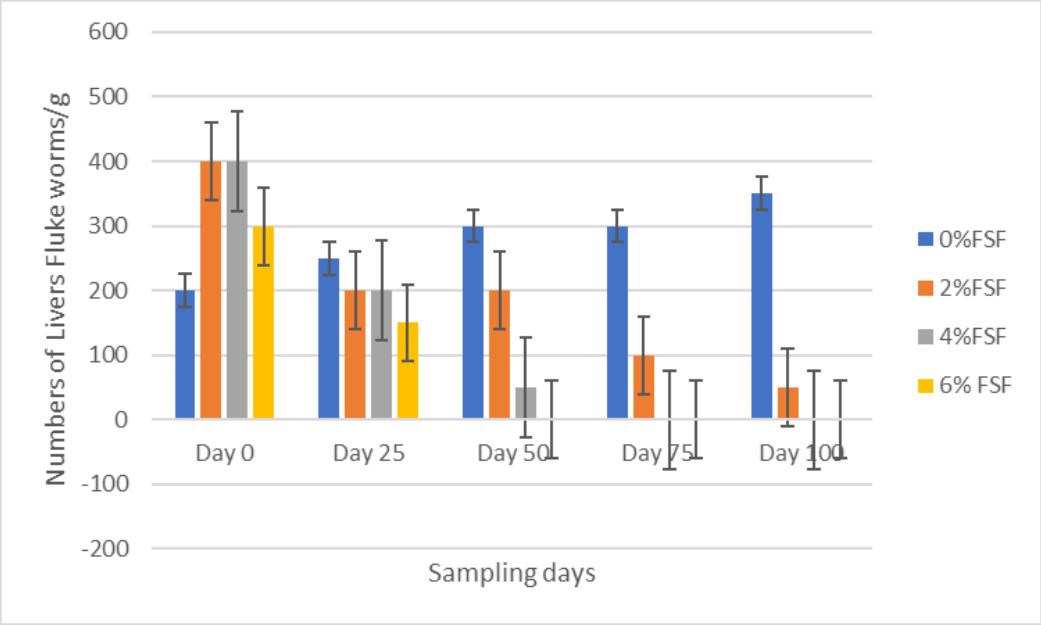

Figure 4: Fecal egg counts of liver fluke worms in Dohne Merino wethers fed different levels of FSF over 0, 25, 50, 75, and 100 days, presented as means ± standard errors.

Discussion

The white and red blood cell counts provide crucial insights into the overall health and wellbeing of sheep in production. In this study, an increase in WBC counts was observed in wethers fed diets with 4% and 6% FSF from day 50 onwards, whereas WBC counts in the 0% and 2% FSF groups significantly declined during the same period ($P<0.05$). These findings confirm previous experiments, which also demonstrated that adding 4% (T3) and 6% (T4) FSF to diets enhanced white blood cell counts in wethers after 50 days. However, previous studies noted that these effects were not statistically significant ($p > 0.05$). In alignment with Emeruwa (2016), the results of the present study show a comparable pattern of WBC control across different FSF treatment levels in sheep.

The increase in circulating WBC observed in wethers fed 4% and 6% FSF from day 25 to 100 suggests an active immune response, potentially due to infection or inflammation caused by tissue damage. WBCs are known to combat foreign bodies in the system (Babeker and Bdalbagi et al., 2015). This finding aligns with Weaver et al. (2013), who reported that FSF in the diets of young gilts positively affects the immune system against Aflatoxin.

Key red blood cell (RBC) parameters such as mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), red blood cell distribution width (RDW), and mean corpuscular hemoglobin concentration (MCHC) are critical for diagnosing anemia. MCHC specifically measures the hemoglobin concentration within RBCs. In this study, the values for RBC, MCV, MCH, hemoglobin (Hgb), and hematocrit indicate that FSF inclusion in Dohne Merino wethers' diets improves hematological parameters. This supports the notion that FSF can enhance the blood's oxygen-carrying capacity.

The increase in RBC in FSF-fed wethers may be attributed to the iron content in FSF, as iron and other minerals in FSF promote RBC production. The addition of FSF likely provides essential minerals for enzymes such as sulfite oxidase, xanthine dehydrogenase, and

aldehyde oxidase, which are crucial for RBC improvement. These findings are consistent with Weaver et al. (2013).

Blood urea levels reflect the state of protein metabolism and dietary amino acid balance in animals (Tao et al., 2018). As Jiwuba et al. (2017) noted, high blood urea levels are linked to poor protein quality in animal feed. The consistent blood urea values between the 0% FSF diet and FSF-supplemented diets throughout this study indicate that FSF addition did not negatively impact the protein quality in the feed of Dohne Merino wethers. However, wethers on the 4% FSF diet tended to increase blood urea from day 50 to 100, though not statistically different from those on the 0% and 2% FSF diets, but different from the 6% FSF diet. This suggests that including more than 4% FSF in the diet could lead to higher blood urea levels, possibly due to increased protein concentration in the rumen, leading to excess protein breakdown into ammonia, which is then converted to urea in the blood. This observation aligns with Chaves et al. (2011) and Bahrami-Yekdangi et al. (2015), who reported a negative correlation between rumen ammonia nitrogen and blood urea.

The concentrations of total protein, albumin, bilirubin, Na, K, glucose, cholesterol, and liver enzymes were within the normal range for wethers, indicating good health and no adverse effects from FSF supplementation (Ikusika et al., 2019a). The observed improvement in total protein levels in FSF-fed wethers compared to the control suggests increased nitrogen availability at the tissue level due to FSF. Zinc in FSF is known to stimulate appetite, leading to increased protein intake through higher feed consumption. However, no statistical difference was noted between FSF-supplemented and non-supplemented diets, consistent with Emeruwa (2016), who found no significant differences in West African dwarf sheep fed varying FSF amounts.

Serum creatinine levels were significantly lower in wethers on the 4% FSF diet compared to those on 0%, 2%, and 6% FSF diets on day 100, indicating a positive health impact at 4% inclusion. Creatinine, a waste product of protein metabolism, was reduced, suggesting a lower catabolism rate in wethers on the 4% FSF diet (Paswan et al., 2016).Serum glucose levels decreased on all sampling days in wethers on the 0% FSF diet, initially dropped in those on the 4% and 6% FSF diets but increased in wethers on the 2% FSF diet. Although there was no significant variation among the different FSF inclusion levels except at 75 and 100 days, wethers on the 0% FSF diet had significantly lower serum glucose levels ($P<0.0001$) from day 75 onwards compared to the FSF-supplemented groups. This suggests that continuous FSF supplementation for over 75 days can improve serum glucose levels, possibly due to Na, Cl, and K elements in FSF stimulating appetite and increasing feed intake. However, this contrasts with Emeruwa (2016), who reported higher serum glucose in the control group compared to the FSF-supplemented group, likely due to differences in breed, climate, and diet.

Gastrointestinal parasites cause significant economic losses in small ruminant production in semi-arid and arid regions. Haemonchus contortus is known to cause anemia, hematological and biochemical disturbances, low immunity, poor wool quality, and sometimes mortality in sheep (Bennett et al., 2011). This study found that FSF effectively reduces various gastrointestinal parasites, including wireworms, long-necked worms, liver flukes, conical flukes, and coccidia. Wethers fed 4% and 6% FSF showed a significant reduction in worm counts by day 50, while those on 2% FSF showed a decrease by day 75. This confirms reports by McLean et al. (2005) and Bennett et al. (2011), who observed that FSF inclusion improves sheep performance and reduces gastrointestinal parasites, requiring

at least 75 days for significant reduction. Similarly, Bernard et al. (2009) observed significant fecal egg count reductions in Spanish/Boer cross goats fed FSF at varying inclusion levels of 1.77g, 3.54g, and 5.31g per kg. The effectiveness of FSF against parasites is likely due to its surface characteristics, such as abrasiveness, acidity, solubility, hydrophobicity, and absorptive properties, which dehydrate and cut the worms. Akin et al. (2000) and Yuan et al. (2010) suggested that both the physical and chemical properties of diatomaceous earth (FSF) contribute to its effectiveness against gastrointestinal parasites, supporting the fecal egg count reduction observed in this study.

Conclusion

The results indicate that supplementing Dohne Merino wethers' diets with FSF at a 4% inclusion rate has beneficial effects on serum urea, total protein, and glucose levels without altering other serum parameters. Additionally, the inclusion of FSF at this rate improves RBC, WBC, platelet counts, hemoglobin, RDW, and hematocrit, suggesting enhanced immunity and overall health. The significant reduction in fecal egg counts among FSF-fed wethers suggests FSF's potential as an effective anthelmintic for sheep in semi-arid regions. However, further research is needed to explore the impact of FSF inclusion on wool parameters, body condition scores, and feed preferences in Dohne Merino wethers.

Funding

South African Medical Research Council.

Data availability statement

Data will be available on request

Acknowledgment

The author wishes to acknowledge the Department of Livestock and Pasture Science, GMRDC, University of Fort, and the South African Medical Research (SAMRC), for providing the platform and enabling environment for this research

CONFLICT OF INTEREST

The author reports no conflict of interest.

References

Akin S, Schembre, JM, Bhat SK, Kovscek AR. (2000) Spontaneous imbibition characteristics of diatomite. Journal of Petroleum Science and Engineering 25,149 165

Anassori E, Dalir-Naghadeh B, Pirmohammadi R, Hadian M. (2015). Changes in blood profile in sheep receiving raw garlic, garlic oil, or monensin. Journal of Animal Physiology and Animal Nutrition 99,114–122.

Association of Official Agricultural Chemists (AOAC). (2012) edition

Bahrami-Yekdangi H, Khorvash M, Ghorbani GR, Alikhani M, Jahanian R, Kamalian, E.(2015) Effects of decreasing metabolizable protein and rumen-undegradable protein on milk production, composition, and blood metabolites of Holstein dairy cows in early lactation. *Journal of Dairy Science* 97, 3707-3714.

Bennett DC, Yee R, Rhee YJ, Cheng KM (2011). Effect of diatomaceous earth on parasite load, egg production, and egg quality of free-range organic laying hens. Poultry Science 90,1416–1426.

Bernard G, Worku M, Ahmed M.(2009). The Effects of Diatomaceous Earth on Parasite Infected Goats. Bulletin of the Georgian National Academy of Sciences 3,129–135.

Catorci A, Gatti R, Cesaretti S (2012). Effect of sheep and horse grazing on species and functional composition of sub-Mediterranean grasslands. Applied Vegetation Science 15,459–469.

Chaves A, Stanford K, Dugan M, Gibson L, McAllister T, Vanherk F, Benchaar C.(2011). Effects of cinnamaldehyde, garlic and juniper berry essential oils on rumen fermentation, blood metabolites, growth performance, and carcass characteristics of growing lambs. Livestock Science 117,215–224

Douglas JW, Harold T (2012) The Complete Blood Count, Bone Marrow Examination, and Blood Banking: General Comments and Selected Techniques.In: Michael D. Willard HT, editor. Small Animal Clinical Diagnosis by Laboratory Methods. Fifth. Saunders: Elsevier Science and Technology. p. 12–37.

Emeruwa CH.(2016). Growth Performance of West African Dwarf Sheep Fed Diets Supplemented with Fossil shell flour. PhD Thesis submitted to The Department of Animal Science, University of Ibadan, Nigeria.

Etim NN, Williams ME, Akpabio U, Offiong EA. (2014). Haematological Parameters and Factors Affecting Their Values. Agricultural Science2,37–47.

Foreyt WJ. (2013). Veterinary parasitology reference manual. 5th Editio. Iowa: Blackwell publishing professional, United States.

Gochman N, Schmitz JM (1972). Application of a new peroxide indicator reaction to the specific, automated determination of glucose with glucose oxidase. Clinical Chemistry 18,943–950.

Gwaze FR, Chimonyo M, Dzama K (2012). Effect of season and age on blood minerals, liver enzyme levels, and faecal egg counts in Nguni goats of South Africa. Czech Journal of Animal Science 57,443–453.

Ikusika OO, Mpendulo CT, Zindove TJ, Okoh, AI. (2019a). Fossil Shell Flour in Livestock Production: A Review. Animals 9,1–20.

Ikusika OO, Mpendulo CT, Zindove TJ, Okoh AI. (2019b). Effect of Varying Inclusion Levels of Fossil Shell Flour on Growth Performance, Water Intake, Digestibility, and N Retention in Dohne-Merino Wethers. Animals 9,565

Jiwuba PC, Ahamefule FO, Ogbuewu IP, Ikwunze K(2017). Blood chemistry and haematology of West African Dwarf goats fed Moringa oleifera leaf meal (MOLM) in their diet. Comp Clin Pathol 26:621–624.

John WH (2012) Haematology Procedures. In: Veterinary Hematology. 11th–32nd ed. Florida: Elsevier Science and Technology p. 11–32.

Levecke B, Rinaldi L, Charlier J, Maurelli MP, Bosco A, Vercruysse J, Cringoli G (2012). The bias, accuracy and precision of faecal egg count reduction test results in cattle using McMaster, Cornell-Wisconsin and FLOTAC egg counting methods. Veterinary Parasitology 188,194– 197.

Malekkhahi M, Tahmasbi AA, Naserian AA, Mesgaran MD, Kleen JL, Parand AA.(2015). Effects of essential oils, yeast culture and malate on rumen fermentation, blood metabolites, growth performance and nutrient digestibility of Baluchi lambs fed high-concentrate diets. Animal physiology and Animal Nutrition 99,221–229

McLean B, Frost D, Evans E, Clarke R, Griffiths B (2005). The inclusion of diatomaceous earth in the diets of grazing ruminants and its effect on gastrointestinal parasite burdens. In: International Scientific Conference on Organic Agriculture, Adelaide, Australia, International Scientific Conference of Organic Agriculture Research, Bonn, Germany.

National Research Council (NRC). (2007). Nutrient requirements of small ruminants: sheep, goats, cervids, and new world camelids. Washington, D.C.USA.

Odoi, A., Gathuma, J.M., Gachuiri, C.K.,& Omore, A.(2007). Risk factors of gastrointestinal nematode parasite infections in small ruminants kept in smallholder mixed farms in Kenya. BMC Veterinary Research 3,1–11.

Paswan JK, Kumar K, Kumar S, Kumar A (2016). Effect of feeding Acacia nilotica pod meal on hematobiochemical profile and faecal egg count in goats. Veterinary World 9,1400– 1406

Ruiz-Garcia L, Lunadei L.(2011). The role of RFID in agriculture: Applications, limitations and challenges. Computers and Electronics in Agriculture 79,42–50

Schröder UJ, Staufenbiel R (2010). Invited Review: Methods to Determine Body Fat Reserves in the Dairy Cow with Special Regard to Ultrasonographic Measurement of Backfat Thickness. Journal of Dairy Science 89,1–14

Soulsby EJ (1982). Helminths, Arthropods and Protozoa of Domesticated Animals. Seventh Edition. Pp 291. Published by Bailliere Tindall and Cassel Ltd, London, UK.

Tao H, Guo F, Tu Y, Si B, Xing Y, Huang D, Diao Q (2018). Effect of weaning age on growth performance, feed efficiency, nutrient digestibility and blood-biochemical

parameters in Droughtmaster crossbred beef calves. Asian-Australas J Anim Sci 31:864–872.

Tietz NW (1995) Fundamentals of Clinical Chemistry. 3rd Ed. W.B. Saunders, Philadelphia, USA.

Uhlinger CA (1991). Equine, small strongyles: epidemiology, pathology, and control. Compend Contin Educ Pract Vet 13,863–869

Wang SP, Wang WJ.(2016). Effects of dietary supplementation of Chinese herb medicine mixture on rumen fermentation, nutrient digestion and blood profile in goats. South African Journal of Animal Science 46 (3),247-260.

Weaver AC, Todd SM, Hansen JA, Kim YB, De Souza ALP, Middleton TF, Kim SW.(2013). The use of feed additives to reduce the effects of Aflatoxin and deoxynivalenol on pig growth, organ health, and immune status during chronic exposure. Toxins 5:1261,– 1281.

Whitlock VY(1948). Some modifications of the McMaster helminth egg-counting technique and apparatus. Journal of the Council for Scientific and Industrial Research Australia 21,177–180.

Yuan D, Liu D, Fan M, Yang D, Zhu R, Ge F, Zhu X, He H.(2010). Removal of hexavalent chromium [Cr(VI)] from aqueous solutions by the diatomite-supported/unsupported magnetite nanoparticles. Journal of Hazardous Materials 173,614–621