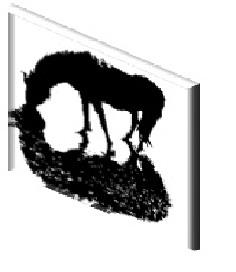
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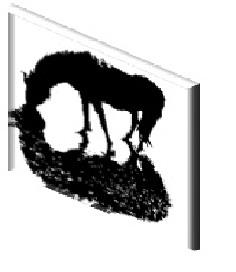
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**Effect of Fossil Shell Flour Supplementation on Hematobiochemical Profiles and Parasitic Loads in Dohne Merino Wethers**

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**ABSTRACT**

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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

**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  25  50  75  100 | 144.0bc 145.66a  143.0a  144.0a  143.0a | 146.33ab  144.0a  145.0a  142.66a  141.66a | 151.0a  147.66a  142.0a  144.33a  143.33a | 146.0ab 144.66a  143.0a 143.33a  142.33a | 0.579 | 0.048 | 0.000 | 0.065 |
| K (mmol/l) | 0  25  50  75  100 | 4.96bc  5.10a  5.60ab  5.00ab  5.00ab | 5.00b  4.90a  5.66a  5.033ab  5.033ab | 7.03a  4.56b  5.0bc  4.86ab  4.86ab | 4.833bc  5.2a  4.96bc  4.63c  4.63c | 0.0141 | 0.125 | 0.011 | 0.001 |
| Urea (mmol/L | 0  25  50  75  100 | 6.66a  6.06a  6.40a  6.10a  6.10a | 6.60a  6.05a  6.35a  6.06a  6.08a | 4.93bc  6.08a  5.73c  5.79a  5.83a | 5.23ab  6.16a  6.20b  5.10b  4.80b | 0.176 | 0.000 | 0.004 | 0.158 |
| Triglycer (mmol/L) | 0  25  50  75  100 | 0.11b  0.20c  0.20b  0.17c  0.15d | 0.10b  0.19d  0.22a  0.24b  0.26b | 0.18a  0.38a  0.19c  0.41a  0.43a | 0.18a  0.33b  0.12d  0.15d  0.18c | 0.0172 | 0.000 | 0.0001 | 0.0001 |
| Cholest. (mmol/L) | 0  25  50  75  100 | 1.45ab  1.37a  0.98a  1.01a  1.13a | 1.42ab  1.27a  1.06a  1.09a  1.10a | 2.14a  1.34a  1.13a  1.31a  1.33a | 1.56ab  1.24a  1.21a  1.27a  1.26a | 0.0711 | 0.012 | 0.000 | 0.444 |
| ALP (U/L) | 0  25  50  75  100 | 52.66b  141.33b  163.0b  129.66b  125.66b | 58.33b  91.66c  126.0c  146.0b  145.0b | 145.66a  210.33a  211.33a  223.33a  220.3a | 54.33b  84.33c  73.66d  72.66c  70.66c | 11.5 | 0.000 | 0.001 | 0.684 |
| Bilirubin (μmol/L) | 0  25  50  75  100 | 6.66a  7.66a  6.0a  5.66a  5.66a | 7.0a  6.0c  5.12b  4.0c  4.0c | 5.0b  6.66b  4.42c  3.33d  3.33d | 6.66a  4.66d  3.66d  5.0b  4.66b | 0.298 | 0.001 | 0.000 | 0.000 |
| Albumin (g/L) | 0  25  50  75  100 | 12.66a  13.0a  12.66a  13.0a  12.50a | 13.0a  12.0b  12.0b  12.66a  12.16a | 12.66a  13.33a  12.33a  13.0a  12.85a | 12.33a  12.0b  12.33a  13.0a  12.90a | 0.171 | 0.137 | 0.103 | 0.501 |
| Glucose(mmol/L) | 0  25  50  75  100 | 2.53b  2.76a  2.60a  2.25c  2.20c | 2.30bc  2.65a  2.76a  3.66a  3.97a | 2.63b  2.60a  2.53a  2.96b  3.02b | 4.06a  2.50a  2.52a  2.73b  2.76b | 0.151 | 0.224 | 0.210 | 0.008 |
| Protein (g/L) | 0  25  50  75  100 | 61.0b  57.66ab  59.0a  57.66b  60.66b | 54.33c  61.0a  53.66b  58.33b  63.33b | 54.0c  57.33ab  58.2a  58.50b  61.0b | 68.66a  56.0bc  62.0a  65.33a  68.33a | 1.98 | 0.001 | 0.738 | 0.094 |
| creatinine (μmol/L) | 0  25  50  75  100 | 105.33a  79.33a  61.33a  61.0b  60.80b | 91.66b  71.66b  54.66b  69.33a  69.93a | 78.33c  60.66c  51.33b  53.33c  52.12c | 64.33d  55.0d  48.33b  63.33b  65.10ab | 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\* Treat** |
| MCHC (((g/d(g/L) | 0  25  50  75  100 | 48.96b  33.53b  57.10a  55.60a  56.46a | 41.80c  53.00a  51.87a  56.07a  56.93a | 66.80a  51.17a  56.26a  42.43b  41.57b | 37.06c  37.83b  42.13b  45.36b  46.23b | 3.479 | 0.031 | 0.0001 | 0.0001 |
| RDW (fL) | 0  25  50  75  100 | 34.17b  33.07b  29.77c  32.90c  31.90c | 36.63a  34.93ab  35.60a  35.40ab  36.00ab | 32.97bc  35.67a  33.93b  37.20a  37.80a | 38.66a  30.86c  33.93b  35.53ab  36.53ab | 1.078 | 0.004 | 0.0004 | 0.0063 |
| MPV (fL. µm3. ) | 0  25  50  75  100 | 6.83a  7.27a  6.50b  5.63c  4.63c | 5.77c  5.70c  7.73a  7.73a  8.73a | 6.27b  6.53b  6.00c  6.47b  7.47a | 6.06bc  6.33b  6.50b  6.53b  7.53d | 0.249 | 0.116 | 0.0012 | 0.0001 |
| Platelet (×103/μl) | 0  25  50  75  100 | 53.6bcd  30.6cd  25d  27d  23d | 55.5bc  85.3b  79.6bc  74.3c  71.3c | 59.3b  61.6c  93.6b  103.6b  108.6b | 95.6a  108a  146a  183.6a  198.6a | 8 | 0.000 | 0.0001 | 0.0001 |
| WBC (cells/L | 0  25  50  75  100 | 59.3a  60.2a  54.0a  53.6a  50.6a | 30.8b  30.6b  25.6b  13.9cd  15.9cd | 19.2bcd  18.3bc  18.8bcd  22.2bc  25.2bc | 25.2bc  29.8b  22bc  34.4b  38.4b | 6.7 | 0.278 | 0.0003 | 0.5400 |
| Haemoglo.  (mmol/L) | 0  25  50  75  100 | 10.36ab  9.90a  8.43a  8.60a  8.80a | 10.56a  8.43b  7.93bc  7.80a  7.70b | 9.50b  8.5b  7.23bcd  8.10a  8.40ab | 10.63a  8.33bc  8.17b  8.37a  8.45ab | 0.502 | 0.001 | 0.045 | 0.842 |
| Heamotocri.  (L/L) | 0  25  50  75  100 | 0.21bc  0.25a  0.15cd  0.14c  0.13dc | 0.24ab  0.20ab  0.18abc  0.20ab  0.22ab | 0.16d  0.19bc  0.20ab  0.23a  0.25a | 0.27a  0.23ab  0.22a  0.20ab  0.19bc | 0.017 | 0.002 | 0.003 | 0.0003 |
| MCV (L/L) | 0  25  50  75  100 | 43.57a  35.77c  45.43a  45.33a  45.13a | 41.57bc  45.17a  43.10b  44.00a  45.00a | 40.20c  43.73a  44.60ab  41.60bc  40.70c | 41.80b  38.80b  41.66c  42.67b  42.87b | 0.750 | 0.000 | 0.002 | 0.0001 |
| MCH (g/L) | 0  25  50  75  100 | 21.83b  12.03c  25.73a  25.37a  25.60a | 18.03c  24.03a  22.40b  21.66b  20.067b | 27.00a  22.13a  22.80ab  17.17cd  15.43c | 15.93d  15.63b  18.00c  19.43bc  20.37b | 1.469 | 0.000 | 0.0001 | 0.0001 |
| RBC (109/L) | 0  25  50  75  100 | 5.05ab  6.72a  4.41bc  3.53bc  3.5bc | 5.99a  5.46bc  4.19b  4.37b  4.37b | 3.8bc  3.86bc  6.02a  6.1a  6.13a | 6.6a  5.6b  6.06a  5.44ab  5.44ab | 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).

Chart, bar chart

Description automatically generated

**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

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**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.

Chart, bar chart

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**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.

Chart, bar chart

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**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

Chart, bar chart

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**Figure 5**: 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.

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**Data availability statement**

Data will be available on request

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**CONFLICT OF INTEREST**

The author reports no conflict of interest.

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