Effect of increasing fossil shell flour levels on digestive and metabolic utilization, health, body weight change and wool production, and quality in Dohne-Merino wethers.

BY

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February 2020

DECLARATION

I, the undersigned, declare that this thesis entitled "Effect of increasing levels of fossil shell flour on digestive and metabolic utilization, health, body weight change and wool production and quality in Dohne-Merino wethers" submitted to the University of Fort Hare for the degree of Doctor of Philosophy in Animal Science in the Faculty of Science and Agriculture is my original work with exemption to the citations and that this work has not been submitted to any other university in partial or entirely for the award of any degree.

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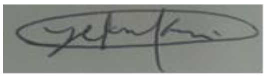
Signature 

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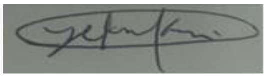
Signature…  ………………………… Date……………… February 05, 2020…………….

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This thesis entitled "Effect of increasing levels of fossil shell flour on digestive and metabolic utilization, health, body weight change and wool production and quality in Dohne-Merino wethers" meets the requirements governing the award of the degree of Doctor of Philosophy by the University of Fort Hare. This is approved for its contribution to scientific knowledge and scholarly presentation.

--- -----------------10 February 2020----------

Dr. C.T Mpendulo Date

Major Supervisor

DEDICATION

This thesis is dedicated to God of **The Redeemed Christian Church of God**, who gave me the grace to complete this doctoral research. To Him alone be all praises, honour, and adoration forever.

LIST OF ACRONYMS

FSF	Fossil shell flour
DMI	Dry matter intake
DM	Dohne-Merino
BCS	Body condition score
GLM	Generalized linear model
ANOVA	Analysis of Variance
SAS	Statistical Analysis Systems
S.E.	Standard Error
ADG	Average daily gain
CH_4	Methane
CO_2	Carbon Dioxide
EDTA	Ethylene Diamine Tetra Acetic acid
FAO	Food and Agricultural organization
GC	Gas Chromatography
GHG	Greenhouse Gas
LMD	Laser Methane Detector.
mg/kg	Milligrams per Kilogram
NRC	National Research Council
OM	Organic Matter
EE	Ether extract

CP	Crude Protein
CF	Crude Fibre
NDF	Neutal Detergent Fibre
ADF	Acid Detergent Fibre
ADFL	Acid Detergent Fibre Lignin
SAMRC	South African Medical Research Council
SPSS	Statistical Packages for Social Sciences
USA	United States of America
USEPA	United States Environmental Protection Agency

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Abstract

The study's broad objective was to assess growth performance, blood and parasitic profiles, wool parameters, methane emission, and nutritional status of Dohne-Merino wethers fed diets supplemented with varying levels of fossil shell flour. Twenty-four Dohne-Merino wethers, averagely weighing 20.0±1.50 kg, were divided into four groups and used in this study. The effects of varying inclusion levels of fossil shell flour (FSF) (0, 2, 4, and 6 %) on feed intake, water intake, nutrient digestibility, N-retention, hematobiochemical and parasitic profiles, body condition scores, feed preference, wool parameters and methane output were determined. The influence of FSF's varying inclusion levels on fermentation parameters, *in vitro* true digestibility, and relative feed values were also determined.

Wethers fed with 4% FSF inclusion level diet showed the highest ($P<0.05$) values for dry matter intake, average daily weight gain, N retention, (Nitrogen retention) and apparent digestibility of crude protein (CP), Ether Extract (EE) and Ash 6 % ($P < 0.05$). The urinary N and fecal N were lowest in wethers fed 4% FSF inclusion level and highest in those fed on diets without FSF ($P < 0.05$). Water intake was highest in wethers fed 0% FSF diet, followed by those fed on 4% and lowest in 6% FSF ($P< 0.05$). There was a significant increase in white blood cell counts in wethers fed on a diet with 4% and 6% FSF inclusion levels from day 10 to 100 compared to wethers fed on a diet with 0% and 2% FSF ($P <0.05$). Red blood cell counts also increased significantly in wethers fed on a diet containing 4% FSF, but a slight increase in wethers with 6 % FSF diet, throughout the experimental period ($P < 0.05$). Blood urea was highest in wethers fed 0% FSF inclusion level and lowest in 6% at day 30 to day 100. Wethers on 4% FSF diet showed an increase in blood urea from day 30 to day 100 ($P < 0.05$) while wethers on 0% and 2% remained unchanged ($P<0.05$) during this period and not different ($P > 0.05$) from wethers on 4% FSF as well. The total protein concentration, albumin, total bilirubin, Na, K, glucose, cholesterol, and liver enzymes were normal for wethers. However, serum creatinine level was lower in wethers fed on 4% FSF than those on 0% FSF ($P < 0.01$). Haemonchus and Coccidian fecal egg counts were low in wethers fed diets with 2%, 4%, and 6 % FSF ($P < 0.01$) compared

with wethers fed with 0% FSF inclusion level during the same period. The body condition score of wethers fed on diets with 2%, 4%, and 6% FSF inclusion levels were higher than those on 0% ($P < 0.05$). Wool yield, staple length, the coefficient variation of the fibre diameter, and fibre of wethers fed on diets with FSF were higher than those without FSF ($P < 0.05$). The fibre diameter of the wethers fed on FSF supplemented diets was the same as those on 0 % FSF ($P > 0.05$). The wethers spent more time on FSF supplemented diets and consumed more feed compared to that without FSF supplemented ($P < 0.05$). The levels of FSF inclusion in the diet affected the enteric methane output (ppm-m), where 4 % FSF had the highest enteric methane output while wethers on 2 % FSF had the lowest methane output ($P < 0.05$). Resting wethers produced more methane (g/day) than those feeding or standing ($P < 0.05$). Increasing levels of FSF did not affect ruminal temperature and pH. Ammonia-N increased with increasing FSF except in wethers fed on a diet with 4% FSF ($P < 0.01$). The total molar concentrations of the wethers' volatile fatty acids decreased with increasing FSF levels ($P > 0.05$). The acetic propionic ratio of the wethers also decreased except at a 4% inclusion level. The *in vitro* true digestibility dry matter (IVTDDM), *in vitro* true digestibility neutral detergent fibre (IVTDNDF), and *in vitro* true digestibility acid detergent fibre (IVTDADF) of the wethers decreased up to 4% FSF inclusion but tended to increase at 6% inclusion. This study's result as one of the pioneer studies in Dohne-Marino wethers showed that FSF treatment has the potential to improve the nutritional status of the animal and the animal performance and wool quality. Health-wise, FSF decrease nematode population and boost animal immunity as seen in RBC and WBC counts. It also can play a major role in protecting the environment, as seen in its reduction in feacal and urinary nitrogen, which is heavily involved in environmental pollution. Result also confirmed that the best period to target for methane mitigation in ruminants is the resting period. In conclusion, the inclusion of FSF in the diet of Dohne-Merino wethers has the potential to improve the overall performance, with a 4% FSF inclusion level having optimal productivity. However, future research is required to investigate FSF's effect on meat quality, rumen microbial community, *in-vivo* digestibility, and milk production.

Keywords: Fossil shell flour, varying levels, productivity, nutritional status, Dohne-Merino wethers.

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Chapter 1
General Introduction

1.1 Background

Sheep (*Ovis aries*) is a versatile and adaptable species in man's company since their domestication in the pre-agricultural age (Meadows, 2014). Today sheep farming is practiced in almost all the inhabited continents of the world, being easily adaptable and instrument to several civilizations (Colledge et al.,2005; Skapetas and Kalaitzidou, 2017). According to FAO (2017), over 1.2 billion sheep in the world provide employment and food to millions of people both in the developed and developing nations of the world. In sub-Sahara Africa, sheep are found in all the 41 countries that make up this region (Chessa et al.,2009; Lv et al.,2015). According to Locke and O'Connor (2017), there are over 351 million sheep in this region where they are exploited for various uses, including food, religious ceremonies, source of income, means of saving and insurance, and as a matter of prestige. The sub-Sahara is characterized by prolonged drought due to low rainfall (Ben-Salem and Smith, 2008; Ruiz-Garcia et al.,2011). This brings about feed shortage, and sheep are left with the option of feeding on rejected crop residue and low nutritious grasses that leads to a decrease in nutrient intake (Scherf, 2000; Ryder,2007).

Consequently, sheep production in the region performs below expected productivity. In South Africa, over 28.8 million sheep contribute notably to Livestock Gross Domestic Product through meat, wool, and pelt production. According to DAFF (2017), wool and meat from sheep contributed over R3.3b and R4.16 b respectively to the economy of South Africa. Generally, there are over twenty breeds of sheep in South Africa, Dohne Merino being one. The Dohne Merino breed accounts for 27.8% of the South African small stock genetic resource portion as represented by weaning weight records submitted to the National Small Stock Improvement Scheme (NSSIS) (Snyman, 2014; Mc Master, 2015; Naidoo., et al., 2016). Despite these contributions, sheep production is far below optimal productivity and unable to meet demand in this region.

The global demand for livestock products will continue to increase due to an increase in the human population (FAO, 2012). According to FAO (2017). The world population in 2050 may rise to 9.8 billion from its current 7.6 billion. This will make the demand for food more competitive and, thus, the need for livestock products will also increase. Sheep can be an essential part of food security because of their ease of adaptation, low maintenance cost, and high fecundity.

Moreover, a ewe can lamb about two times a year compared to a cow, which can only calve yearly; hence, sheep have high turnover. Sheep adapt easily due to their small frame and ability to utilize forbs, buds, twigs, dry leaves, fruit, and flowers of woody plants during drought, and they also have less water requirements (Petrovic *et al.*, 2011). Sheep production, however, has its constraints. The major constraints to sheep production are diseases and gastrointestinal parasites (Sejian *et al.*, 2012). Although sheep thrive under poor quality natural rangelands, productivity usually suffers when optimum nutrition requirements are not met. Nutrition has a profound influence on the ability of sheep to produce quality mutton and wool. Hoffman et al. (2016) reported vital increases in growth performance when sheep are fed with diets containing high protein levels.

Sheep usually picks up many gastrointestinal parasites and other parasites causing diseases because of their feeding habits, such as grazing near the ground. This, coupled with low-quality feeds available in the sub-Saharan natural rangelands, results in compromised feed conversion efficiency and, thus, growth rate (Sejian et al., 2012). Sheep farmers usually supplement the feed with additives such as antibiotics to promote the more efficient conversion of feed to mutton and wool and growth rate. Feeds additives are products used in animal nutrition to improve the quality of feed and, thus, performance and health (Frater, 2014). In the last few decades, there has been a general reduction in the burden of sheep's diseases (Sejian *et al.*, 2012). This is because of the availability and effectiveness of drugs and vaccines and improvement in diagnostics that lead to antibiotics used in animal nutrition.

This antibiotic improves the quality of food from animal origin and improves the animals' performance and health (Frater, 2014). Chemical-based feed additives for sheep are efficient in stimulating feed digestion and consequently increasing sheep performances. However, chemical-based additives have resulted in antibiotic resistance and the spread of resistant bacteria to other animals and human beings (Chang *et al.*, 2015). This has brought about recent concern by consumers of the risk of consuming mutton and milk of sheep fed on diets with these additives. Sequel to this, many developed nations have promulgated laws that prohibit the use of such chemicals-based additives in farm animal production (Lance, 2016). There is, therefore, the need to replace antibiotics with natural promoters such as probiotics, prebiotics, feed enzymes, organic acids, and herbal extracts. Such restriction will promote healthy meat and still achieve optimum mutton, milk, and wool production. One of such additives can be fossil shell flour.

Fossil shell flour is a fossilized deposit of microscopic shell created by simple one-celled plants called diatoms. According to Dolatabadi et al. (2011), fossil shell flour is a geological deposit made up of the fossilized skeletons of diatoms, which are unicellular algae that live in ponds, streams, lakes, and seas and contains 14 trace minerals including Copper, Zinc, Magnesium, Sodium, Calcium, Sulphur, and aluminum (Eldernawi et al., 2014). These trace elements can be of importance to sheep performance (Martin, 2013). Previous studies have shown that fossil shell flour reduces fecal egg counts (FEC) in grazing sheep and cattle (Bernard et al., 2009a). It contains a large amount of silicon and is beneficial for human consumption (Koster, 2013). Traces of fossil shell flour in mutton from sheep fed with a diet supplemented with fossil shell flour will not pose a health hazard to human beings (Ikusika *et al.*, 2019). Fossil shell flour is rich in minerals that are not excessively available in today's feed crops, and it may bind to toxic metals such as Lead and Mercury build-up and help rid it from the sheep body (Adebiyi et al., 2009). The health improvements observed in sheep-fed diets supplemented with fossil shell flour appear to be due to three primary actions of fossil shell flour, which include eliminating parasites, reducing physiological stress, and increasing assimilation of nutrients from feed

(McLean et al., 2005; Koster, 2013). Fossil shell flour has antimicrobial properties, enabling it to function similarly to sheep production antibiotics (Eldernawi et al., 2014).

Natural feed additives such as fossil shell flour in livestock production are of global interest compared to inorganic feed additives. However, a paucity of works has been conducted on the use of fossil shell flour in animal performance. Some of these include assessing the effects of fossil shell flour on the growth performance of cockerel by Adebiyi et al. (2009) and the effect of fossil shell flour on growth performance and broiler meat's carcass characteristics Adeyemo (, 2013). Also, the effect of Fossil shell flour on West African dwarf sheep's growth performance by Emeruwa (2016). However, these cannot be substantially used to delineate FSF's effects in a dual-purpose breed of sheep such as Dohne Merino that are found in different climatic regions. Therefore, it is essential to understand the possible effect of FSF on the growth performance of Dohne-Merino sheep found in the semi-arid and arid regions. Moreso, it is important to consider FSF's effect on wool parameters, feed preferences, rumen fermentation parameters, methane output, and other studies carried out in this research, which to the best of my knowledge is the first of its kind when taken holistically. Besides, this study determined the optimal level of FSF inclusion that can successfully replace inorganic feed additives in sheep's diets for optimal productivity.

1.2 Justification

The use of feed additives in animal feeds to enhance the absorption of nutrients and affect sheep's growth has been in operation for the last four decades (Thornton, 2010). The study will shed light on the efficient utilization of affordable feeds by adding fossil shell flour to sheep diets. This study's outcomes are useful to both the commercial and smallholder farmers to improve their sheep's growth performance and health status. Improvement could be to promote feed intake by adding fossil shell flour as a feed supplement and facilitating the growth of Dohne-Merino sheep to reach market weight earlier than expected, thereby increasing the farmers' profit. Enlightening on the effects of FSF on wool quality will help farmers to boost their productivity. Findings from the current study will be advantageous to sheep farmers because market weight, mutton, and wool quality are commercial value and profit made by farmers. The study will find a replacement for chemical-based feed additives (and its challenges), bring about efficient feed utilization, and improve production performance through fossil shell flour. This study's findings will shed light on the cost-effectiveness of production through disease management, better market weight, and improved meat quality of the sheep industry in South Africa through higher income generation. There is hopeful optimism that mutton and milk will be free from hazardous residue emanating from the use of antibiotics as feed additives, thereby improving the consumers' quality of life.

1.3. Aim and objective

This study's broad aim is to assess the growth performance, nutritional status, wool parameters, methane emission, blood and parasitic profiles of Dohne-Merino sheep fed with a diet supplemented with varying levels of fossil shell flour.

To achieve this aim, the following specific objectives were set to:

1. Investigate the effect of varying inclusion levels of fossil shell flour on growth performance, water intake, digestibility, and N retention in Dohne-merino wethers.
2. Determine the hematobiochemical and parasitic profiles of Dohne merino wethers fed with a diet supplemented with varying amounts of Fossil shell flour.
3. Assess the influence of fossil shell flour supplementation on wool parameters, feed preference, and body condition scores of Dohne-merino wethers.

4. Examine the effect of dietary supplementation with fossil shell flour on enteric methane output and position-dependent variations in Dohne-merino wethers.
5. Evaluate the effect of dietary Fossil shell flour supplementation on Dohne-merino wethers rumen fermentation parameters, *in vitro* true digestibility, and relative feed values.

1.4 Hypotheses

The hypotheses tested were that:

1. Varying inclusion levels of FSF positively affect the growth performance, water intake, digestibility, and N retention of Dohne-merino wethers.

2. Varying amount of Fossil shell flour in the diets improve the hematobiochemical and parasitic profile of Dohne merino wethers

3. Influence of Fossil shell flour supplementation improves wool parameters, feed preference, and body condition scores of Dohne-merino wethers.

4. Dietary supplementation with Fossil shell flour determines the enteric methane output and position-dependent variations in Dohne-merino wethers.

5. Dietary Fossil shell flour supplementation on Dohne-merino wethers basal diet improves rumen fermentation parameters, *in vitro* true digestibility, and relative feed values**.**

1.5. References

Adebiyi O.A., Sokunbi O.A., Ewuola E.O. **2009**. Performance Evaluation and Bone Characteristics of Growing Cockerel Fed Diets Containing Different Levels of Diatomaceous Earth. Middle-East Journal of Scientific Research. 4:36–39.

Adeyemo, G.O. 2013. Growth performance of broiler chicken fed Fossil shell flour growth promoter. Food and nutrition science 4:16-19.

Ben Salem, H., Smith, T. 2008. Feeding strategies to increase small ruminant production in dry environments. Small Ruminant Research 77, 174–194.

Bernard, G., Worku, M., Ahmedna, M. 2009a. The Effects of Diatomaceous Earth on

Parasite Infected Goats.Bull. Georg. Natl. Acad. Sci. 2009, 3, 129–135**.**

Chang, Q., Wang., Regev-Yochay, G., Lipsitch, M., Hanage, W.P.2015. Antibiotics in agriculture and the risk to human health: how worried should we be? Evolutionary Applications. 8:240-247.

Chessa, B., Pereira, F., Arnaud, F., Amorim, A., Mainland, I., Kao, R.R., Pemberton, J.M., Beraldi, D., Stear, M.J., Alberti, A., Pittau, M., Iannuzzi, L., Banabazi, M.H., Kazwala, R.R., Zhang, Y., Arranz, J.J., Ali, B.A., Wang, Z., Uzun, M., Dione, M.M., Olsaker, I., Holm, L., Saarma, U., Marzanov, N., Chessa, B., Pereira, F., Arnaud, F., Amorim, A., Goyache, F., Mainland, I., Kao, R.R., Pemberton, J.M., Beraldi, D., Stear, M.J., Alberti, A., Pittau, M., Banabazi, M.H., Kazwala, R.R., Zhang, Y., Arranz, J.J., 2009. Revealing the History of Sheep Domestication Using Retrovirus Integrations. Science 324, 22–27.

Colledge, S., Conolly, J., Shennan, S. 2005. The evolution of Neolithic farming from SW Asian origins to NW European limits. European Journal of Archaeology 8, 137–156.

Department of Agriculture, Forestry and Fisheries (DAFF). 2017. A Profile of the South African Wool Market Value Chain, A Profile of the South African Wool Market Value Chain. Arcadia, South Africa. https://doi.org/10.1017/CBO9781107415324.004.

Dolatabadi, J. E., Guardia M. 2011. Applications of diatoms and silica nanotechnology in biosensing, drug and gene delivery, and complex metal nanostructures are formed. Trends Analyt Chem. 30:1538–1548.

Eldernawi A.M., Rious M.J., Al-Samarrai K.L. 2014. Chemical, Physical and Mineralogical, Characterization of\nAl-Hishal Diatomite at Subkhah Ghuzayil Area, Libya. International Journal of Research in Applied, Natural and Social Sciences 2:165-174.

Emeruwa, C.H. 2016. Growth Performance of West African Dwarf Sheep Fed Diets Supplemented with Fossil shell flour. University of Ibadan, Nigeria.

Food and Agriculture Organization (FAO). 2017. The future of food and agriculture: Trends and challenges. United Nations. https://doi.org/ISBN 978-92-5-109551-5.

Food and Agriculture Organization (FAO). 2012. The State of Food and Agriculture 2012: Investing in Agriculture for a Better Future, Food and agricultural organization of the united nations. https://doi.org/9789251073179.

Frater, P., 2014. Feed additives in ruminant nutrition. Can feed additives reduce methane and improve performance in ruminants? Agriculture and Horticulture Development Board. www.beefandlamb.ahdb.org.uk. (accessed on 18/08/2018)

Hoffman, M.L; Peck, K.N; Forella M.E; Fox, A.R; Govoni, K.E and S. A. Zinn, S.A. 2016. The effects of poor maternal nutrition during gestation on postnatal growth and development of lambs. Journal of Animal Sci.94:789–799.

Ikusika, O.O., Mpendulo, C.T., Zindove, T.J., Okoh, A.I. 2019. Fossil Shell Flour in Livestock Production: A Review. Animals. 9:1–20.

Koster, H., 2013. Diatomite in Animal Feeds. (Online) Available from: Agrisilica.Co. Za/Pdf/Eng/Diatoms%20in%20Animal%20Feeds%20HH%20Koster.Pdf. (Accessed: June 20, 2018).

Lance Roasa. 2016. The Veterinary Feed Directive Rules for Veterinarians. A Practical Guide for the Practicing Veterinarian. An Agricultural & Food Law Consortium Project.Vol. 1:56

Locke, Rebecca, and Jack O'Connor, 2017. Sheepmeat's unique global position. mla market information. Meat and livestock Report, NSW, Australia.mla.com.ua (Accessed on 6/08/2018).

Lv, F.H., Peng, W.F., Yang, J., Zhao, Y.X., Li, W.R., Liu, M.J., Ma, Y.H., Zhao, Q.J., Yang, G.L., Wang, F., Li, J.Q., Liu, Y.G., Shen, Z.Q., Zhao, S.G., Hehua, Ee., Gorkhali, N.A., Farhad Vahidi, S.M., Muladno, M., Naqvi, A.N., Tabell, J., Iso-Touru, T., Bruford, M.W., Kantanen, J., Han, J.L., Li, M.H., 2015. Mitogenomic meta-analysis identifies two phases of migration in the history of Eastern Eurasian sheep. Molecular Biology and Evolution 32, 2515–2533.

Martin, K., 2013. Silicon: the health benefits of a metalloid. Met Ions Life Sci. 13, 451–471.

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

McMaster, C., 2015. Birth of a breed. The Dohne Merino Story. Simon Says Advertising, Port Elizabeth, RSA.

Meadows J.R.S. 2014. Sheep: Domestication. In: Smith C. (eds) Encyclopedia of Global Archaeology. Springer, New York, NY.

Naidoo, P., Olivier, J.J.J., Morris, J., Cloete, S.W.P., 2016. Does selecting for finer wool result in a higher incidence of creeping belly in the South African Dohne Merino sheep breed? Elsenburg Journal 13(2):49-53.

Petrovic, M.P., Petrovic, M.M., Ruzic-Muslic, D., Caro-Petrovic, V., Maksimovic, N., Ilic, Z., Vuckovic, S. 2011. Opportunities and challenges for sustainable sheep production in Serbia. Biotechnology in Animal Husbandry 27, 463–472.

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

Ryder L.M. 2007. Sheep and man., in Sheep and Man. Duckworth and Co Ltd. UK. Longman. pp.63-84

Scherf B.D. 2000. World watch list for domestic animal diversity. In: Food and Agriculture Organization of the United Nations. Rome: Food and Agriculture Organization of the United Nations.

Sejian, V., Maurya, V. P., Naqui S.M.K. 2012. Adaptability and growth of Malpura ewes subjected to thermal and nutritional stress. Tropical Animal Health and Production. 42:1763,,–1770.

Sejian, V., Bahadur, S., Naqvi, S.M.K., 2014. Effect of nutritional restriction on growth, adaptation physiology, and estrous responses in Malpura ewes 64, 189–205.

Skapetas, B., Kalaitzzidou, M., 2017. Current Status and Perspectives of Sheep Sector in the World. Livestock Research for Rural Development. 29:115-117.

Snyman, M., 2014. South African sheep breeds: Dorper. Weekly magazine on Dorper sheep. Vol.45:1–2.

Thornton, P.K., 2010. Livestock production: Recent trends, prospects. Philosophical Transactions of the Royal Society. Biological Sciences 365, 2853–2867.

Chapter 2
LITERATURE REVIEW

Papers that emanated from this literature review are:

1. Fossil shell flour in livestock production-A review (Published in ***Animals*** (2019), 9 (70): 1-20)

2. Prospect of Dohne-Merino for food security-A review (Under review in Journal of Applied Animal Research).

2.1 Introduction

Sheep have spread and established themselves too far-reaching geographical regions due to their adaptability to relatively poor- nutrients diets, tolerance to extreme climate conditions, and also the small body frame for better management (Kijas and Townley 2009). Sheep are adapted due to their small frame and ability to utilize forbs, buds, twigs, dry leaves, fruit, and flowers of woody plants during drought, and they also have fewer water requirements (Geoffrey and Hinch, 2017). In addition, sheep have low feed and area requirements, a higher reproductive rate, and are small enough to be consumed by an average rural family in a day or two; hence no refrigeration facilities are needed (Tibbo 2006). Sheep produce meat, milk, fiber, and hides, while feacal pellets from sheep generate economic relevance due to their use in organic farming. More than 60 % of the area is prone to drought and famine in the arid and semi-arid regions. Rearing of sheep in such regions contribute towards risk reduction and adaptation in climate change (Shinde and Sejian 2013). However, sheep production has its constraints, including diseases and gastrointestinal parasites (Sejian et al.,2012; Begg et al., 2017). Although sheep thrive under poor quality natural rangelands, productivity usually suffers when optimum nutrition requirements are not met.

Nutrition profoundly influences sheep's ability to produce quality mutton and wool (Xu et al., 2017). Hoffman et al. (2016) reported vital increases in growth performance when sheep are fed diets with high protein levels. Sheep picks up many gastrointestinal parasites and other diseases causing parasites because of their feeding habits, such as grazing near the ground. As a result, farmers use diets supplemented with feed additives to promote efficiency. Antibiotic feed additives are some of the commonly used. Still, these chemical-based additives are expensive and leave residue on animal products, which have been established to have health implications on consumers (Zhang et al.,2015). Besides, the continuous use of antibiotics has caused the spread of antibiotic-resistant bacteria to animals and humans. Therefore, there is a need to replace antibiotics with naturally- occurring feed additives such as prebiotics, probiotics, feed enzymes, herbal extracts, and organic acids to have healthier meat and still achieve optimum production (Ruiz- Garcia and Lunadei, 2011). One of such alternatives is fossil shell flour (FSF).

Fossil shell flour (FSF), or diatomaceous earth, has numerous uses, including purification of water, performance enhancer in livestock, mycotoxin binder, and in stored grain pest control. It can also be used as a dietary supplement for animals and other agricultural applications (Bennett et al., 2011; Koster, 2013), thus contributing to livestock production and, consequently, food security and safety. According to Dolatabadi (2011), diatomaceous earth consists of geologically-deposited fossilized skeletal remains of siliceous marine organisms and freshwater unicellular species, particularly algae and other diatoms. Diatoms can be described as a minute, single-celled water organisms. These minute organisms are confined by a glassy crust. This crust is formed from the silicon dioxide in its source water. Diatomaceous earth is differentiated into two types based on the source. One originates from the sea and the other from freshwater such as lakes. Diatomaceous earth from freshwater is preferred because it is richer in silicon dioxide. The diatomaceous earth (food grade) usage in livestock must be crushed until a fine flour is formed to enable a large surface area for absorption. The outcome is referred to as amorphous silica or fossil shell flour. When viewed with a powerful scientific microscope, the miniature sharp edges can be seen, but it physically feels like chalk dust.

Many of these fossilized sedimentary layers have been in existence for a minimum of twenty million years in the Eocene and Miocene epochs lakes and seas (Bakr et al., 2010). The physical and chemical properties of fossil shell flour enable it to play a vital role in livestock production. Previous authors (Aw et al., 2013) have stated that diatoms' surfaces possess many porous nanostructure silica cell walls or frustules, enlarging its surface area and enabling it to be used as a substance carrier. It has been acknowledged as an animal's natural health and sustenance product (Weaver et al., 2013). It is a fine and pale color silica dust that can absorb liquids with a definite abrasive characteristic obtained after quarrying, crushing, and milling (Korunic 1998; Eldernawi et al., 2014). Due to its abrasive property, FSF has been effective when used as vector and gastrointestinal parasite control in ruminants and poultry (Hussain and Usmani 2006; Bernard et al., 2009a; Miller et al.,2013). This review discusses the overview of sheep production, Dohne-Merino breed, fossil shell flour, its composition, availability, it's potential in performance, health status, wool parameters, and nutritional status of livestock production.

2.2. Sheep production: Industry overview

Sheep (Ovis aries) are believed to have been among the first animals to be domesticated. It played a major role in food and material production in the human community and has spread globally, following human movements (Colledge et al.,2005; Chessa et al., 2009). Table 1 shows some sheep's breeds, their main purpose, and where they are found in the world. Sheep domestication started probably during the hunting era before the settled agricultural age. Most of the present-day sheep emanated from Western Asia, from where it is believed that domestication of sheep began. Some of the original breeds are Bighorn, the Argali, the Urial, which is found in Asia, and Mouflon in Europe (Ryder 2007; Lv et al., 2015). Urial, which presently is in central Asia, Iran, and China, is believed to be the ancestor of the majority of today's domestic sheep. (Scherf, 2000; Lv et al., 2015). Also, the wool sheep found in Africa and Asia are believed to be descendants of the Urial breed. Pieces of information from archaeology and molecular biology showed that the domestication of sheep commenced in the Fertile Crescent region spanning between Central Anatolia to the north of Zagros mountains (Tapio et al., 2006; Zeder 2008). The Food and Agriculture Organization–Domestic Animal Diversity (FAO 2017) database showed that, out of 1311 breeds of sheep worldwide, 758 are found in Europe and Caucasus, followed by Asia and Africa with 318 and 122 breeds, respectively. Asia has nearly half of the world sheep population of about 1.2bilion with a high percentage of them in China, India, and Iran.

In Africa, sheep are generally classified morphologically into four types: thin-tailed with hair, thin-tailed with wool, fat-tailed, and fat-rumpled (Epstein 1971; Soma et al., 2012). In Africa, there are 122 recognized breeds of sheep as of the year 2000 (Taberlet et al. 2008). Archaeological records reported that sheep's migration to the southern part of Africa was about 2000 years ago (Plug and Badenhorst 2001).

2.3. The Dohne-Merino Sheep

Dohne Merino is a synthetic dual-purpose (wool and meat) South African sheep developed in 1939 locally by the South African Department of Agriculture. The breed is a cross between South African merino ewes and German Mutton Merino rams (Casey and Wilson 2016). The limitation to merino sheep farming of that period was low fertility, high mortality, susceptibility to fleece rot, and blowfly strike as a result of the excessive skin fold. Also, the grazing habits of merino are of intensive management that leads to high input cost, which compromised farm profitability. Dohne Merino, a hardy and adaptable breed, was developed to meet the quest for a more versatile and more significant income generation through mutton and high fecundity breed (Swanepoel 2006; Van Wyk 2011). Originally, it was bred for semi-intensive farming in the Eastern Cape grassland regions, but the Dohne Merino's capability to succeed under various conditions resulted in the breed's fame and expansion to other provinces in South Africa and beyond (Cloete et al., 1998; Van Wyk et al., 2008; Van Wyk 2011). The Dohne-Merino is one of the leading woolled sheep breeds in South Africa. Along with its country of origin, Dohne-Merino has also been exported to other major sheep producing countries such as Australia, New Zealand, Peru, Uruguay, Argentina, China, and Russia.

Table 2 1: Some breeds of sheep, their main purpose and location in the world.

Type of breed	Function/purpose	Location	References
Merino	Wool	Originated in Spain. Have spread to Australia, New Zealand, China, South Africa	(Cloete et al., 2014)
Dorper	Meat	South Africa	(Snyman 2014)
Karakul	Meat	Central Asia, Turkey	(Snyman 2014)
Red Maasai	Meat	East Africa, especially Kenya, Uganda, and Tanzania.	(Synman et al., 2014)
Barbados black belly	Meat	Caribbean region, in Peru and in Mexico	(USEPA 2009)
East Friesian	Milk and meat	Germany, Holland, New Zealand, Sweden	(David et al., 2017)
Fat-tailed Awassi	Milk and meat	South-west Asia, Iraq, Syria, Jordan, Israel, Lebanon	(David et al., 2017)
Assaf breed	Milk and meat	Israel	(David et al., 2017)
Chios breed	Milk and meat	Greece	(David et al., 2017).
Soviet Merino	Fine wool	Russia, Siberia	(Deniskova et al., 2018)
Poll Dorset	Meat	Australia	(Begg et al., 2017)
Suffolk	Meat	The United Kingdom is Origin. Presence notable in all European countries and beyond.	(Villagra-Blanco et al., 2015)
Texel	Meat	Originating in the Netherlands but are also popular in Australia, Europe, New Zealand, United States, and Uruguay.	(Carneiro et al., 2010)
Hampshire	Meat	England and many parts of Europe	(Carneiro et al.., 2010)
Corriedale	wool and meat	Originated from New Zealand but are found in Australia, South America.	(Carneiro et al., 2010)
South African Mutton Merino (German Merino)	Wool and meat	South Africa	(Cloetete et al., 2014)
Dohne Merino	meat and wool	South Africa, Australia, New	(Naidoo et al.,

		Zealand, South America.	2016)
Turkidale	Wool and meat	Tuki Tuki in New Zealand and Australia	(Jawasreh et al., 2018)
Superfine wool	Wool	Higher rainfall area of Australia (Northern, central and southern Tableland of New South Wales, parts of Victoria and most of Tasmania)	(Jawasreh et al., 2018)
Dormer	Meat	South Africa	(Chulayo and Muchenje 2013)
Black-headed Persian	Meat and leather	South Africa, Somalia, Saudi Arabia.	(Chulayo and Muchenje 2013)
West Africa Dwarf	Meat	West and Central Africa (Senegal to Chand, Gabon, Nigeria, Cameroon, and the Republic of the Congo	(Jiwuba et al., 2017)
Criollo	Meat	Central America	(Ocampo et al., 2017)
Tefrom	wool and meat	New Zealand	(Díaz et al., 2015)
Waziri	wool and meat	Waziristan area and Bannu district in NWF Province in Pakistan.	(Ahmed et al., 2017)
Van Rooy	Meat and good leather.	South Africa, especially in Bushveld part of Gauteng Province	(Molotsi et al., 2017)
Polwarth	wool and meat	Originated from Australia but they are found in New Zealand and South America	(Salehian et al., 2015)
Nilgiri	Wool	Nilgiri district of Tamil Nadu State in India	(Saxena et al., 2015)

The Dohne Merino sheep are mainly white in color, with rams and ewes usually polled. The lambs grow relatively faster and reach around 25 to 35 kg live weight within their 100 days of age. The average live body weight of the mature Dohne Merino ewes is between 50 and 65 kg, while the mature ram's average live weight varies from 80 to 100 kg (Cloete and Cloete 2014). This breed's fleece production is between 3.5 and 5 kg per year (Mvinjelwa et al., 2014), while their wool has an average fiber diameter of 17 to 21 microns. The plain bodied, open-faced, and breech-free outlook of Dohne-merino (DM) improves reproduction efficiency and reduces flystrike/glass seed issues. This also makes it to attract powerful welfare and animal activist lobbyists who intend to undermine and control livestock production globally.

Dohne-merino has developed a unique combination of adaptive traits that best respond to pressures to the local environment (Peters et al., 2010). This includes disease tolerance, fluctuation in nutrient availability and quality, extreme and harsh climate conditions, and ability to survive and reproduce for a long time, sometimes with poor quality feed (Nsoso et al., 2004; Sejian et al., 2010). Lean (2016) observed that, under high, medium, or low rainfall environments, DM is more productive in weaning weight, fecundity, higher survival rate, and profitability than fine wool merinos and a crossbreed prime lamb enterprise. Therefore, farming with livestock such as Dohne Merino sheep that is robust and adaptable to harsh environments is important in developing countries, especially in semi-arid and arid environments.

2.4. Fossil shell flour (FSF)

Fossil shell flour (FSF), also known as Diatomaceous earth, or diatomite, consists of amorphous silicates with important physical and chemical characteristics. These include porosity and permeability, low density and thermal conductivity, tiny particle size, high surface area, solubility, hydrophobia, and absorption capabilities, which are molecular filter actors, substituting their integral cations without physical changes. The substance is non-toxic, cheap, and readily available in large quantities in many countries. Recently, FSF has been modified as additives for several uses. Recent studies have supported its use as an animal growth promoter, vaccine adjuvant in livestock, water purifier, mycotoxin binder, inert dust applications in stored-pest management, pesticide, animal feed additive, as a natural source of silicon in livestock and as a natural anthelmintic. The advantages of FSF include its low-cost and availability, nontoxic characteristics, and the fact that food grade diatomaceous earth is safe for human consumption.

2.4.1 Availability and Accessibility

Fossil shell flour has been known for decades, and several countries are actively involved in the mining, milling, and transformation of this compound. The world production of diatomite in 1981 was 1.5 million tons, and half of which was said to come from North America (Crangle, 2010). Table 2.2 shows the major world producers of diatomite, and the estimated annual quantity produced. The resources of crude diatomite all over the world are sufficient for the anticipatable future with currents percentage applications in absorbents (9%), fillers (14 %), cement (21%), filter aids (55%), other uses (1%) including specific pharmaceutical, biomedical, and agriculture uses.

2.4.2. Physical and Chemical Characteristics of FSF

Fossil shell flour (FSF) is fine, soft, lightweight, pale coloured, biogenetic sources, and comprised mainly of amorphous silicon (SiO_2NH_2O), derived from the skeletons of diatoms. It is abundantly available on the planet earth. It has distinctive physical properties, such as porosity (35–65%), permeability (0.1–10 MD), low density and thermal conductivity, tiny particle size (Al-Ghouti *et al.*, 2005), and large surface area (Akin *et al.*, 2000). The characteristics of the surface of diatomites, such as acidity, solubility, hydrophobicity, ion exchange, and absorption functionalities, are primarily controlled by the presence of water. This is partly and morphologically connected with the diatomaceous earth's crystal structure, thereby resulting in active hydroxyl groups (Yuan *et al.*, 2010). Its unique porosity (typically 10–200 μm), minute particle size, extensive surface area, high permeability, poor thermal conducting properties, and chemical inertness make it of great interest among naturally- occurring materials (Gao *et al.*, 2005; Pookmanee *et al.*, 2008).

Table 2 2: World diatomaceous earth production (in thousand metric tons)

Country	**2014**	**2015**	**2016**	**2017**
USA	901	925	850	700
Argentina	100	55	200	200
China	420	420	420	420
Czech Republic	49	50	450	450
Denmark	95	95	440	440
France	75	75	75	75
Japan	90	100	100	100
Mexico	88	80	80	90
Peru	125	125	150	120
Russia	70	70	70	70
Spain	36	36	50	50
Turkey	85	90	60	60
Other countries	122	170	120	120

Sources: Crangle (2010); USAEPA (2016)

Table 2.3 shows the elemental composition of natural FSF, and Table 2.4 (Angela *et al.*, 2012) compares the chemical composition of fossil shell flour from various locations. Kilpinen and Steenberg (2009) reported that FSF from different sources differs in therapeutic strength against parasites, especially in poultry.

Natural fossil shell flour can be changed by treating it with hydrochloric acid to purify the silica surface. This helps to significantly mitigate the input of detrimental calcium, iron, aluminum, magnesium, and alkaline rudiments while dissolving little of the silica (Goren *et al.,* 2002; Pookmanee *et al.,* 2008; Bello *et al.,* 2014). Table 2.5 shows the chemical characteristics of modified and natural FSF, obtained through X-ray fluorescence spectrometer. The composition reveals that silicon dioxide (SiO_2) is the principal constituent while small amounts of iron oxide (Fe_2O_3) and aluminum oxide (Al_2O_3) are also present (Pookmanee *et al.,* 2010; Bello *et al.,* 2014). After modification, fossil shell flour can remove substances such as Lead (Pb^{2+}), Copper (Cu^{2+}), and other heavy metals optimally (Belloet al., 2014). Due to its absorptive characteristic, Magnesium oxide is one of the elements used in diatomite modification, which is achieved by treatment with sodium hydroxide and manganese chloride (Crangle 2010).

2.4.3 Fossil shell flour as a Feed additive

Diatomaceous earth can be a perfect animal feed additive for all livestock, with many benefits from preserving feed quality to improving livestock health and performance through better digestibility, acceptability, and overall bioavailability Koster, 2013). Diatomaceous earth will also provide cost-benefit advantages by improving mixing properties and increasing the bulk density of some ingredients. Fossil shell flour functions as a natural preservative for the feed, absorbing the moisture that may cause fungus, mold, or rot (Bennett et al., 2011). In addition, due to its moisture reduction capacity, it reduces clumping and prevents caking of the feed. This helps preserve feed without the need for chemicals, making it more acceptable to the animals during feeding and increasing processing and delivery efficiency (Korunic, 1998; Lakkawar et al., 2016).

Table 2.3: Composition of natural diatomite

Chemical (% Weight)	Diatomite content
SiO_2	82.16
Al_2O_2	4.89
FeO_2	1.46
CaO	1.23
MgO	0.89
MnO_2	0.52
KiO	0.54
NaO	0.43
TiO_2	0.19
P_2O_5	0.12
Loss of Ignition	7.55

Sources: Adebiyi et al., (2009); Pirsaraei et al., (2015)

Table 2 4: Chemical composition of different sources of diatomite (%)

Country/Sample	SiO_2	Al_2O_3	Fe_2O_3	TiO_2	Na_2O	KO_2	CaO	MgO	L. Others
China	82.95	5.75	1.41	0.69	0.06	0.06	0.24	0.21	7.95
Turkey	76.5	7.25	3.85	0.5	0.45	0.85	-	-	0.43
Egypt	83.6	2.24	1.07	0.17-	-	0.53_	6.17	_	4.86
Algeria	72.1	5.3	3.8	0.37	0.65	0.54	7.2	2.6	7.44
Jordan	7.25	11.42	5.81	_	7.21	0.69	1.48	0.25	0.66
Mexico	70.38	13.52	3.37	_	0.17	0.3	0.66	0.42	11.18
Guangdong Chin	90.1	_	0.3	0.4	_	_	0.5	0.2	8.5
Shengzhon, China	65	17.50	4.8	_	0.5	_	1.1	_	11.1
Morocco	62.8	9.7	11.4	_	7.3	_	_	_	8.8
China	72	7.3	4.3	_	1.8	1.2	10	1	2.4
Suizhon, China	71.35	13.26	5.5	0.08	6.7	0.11	1.94	0.15	0.91
Caldiran, Lake Van Basin, Turkey	69.7	11.5	0.65	0.65	0.08	1.4	_	_	15.3
United States	79.55	8.18	2.62	0.70	0.25	1.30	1.31	_	3.8
Kenya	84.5	3.06	1.86	0.17	1.80	0.39	1.19	0.91	6.08
Spain	88.60	0.62	0.20	0.05	3.0	0.81	0.50	0.39	5.20
Russia	79.92	6.58	3.56	0.48	1.43	0.98	0.65	0.72	4.91
Canada	89.7	3.7	1.09	0.10	0.30	0.55	0.31	0.41	3.70
Japan	86.0	5.8	1.6	0.22	0.07	0.29	0.48	0.53	4.4
Nevada	86.0	5.27	2.12	0.21	0.34	0.39	0.24	0.29	4.90
Shenzhou, China	89.6	2.5	1.8	_	1.5	_	1	_	4.5

Sources: Barakat (2011); Caliskan et al., (2011); Sheng et al., (2011); Koyuncu (2012); Safa et al., (201

Table 2 5: Chemical composition of natural & modified diatomite (%)

Compound	Natural Diatomite	Modified Diatomite
SiO	63.31	56.79
Al_2O_3	13.42	12.15
Fe_2O_3	12.58	10.11
Na_2O	0.74	2.37
CaO	0.49	0.08
Cl	<0.019	9.12
Loss on Ignition	6.54	6.73

Sources: Pookmanee et al., (2010); Bello et al., (2014) Diatomaceous earth also helps to increase stock health, feed conversion, and, ultimately, its performance. It is known to reduce internal and external parasites, especially worms. Also. Its residual mineral content may also give an animal a shinier coat. Therefore, using fossil shell flour as an animal feed additive will increase return on investment by keeping the animal healthier, improving their feed conversion rates, and ensuring the feed quality is maintained.

2.4.4. Fossil Shell Flour Can Be a Replacement of Antibiotic in Animal Feed

Fossil shell flour might be able to replace antibiotics as a growth promoter (Bennett et al., 2011; Martel-Kennes et al., 2016; Sarijit et al., 2016), as a gastrointestinal nematodes eradicator (Ahmed et al., 2013; Baltran and Martins 2015) and as an eliminator of dangerous bacteria in the body system of an animal (Chen et al., 2016; Zhang et al., 2016). Most of the chemical constituents of FSF have a strong negative charge. Just like a magnet, the negatively charged elements of FSF attract all positive bodies that are sufficiently tiny to pass through the porous openings of FSF. These strong charges can attract many oppositely charged substances, whether the element, bacteria, or viruses. Therefore, when animals consume FSF, it has the potential to remove dangerous substances (positively charged) from the body system as it flows through the gastrointestinal tract.

When diatomite is added to the feed of animals, Gram-positive bacteria, which are normally targeted in ruminant animals through antimicrobial feed additives, may also bind to the negatively charged shells. This could promote FSF as an efficient replacement for antimicrobial and antibiotic products (James et al., 2011). Zhang et al. (2016) evaluated the impact that diatomite (2 % concentration) has on the adsorption of *Escherichia coli* (ATCC 25922). They reported that the highest adsorption capability of 10.99 mg g^{-1} was realized within 26 h with a solution of 12 mg L^{-1} As (III) at pH 4. Another study carried out previously (Chen and Liu 2016) on the adsorption ability of diatomite also demonstrated the superior capacity of diatomite to neutralize bacteria, comprising Gram-positive *Staphylococcus aureus* and Gram-negative *E.*

coli.

2.5. Potentials of Fossil Shell Flour

2.5.1 Potential of Fossil Shell Flour in Feed Storage and Preference

Over one-third of the total annual grains produced globally are lost due to pests during storage (Singh et al., 2009). In sub-Saharan Africa, farmers suffer significant losses to their products as a result of insect damage. These losses undermine their livelihood, food security, reduction in market returns, and an indirect effect on animals' feeding (Stathers et al., 2008). This loss often increases the cost of production in livestock industries due to an increase in the cost of feeds. Different traditional strategies have been adopted to prevent these losses, including mixing grain with ashes or plant materials and the use of synthetic chemicals (insecticides). Residual synthetic chemicals are the most frequently used protectants against stored-product pests in stored grain. They are often applied directly to the product and protect against stored-grain pests as long as these insecticides' effect remains (Arthur 1999).

Nevertheless, current frequently used protectants possess numerous disadvantages because of their toxicity to mammals and the fact that they leave deposits in the product; many species of insects are resilient to some current protectants (Arthur 1999). These shortcomings have made researchers assess different control methods such as insect growth regulators, botanicals, biological control, microbial control, and inert dust. Fossil shell flour (FSF) is the most promising alternative that can replace insecticides. This is because the absorption of epicuticle lipids and fatty acids by FSF results in arthropods' dehydration. Fossil shell flour comes into direct contact with the insect bodies and absorbs the waxy cuticle of the outer layer, after which the insect loses water and dies (Ebeling 1971; Sabbour et al., 2012; Sabbour et al., 2014). Fossil shell flour needs no specialized kit or techniques for application on grains but simply spreading it. Shah and Khan (2014) reported that FSF is tenacious in its mode of operation, poses little or no pest resistance problems, and leaves no residue. Still, its efficacy depends on the influences of temperature, provenance, humidity, and particular pests and substratum individuality. Sabbour et al. (2014) reported that calcium hydroxide modified diatomite (Ca-DE) and those modified with sodium hydroxide (Na-DE) were the most effective antidote against insects. Ca-DE yielded better results and accomplished the highest mortality percentages recorded as 88% and 96 % for 1.0 FSF treatments against *R. dominica* and *B. incarnatus,* respectively.

The lowest mortality rate was recorded for Al-DE at a concentration of 0.5% and achieved 21 and 15% mortality for *R. dominica* and *B. incarnatus*, respectively. In a similar study conducted by the same researchers on FSF in pest management in stored-grain, they reported that the reproduction rate of tested insects was significantly reduced by FSF and modified FSF. Egg production by the tested insect was reduced considerably by modified-FSF under stored conditions. The average quantity of eggs laid and the percentage of adult emergence (F1) of each tested insect were appreciably affected by both natural FSF and modified-FSF in comparison to the control. The same authors found that Nano-FSF strongly reduced the number of eggs laid for *T. confusum* but reduced to a lesser extent for *T. castaneum* (3.8 ± 1.5, 17.8 ± 7.5, 26.6 ± 3.5 eggs/female and (13.8 ± 1.5, 37.8 ± 7.5, 46.6 ±3.5 eggs/female) after 20, 90, and 120 days of storage interval respectively.

The constant effect of nanoparticles showed numerous unusual modes of action such as falling oviposition, immature emergence (F1), and influx percentages of hardened insects. It can be concluded that FSF-nanoparticles and natural FSF can be a good alternative in pest management programs for *T. confusum* and *T. castaneum*. Badii et al. (2014), in their studies on the prevention of *C. maculates* infestation in stored Kersting's groundnut, reported that mortality of adults C. maculates increased gradually with the increased quantity of FSF and the contact period. Grains treated at 2.00 or 1.50 $g\ kg^{-1}$ documented considerably fewer eggs and a lower rate of F1 emergence than dosages with lower quantity. An increase in FSF concentration continuously decreased produce weight due to low beetle numbers; however, a significant difference in seed viability was not recorded. FSF was more efficient at 50 % relative humidity than at 80 % relative humidity.

FSF's potential can be explored by communal farmers, animal feed mill operators, and commercial farmers to preserve grains for their animals during surplus to the time of scarcity.

This is because it is cheap, non-toxic, readily available, and requires no equipment for administration. FSF likewise has broad uses as an anticaking agent in the storage of grain and feed mixing. This enables better flowability, mixing and handling, and prevention of particles from clumping together. According to Bennett et al. (2011), food-grade fossil shell flour placed in livestock feed may help discourage the growth of fleas and other dangerous organisms.

Also, Forde (2016) reported that factors such as flavor, the physical outlook of the feeds, taste, and particle size of the diets influenced feed preference by sheep. Sheep requires 25 to 40 ounces per head per day of NaCl and will eat less feed when deprived of NaCl (Digby et al., 2011). Likewise, it has been observed that minerals such as Na, Ca, K, and Mg increase the palatability of diets (Desimone et al., 2013; Garmyn et al., 2011). Slamova et al. (2011) reported that FSF, Zeolite, bentonite, and some dietary clays are rich in minerals that could increase livestock diets' palatability.

2.5.2. Potential of Fossil Shell Flour as Animal Performance Enhancer and Digestibility

Growth promoters and enhancers are materials that facilitate farm animals' growth, particularly swine, poultry, and livestock, for better market value. The market for animal growth promoters and enhancers is large and growing. The most widely used group of growth promoters in animal feed is antibiotics (Li et al., 2013). Other growth promoters include probiotics, prebiotics, essential oils, antibodies, bacteriophages, antimicrobials, innate defense molecules, immune enhancers, and combinations thereof (El-Tawab et al., 2016). The development of new growth enhancers, particularly those that can be made cost-effectively, is important. In line with this, El-Tawab et al. (2016) and Sarijit et al. (2016) conducted a study using spent filter media containing ~35% moisture, 32 % diatomaceous earth, 22 % organic carbon, and 11% activated carbon, and reported that although their results were not definitive, spent filter media improved daily feed intake, weight gain, and efficiency.

It is believed that the use of spent filter media may advantageously increase the growth of the animals daily compared to identical amounts of conventional animal feed compositions, although this result is believed to be affected by other conditions (Schingoethe et al., 2009; and

Sarijit et al., 2016). In addition, El-Tawab et al. (2016) and Sarijit et al. (2016) also noted that these animal feed compositions did not adversely affect the animals' lean mass percentage and did not appear to exhibit any harmful effects to the growth or the health of the animals. Likewise, Adebiyi et al. (2009) reported a substantial improvement in average weight gain, feed conversion efficiency, and bone development of cockerel fed 6 % fossil shell flour inclusion level as feed additives the 16 weeks of the trials. This could be because FSF's addition increases the average daily feed intake by increasing the palatability of the feed, thereby making more nutrients available at the tissue level for growth and weight gain. Similarly, fossil shell flour significantly improved intake of feed (7.44 %), body weight gain (9.51%), and the improved ratio of feed conversion (2.08%), as well as the productive efficiency index (5.48%) in cockerel exposed to aflatoxin B1 (Adebiyi et al., 2009). Fossil shell flour also improved serum albumin (2.26 %) and the action of serum LDH (44.4 %) in a previous study (Modirsanei et al., 2008).

Modirsane et al. (2016) supplemented the feed of 252 piglets with silicon dioxide (a major component (79%) of FSF) and found improved feed intake by 4.13% and an improved mean daily gain by 3.26 % during the early general period, compared to groups not given silicon dioxide. FSF's addition led to an increment of 2.2 % in the piglets' weight at the expiration of the post-weaning phase (24.52 kg vs. 23.99 kg). It concluded that the addition of 0.02 % crystalline silicon dioxide (fossil shell flour) to piglet feed increased daily feed intake, growth rate, and piglet weight. Therefore, it is clear that FSF could offer a prospective financial gain to livestock farmers.

2.5.3. Potential of Fossil Shell Flour as Water Purifier for Livestock Usage

The provision of poor-quality drinking water for livestock in semi-arid and arid regions for several months of the year is ubiquitous (NRC 2007; Garcia et al., 2010). These supplies originate from streams, canals, small wells, or boreholes used for irrigation (Schlink et al., 2010). This water is often high in salt and toxic elements such as lead, copper, selenium, mercury, and arsenic (Bello et al., 2014). They are hazardous to animal health and may cause physiological upset or render the animal products unsafe or unfit for human consumption and possibly cause the death of the animal (Pick 2011). The bulk of heavy metals are identified to be poisonous and cancer-causing agents. Problems emanating from water toxicity are aggravated by the irrigation of forage with the same potentially toxic water. Plants' absorption of these chemicals can be transferred into the animal body, thus increasing the animal's risk and toxicity. Sources of these toxins and salts are animal feces, birds, animal carcasses, intensive livestock industry runoff from bare paddocks, sewerage waste, veterinary antibiotics, herbicides, and pesticide residues (Bello et al., 2014). Diatomite that has been modified with manganese- oxide is an active and good adsorbent for removing heavy metal ions such as, Pb^{2+}, Cu^{2+}, Cd^{2+} Ni^{2+}, and Hg^{2+} (Bello et al., 2014). The negative charges on the modified surface is an attribute that enhances adsorption performance. In addition, the enlargement of the surface area is believed to play a vital role in the overall removal process.

Therefore, lower loading of diatomite has a superior performance over higher loading of other adsorbents (Al-Degs et al., 2000). Similarly, Al-Degs et al. (2000) and Moleshi and Nahid (2015) reported that heavy metal ions such as Pb, Ni, and Cu were removed from wastewater using manganese-oxide modified Iranian diatomite, which is considered as an adsorbent and a filtration substance. Also, organic and inorganic substances can be removed from wastewater and surface water through the absorption of diatomite modified with Mn (Walker and Weatherley 1999). Bello et al. (2014) reported that modified diatomite has a greater removal capacity for heavy metals from water than unmodified diatomite. Danil de Namor et al. (2012) established that the use of diatomaceous earth is a promising technique in removing heavy metals from wastewater and surroundings. Therefore, FSF can treat water/wastewater for animal use, hence reducing the risk of heavy metal contamination arising from meat consumption. Also, FSF has significant potentials in mitigating water scarcity and turning waste into wealth.

2.5.4. Potential of Fossil Shell Flour as Source of Minerals for Livestock

Minerals are naturally-occurring substances generally required in microscopic amounts within

1 to 2500 mg per day, depending on the mineral (Oana et al.,2016). Like vitamins and other essential food nutrients, mineral requirements vary with animal species, age, physiological status, and purpose for which the animal was raised. Large mammals require large amounts of calcium to build and preserve bones and the standard function of nerves and muscles (Allison-Silva et al., 2016). Phosphorus is an important constituent of adenosine triphosphate (ATP) and nucleic acid, is also vital for acid-base balance and the formation of teeth and bones. Red blood cells cannot function properly without iron in hemoglobin: the oxygen-carrying pigment of red blood cells. Iron is also an important component of the cytochromes that function in cellular respiration. Molybdenum, manganese, copper, zinc, magnesium, selenium, and iron are important cofactors found in certain enzymes' structure and are crucial in many biochemical pathways.

Mammals need iodine to synthesize thyroid hormones. Sodium, potassium, and chlorine are imperative in upholding osmotic balance between cells and the interstitial fluid (Soetan et al., 2010). According to Galyean et al. (1999), different microminerals, for example, Se, Mn, Zn, and Cu, are essential for the optimal performance of the immune structure and resilience against pathogens. Cobalt deficiency decreases the resistance of animals against helminth infections (Ferguson et al.,1989). Molybdenum also plays a vital role in immunity against endoparasite (McClure 2003) and can decrease the worm burden in lambs (Miller et al.,2013; Qudoos et al., 2017). Natural food-grade FSF contains 15 macro and microminerals, which are essential to animal diets. The inclusion of natural FSF to poultry diets has continuously revealed weight gains in production. This gain could be connected to a combination of factors such as the ability of FSF to decrease parasite populations, promotes a reduction in stress on the animal, and improved nutrients assimilation (Adebiyi et al., 2009; Adeyemo 2013). Fossil shell flour contains a wide range of naturally present chelated minerals such as calcium, magnesium, iron, phosphate, sodium, titanium, and potassium. The mineral constituents that FSF provides may replace a small proportion of the entire mineral premix or complex.

The ability to supplement both micro and macro minerals (particularly the effects of fossil shell flour on improved general mineralization such as bone), may also be the reason for enhanced livestock performance (Carlisle 1976; Seaborn and Nielsen 1994; Mertz 2012). A study conducted at the University of California reported that the silicon inclusion promoted leg thickness and more prominent combs of chicken relative to their body size than the group without silicon supplementation (Carlisle 1976; Adebiyi et al., 2009). Adebiyi et al. (2009) also reported that growing cockerels fed 6 % inclusion levels of FSF had the highest Ca, P, and Ash values than the control and other treatments. However, tibiotarsi weight, length, and robusticity index were not affected significantly. Livestock production and health problems are often associated with calcium deficiency in many species of animal. The addition of natural food-grade FSF to diets could avert any calcium associated performance or health issues. Minerals present in natural food-grade FSF can also help to meet the mineral constituents of lactating animals (Oana et al., 2016). Table 2.6 shows the mineral constituent of food-grade FSF, including major and trace elements needed by livestock for growth performance.

Fossil shell flour is the most abundant organic amorphous silica in the world (79% to 94 % of silicon) (Martin 2013). It exists in silicon dioxide form; it is bioavailable and is essential for good bone growth and nutritionally crucial for preventing certain conditions of chronic diseases associated with aging (Martin 2007; Martin 2013). Humans, animals, and plants have an essential need for silicon to sustain life, and regrettably, in today’s world, our diets can easily become deficient in silicon.

Table 2.6: Mineral constituent of fossil shell flour (FSF)

Element	Quantity
Calcium (Ca)	0.40
Sodium (Na)	0.26
Manganese (Mn)	0.0052
Iron (Fe)	0.72
Copper (Cu)	0.0019
Vanadium (V)	ppm 43.8
Sulfate Sulfur (S)	0.062
Phosphorus (as P205)	0.037
Potassium (K)	0.16
Chloride	0.074 % or 740 ppm
Zinc (Zn)	0.0022
Titanium (Ti)	ppm 420
MgO (calculated from % Mg)	0.34
Strontium (Sr)	ppm 59.9
Boron (B)	0.0023
Magnesium (Mg)	0.21
% CaO (calculated from % Ca)	0.55
Aluminum (Al) %	0.65

Sources: Adebiyi et al. (2009)

This is because Silicon is essential for the growth and bone calcification in mammals. Also, it is a biological crosslinking agent of connective-tissue-based membrane structures. Silicon is known to benefit several human disorders, such as osteoporosis, aging of skin, hair, and nails, and atherosclerosis.

2.5.5. Fossil shell flour and blood metabolites of sheep

The productive performance of sheep largely depends on their health status. The most accurate and precise way of accessing health status is to have a picture of the animal's blood profile (Schroder and Staufenbiel, 2010). Damptey et al. (2014) reported that blood metabolites could be used to determine an animal's well-being and to detect the adequacy of feed offered to such an animal. According to Antunovic et al. (2011), a sheep's nutritional status could reliably be assessed through the blood metabolic profile and is independent of the sheep's physiological condition. In a study conducted by Novoselec et al. (2017),

it was reported that serum urea, total plasma protein, albumin, globulin, and creatine could be used to evaluate the crude protein in the diet and also the protein status of the animal. Likewise, haematological indices, especially the white blood cells and the red blood cells, are great indicators of sheep production's health status (Ceriac et al., 2017). Weaver et al. (2013) reported that FSF's addition into the feeds of young gilts improves the immunity system against Aflatoxin. Similarly, Emeruwa (2016) observed an improvement in RBC, WBC, blood glucose, total serum protein, and blood minerals of sheep fed FSF at 2 % inclusion levels. Adeyemo (2013) concluded that because of the richness of FSF in trace elements, it promotes feed intake and improves blood glucose and total blood protein.

2.5.6. Potential of FSF in Livestock Parasite Control

Parasitism in small ruminants is a major problem for farmers, especially for smallholders. By nature, small ruminants, especially sheep, graze close to their dung, which exposes them to parasitic ova and subsequently parasite load (Osweiler and Carson 1997). Consequently, parasitic gastroenteritis continues to be a health risk and constraint to the production of small stocks due to associated disease, mortality, control measures, and cost of treatment at clinical as well as subclinical levels (Nwosu *et al.*, 2007). Economically, a reduction in profitability levels of ~15%, including weight loss of ~50 % on account of intestinal parasites, has been reported by Bhat *et al. (*2011); Shahnawaz *et al. (*2011). This loss results from low production on account of poor growth, low weight gains, and poor utilization of feed (Ijaz *et al.,* 2009). Parasites can cause hematological and biochemical turbulences in sheep (Ijaz *et al.*, 2009) and anorexia, altered water and electrolyte balance, anemia, poor reproductive performance, and weight loss. This can lead to an increase in the mortality rate of lambs (Hussain and Usmani 2006). Studies have revealed that GIT parasites are significant causes of production losses in sheep, particularly in sub-Saharan Africa (Hussain and Usmani 2006; Odoi *et al.*, 2007). At any stage in sheep growth, they can be vulnerable to gastrointestinal nematodes, although lambs and peri-parturient ewes are most epidemiologically affected (Besier *et al.*, 2016). One of the main organisms that economically restrict sheep production worldwide is gastrointestinal nematodes (Miller *et al.*, 2013). The most important factor limiting the control of this parasitic organism is the stable increase of its anthelmintic resistance globally, particularly if animals are underdosed or treated under preventative and suppressive treatment regimes. Therefore, alternative and complementary sustainable control programs such as FSF would be beneficial.

For the past two decades, ' fossil shell flour (FSF) has been used to deworm animals naturally. In a previous study by Koster (2013), a 2 % inclusion level of fossil shell flour in the diet of dairy and beef cattle reduced parasite investigation and increased production of milk by 5%, and improve meat quality. The National Experimental Council and the National Council of Organic Standards in the USA discuss the disadvantages of chemical inoculations in their database of published products and suggest that Diatomite be used as an alternative (Koster, 2013). Similarly, on the efficacy of FSF with ewes, Deutschlander (1993) reported that a combination of FSF and a mineral supplement with no other anthelmintic used at a ratio of 1:1

for three months reduced the incidence of Haemonchus compared to the control.

Lambs fed with 2 % inclusion rate of FSF appeared to have an earlier weight gain, tails that were cleaner, and shining wool (Deutschlander, 1993). A better improvement in the general body condition of lambs was also reported. Deutschlander (1993) also performed a similar study where heifers (five hundred pounds weight) fed FSF pasture on free-choice conditions showed no worms, either mid- or late-season. The heifers consumed FSF of about one pound per week per heifer, and when the dairy cows were not fed FSF for a few days, they craved the substance and consumed several pounds as soon as it was made available to them. Despite the heavy feeding on FSF, no side effects were recorded in the cows, and they remained in good condition. It was concluded that the lambs performed much better than the ewes and cattle while on pasture. In addition, the use of FSF recorded savings of $1.50 per head for both ewes and cattle as a dewormer over the use of conventional medicines. Deutschlander (1993) observed that the heifer and ewes' body conditions were very satisfied when they were withdrawn from the pasture. In another study, Bernard *et al.* (2009) observed that Spanish/Boer cross goats fed varying inclusion levels of FSF at 1.77 g, 3.54 g, and 5.31 g per kg had significant improvement in mean weight gain and reduced fecal egg count as the inclusion levels increased. Similar observations were reported by Bennett *et al.* (2011); Mclean *et al.* (2005) showed that FSF significantly increased body weight, feed conversion efficiency, growth rate, and decreased parasite load of the experimental animals. However, they both postulated that FSF should be fed for an effective and optimal outcome for a more extended period. They hypothesized that the abrasive edges of the diatom particles injured the cuticles of nematodes when in contact, resulting in dryness and eventually the parasite's death. In line with these findings, Ahmed *et al.* (2013) considered the use of biological control of gastrointestinal parasites in sheep, feeding different biological organisms and diatomaceous earth in their diet. They also reported that diatomaceous earth had an efficacy of 61%, although efficacy varied with time.

2.5.6. Fossil shell flour and rumen manipulation in Sheep production.

Sheep can utilize grasses and other fibrinolytic materials because the gastrointestinal tract has a compartment called the rumen. The rumen forms the larger part of the reticulorumen, the first chamber in the ruminant animals' alimentary canal. It contains digestive juices and millions of microorganisms and serves as the primary site for microbial fermentation of ingested feed (Ahmed et al., 2013). Internally the surface is tiny projections, papillae in shape and often described as a "fermentation vat." Through fermentation, microbes within the rumen convert cellulose and fiber, which are useless to the host animal, into volatile fatty acids (VFA), microbial protein, and B vitamins, useful products. The most common VFA produced during the process of fermentation are acetate, propionate, and butyrate. These VFA are sources of 60 – 80% of the ruminant's energy (Atikah et al., 2018). This same process also produced gasses, including methane nitroxides as a byproduct (Kara et al., 2016). Their molar concentration of VFA differs depending on many factors, including the type of diets (Wiedemann et al.,2016). Similarly, methane generated as a byproduct of fermentation in ruminant animals' rumen constitutes about 6-12% of greenhouse gasses globally (Tapio et al., 2017). Rumen has a temperature ranging between 38 oC - 42 oC and a pH of slightly acidic to neutral (6-7). It is an anaerobic condition (Henderson et al., 2015).

Microbes found in sheep's rumen include bacteria and archaea (most dominated), protozoa, and fungi. Among the highly conserved and known methanogenic archaea are Methanosphaera sp, Methanomassiliicoccaceae sp, Methanobrevibacter sp, and Methanobrevibacter sp. Simultaneously, those of bacteria include Prevotella sp, Butyrivibrio sp, Ruminococcus sp, Lachnospiraceae sp, Ruminococcaceae sp, and Bacteroidales sp. (Henderson et al., 2015).

The type, productivity, and functionality of microbes in the rumen of sheep depend on the age of the sheep, type and size of the feed consumed, presence and type of feed additive (Kanber et al., 2016). Dias et al. (2013) reported that both the microbes in the rumen and the host animals require major and trace minerals for growth and development. It was further stated that sulphur is needed to form amino acids such as cysteine and methionine, which are building blocks of proteins (Golder et al., 2014). It was observed that the inclusion of sulphate to the diet of sheep improved microbial counts of all microbes in the rumen with fungi group, which helps in fiber degradation having the highest increase (van Zijderveld et al., 2010). Therefore, FSF could be used to manipulate the microbial species in the rumen of the ruminant animal since FSF is rich in minerals, including Sulphur.

2.5.7. Potential of Fossil Shell Flour on Quality of Wool

In the face of demand for lighter weight fabrics by consumers and the rigorous challenge of competition from synthetic fabrics, producing quality wool that will translate to the consumer's yearning has been of utmost importance to the sheep industry in the past 25 years. When looking for fine, uniform and high-quality wool appropriate for the textile industry, Dohne-merino and other merino sheep come to mind because of the wool quality they produce (Cilek et al., 2015). Wool fiber diameter and fiber length are the key characteristics used to evaluate the processing route and final quality of the finished textile products (Wang et al., 2013). Factors such as genotype, nutrition, age, and sex determine the quantity and quality of fleece from sheep. Esfandyari et al. (2011) reported that the animal's genetic composition greatly influences the fiber diameter, and the proportion of kemp. Fiber diameter variability and proportion of medullated fiber are attributed to the sex of the animal. Cilek et al. (2015) observed that females have a greater measure of fiber diameter variability and proportion of medullated fiber than males and that age on all fleece traits was significant. The effect of sex was statistically noticeable for all fleece traits except for fiber tenacity and comfort factor. It can be generally concluded that younger sheep and rams have higher fleece yield and fleece quality than older sheep and ewes. Similarly, the age of the animal and the fiber diameter are directly proportional to each other, while age and fiber length is not affected by age in merino sheep. Fiber diameter is affected by the quality and quantity of nutrients available to the animal, which is also related to live weight (Allden et al., 1979; Hatche 2005; McGregor et al., 2016).

Variation in staple length relates positively to live weight (Huisman and Brown 2008).

McGregor et al. (2016) observed that the highest correlation between staple length and live weight was observed when measured simultaneously but weaker when measured at different times. Also, fiber diameter variation mostly influences staple strength (Hatcher et al., 2005; Schlink et al., 2010; McGregor et al., 2016). Staple strength and location of a break are greatly influenced by the minimum fiber diameter and variation rate along with the fiber. However, nutrition, disease, stocking rate, genotype, and the animal's physiological state all influence the staple strength. Likewise, climate, altitude, genotype, nutrition and soil, relative humidity, pH within the fleece, the level of perspiration, production of sebum by sebaceous glands, and time of shearing are factors that determine the color of wool (Sumner et al., 2003; Sumner et al., 2004; Sumner 2005).

The effect of gastrointestinal parasites and lice on fleece yield and quality have also been studied. Frequent treatment with anthelmintics increases the fleece yield, fiber diameter, staple length, and strength as well as crimp fleece weight (Van Burgel et al., 2011; James et al., 2011). *Bovicola ovis* (lice) infestation harms wool characteristics, and this is amplified as the number of lice present and the duration of infestation increases. With lice infestation, greasy wool changes color and becomes less bright, staple length is reduced, and staple strength is negatively affected (James et al., 2011). Van Burgel et al. (2011) also reported that sheep with modest to severe infestation produce less sound wool (1.7 vs. 3.0 kg/head) and more cast wool (0.4 vs. 0.1 kg/head) than sheep with very few lice.

Since fossil shell flour improves body weight gain, reduces intestinal parasites, and in general might be expected to suppress ectoparasites, it follows that inclusion of fossil shell flour to the diets of sheep may increase fleece yield and improve the quality of wool greatly for good market prices as well as the international standard. As an alternative to anthelmintic, fossil shell flour (FSF) was evaluated by Baltran and Martins (2015) for its ability to inhibit the migration of *Oesaphagostomum dentatum* larvae using migration and inhibition assays in vitro, in unsheathed and sheathed third-stage larvae. They observed that FSF was more effective in unsheathed larvae at 0.3 mg/mL after 20 h with 61.6 % inhibition. With sheathed larvae, FSF had a significant effect of 1 mg/mL exposed within 24 h with 67.6 % inhibition. This shows that the presence of cuticle could reduce the effectiveness of FSF as anthelmintic. Therefore, researchers need to investigate parasite cuticle abrasion by using equipment such as scanning electron microscopy to examine the cuticle integrity (from treated versus untreated). In a separate experiment, Osweiler et al. (1997) contrarily observed that FSF did not lower the parasite load in lambs.

Fossil shell flour has also been reported to reduce ectoparasites. Dawson (2010) said a significant reduction in bird flea population sizes (*Ceratophyllus sp*) and several blowfly species (*Protocalliphora* spp.) in the same year. Fossil shell flour seemed to be more efficient at reducing the flea populations, perhaps because fleas have body sizes that are smaller and more susceptible to dehydration because of FSF's abrasive action. It was also confirmed, in more recent researches by Martins and Mullens (2012) and Amy (2016), that FSF can suppress the activities of Northern fowl mites (*Acaris macronyssidae*). Kilpinen and Steenberg (2009) reported that FSF is one of the commonly used substitute control methods for poultry red mites (*Dermanyssus gallinae*) in Europe because it kills the target host mainly by desiccation. The authors also observed large differences in the types of FSF versus red mites' mortality. Therefore, there is a need for other researchers to investigate different types of FSF formulations head to head for parasite control.

2.6. Impact of Climate Change and heat stress on Sheep Production and the use of FSF

A long-time alteration of weather on the earth, known as climate change, is caused by greenhouse gasses (GHG). These changes occurred from cooler to warmer, and a rise of 1.8-4 ^{0}C is postulated in the next 100 years, depending on the GHG emission rate (IPCC 2018). Livestock contributes about 18% of the total anthropogenic GHG emission (Thornton 2010). Sheep are seen as animals for the future because they can survive and strive in vulnerable climates such as droughts (Gowane et al., 2017). Though sheep are vulnerable to changing climate, they are relatively more resilient to the impact of climate change than large ruminants. However, because of change in environmental factors such as temperature, humidity, rainfall, availability of forages, and pathogen prevalence, sheep production is affected as a result of feed intake, growth rate, genetic variation, and other traits of importance (Safari et al.,2005; Ghafouri-Kesbi and Notter 2016; Gowane et al., 2017). Based on the climate change scenario, there is an increasing concern about the impact of heatwaves on animal welfare and their production in both temperate and tropical regions (Nardone et al., 2010). There are negative effects of heat stress on animal productivity and health (Bernabucci et al., 2015). The heat stress effects are particularly harmful in high productive animals due to the close relationship between metabolic heat generation and production levels. In the last two decades, there is a growing interest in using supplements or additives to mitigate greenhouse gas emissions in livestock production systems, especially small stock. Thota et al. (2017) reported that the mean enteric methane emissions (l/day) were significantly lower in sheep fed with probiotics supplemented diet than sheep fed on a diet without probiotics supplementation and reduced 21.9 percent as compared to the non-supplemented diets. Similarly, Ma et al. (2016) reported that allicin supplementation effectively reduced daily methane emissions in ewes, probably by decreasing the population of ruminal protozoans and methanogens. There is a paucity of information on the use of FSF for rumen manipulation for the purpose of reducing the production of methane in the rumen of a ruminant animal

2.7. Other Potentials of Fossil Shell Flour in Feed Industry and Livestock Production

2.7.1. Fossil Shell flour as adjuvant vaccine

Deactivated vaccines are normally used in poultry as part of a complete vaccination procedure. These vaccines can stir up antibodies of high titers, capable of protecting against general infections, and transmitted from parent stock to their offspring as maternal antibodies. To boost their immunogenicity, these vaccines have adjuvants. Common adjuvants often used in poultry vaccines are aluminum hydroxide ointment (Alum) and ASO4 (oil-based type) (Schijns 2011; Fox and Haensler 2013). Substances added to a vaccine to accelerate or enhance the body's immune response to the vaccine and reduce the quantity of antigen needed for vaccines are referred to as adjuvants (Mount et al., 2013). According to Waksman (1979) and Hunter (2002), this can be accomplished by three different mechanisms. First, a prolonged immune response is induced by a slow-release due to the deposit of antigens formed at the injection site. Second, particulate antigens are formed, which antigen-presenting cells can detect.

Finally, local inflammation may be caused by adjuvants that initiate system-recognition receptors, activating antigen production. However, the undesirable effects of adjuvants are inflammations at the injection site. This effect is noticeable in farm animals such as poultry, resulting in a reduction of meat quality and escalating condemnation of carcasses (Singh and O'Hagan 2003; Schat 2014). The price of vaccines is also considerably increased by adjuvants (Meeusen et al., 2007). Discovering new, safe, steady, and cheap adjuvants are therefore vital to improving existing vaccines. Fossil shell flour has successfully met this need. Singh and O'Hagan (2003) experimented using fossil shell flour as an adjuvants vaccine for Newcastle Disease Virus (NDV) in poultry, suggesting that fossil shell flour could function as a prospective immunological agent for vaccines against poultry diseases. They established that using fossil shell flour as a vaccine adjuvant causes no harmful effects to hatchability, quality of chicks, body weight, and meat quality. Although an apparent immune response was not observed when the vaccines were applied in vivo, subcutaneous boosters with NDV adjuvanted with diatoms generated NDV specific antibodies, starting at 7 d post the second booster in chickens. The effectiveness of diatoms as an adjuvant for INDV vaccines was similar to the action shown by aluminum hydroxide gel. The researchers proposed that fossil shell flour can be used as poultry adjuvant deactivated vaccines.

A similar study by Nazmi et al. (2017) also looked at the efficacy of diatoms as adjuvants for the Ark-DPI live infectious bronchitis virus (IBV) vaccine after ocular or spray application.

They observed that the addition of diatoms had no detrimental effect on the vaccine virus, hatchability, chick quality, live weight, and meat quality. However, the addition of diatoms to the vaccine did not stimulate higher immunoglobulin (IgG) titers in the serum or immunoglobulin (IgA) titers in tears. It also did not influence the occurrence of monocytes/macrophages in blood and spleen determined by flow cytometry. In addition, protection generated against IBV homologous challenges, measured by a viral load in tears, respiratory signs, and histopathology in tracheas, did not vary when diatoms were present in the vaccine formulation. It was concluded that FSF could be used as a potential adjuvants vaccine in Newcastle Disease Virus (NDV), although its efficacy on the Ark-DPI live infectious bronchitis virus (IBV) needs further research.

2.7.2. Prevention of Scours

Grazing animals in the process of searching for silicon dioxide, which is beneficial for their growth, frequently eat dark dirt, which contains a crystal-like type of silicon dioxide. The farmyard variety of dirt is contaminated with diseased organisms, which can lead to calves and young animals' death. Providing stock with food-grade fossil shell flour that contains at least 80 % pure silicon dioxide can prevent calf scours (Nazmi et al., 2017). The purpose of providing fossil shell flour of free choice to the young animal is to prevent them from eating black dirt in the farmyard or elsewhere. Therefore, fossil shell flour serves as a better substitute for black dirt.

2.8. The future of the sheep industry in relation to the Usage of FSF

There are serious losses to stored grain suffered by communal farmers in sub-Saharan Africa because of insect damage. However, as many of these farmers cannot afford the cost of chemical protectants, they resort to using diatomaceous earth to preserve their products for their household and livestock use (Stathers *et al.,* 2008). Diatomaceous earth is cheap, readily available, effective against insects, and safe to use. All farmers, whether commercial, small-scale, or communal, can make use of FSF. The numerous uses of FSF give room for all types of farmers to explore the various benefits and applications of fossil shell flour. Stathers *et al.* (2008), observed in two different studies that small-scale farmers in Tanzania and Zimbabwe were able to preserve their grains for both their animals and their household by applying diatomaceous earth to the grain at the rate of 0.1% (*w*/*w*). Likewise, Badii *et al.* (2014) reported that diatomaceous earth applied at 1.50 or 2.00 g kg^{-1} at 50 % RH is a feasible substitute for preventing *C. maculates* infestation in stored Kersting's groundnut for commercial and small-scale farmers.

Another opportunity that is open to commercial, small-scale, and even communal farmers in the use of diatomaceous earth is that food-grade diatomaceous earth can be put in a bowl and placed in the pen or barnyard as a mineral lick for the animal (Van Pleltzen 2015). Farmers of different scales of production can therefore make use of diatomaceous earth for their animals to preserve feed ingredients and provide a source of minerals and other numerous uses.

As a result of a global increase in organic farming, from the demand for organic livestock edible products by consumers and food safety campaign programs, fossil shell flour can be a potential attraction to both scientists and commercial farmers. Its use has not yet been adopted in most countries, particularly in sub-Saharan Africa. There are relatively few studies investigating the potential of fossil shell flour in livestock, especially in small stock. Besides an absence of authenticated statistical information from researchers, there is likewise a gap in knowledge concerning applying these substances in an inorganic control program. There is a need for adequate scientific information and infrastructure to produce and continuously supply a naturally-occurring substance such as fossil shell flour for improving livestock production locally and internationally. There is also a need for awareness programs regarding fossil shell flour used in the livestock industry for both farmers and consumers. Additional investigation is needed to motivate for the broader potential of fossil shell flour as an agent of improvement in livestock production. In this context, it can be assumed that diatomaceous earth will achieve a greater role in livestock production and food safety in the near future.

2.9. Summary

As a result of a global increase in organic farming, from the demand for organic livestock edible products by consumers and food safety campaign programs, fossil shell flour can be a potential attraction to both scientists and commercial farmers. Its use has not yet been adopted in most countries, particularly in sub-Saharan Africa. There are relatively few studies investigating the potential of fossil shell flour in livestock, especially in small stock. Besides an absence of authenticated statistical information from researchers, there is likewise a gap in knowledge concerning applying these substances in an inorganic control program. There is a need for adequate scientific information and infrastructure to produce and continuously supply a naturally-occurring substance such as fossil shell flour for improving livestock production locally and internationally. There is also a need for awareness programs regarding fossil shell flour used in the livestock industry for both farmers and consumers. Additional investigation is needed to motivate for the broader potential of fossil shell flour as an agent of improvement in livestock production. In this context, it can be assumed that diatomaceous earth will achieve a greater role in livestock production and food safety in the near future.

2.10 References

Abd El-Tawab, M.M., Youssef, M.I., Bakr H.A., Fthenakis G.C., Giadinis, N.D. 2016. Role of probiotics in nutrition and health of small ruminants. Polish Journal of Veterinary Sciences.19:893–906.

Acheampong-Boateng, O., Bakare, A.G., Nkosi, D.B., Mbatha, K.R. 2017. Effects of different dietary inclusion levels of macadamia oil cake on growth performance and carcass characteristics in South African mutton merino lambs. Tropical Animal Health and Production. 49:733.

Adebiyi, O.A., Sokunbi, O.A., Ewuola, E.O. 2009. Performance Evaluation and Bone Characteristics of Growing Cockerel Fed Diets Containing Different Levels of Diatomaceous Earth. Middle-East Journal of Scientific Research. 4:36–39.

Adeyemo, G.O., 2013. Growth performance of broiler chicken fed Fossil shell flour growth promoter. food and nutrition science. 4:16-19**.**

Ahmed, M.A., Laing, M.D., Nsahlai, I. V. 2013. Studies on the ability of two isolates of Bacillus thuringiensis, an isolate of Clonostachys rosea f. rosea and a diatomaceous earth product to control gastrointestinal nematodes of sheep. Biocontrol Science and Technology.23:1067–1082.

Ahmed, S., Ahmad, H., Nadeem, M.S. 2017. First checklist and distribution of sheep breeds of Khyber Pakhtunkhwa, Pakistan. 5:181–183.

Akin, S., Schembre, J.M., Bhat, S.K., Kovscek, A.R. 2000. Spontaneous imbibition characteristics of diatomite. Journal of Petroleum Science and Engineering. 25:149–165.

Al-Degs, Y.S., Tutunju, M.F., Shawabkeh, R.A. 2000. The feasibility of using diatomite and Mn-diatomite for remediation of Pb2+, Cu2+, and Cd2+from water. Separation Science and Technology. 35:2299,–2310.

Al-Ghouti, M., Khraisheh, M. A. M., Ahmad, M. N. M., Allen, S. 2005. Thermodynamic behaviour and the effect of temperature on the removal of dyes from aqueous solution using modified diatomite: A kinetic study. J Colloid Interface Sci. 287:6-13.

Atikah, N., Alimon, A.R., Yaakub, H., N. Abdullah, N., M. F. Jahromi, M.F., M. Ivan, M., Samsudin, A.A .2018. Profiling of rumen fermentation, microbial population and digestibility in goats fed with dietary oils containing different fatty acids. *BMC Veterinary Research*. BMC Veterinary Research. 14: 1–9.

Alisson-silva, F., Kawanishi, K., Varki, A. 2016. Molecular Aspects of Medicine Human risk of diseases associated with red meat intake: Analysis of current theories and proposed role for metabolic incorporation of a non-human sialic acid. Molecular Aspects of Medicine. 51:16–30.

Allden, W.G., Black, J.L., Reis P. 1979. Feed intake, diet compositions and wool growth: Physiological and environmental limitations to wool growth. In: Proceedings of a national workshop Laura, NSW, University of New England Publishing, Armidale, NSW; p. 61–78.

Amy, C. M. 2016. Studies of Northern Fowl Mite Host-Parasite Interactions and Evaluation of Novel Control Strategies in Poultry Permalink. University of California, Riverside, United States.

Angela, F. D., El-Gamouz, A.; Sofia, F., Vanina M., Liliana, V., Oliver, A.W. 2012. Turning the volume down on heavy metals using tuned diatomite. A review of diatomite and modified diatomite for the extraction of heavy metals from water. J Hazard Mater. 14:241–242.

Antunovic, Z., Maric, I.,Steiner, Z.,Vergara, M., Novoselec, J. 2011. Blood Metabolic

Profile of the Dubrovnik Sheep. Croatian Endangered Breed', *Macedonian Journal of Animal Science*, 1(1): 35–38.

Arthur, F.H. 1999. Grain protectants: current status and prospects for the future. J Stored Prod Res. 32:293–302.

Aw, M. S., Bariana, Y.M., Yu, Addai-Mensah, J., Losic, D. 2013. Surface-functionalized diatom microcapsules for drug delivery of water-insoluble drugs. J Biomat Applic 28:163–174.

Badii, B.K., Adarkwah, C., Obeng-ofori, D., Ulrichs, C. 2014. Efficacy of diatomaceous earth formulations against Callosobruchus maculatus (F.) (Coleoptera: Bruchidae) in Kersting's groundnut (Macrotyloma geocarpumHarms): influence of dosage rate and relative humidity. Journal of Pest Science. 87:285–294.

Bakr, M., Hossam, E., Galal, M. 2010. Diatomite: Its Characterization, Modifications and Applications. Asian Journal of Materials Science. 2:121–136.

Baltran, M.A., Martins, R. 2015. Original article diatomaceous earth inhibited the. Philipp J Vet Anim Sci. 41:135–140.

Barakat, M.A. 2011. New trends in removing heavy metals from industrial wastewater. Arabian Journal of Chemistry. 4:361–377.

Begg, D.J., Purdie, A.C., De Silva, K., Dhand, N.K., Plain, K.M., Whittington, R.J. 2017. Variation in susceptibility of different breeds of sheep to Mycobacterium avium subspecies paratuberculosis following experimental inoculation. Veterinary Research. 48:1–11.

Bello, O.S., Adegoke, K.A., Oyewole, R.O. 2014. Insights into the Adsorption of Heavy Metals from Wastewater using Diatomaceous Earth. Separation Science and Technology.49:1787–1806.

Bennett, D.C., Yee, A., Rhee, Y.J., Cheng, K.M. 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., Ahmedna, M. 2009a. The Effects of Diatomaceous Earth on Parasite Infected Goats. Bulletin of the Georgian National Academy of Sciencesi. 3:129–135.

Bernard, G., Worku, M., Ahmedna, M. 2009b. The Effects of Diatomaceous Earth on Parasite Infected Goats. Bulletin of the Georgian National Academy of Sciencesi. 3:129–135. **Besier, R.B., Kahn, L.P., Sargison, N.D., Van Wyk, J. 2016.** The pathophysiology, ecology and epidemiology of Haemonchus contortus infection in small ruminants. Adv Parasitol. 93:95–143.

Bhat, S. A., Manzoor, R. M., Qadri, S., Allaie, I., Khan, H.M. 2011. comparative resistance of Sheep Breeds to Strongyle spp. In pasture infection in Jammu and Kashmir. Int J Vet Sci Technol. 10:2157–7579.

Caliskan, N., Kul, A.R., Alkan, S., Sogut, E.G., Alacabey, I. 2011. Adsorption of Zinc (II) on diatomite and manganese-oxide-modified diatomite: A kinetic and equilibrium study. Journal of Hazardous Materials. 193:27–36.

Carlisle, E.M. 1976. In vivo requirement for silicon in articular cartilage and connective tissue formation in the chick. J Nutr. 106:478–484.

Cardellino, R.C., Mueller, J.P. 2008. Proc. Symp. on Natural Fibres, In: Proc Symp on Natural Fibres.Uruguay**.**

Carneiro, H., Louvandini, H., Paiva, S.R., Macedo, F., Mernies, B., Mcmanus, C. 2010. Morphological characterization of sheep breeds in Brazil, Uruguay and Colombia. Small Ruminant Research.94:58–65.

Casey, A.E., Wilson, B.C.D. 2016. The Dohne in Australia. In: Cameron Hills, editor. Global Dohne Conference, Dubbo NSW, Dubbo NSW. Dubbo NSW: Dohne Merino Breeders Association, NSW Australia; p. 3–5**.**

Cériac, S., Jayles, C., Arquet,R., Feuillet,D., Y., Félicité, Y., Archimède, H., Bambou,J.C. 2017. The nutritional status affects the complete blood count of goats experimentally infected with Haemonchus contortus, *BMC Veterinary Research*, 13(1):1-11**.**

Chen, Y., Liu, K. 2016. Preparation of granulated N-doped TiO2/diatomites and its application of visible light degradation and disinfection. Power Tech. 303:176–191.

Cilek, S., Breeding, A., Medicine, V. 2015. Determination of Fleece Qualities of Malya Sheep (11/ 16 Akkaraman X 5 / 16 Deutsches Merinofleischschaf) and Effect of Age and Sex on These Qualities. Pak J Agri Sci. 52:533–540.

Crangle, R. J. 2010. Diatomite: U.S. Geological Survey Mineral Commodity Summaries. In: U.s Geological survey summaries; p. 52–53.

Chessa, B., Pereira, F., Arnaud, F., Amorim, A., Mainland, I., Kao, R.R., Pemberton, J.M., Beraldi, D., Stear, M.J., Alberti, A. 2009. Revealing the History of Sheep Domestication Using Retrovirus Integrations. Science. 324:22–27.

Chulayo, A.Y., Muchenje, V. 2013. The effects of pre-slaughter stress and season on the activity of plasma creatine kinase and mutton quality from different sheep breeds slaughtered at a smallholder abattoir. Asian-Australasian Journal of Animal Sciences. 26:1762–1772.

Cloete, S.W.P., Cloete, J.J.E. 2014. Production Performance of Merino and Dohne Merino Ewes and Lambs in Pure or Crossbreeding Systems. 43:217–220.

Cloete, S.W.P., Scholtz, A.J, Aucamp, B. 1998. Environmental effects, heritability estimates and genetic trends in a Western Cape Dohne Merino nucleus flock. South African Journal of Animal Sciences. 28:185–195**.**

Colledge, S., Conolly, J., Shennan, S. 2005. The evolution of Neolithic farming from SW Asian origins to NW European limits. European Journal of Archaeology. 8:137–156.

Danil de Namor, A.F., El Gamouz, A., Frangie, S., Martinez, V., Valiente, L., Webb, O.A. 2012. Turning the volume down on heavy metals using tuned diatomite. A review of diatomite and modified diatomite for the extraction of heavy metals from water. Journal of Hazardous Materials. 30:241–242.

Damptey, J.K., Obese, F.Y., Aboagye, G.S., Ayim-Akonor, M., Ayizanga, R.A. 2014. Blood metabolite concentrations and postpartum resumption of ovariancyclicity in sanga cows', South African Journal of Animal Sciences, 44:10-17.

David, L., George, T., Haenlein, F.W. 2017. Production of Sheep Milk. In: Handbook of Milk of Non-Bovine; p. 102.

Dawson, R.D., Dawson, R.D. 2010. Efficacy of Diatomaceous Earth at Reducing Populations of Nest-Dwelling Ectoparasites in Tree Swallows / Eficacia de diatomáceas terrestres en reducir las poblaciones de ectoparásitos en nidos en Tachycineta bicolor Published by: Wiley on behalf of Assoc. J Field Orni- thol. 75:232–238.

Deutschlander, D. 1993. Evaluating Diatomaceous Earth as a Wormer for Sheep and Cattle Energy and Sustainable Agriculture Program - Minnesota Department of Agriculture, United States.

Deniskova, T.E., Dotsev, A.V., Selionova, M.I., Kunz, E., Medugorac, I., Reyer, H., Wimmers, K., Barbato, M., Traspov, A.A., Brem, G., Zinovieva, N.A. 2018. Population structure and genetic diversity of 25 Russian sheep breeds based on whole-genome genotyping. Genetics Selection Evolution. 50:1–16.

Dias, R.S., López, S., Patiño, R.M., Silva, T.S., Silva Filho, J.C., Vitti, D.M.S.S., Peçanha, M.R.S.R., Kebreab, E., France, J. 2013. Calcium and phosphorus utilization in growing sheep supplemented with dicalcium phosphate. Journal of Agricultural Science. 151:424–433.

Díaz, P., Quílez, J., Prieto, A., Navarro, E., Pérez-Creo, A., Fernández, G., Panadero, R., López, C., Díez-Baños, P., Morrondo, P. 2015. Cryptosporidium species and subtype analysis in diarrhoeic pre-weaned lambs and goat kids from north-western Spain. Parasitology Research. 114:4099–4105.

Dolatabadi, J. E. N., M de la G. 2011. Applications of diatoms and silica nanotechnology in biosensing, drug and gene delivery, and formation of complex metal nanostructures. Trends Analyt Chem. 30:1538–1548.

Ebeling, W. 1971. Sorptive dust for pest control. Ann Rev Entomol. 16:123–158.

Eldernawi A.M., Rious M.J., Al-Samarrai K.L. 2014. Chemical, Physical and Mineralogical, Characterization of\nAl-Hishal Diatomite at Subkhah Ghuzayil Area, Libya. International Journal of Research in Applied, Natural and Social Sciences 2:165-174.

Emeruwa, C.H. 2016. Growth Performance of West African Dwarf Sheep Fed Diets Supplemented with Fossil shell flour. University of Ibadan, Nigeria

Epstein, H. 1971. The Origin of the Domestic Animals of Africa. African Publishing Corporation, New York. In: The Origin of the Domestic Animals of Africa.

Esfandyari, H., Aslaminejad, A.A., Rafat, S.A. 2011. Wool characteristics in the third generation of Arkharmerino × Ghezel and Arkharmerino × Moghani crossbreed sheep. Tropical Animal Health and Production. 43:1337–1343.

Food and Agriculture Organization (FAO). 2012. The State of Food and Agriculture 2012: Investing in Agriculture for a Better Future.Rome, Italy.

Food and Agricultural Organization (FAO). 2017. The future of food and agriculture: Trends and challenges. Rome. Available from: http://www.fao.org/3/a-i6583e.pdf.(accessed on 18/06/2018).

Ferguson, E.G.W., Mitchell, G.B.B., MacPherson, A. 1989. Cobalt deficiency and Osterfagia circumcincra infection in lambs. In: Vet Rec,124. p. 20.

Fox, C.B, Haensler, J. 2013. An update on safety and immunogenicity of vaccines containing emulsion-based adjuvants. Expert Review of Vaccines. 12:747–758.

Galyean, M.L., Perino, L.J., Duff, G.C. 1999. Interaction of cattle health/immunity and nutrition. Journal of Animal Science. 77:1120–1134.

Gao, B., Jiang, P., An, F., Zhao, S., Ge, Z. 2005. Studies on the surface modification of diatomite with polyethyleneimine and trapping effect of the modified diatomite for phenol. Applied Surface Science. 250:273–279.

Garcia, G., Araújo, L., De Voltolini, T.V., Chizzotti, M.L., Turco, H.N., Fernando, F., Carvalho, R. D. 2010. Revista Brasileira de Zootecnia Water and small ruminant production. 2010:326–336.

Geoffrey, N., Hinch. 2017. Understanding the natural behaviour of sheep. :1–15. Available from: http://www.naturalstep.ca/understanding-the-problem (Acessed on 9/11/2018)**.**

Ghafouri-Kesbi, F., Notter, D.R. 2016. Sex influence on genetic expressions of early growth in Afshari lambs. Archives Animal Breeding. 59:9–17.

Golder, H.M., Celi, I.P., Rabiee, A.R., Lean, I.J. 2014. Effects of feed additives on rumen and blood profiles during a starch and fructose challenge. J. Dairy Sci. 97 :985–1004

Goren, R., Baykara, T., Marsoglu. M. 2002. A study on the purification of diatomite in HCl. Scandinavian Journal of Metallurgy. 31:115–119.

Gowane, G., Gadekar, P., Prakash, V., Kadam, V., ChopraL, L.P. 2017. Climate Change Impact on Sheep Production: Growth, Milk, Wool, and Meat. In: Sheep Production Adapting to Climate Change. p. 31–69.

Hatcher, S., Atkins, K.D., Thornberry, K. 2005. Age changes in wool traits of Merino sheep in western NSW. Pro Assoc Adv Anim Breed Genet. 16:316–317.

Inter-government Panel on Climate Change (IPCC). 2018. 2018/24/PR IPCC PRESS RELEASE 8 October 2. Geneva, Switzerland. (Accessed on 6/04/2019)

Henderson, G., Faith Cox, F., Ganesh, S., Jonker, A., Young, W., Janssen, P.H. 2015. Rumen microbial community composition varies with diet and host, but a core microbiome is found across a wide geographical range. *Scientific Reports*, 5(October): 14567lhafid

Hoffman, M.L; Peck, K.N; Forella M.E; Fox, A.R; Govoni, K.E and S. A. Zinn, S.A. 2016. The effects of poor maternal nutrition during gestation on postnatal growth and development of lambs. Journal of Animal Sci.94:789–799.

Huisman, A.E., Brown D. 2008. Genetic parameters for bodyweight wool, and disease resistance and reproduction traits in Merino sheep 2. Genetic relationships between bodyweight traits and other traits. Aust J Exp Agric. 48:1186–1193.

Hunter, R.L. 2002. Overview of vaccine adjuvants: Present and future. Vaccine. 20:7–12**.**

Hussain, H.U., RH Usmani. 2006. Livestock of Pakistan. 1st Ed., Livestock Foundation, Islamabad.

Ijaz, M., M. Khan, M., Avais, K., Ashraf, M., Ali, M. 2009. Infection rate and chemotherapy of various helminths in diarrhoeic sheep in and around Lahore. Journal Animal Sciences, 19:13–16.

James, P.J., Horton, B.J., Campbell, N.J., Evans, D.L., Winkleman, J., McPhie, R. 2011. Population dynamics and production effects of sheep lice (Bovicola ovis Schrank) in extensively grazed flocks. Animal Production Science. 51:753–762.

Jawasreh, K.I., Ababneh, M.M., Ismail, Z.B., Younes, A.M., Al Sukhni, I. 2018. Genetic diversity and population structure of local and exotic sheep breeds in Jordan using microsatellites markers. Veterinary World. 11:778–781.

Jiwuba, P.D.C., Jiwuba, L.C., Onyekwere, M.U. 2017. Growth performance, haematology and serum biochemistry of West African dwarf sheep fed cassava peel - oil palm leaf meal-based diets in a hot humid tropic. Agricultura. 14:37–44.

Kara, K., Aktug, E., Ozkaya, S. 2016. Ruminal digestibility, microbial count, volatile fatty acids and gas kinetics of alternatives forgae sources for arid and semi-arid areas as *in vitro*. Italian J. Anim. Sci. 15(4), 673-680

Kilpinen, O., Steenberg, T. 2009. Inert dusts and their effects on the poultry red mite. Expermental and Applied Acarology. 48:51–62.

Kijas, J., Townley, D.B. 2009. A genome wide survey of SNP variation reveals the genetic structure of sheep breeds. PLoS One. 4:4668.

Korunic, Z. 1998. Diatomaceous earths, a group of natural insecticides. Journal of Stored Products Research. 34:87–97.

Koster. H. 2013. Diatomite in Animal Feeds.Available from agrisilica.co.za/pdf/eng/Diatoms%20in%20Animal%20Feeds%20HH%20Koster.pdf. (Accessed: 20th June 2018).

Koyuncu, M. 2012. Color removal from aqueous solution of tar- chromium green 3G dye using natural diatomite. Physicochem Probl Miner Process. 48:485.

Lakkawar, A.W., Sathyanarayana, M.L., Narayanaswamy, H.D., Sugunarao, O., Yathiraj, S. 2016. Efficacy of diatomacious earth in amelioration of aflatoxin induced toxicity in broiler chicken. Indian Journal of Animal Research. 50:529–536.

Lean, G.R. 2016. How the Dohne stack up financially. Proceeding in Global Dohne Conference. Dubbo, NSW, Australia. p. 7–9.

Li, Y.X., Zhang, X.L., Li, W., Lu, X.F., Liu, B., Wang, J. 2013. The residues and environmental risks of multiple veterinary antibiotics in animal faeces. Environmental Monitoring and Assessment. 185:2211–2220.

Lv, F.H., Peng, W.F., Yang, J., Zhao, Y.X., Li, W.R., Liu, M.J., Ma, Y.H., Zhao, Q.J., Yang, G.L., Wang, F.2015. Mitogenomic meta-analysis identifies two phases of migration in the history of Eastern Eurasian sheep. Molecular Biology and Evolution. 32:2515–2533.

Ma, G. W., Chu, Y. K., Zhang, W. J., Qin, F. Y., Xu, S. S., Yang, H., Rong, E. G., Du, Z. Q., Wang, S. Z., Li, H., Wang, N. 2017. Polymorphisms of FST gene and their association with wool quality traits in Chinese Merino sheep. PLoS ONE. 12:1–13.

Martel-Kennes, Y., Lévesque, J., Decaux, C. 2016. Effect of crystalline silicon dioxide in piglet feed on growth performance with different levels of growth promoters. Journal of Animal Science, 94:488.

Martin, C., Mullen, B. 2012. Housing and dustbathing effects on northern fowl mites (Ornithonyssus sylviarum) and chicken body lice (Menacanthus stramineus) on hens. Advances in Veterinary Technology and Procedures: A Multi Journal of Virtual Issues. 26:323–333.

Martin, K. 2007. chemistry of silica and its potential health benefits. J Nutri Health Aging. 11:94–97.

Martin, K. 2013. Silicon: the health benefits of a metalloid. Met Ions Life Sci. 13:451–471.

McClure, S.J. 2003. Mineral nutrition and its effects on gastrointestinal immune function of sheep. Aust J Exp Agric. 43:1455–1461.

McGregor, B.A., De Graaf, S.P., Hatcher, S. 2016. On-farm factors affecting physical quality of Merino wool. 1. Nutrition, reproduction, health and management. Small Ruminant Researc137:138–150.

Meeusen, E. N. T., Walker, J., Peters, A., Pastoret, P.P., Jungersen G. 2007. Current status of veterinary vaccines. Clin Microbiol Rev. 20:489–510.

Mertz, W. 2012. Trace Elements in Human and Animal Nutrition. In: Trace Elements in Human and Animal Nutrition, Volume 2 Academic Press Inc, Hacourt Brace Jovanovich Publishers, Orlando, Florida 32887. [place unknown]; p. 133–154.

Miller, P. J., Afonso, C.L., Attrache, J.E., Dorsey, K.M., Courtney, S.C., Guo, Z.J., Kapczynski D. 2013. Effects of Newcastle disease virus vaccine antibodies on the shedding and transmission of challenge viruses. Dev Comp Immunol. 41:505–507.

Modirsane, M., Mansoori, B., Khosravi, A.R., Kiaei, M.M., Khazraeinia, P., Farkhoy, M., Masoumi, Z. 2008. Effect of diatomaceous earth on the performance and blood variables of broiler chicks during experimental aflatoxicosis. 632:626–632.

Möhner, M., Pohrt, A., Gellissen, J. 2017. Occupational exposure to respirable crystalline silica and chronic non-malignant renal disease: systematic review and meta - analysis. International Archives of Occupational and Environmental Health. 90:555–574.

Moleshi, P., Nahid, P. 2015. Heavy metal removal from water and wastewater using raw and modified diatomite. International journal of Engineering transaction B: Application. 20:141–146.

Molotsi, A., Dube, B., Oosting, S., Marandure, T., Mapiye, C., Cloete, S., Dzama, K. 2017. Genetic traits of relevance to sustainability of smallholder sheep farming systems in South Africa. Sustainability. 9:1–19.

Mount, A., S., Koernig, A., Silva, D., Drane, E., Maraskovsky, Morelli, A. 2013. Combination of adjuvants: the future of vaccine design. Exp Rev Vaccines. 12:733–746.

Mvinjelwa, S.A., Mapekula, M., Maphosa, V., Muchenje, V. 2014. Body and fleece weights of woollen sheep in veld communal rangelands of the Eastern Cape Province. Tropical Animal Health and Production. 46:913–918.

Naidoo, P., Olivier, J.J., Morris, J., Cloete, S.W.P. 2016. Does selecting for finer wool re sult in higher incidence of creeping belly in the South African Dohne Merino sheep breed? 13:49–53.

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

Nazmi, A., Hauck, R., Davis, A., Hildebrand, M., Corbeil, L.B., Gallardo, R.A. 2017. Immunology, health, and disease: Diatoms and diatomaceous earth as novel poultry vaccine adjuvants. Poultry Science. 96:288–294.

Novoselec, J., Šperanda, M., Klir1, Z., Mioč, B., Steiner, Z., Antunović, Z. (2017). Blood biochemical indicators and concentration of thyroid hormones in heavily pregnant and lactating ewes depending on selenium supplementation. Acta Vet. Brno. 86: 353–363

Nwosu, C.O., Madu, P.P., Richards, W.S. 2007. Prevalence and seasonal changes in the population of gastrointestinal nematodes of small ruminants in the semi-arid zone of north-eastern Nigeria. Veterinary Parasitology. 144:118–124.

Nsoso, S.J., Podisi, E., Otsogile, B. S., Mokhutshwane, B.A. 2004. Phenotypic Characterization of Indigenous Tswana Goats and Sheep Breeds in Botswana: Continuous Traits. Tropical Animal Health and Production. 36:1573–7438.

Oana, C., Tănaselia, C., Miclean, M., Levei, E., Senilă, M., Senilă L. 2016. Analysis of minor and trace element in Cow, Goat and Sheep milk in NW part of Romania. Pro-Environment. 9:87–90.

Ocampo, R.J., Martinez, R., Rocha, J.J., Cardona, H. 2017. Genetic characterization of Colombian indigenous sheep. Revista Colombiana de Ciencias Pecuarias. 30:116–125.

Osweiler, G.D., Carson, T.L. 1997. Evaluation of diatomaceous earth as an adjunct to sheep parasite control in organic farming. Leopold Center for Sustainable Agriculture Progress Reports. 6.

Peters, F.W., Kotze, A., van der Bank, F.H., Soma, P., Grobler, J.P. 2010. Genetic Profile of the Locally Developed Meatmaster Sheep Breed in South Africa Based on Microsatellite Analysis. Small Rumin Res. 90:101–108.

Pick, T. 2011. Assessing Water Quality for Human Consumption, Agriculture, and Aquatic LifeUses.Availablefrom:https://www.nrcs.usda.gov/Internet/FSE_DOCUMENTS/nrcs144p2_051302.pdf. (Accessed on 10/8/2018)**.**

Pirsaraei, A., Reza, S., Hasan, A.M., Ahmad, J.J., Zohreh, F., Jafar, T. 2015. The Effect of Acid and Thermal Treatment on a Natural Diatomite. Chemistry Journal. 1:144–150.

Plug, I., Badenhorst, S. 2001. The distribution of macromammals in Southern Africa over the past30000 years. Transvaal Museum, Pretoria, South Africa.

Pookmanee, P., Jansanthea, P., Phanichphant, S. 2008. Adsorption of heavy metals onto natural and modified diatomite KMIT. Science Journal. 8 (2B):1–8. 8:1–8.

Pookmanee, P., Thippraphan, P., Phanichphant S. 2010. Adsorption of copper ion (II) by manganese chloride modified diatomite via hydrother- mal method. In: The proceeding of 36th Congress on Science and Technology.Texas, United States. pp 26–28.

Qudoos, A., Khan, M.N., Sajid, M.S., Muhammad, G. 2017. Correlation of trace mineral profiles with gastrointestinal worm burden in rangeland sheep of Chakwal District, Punjab, Pakistan. International Journal of Agriculture and Biology. 19:140–144.

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

Ryder, L.M. 2007. Sheep and man. In: Sheep and man. Duckworth and Co Ltd UK**.**

Sabbour, M. M., Abd-El-Aziz, S.E. 2014. Control of Bruchidius incarnatus and Rhyzopertha Dominica using two entomopathogenic fungi alone or in combination with modified diatomaceous earth. Elixir Entomology. 68:22239–22242.

Sabbour, M.M., Abd-el-aziz, S.E., Sherief, M.A. 2012. Efficacy of Three Entomopathogenic Fungi Alone or in Combination with Diatomaceous Earth Modifications for the Control of Three Pyralid Moths in Stored Grains. Journal of Plant Protection Research. 52:360–362.

Safa, M., Larouci, M., Meddah, B., Valemens, P. 2012. The sorption of lead, cadmium, copper and zinc ions from aqueous solutions on raw diatomite from Algeria. Water Sci Technol.65:1729.

Safari, E., Fogarty, N.M., Gilmour, A.R. 2005. A review of genetic parameter estimates for wool, growth, meat and reproduction traits in sheep. Livestock Production Science. 92:271– 289.

Salehian, Z., Naderi, N., Souri, M., Mirmahmoudi, R., Hozhabri, F. 2015. Seasonal variation of fibre follicle activity and wool growth in fat-tailed Sanjabi sheep in west Iran. Tropical Animal Health and Production. 47:567–573.

Sarijit, J., Steve, S., Micheal, E., Rodney, D. 2016. Animal feed compositions including spent filter media containing diatomaceous earth. US20160295884A1US Application. US20160295.

Saxena, V.K., Jha, K.B., Meena, S.A., Naqvi, S.M. 2015. Characterization of MTNR1A gene in terms of genetic variability in a panel of subtemperate and subtropical Indian sheep breeds. Journal of Genetics. 94:715–721.

Schat, K.A. 2014. Vaccines and vaccination practices: key to sustainable animal production. Encyclopedia of Agriculture and Food Systems Elsevier Inc, San Diego vol5.

Scherf, B.D. 2000. World watch list for domestic animal diversity. In: Food and Agriculture Organization of the United Nations. Rome: Food and Agriculture Organization of the United Nation.

Sejian, V., Maurya, V. P., Naqui, S.M.K. 2012. Adaptability and growth of Malpura ewes subjected to therma and nutritional stress. Tropical Animal Health and Production. 42:1763–1770.

Sejian, V., Maurya, V.P., Naqvi, S.M.K., Kumar, D., Joshi, A. 2010. Effect of induced body condition score differences on physiological response, productive and reproductive performance of Malpura ewes kept in a hot, semi-arid environment. Journal of Animal Physiology and Animal Nutrition. 94:154–161.

Schijns, V.E., Lavelle, E.C. 2011. Trends in vaccine adjuvants. Exp. Rev. Vaccines. 10:539–550. ExpRevVaccines. 10:539–550.

Schingoethe, D.J., Kalscheur, K.F., Hippen, A.R., Garcia, A.D. 2009. Invited review: The use of distillers' products in dairy cattle diets. Journal of Dairy Science. 92:5802– 5813.

Schlink, A.C., Nguyen, M., Viljoen, G.J. 2010. Water requirements for livestock production: Water requirements for livestock. Rev.Sci.tech.. 29:603–619.

Schröder, U. J., 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): 1–14.

Seaborn, C.D., Nielsen, F.H. 1994. Boron and silicon: Effects on growth, plasma lipids, urinary cyclic amp and bone and brain mineral composition of male rats. Environmental Toxicology and Chemistry. 13:941–947.

Sejian, V., Maurya, V.P., Naqui, S.M.K. 2012. Adaptability and growth of Malpura ewes subjected to therma and nutritional stress. Tropical Animal Health and Production. 42:1763–1770.

Shah, M.A., Khan, A.A. 2014. Use of diatomaceous earth for the management of stored-product pests. International Journal of Pest Management. 60:100–113.

Shahnawaz, M., Shahardar, R. A.and Wani ZA. 2011. Seasonal prevalence of platyhelminthosis of sheep in gandarbal area of Kashmir Vally. Vet Parasitol. 25:59–62**.**

Sheng, G., Yang, S., Sheng, J., Hu, J., Tan, X., Wang, X. 2011. Macroscopic and microscopic investigation of Ni (II) sequestration on diatomite by batch, XPS and EXAFS techniques. Environ Sci Technol. 45:7718.

Shinde, A.K., Sejian, V. 2013. Sheep husbandry under changing climate scenario in India: An overview. Indian Journal of Animal Sciences. 83:998–1008.

Singh, C.B., Jayas, D.S., Paliwal, J., White, N.D.G. 2009. Detection of insect-damaged wheat kernels using near-infrared hyperspectral imaging. Journal of Stored Products Research. 45:151–158**.**

Singh, M., O'Hagan, D.T. 2003. Recent advances in veterinary vaccine adjuvants. International Journal for Parasitology. 33:469–478.

Slamova, R., Trckova, M., Vondruskova, H., Zraly, P.I. 2011. Clay minerals in animal nutrition. Appl Clay Sci 51:395–398.

Soetan, K.O., Olaiya, C.O., Oyewole, O.E. 2010. The importance of mineral elements for humans, domestic animals and plants: A review. African Journal of Food Science. 4:200–222.

Stathers, T.E., Riwa, W., Mvumi, B.M, Mosha, R., Kitandu, L., Mngara, K., Kaoneka, B., Morris, M. 2008. Do diatomaceous earths have potential as grain protectants for small-holder farmers in sub-Saharan Africa? The case of Tanzania. Crop Protection. 27:44–70.

Streit, E., Schatzmayr, G., Tassis, P., Tzika E., Marin, M., Taranu, I., Tabuc, C., Nicolau, A., Aprodu, I., Puel O. 2012. Current situation of mycotoxin contamination and co- occurrence in animal feed—Focus on Europe. toxins. 4:788–809.

Sumner, R.M.W., Ashby, M.G., Craven AJ. 2004. Relation between sweating and wool yellowing in Merino and Romney sheep. In: Proceedings of the New Zealand Society of Animal Production. p. 287.

Sumner, R.M.W., Young, S.R., Upsdell, M. 2003. Wool yellowing and pH within Merino and Romney fleeces. In: Proc New Zeal Soc Anim. 65: 155–159.

Sumner, R.M.W. 2005. Relationship of mean fibre diameter and time of shearing with wool yellowing in Merino sheep farmed under North Island conditions. Proc New Zeal Soc Anim. 65:191–196.

Snowder, G.D. 2009. Composite trait selection to improve reproduction and ewe productivity: a review. Anim Prod Sci. 49:9–16.

Snyman, M. 2014. South African sheep breeds: Dorper: Dorper sheep: Monthly magazine March.1–2.

Soma, P., Kotze, A., Grobler, J.P., van Wyk, J.B. 2012. South African sheep breeds: Population genetic structure and conservation implications. Small Ruminant Research. 103:112–119.

Swanepoel, J.W. 2006. A Genetic Evaluation of the Dohne Merino. Dissertation submitted to the Faculty of Natural and Agricultural Sciences, Department of Animal, Wildlife and Grassland Sciences, University of the Free State, South Africa.

Taberlet, P., Valentini, A., Rezaei, H.R., Naderi, S., Pompanon, F., Negrini, R., Ajmone-Marsan, P. 2008. Are cattle, sheep, and goats endangered species? Molecular Ecology. 17:275–284.

Tapio, M., Marzanov, N., Ozerov, M., Ćinkulov, M., Gonzarenko, G., Kiselyova, T., Murawski, M., Viinalass, H., Kantanen, J. 2006. Sheep mitochondrial DNA variation in European, Caucasian, and Central Asian areas. Molecular Biology and Evolution. 23:1776–1783.

Tapio,I., Snelling, T.J., Strozzi, F., Wallace,R.J. I. 2017. The ruminal microbiome associated with methane emissions from ruminant livestock. Journal of Animal Science and Biotechnology, 8(1):0–11.

Thornton, P.K. 2010. Livestock production: Recent trends, future prospects. Philosophical Transactions of the Royal Society B: Biological Sciences. 365:2853–2867.

Thota, P., Mahender, S.C., Ramana, D. B. V. 2017. Effect of Probiotic Supplementation on Nutrient Digestibilities, Growth Performance and Enteric Methane Emissions in Deccani Ram Lambs. J. Anim. Res. 7:1009–1017.

Tibbo, M. 2006. Productivity and health of indigenous sheep breeds and crossbreds in the central Ethiopian highlands. Doctoral thesis, Department of Animal Breeding and Genetics, Swedish University of Agricultural Sciences Uppsala, Swedden. 76pp**.**

United States Enviromental Protection Agency (USEPA). 2009. National Primary Drinking Water Regulations. Usepa. 1:7. Available from: https://www.epa.gov/ground-water-and-drinking-water/national-primary-drinking- water-regulations. (Accessed on 11/2/2018).

Van Burgel, A.J., Oldham, C.M., Behrendt, R., Curnow, M., Gordon, D.J., Thompson, A.N. 2011. The merit of condition score and fat score as alternatives to liveweight for managing the nutrition of ewes. Animal Production Science. 51:834–841.

Van Pleltzen. 2015. An Award-wining Merino sheep enterprise with the use of diatomaceous earth. Farmer's weekly. July 30.

Van Wyk, J. B., Swanepoel, J.W., Cloete, S.W.P., Olivier, J.J., Delport, G. 2008. Across flock genetic parameter estimation for yearling body weight and fleece traits in the South African Dohne Merino population. South African Journal of Animal Science. 38:31–37.

Van Wyk, D.N. 2011. A quantitative analysis of supply response in the Namibian mutton industry. A thesis submitted to Department of Animal Sciences, Stellenbosch University, South Africa.256pp**.**

van Zijderveld, S. M., Gerrits, W.J., Apajalahti, J.A., Newbold, J.R., Dijkstra, J., Leng, R.A., Perdok, H.B. 2010. Nitrate and sul- fate: Effective alternative hydrogen sinks for mitigation of ruminal methane production in sheep. J. Dairy Sci. 93:5856–5866.

Villagra-Blanco, R., Dolz, G., Montero-Caballero, D., Romero-Zuniga, J.J. 2015. Detection of antibodies against Chlamydophila abortus in Costa Rican sheep flocks. Open Veterinary Journal. 5:122– 126.

Waksman, B.H. 1979. Adjuvants and immune regulation by lymphoid cells. Springer Seminars in Immunopathology. 2:5–33.

Walker, G. M., Weatherley, L.R. 1999. Kinetic of acid dye adsorption on GAC. Water Res. 33:1895.

Wang, Y., J. Cai, Y. G., Jiang, X. G., Jiang, D. Y., Zhang, D. 2013. water-insoluble drugs. J Biomat Applic. 28:163–174.

Weaver, A.C., Todd, S. M., Hansen, J.A., Kim, Y.B., De Souza, A.L.P., Middleton, T.F., Kim, S.W. 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.

Wiedemann, S. G., Yan, M. J., Henry, B. K., Murphy, C. M. 2016. Resource use and greenhouse gas emissions from three wool production regions in Australia. J. Clean. Prod. 122: 121–132.

Xu, T., Xu, S., Hu, L., Zhao, N., Liu, Z., Ma, L., Liu, H., Zhao, X. 2017. Effect of dietary types on feed intakes, growth performance and economic benefit in tibetan sheep and yaks on the qinghai-tibet plateau during cold season. PLoS ONE. 12:1–16.

Yuan, P., Liu, D., Fan, M., Yang, D., Zhu, R., Ge, F., Zhu, J.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.

Zeder, M.A. 2008. Domestication and early agriculture in the Mediterranean Basin: Origins, diffusion, and impact. Proceedings of the National Academy of Sciences [Internet]. 105:11597–11604**.**

Zhang, Q., Shu, X., Guo, X., Mo, D., Wei, S., Yang, C. 2016. Effect of ions on sorption of tylosin on clay minerals. RSC Adv. 6.

Chapter 3

Effect of varying inclusion levels of fossil shell flour on growth performance, water intake, digestibility, and N retention in Dohne-Merino wethers

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Abstract

The current study determined the effects of varying levels of Fossil shell flour (FSF) supplementation on growth performance, water intake, digestibility, and N retention in Dohne Merino wethers. Twenty-four Dohne-Merino wethers (20 ± 1.5 kg body weight) were used in a complete randomized design with six wethers per treatment. The wethers were fed a basal diet without FSF inclusion (control), or with the addition of FSF (2%), (4 %), or (6%) of the diet for 105 days. Wethers fed on a diet with 4 % FSF had the highest values for dry matter intake, total weight gain, N retention as well as for most of the apparent digestibility nutrients (CP, EE, and Ash) compared to those fed on (P < 0.05). The urinary and fecal N excretion also significantly decreased in the FSF treated diets compared to those fed on a diet with 0% FSF (P< 0.05). Water intake values were highest in wethers fed on a diet containing 0% FSF and differed (P < 0.05) from those fed on diets with 2% and 6% FSF but not those on a diet with 4% FSF. It was concluded that a 4% inclusion rate of FSF would give the best improvement on growth performance, diet digestibility, and N retention of Dohne- Merino wethers. Besides, FSF inclusion in sheep's diets is a safe, natural additive that can help reduce environmental pollution by reducing fecal and urinary N excretion.

Keywords: Fossil shell flour, growth performance, digestibility, nitrogen utilization, Dohne-Merino

3.1. INTRODUCTION

The use of growth promoters in farm animal diets is growing at an increasing rate (Frater, 2014). These promoters are used to improve feed efficiency and the growth performance of farm animals. Rumen regulation through growth promoters is one of the most important methods for improving feed efficiency and, thus, growth performance. Likewise, feed additives in ruminant’s nutrition have the potential to increase dry matter intake (DMI), feed conversion efficiency (FCE), and animal productivity (Chaturvedi et al., 2014; Frater, 2014). There is a wide range of feed additives that include antibiotics, probiotics, antioxidants, enzymes, prebiotics, organic acids, mycotoxin binders, hormones, beta-agonist, defaunation agents, essential oil, and herbal feed additives, most of which are chemical-based (Chaturvedi et al., 2014)

Although the use of growth promoters as feed additives has been a hallmark of modern animal husbandry, in recent years, there have been increased concerns on chemical residues left in meat and other animal products as a result of its chemical-based feed additives (Tedeschi et al., 2011; Jalaal et al., 2015). There is also an increase in ecological risk because of the accumulation of veterinary antibiotics residue in animal manure (Li et al., 2013), bodies of water, sediments, and soils (Hu et al., 2010; Watanabe, 2010). Arikan et al. (2009) reported that antibiotics administered to farm animals either as growth promoters or medication were usually excreted without metabolism. Similarly, Pils (2005) observed that between 70 to 90 % of tetracycline might be excreted as parent compounds through urine and feaces.

As a result of the possible risks of chemical-based growth promoters, there have been increased interest and promotion of natural growth promoters (NGPs). Several plants and plant extracts, enzymes, organic acids, and oils have recently received considerable attention as possible NGPs that are eco-friendly (Valero et al., 2014). One of the major drawbacks to the use of these NGPs is the time and cost involved in harvesting them. One NGP that could be useful as a cost-effective, readily available, healthy, and eco-friendly feed additive is fossil shell flour. Fossil shell flour is a naturally occurring silicate-rich substance with important physical and chemical characteristics that enable it uses recently as a feed additive in livestock production. The substance is non-toxic, cheap, and readily available in many countries (Ikusika et al., 2019). The mineral constituent of dietary fossil shell flour includes Sodium, Copper, Zinc, Iron, Magnesium, Calcium, 2% Magnesium, Potassium, Aluminum, Sulfate sulphate, 2 %Boron, and Vanadium (Adebiyi et al., 2009; Adeyemo 2013, Lakkawar *et al*., 2016).

In the study conducted by Emeruwa (2016), using West Africa dwarf sheep, it was observed that inclusion of fossil shell flour in the diet improves their weight gain with sheep fed 4 % inclusion level of fossil shell flour growing faster than 6%. Likewise, Sarijit *et al*. (2016) reported that FSF's addition to heifers' feeds at 2% levels improves daily feed intake, weight gain, and feed conversion efficiency. Emeruwa (2016) reported an optimum inclusion level of 4 % FSF on digestibility crude protein and 2% FSF on N retention in sheep.

Although few research works have been documented in the literature on the effect of FSF on the growth performance of West African Dwarf sheep in a tropical region, it is also very necessary to confirm its effects on a dual-purpose breed of sheep domiciled in a semi-arid region. Moreso, there is a paucity of information on feedlot performance of Dohne-Merino supplemented with varying levels of FSF. Still, none reported on the effect of FSF on the water intake of Dohne- Merino. This is important because water and feed resources have a direct relationship (Mpendulo, 2016). It is also essential to understand the possible effect of FSF on the water consumption of sheep since the sub-Sahara region is known for water scarcity (Ran et al., 2016). Considering that FSF's use can be cost-effective, healthy, and eco-friendly if the effectiveness of FSF in improving the growth performance of sheep is ascertained, farmers, especially poor-resources ones, can increase their productivity through FSF. This will also be communicated to the policymakers to promote FSF as a feed additive instead of antibiotics. Therefore, the objective of this study was to assess the effects of four levels of FSF inclusion on growth performance, water intake, digestibility, and N retention of Dohne- Merino sheep. It was then hypothesized that the increase in FSF inclusion in diets increases the growth performance, digestibility of nutrients, and N retention in Dohne-Merino sheep.

3.2 Materials and Methods

3.2.1. 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)].

3.2.2. Study site description

The experiment was conducted at the small ruminant unit of the University of Fort Hare teaching and research farm, Alice, Eastern Cape, South Africa. The research farm lies at a longitude 26º 50' E and latitude of 32°46' S. The annual rainfall is between 480-490 mm and a temperature range between 24.6 °C and 11.1 °C (average is 17.8 °C) at an altitude of 535 meters above sea level.

3.2.3. Animal, experimental design and management

Twenty-four five months old Dohne-Merino wethers weighing 20 ± 1.5kg on average were selected from a commercial farm in Mitford village, Tarkastad, Eastern Cape province, South Africa. All the 24 wethers were bought from the same farm. 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. The pens had a similar temperature, relative humidity, and sunlight conditions. The experiment lasted for 105 days, excluding 14 days of the adaptation period. The wethers had access to sufficiently clean and fresh water ad libitum on a daily basis. Each wether was ear-tagged and labeled for identification on a diet basis.

3.2.4. Experimental Diets

The diets for the wethers consisted of concentrate and hay at a 40:60 ratio. The concentrate was made up of maize (8%), sunflower oil cake (10 %), molasses (5%), wheat offal (15%), limestone (1.5%), salt 0.3%, and sheep mineral-vitamin premix (0.2%), whereas the hay consisted of 30 % teff and 30 % Lucerne. The ingredients for concentrate were purchased from Monti Feeds (pty) Ltd, East London, South Africa. In contrast, the teff and Lucerne were purchased from Umtiza Agricultural products (Pty) Ltd, Kwantu shopping mall, Alice, South Africa. All ingredients were thoroughly milled and mixed evenly together to form the basal diet. The feed was formulated to meet the nutritional (energy and protein) requirements of the used sheep (NRC, 2007). The four dietary groups were: basal diet (0%); basal diet +2% FSF; basal diet +4 % FSF and basal diet + 6% FSF. The wethers were fed at 8:00h and 15:00h at 4 % of the bodyweight (on dry matter (DM) basis). The food-grade Fossil shell flour was purchased from Eco-Earth (Pty) Ltd, Port Elizabeth, South Africa, which produces this product under a license by the Department of Agriculture, Forestry and Fisheries of South Africa.

3.2.5. Analytical procedures

3.2.5.1 Proximate analysis of the experimental diets, orts, and fecal sample

The proximate composition of the experimental diet is presented in Table 3.1. Dry matter content of the diets, orts, and fecal samples was measured by drying samples in an air-forced oven at 135°C for 24 h (method 930.15; AOAC 2005). Ash content was measured by placing samples into a muffle furnace at 550°C for five h (method 938.08; AOAC 2005). Organic matter (OM) was calculated as the difference between DM and the ash content. Nitrogen (N) was measured by the Kjeldahl method using Selenium as a catalyst, and crude protein (CP) was calculated as 6.25 × N. Gross energy (GE) was measured using a bomb calorimeter (C200, IKA Works Inc., Staufen, Germany). Ether extracts (EE) were measured by weight loss of the DM on extraction with diethyl ether in Soxhlet extraction apparatus for 8 h (method 920.85; AOAC 2005). The crude fibre was determined by allowing the sample to boil with 1.25% dilute H2SO4, washed with water, further boiled with 1.25% dilute sodium hydroxide, and the dried residue (65 ^{o}C for 3 hours) after digestion was taken as crude fibre (method 978.10) as described by Nancy (2009).

3.2.5.2. Mineral analyses

The mineral composition of the dietary FSF used is shown in Table 3.2. In determining the FSF's mineral content, 5.0 g of the sample was weighed in triplicate and burnt at 550 ^{o}C in a muffle furnace for 5.5 hours. The residues were cooled in a desiccator, before

Table 3 1: Proximate analysis of the experimental diets

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

Table 3 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 P_2O_5)	0.037
Sulfate Sulfur (S)%	0.062
Aluminum (Al) %	0.065
Vanadium (V) %	0.00438
Boron (B) %	0.0023

They were dissolving in 100 ml of deionized water. Suitable salts of the elements were used to make their standards. The standard mineral solutions were injected into the atomic absorption spectrophotometer (Jenway, FPSP 210 model 6305, United Kingdom), and concentration was obtained. These standards were used to determine Mg, Zn, Fe, Cd, Ca, Al, Mn, and B in an unknown feed sample. The concentration of Na and K were determined using a flame photometer (Jenway Models PFP7 and PFP7/C, Cole-Parmer, United Kingdom).

3.2.6. Feed intake and growth performance

During the 105 days of successive feeding, data on fed feed to each wether and the corresponding orts were recorded daily to estimate voluntary intake of DM and nutrients. Feed intake was determined by weighing the feed leftover in feed troughs, including feed refusals every day at 0800 h. Amounts of feed that disappeared were considered to be feed ingested by the wethers. The way the feeding troughs were built does not permit feed spillages. Weights of feed leftover were subtracted from the total weight of the feed allocated to each wether and divided by 7 to determine average daily feed intake (ADFI) (Tefera et al., 2015). Dry matter intake was determined by multiply the percentage dry matter of the feed with the average daily feed intake.

Samples of feeds offered and orts were oven-dried at 65 °C until constant weight to determine DM concentration. Ground samples (to passed through 1-mm sieve) (Wiley mill; Thomas Scientific, Philadelphia, USA) was used to analyzed for organic matter (OM), CP, EE, and CF by the procedures described in section 3.2.5.1. The body weight (BW) of each wether was recorded at the beginning of the trial, on a weekly basis throughout the trial, and at the end of the experiment before the morning feeding using RUUDWEIGH, KM-2E electronic weighing system with 0.05 precision (RUUDSCALE, Durbanville, South Africa). Feed intake, average daily weight gain (ADG), and feed efficiency were calculated from the data obtained.

3.2.7. Apparent nutrients digestibility and N retention

Apparent digestibility coefficients of DM, OM, CP, EE, and CF were determined by the total fecal collection method (Gebrehiwot et al., 2017). On day 91, four animals per treatment were placed into individual metabolism crates (0.5 m × 1.2 m), allowing feces and urine to be collected. The digestibility trial lasted for 14 days (7 days for adaptation to metabolism crates and 7 days for the sample collection).

The amount of feed fed, refusal and feces were weighed daily and homogenized. A 10% sample of total feces was collected during a 7-day collection period as described by Ma et al. (2017). Urine was collected daily in buckets containing 100 mL of 10 % (v/v) H_2SO_4. The volume was measured, and a sample (10 % of total volume) was collected and stored at −20°C until analysis. Samples of feed, orts, feces, and urine were pooled to form a composite sample for each wether. Urinary N was analyzed by the Kjeldahl method (AOAC 2005), and N retention was calculated as daily N excretion (urinary N plus fecal N) subtracted from daily N intake.

3.2.8. Statistical analyses

The data on apparent digestibility, feed and water intake, growth parameters, and N retention were analyzed using the PROC MIXED of SAS 9.4 (SAS Institute, 2012). The model included the fixed effects of FSF inclusion level, week, and their interactions FSF inclusion level. Repeated measures were used to analyze data on. The effects of were. The following model was used: The PROC REG of SAS 9.4 (SAS Institute, 2012) was used to determine relationships between FSF and apparent digestibility, feed, and water intake, growth parameters, and N retention

3.3. Results

3.3.1. Feed and water intake

As shown in Table 3.3, the inclusion of FSF in the diet of Dohne-Merino wethers had a significant effect on feed intake ($P < 0.05$). Wethers on diets containing 4 % and 6 % FSF (linear p = 0.04; quadratic p = 0.02) consumed more feed than wethers fed on a diet with 0 % FSF while wethers fed on a diet with 2 % consumed the least feed. Feed efficiency was highest in wethers fed a diet containing 4% FSF, while wethers on 0% FSF diet had the lowest value ($p < 0.05$). The wethers on the 2% FSF diet were not different from wethers on 6 % FSF diet. The highest feed intake in wethers with 4 % FSF was noticeable from week 7 until the end of the trial (Fig 3.1).

Water intake decreased linearly as FSF's inclusion levels increased in the diets, except for 4 % FSF inclusion level ($P < 0.01$; Table 3.3). The wethers on a diet with 0% FSF had the greatest water intake, while those on 6% FSF showed the lowest value. The wethers on 0% FSF diet tended to drink more water than wethers on 4% FSF diet ($P > 0.05$).

3.3.2. Growth Performance

The effects of varying inclusion levels of FSF on growth performance are shown in Table 3.3. Average daily weight gain and final weight increased with increasing FSF inclusion levels ($P < 0.05$). The average daily weight gain (ADWG) was lowest for wethers fed on 0 % FSF and greatest at 4 % FSF supplementation ($P<0.05$). Figure 3.1 showed BW's evolution over the trial and showed that wethers on 4% FSF diet had the greatest BW at the end of the experiment.

3.3.3. Nitrogen utilization and nutrients apparent digestibility

Table 3.3 shows the nitrogen utilization and diet apparent digestibility of the diets by Dohne-Merino wethers. Nitrogen intake, urinary N, and N balance were not affected ($P>0.05$) by FSF inclusion levels. Fecal N decreased as inclusion levels of FSF increased ($P<0.05$).

Table 3 3: Effects of varying levels of FSF on growth performance and water intake on.

Parameter	Level of FSF in the diet (% of DM)				SEM	P-Values	
	0	2	4	6		Linear	Quadratic
Average daily feed intake(g)	593	572	694	648	39.9	0.045	0.024
Feed Efficiency (g/g)	0.14	0.18	0.19	0.17	0.01	0.011	0.023
ADG (g/d)	84.7	92.9	121.4	105.4	9.53	0.000	0.011
Average daily water intake (L)	1.95	1.67	1.8	1.45	0.10	0.000	0.113
Feed: Gain ratio	19.2	19.4	19.5	19.4	0.43	0.667	0.221
Final weight (Kg)	28.0	28.6	31.3	29.7	0.48	0.043	0.322
Total weight gain (Kg)	8.32	9.20	12.0	10.3	0.38	0.042	0.232

Dohne-Merino wethers.

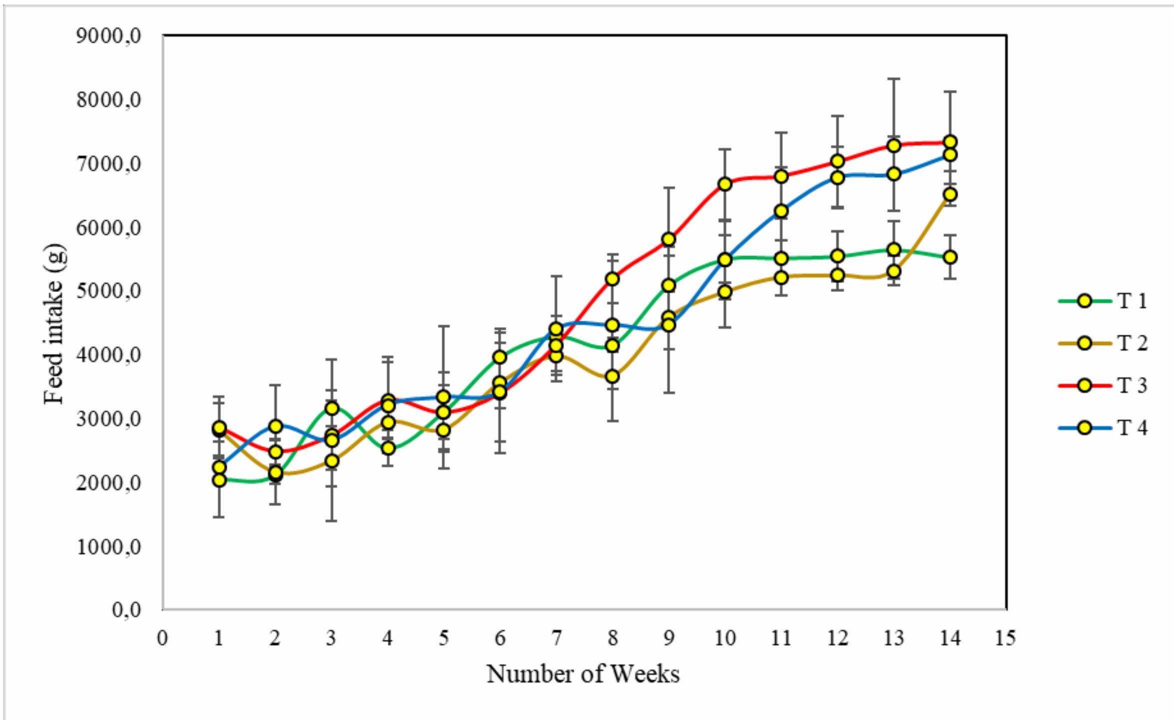

Figure 3.1: Average daily feed intake of Dohne-Merino wethers fed varying levels of Fossil shell flour (FSF).

Increasing the inclusion levels of FSF had no effects on DM, OM, EE, and CF digestibility (Table 3.4), but it has a quadratic effect on apparent CP digestibility ($p = 0.01$). Wethers fed on a diet supplemented with 4% FSF showed the greatest value for all nutrient's digestibility and were significantly different ($P< 0.05$) from those fed on 0% FSF for all nutrients. However, there were no differences ($P>0.05$) among wethers fed on FSF supplemented diets for CP and EE digestibility (Table 3.4).

3.4. Discussion

The result obtained in the study was different from the results obtained by Emeruwa (2016), who recorded the lowest feed intake in the group fed 2% of FSF (900 ± 9.0 g/d) and the highest value for the 6 % inclusion group (1009 ± 9.0 g/d) in West African Dwarf Sheep. This could result from the difference in the basal dietary composition of experimental diets used in this current study. In this study, a combination of concentrate and two different hay was used, while Emeruwa (2016) used only concentrate different from the one used in this study. The slight declined in the feed intake at 6 % FSF inclusion level compared to 4 % FSF could probably due to a change in taste that leads to a reduction in acceptance of diets with 6 % FSF as a result of higher levels of mineral content. Also, McLean et al. (2005) reported significant variations in calves' feed intake fed varying levels of FSF. Sheep requires about 25 to 40 ounces per head per day of NaCl and will eat less feed when deprived of NaCl (Digby et al., 2011; Van Pleltzen 2015). Similarly, it has been observed that minerals such as Na, Ca, K, and Mg increase the palatability of the diet and the feed intake of the animal (Garmyn et al., 2011; Desimone et al.,2013). This could be why wethers fed FSF- supplemented diets in this experiment had greater feed intakes than the control, which is not supplemented.This is because FSF had been reported to be rich in minerals, including Na (923 ppm), Ca (0.22%), Mg (0.11%), and K (0.11%) (Adebiyi et al., 2009; Adeyemo 2013; Lakkawar et al., 2016). The absence of quadratic effect observed for various inclusion levels of FSF on nutrients intake shows that adding increasing levels of FSF up to 6% inclusion levels of the diets DM had no adverse effects on their consumption by the wethers. Water has been described as a vital component of sheep production (Schlink et al., 2010). The results from this experiment align with the observation of Mpendulo et al. (2017), which stated

Table 3 4: Nitrogen utilization (g/d) and apparent digestibility (%) of Dohne Merino wethers fed varying levels of fossil shell flour

Parameter	Level of FSF in diet, % of DM				SEM	P-Values	
	0	2	4	6		Linear	Quadratic
N Retention(g/d)							
N intake	9.83	11.07	13.96	10.24	0.44	0.433	0.954
Fecal N	1.94	1.31	1.23	1.48	0.47	0.033	0.033
Urinary N	0.79	0.32	0.41	0.58	0.07	0.856	0.842
N balance	7.15	9.41	12.35	8.18	0.46	0.454	0.965
Apparent digestibility (%)							
Dry matter	64.50	63.83	72.92	62.83	2.74	0.375	0.311
Organic matter	65.58	66.24	73.15	63.13	2.81	0.353	0.367
Crude protein	70.62	81.83	85.24	77.16	1.65	0.192	0.011
Ether extract	85.54	89.69	93.01	90.19	1.41	0.247	0.222
Crude fibre	51.10	51.17	64.79	52.73	3.88	0.712	0.312

That feed intake of Nguni goats increased with water consumption. Also, it agreed with the report of Prasetiyono et al. (2000) and Abioja et al. (2010), which says that increased water intakes usually encourage sheep to eat more feed.

The decreased fecal N values with increasing FSF inclusion levels indicate that FSF could be used to reduce environmental pollution by reducing fecal N excretion. This is because the accumulation of nitrogen in litter causes high ammonia and smelling environment that can cause respiratory diseases both to animal and human beings. Nitrous oxide, which is toxic to our environment, could also originate from high nitrogen. This result was similar to the report obtained by Rajabi et al. (2017) using pomegranate peel extract in fattening lambs as a feed supplement. Vallejo et al. (2016) reported that the addition of natural feed additive such as xylanase (at 3 µl/g) to sheep diets could reduce excretion of N through fecal and urine.

The linear positive effect of the diets on daily N balance in the present study was similar to that reported by Soroor et al. (2013) when fattening Mehraban lambs were fed Echium ammonium extract up to 1.5 mL/ kg of diet DM. Conversely, this result was not in agreement with the result observed by Emeruwa (2016) using FSF as a supplement in West African dwarf sheep, as they observed that N retention was greater in control compared to the treatments. However, both in this study and in the study reported by Adebiyi et al. (2009), FSF has no significant effect on N balance and N retention. The differences among studies could be due to different experimental conditions. This study was conducted in a semi-arid region, while Emeruwa (2016) conducted their trial in a tropical region. Other factors, as dietary and breed differences, could have also influenced the results.

These results on apparent digestibility indicated that FSF enhanced the absorption of amino acids by the wether hence making it available at tissue levels (Bahrami-yekdangi et al., 2015). These results are contrary to those obtained by Emeruwa (2016), who found that West African Dwarf sheep fed diets containing FSF at the same levels of inclusion than in the present study had numerically greater CP and EE digestibility values for control compared to other treatments, though there was no significant difference. This difference in the results could be due to differences in breeds and diet composition. The lack of no significant effect of FSF on DM, OM, EE, and CF, may be attributed to similarity in the digestibility of the experimental diets, particularly crude fibre digestibility (Van Soest 1994). Additionally, the relative similarity in the physical characteristics of the experimental diets may be the reason(s) for no intake differences among the experimental sheep. A study by Riaz et al. (2014) on experimental animals demonstrated that feeds' physical characteristics could not be underestimated as it plays a huge role in feed intake. They went forward to explain that physical properties are as important as the nutrients composition of diets since it will influence palatability and promote intake (Riaz et al., 2014).

3.5. Conclusion

This study showed that adding 4 % FSF to the diets of Dohne-Merino wethers gives a better improvement than other levels of FSF inclusion on DMI, feed efficiency, ADG, total weight gain, N retention, and apparent nutrient digestibility of most of the nutrients. In addition, the wethers on this treatment consumed the lowest volume of water, making them more suitable in South Africa with the resist water crisis (draught). These results suggested that FSF's inclusion at 4 % in the diet could be potentially used as a performance enhancer by both smallholders and commercial farmers. Therefore, larger-scale experimentation and in communities could be possible studies to established the supplements. Also, FSF's effect on blood parameters and potential anthelmintic will be very important to understand its biochemistry.

3.6. References

Abioja, M.O., Osinowo, O.A., Adebambo, O.A., Bello, N.J., Abiona, J. A.2010. Water restriction in goats during hot-dry season in the humid tropics: feed intake and weight gain Nigerian livestock industries experience. Arch. Zootec. 59: 195–203.

Adebiyi, O.A., Sokunbi, O.A., Ewuola, E.O. 2009. Performance Evaluation and Bone Characteristics of Growing Cockerel Fed Diets Containing Different Levels of

Diatomaceous Earth. Middle-East Journal of Scientific Research. 4: 36–39.

Adeyemo, G. O.2013 Growth performance of broiler chicken fed Fossil shell flour growth promoter. food and nutrition science. 4:16-19

AOAC. 2005. Official method of Analysis Association of Officiating Analytical Chemist; 18th Editi.; Washington DC, United States.

Arikan, O. A., Mulbry, W., Rice, C.2009. Management of antibiotic residues from agricultural sources: Use of composting to reduce chlortetracycline residues in beef manure from treated animals. Journal of Hazardous Materials.164:483–489.

Bahrami-yekdangi, G. R., Ghorbani, M., Khorvash, M. A., Ghaffari, M.H.2015. Reducing crude protein and rumen degradable protein with a constant concentration of rumen undegradable protein Journal of Anim. Sci. 94:1–8.

Chaturvedi, I., Dutta, T. K., Singh, P. K., Sharma, A., Kumar, M., Rao, B. 2014. Effect of Herbal Feed Additives on IVDMD, Methane and Total Gas Production Via in-vitro Study. Journal of Agroecology and Natural Resource Management.1: 2394– 786.

Desimone, J. A., Beauchamp, G. K., Drewnowski, A., Johnson, G. H. 2013. Sodium in the food supply: Challenges and opportunities. Nutrition Reviews.71:52–59.

Digby, S. N., Chadwick, M. A., Blache, D. 2011. Salt intake and reproductive function in sheep. Animal. 5:1207–1216.

Emeruwa, C. H. 2016. Growth Performance of West African Dwarf Sheep Fed Diets Supplemented with Fossil shell flour., University of Ibadan, Nigeria.

Frater, P. 2014. Feed additives in ruminant nutrition. Can feed additives reduce methane and improve performance in ruminant? Agriculture and Horticulture Development, Board.www.beefandlamb.ahdb.org.uk

Garmyn, A. J., Hilton, G. G., Mateescu, R. G., Morgan, J. B., Reecy, J. M., Tait Jr, R. G., Beitz, D. C., Duan, Q., Schoonmaker, J. P., Mayes, M. S., Drewnoski, M., Liu, Q., VanOverbeke, D. L. 2011. Estimation of relationships between mineral concentration and fatty. Journal of Animal Science. 89:2849–2858.

Gebrehiwot, G., Negesse, T., Abebe, A. 2017. Effect of Feeding Leucaena leucocephala Leaves and Pods on Feed Intake, Digestibility, body Weight Change and Carcass Characteristic of Central-Highland Sheep Fed basal Diet Wheat bran and Natural Pasture Hay in tigray, Ethiopia. International Journal of Agriculture, Environment and Biotechnology. 10: 367.

Hu, X., Zhou, Q., Luo, Y. 2010. Occurrence and source analysis of typical veterinary antibiotics in manure, soil, vegetables and groundwater from organic vegetable bases, northern China. Environmental Pollutio. 158:2992–2998.

Ikusika, O. O., Mpendulo, C. T., Zindove, T. J., Okoh, A.I. 2019. Fossil Shell Flour in Livestock Production: A Review. Animals. 9:18–20.

Jalaal, M., Balmforth, N.J., Stoeber, B. 2015. Slip of Spreading Viscoplastic Droplets. Langmuir. 31: 12071–12075.

Lakkawar, A. W., Sathyanarayana, M. L., Narayanaswamy, H. D., Sugunarao, O., Yathiraj, S. 2016. Efficacy of diatomacious earth in amelioration of aflatoxin induced toxicity in broiler chicken. Indian Journal of Animal Research. 50:529–536.

Li, Y. X., Zhang, X. L., Li, W., Lu, X. F., Liu, B., Wang, J. 2013. The residues and environmental risks of multiple veterinary antibiotics in animal faeces. Environmental Monitoring and Assessment. 185:2211–2220.

Ma, G. W., Chu, Y. K., Zhang, W. J., Qin, F. Y., Xu, S. S., Yang, H., Rong, E. G., Du, Z. Q., Wang, S. Z., Li, H., Wang, N. 2017. Polymorphisms of FST gene and their association with wool quality traits in Chinese Merino sheep. PLoS ONE. 12:1–13.

Nancy, J. 2009. Thiex Evaluation of Analytical Methods for the Determination of Moisture, Crude Protein, Crude Fat, and Crude Fiber in Distillers Dried Grains with Solubles. J. AOAC Int. 92, 61–73.

Tefera, G., Tegegne, F., Mekuriaw, Y., Melaku, S., Tsunekawa, A .2015. Effects of different forms of white lupin (Lupinus albus) grain supplementation on feed intake, digestibility, growth performance and carcass characteristics of Washera sheep fed Rhodes grass

(Chloris gayana) hay-based diets. Trop Anim Health Prod. 47:1581–1590.

Mpendulo, C.T., Chimonyo, M., Zindove, T.J. 2017. Influence of water restriction and salinity on feed intake and growth performance of Nguni does. Small Ruminant Research. 149:112–114.

Nancy J. T. 2009. Evaluation of Analytical Methods for the Determination of Moisture, Crude Protein, Crude Fat, and Crude Fiber in Distillers Dried Grains with Solubles. Journal of AOAC International. 92:61–73.

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

Prasetiyono, B.W.H.E., Sunagawa, K., Shinjo, A., Shiroma, S. 2000. Physiological relationship between thirst level and feed intake in goats fed on alfalfa hay cubes. Journal of Anim. Sci. 13:1536–1541.

Pils, J.R.V.2005. The Role of Soil Clays and Clay-Humic Complexes in Processes Controlling Colloidal Stability and the Sorption and Degradation of Tetracyclines Recommended Citation. Ph.D. Thesis, Iowa State University, Ames, IA, USA.

Rajabi, M., Rouzbehan, Y., Rezaei, J. 2017. A strategy to improve nitrogen utilization, reduce environmental impact, and increase performance and antioxidant capacity of fattening lambs using pomegranate peel extract. Journal of Animal Science. 95:499–510.

Ran, Y., Lannerstad, M., Herrero, M., Van Middelaar, C.E., De Boer, I.J.M. 2016. Assessing water resource use in livestock production: A review of methods. Livestock Science.187:68-79

Riaz, M. Q., Südekum, K. H., Clauss, M., Jayanegara, A. 2014. Voluntary feed intake and digestibility of four domestic ruminant species as influenced by dietary constituents: A meta-analysis. Livestock Science. 162:76–85.

Schlink, A.C., Nguyen M., Viljoen, G.J. 2010. Water requirements for livestock production: Water requirements for livestock. RevSci.tech.off.intEpzi. 29:603–619.

Soroor, E. N., Rouzbehan, Y., Alipour, D. 2013. Effect of Echium amoenum extract on the growth rate and fermentation parameters of mehraban lambs. Animal Feed Science and Technology. 184:49–57.

Tedeschi, L. O., Callaway, T. R., Muir, J. P., Anderson, R. C. 2011. Potential environmental benefits of feed additives and other strategies for ruminant production. Revista Brasileira de Zootecnia. 40:291–309.

Valero, M. V., Prado, R. M., Zawadzki, F., Eiras, C. E., Madrona, G. S., Prado, I. N. 2014. Propolis and essential oils additives in the diets improved animal performance and feed efficiency of bulls finished in feedlot. Acta Scientiarum. Animal Sciences. 36:419.

Vallejo, L. H., Salem, A. Z. M., Camacho, L. M., Kholif, A. M., Mariezcurrena, M. D., Cipriano, M., Alonso, M. U., Olivares, J., Lopez, S. 2016. Effects of xylanase supplementation on feed intake, digestibility and ruminal fermentation in Rambouillet sheep. Journal of Agricultural SciencE. 154:1110–1117.

Van Pleltzen.2015. An Award-wining Merino sheep enterprise with the use of diatomaceous earth. Farmer’s weekly. July 30.

Van Soest, P. J. 1994. Nutritional ecology of the ruminant.; 2nd ed.; Cornell Univ. Press: Ithaca, NY.

Watanabe, B.A 2010. Use and environmental occurrence of antibiotics in freestall dairy farms. 44:6591–6600.

Chapter 4

Hematobiochemical and parasitic profiles of Dohne Merino wethers fed diet supplemented with varying inclusion levels of fossil shell flour

(Submitted to Journal of Applied Animal Research)

Abstract

The current study investigated the effects of feeding different concentrations of FSF on hematobiochemical profiles and the gastrointestinal parasitic loads of Dohne Merino wethers. A total of twenty-four Dohne Merino wethers weighing 20 ± 1·5 kg were used in a complete randomized design with six wethers per treatment. The wethers were fed a basal diet, 0% FSF, basal diet +2%FSF, basal diet +4% FSF or basal diet + 6% FSF of diet DM for 105 days. Blood and faecal samples were collected on day 0, 10, 30, 70, and 100 of the feeding trials for the examination of different haematological parameters, serum biochemical indices, and faecal egg count using McMaster Technique. The white blood counts increased in wethers fed diets containing 4 % and 6% FSF from day 10 to 100 compared to those on 0% and 2 % FSF ($P < 0.05$). Red blood cell counts also increased in wethers on a diet with 4% FSF, and partially in those with 6% FSF throughout the experimental period compared to wethers fed on a diet with 0% (control) ($P<0.05$). In serum biochemistry, blood urea was highest in wethers with 0% FSF and lowest in 6% ($P<0.05$). Wethers fed on a diet with 4%FSF showed an increase in blood urea from day 30 to day 100 but not different from those on 0% and 2% FSF inclusion levels ($P < 0.01$). The total protein concentration, albumin, total bilirubin, Na, K, glucose, Cholesterol, and liver enzymes were all within the normal range. Serum creatinine level was significantly lower in wethers on a diet with 4% FSF compared to those on 0 % FSF ($P<0.05$). Haemonchus and Coccidian egg count was low in wethers fed diets with 2%, 4%, and 6% FSF ($P< 0.01$)

While they remain unchanged in wethers fed with 0% FSF inclusion levels, it was concluded that the inclusion of FSF at 4% improves haematological and serum biochemistry parameters were leading to a remarkable reduction in parasite loads. Supplementing FSF at 4% inclusion concentration can be an alternative to inorganic anthelmintic in sheep production.

Keywords: Fossil shell flour, Blood haematology, Blood biochemistry, Fecal egg count, and Dohne-merino.

4.1. Introduction

The productive performance of sheep largely depends on their health status. Therefore, assessing the health of the sheep is essential in addressing anomalies that might negatively affect their performance (Gwaze et al., 2012). Growth rate and body condition scoring are some of the past methods to evaluate the health status of small stock (Douglas et al., 2012). However, these methods cannot give an immediate, accurate, and precise picture of the health status like blood profile and faecal egg count analysis (Schröder and Staufenbiel, 2010). Several factors, such as age, sex, and breed, dictate the blood constituents of farm animals. Other factors include the quantity and quality of feed, presence of antinutritional factors in the feed, and types of feed additives in the feed (Etim et al., 2014; Babeker and Bdalbagi, 2015).

Endo-parasites constitute a significant constraint to sheep production (Catorci et al., 2012). This is because sheep pick up many gastrointestinal parasites and other diseases causing parasites because of their feeding habits such as grazing near the ground. Thus, gastrointestinal parasites impede sheep's performance by interfering with host physiologic and metabolic processes leading to hematobiochemical imbalances and weight loss (Odoi et al. 2007). Recently, the use of feed additives in ruminant diets has received substantial attention, especially as growth enhancers and rumen manipulators for mitigating methane production (Anassori et al., 2015). These substances are added to improve diet quality and animal performance (Frater, 2014). Studies have shown that the inclusion of feed additives, such as organic additives, affects the composition of the animals' blood profile (Wang et al. 2016). Other importance of feed additives includes antimicrobial activity, manipulation of rumen microbes against dietary energy loss, and methane production, among many other things. Wang and Wang (2016) reported that the inclusion of Chinese herbal medicine (plant extracts) improves the functions of the immune system and antioxidation, as well as the digestion and metabolism of goats. Several additives have been used in ruminant feeds, including antibiotics, probiotics, prebiotics, plant extract, herbs, and organic substances. However, chemical-based additives have resulted in antibiotic resistance and the spread of resistant bacteria to other animals and human beings (Chang et al., 2015). Also, antibiotics, prebiotics, probiotics, enzymes, and herbal extracts are expensive and sometimes not reachable and affordable to communal farmers. There is, therefore, a need to replace antibiotics and these feed, as mentioned above additives with organic feed additives that are cheap, available, and able to produce healthier meat and still achieve optimum production (Ruiz Garcia et al., 2011). One of such alternatives can be fossil shell flour.

Emeruwa (2016) reported that serum chemistry in terms of urea, cholesterol, total protein, and alanine aminotransferase (ALT) of West Africa Dwarf Sheep fed diets supplemented with varying amounts of fossil shell flour were not significantly different among the dietary groups. However, a significant decrease was recorded for albumin (g/dL) and glucose (mg/dL) for sheep on 0 % inclusion level of fossil shell flour than diets supplemented with fossil shell flour ($P < 0.05$). This implies that more energy and protein will be available for productive and reproductive performance in animals supplemented with FSF, thereby increasing the animal's general output. Though research on FSF's effect on some blood parameters of West Africa Dwarf Sheep had been done, it is imperative to note that these findings may not be true for other breeds of sheep, especially those in a different climatic region. Moreso, there is a need to carry out a complete heamatobiochemical profile of sheep fed varying FSF levels. Also, FSF's effects on prevalent endo-parasites that affect sheep production in the Eastern Cape of South Africa needs to be verified. Hence, this study using Dohne Merino in a semi-arid region.

Examination of a faecal sample of a sheep also indicated the sheep's internal condition, especially the gastrointestinal tracts. Endo-parasites are a major constraint to small ruminant production (Catorci et al., 2012). Thus, gastrointestinal parasites impede sheep's performance by interfering with host physiologic and metabolic processes leading to hematobiochemical imbalances and weight loss (Odoi et al., 2007). In the past, chemical-based dewormers that animals had recently developed resistance to has been used to control gastrointestinal parasites in livestock. However, fossil shell flour (FSF) has been used to deworm livestock naturally in recent times. Koster (2013) reported that the addition of 2% FSF to heifer diets inhibits the growth of internal parasites and worms. Similarly, Bennett et al. (2011) showed that the inclusion of 2% FSF into the diets of laying birds significantly increase total weight gain, and feed efficiency, egg-laying and decrease parasite load in 2 breeds of commercial egg layers: Bovan Brown (BB) and Lowmann Brown (LB).

Among the lists of feed additives, the most recently sought for are the organic feed additive such as fossil shell flour. It is a naturally occurring, silicon-rich sedimentary rock from a single-celled plant called diatoms (Koster 2013; Ikusika et al. 2019). The FSF is readily available, cost-effective, healthy, and eco-friendly for sheep (Ikusika et al., 2019). Given the importance of heamatobiochemical and parasitic profile in determining the health status of sheep, which is still a concern with sheep farmers, it is crucial that as a substitute is being sought for chemical-based feed additive by using FSF, sheep farmers will have knowledge of quantity and duration of feeding FSF in the diets of sheep. Also, suppose the replacement of antibiotics with FSF in the diets of sheep could be established. In that case, sheep farmers will save more money, consumers of mutton can have healthier meat, and our environment can be void of pollutants. Therefore, the current study was conducted to evaluate the effects of varying inclusion levels of FSF in Dohne -merino sheep diets on blood parameters and gastrointestinal parasite loads. It was hypothesized that fossil shell flour in Dohne Merino wethers' diets would improve blood parameters and gastrointestinal parasite loads.

4.2. Materials and Methods

4.2.1. Study site description

The study site was described in section 3.2.2.

4.2.2. Animal, experimental design and management

The animal, experimental design, and management are the same as described in section 3.2.3.

4.2.3. Experimental Diets

The processing and composition of the experimental diets is the same as described in

section 3.2.4

4.2.4. Analytical procedures

4.2.4.1. Mineral analyses

Mineral analyses were as described in 3.2.5.2

4.2.4.1.1. Proximate analysis of the experimental diets

The procedures and the methods were as described in section 3.2.5.1

4.2.5. Blood sample collection and analyses

Blood samples from five wethers per treatment were collected on day 0, 10, 30, 70, and 100 at 0800 hours before feeding. Blood was collected from the jugular vein into a bijou bottle. From each wether, 10 mL of blood samples were collected into three separately labeled sterile bijou bottles. A blood sample of about 5 ml was deposited into a bottle containing ethylenediaminetetraacetic acid (EDTA) solution (concentration) for heamatological analyses. For serum biochemical studies, 3ml of the blood sample was deposited in the second sterile bottle, which was anticoagulant free, and 2 mL of blood samples were collected in sodium oxalate fluoride bottles for glucose concentration determination. The tubes were labeled with the wethers identification number and put in a cooler box containing ice packs, and transported to the laboratory within two hours.

Analyses on heamatological were done the same day the blood samples were collected. The total red blood cells (RBC) counts were determined by a microscopic method using a counting chamber after dilution with Eayemis solution. Total white blood cells (WBC) count was carried out using the improved Neubauer haemocytometer chamber with 2 % acetic acid as diluents as described by John (2012). Blood platelet counts were determined by diluting a small blood smear with 1% ammonium oxalate before counting in a counting chamber (heamocytometer) using light microscopy, according to Douglas and Harold (2012). The packed cell volume (PVC) was done by the microhaematocrit method, while haemoglobin (Hb) estimation was done using the alkali-haematin method as described by John (2012). The mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC), mean corpuscular volume (MCV) were calculated from RBC, Hb, and HCT values as described by John (2012).

Blood samples in the anticoagulant-free bottles were allowed to coagulate at room temperature before centrifuged at 3500 rounds per minute (rpm), at 10°C for 15 minutes (Model 5403 centrifuge, Gatenbay Eppendorf GmbH, Engelsdorp, Germany). This enables the separation of serum from the blood cells' components. The serum samples were then transferred to 1.8 ml cryovials and frozen at -20°C until blood metabolites were determined. Serum samples were analyzed for total protein (TP), albumin, and creatinine by the methods described by (Lowry et al. (1951), Tietz (1995), and Wu (2006), respectively. Serum urea concentration was also determined following the method described by Tietz (1995). Globulin was calculated as the difference in concentration between total protein and albumin. Analysis for glucose was carried out following the method described by Gochman and Schmitz (1972).

4.2.6. Determination of fecal egg counts

Fecal samples were collected from five wethers per treatment, directly from the rectum using a glycerine lubricated latex glove with the middle finger. One hundred grams of fecal per wether were collected on days 0, 10, 30, 70, and 100. Samples were collected and immediately kept in a labeled polythene bag and stored in a cooler box at 4 °C and were transported to the laboratory for analysis the same day. Sodium chloride was used as the floating medium, and modified Mcmaster techniques were used to determine fecal egg count. Fecal samples (4 g) were thoroughly mixed with NaCl's saturated solution (56 mL). The number of nematode eggs per gram (g) of fecal was obtained by multiplying the total number of eggs counted in the McMaster slide's two squares by the dilution factor of 50 (Whitlock 1948). The modified McMaster technique detects 50 or more eggs per g of fecal. This technique is the most widely employed method for this purpose. It is quick, and the eggs are floated free of debris before counting.

Also, the method is robust and accurate (Levecke et al., 2012). The keys developed for nematode egg types identification (Uhlinger, 1991; Foreyt, 2013) were used to identify four common species of worms and protozoa using the sedimentation method described by Soulsby (1982).

4.2.7. Statistical analyses

Data were analyzed using the general linear model procedure of SAS (2011). The effects of varying inclusion levels of FSF inclusion and week on faecal egg counts (FEC) and blood profile were determined. Terms in the model were treatments (0%, 2%, 4% and 6%), days of sampling (0,10,30,70, and 100), and the interaction between days and treatments. The FEC was transformed using $\log_{10}$ (FEC + 1) to normalize the data. Turkeys' studentized range test was used to test the significant differences between means when the F-test was significant at P <0.05.

4.3 Results

4.3.1. Serum biochemistry

The concentration of glucose in wethers fed on diets with 2%, 4%, and 6% was higher than those fed with 0% FSF diet (P<0.05). Wethers fed on a diet with 2% FSF had the highest concentration of glucose at day 70 and day 100, which was higher than wethers fed with 0%,4%, and 6% FSF diets at the said days of sampling (P < 0.05). Glucose concentrations of wethers on 4% and 6% FSF diets were higher than wethers on 0% FSF diet at the last 2 days of the sampling period (P < 0.05). The total protein concentration was lesser in wethers fed on a diet with 0 % FSF compared to wethers fed with 2%, 4%, and 6% FSF at all the sampling days except day 30 (P<0.05). There were no significant differences among them from day 30 to day 100 except in wethers fed on a diet with 6% FSF. For urea concentration, all the values in wethers fed on diets with 2%, 4%, and 6% FSF were lesser than the values in wethers fed on a diet with 0% FSF at all the sampling days(P<0.05). However, urea concentration for wethers on 0 % and 2% FSF was not different statistically, but they were significantly different from wethers on 4% and 6% FSF diets (P < 0.05). There was no much variation among the wethers on all the minerals concentration diets (Na and K), especially at day 100. For ALT concentration, wethers fed on diets with 2%,4 % and 6 % FSF were significantly higher (P < 0.05) than wethers on 0% FSF diet at days 30 ,70 and 100 (Table 4.1).

In the serum biochemistry parameters, a negative correlation exists between the serum minerals (Na^+= -0.184 and K^+= - 0.236) and total protein (Table 4.3). However, serum urea and total protein showed a positive correlation (P = 0.365). Similarly, Bilirubin had a positive correlation with total protein.

4.3.2. Hematological parameters

The white blood cell (WBC) counts of wethers fed diet containing 0%, and 2% FSF were greater (P<0.05) than wethers fed on diets with 4% and 6% FSF at all the sampling days (Table 4.2). Though lower WBC counts were observed at 0 and 10 day in wethers fed diets with 4 % and 6% FSF, an increase was noticed for WBC in wethers on diets with 4% at day 10 and 6 % FSF at day 30. There were statistical variations (P=0.0003) among the wethers on 2%, 4%, and 6% FSF diets, as well as 0 % FSF diet. Wethers fed on a diet with 4% FSF had higher red blood cell (RBC) counts compared to wethers on 0 %, 2%, and 6% FSF diets at day 30, 70, and 100. Likewise, there were significant variations in MCH, Hematocrit, Platelet, RDW, MCHC, and Hgb in wethers on 0% FSF diet and fed 2 %, 4%, and 6% FSF (P <0.05; Table 4.2).

4.3.3. Fecal egg count

The addition of FSF to the diets of wethers at 4% and 6% inclusion rate eradicates (P < 0.05) coccidia protozoa at day 30. In comparison, 2% FSF inclusion could only reduce to a lower number but could not eliminate the parasite (Fig 4.1). At 2% FSF inclusion, wireworm was reduced to zero levels at day 30, while 0% of FSF supplementation remained unchanged in the population during the same period. FSF's effect on conical and liver fluke worms is demonstrated in Fig 4.2, 4.3, 4.4, and 4.5. Addition at 2% inclusion gives total eradication

Table 4.1: 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
	10	145.66[a]	144.0[a]	147.66[a]	144.66[a]				
	30	143.0[a]	145.0[a]	142.0[a]	143.0[a]				
	70	144.0[a]	142.66[a]	144.33[a]	143.33[a]				
	100	143.0[a]	141.66[a]	143.33[a]	142.33[a]				
K (mmol/l)	0	4.96[bc]	5.00b	7.03a	4.833[bc]	0.0141	0.125	0.011	0.001
	10	5.10a	4.90[a]	4.56[b]	5.2[a]				
	30	5.60[ab]	5.66[a]	5.0[bc]	4.96[bc]				
	70	5.00[ab]	5.033[ab]	4.86[ab]	4.63[c]				
	100	5.00[ab]	5.033[ab]	4.86[ab]	4.63[c]				
Urea (mmol/L	0	6.66[a]	6.60[a]	4.93[bc]	5.23[ab]	0.176	0.000	0.004	0.158
	10	6.06[a]	6.05[a]	6.08[a]	6.16[a]				
	30	6.40[a]	6.3[5a]	5.73[c]	6.20[b]				
	70	6.10[a]	6.06[a]	5.79[a]	5.10[b]				
	100	6.10[a]	6.08[a]	5.83[a]	4.80[b]				
Triglycer (mg/dl)	0	0.11[b]	0.10[b]	0.18[a]	0.18[a]	0.0172	0.000	0.0001	0.0001
	10	0.20[c]	0.19[d]	0.38[a]	0.33[b]				
	30	0.20[b]	0.22[a]	0.19[c]	0.12[d]				
	70	0.17[c]	0.24[b]	0.41[a]	0.15[d]				
	100	0.15[d]	0.26[b]	0.43[a]	0.18[c]				
Cholest. (mmol/L)	0	1.45[ab]	1.42[ab]	2.14[a]	1.56[ab]	0.0711	0.012	0.000	0.444
	10	1.37[a]	1.27[a]	1.34a	1.24[a]				
	30	0.98[a]	1.06[a]	1.13[a]	1.21[a]				
	70	1.01[a]	1.09[a]	1.31[a]	1.27[a]				
	100	1.13[a]	1.10[a]	1.33[a]	1.26[a]				
ALP (U/L)	0	52.66b	58.33b	145.66a	54.33b	11.5	0.000	0.001	0.684
	10	141.33b	91.66c	210.33a	84.33c				
	30	163.0[b]	126.0[c]	211.33[a]	73.66[d]				
	70	129.66[b]	146.0[b]	223.33[a]	72.66[c]				
	100	125.66[b]	145.0[b]	220.3[a]	70.66[c]				

Bilirubin (mg/dl)	0	6.66^{a}	7.0^{a}	5.0^{b}	6.66^{a}	0.298	0.001	0.000	0.000
	10	7.66^{a}	6.0^{c}	6.66^{b}	4.66^{d}				
	30	6.0^{a}	5.12^{b}	4.42^{c}	3.66^{d}				
	70	5.66^{a}	4.0^{c}	3.33^{d}	5.0^{b}				
	100	5.66^{a}	4.0^{c}	3.33^{d}	4.66^{b}				
Albumin (g/L)	0	12.66^{a}	13.0^{a}	12.66^{a}	12.33a	0.171	0.137	0.103	0.501
	10	13.0^{a}	12.0^{b}	13.33^{a}	12.0^{b}				
	30	12.66^{a}	12.0^{b}	12.33^{a}	12.33^{a}				
	70	13.0a	12.66^{a}	13.0a	13.0^{a}				
	100	12.50^{a}	12.16^{a}	12.85^{a}	12.90^{a}				
Glucose(mmol/L)	0	2.53^{b}	2.30^{bc}	2.63^{b}	4.06^{a}	0.151	0.224	0.210	0.008
	10	2.76^{a}	2.65a	2.60a	2.50^{a}				
	30	2.60^{a}	2.76^{a}	2.53^{a}	2.52^{a}				
	70	2.25^{c}	3.66^{a}	2.96^{b}	2.73^{b}				
	100	2.20^{c}	3.97^{a}	3.02^{b}	2.76^{b}				
Protein (g/L)	0	61.0^{b}	54.33^{c}	54.0^{c}	68.66^{a}	1.98	0.001	0.738	0.094
	10	57.66^{ab}	61.0^{a}	57.33^{ab}	56.0^{bc}				
	30	59.0^{a}	53.66^{b}	58.2^{a}	62.0^{a}				
	70	57.66^{b}	58.33^{b}	58.50^{b}	65.33^{a}				
	100	60.66^{b}	63.33^{b}	61.0^{b}	68.33^{a}				
Creatine (mg/dl)	0	105.33^{a}	91.66^{b}	78.33^{c}	64.33^{d}	3.29	0.001	0.000	0.194
	10	79.33^{a}	71.66^{b}	60.66^{c}	55.0^{d}				
	30	61.33^{a}	54.66^{b}	51.33^{b}	48.33^{b}				
	70	61.0^{b}	69.33^{a}	53.33^{c}	63.33^{b}				
	100	60.80^{b}	69.93^{a}	52.12^{c}	65.10^{ab}				

^{abc}mean values with different superscript 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 4.2: The hematological parameters of sheep fed varying amounts of FSF.

Parameter	Day	Treatment				SEM	P-value		
		1	2	3	4		Day	Treat	Day* Treat
MCHC (g/dl)	0	48.96^{b}	41.80^{c}	66.80^{a}	37.06^{c}	3.479	0.031	0.0001	0.0001
	10	33.53^{b}	53.00^{a}	51.17^{a}	37.83^{b}				
	30	57.10^{a}	51.87^{a}	56.26^{a}	42.13^{b}				
	70	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 (%)	0	34.17^{b}	36.63^{a}	32.97^{bc}	38.66^{a}	1.078	0.004	0.0004	0.0063
	10	33.07^{b}	34.93^{ab}	35.67^{a}	30.86^{c}				
	30	29.77^{c}	35.60^{a}	33.93^{b}	33.93^{b}				
	70	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 (%)	0	6.83^{a}	5.77^{c}	6.27^{b}	6.06^{bc}	0.249	0.116	0.0012	0.0001
	10	7.27^{a}	5.70^{c}	6.53^{b}	6.33^{b}				
	30	6.50^{b}	7.73^{a}	6.00^{c}	6.50^{b}				
	70	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	53.6^{bcd}	55.5^{bc}	59.3^{b}	95.6^{a}	8	0.000	0.0001	0.0001
	10	30.6^{cd}	85.3^{b}	61.6^{c}	108^{a}				
	30	25^{d}	79.6^{bc}	93.6^{b}	146^{a}				
	70	27^{d}	74.3^{c}	103.6^{b}	183.6^{a}				
	100	23^{d}	71.3^{c}	108.6^{b}	198.6^{a}				
WBC (×103/µl)	0	59.3^{a}	30.8^{b}	19.2^{bcd}	25.2^{bc}	6.7	0.278	0.0003	0.5400
	10	60.2^{a}	30.6^{b}	18.3^{bc}	29.8^{b}				
	30	54.0^{a}	25.6^{b}	18.8^{bcd}	22^{bc}				
	70	53.6^{a}	13.9^{cd}	22.2^{bc}	34.4^{b}				
	100	50.6^{a}	15.9^{cd}	25.2^{bc}	38.4^{b}				
Haemoglo. (g/dl)	0	10.36^{ab}	10.56^{a}	9.50^{b}	10.63^{a}	0.502	0.001	0.045	0.842
	10	9.90^{a}	8.43^{b}	8.5^{b}	8.33^{bc}				
	30	8.43^{a}	7.93^{bc}	7.23^{bcd}	8.17^{b}				
	70	8.60^{a}	7.80^{a}	8.10^{a}	8.37^{a}				
	100	8.80^{a}	7.70^{b}	8.40^{ab}	8.45^{ab}				
Heamotocri. (%)	0	0.21^{bc}	0.24^{ab}	0.16^{d}	0.27^{a}	0.017	0.002	0.003	0.0003
	10	0.25^{a}	0.20^{ab}	0.19^{bc}	0.23^{ab}				
	30	0.15^{cd}	0.18^{abc}	0.20^{ab}	0.22^{a}				
	70	0.14^{c}	0.20^{ab}	0.23^{a}	0.20^{ab}				
	100	0.13^{dc}	0.22^{ab}	0.25^{a}	0.19^{bc}				
MCV (fl)	0	43.57^{a}	41.57^{bc}	40.20^{c}	41.80^{b}	0.750	0.000	0.002	0.0001
	10	35.77^{c}	45.17^{a}	43.73^{a}	38.80^{b}				
	30	45.43^{a}	43.10^{b}	44.60^{ab}	41.66^{c}				
	70	45.33^{a}	44.00^{a}	41.60^{bc}	42.67^{b}				
	100	45.13^{a}	45.00^{a}	40.70^{c}	42.87^{b}				

MCH (pg)	0	21.83^{b}	18.03^{c}	27.00^{a}	15.93^{d}	1.469	0.000	0.0001	0.0001
	10	12.03^{c}	24.03^{a}	22.13^{a}	15.63^{b}				
	30	25.73^{a}	22.40^{b}	22.80^{ab}	18.00^{c}				
	70	25.37^{a}	21.66^{b}	17.17^{cd}	19.43^{bc}				
	100	25.60^{a}	20.067^{b}	15.43^{c}	20.37^{b}				
RBC (M/µl)	0	5.05^{ab}	5.99^{a}	3.8^{bc}	6.6^{a}	0.594	0.399	0.030	0.000
	10	6.72^{a}	5.46^{bc}	3.86^{bc}	5.6^{b}				
	30	4.41^{bc}	4.19^{b}	6.02^{a}	6.06^{a}				
	70	3.53^{bc}	4.37^{b}	6.1^{a}	5.44^{ab}				
	100	3.5^{bc}	4.37^{b}	6.13^{a}	5.44^{ab}				

^{abc}mean values with different superscript 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 3: 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

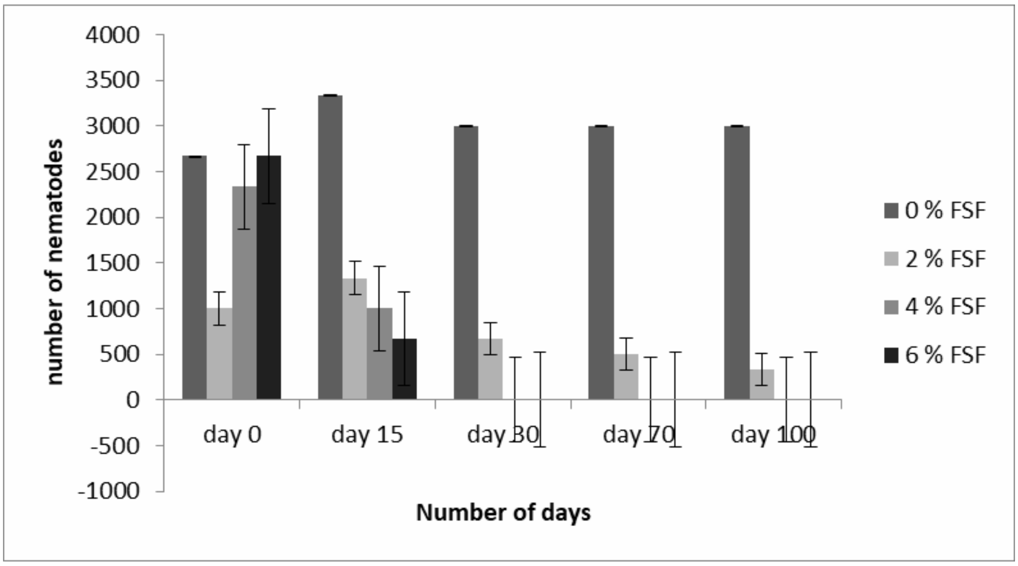

Figure 4 1: Counts of coccidia in Dohne-merino wethers fed varying levels of FSF for 100 days shown as means ± standard errors

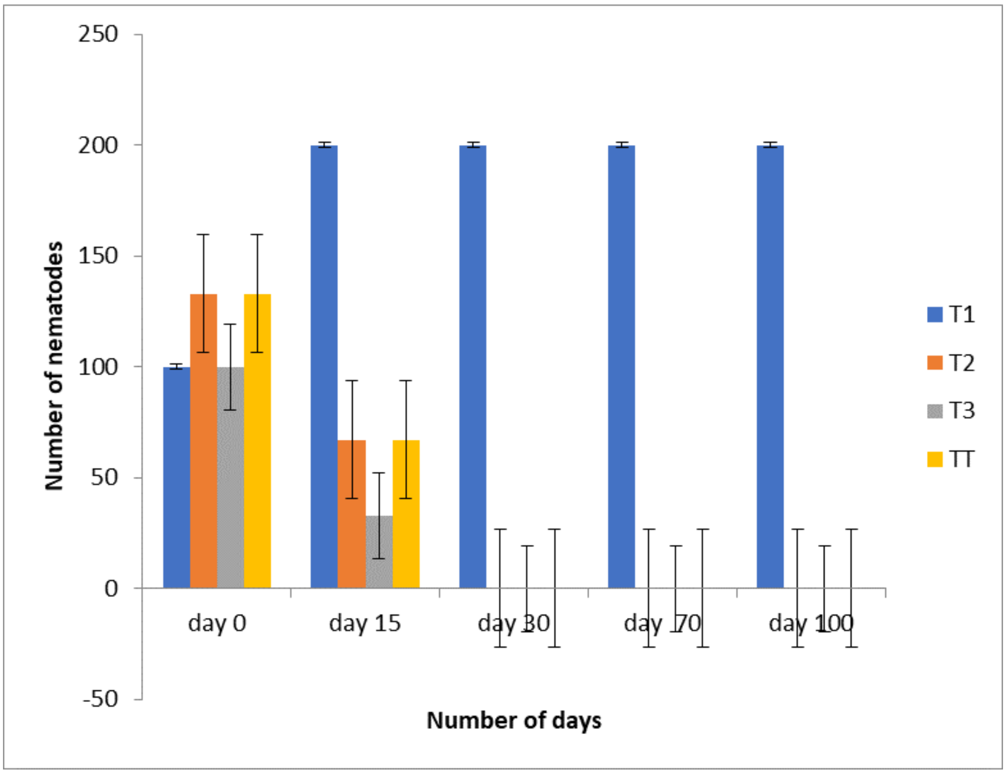

Figure 4 2: Counts of wireworm in Dohne-merino wethers fed varying levels of FSF for 100 days shown as means ± standard errors

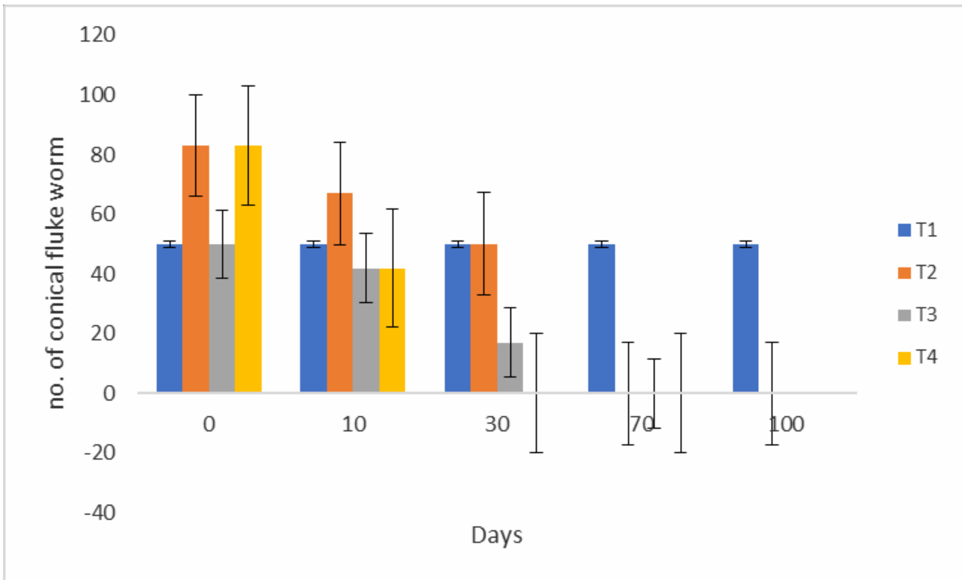

Figure 4 3: Counts of conical fluke wormin Dohne-merino wethers fed varying levels of FSF for 100 days shown as means ± standard errors.

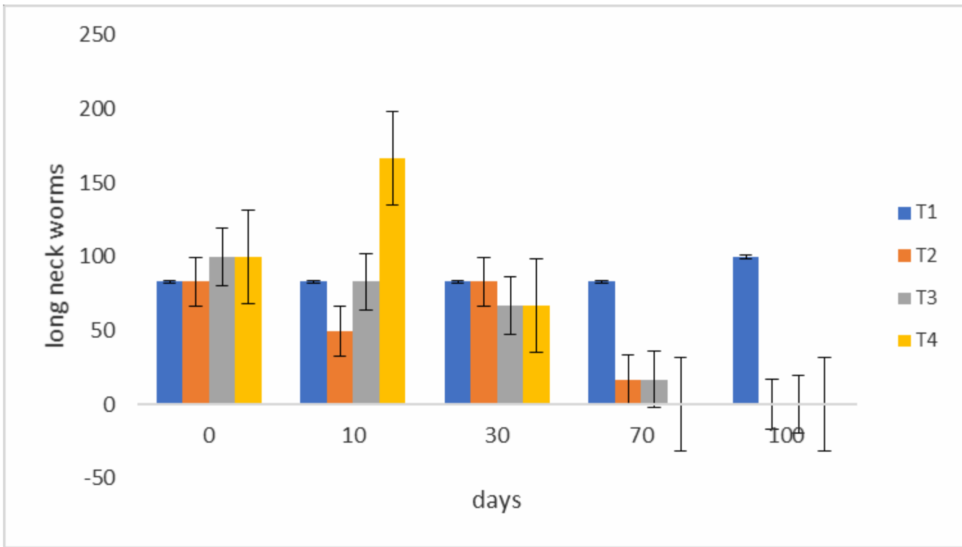

Figure 4 4: Counts of Long-necked worms in Dohne- merino wethers fed varying levels of FSF for 100 days shown as means ± standard errors

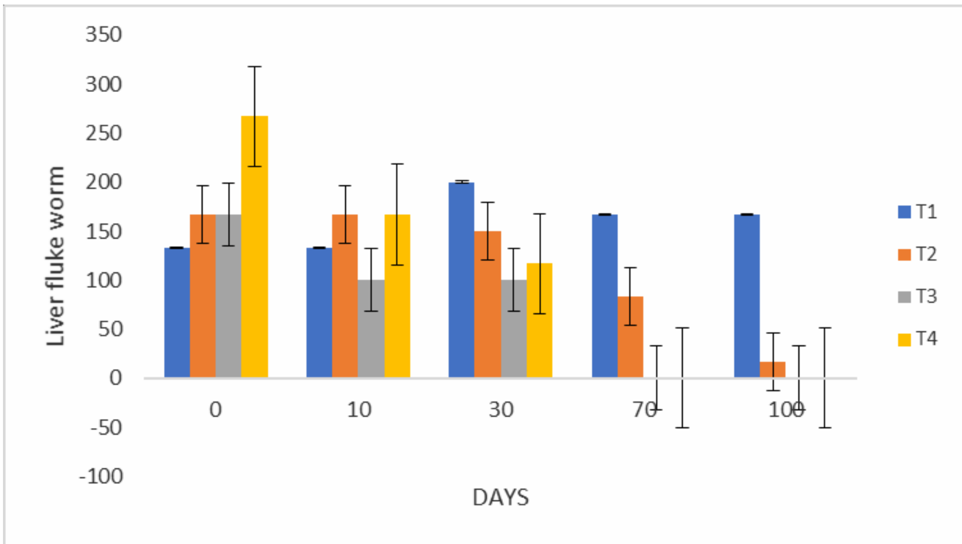

Figure 4 5: Counts of Liver flukes' worm in Dohne-merino wethers fed varying levels of FSF for 100 days shown as means ± standard errors.

of these worms at 100 day. Fossil shell inclusion at 2% did not eradicate long-necked worms at 100 days except on a higher inclusion level of 4% and 6% inclusion rate (Fig 4).

4.4. Discussion

Haematological indices, especially the white blood cell and the red blood cell, are great indicators of sheep production's health status. There was an increase in WBC counts in wethers fed 4% and 6% FSF from day 30, while a decrease was observed in wethers on 0% and 2% FSF diets during the same period. This showed that the addition of FSF at 4% (T3) and 6% (T4) to the diets improved the wethers' white blood cell from 30 days. However, no significant effect was recorded ($p>0.05$) among them. The findings observed in this present study agreed with Emeruwa (2016) report, who reported no significant difference between the control and the other treatments in sheep fed on different amounts of FSF. The increase in circulating WBC observed in wethers fed 4% and 6% FSF from day 10 to 100 could indicate heightened functions of the sheep immune system. The WBC is known to fight foreign bodies in the system (Babeker and Bdalbagi 2015). This observation agrees with the findings of Weaver et al. (2013), who reported that the addition of FSF into the feeds of young gilts improves the immunity system against Aflatoxin.

The mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), red blood cell distribution width (RDW), and mean corpuscular haemoglobin concentration (MCHC) are related to individual RBC and are important for the diagnosis of anaemia. Mean corpuscular haemoglobin concentration (MCHC) is the concentration of haemoglobin in red blood cells. In all the treatments considered in this study, RBC, MCV, MCH, Hgb, and Hematocrits values indicate that inclusion of FSF into Dohne Merino wethers' diets improves the hematological blood parameters. This buttresses the point that FSF inclusion in feed has the capability of supporting the high oxygen-carrying capacity of the blood. This finding is in agreement with the reports of Babeker and Bdalbagi (2015).

Blood urea is an indicator of protein metabolism conditions and dietary amino acid balance in animals (Tao et al., 2018). Jiwuba et al. (2017) associated high blood urea levels with poor protein quality in the animal feed. The similarity in the values of blood urea observed between the 0% FSFdiet and FSF supplemented diets in this study indicates that FSF's addition in the diets of Dohne -Merino wethers did not negatively affect the quality of the protein in the feed. Though, wethers on 4 %FSF diet tended to increase in blood urea at day 30 to 100 yet not different from wethers on 0 % and 2 %FSF diets but different from wethers on 6 % FSF diet. This implies that FSF's addition in the diets of Dohne-Merino, wether more than 4 % inclusion levels, will lead to high levels of urea in the blood. This could be because FSF increases protein concentration in the rumen of sheep, and excess protein may be broken down to ammonia in the rumen, which will be transported to renal organs through the blood in the form of urea. Hence, there will be more urea in the blood. This is in agreement with the findings of Chaves et al. (2011) and Baharami et al. (2015), who all reported a negative correlation between rumen ammonia nitrogen and blood urea.

The total protein concentration, albumin, total bilirubin, Na, K, glucose, cholesterol, and liver enzymes were normal for wethers. This suggests that the experimental wethers were in good health conditions and the FSF supplementation had no adverse effects on their productivity (Ikusika et al., 2019). The total protein level improvement observed for wethers on 2 %, 4 %, and 6 %FSF diets compared to wethers on 0 % FSF diet shows that more nitrogen was available at the tissue level due to FSF supplementation. However, no statistical difference was observed among the FSF supplemented diets and non-supplemented diets. This is similar to Emeruwa (2016) report, which found no significant differences between control and treatment fed with varying amounts of FSF in West Africa dwarf sheep.

Serum creatinine level was significantly lower in wethers on 4 % FSF diet as compared to wethers on 0 %, 2 %, and 6 % FSF diets at day 100 of the experiment. This low serum creatinine level in T3 (4 %) indicates FSF's good health impact at 4 % inclusion amounts. Creatinine is a waste molecule resulting from protein metabolism, and its low concentration depicts reduced catabolism rate in wethers on a 4 % FSF diet (Paswan et al. 2016).

Serum glucose witnesses' reduction at all the sampling days in wethers on 0% FSF diet, initial retardation in wethers with 4% and 6% FSF diets but increased in wethers on 2% FSF diet at all the sampling days. However, there was no significant variation among wethers on all the varying inclusion levels of FSF except at 70 days and 100 days. Wethers on 0% FSF diet were statistically lower ($P<0.0001$) than diets supplemented with the FSF group. This agreed with Malekkhahi et al. (2015), which reported higher values for blood glucose in Baluchi lambs fed on diets supplemented with essential oil, yeast culture, and malate compared with the control. Conversely, the result is contrary to the result obtained by Emeruwa (2016), who reported higher serum glucose for control compared to the FSF supplemented group. This could be due to breed, climate, and diet differences.

Economic losses resulting from gastrointestinal parasites are major problems in small ruminant production in semi-arid and arid regions. Haemonchus contortus has been reported to cause anaemia, hematological and biochemical turbulences, low immunity, poor wool quality, and sometimes mortality in sheep (Sanmarie Vermaak 2019).

From this study, FSF seems to effectively reduce wireworm, long-necked worm, liver fluke worm, conical fluke worm, and coccidia. Wethers fed on diets with 4% and 6% FSF, at day 30, had a remarkable reduction in counts of various worms considered while wethers fed 2% FSF had decreased in the counts of worms investigated day 70. This finding confirmed the report of Deutschlander (1993); McLean et al. (2005), and Bennett et al. (2011), who all reported that inclusive of 2% FSF to the diets of lambs improve sheep performance and reduce gastrointestinal parasites. They also reported that it takes a minimum of 75 days of regular inclusion of FSF to reduce gastrointestinal parasites. Similarly, Bernard et al. (2009) observed that Spanish/Boer cross goats fed on varying inclusion amounts of FSF at 1.77 g, 3.54 g, and 5.31 g per kg had significant mean weight gain fecal egg count reduction as the inclusion amounts increased. The action of FSF against these parasites could be because the characteristics of the surface of fossil shell flour such as abrasiveness, acidity, solubility, hydrophobicity, absorption functionalities with a crystal structure of FSF dehydrate worms and at the same time, the abrasive nature cut the worms thereby euthanizing them. Akin et al. (2000) and Yuan et al. (2010) suggested that both the physical and chemical properties of diatomaceous earth (FSF) are effective against gastrointestinal parasites, which is believed to be the reason for feacal egg count reduction in this study.

4.5. Conclusion

The results show that supplementing Dohne Merino wethers on diets with FSF at a 4% inclusion rate positively affects the serum urea, total protein, and glucose but does not alter the other parameters' normal serum range. Similarly, the RBC, WBC, Platelet, Hgb, RDW, and Hematocrit were improved with 4% inclusion amounts in the diets of Dohne- merino which indicates good immunity and good health effect of FSF for wethers on 4%. The remarkable reduction in faecal egg count for 2%, 4%, and 6%FSF inclusion levels showed that FSF could be an effective anthelmintic for Dohne-Merino wethers in the semi-arid region. However, the effect of the improvement observed on parasitic load, and blood profile in this chapter due to FSF inclusion needs to be investigated on the wool parameters, body condition scores, and feed preference in Dohne Merino.

4.6 References

Akin, S., Schembre, J.M., Bhat, S.K., Kovscek, A.R. 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.

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

Babeker and Bdalbagi, E.A. 2015. Effect of Feeding Different Levels of Moringa Oleifera Leaves on Performance, Haematological, Biochemical and Some Physiological Parameters of Sudan Nubia Goats. Online Journal of Animal and Feed Research. 5:50–61.

Chaves, A., Stanford, K., Dugan, M., Gib- son, L., McAllister, T., Vanherk, F., Benchaar, C.2011. Effects of cinnamal- dehyde, garlic and juniper berry essential oils on rumen fermentation, blood metabolites, growth performance, and carcass characteristics of growing lambs. Livestock Science. 117: 215–224**.**

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

Bennett, D.C., Yee, A., Rhee, Y.J., Cheng, K.M. 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., Ahmedna, 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.

Chang, Q., Wang., Regev-Yochay, G., Lipsitch, M., Hanage, W.P.2015. Antibiotics in agriculture and the risk to human health: how worried should we be? Evolutionary Applications. 8:240-247

Deutschlander, D. 1993. Evaluating Diatomaceous Earth as a Wormer for Sheep and Cattle. Available from: https://www.a-b-c-plus.com/Images/PDFs/FPD291- DE_Literature.pdf

Douglas, J.W., 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, C.H. 2016. Growth Performance of West African Dwarf Sheep Fed Diets Supplemented with Fossil shell flour. University of Ibadan, Nigeria.

Etim, N.N., Williams, M.E., Akpabio, U., Offiong, E.A. 2014. Haematological Parameters and Factors Affecting Their Values. Agricultural Science. 2:37–47.

Frater, P. 2014. Feed additives in ruminant nutrition. Can feed additives reduce methane and improve performance in ruminant? Agriculture and Horticulture Development Board. www.beefandlamb.ahdb.org.uk (Accessed on 8/18/2018)

Foreyt, W.J 2013. Veterinary parasitology reference manual. 5th Editio. Iowa: Blackwell publishing professional, United States.

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

Gwaze, F.R., 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, O.O., Mpendulo, C.T., Zindove, T.J., Okoh, A.I. 2019. Fossil Shell Flour in Livestock Production: A Review. Animals. 9:1–20.

Jiwuba, P.C., Ahamefule, F.O., Ogbuewu, I.P., 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, W. H. 2012. Hematology Procedures. In: Veterinary Hematology. 11th–32nd ed. Florida: Elsevier Science and Technology; p. 11–32.

Koster.H.2013.Diatomite in Animal Feeds.Availablefrom agrisilica.co.za/pdf/eng/Diatoms%20in%20Animal%20Feeds%20HH%20Koster.pdf.

Levecke, B., Rinaldi, L., Charlier, J., Maurelli, M.P., 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.188:194–199.

Lowry, O H., Rosebrough, N.J., RRJ. 2014. Protein measurementwith the Folin phenol reagent. J. Biol. Chem.193:265, 1951. Encyclopedia of Quality of Life and Well-Being Research. 1:921–921.

Malekkhahi, M., Tahmasbi, A.M., Naserian, A.A., Mesgaran, M.D., Kleen, J.L., Parand, A.A. 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, A., 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 requeriments of small ruminants: sheep, goats, cervids, and new world camelids. Washington, D.C.

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, J.K., Kumar, K., Kumar, S., Kumar, A. 2016. Effect of feeding Acacia nilotica pod meal on hematobiochemical profile and fecal 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.

Sanmarie.V.2019.Wireworm/Haarwurm.MohairGrowers (https://www.angoras.co.za/page/wireworm).Accessed on 10/11/2019

SAS, 2011. SAS/STAT User's Guide (Version 9.3). SAS Inst. Inc., Cary, NC.

Schröder, U.J., 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, E.J. 1982. Helminths, Arthropods and Protozoa of Domesticated Animals. Seventh Edition. Pp 291. Published by Bailliere Tindall and Cassel Ltd, London, Uk.

T N, M C., A IO., V M., K D., J G R. 2007. Assessing the nutritional status of beef cattle: current practices and future prospects. African Journal of Biotechnology. 6:2727–2734.

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 N.W.1995. Fundamentals of Clinical Chemistry. 3rd Ed. W.B. Saunders, Philadelphia, USA

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

Wang, L.Z., Zhou, M.L., Wang, J.W, Wu D, Yan T. 2016. The effect of dietary replacement of ordinary rice with red yeast rice on nutrient utilization, enteric methane emission and rumen archaeal diversity in goats. PLoS ONE. 11:1–15.

Wang, S.P and Wang, W.J. 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, A.C., Todd, S. M., Hansen, J.A., Kim, Y.B., De Souza, A.L.P., Middleton, T.F., Kim, S.W. 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, H.V., 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.

Wu, A., 2006. Clinical Guide to Laboratory Tests. 4th Edition Elsevier, San Francisco. Pp 1856.

Yuan, P., Liu, D., Fan, M., Yang, D., Zhu, R., Ge, F., Zhu, J.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.

Chapter 5

Influence of fossil shell flour supplementation on feed preference, body condition scores and wool parameters of Dohne Merino wethers

Abstract

The abundance of fossil shell flour (FSF) globally has increased interest in its use as a feed additive in sheep diets. This study evaluated the effects of FSF's varying inclusion levels on feed preference, body condition scores, and wool parameters of Dohne Merino (DM) wethers. Twenty-four DM wethers, weighing 20 ± 1·5 kg on average were fed dietary food-grade fossil shell flour in a completely randomized design of four treatment with six wethers in each treatment. The wethers were fed a basal diet without FSF addition (control, 0%), or FSF (2%, 4%, or 6%) into the diet DM for 105 days. Average daily weight gain and body condition score of 2%, 4%, and 6 % FSF inclusion levels were higher ($P < 0.05$) than the control 0%. Wool yield, staple length, a coefficient variation of the fibre diameter, and fibre <15% of wethers supplemented with FSF were higher ($P < 0.05$) compared to the diet without FSF. The fibre diameter of the wethers supplemented with FSF was the same ($P>0.05$) as the control. Dohne Merino wethers preferred FSF supplemented diets to those without FSF supplement. The inclusion of FSF in DM wethers' diet up to 6% DM showed improvement in the average daily gain and body condition scores, feed intake through preference, wool yield, and quality. Thus, the addition of FSF in the diets could be advantageous in Dohne-Merino wethers' performance and production with the potential of increasing both the quantity and quality of wool.

Keywords: Fossil shell flour, body condition scores, feed preference, wool parameters, Dohne-Merino

5.1. Introduction

Sheep plays a major part in the global agricultural economy by producing wool and mutton, with the world's sheep population estimated to be over 1.173 billion (FAO, 2016). In South Africa, sheep contributes about 10% of the total income from animal products, and wool contributes more than half of the 10% (Naidoo et al., 2016). The most used natural animal fiber for fabrics, carpets, upholstery, saddle cloths, and horse rugs (IWTO, 2015). Common wool breeds include Merino, Superfine wool, Nilgiris, Chinese Merino, Corriedale, Dohne Merino (Cloete *et al.*, 2012; Saxena *et al.*, 2015). Among these, the Dohne Merino is known to produce uniform, high quality, and fine wool (Esfandyari et al., 2011). Around 40 % of the total South African wool sheep are Dohne Merino breed (DAFF, 2016).

Over the last three decades, the demand for lighter-weight fabrics by consumers and competition from synthetic fabrics has made the production of quality wool of utmost importance to the sheep industry (Mortimer et al., 2017). Fiber diameter, fibre diameter coefficient of variation, comfort factor, fibre curvature, spinning fineness, staple length, staple strength, and clean fleece yield are the major determinants of wool quality (Cottle et al., 2013). The quality and quantity of wool are determined by factors such as sheep, nutrition, gender, and nematode infection (Salehian et al., 2015). The health conditions of a sheep affect the volume of wool being produced, staple length (SL), staple strength (SS) and, fibre diameter (FD) (Naderi et al., 2015). Reduction and pigmentation of fibre diameter and clean fleece weight have been caused by nematode infection (Sales, 2011).

Strategies such as dosing with anthelmintic have been widely used to control nematodes in sheep and improve wool quality and quantity. McGregor et al. (2016) observed that reduced worm load due to frequent treatment by anthelmintic gives rise to better greasy fleece weight (GFW) and FD. However, chemical-based anthelminthic has increased anthelmintic resistance, especially in South Africa, where sheep farming is becoming unsustainable. Anthelmintic resistance is, thus, fast becoming one of the biggest challenges to wool production. Therefore, there is a need to employ more sustainable worm control strategies to reduce reliance on routine anthelmintic. One such way could be the use of natural anthelmintics such as fossil shell flour (FSF), Zeolites, and $LaCl_3$(Mclean et al.,2005). The FSF is natural, and parasites may not develop resistance against it because of certain properties it possesses (As observed and recorded in chapter 4). There are suggestions that FSF powder is characterized by abrasive action that pierces or scratches the outer protective layer of invertebrates, including internal parasites resulting in death by dehydration (Ahmed et al., 2013).

Gender and physiological status also play a significant role in the wool parameters of sheep. Cilek *et al.* (2015) reported that higher volumes of fleece were found in wethers than ewes. There have been suggestions that because ewes and rams have low sulphur-containing amino acids such as cysteine and methionine, they do not produce much wool compared to wethers (Pal *et al.*, 2010; Fadayifar *et al.*, 2012). As a result, many Dohne Merino producers keep a large proportion of their flock as wool production wethers. Nutrition is another major factor that determines the wool characteristics of sheep (McGregor et al., 2016). Rangel and Gardiner (2009) and Ramos et al. (2019) observed that sheep diets with an average of 15% CP improved wool fibre diameter, staple length, and growth rate. Body condition scoring (BCS), a strong proxy of sheep's nutritional status, has been reported to have a linear relation to wool growth and fibre diameter (Van Burgel et al., 2011). High BCS results in greater wool yield. Fibre diameter responds to changes in nutrient availability and average weight gain. Both the macro and micro-minerals have been found to play a major role in wool quality and quantity (Fadayifar et al., 2012). An adequate supply of minerals such as Zn, Cu, Fe, and pyridoxine is required for the production of good quality wool (Silva et al.,2015)

Pastures in semi-arid and arid regions have low levels of minerals such as Zn, S, Cu, and Fe.

These low levels of Zn, S, Cu, and Fe results in low wool growth rate and poor wool quality (NRC,2007). Harsh environmental conditions and redirection of grains from feed to fuel have made supplementation feeding increasingly expensive (FAO, 2009; Li *et al.*, 2013). There is, therefore, a need to explore supplements that are readily available, cheap, and of good nutritional value that can meet this requirement. The FSF, rich in trace elements such as Zn, S, Cu, and Fe, is a possible solution (Martin, 2013). Therefore, the application of FSF may enhance the nutritional status of Dohne-Merino sheep.

Fossil shell flour is a deposit of unicellular algae made up of fossilized skeletons of diatoms found in ponds, streams, lakes, or seas (Bernard *et al.*, 2009). Fossil shell flour comprises 14 trace minerals such as Mg Zn, S, Cu, Fe, Ca, Mn, which can be fundamental for improving feed preference, body condition scores, and wool quality of sheep. Fossil shell flour is readily available, cheap, and has no adverse effects on sheep for now (Ikusika et al., 2019; Chapter 2). Feed preference determines the acceptance of feed hence the intake. Low intake results in reduced wool growth and low staple length, poor fibre <15%, and poor fibre diameter (Distel and Villalba, 2018). Against this background, the study investigated the impact of the inclusion of different FSF levels in the diet of Dohne-Merino wethers on wool growth and quality, body condition score, and feed preference. It was hypothesized that the influence of fossil shell flour supplementation would increase wool parameters, feed preference, and body condition scores of Dohne-Merino wethers.

5.2. Materials and methods

5.2.1. Ethical approval

As described in 3.2.1.

5.2.2. Description of study site

The study was described in section 3.2.2.

5.2.3. Sheep and housing

Twenty-four Dohne Merino wethers were selected from a commercial farm in Mitford village, Tarkastad, Eastern Cape of South Africa. The wethers were about 5 months old and weighed 20 ± 1.5 kg on average. The wethers were used in a completely randomized design of four treatments with six wethers per treatment. All the 24 wethers were raised at the same housing and equipment facilities in the same area under the same average environmental condition. The pens had a similar temperature (23.29 °C), relative humidity (76.75%), dry-bulb temperature (24.82 °C), and wet bulb temperature (21.15 °C). They were individually housed in a 1.5 m × 1.5 m well-ventilated roofed building with concrete flooring for each sheep. The experiment lasted for 105 days, excluding 14 days of the adaptation period. The sheep had access to clean and fresh water *ad libitum*.

5.2.4. Diets and feeding

The processing and composition of the experimental diets are the same as described in section 3.2.4.

5.2.5 Analytical procedures

5.2.5.1 Mineral analyses

The mineral analysis was described in 3.2.5.2

5.2.5.2. Proximate analysis of the experimental diets

The procedures and the methods were described in section 3.2.5.1

5.2.6. Measurements

5.2.6.1. Wool samples collection and measurement

An area of approximately 10 cm × 10 cm was shorn on the mid-side left of each wether with a clipper (Oster clippers, No 47cutting head, Germany) on day 0 of the experiment. At the end of the experiment (day 105), a second patch was clipped, and wool removed from the same spot. Heron's formula was used to measure and determine the patch area described by De Barbieri et al. (2017). The formula stated that the area of triangle=$\sqrt{(s-a)(s-b)(s-c)}$, where a, b and c are the sides of the triangle. S is the semi perimeter of the triangle, calculated as $S=a+b+c/2$. The clipped wool was dried in an oven for 48 h at 60 °C and weighed to determine the total wool weight. The equation determined wool growth: greasy weight (mg)/ area (10×10 cm) divided by the number of experimental days. Greasy wool is the wool in its natural state, after removing it from the sheep, before any commercial processing. It contains vegetable matters, extraneous soil, yolk, moisture, and suint. Greasy weight was determined by the near-infrared method (NIR). The NIR is a spectrophotometer, which uses near-infrared energy to measure the amount of grease remaining in a sample of scoured wool. Samples were then washed at 90 °C with Clean Plus (a mild soap produced by Clean Plus Chemical, Australia), and rinsed twice with cold distilled water to remove impurities and wool grease. This was followed by oven drying at 110 °C for 4 h. Samples were then reweighed at a relative humidity of 65% to calculate the clean wool weight (Charles et al., 2012). The wool yield was then calculated by determining the percentage of clean wool weight relative to greasy fleece weight, after which they were packed in a labeled plastic bag and sent to a commercial laboratory (BKB Wool (PTY) Ltd, Port Elizabeth, South Africa) where fibre diameter, SD, FDCV, CEM, comfort factor, staple length, FD Dev and number fibre <15% were measured according to IWTO 12 norms as described in detail by Charles *et al.* (2012).

5.2.6.2. Body condition score

The BCS was carried out weekly before the morning feeding by a trained person. The BCS chart was developed based on the system described by Khan *et al.* (2011) and Kenyon *et al.* (2014). There are 0.5 intervals for scores ranging from 1 (emaciated) to 5 (obese) in this method. Scoring was done by touching redundancy and crossing the amount of muscling and fat deposition over and around the vertebrae in the loin region.

5.2.6.3. Feed preference

For the feed preference test, twenty-four wethers from the section were individually housed in pens (5 × 7 m), having four feeding troughs with identification in a completely randomized design with six wethers per diet. The feeding troughs were distributed at an equal distance of 0.7 m from one another and positioned beside one facing the pens' entrance. Before the trial commencement, all the wethers were allowed to acclimatize themselves to the pen and feeders for 5 days. After the adaptation period and sequel to an overnight fast, 200 g of each diet 0%, 2%, 4%, and 6% FSF, was put into the four different feeding troughs in each pen. Each wether in each pen with four different feeding troughs containing four different diets was allowed to access the feeding troughs for ten minutes daily for a period of 7 days. Daily, the feed was rotated to a different feeding trough, and each wether was introduced into the pen for 10 minutes, and the order they accessed the various feeding trough was noted. The number of times the wether visit each feeding trough within the stipulated period were determined by counting and recorded as the number of visits (NV). The number of bites per visit by wether for each diet was determined by counting and recorded as the number of bites per visit (NBV). Time spent on a visit by each wether on each diet was determined using a stopwatch (EMC, Zhejiang, China) and recorded as time spent visit (TSV). The dry matter intake (DMI) for each diet was calculated by multiplying the percentage dry matter of the feed with the average daily feed intake. At the end of the 10 minutes, the feed refusal for each feeding trough was weighed to enable the determination of feed consumed. At the end of the preference test, animals received 1 kg of a mixture of leucine and teff hay.

5.2.6.4. Feed intake

Feed intake was determined by weighing the feed leftover in feed troughs, including feed refusals every day at 0800 h. Amounts of feed that disappeared were considered to be feed ingested by the wethers. The feeding troughs are designed to reduce feed spillages. Weights of feed leftover were subtracted from the total weight of the feed allocated to each wether and divided by 7 to determine average daily feed intake (ADFI) (Tefera et al., 2015). Dry matter intake was determined by multiply the percentage dry matter of the feed with the average daily feed intake.

5.2.6.5. Average daily gain

Average daily gain (ADG) was measured by weighing the wethers every week throughout the experiment period. The difference in weight of wethers at the beginning and end of each week divided by 7 determined the ADG (Sun et al., 2015). To determine body weight gain (BWG), the wethers were weighed weekly, over 14 weeks, using RUUDWEIGH, KM-2E electronic weighing system with 0.05 precision (RUUDSCALE, Durbanville, South Africa).

5.2.7. Statistical analyses

The general linear model of SAS 9.4 (SAS Institute, 2012) was used to analyze the effect of FSF inclusion levels on body condition scores, average daily weight gain, fibre diameter, staple length, comfort factor, SD, FDCV, CEM, wool growth, number of visits, number of bites per visit, time spent per visit, and dry matter intake. The model included different FSF inclusion levels as fixed effects for body condition score, wool parameters, and feed preference. The following model was used:

$$Y_{ij}= \mu+\beta_i+W_j + (WxB)_{ij}+ E_{ij}.$$

Where:

Y_{ij} is the dependent variable (BCS, ADG, FD, SL)

μ is the overall mean

β_i the effect different inclusion levels of FSF (j = 0 % ,2 % ,4 % and 6 %)

W_j is the effet of the jth week;

$(WxB)_{ij}$ is the interaction between week and inclusion levels of FSF;

E_{ij} is residual error ~ N (0; Iσ2).

Turkeys' studentized range test was used to test the significant differences between means.

5.3. Results

5.3.1. Body condition scoring

In chapter 3, the growth performance of wethers fed varying levels of FSF was determined. The result showed that growth performance had a linear relationship with body condition scores (Table 5.1). There was a significant effect of the FSF inclusion levels on the wethers' body condition score ($p < 0.05$). Wethers fed on FSF-based diets had the highest BCS than those fed on 0 % FSF ($p < 0.05$). Wethers fed on 4 % FSF had higher BCS than those fed on 2% FSF and 6 % FSF ($P > 0.05$, Table 5.1).

5.3.2. Wool quality parameters

The effects of FSF inclusion levels on wool characteristics are presented in Table 5.2. Wethers supplemented with FSF had higher wool growth than those fed on a diet with 0 % ($P < 0.05$). Wethers fed on a diet with a 6% FSF inclusion level had the highest wool growth, followed by 4%, 2%, and 0% FSF ($P < 0.05$). There was no significant difference in wool fibre diameter between wethers fed on diets with 0% and 4% FSF. Wethers fed on 6% inclusion level had lower wool diameter than those fed on 0% and 4% FSF ($P < 0.05$), but were similar to those fed on 2% FSF ($P > 0.05$). The staple length of wool from wethers fed on a diet with 0% FSF was shorter compared to wethers supplemented with FSF ($P < 0.05$). Wethers fed on 6% FSF had the highest value, followed by those on 4%, 2%, and the least was those fed on 0% FSF ($P < 0.05$).

5.3.3. Feed preference

The wethers' preference for diets is presented in Table 5.3. The number of visits (NV), number of bites per visit (NBV), time spent on the visit (TSV), and the dry matter intake (DMI) was higher ($P < 0.05$) in wethers fed on diets with 2%, 4% and 6% FSF than those on 0% FSF. The feed refusal (FR) was higher in wethers fed a diet with 0% FSF than those fed on 2%, 4%, and 6% inclusion levels ($P < 0.05$).

Table 5 1: The initial body weight, final body weight, total body weight gain, and body condition score of wethers for each treatment.

Parameters	Treatments				SEM	P-Value
	0 %	**2 %**	**4 %**	**6 %**		
Initial weight (kg)	19.6	19.4	19.5	19.43	0.428	
Final weight (kg)	27.97^{c}	28.57^{b}	31.30^{a}	29.66^{b}	0.4839	0.004
Average daily weight gain (g/d)	84.69^{c}	92.86^{bc}	121.42^{a}	105.35^{b}	9.53	0.011
DMI, g/d	576.29^{c}	546.11^{d}	665.76^{a}	618.84^{b}	11.84	0.0001
Body condition scores (units)	2.87^{b}	3.1^{a}	3.25^{a}	3.13^{a}	0.077	0.1088

^{abc}mean values with different superscript 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 5 2: Effect of different inclusion levels of FSF on wool parameters of Dohne Merino wethers.

Parameters	FSF inclusion levels (%)				SEM	P-Value
	0 %	2 %	4 %	6 %		
Wool growth (mg/100cm^2/day)	81.4^{c}	96.5^{b}	98.3^{b}	108.7^{a}	2.700	0.042
FD^{2}µm	18.77^{ab}	18.17^{bc}	19.73^{a}	17.46^{c}	0.557	0.110
FD Dev	0.232^{a}	0.96^{a}	0.93^{a}	0.33^{a}	0.509	0.882
SD Dev. (µm)	3.96^{a}	3.300^{c}	3.77^{a}	3.63^{a}	0.225	0.310
FDCV% (µm)	21.23^{a}	19.4^{ab}	18.80^{c}	20.83^{a}	0.624	0.073
Fibre<15 (mm%)	13.66^{b}	14.43^{b}	8.3^{c}	22.73^{a}	0.473	0.05
Comfort factor%	99.46^{ab}	99.66^{ab}	98.8^{b}	99.87^{a}	0.474	0.460
Staple length (mm)	46^{c}	51.32^{b}	51.67^{b}	58.33^{a}	1.170	0.0003
CEM	7.5	6.47	6.8	6.83	2.950	0.511

^{abc}mean values with different superscript 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 5 3: Counts of visit by the animal, number of bits per visit, time spent per visit, dry matter intake and feed refusal of wethers for different inclusion levels of FSF.

Parameters	FSF inclusion levels (%)				SEM	P-value
	0 %	**2 %**	**4 %**	**6 %**		
NV	2.96^{c}	3.96^{bc}	4.64^{b}	6.75^{a}	0.455	0.0001
NBV	3.17^{c}	4.43^{c}	8.00^{b}	11.54^{a}	0.889	0.0001
TSF	1.96^{c}	2.89^{b}	3.36^{b}	4.82^{a}	0.27	0.0001
DMI	18.82^{c}	36.85^{bc}	45.10^{b}	73.46^{a}	6.576	0.0001
FR	381.18^{a}	363.14^{ab}	354.54^{b}	328.68^{c}	6.751	0.0001

^{abc}mean values with different superscript 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. NV=number of visits; NBV=number of bites per visit; TSF=time spent per visit; FR=feed refusal; DMI=dry matter intake.

5.4. Discussion

Body condition scores (BCS) is a good predictor of fat reserves. An increase in body condition scores from 3 to 4 or 4 to 5 units results in a greater fat deposit rate at a subcutaneous level (Chay-Canul et al., 2016). Several studies have shown a direct relationship between body weight and body condition scores, but there is variation in body weight as BCS increases in different breeds of animals (Kenyon et al., 2014). In this study, the BCS followed the trend reported by Kenyon *et al.* (2014), where the BCS of the wethers fed with FSF supplemented diets were different ($P < 0.05$) from the control. The live weight gain comes at an energy cost, and BCS gain also has an energy cost. Therefore, it implies that wethers in FSF supplemented diets will have more energy reserves for productivity, physiological, and metabolism purposes than those on non-supplemented diets. Ramos *et al.* (2019) reported that wool growth rate and wool quality depend largely on the dietary protein available to the sheep. This present study observed a normal range of protein content for wool growth, as recommended by the National Research Council (NRC, 2007). Also, the high content of Zn in FSF helps in the synthesis of sulphur containing amino acid that promotes wool growth (Fadayifar *et al*., 2012). Similarly, FSF increases the DMI of the feed, which in turn increases the availability of protein for the sheep. It could be observed from our study that wethers fed on a diet with the highest DMI (g/day) (T3 (4 %) and T4 (6 %) had the highest wool growth. This aligns with the report of Li et al. (2013), who observed that the addition of mineral supplements to the diet of sheep increased the clean weight growth even in sheep not selected for wool growth. In essence, wool growth trends were similar to those of body weights, which were consistent with the results of McGregor et al. (2016) in which different diets were compared. A slight small change was noticed in fibre diameter between the wool of wethers fed on a diet with 0 % FSF and those on supplemented diets, while a considerable difference was observed between the wool of wethers on 0 % FSF and those on supplemented diets in staple length, FDCV and fibre>15%. This is in agreement with Frey et al. (2007), who established that finer wool from ewes showed less variations in the fibre diameter change. The difference in staple length observed in this study could be due to mineral elements such as Zn, Cu, and Fe that are present in FSF, which has been established to have a major impact on wool follicle growth and quality (Sahoo, 2011; Hatcher *et al.*, 2005).

Copper and zinc function directly in the process of wool growth. Copper is involved in keratinization, while rapidly proliferating tissues specifically require zinc. The two elements play a vital role in protein synthesis (White et al., 1994; Shinde et al., 2013). Shinde *et al*. (2013) reported that Zn concentration of 0.5 ppm in Merino lambs' plasma supported wool growth. It has been observed that Cu and Zn are involved in continuous and adequate manufacturing of Sulphur-containing amino acids (cysteine and methionine) that are needed by the wool follicles to sustain wool growth (Fadayifar et al., 2012; Pal et al., 2010; Reis, 1989). Methionine functions primarily in cysteine provision via the transulphuration pathway or by catabolism through the transamination pathway. However, comfort factor, CEM, FDSD, and FD Dev were not affected by the dietary treatments. This aligns with the observations of Grace and Lee (1992) and Dove (2009) that high Zn intake had no effect on the comfort factor, coarse edge micron (CEM), or fiber diameter coefficient of variation.

Wethers fed with the highest amount of FSF produced wool with FD of 17.46 μm. This can be classified as superfine wool; according to the Australian Wool Corporation (1990), FD between 15 and 18.75 μm is classed as such. This could be attributed to the high mineral content of diet at 6 % FSF, especially Copper, Zinc, and Iron that affect wool growth and fibre diameter (Schlink *et al*., 2010).

The wethers visited diets supplemented with FSF more than the control. Also, among the supplemented diets, the number of visits increased as the amount of FSF increases in the diets. This also made the number of bites per visit (NBV) and the DMI in the FSF supplemented diets to be statistically higher than the control. Among the factors that might have influenced feed preference by sheep are flavor, the physical outlook of the feeds, taste, and particle size of the diets (Forde, 2016; Dwain Horrocks, 1999). Sheep requires 25 to 40 ounces per head per day of NaCl and will eat less feed when deprived of NaCl (Digby et al., 2011). Similarly, it has been observed that minerals such as Na, Ca, K, and Mg increase the palatability of diets (Desimone et al., 2013; Garmyn et al., 2011). Also, FSF, Zeolite, bentonite, and some dietary clays are rich in minerals that could increase livestock diets' palatability (Slamova et al., 2011). This corroborates the result in this current study that FSF is rich in minerals (Na: 923 ppm; Ca: 0.22%; Mg: 0.11%; K: 0.11%) (Table1). Hence, the increase in the number of visits, number of bites by the wethers in the supplemented diets compared to the control could be attributed to the good taste impacted by its richness in dietary minerals including Na, Ca, K, and Mg. It has been reported that Na in the form of sodium chloride in the diets of sheep influences the feed preference through the taste and flavor of the diets (Merrill, 2013; Villalba *et al*., 2011). In the same way, Dias et al. (2013) and Abarghani et al. (2012) reported that Mg, which cannot be stored in the body are involved in energy generating reaction in the tissue of sheep, while Calcium and Potassium are involved in metabolism and body electrolytes balance that secure the health status of the animal vis-à-vis feed intakes. This agrees with the findings of Silva et al. (2015) and Moyo et al. (2019), who both reported that mineral supplementation has positive effects on feed preference vis-a-vis the feed intake and the DMI. Sahoo and Soren (2011) also reported that the addition of mineral supplements to sheep's diets increases the animal's preference for such feed.

5.5. Conclusion

Using fossil shell flour supplementation in the diets (2%, 4 %, and 6%) improved dry matter intake, average daily weight gain, body condition scores, and influenced feed preference and wool production and quality of Dohne-merino wethers.

Among supplemented diets, wethers fed on a diet with a 4% FSF inclusion level gave the most significant improvement in respect of average daily weight gain, dry matter intake, and body condition scores, while wethers on 6% FSF diet gave the highest wool yield, staple length, fibre <15% and was the most preferred diet by the wethers. Fibre diameter tended to improve but insignificantly, while FSF inclusion made no difference statistically on comfort factors, FD coefficient of variation, the standard deviation of fibre diameter, and coarse edge micron (CEM). It is therefore suggested that FSF should be included in the diets of Dohne-merino as it has the potential to improve wool growth, growth performance, and some wool qualities without adversely affecting the sheep. However, it is necessary to understand FSF's possible effect on sheep's methane production, especially with the recent global concern on greenhouse gasses.

5.6 References

Abarghani, A., Mostafaei, M., Alamisaed, K., Ghanbari, A., Sahraee, M., Ebrahimi, R., 2012. Investigation of calcium, phosphorous, and magnesium status of grazing sheep in Sabalan region, Iran. Journal of Agricultural Science and Technology 15:65–76.

Ahmed, M.A., Laing, M.D., Nsahlai, I. V., 2013. Studies on the ability of two isolates of Bacillus thuringiensis, an isolate of Clonostachys rosea f. rosea and a diatomaceous earth product to control gastrointestinal nematodes of sheep. Biocontrol Science and Technology 23:1067–1082.

Bernard, G., Worku, M., Ahmedna, M., 2009. The Effects of Diatomaceous Earth on Parasite Infected Goats. Bulletin of the Georgian National Academy of Sciencesi 3:129–135.

Charles, E.K.R., Jonas, E., Chaves, A. V., 2012. Diet preference of lambs offered a choice of concentrate diets containing different proportions of wheat dried distiller's grain with solubles. Small Ruminant Research 108:67–72.

Chay-Canul, A.J., Garcia-Herrera1, R., Meza-Villalvazo, V.M., Gomez-Vazquez, A., Cruz- Hernandez, A., Magaña-Monforte, J.G., Ku-Vera, J.C. 2016. Body fat reserves and their relationship to ultrasound back fat measurements in Pelibuey ewes. Ecosistemas Pecursor Agropecuarios 3:407–413.

Cilek, S., Breeding, A., Medicine, V. 2015. Determination of Fleece Qualities of Malya Sheep (11 / 16 Akkaraman X 5 / 16 Deutsches Merinofleischschaf) and Effect of Age and Sex on These Qualities. Pak. J. Agri. Sci. 52:533–540.

Cloete, J.J.E., Hoffman, L.C., Cloete, S.W.P. 2012. A comparison between slaughter traits and meat quality of various sheep breeds: Wool, dual-purpose and mutton. Meat Science 91:318–324.

Cottle, D., Gaden, C.A., Hoad, J., Lance, D., Smith, J., Scott, J.M.2016. The effects of pasture inputs and intensive rotational grazing on superfine wool production, quality and income. Animal Production Science. 53:750–764.

Department of Agriculture, Forestry and Fisheries (DAFF). 2016. A Profile of the South African Wool Market Value Chain, A Profile of the South African Wool Market Value Chain.Arcadia, South Africa.

De Barbieri, I., Montossi, F., Ciappesoni, G. 2017. Ewe reproduction affected by crossbreeding Corriedale and Dohne Merino. Book of abstracts of the 68th annual meeting of the European federation of animal science: 158.

Desimone, J.A., Beauchamp, G.K., Drewnowski, A., Johnson, G.H. 2013. Sodium in the food supply: Challenges and opportunities. Nutrition Reviews 71:52–59.

Dias, R.S., López, S., Patiño, R.M., Silva, T.S., Silva Filho, J.C., Vitti, D.M.S.S., Peçanha, M.R.S.R., Kebreab, E., France, J. 2013. Calcium and phosphorus utilization in growing sheep supplemented with dicalcium phosphate. Journal of Agricultural Science. 151:424–433.

Digby, S.N., Chadwick, M.A., Blache, D. 2011. Salt intake and reproductive function in sheep. Animal 5:1207–1216.

Distel, R.A., Villalba, J.J. 2018. Use of unpalatable forages by ruminants: The influence of experience with the biophysical and social environment. Animals 8:56

Dove, H.2009. Supplementary Feeding for Wool Growth and Reproduction, in: Nutrition Management for Grazing Animals. pp. 1–10.

Dwain, R., Horrocks, J.F.V. 1999. Forage quality in: Harvested Forages. pp. 17–47.

Esfandyari, H., Aslaminejad, A.A., Rafat, S.A. 2011. Wool characteristics in the third generation of Arkharmerino × Ghezel and Arkharmerino × Moghani crossbreed sheep. Tropical Animal Health and Production. 43:1337–1343.

Fadayifar, A., Aliarabi, H., Tabatabaei, M.M., Zamani, P., Bahari, A., Malecki, M., Dezfoulian, A.H. 2012. Improvement in lamb performance on barley-based diet supplemented with zinc. Livestock Science 144:285–289.

Food and Agriculture Organization of the United Nations (FAO). 2009. Statistical Yearbook. Rome, Italy.

Food and Agriculture Organization of the United Nations (FAO). 2016. Statistical Yearbook. Rome, Italy

Frey, A., Martín, N., Caro, A. D., Ugarte, D.Á., Elvira, M. 2007. Variación del diámetro promedio de fibras en ovejas Merino trasladadas desde la Patagonia a la región Pampeana en Argentina. Fiber diameter change in Patagonian Merino ewes moved to Pradera Pampeana Region in Argentina. Sitio Argentino de Producción Animal 17:1–6.

Forde, G. C., 2016. Flavor perception and satiation, in: From Food to Behaviors, Wellbeing and Health. Woodhead Publishing Series in Food Science, Technology and Nutrition. Pp. 251–276.

Garmyn, A.J., Hilton, G.G., Mateescu, R.G., Morgan, J.B., Reecy, J.M., Tait Jr, R.G., Beitz., D.C., Duan, Q., Schoonmaker, J.P., Mayes, M.S., Drewnoski, M.., Liu, Q., VanOverbeke, D.L. 2011. Estimation of relationships between mineral concentration and fatty ... Journal of Animal Science 89:2849–2858.

Grace, N.D., Lee, J., 1992. Influence of high zinc intakes, season, and staple site on the elemental composition of wool and fleece quality in grazing sheep. New Zealand Journal of Agricultural Research 35:367–377.

Hatcher, S., Atkins, K.D., Thornberry, K..2005. Age changes in wool traits of Merino sheep in western NSW. Pro. Assoc. Adv. Anim. Breed. Genet 16:316–317.

Ikusika, O.O., Mpendulo, C.T., Zindove, T.J., Okoh, A.I. 2019. Fossil Shell Flour in Livestock Production: A Review. Animals. 9:1–20.

Internation Wool and Textile Organization (IWTO). 2015. Global wool production. 12 Edition 1–5.

Kenyon, P.R., Maloney, S.K., Blache, D. 2014. Review of sheep body condition score in relation to production characteristics. New Zealand Journal of Agricultural Research 57: 38–64.

Khan, S.A., Epstein, J.H., Olival, K.J., Hassan, M.M., Hossain, M.B., Rahman, K.B.M.A., Elahi, M.F., Mamun, M.A., Haider, N., Yasin, G., Desmond, J. 2011. Hematology and serum chemistry reference values of stray dogs in Bangladesh. Open Veterinary Journal 1:13–20.

Li, L., Silveira, C.I., Nolan, J. V, Godwin, I.R., Leng, R.A., Hegarty, R.S. 2013. Effect of added dietary nitrate and elemental sulfur on wool growth and methane emission of Merino lambs. Animal ProductionScience.53:1195–1201.

Martin, K. 2013. Silicon: the health benefits of a metalloid. Met Ions Life Sci. 13:451–471.

McGregor, B.A., de Graaf, S.P., Hatcher, S. 2016. On-farm factors affecting physical quality of Merino wool. 1. Nutrition, reproduction, health and management. Small Ruminant Research 137: 138–150.

McLean, B., Frost, D., Evans, E., Clarke, A., 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

Meale, S.J., Chaves, A. V, Ding, S., Bush, R.D., Mcallister, T.A. 2014a. Effects of crude glycerin supplementation on wool production, feeding behavior, and body condition of Merino ewes 1. J. Anim. Sci 91:878–885.

Meale, S.J., Chaves, A. V, He, M.L., Mcallister, T.A. 2014b. Dose – response of supplementing marine algae (Schizochytrium spp.) on production performance, fatty acid profiles, and wool parameters of growing lambs 1. J. Anim. Sci 92:2202–2213.

Merrill, C., 2013. the Evaluation of a Flavor Enhancer on Intake and Production of High Producing Dairy Cow. A thesis submitted to the Department of Animal Science, University of Delaware.

Moyo, M., Adebayo, R.A., Nsahlai, I.V. 2019. Effects of diet and roughage quality, and period of the day on diurnal feeding behaviour patterns of sheep and goats under subtropical conditions. Asian-Australasian Journal of Animal Sciences 32:675–690.

Mortimer, S.I., Hatcher, S., Fogarty, N.M., Van Der Werf, J.H.J., Brown, D.J., Swan, A.A., Jacob, R.H., Geesink, G.H., Hopkins, D.L., Hocking Edwards, J.E. 2017. Genetic correlations between wool traits and meat quality traits in Merino sheep. Journal of Animal Science. 95:4260–4273.

Naidoo, P., Olivier, J.J., Morris, J., Cloete, S.W.P. 2016. Does selecting for finer wool result in in the South African Dohne Merino sheep breed. South Africal Journal of Animal Science. 13:49–53.

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

States.

Pal, D.T., Gowda, N.K.S., Prasad, C.S., Amarnath, R., Bharadwaj, U., Suresh Babu, G., Sampath, K.T. 2010. Effect of copper- and zinc-methionine supplementation on bioavailability, mineral status and tissue concentrations of copper and zinc in ewes. Journal of Trace Elements in Medicine and Biology 24:89–94.

Ramos, Z., De Barbieri, I., van Lier, E., Montossi, F. 2019. Body and wool growth of lambs grazing on native pastures can be improved with energy and protein supplementation. Small Ruminant Research 171:92–98.

Rangel, J.H., Gardiner, C.P 2009. Stimulation of wool growth by Desmanthus spp. as a supplement in a Mitchell grass hay. Tropical Grasslands 43:106–111.

Salehian, Z., Naderi, N., Souri, M., Mirmahmoudi, R., Hozhabri, F. 2015. Seasonal variationof fibre follicle activity and wool growth in fat-tailed Sanjabi sheep in west Iran. Tropical Animal Health and Production 47:567–573.

Sales, J. 2011. Effects of Saccharomyces cerevisiae supplementation on ruminal parameters, nutrient digestibility and growth in sheep: A meta-analysis. Small Ruminant Research 100: 19–29.

Sahoo, A., Soren, N.M. 2011. Nutrition for Wool Production.WMC002384. 2(10):1-11.

Saxena, V.K., Jha, K.B., Meena, S.A., Naqvi, S.M. 2015. Characterization of MTNR1A gene in terms of genetic variability in a panel of subtemperate and subtropical Indian sheep breeds. Journal of Genetics 94:715–721.

Schlink, A.C., Nguyen, M., Viljoen, G.J., 2010. Water requirements for livestock production: Water requirements for livestock. Rev.Sci.tech.off.int.Epzi 29: 603–619.

Shinde, A.K., Sankhyan, S.K., Meena, R., Regar, R.K.2013. Effect of feed supplementation with copper- and zinc salts on the growth, wool yield, nutrient utilization, blood constituents and mineral profile of Malpura lambs, in: Agricultural Science Research. pp. 284–291.

Silva, T.P.D., Marques, C.A.T., Torreão, J.N.C., Bezerra, L.R., Araújo, M.J., Gottardi, F.P., Edvan, R.L., Oliveira, R.L. 2015. Ingestive behaviour of grazing ewes given two levels of concentrate. South African Journal of Animal Sciences 45:180–187.

Slamova, R., Trckova, M., Vondruskova, H., Zraly, P.I. 2011. Clay minerals in animal nutrition. Appl Clay Sci 51:395–398.

Van Burgel, A.J., Oldham, C.M., Behrendt, R., Curnow, M., Gordon, D.J., Thompson, A.N. 2011. The merit of condition score and fat score as alternatives to liveweight for managing the nutrition of ewes. Animal Production Science 51:834–841.

Villalba, J.J., Bach, A., Ipharraguerre, I.R. 2011. Feeding behavior and performance of lambs are influenced by flavor diversity. Journal of Animal Science 89:2571–2581.

White, C.L., Martin, G.B., Hynd, P.I., Chapman, R.E. 1994. The effect of zinc deficiency on wool growth and skin and wool follicle histology of male Merino lambs. British Journal of Nutrition 71:425–435.

Chapter 6

Effect of dietary supplementation with fossil shell flour on enteric methane output and position-dependent variations in Dohne-merino wethers

Abstract

This study's objective was to evaluate the effect of varying inclusion levels of fossil shell flour (FSF) on Dohne-Merino wethers' position on enteric methane output. Dietary food-grade fossil shell flour was fed to twenty-four Dohne Merino wethers, weighing 20.0±1.50 kg averagely, using a completely randomized design with six wethers in each treatment. The wethers were fed on a basal diet without FSF addition (control, 0 %) or FSF (2%, 4%, or 6%) of diet DM for 12 weeks. Enteric methane production was measured when the wethers were feeding, standing, and resting, using a portable Laser Methane Detector (LMD). Wethers fed on a diet with 4% showed the highest enteric methane output, followed by 6%, 0%, and 2% FSF. Higher enteric methane emission was observed for resting wethers than those feeding and standing ($P < 0.05$). Inclusion of fossil shell flour in Dohne-merino wethers' diets at 4% and 6% increases enteric methane output ($P<0.05$). Dohne-merino sheep tend to emit more enteric methane when resting than when feeding or standing idle.

Keywords: Fossil shell flour, enteric methane, animal position, Dohne-merino ram.

6.1. Introduction

In the last two decades, there has been great concern about global warming due to an increase in the concentration of many atmospheric gasses, which leads to an increase in atmospheric temperature (IPPC, 2018). These gasses include methane, carbon dioxide, and nitrous oxide often referred to as greenhouse gases. It has been projected that greenhouse effects in the next century will bring about the distribution of new deserts in the world

, and change the range of pest that affects plants, which may threaten the existence of animals and human health (Moss et al., 2000). The emission of GHG from livestock and their impact on climate change is a major concern worldwide (Aemu et al., 2011).

Emissions from livestock contribute 98% of methane emissions from the agricultural sector (Otter, 2010). Ogino et al. (2007) observed that enteric methane constituted about 50% to 60 % of GHG emitted in ruminant production at the farm scale. Methane emissions from livestock are estimated to be 2.2 billion tons of carbon dioxide equivalent, accounting for 35% of the global anthropogenic methane emissions (FAO, 2013; Knapp et al., 2014). However, it is expected to increase, particularly in developing countries (Tubiella et al., 2013) further.

Enteric methane is a natural by-product of the fermentation processes in the large intestine of ruminant animals and is released into the atmospheric environment through breathing (Wiedemann et al., 2016). Methane and nitrous oxide have higher global warming potentials than carbon dioxide (Fraser et al., 2015). Methane is 21 to 25 times more effective in trapping heat in the atmosphere, while nitrous oxide has a global warming potential of 296 to 310 times that of CO_2 (IPCC, 2006; ANIR, 2009). Depending on the level of feed intakes and rumen activity, the rate of enteric methane production varies with individual animals (Chaturvedi et al.,2014). Apart from its negative effect on global warming, methane accounts for a significant amount of animals' energy loss during grazing or browsing. The energy losses range from 2% to 12% of gross energy (GE) intake, thereby lowering livestock productivity and economic efficiency of ruminant production (Sallaku et al., 2011; IPCC, 2018). Reducing the production of enteric methane in ruminants without altering animal production is, therefore, desirable both as a strategy to reduce global GHG emissions and as a means of improving feed conversion efficiency.

Sheep has the largest population among the small stock globally (FAO, 2017); hence their contribution to GHG is high (IPPC, 2018). The Dohne-Merino has been one of the fastest breeds of sheep spreading across many continents (Mcmaster, 2015; Cardellino, 2016) account for over 36 % of the GHG emissions from livestock species. Methane emissions by sheep are produced in the rumen during microbial fermentation of feed, especially carbohydrates (Sallaku et al., 2011). Methane production in sheep is influenced by feed intake, diet composition, digestibility and quality of forage, forage species breed, and purposes of the animal. Also, activities of the animals, such as resting, standing, or feeding, as well as feed additives, have been reported to affect the volume of methane output in other livestock (Scholtz et al., 2013). The animals' position and the activities have been reported to affect the amount of methane being generated. Roessler *et al*. (2018) reported that when a goat is lying, it generates more enteric methane than when standing. Likewise, Washaya et al. (2018) observed that when goats are ruminating or resting after a long time of journey, higher methane was being generated than when the animal is standing after feeding.

There is a growing interest in using supplements or additives to mitigate the emission of greenhouse gas in livestock production systems, especially small stock. Thota et al. (2017) reported that the mean enteric methane emissions (l/day) were significantly lower in sheep fed with probiotics supplemented diet than sheep fed on a diet without probiotics supplementation and reduced 21.9 percent as compared to the non-supplemented diets. Similarly, Ma et al. (2016) reported that allicin supplementation effectively reduced daily methane emissions in ewes, probably by decreasing the population of ruminal protozoans and methanogens. These chemicals, however, are either toxic to the cow or exhibit only transient effects on methanogens (Moss et al., 2000). The use of natural products as additives to mitigate the emission of greenhouse gas in livestock is beneficial to both livestock and consumers.

The most recently sought for use as feed additives is fossil shell flour (FSF), among the common natural products used as feed additives. In chapters 3, 4, and 5, it had been observed that FSF as a feed additive benefits sheep production in terms of growth performance, feed preference, and wool quality. A few studies have been conducted on the impact of FSF in sheep diets on methane gas production. Fossil shell flour is a naturally occurring, silicon-rich sedimentary rock made up of fossilized remains of millions of diatoms, a type of hard-shelled plant algae originally deposited millions of years ago in the earth from dried up seas and lakes (Ikusika et al., 2019; Koster, 2013). They are readily available, cost-effective, healthy, and eco-friendly for both animal and human beings. Because of the antimicrobial activities that have been reported of FSF (Chen and Liu, 2016), it is believed that it would reduce methane production by militating methanogenesis microbes.

Several techniques have been developed to measure the enteric methane emissions in ruminant animals (Patra, 2016). The respiration chamber technique is considered the standard gold method to quantify ruminant animals' enteric methane output. However, estimates obtained from chamber measurements are not transferable to grazing ruminants because they are obtained in an artificial environment (Storm et al., 2012). Also, this technique is very expensive and time-demanding. The portable laser methane detector (LMD) technique may offer a reasonable alternative because it allows the quantification of CH_4 from animals in their natural environment without interfering with the animal's behavior (Chagunda et al., 2009; Ricci et al., 2014). Unlike the chamber technique, it is easy to handle, cheap, and time saver.

Up to date, there have been few pieces of literature on the use of FSF as an additive to reduce methane emissions in sheep with inconclusive results. Further studies are required to evaluate and ascertain the effect of FSF's varying dietary inclusion on enteric methane emissions. Besides, it is a requirement for feed additives, which should have no negative effect on feed utilization. This will help to enlighten policymakers, farmers, and non-governmental organizations on another strategy that will reduce greenhouse gasses, thereby preserving our environment. Against this background, this study's objective was to investigate the effect of a diet supplemented with varying levels of FSF at a different animal position on enteric methane production by Dohne-Merino wethers. It was hypothesized that the inclusion of fossil shell flour into the diet of Dohne-merino wethers would decrease the production of enteric methane.

6.1. Materials and Methods

6.1.1. Ethical approval

The ethical approval was described in section 3.2.1

6.1.2. Study site

The study site was described in section 3.2.2.

6.1.3. Sheep and housing

The animals and housing, experimental design, and management are the same as described in section 3.2.3.

6.1.4. Diets and feeding

The processing and composition of the experimental diets is the same as described in section 3.2.4

6.1.5. Analytical procedures

6.1.5.1. Mineral analyses

Mineral analyses were described in 3.2.5.2

6.1.5.2. Proximate analyses of the experimental diets

The procedures and methods were described in section 3.2.5.1.

6.1.6. Measurement of methane production

The measurement of methane was done using laser methane detector LMD (Crowcon Detection Instruments Ltd., Oxford shire, United Kingdom). Measurements were carried out weekly from the inception of the trial during three different wethers' activities, which includes resting, feeding, and standing. Also, during the last 7 days of the experiment, methane output was measured daily for the same three activities of the wethers. Methane gas column density was measured by directing the hand-held LMD machine targeting (visible HeNe) at the nostrils of wethers for a period of 25s per wethers at a distance of 2 m. The 2-m distance was considered safe to prevent the disturbance of the animal's activity as described by Chagunda et al. (2009) and Washaya et al. (2018). The effect of methane that existed in the atmosphere from the measured results was discounted using the offset function of the LMD. All measurements were taken at approximately the same time of day (1000h-1100h). Three measurements were taken from individual wethers during each activity. Methane eructed was determined per each activity using standard respiratory coefficients per activity, and then translated to an equivalent emission per day. Methane production was also evaluated in relation to dry matter intake (DMI). Laser methane detector (LMD) measures methane emission in ppm-m, which is not equivalent to g/kg/d. Therefore, to know how much of methane is being produced per wether, methane was determined on a DMI basis.

Methane eructed during activity MTV = MMD × T_{Vr} / 106ml (Chagunda et al., 2009)

Where: MTV is the enteric methane in breath in ml during ruminating; MMD is the enteric methane detected by LMD converted from ppm-m to ml.

TVr is the tidal volume during different activities

Tidal volume (feeding) = 3100 ml, tidal volume (standing) = 3800 ml for dairy animals. These were then converted using livestock units to represent sheep, where 0.5 LU cow is equivalent to 0.1 livestock unit (LU) sheep (Chilonda and Otte, 2006) for sub-Saharan Africa.

The TVr for sheep were, therefore: TVr feeding = 620 ml, TVr standing = 760 ml, TVr ruminating = 760 ml

Methane eructed per activity per day MTTA = MTV × RTA (Chagunda et al., 2009)

MTTA is the amount of enteric methane produced during an activity (rumination, feeding, just standing).

Methane eructed per day MD = MTTA x (TD × RTA) ml/day (Chagunda et al., 2009) Where:

MD is daily enteric methane

TD is daytime in seconds

RTA is the total time spent on an activity

RTA standing = 1440, RTA feeding = 2880, and RTA ruminating = 7200 By substitution and use of specific density conversion factor, daily enteric methane in grams (MDG) is:

MDG (g/day) = MD x 0.00066715 (CH_4 density in g/ml) (Chagunda et al., 2009)

Methane (l/day) = 0.0305 DMI(g/day) – 4.441 (Shibata et al., 1992) M (kg/head/day) = DMI x 0.0188 + 0.00158 (Howden & Reyenga, 1999).

6.1.7. Statistical analyses

The PROC MIXED procedure of Statistical Analysis Systems Institute (SAS, 2012) for repeated measures was used to test for significance of inclusion level of FSF and position of wethers on methane volume. Turkey's studentized range test was used to test the significant differences between means. The statistical model used was:

$Y_{ijk} = \mu + T_j + B_j + D_k + (TxBxD)_{jjk} + e_{ijk}$ Where:

Y_{ijk} is methane volume μ is the overall mean

T_{js} the effect of diet (i = 1,2,3,4) B_j the effect of position (i=1,2,3)

D_k is the effect of week (k, = 1,2,3,4,5,6)

$(TxBxD)i_{jk}$ is the interaction effect between treatment, week, and position

e_{ijk} is the error term.

6.2. Results

Table 6.1 shows enteric methane emission from wethers fed diets with varying FSF levels during different activities. Enteric methane output was lowest in the wethers fed on a diet with 0% FSF and highest on those feds with 6% FSF during standing, feeding, and resting ($P < 0.05$). As the FSF inclusion level increased, enteric methane output also increased except for feeding and resting wethers fed on diets with 2 % and 4 % FSF ($P<0.05$). Across the diets, there were no significant differences for all the activities ($P > 0.05$). The wethers released the highest methane volume when they were resting and the least when feeding ($P < 0.05$).

Table 6.2 shows the methane emission, average daily feed intake, and the dry matter intake in the last 7 days of the feeding trial measured consecutively. Both the ADFI and the DMI had a linear relationship with the amount of methane produced. Wethers fed 4 % FSF had the highest ADFI and DMI values and produced the highest ($P<0.05$) methane value. The amount of methane produced by wethers fed on a diet with 0% FSF was significantly lesser compared to the amount generated by wethers on 4% and 6% FSF, but not from wethers on 2% FSF ($P < 0.05$). Wethers on a 4%FSF diet emitted more methane than the wethers on 0% FSF, and other FSF supplemented treatment ($P < 0.05$). In all the diets, wethers generated more methane (g/day) when they are resting than feeding or just standing ($P < 0.05$). In all the activities together, wethers fed on a diet with 4 % produced more methane than those on 0 % FSF and other FSF supplemented diets ($P < 0.05$).

Figure 6.1 shows the amount of methane generated by the wethers on varying amounts of FSF over a period of 12 weeks. From week 1-3, the volume of methane produced by wethers fed on 0 %, 2%,4 %, and 6% FSF of the diets was the same ($P<0.05$). From week 4-6, the volume of methane emitted by wethers on 0% FSF began to be lesser compared with those emitted by wethers on 2%, 4%, and 6% FSF of the diets. From week 7 -12, the volume of methane emitted was very higher in the FSF supplemented diets compared to 0% FSF diet ($P<0.05$).

Table 6 1: Enteric methane emission from Dohne- merino wethers fed on varying FSF levels during different activities in the last seven days of the trial.

	Levels of FSF inclusion				
Activity	**0 %**	**2 %**	**4 %**	**6 %**	**SEM**
Standing	17.74[a]	18.54[a]	21.86[a]	25.71[a]	4.66
Feeding	15.83[a]	22.52[a]	13.24[a]	19.31[a]	4.81
Resting	38.63[a]	42.46[a]	42[a]	47.50[a]	11.25

[abc] mean values with different superscripts across the row are significantly different (P < 0.05).

Table 6 2: Enteric methane emission from Dohne- merino wethers fed on varying inclusion levels of FSF (grams /day).

Activity	Levels of FSF inclusion				SEM
	0 %	2 %	4 %	6 %	
ADFI (g)	84.69[c]	92.86[bc]	121.42[a]	105.35[b]	9.53
DMI (g)	576.29[c]	546.11[d]	665.76[a]	619.84[b]	11.84
Methane (l/day)	17.27	16.66	20.30	18.90	0.32
Methane kgDMI/year/	3,887.25	3,748.55	4569.8	4252.25	81.22
Methane (g/kg DMI)	10.65	10.27	12.52	11.65	0.218
Methane (g/day					
Standing	0.0046	0.0125	0.0198	0.023	0.000147
Feeding	0.0114	0.0017	0.0124	0.0141	0.0024
Resting	0.036	0.0511	0.301	0.0438	0.00041

[abc] mean values with different superscripts across the row are significantly different ($P < 0.05$).

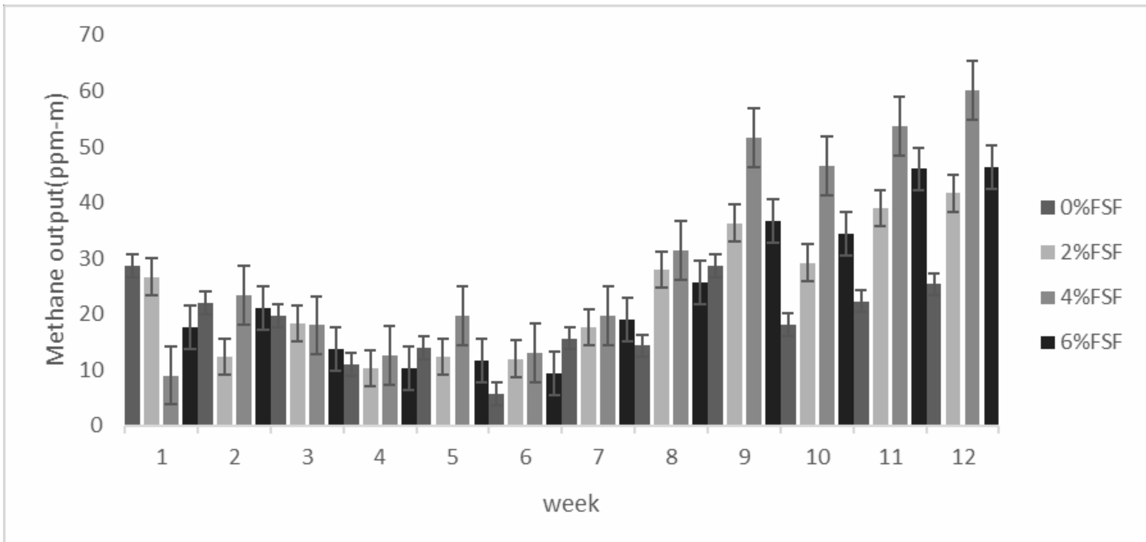

Figure 6 1: Methane emission of Dohne-merino wethers at varying Fossil shell flour levels measured for 12 weeks shown as means ± standard errors. 0 % FSF, 2 %, FSF, 4 % FSF, and 6 % FSF.

Figures 6.2 shows the effect of different activities of wethers on methane output at 0%, 2%,4 % and 6% FSF diets. Methane output was highest ($P<0.05$) during resting and lowest during feeding at the varying inclusion levels of FSF. The methane emitted during resting were significantly different ($P<0.05$) from the volume emitted during feeding activities. In all the activities, methane output increased as the FSF inclusion levels increased up to 4% FSF levels and declined thereafter.

6.3. Discussion

The current study found that daily methane emissions (ppm-m) increased as the FSF inclusion levels increases. This was also true of both the ADFI and DMI, which increased as the FSF inclusion increases. Previous studies have shown that as animals consume more feed, they produce significantly more gas than their control (Scholtz et al., 2013; Tao et al., 2018). Ramin and Huhtanen (2013) and Du Toit et al. (2013) reported that the total methane emitted by an animal is determined mainly by the DMI of the feed consumed by that animal. The results of this study align with the report from these authors. The DMI of FSF supplemented treatments were higher than the DMI of the wethers on 0 %FSF. Hence, the methane output of the supplemented diets was higher than those wethers on 0 %FSF. This could be because FSF increased the feed intake of the wethers, thereby increasing the DMI (g/kg) hence more feed content for fermentation. The higher methane output in wethers on FSF supplemented diets compared to those on 0 % FSF observed in this study agrees with Emeruwa's (2016) report, which considered the effect of varying levels of FSF on in vitro gas production from West Africa Dwarf sheep. This result suggests that FSF promotes methanogen or protozoan populations. Newbold et al. (1995) and Ma et al. (2016) reported that endo- and ecto-symbiotic methanogens of protozoans could contribute up to 25% of rumen fluid methane emissions in sheep.

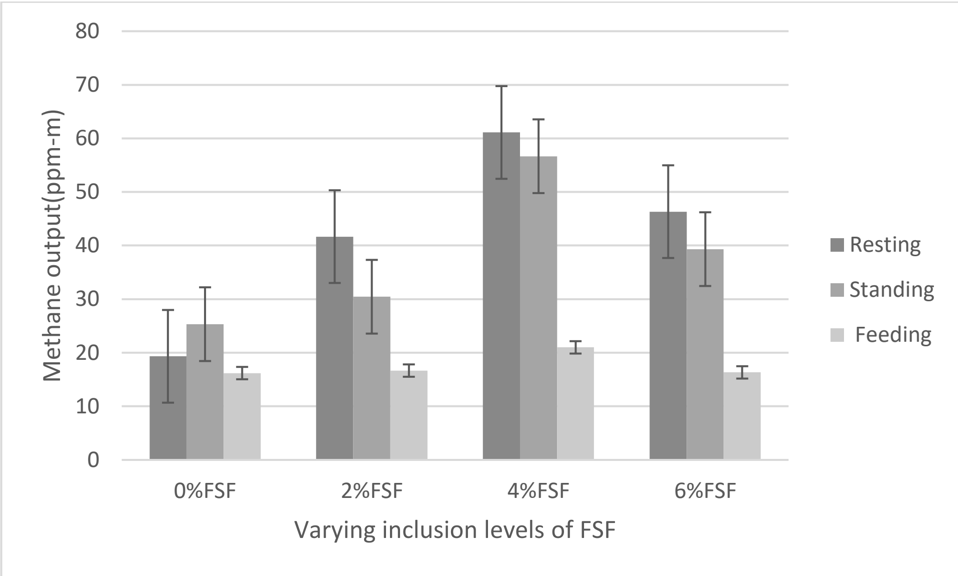

Figure 6 2: Methane emission at different position of Dohne-merino wethers fed basal diet+4

Though the result obtained for wethers on FSF supplemented diets (between 10.27 to 12.52 g/kg DM) were higher compared with wethers of FSF non-supplemented diet (10.65 g/kgDM), it is still lower than estimation given for South Africa commercial sheep by Du Toit et al. (2013). This study also observed that; more methane was emitted during the 8th and 12th week compared to the 1st to 7th week. Methane output was inconsistent at the early period of the trial. Hence, methane from wethers on 0 % FSF was higher than those wethers on 2 %,4 %, and 6 % FSF during the 1st and 3rd week. However, wethers on FSF supplemented diets emitted far higher methane volume compared to 0 % FSF during the last 5 weeks of the trial. This could be because FSF has increased the palatability of the FSF supplemented diets, thereby increasing the average daily feed intake, which increases the amount of methane generated from such wethers.

An animal's position at different times of the day affected the amount of enteric methane being generated at a particular time (Chagunda et al., 2009; Chagunda, 2013). Grobler et al. (2014) reported a positive relationship between lying behavior and rumination activity for dairy cattle using a distance of 3 m between the measurement device and the animal. Animal enteric methane production is relative to activities by the animal (Washaya et al., 2018). During the day, an animal is either eating, standing, or resting (during which they ruminate what they have eaten), and these 3 different positions were considered. Chagunda et al. (2009) and Washaya et al. (2018) reported that when an animal is quiet and relaxed during rumination, methane emission is higher than when an animal is eating or standing. The result obtained from this study agreed with these authors' reports, in that the wethers emitted more methane output during resting than when standing or feeding. The explanation of this could be that when an animal is eating (feeding), lesser microbial activities in the rumen (reservoir of microbes) are going on compared to when the wethers are resting. When wethers are eating, most of the activities take place in the mouth. At this stage, enzymes and very few counts of microbes that are contained in the saliva are involved. Also, continued dilution of the rumen during eating and peristaltic contractions for disturbance of microbial activities compared to resting period decreases methane production. During the eating period, particles are also larger, thereby decreasing microbial activity. However, when wethers are resting, one of the characteristics they exhibit is called regurgitation. This involves bringing back from the rumen to the mouth, feeds they have previously swallowed while feeding for proper chewing, grinding, and mixing. Regurgitation breaks down, feeds into small particles, increases surface area for rapid fermentation, and releases more soluble locked in crystalline structures hence making them available. Therefore, more methane is produced due to more soluble that gave room to increase in microbial growth and population.

6.4 Conclusion

In this study, methane production from Dohne Merino wethers was relative to the animal activity, with resting producing more gas than when feeding or standing. Diets supplemented with FSF produce more methane gas than non-supplemented diets. When feeding of FSF goes beyond 5 weeks, a greater volume of methane may be generated because of an increase in average daily feed intake promoted by continuous addition of FSF. Enteric methane production is positively related to ADFI and DMI of the wether. Since methane is generated in sheep as a result of fermentation in the rumen, there is, therefore, a need to investigate the effect of varying levels of FSF on the rumen fermentation parameters, in vitro true digestibility, and relative feed value in Dohne Merino wethers.

6.5 References

Alemu, A. W., Dijkstra, J., Bannink, A.; France, J., Kebreab, E. 2011. Rumen stoichiometric models and their contribution and challenges in predicting enteric methane production. Anim. Feed Sci.Technol.*166–167*:761–778.

Australian National Greenhouse Accounts: National Inventory Reports (ANIR). 2009. Climate Change and Energy Efficiency, Commonwealth of Australia, Canberra, ACT.

Blignaut, J. N., Mabugu, R. M., Chitiga-Mabugu, M. R., 2005. Constructing a greenhouse gas emissions inventory using energy balances: the case of South Africa. J. Energy South. Africa *16*: 21–32.

Cardellino, R. 2016. The Dohne In South America R.C. Cardellino Delta Consultants, Montevideo, Uruguay

Chagunda, M. G. G., Ross, D., Roberts, D. J., 2009. On the use of a laser methane detector in dairy cows. Comput. Electron. Agric *68*:157–160.

Chagunda, M. G. G. 2013. Opportunities and challenges in the use of the Laser Methane Detector to monitor enteric methane emissions from ruminants. Animal *7*:394–400.

Chaturvedi, I., Dutta, T. K., Singh, P. K., Sharma, A., Kumar, M., Rao, B. 2014. Effect of Herbal Feed Additives on IVDMD, Methane and Total Gas Production Via in-vitro Study. J. Agroecol. Nat. Resour. Manag. 1:2394–786.

Chen, Y., Liu, K. 2016. Preparation of granulated N-doped TiO2/diatomite's and its application of visible light degradation and disinfection. Power Tech. 303:176–191.

Chilonda, P., Otte, J. 2006. Indicators to monitor trends in livestock production at national, regional and international levels. Livestock Research for Rural Development. Vol.18, Article #117. (Accessed on 21/11/2019).

Du Toit, C. J. L., van Niekerk, W. A., Meissner, H. H. 2013. Direct greenhouse gas emissions of the South African small stock sectors. South African J. Anim. Sci. *43*:340–361.

Emeruwa, C. H. 2016. Growth Performance of West African Dwarf Sheep Fed Diets Supplemented with Fossil shell flour., University of Ibadan, Nigeria.

Food and Agriculture Organization (FAO). 2013. Statistical Yearbook. Rome, Italy.

Food and Agriculture Organization (FAO). 2017. Statistical Yearbook. Rome, Italy

Fraser, M. D., Fleming, H. R., Theobald, V. J., Moorby, J. M. 2015. Effect of breed and pasture type on methane emissions from weaned lambs offered fresh forage. J.

Agric. Sci. *153*:1128–1134.

Intergovernment Panel on Climate Change (IPCC).2018. Press Release 8 October 2018. (Accessed on 11/04/2019)

Grobler, S. M., Scholtz, M. M., van Rooyen, H., Mpayipheli, M., Neser, F. W. C. 2014. Methane production in different breeds, grazing different pastures or fed a total mixed ration, as measured by a laser methane detector. South African J. Anim. Sci. *44*: S12– S16.

Howden, S.M., Reyenga, P.J. 1999. Methane emissions from Australian livestock: implications of the Kyoto Protocol. Aust. Journal of Agric. Res. *50*:1285–1292.

Ikusika, O.O., Mpendulo, C.T., Zindove, T.J., Okoh, A.I. 2019. Fossil Shell Flour in Livestock Production: A Review. Animals. 9:1–20.

Intergovernment Panel on Climate Change (IPCC). 2006. guidelines for National Greenhouse Gas Inventories. Prepared by the National Greenhouse Gas Inventories Programme. Eds: Eggleston, H.S., Buendia, L., Miwa, K., Ngara, T. & Tanabe, K., Published: IGES, Tokyo, Japan

Knapp, J. R., Laur, G. L., Vadas, P. A., Weiss, W. P., Tricarico, J. M. 2014. Invited review: Enteric methane in dairy cattle production: Quantifying the opportunities and impact of reducing emissions. J. Dairy Sci. *97*: 3231–3261.

Koster, H. 2013.Diatomite in Animal Feeds.Availablefrom agrisilica.co.za/pdf/eng/Diatoms%20in%20Animal%20Feeds%20HH%20Koster.pdf.

Ma, T., Chen, D., Tu, Y., Zhang, N., Si, B., Deng, K., Diao, Q. 2016. Effect of supplementation of allicin on methanogenesis and ruminal microbial flora in Dorper crossbred ewes. Journal of Animal Science and Biotechnology 7:1-7

McMaster, C. 2015. Birth of a breed. The Dohne Merino Story.Simon Says Advertising, Port Elizabeth, RSA.

Moss, A.R., Jouany, J.P., Newbold, J. 2000. Methane production by ruminants: its contribution to global warming. Annales de Zootechnie 49:231–253.

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

Newbold, C.J., Lassalas, B., J.P. 1995. The importance of methanogens associated with ciliate protozoa in ruminal methane production in vitro. Lett. Appl. Microbiol. *21*: 230–234.

Ogino, A., Orito, H., Shimadad, K., Hirooka, H. 2007. Evaluating environmental impacts of the Japanese beef cow– calf system by the life cycle assessment method. Anim. Sci. J., 78: 424–432.

Otter, L. 2010. The South African agricultural GHG inventory for 2004. Department of Agriculture, Forestry and Fisheries, South Africa.

Patra, A.K., 2016. Recent advances in measurement and dietary mitigation of enteric methane emissions in ruminants. Front. Veterinary Sci. 3:39

Ramin, M., Huhtanen, P. 2013. Development of equations for predicting methane emissions from ruminants. J. Dairy Sci. *96*:2476–2493.

Ricci, P., Chagunda, M.G.G., Rooke, J., Houdijk, J.G.M., Duthie, C.-A., Hyslop, J., Roehe, R., Waterhouse, A. 2014. Evaluation of the laser methane detector to estimate me- thane emissions from ewes and steers. J. Anim. Sci. 92:5239–5250.

Roessler, R., Chefor, F., Schlecht, E. 2018. Using a portable laser methane detector in goats to assess diurnal, diet- and position-dependent variations in enteric methane emissions. Comput. Electron. Agric. 150: 110-117

Sallaku, E., Vorpsi, V., Jojic, E., Sallaku, F., Dodona, E. 2011. Evaluation of methane emissions from animal farms in Shkodra district-Albania. Res. J. Agric. Sci. *43*:484–489.

Scholtz, M. M., Van Ryssen, J. B. J., Meissner, H. H., Laker, M. C. 2013. South African perspective on livestock production in relation to greenhouse gases and water usage. South African Journal of Animal Science. 43(3):247-254.

Shibata, M., Terada, F., Iwasaki, K., Kurihara, I., Nishida, T. 1992. Methane production in heifers, sheep and goats consuming diets of various hay concentrate ratio. Anim. Sci. Technol. 64:790-796.

Storm, I.M.L.D., Hellwing, A.L.F., Nielsen, N.I., Madsen, J. 2012. Methods for measuring and estimating methane emission from ruminants. Animals 2:32-35.

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.

Thota, P., Mahender, S.C., Ramana, D. B. V. 2017. Effect of Probiotic Supplementation on Nutrient Digestibilities, Growth Performance and Enteric Methane Emissions in Deccani Ram Lambs. J. Anim. Res. *7*:1009–1017.

Washaya, S., Mupangwa, J., Muchenje, V. 2018. Chemical composition of *Lablab purpureus* and Vigna unguiculata and their subsequent effects on methane production in Xhosa lop-eared goats. S. Afr. J. Anim. Sci. *48*: 445.

Wiedemann, S. G., Yan, M. J., Henry, B. K., Murphy, C. M. 2016. Resource use and greenhouse gas emissions from three wool production regions in Australia. J. Clean. Prod. 122: 121–132.

Chapter 7

Effect of dietary fossil shell flour supplementation on Dohne-Merino wethers basal diet rumen fermentation parameters, *in vitro* true digestibility, and relative feed values.

Abstract

This study investigated the effects of various fossil shell flour (FSF) levels on rumen parameters of Dohne-Merino wethers, *in vitro* digestibility, and relative feed values of the feed used in feeding Dohne-Merino wethers. Twenty-four fistulated wethers (20 ± 1·5 kg body weight each) in a complete randomized design of four treatments having six wethers per treatment, were fed a basal diet without fossil shell flour (control, 0%), or with the addition at 2% FSF (T2), 4% FSF(T3) and 6% FSF (T4) of diet DM for 105 days excluding 14 days adaptation period. Ruminal temperature, pH, ammonia nitrogen concentration, and volatile fatty acids (VFA) were determined. *In vitro* true digestibility of feed (IVTD$^{DM),}$ organic matter digestibility (IVTD^{OM}), neutral detergent fibre (IVTD^{NDF}), acid detergent fibre (IVTD^{ADF}), and acid detergent lignin (IVTD^{ADL}) of the four dietary treatments were determined using Daisy^{200} incubator while relative feed values (RFV) were calculated from feed intake and chemical components of the feeds. The results showed that increasing FSF levels had no effect on ruminal T^{0}C or pH, but Ammonia-N increased ($p<0.01$) with increasing FSF. The total molar concentrations of volatile fatty acids (VFA) decreased ($p<0.05$) with increasing levels of FSF. Acetic: propionic ratio decreased except at 4 % inclusion level. IVTD^{DM}, IVTD^{NDF} and IVTD^{ADF} decreased up till 4% FSF inclusion but tended to increase ($P = 0.06$) at 6% inclusion. Relative feed values of the diets tended to increase ($P=0.07$) by adding fossil shell flour. In conclusion, the addition of FSF

to diets of Dohne-Merino wethers up to 6% FSF inclusion rates did not improve $IVTD^{DM}$, IVTDNDF, and $IVTD^{ADF}$ but for a small increment of rumen nitrogen with no adverse effects on the rumen parameters. The RFV moved the feed from good to premium when supplemented, hence increasing experimentation to exploit ruminant nutrition.

Keywords: Fossil shell flour, rumen parameters, in vitro true digestibility, and feed quality.

7.1. Introduction

Small ruminants, especially sheep, are reared and maintained in most sub-Sahara African countries on high roughage-based diets with limited access to concentrate supplements (Yang et al., 2018). This has led to reduced production performance, low meat quality, and subsequent economic losses to farmers and the nation. Therefore, improving feed utilization in small ruminant nutrition is one of the essential features that can improve farming profitability. One of the most popular strategies for improving feed utilization is the use of feed additives, including antibiotics, live yeast, enzymes (both fungal and bacterial sources), plant extracts, algae, bentonites, and fossil shell flour (Abdel-Aziz et al., 2015; Durmic et al., 2014; Valdes et al., 2015). Chapter three of this thesis observed that fossil shell flour increased feed intake, average daily weight gain, and Dohne Merino sheep's overall performance.

Similarly, Frater (2014) reported that feed additives had been shown to stabilize rumen pH, reduce risk of acidosis, increase dry matter intake (DMI), reduce methanogenesis, enhance rumen development, and improve meat quality. Manipulation of the rumen microbial ecosystem to improve ruminant's performance are essential issues for animal nutritionists. Thus, the need to identify feed additives with the potential to modify rumen fermentation and efficiency of feed utilization while decreasing nitrogen excretion is essential.

The removal of antibiotic growth-promoters as feed additives in ruminant nutrition in most advanced countries of the world have led to the use of plant extracts, live yeast, enzymes or other organic additives to manipulate ruminal fermentation (Martin and Kadokawa, 2006; McGrath et al., 2018). These manipulations can promote feed efficiency and animal growth, prevent, control, and treat diseases (Wang and Wang, 2016). The availability and cost implication of plant extracts, live yeast, enzymes, and other bioorganic feed additives have been a challenge. However, in recent times the use of organic feed additives such as fossil shell flour in the diets of livestock is becoming popular because it is assumed to leaves no residue in the products of animal been consumed, abundantly available, and relatively cheap compared to inorganic feed additives (Chapter 2 of the thesis). Besides, fossil shell flour (FSF) as feed additives reduces both human and environmental pollution by reducing faecal and urinary nitrogen output (Chapter 3 of this thesis). Fossil shell flour has been used as performance enhancers. It improves feed utilization and efficiency in livestock production, especially in non-ruminant, because of its richness in minerals (majorly silicon-oxide) (Chapter 2 of this thesis).

Chapter 3 of this study observed and reported that FSF improved Dohne Merino sheep's growth performance by increasing the dry matter intake. However, it is yet to be investigated if various FSF inclusion levels improve the rumen environmental and fermentation parameters, in vitro true digestibility, and relative feed value. This is because paucity information in the literature is available on the use of FSF to improve rumen fermentation parameters and true digestibility for efficient feed utilization in Dohne Merino sheep. Likewise, little information of varying levels or correct additive concentration is available. Therefore, the objective of the present study was to investigate the effects of different inclusion levels of fossil shell flour on feed quality, *in vitro* true digestibility, and ruminal fermentation parameters in Dohne-Merino wethers. It was hypothesized that the presence of fossil shell flour in Dohne Merino wethers' diets would improve feed quality, *in vitro* true digestibility, and ruminal fermentation parameters.

In chapter 6, it was found that methane emission increased with an increase in FSF inclusion levels. In this study, rumen fluid was obtained from wethers fed on various levels of FSF

Inclusion and rumen environmental indicators, rumen fermentation parameters, and in vitro true digestibility were investigated and compared. Secondly, the effect of varying levels of FSF on relative feed value was determined. This broadens the knowledge of rumen activities in sheep fed with different amounts of FSF.

7.2. Materials and methods

7.2.1. Ethical approval

The University of Fort Hare approved the handling and use of animals (Ethically approved number - MPE041IKU01).

7.2.2. Study site description

The Experiment was conducted at the Small Ruminant Unit of the University of Fort Hare Teaching and Research Farm (Animal Section), Alice, Eastern Cape, South Africa. The research farm is located along Alice-Kings Williams's town, Alice, which lies at longitude 26^{o}50' E and latitude of 32°46' S. The annual rainfall is between 480-490 mm and a temperature range between 24.6 ^{o}C and 11.1 ^{o}C (average is 17.8 ^{o}C) at the altitude of 535 meters above sea level. The in vitro true digestibility using Daisy^{200} incubator was carried out at the Ruminant Nutritional Laboratory of the University of Zululand, Empangeni, KwaZulu Natal Province, South Africa.

7.2.3. Animals, experimental design and management

A total of twelve single-stage rumen-cannulated Dohne-Merino wethers were randomly allotted into four different diets in a completely randomized design with three wethers per diet. The donor wethers were fed a basal diet without FSF addition 0 % or FSF 2 %, 4%, or 6% of diet DM. The wethers were fed the experimental diets 35 consecutive days before the rumen fluid collection. The ruminal fluid was collected from the cannulated wethers before morning feeding (7:30h). Approximately 500ml of rumen fluid were obtained from each fistulated wethers by allowing manually collected ruminal digesta to strained through 4 layers of cheesecloth.

7.2.4. Experimental Diets

The processing and composition of the experimental diets are the same as described in section 3.2.4 and given in Table 3.1

7.2.5. Determination of *in vitro* True digestibility

Rumen samples were collected from the twelve wethers through the fistulation. One -stage techniques for ruminal fistulation described by Ghazy (2017) were used to collect ruminal fluid. The rumen liquor was collected from the sheep and transferred into a thermos flask pre-warmed to a temperature of 39 ^{o}C and transported immediately to the nutritional laboratory. The incubation procedure was carried out using the DAISY$^{200\ Incubator}$ ANKOM Technology (2017) method. Each empty F57 bag was weighed (W_1) and recorded. Feed samples (≤0.5g each) from each treatment were carefully put into the F57 bags and recorded as W_2. Each bag was then heat-sealed, labeled, and evenly distributed on both sides of the Daisy^{200} incubator digestion jar with one sealed blank bag in all jars as correction factor (C_1); after that, the samples were pre-warmed at 39 ^{o}C with 1330 ml of buffer solution A (10.0 KH_2PO_4 + 0.5 $MgSO_4.7H_2O$ +0.5 NaCl + 0.1 $CaCl_2.2H_20$ + 0.5 Urea), 270 ml of buffer solution B (15.0 Na_2CO_3 + 1.0 $Na_2S.9H_2O$) and 400ml of four-layer cheesecloth filtered rumen fluid. This was poured into the digestion jar, purged with CO_2 for 30 s, and placed into the Daisy incubator at 39 ^{o}C for 48 hours. The Daisy incubator temperature was maintained at 39.5 ^{o}C ± 0.5. After incubation, the jars were removed from the incubator, water drained, and bags were thoroughly rinsed with cold tap water until the water was clean using minimal mechanical agitation. The bags were then dried at 60 ^{o}C until constant mass. These samples were further analyzed for fibre using the ANKOM^{200} Fiber Analyzer to determine the NDF using the procedure described by Van Soest et al. (1991). *In vitro* NDF weight was recorded as W_3. These values were used to calculate IVTD^{DM} (Invitro true digestibility on a dry matter basis). This formula is taken from the Ankom^{200} method.

$$\%\text{IVTD} = \frac{100 - (W_3 - (W_1 \times C_1) \times 100}{W_2}$$

$$\%IVTD^{DM}(DM\ basis) = \frac{100 - (W_3 - (W_1 \times C_1) \times 100}{(W_2 \times DM)}$$

Where W1= empty bag weight

W2= sample weight

W3= final bag weight after in vitro and sequential ND treatment

C1 =Blank bag correction (final oven-dried weight/original blank bag weight).

7.2.6. Determination of relative feed values

Relative feed value is defined as estimating the digestibility of the forage dry matter and how much the sheep can consume based on its "satisfying" capacity. This was developed in America by Hay Marketing Task Force. It is a key tool in forage marketing and forages quality education. Using the method or equation of Rohweder et al. (1978), the relative feed value (RFV) of the diets was calculated as follows:

DMI = Dry matter intake (Live weight = LW %) = 120/ (NDF %); DMD = Dry matter digestibility (%) = 88.9 - (0.779×ADF %); RFV = Relative feed value = (DMD×DMI)/1.29.

Based on the Quality Grading Standard assigned by the Hay Marketing Task Force of the American Forage and Grassland Council, the RFV was assessed as roughages based on prime >151, 1 (premium) 151-125, 2 (good) 124- 103, 3 (fair) 102-87, 4 (poor) 86-75, 5(reject) < 75.

7.2.7. Determination of rumen fluid parameters (PH, temperature, and volatile fatty acid)

Rumen fluid was collected from twelve wethers through a fistula, and the pH was immediately measured using a digital pH meter (Sartorius PT-10; Sartorius AG, Göttingen, Germany) and they were recorded for each wether. Ammonia-N was determined from 5-mL of strained (through 4 layers of cheesecloth) rumen fluid pipetted with 1 ml of H_2SO_4 (20% Conc) for preservation before storing at -20 °C. It was analyzed using the phenol hypochlorite assay (Galyean, 2006).

To analyze ruminal VFA, 5 mL of strained rumen fluid-preserved with 1 mL of an acid solution containing 20 % orthophosphoric acid and 0.5 formic acids (5% conc.) and stored at - 20°C was used. After thawing, rumen fluid samples were centrifuged (15,000 × g for 15 min at 4 °C), and the supernatant was used to quantify the VFA. The concentration of acetic acid, butyric acid, propionic acid, iso-butyric, valeric acid, and iso-valeric was measured by a gas chromatograph (UNICAM 4600; SB Analytical, Cambridge, UK) equipped with a flame ionization detector (250 °C), split-injection port (1.0 µL injection), capillary column (Agilent J&W HP-FFAP, 10 m by 0.535 mm by 1.00 m, 19095F-121; Agilent Technologies, Santa Clara, CA), and helium as carrier gas (column head pressure of 68.94 kPa), and the working temperatures of the injector and detector were 250 °C and 300 °C, respectively following the procedures described by Erwin et al., (1961). The initial column temperature was set at 80 °C for 1 min and then increased by 20 °C/min to 120 °C, followed by 6.2 °C/ min to 140
°C and after that by 20 °C/min to 205 °C.

7.2.8. Statistical analysis

Analysis of variance by one-way experimental design within the groups was used to analyze the chemical composition, *in vitro* true digestibility of each component, relative feed values, and rumen parameters (GLM of SAS, 2018). Duncan's multiple range test was done to compare the means, and differences were considered when $P < 0.05$.

7.3. Results

The effects of FSF's varying inclusion levels in diets of Dohne-merino rams on pH, temperature, ammonia-N, and VFA are presented in Table 7.1. Rumen pH tended to increase slightly with FSF but was not different ($P>0.07$) within the FSF inclusion levels.

Table 7 1: Effects of varying inclusion levels of FSF in diets on rumen parameters of Dohne-merino.

Parameter	0 %FSF	2 %FSF	4 %FSF	6 %FSF	SEM	P-Value
NH_3 -N	4.94^{b}	8.43^{a}	4.22^{b}	5.33^{b}	1.12	0.01
pH	6.67^{a}	6.72^{a}	6.74^{a}	6.88^{a}	0.03	0.07
T°C	34.17^{a}	34.33^{a}	34.33^{a}	34.63^{a}	0.13	0.03
Total VFA mM	64.72^{a}	58.18^{a}	54.45^{a}	52.91^{a}	20.30	0.52
Acetic mmol/l	33.48^{a}	29.30^{a}	27.69^{a}	23.62^{a}	13.67	0.10
Butyric	13.66^{a}	11.60^{a}	11.53^{a}	13.09^{a}	10.23	0.144
Propionic	16.61^{a}	15.60^{a}	13.04^{a}	15.55^{a}	10.69	0.46
Iso-butyric	0.65^{a}	0.42^{a}	0.96^{a}	0.04^{a}	0.84	0.95
Valeric	0.12^{a}	0.46^{a}	0.70^{a}	0.46^{a}	0.27	0.46
Isovaleric	0.20^{a}	0.53^{a}	0.53^{a}	0.14^{a}	0.02	0.13
Acetate: propionic ratio	2.015^{a}	1.846^{a}	2.123^{a}	1.518^{a}	0.82	0.23

The NH_3-N concentration was greater ($P<0.01$) in 2% and 6% FSF than 0% and 4%. The molar total VFAs, acetic, butyric, and iso-butyric values decreased ($P < 0.05$) with an increase in FSF inclusion levels. The 4% FSF had lesser propionic value compared to 2%, 6%, and 0 % FSF ($P < 0.05$). Molar iso-valeric acid in rumen fluid also did not change with FSF inclusion ($P > .05$). The acetate to propionate ratio was highest at 4% FSF and lowest in 6% FSF.

Table 7.2 shows the nutrient composition of the four diets due to varying inclusion levels of FSF. The non-significant difference of all components and crude protein showed that FSF inclusion up to 6% does not affect the nutrient content.

Table 7.3 shows the *in vitro* true digestibility of dry matter ($IVTD^{DM}$) after adding three amounts of FSF to the diets, which consistently decreased ($P < 0.05$). $IVTD^{OM}$ %, $IVTD^{NDF}$%, $IVTD^{ADF}$% and $IVTD^{ADL}$% all decreased at different levels of FSF supplementation compared to the control (0 %).

$IVTD^{DM}$: in vitro true dry matter digestibility; $IVTD^{OM}$: in vitro true organic matter digestibility $IVTD^{NDF}$: in vitro true neutral detergent fibre digestibility; $IVTD^{ADF}$: in vitro true acid detergent fibre digestibility; $IVTD^{ADL}$: in vitro true acid detergent lignin digestibility.

The effects of including various FSF amounts into Dohne-merino wether diets on in vitro true digestibility and feed quality as defined by their RFV content are presented in Table 7.3. Relative feed value (RFV) and DMI tended to increase ($P \leq 0.05$) as the amounts of FSF added to the diets increased (Table 7.4).

Table 7 2: Chemical composition of the diets with varying FSF inclusion levels.

Levels of FSF inclusion	DM	OM	CP	EE	ASH	NDF	ADF	ADL
0 %FSF	95.52±1.02	88.27±1.4	14.72±1.05	1.55±1.03	11.72±0.60	44.26±0.9	40.16±5.94	11.07±1.2
2 %FSF	95.49±1.02	90.54±1.4	14.57±1.05	1.72±1.03	9.44±0.60	39.2±0.9	31.52±5.94	11.52±1.2
4 %FSF	95.89±1.02	90.47±1.4	14.62±1.05	1.90±1.03	9.51±0.60	39.53±0.9	31.64±5.94	11.30±1.2
6 %FSF	95.56±1.02	89.74±1.4	15.51±1.05	1.99±1.03	10.29±0.60	38.74±0.9	31.08±5.94	11.34±1.2

DM= dry matter, OM=organic matter,CP= crude protein,EE =ether extract,NDF =neutral detergent fibre, ADF=acid detergent fibre, ADL= acid detergent Lignin.

Table 7 3: In vitro true digestibility of Dohne-merino diets with various levels of Fossil shell flour.

Parameter	0 %FSF	2 %FSF	4 %FSF	6 %FSF	SEM	P-Value
$IVTD^{DM}$ %	62.38^{a}	58.76^{b}	58.13^{b}	55.4^{c}	1.19	0.05
$IVTD^{OM}$ %	64.38^{a}	60.83^{b}	59.98^{b}	57.21^{c}	1.14	0.001
$IVTD^{NDF}$%,	39.03^{a}	34.79^{a}	36.67^{a}	38.26^{a}	3.12	≥0.05
$IVTD^{ADF}$%	34.44^{a}	31.87^{a}	34.58^{a}	35.82^{a}	2.84	≥0.05
$IVTD^{ADL}$%	12.60^{b}	12.43^{b}	19.02^{a}	16.66^{ab}	2.16	0.0001

$IVTD^{DM}$ =In-vitro tru digestibility dry matter, $IVTD^{OM}$ =In-vitro tru digestibility organic matter, $IVTD^{NDF}$ = In-vitro tru digestibility neutral detergent fibre, $IVTD^{ADF}$= In-vitro tru digestibility acid detergent fibre $IVTD^{ADL}$= In-vitro tru digestibility acid detergent lignin

Table 7 4: Effects of FSF inclusion levels on diets quality.

Levels of FSF inclusion	DMD%	DMI(%LW)	RFV	RFV -Quality	%INVTD
0 %	62.07[ab]	3.10[a]	149.95[a]	1-good	83.44[a]
2 %	64.07[a]	3.50[a]	175.60[a]	Prime	87.48[a]
4 %	58.07[b]	3.47[a]	155.99[a]	Prime	84.53[a]
6 %	60.99[ab]	3.15[a]	150.08[a]	1-Premium	86.85[a]
SEM	2.21	0.32	17.90		2.89
P-value	0.05	0.08	0.07		0.05

DMD= dry matter digestibility, DMI= dry matter intake, RFV= relative feed value

7.4. Discussion

Mean ruminal pH and temperature values were within the normal physiological range of 6.1 to 6.8 in all experimental lambs, as observed by Van Soest (1994). Imani Rad et al. (2016) and McDonald et al. (2011) reported that factors such as DMI, type of diets, addition or non-addition of an additive, hay to concentrate ratio, the physical structure of the diets, digestibility, and feeding method affect rumen pH. In this study, these factors were not different among the dietary treatments; thus, there was no change ($P \geq 0.05$) in the rams' rumen pH. This means that the inclusion of FSF in the diets of Dohne-merino wethers maintained the ruminal pH and ruminal metabolism. The reason for this could be that FSF has a buffering capacity, which helps to stabilize the rumen pH. The mean ammonia concentration for 2% and 6% FSF values obtained from this study were within the minimum concentration of 5 mg/dL required for maximal growth of microbes in the rumen of animals (Sinclair DA, Dawes IW, 1993). The concentration of ammonia-N was affected by the inclusion of FSF at 2% and 6% in the diets of Dohne -merino wethers, compared to the 0 % level ($P < 0.05$). The reason could be that since FSF increased protein available in the rumen (McDonald et al., 2011), it follows that there would be degradation of excess crude protein in the rumen, leading to ammonia accumulation in the rumen. This will enhance rumen protozoa growth. This aligns with the study of Pal et al. (2010), who reported that when feed protein degradation is depressed in the rumen, protozoa growth is hindered as a result of a decrease in rumen ammonia concentration.

The main supply of energy to ruminants comes from the end products of fermentation by various microorganisms in the rumen, which is VFA (Imani-Rad et al., 2016; Van Soest, 1994). Hence, sheep performance and productivity can be enhanced through diets that promote higher production of VFA compared to lesser amounts (Penner, 2014). Though the amount of total molar VFA observed in this study was reducing as FSF inclusion levels increased, it still falls within the normal ranges of 60 to 150 mmol/L as reported by Imani-Rad et al. (2016) and

Martínez-Fernández et al. (2014). Also, the lowering of total VFAs, acetic acid, butyric, and iso-butyric acid concentration as the inclusion of FSF increases were similar to Partanen and Jalava's (2005) findings. The reduction in molar total VFAs and individuals in rumen fluid may be due to FSF's antimicrobial effects on rumen bacteria. There might be a change in the metabolic pathway through which microbes utilize substrates, reducing the typical VFA by-products (Baltran and Martins, 2015; Fernandez et al., 1998; Ikusika et al., 2019). However, the net reduction is very small, as the VFA concentration was still within the normal VFA mM ratio.

The diet consisted of maize, sunflower oil cake, wheat bran, molasses, limestone, salt, and premix, while the hay was a combination of teff and Lucerne in equal proportion. Teff and Lucerne have been reported to be the right combination for sheep production, positively impacting performance (Kafilzadeh et al., 2012). The NDF, ADF, and ADL observed in this study are within the normal range reported by Gebremariam et al. (2014) on teff and Lucerne. The diet CP% (14.7) was above the average CP% (~7.5) for ruminant nutrition, as reported by Fitwi and Tadesse (2013). The supplemented feed CP% varied from 15.52 compared to the 55 g/kg DM for teff and 118.0 g/kgDM for Lucerne reported by Ghasemi et al. (2012). This could be due to the addition of concentrate to the diets. The major component of the concentrate, such as sunflower cake and maize, were richer in crude protein than teff and Lucerne. The similarity in the values of the various components shows uniformity in the basal diets, and the only source of variation can simply be FSF levels. However, the addition of FSF at various inclusion levels in this study did not affect the diets' chemical composition.

The use of different feed additives to improve fibrous feeds' digestibility in ruminants has been reported severally (Aboagye et al., 2015; Kondratovich et al., 2017; Salman et al., 2017). However, information available on using fossil shell flour as additives for improving rumen parameters and influence on true digestibility and relative feed values in sheep was very few and not Dohne Merino wethers. Emeruwa (2016) reported a significant difference in sheep supplemented with varying amounts of FSF and the control for *in vitro* organic matter digestibility. It was observed that treatment with 2 % inclusion levels had the highest $INVTD^{OM}$ % (62.19%) among all the treatments. This was contrary to the present study's findings, which show that the control treatment had the highest $INVTD^{OM}$ % values. The difference could be attributed to the different feed ingredients used in the two experiments. In this trial, both concentrate and hay were used, while Emeruwa (2016) used concentrate feed only.

The $IVTD^{NDF}$% is an essential parameter for determining feed intake and quality (Oba and Allen, 2011; Salman et al., 2017). In this study, the values of $IVTD^{NDF}$% tended to be higher in the 0 % inclusion level than 2%, 4%, and 6% FSF. This means that digestibility decreased with increased FSF. This could be as a result of the antimicrobial properties of FSF that might have reduced the activities of the ruminal microorganism such as *Ruminococcus albus*, *Ruminococcus flavefaciens*, and *Butyrivibrio fibrisolvens* that degrade fibrous feeds. The mechanism of action was not known but could be associated with FSF's ion exchange and absorption property, which can alter the cells' electrolytes balance. This interruption can disturb the trans-membrane movement and intracellular equilibrium of ions, exhausting and causing some microorganisms' death.

The observation in this study was similar to the reduction in NDF and ADF values obtained in a trial conducted by Anassori et al. (2012). They investigated the effects of different feed additives on $INVTD^{NDF}$% and $INVTD^{ADF}$ %, and it was reported that Monessin and essential oil decreased NDF and ADF degradability in the $Daisy^{200}$ digestion method. The reason is that Monessen has an inhibiting effect on gram-positive microorganisms, which include most of the cellulolytic bacteria, capable of hydrolyzing fiber, thereby reducing NDF degradability. This gave a strong backing of FSF's antimicrobial potential as indicated in the previous experimental chapter hence the decrease in INVTD observed in this study.

The feed quality improved with an increase in FSF addition in the diets, and all the treatments proved to have premium feed quality generally, as demonstrated by the relative feed value. There was no significant difference between them. The premium rated feed values obtained in this study resulted from an increase in DMD and DMI values. The inclusion of FSF increases the feed intake of withers as well as dry matter intake and dry matter digestibility. The mechanism explaining this was not clear but was associated with FSF richness in minerals such as Na, Ca, Mg, Cu, and Fe (Adebiyi et al., 2009).

Moreover, these minerals seem to increase the palatability of the diets (Desimone et al., 2013; Garmyn et al., 2011); hence, the high feed intake value. This agreed with the finding of Silva et al. (2015) and Moyo et al. (2019), who both reported that mineral supplementation has positive effects on feeding acceptance vis-a-vis the feed intake and the DMI. Similarly, Sahoo (2011) reported that the addition of mineral supplements to sheep's diets increases the animal's preference for such feed. The addition of FSF in the diets also increased INVTD%. Diets supplemented with FSF has higher INVTD numerical values than the non-supplemented diets (0%). Nevertheless, no significant difference was observed.

7.5. Conclusion

This study showed that adding FSF at varying levels to diets of wethers containing concentrate and hay (teff and Lucerne) has no adverse effects on ruminal temperature and pH. Also, the increasing level of FSF did not have any major changes in total molar VFA but with a relative increment in ammonia nitrogen concentration at 2 % inclusion level. *In vitro* digestibility results showed no adverse effects on the diets. Fossil shell flour, being rich in minerals, improved feed intake, and feed values of the diets. Therefore, FSF has the potential to enhance the production performance of Dohne Merino wethers. Though $IVTD^{NDF}$% decreased with an increase in FSF levels. $INVTD^{ADF}$% also decreased up till 4 % inclusion levels. However, there is a need for further investigation using *in vivo* digestibility techniques with FSF to show the effects of different levels of FSF supplementation to support the *in vitro* true digestibility findings in the current study.

7.6 References

Abdel-Aziz, N.A., Salem, A.Z.M., El-Adawy, M.M., Camacho, L.M., Kholif, A.E., Elghandour, M.M.Y., Borhami, B.E. 2015. Biological treatments as a means to improve feed utilization in agriculture animals-An overview. Journal of Integrative Agriculture 14: 534–543.

Aboagye, I.A., Lynch, J.P., Church, J.S., Baah, J., Beauchemin, K.A. 2015. Digestibility and growth performance of sheep fed alfalfa hay treated with fibrinolytic enzymes and a ferulic acid esterase producing bacterial additive. Animal Feed Science and Technology 203:53–66.

Adebiyi, O.A., Sokunbi, O.A., Ewuola, E.O. 2009. Performance Evaluation and Bone Characteristics of Growing Cockerel Fed Diets Containing Different Levels of Diatomaceous Earth. Middle-East Journal of Scientific Research 4: 36–39.

Anassori, E., Dalir-Naghadeh, B., Pirmohammadi, R., Taghizadeh, A., Asri-Rezaei, S., Farahmand-Azar, S., Besharati, M., Tahmoozi, M. 2012. In vitro assessment of the digestibility of forage-based sheep diet, supplemented with raw garlic, garlic oil, and monensin. Veterinary research forum: an international quarterly journal 3: 5–11.

Beltran, M.A., Martins, R., 2015. Original article diatomaceous earth inhibited the. Philipp J Vet Anim Sci 41: 135–140.

Desimone, J.A., Beauchamp, G.K., Drewnowski, A., Johnson, G.H. 2013. Sodium in the food supply: Challenges and opportunities. Nutrition Reviews 71:52–59.

Durmic, Z., Moate, P.J., Eckard, R., Revell, D.K., Williams, R., Vercoe, P.E. 2014. In vitro screening of selected feed additives, plant essential oils, and plant extracts for rumen methane mitigation. Journal of the Science of Food and Agriculture 94:1191–1196.

Emeruwa, C.H., 2016. Growth Performance of West African Dwarf Sheep Fed Diets Supplemented with Fossil shell flour. University of Ibadan, Nigeria.

Erwin, E.S., GJ Marco, G.J. 1961. Volatile Fatty Acid Analyses of Blood and Rumen Fluid by Gas Chromatography. Journal of Dairy Science 44: 1768–1771.

Fernandez, M.I., Woodward, B.W., Stromberg, B.E. 1998. Effect of diatomaceous earth as an anthelmintic treatment on internal parasites and feedlot performance of beef steers. Anim. Sci. 66: 635–641.

Fitwi, M., Tadesse, G., 2013. Effect of sesame cake supplementation on feed intake, body weight gain, feed conversion efficiency, and carcass parameters in the ration of sheep fed on wheat bran and teff (Eragrostis teff) straw. Momona Ethiopian Journal of Science 5: 89.

Frater, P., 2014. Feed additives in ruminant nutrition. . Can feed additives reduce methane and improve performance in ruminants? Agriculture and Horticulture Development Board. www.beefandlamb.ahdb.org.uk (Accessed on 18/08/2018)

Garmyn, A.J., Hilton, G.G., Mateescu, R.G., Morgan, J.B., Reecy, J.M., Tait Jr, R.G., Beitz., D.C., Duan, Q., Schoonmaker, J.P., Mayes, M.S., Drewnoski, M.., Liu, Q., VanOverbeke, D.L. 2011. Estimation of relationships between mineral concentration and fatty. Journal of Animal Science 89:2849–2858.

Galyean, L., 2006. Effects of an artificial sweetener on health, performance, and dietary preference of feedlot cattle. J. Anim. Sci. 84:2491–2500.

Ghasemi, S., Naserian, A.A., Valizadeh, R., Tahmasebi, A.M., Vakili, A.R., Behgar, M. 2012. Effects of pistachio by-product in the replacement of lucerne hay on microbial protein synthesis and fermentative parameters in sheep's rumen. Animal Production Science 52: 1052.

Ikusika, O.O., Mpendulo, C.T., Zindove, T. J., Okoh, A.I. 2019. Fossil Shell Flour in Livestock Production: A Review. Animals 9, 1–20.

Imani Rad, M., Rouzbehan, Y., Rezaei, J. 2016. Effect of dietary replacement of alfalfa with urea-treated almond hulls on intake, growth, digestibility, microbial nitrogen, nitrogen retention, ruminal fermentation, and blood parameters in fattening lambs. Journal of Animal Science 94:349–358.

Kafilzadeh, F., Heidary, N., Bahraminejad, S. 2012. Variety effect on composition, the kinetics of fermentation, and in vitro digestibility of oat (Avena sativa L.) Straw and its neutral detergent fibre. South African Journal of Animal Sciences 42:406–415.

Kondratovich, L.B., Sarturi, J O., Ballou, M A., Sugg, D., Campanile, P R B., Reis, B Q., Melo, A-C, B., Pellarin, L A., Ovinge, L.A.2017. Effects of the fibrinolytic enzyme on true in-vitro digestibility of by-products commonly fed to cattle. Journal of Animal Science; Champaign 95: 270.

Martin, G.B., Kadokawa, H. 2006. "Clean, green and ethical" animal production. Case study: reproductive efficiency in small. Small ruminants. J. Reprod. Dev. 52:145–152.

Martínez-Fernández, G., Abecia, L., Arco, A., Cantalapiedra-Hijar, G., Martín-García, A.I., Molina-Alcaide, E., Kindermann, M., Duval, S., Yáñez-Ruiz, D.R. 2014. Effects of ethyl-3-nitrooxy propionate and 3-nitrooxypropanol on ruminal fermentation, microbial abundance, and methane emissions in sheep. Journal of Dairy Science 97:3790–3799.

McDonald, P., R.A. Edward, J.F.D. Greenhalgh, C.A. Morgan, L.A.S. 2011. Animal Nutrition, Animal Nut. ed. Prentice-Hall/Pearson Education Ltd, Harlow, UK.

McGrath, J., Duval, S.M., Tamassia, L.F.M., Kindermann, M., Stemmler, R.T., de Gouvea, V.N., Acedo, T.S., Immig, I., Williams, S.N., Celi, P. 2018. Nutritional strategies in ruminants: A lifetime approach. Research in Veterinary Science 116: 28–39.

Moyo, M., Adebayo, R.A., Nsahlai, I.V., 2019. Effects of diet and roughage quality, and period of the day on diurnal feeding behaviour patterns of sheep and goats under subtropical conditions. Asian-Australasian Journal of Animal Sciences 32:675–690.

Oba, M., Allen, M., 2011. The Impact of Improving NDF Digestibility of Corn Silage on Dairy Cow Performance. Ruminant Nutrition Symposia. pp105–117.

Pal, D.T., Gowda, N.K.S., Prasad, C.S., Amarnath, R., Bharadwaj, U., Suresh Babu, G., Sampath, K.T. 2010. Effect of copper- and zinc-methionine supplementation on bioavailability, mineral status, and tissue concentrations of copper and zinc in ewes. Journal of Trace Elements in Medicine and Biology. 24:89–94.

Partanen, K., Jalava, T. 2005. Effects of some organic acids and salts on microbial fermentation in the digestive tract of piglets estimated using an in vitro gas production technique. Agricultural and Food Science 14:311–324.

Penner, G. B., 2014. Mechanisms of volatile fatty acid absorption and metabolism and maintenance of a stable rumen environment. In: Proc. 25th Annu. Florida Rumin. Nutr. Symp., Feb. 4–5, Univ. Florida, Gainesville, FL. p. 92–106.

Rohweder, D.A., Barnes, R.F., Jorgensen, N. 1978. Proposed hay grading standards based on laboratory analyses for evaluating quality. J. Anim. Sci., 47: 747-759.

Sahoo, A., Soren, N.M., 2011. Nutrition for Wool Production. WMC002384. 2(10):1-11

Salman, M., Cetinkaya, N., Selcuk, Z., Genc, B., Acici, M., 2017. Effects of various inulin levels on in vitro digestibility of corn silage, perennial ryegrass (*Lolium perenne* L.), and common vetch (*Vicia sativa* L.)/oat (*Avena sativa* L.) hay. South African Journal of Animal Science 47: 723.

Sarijit, J., Steve, S., Micheal, E., Rodney, D. 2016. Animal feed compositions, including spent filter media containing diatomaceous earth. US20160295884A1.US Application US20160295.

Silva, T.P.D., Marques, C.A.T., Torreão, J.N.C., Bezerra, L.R., Araújo, M.J., Gottardi, F.P., Edvan, R.L., Oliveira, R.L. 2015. Ingestive behaviour of grazing ewes given two levels of concentrate. South African Journal of Animal Sciences 45:180–187.

Sinclair, D.A., Dawes, IW., D.J. 1993. Purification and characterization of the branched-chain alpha-ketoacid dehydrogenase complex from Saccharomyces cerevisiae. Biochem Mol Biol Int 31: 911–22.

Valdes, K.I., Salem, A.Z.M., Lopez, S., Alonso, M.U., Rivero, N., Elghandour, M.M.Y., Domínguez, I.A., Ronquillo, M.G., Kholif, A.E. 2015. Influence of exogenous enzymes in the presence of Salix babylonica extract on digestibility, microbial protein synthesis, and performance of lambs fed maize silage. Journal of Agricultural Science 153:732–742.

Van Soest, P.J. 1994. Nutritional ecology of the ruminant., 2nd ed. ed. Cornell Univ. Press, Ithaca, NY, United States.

Wang, S.P., Wang, W.J., 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 46:49-54.

Yang, C., Gao, P., Hou, F., Yan, T., Chang, S., Chen, X., Wang, Z., 2018. Relationship between the chemical composition of native forage and nutrient digestibility by Tibetan sheep on the Qinghai–Tibetan plateau. Journal of Animal Science 96:1140–1149.

Chapter 8

General discussion, conclusions, and recommendations

8.1. General discussion

Livestock production is key to tracking food security, and sheep production can play a major role in improving food security globally. In the recent century, sheep farming has been practiced in almost all places where human beings are found and are key to many civilizations (Skapetas and Kalaitzzidou, 2017). This is because sheep are very adaptable to relatively nutrient-poor diets, tolerance to extreme climates (from very cold to very hot and humid), and can easily be managed because of their small body frame. Likewise, sheep required a small area and feed compared to large animals. Sheep has high fecundity and is small enough to be consumed by an average rural family in a day or two; hence no refrigeration facilities are needed. The sheep industries are a source of employment to millions of people worldwide, especially in developing and underdeveloped nations. Sheep provides meat, milk, wool, hides, and some other by-products consumed by man and instrumental to the economic growth of many countries. In meeting these needs, sheep farmers have employed inorganic feed additives to boost sheep's production performance. However, many countries have promulgated laws that prohibit using such inorganic feed additive for farm animals because of the health challenges it poses to the consumer. Therefore, scientists and farmers have moved to the use of organic feed additives for sheep. Among the recently used organic feed additives for sheep, production is fossil shell flour. That is why the broad objective of this study was to assess the growth performance, nutritional status, wool parameters, blood, and parasitic profile of Dohne-Merino sheep fed on a diet supplemented with varying levels of fossil shell flour. The main hypothesis tested was that varying inclusion levels of fossil shell flour would influence the growth performance, nutritional status, wool parameters, blood, and parasitic profile of Dohne-Merino wether.

Chapter 3 hypothesized that varying inclusion levels of Fossil shell flour affect the growth performance, water intake, digestibility, and N retention of Dohne-merino wethers. The results obtained in chapter 3 confirmed the hypothesis tested. One of the major purposes why farmers (both commercial and communal) keep sheep is for sale. The price depends on the size and weight of the sheep. Sheep can utilize poor nutrients diets, but feed additives have been shown to improve their growth performance. The results agreed with the hypothesis that the addition of FSF has effects on the growth performance, water intake, digestibility, and N- retention of Dohne-Merino sheep. The inclusion of FSF in Dohne merino wethers' diet had a significant effect on the feed intake. It was observed that the addition of FSF increased the feed intake and thereby increased the average daily gain (g/l) of the wethers, especially wethers fed on diets at 4 % FSF. This finding suggests that FSF could be a performance enhancer, which will make Dohne -Merino reach market weight earlier than expected. Also, the inclusion of FSF in the diets of whether will reduce production cost. This is because FSF is cheaper compared to inorganic or any other feed additive used in sheep production. The effect of FSF on water intake showed that wethers on a diet with 0%FSF had the highest water intake per day while wethers fed on a diet with 6% had the least water intake. On the part of digestibility, FSF's effect was very significant for CP intake between FSF supplemented diets and the control. This indicates that FSF enhanced the absorption of protein into the animal system, making it available at tissue levels. The N-retention increased as the rate of inclusion of FSF in the diets goes higher. This outcome showed fecal N and urinary N were higher in control compared to FSF supplemented diets. Inclusion levels had significant effects on fecal N but not on urinary N. This suggests that FSF could reduce environmental pollution through the reduction in fecal and urinary N-excretion by Dohne-Merino sheep.

Experimental chapter 4, assessed the hematobiochemical and parasitic profile of Dohne-Merino sheep fed diet supplemented with varying amounts of fossil shell flour**.** The hypothesis tested was that varying amounts of fossil shell flour in the diets will not influence the hematobiochemical and parasitic profile of Dohne merino wethers. The tested hypothesis was rejected as it affected the hematobiochemical and parasitic profile of Dohne-Merino sheep supplemented with varying amounts of fossil shell flour. The results showed that FSF's addition at 4% (T3) and 6% (T4) to the diets improved the white blood cell of the wethers as from 30 days of adding FSF to the diets. An increase in the white blood cell of the wethers due to FSF supplementation suggests that the ability of the wethers to fight the invasion of any foreign body had improved. This means that the immunity system of wethers can be built through FSF's addition in their diets (Weaver et al., 2013). In an environment where diseases are prevalent or during the rainy season when pathogens easily multiply in the body of sheep, supplementation of sheep diets with Fossil shell flour could alleviate this challenge. Likewise, in all the treatments in this study, RBC, MCV, MCH, Hgb, and Hematocrits values indicate that inclusion of FSF into Dohne Merino wethers' diets improves the hematological blood parameters. This indicates that FSF inclusion in feed has the capability of supporting the high oxygen-carrying capacity of the blood (Babeker and Bdalbagi, 2015). Improvement in total protein level observed for wethers on 2%, 4%, and 6% FSF diets compared to wethers on 0% FSF diet shows that more nitrogen was available at the tissue level as a result of FSF supplementation. Also, blood urea, which was an indicator of protein metabolism conditions and dietary amino acid balance in the animals, was observed (Tao et al., 2018). The results showed that the addition of FSF up to 6% inclusion rate brings about high urea levels in the bloodstreams. This implies that FSF's addition in the diets of Dohne- Merino wethers more than 4% will lead to high levels of urea in the blood. This could be because FSF increases protein concentration in the rumen of sheep, and excess protein may be broken down to ammonia in the rumen, which will be transported to renal organs through the blood in the form of urea. The addition of FSF also influenced serum creatine. The concentration of creatinine was least in wethers on 4% FSF diet compared to others. Creatinine is a waste molecule resulting from protein metabolism, and its low concentration suggests reduced catabolism rate in wethers on 4% FSF diet (Paswan et al., 2016). Serum glucose also witnessed FSF influence. Wethers fed on diets with 0% FSF were lower than wethers on FSF supplemented diets. Finally, in this chapter, FSF effects on wireworm, long-necked worm, liver fluke worm, conical fluke worm, and coccidia were examined at days 0, 10, 30,7 0, and 100. The results showed that wethers fed 4% and 6% FSF supplements, at day 30, had a remarkable reduction in counts of various worms considered while wethers fed 2% FSF had a decrease in the counts of worms investigated at day 70. This suggests that the economic losses resulting from gastrointestinal parasites in sheep production in the semi-arid and arid regions can be greatly reduced by adding FSF to the diets at a minimum of 4% for quick action. The increase in average daily feed intake and average daily weight gained as FSF inclusion levels increases in experimental chapter 3 could result in an FSF positive effect on the wethers' blood and parasite profile.

In chapter 5, varying levels of fossil shell flour were adopted to test whether they can influence feed preference, body condition scores, and wool parameters of Dohne-Merino wethers. This hypothesis was found to be true. The body condition scoring, average daily weight gain, and dry matter intake were influenced by FSF levels in the diets and were peaked at 4% FSF inclusion level and later declined a little at 6% FSF inclusion level. The wethers visited diets supplemented with FSF more than the control. FSF's effectiveness in killing nematodes might have influenced animal behavours towards their consumption, hence reducing worms in FSF supplemented diets. Also, among the supplemented diets, the number of visits increased as FSF increases in the diets. Diets with the highest time of visit have lower nematodes and higher WBC counts compared with the control. A linear correlation exists between dry matter intake and wool growth. It was noticed that wethers fed on a diet with 6% FSF had the highest wool growth than at 0% FSF supplementation. This implies that FSF got rid of the nematode infection, and the energy was channeled to wool growth. Also, an increase in WBC in FSF supplemented diets, as recorded in chapter 4, suggests less infection in wethers fed FSF supplemented diets. A slight change was noticed in fibre diameter between the control and the supplemented diets, while a considerable difference was observed between the control and the supplemented diets in staple length, FDCV, and fibre>15%. Therefore, it implies that the amount of FSF in Dohne-Merino wethers' diets influenced wool growth, staple length, slightly fibre diameter, FDCV, and fibre>15%.

Chapter 6, investigated the effect of dietary supplementation with fossil shell flour on enteric methane output and position-dependent variations in Dohne-merino wethers. The hypothesis tested was that dietary supplementation with fossil shell flour would reduce the enteric methane output and is not position-dependent in Dohne-merino wethers. The hypothesis was not accepted for methane output and position-dependent in Dohne-Merino wethers. This study showed that daily methane emissions (ppm-m) increased as the FSF inclusion increases. This could be because FSF increases the wethers' feed intake, thereby increasing the DMI (g/kg). Wethers on 4% FSF diets had the highest dry matter intake, and likewise, the daily methane output was peaked at that level of FSF inclusion. This result suggests that FSF promotes methanogen or protozoan populations in the absence of parasitic microbes in the rumen. As seen in the previous chapter, FSF can deworm or has antimicrobial potential. Finally, in this chapter, it was observed that the amount of methane generated during resting was higher than the amount generated during feeding or standing. This could be as a result of many movements and displacement in the digestive system during the process of regurgitation that happens during resting of the wethers, which could lead to more escape of methane gas from the rumen to the atmosphere through the nostril. Healthier rumen means more microbes more digestion of the much-reduced particle size from regurgitation leading to high digestion and then more methane

Chapter 7 examined the effect of dietary Fossil shell flour supplementation on Dohne-merino wethers' basal diet rumen fermentation parameters, *in vitro* true digestibility, and relative feed values. It was hypothesized that dietary fossil shell flour supplementation on Dohne- merino wethers basal diet will decrease rumen fermentation parameters, in vitro true digestibility, and relative feed values. This hypothesis was accepted as rumen fermentation parameters, in vitro true digestibility, and relative feed values affected by supplementation of basal diets with fossil shell flour. Ruminal ammonia-N concentration increased by the inclusion of FSF at 2% and 6% in the diets of Dohne -merino wethers, compared to the 0% level but decreased at 4% FSF supplementation. The reason could be that since FSF increased protein available in the rumen (McDonald et al., 2011), it, therefore, follows that there would be degradation of excess protein in the rumen, leading to ammonia accumulation in the rumen. This will enhance rumen protozoa growth. Chapter 7 complements Chapter 6 that FSF promotes archaeal and protozoa growth that generates more methane in the rumen. Total VFAs, acetic acid, butyric, and iso-butyric acid concentration reduced as the inclusion of FSF increases. This may be due to FSF's antimicrobial effects on rumen bacteria because most of the volatile fatty acids are being produced by fibrolytic bacteria activities. The $IVTD^{NDF}$% tended to be higher in the 0% FSF inclusion level than 2%, 4%, and 6% FSF inclusion levels groups. This means that digestibility decreased with increased FSF. This could result from a decrease in the activities of fibrolytic rumen bacterial by the antimicrobial properties of fossil shell flour. The relative feed value improved with an increase in FSF addition in the diets, and all the treatments proved to have premium feed quality generally. This could not be associated with fossil shell flour richness in minerals such as Na, Ca, Mg, Cu and Fe, but to the decrease in nematode counts, increase in DMI, and increased nutrient content. Finally, diets supplemented with FSF had higher INVTD % numerical values than non-supplemented diets.

8.2. Conclusion

The inclusion of fossil shell flour in the diets of wethers influenced the growth performance, nutritional status, hematobiochemical and parasitic profile, and wool parameters of Dohne-Merino wethers. The addition of FSF to the diet of Dohne-Merino diet did improve not only the weight gain and nutrition status but also the immunity system of the sheep. It also reduces gastrointestinal parasite load; hence better wool quality is being produced. As a result, commercial and smallholder farmers can increase their productivity as well as profit. To ensure

FSF resourceful utilization, Dohne- Merino wethers can be fed on diets with 4% FSF. This is because 4% FSF supplementation gave the best in terms of growth performance, nematodes reduction capacity, WBC counts, low blood urea, good wool parameters, and feed preference. Likewise, 4 % FSF did not give the highest methane output nor performed badly in digestibility. Therefore, the optimal level for fossil shell flour inclusion in the diets of Dohne-Merino can be set at 4 %, so that the overall performance of Dohne-Merino wethers is not compromised.

8.3. Recommendations

- Inclusion of FSF into the diets of sheep would yield desirable live weights and BCS, therefore, farmers who dispose of their sheep through market channels that pay based on weights are encouraged to add FSF to their diets, especially at 4 % inclusion level, to realize bigger profit margins.
- In a period of disease prevalence, the addition of FSF to the diets of sheep could boost the immunity system of the sheep.
- The use of FSF as natural anthelmintics had been ascertained in this study; however, for FSF to be effective as anthelmintic, at least 4 % inclusion rate should be incorporated into the sheep's diet and be fed continuously for a minimum of 30 days.
- It had been established in this study that the addition of FSF up to 6 % inclusion level to the diet of sheep could increase wool yield; hence, commercial and smallholders' wool farmers can increase their wool yield and quality by adding FSF to the diets of their sheep, 3 to 4 months before shearing to boost the wool yield and quality.
- Preference to a diet containing FSF by sheep had been ascertained; therefore, FSF could be used to enhance the palatability of sheep suffering from anorexia.
- Since it had been ascertained in this study that more methane is emitted when sheep are resting than when feeding or standing idle, sheep could be allowed to rest in an enclosed place where the methane can be captured, and prevention to the atmosphere are prevented.

8.4. Prospects

Further research on the use of FSF in livestock production is necessary to broaden knowledge on FSF. Possible areas are:

- The effects of FSF on methane output needs to be investigated in vivo.
- In the same way, the effects of FSF on the microbial communities needs to be examined.
- The use of other species of animal and breeds of sheep, especially milk-producing breeds, other than the Dohne-Merino breed used in this study, needs attention. This broadens the scope of fossil shell flour in livestock production and investigates its effect on milk yield and constituents.
- It is also significant to investigate the meat quality to help ensure consumers' acceptance and perspective.

These gaps will form key components of the projects of my postdoctoral research.

8.5 References

Babeker, E.A., Bdalbagi, Y.M.A., 2015. Effect of Feeding Different Levels of Moringa Oleifera Leaves on Performance, Haematological, Biochemical, and Some Physiological Parameters of Sudan Nubia Goats. Online Journal of Animal and Feed Research 5:50– 61.

McDonald, P., R.A. Edward, J.F.D. Greenhalgh, C.A. Morgan, L.A.S. 2011. Animal Nutrition, Animal Nut. ed. Prentice-Hall/Pearson Education Ltd, Harlow, UK

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

Skapetas, B., Kalaitzzidou, M. 2017. Current Status and Perspectives of Sheep Sector in the World. Livestock Research for Rural Development 29:16 -19.

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.

Weaver, A.C., ToddSee, M., Hansen, J.A., Kim, Y.B., De Souza, A.L.P., Middleton, T.F., Kim, S.W. 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.

Appendix

Ethical Clearance Certificate

University of Fort Hare

Together in Excellence

ETHICAL CLEARANCE CERTIFICATE

AREC-150311-008

Certificate Reference Number:	MPE041SIKU01
Project title:	**Assessment of growth performance, nutritional status and blood profile of Dohne merino sheep fed diet supplemented with fossil shell flour.**
Nature of Project	PHD in Animal Science
Principal Researcher:	Olusegun Oyebade Ikusika
Supervisor:	Dr C.T Mpendulo
Co-Supervisor:	Dr. T.J Zindove; Prof. C.T Kadzere

On behalf of the University of Fort Hare's Animal Research Ethics Committee (AREC) I hereby give ethical approval in respect of the undertakings contained in the above-mentioned project and research instrument(s). Should any other instruments be used, these require separate authorization. The Researcher may therefore commence with the research as from the date of this certificate, using the reference number indicated above.

Special conditions: None

Please note that the AREC must be informed immediately of

- Any material change in the conditions or undertakings mentioned in the document;
- Any material breaches of ethical undertakings or events that impact upon the ethical conduct of the research.

The Principal Researcher must report to the AREC in the prescribed format, where applicable, annually, and at the end of the project, in respect of ethical compliance. The AREC retains the right to

- Withdraw or amend this Ethical Clearance Certificate if
  - Any unethical principal or practices are revealed or suspected;
  - Relevant information has been withheld or misrepresented;
  - Regulatory changes of whatsoever nature so require;
  - The conditions contained in the Certificate have not been adhered to.

- Request access to any information or data at any time during the course or after completion of the project.

- In addition to the need to comply with the highest level of ethical conduct principle investigators must report back annually as an evaluation and monitoring mechanism on the progress being made by the research. Such a report must be sent to the Dean of Research's office.

The Animal Research Ethics Committee wishes you well in your research.

Yours sincerely

Dr. Craig Tambling
AREC Chairperson

15 October 2018

Animals

Review

Fossil Shell Flour in Livestock Production: A Review

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Simple Summary: Fossil shell flour or Diatomaceous earth is made up of amorphous silicates with important physical and chemical characteristics that enable it to be used for different purposes including uses in livestock production. The substance is nontoxic, cheap, and readily available in large quantity in many countries. Recently, fossil shell flour has been modified as additives for several uses. Recent studies have supported its use as animal growth promoter, vaccine adjuvant in livestock, water purifier, mycotoxin binder, inert dust applications in stored-pest management, pesticide, animal feed additive, as a natural source of silicon in livestock, and as natural anthelmintic. Numerous advantages of fossil shell flour include its low-cost and availability, its nontoxic characteristics, and the fact that food grade diatomaceous earth is safe for human consumption. Likewise, all farmers, whether commercial, small-scale, or communal, can make use of fossil shell flour. The numerous uses of fossil shell flour give room for all types of farmers to explore the various benefits and applications of fossil shell flour. It is believed that through this publication, the potential of fossil shell flour will be exposed and explore by many countries

Abstract: Fossil shell flour (FSF), also known as Diatomaceous earth, or diatomite, consists of amorphous silicates with important physical and chemical characteristics, including porosity and permeability, low density and thermal conductivity, tiny particle size, high surface area, solubility, hydrophobia, and absorption capabilities, which are molecular filter actors, substituting their integral cations without physical changes. The substance is nontoxic, cheap, and readily available in large quantity in many countries. Recently, FSF has been modified as additives for several uses. Recent studies have supported its use as animal growth promoter, vaccine adjuvant in livestock, water purifier, mycotoxin binder, inert dust applications in stored-pest management, pesticide, animal feed additive, as a natural source of silicon in livestock and as natural anthelmintic. Numerous advantages of FSF include its low-cost and availability, its nontoxic characteristics, and the fact that food grade diatomaceous earth is safe for human consumption. In this paper, we review the main uses of FSF in the livestock industry, with reference to similar works earlier published that elucidate their important roles.

Keywords: fossil shell flour; parasites control; performance enhancer; mycotoxin control; stored grain pest control; water purifier

1. Introduction

Increase in the world's population has placed ongoing demand on agriculture, especially in the livestock sector, for food security [1]. However, livestock farming faces challenges due to increases in the prevalence of gastrointestinal parasites and other disease-causing parasites, as well as low quality feeds which has resulted in compromised feed conversion efficiency and thus growth rates [2]. As a result, farmers use diets supplemented with feed additives in order to promote efficiency. Antibiotic feed additives are commonly used. This chemical-based feed additive is expensive and the residue on animal products has health implications for the consumer. In addition, the persistent use of antibiotics has caused the spread of antibiotic-resistant bacteria to animals and humans. Therefore, there is a need to replace antibiotics with naturally-occurring feed additives such as prebiotics, probiotics, feed enzymes, herbal extracts, and organic acids, in order to have healthier meat and still achieve optimum production [3]. One such alternative is fossil shell flour (FSF).

Fossil shell flour (FSF), or diatomaceous earth, has numerous uses, including in water purification, as a performance enhancer in livestock, as a mycotoxin binder, and in stored grain pest control. In addition, it can be used as a dietary supplement for animals as well as other agricultural applications [4,5], thus contributing to livestock productivity and consequently food security and safety. According to the authors of a past work [6] diatomaceous earth consists of geologically-deposited fossilized skeletal remains of siliceous marine organisms and freshwater unicellular species, particularly algae and other diatoms. Diatoms can be described as minute, single-celled water organisms. These minute organisms are confined by a glassy crust. This crust is formed from the silicon dioxide in its source water. Diatomaceous earth is differentiated into two types based on source. One originates from the sea and the other from fresh water such as lakes. Diatomaceous earth from fresh water is preferred because it is richer in silicon dioxide. Diatomaceous earth (food grade) for use in livestock must be crushed until a fine flour is formed. It is then referred to as amorphous silica or fossil shell flour. When viewed with a powerful scientific microscope the miniature sharp edges can be seen, but it physically feels like chalk dust. Many of these fossilized sedimentary layers have been in existence for a minimum of twenty million years in the Eocene and Miocene epochs lakes and seas [7]. The physical and chemical properties of fossil shell flour enable it to play a vital role in livestock production. Previous authors [8,9] have stated that the surfaces of diatoms possess many porous nanostructure silica cell walls or frustules, enlarging its surface area and enabling it to be used as a substance carrier. It has been acknowledged as an animal natural health and sustenance product [10,11], and is described as a fine and pale color silica dust which has the ability to absorb liquids with a definite abrasive characteristic obtained after quarrying, crushing and milling [12,13]. Due to its abrasive property, FSF has been effective when used as vector and gastrointestinal parasite control in ruminants and poultry [14–16]. In this paper, we carried out a review of the main uses of FSF in the livestock industry and other areas of human endeavors.

2. Physical and Chemical Characteristics of FSF

Fossil shell flour (FSF) is fine, soft, lightweight, pale colored, is of biogenetic sources, and is comprised mainly of amorphous silicon (SiO_2NH_2O), derived from the skeletons of diatoms. It is abundantly available on the planet earth and has distinctive physical properties, such as porosity (35–65%), permeability (0.1–10 MD), low density and thermal conductivity, tiny particle size [17], and large surface area [18]. The characteristics of the surface of diatomite, such as acidity, solubility, hydrophobicity, ion exchange, and absorption functionalities, are largely controlled due to the presence of water, which is partly and morphologically connected with the crystal structure of the diatomaceous earth, thereby resulting in vigorous hydroxyl groups [19]. Its unique porosity (typically 10–200 μm), minute particle size, extensive surface area, high permeability, poor thermal conducting properties, and chemical inertness makes it of great interest among naturally-occurring materials [20,21]. Table1 shows the elemental composition of natural FSF and Table2[22] compares the chemical composition of fossil shell flour from various locations. Kilpinen and Steenberg [23] reported that FSF from difference sources have difference therapeutic strength against parasites especially in poultry

Table 1. Composition of natural diatomite [24,25].

Chemical Content (% Weight)	Natural Diatomite
SiO_2	82.16
Al_2O_2	4.89
FeO_2	1.46
CaO	1.23
MgO	0.89
MnO_2	0.52
KiO	0.54
NaO	0.43
TiO_2	0.19
P_2O_5	0.12
Loss of Ignition	7.55

Table 2. Chemical composition of different sources of diatomite (%) [26–30].

Country/Sample	SiO_2	Al_2O_3	Fe_2O_3	TiO_2	Na_2O	KO_2	CaO	MgO	L. Others
China	82.95	5.75	1.41	0.69	0.06	0.06	0.24	0.21	7.95
Turkey	76.5	7.25	3.85	0.5	0.45	0.85	-	-	0.43
Egypt	83.6	2.24	1.07	0.17-	-	0.53_	6.17	_	4.86
Algeria	72.1	5.3	3.8	0.37	0.65	0.54	7.2	2.6	7.44
Jordan	7.25	11.42	5.81	_	7.21	0.69	1.48	0.25	0.66
Mexico	70.38	13.52	3.37	_	0.17	0.3	0.66	0.42	11.18
Guangdong Chin	90.1	_	0.3	0.4	_	_	0.5	0.2	8.5
Shengzhon, China	65	17.50	4.8	_	0.5	_	1.1	_	11.1
Morocco	62.8	9.7	11.4	_	7.3	_	_	_	8.8
China	72	7.3	4.3	_	1.8	1.2	10	1	2.4
Suizhon, China	71.35	13.26	5.5	0.08	6.7	0.11	1.94	0.15	0.91
Caldiran, Lake Van Basin, Turkey	69.7	11.5	0.65	0.65	0.08	1.4	_	_	15.3
United States	79.55	8.18	2.62	0.70	0.25	1.30	1.31	_	3.8
Kenya	84.5	3.06	1.86	0.17	1.80	0.39	1.19	0.91	6.08
Spain	88.60	0.62	0.20	0.05	3.0	0.81	0.50	0.39	5.20
Russia	79.92	6.58	3.56	0.48	1.43	0.98	0.65	0.72	4.91
Canada	89.7	3.7	1.09	0.10	0.30	0.55	0.31	0.41	3.70
Japan	86.0	5.8	1.6	0.22	0.07	0.29	0.48	0.53	4.4
Nevada	86.0	5.27	2.12	0.21	0.34	0.39	0.24	0.29	4.90
Shengzhou, China	89.6	2.5	1.8	_	1.5	_	1	_	4.5

Natural fossil shell flour can be changed by treating it with hydrochloric acid to purify the silica surface. This helps to significantly mitigate the input of detrimental calcium, iron, aluminum, magnesium, and alkaline rudiments while dissolving little of the silica [21,31,32]. Table3shows the chemical characteristics of modified and natural FSF, obtained through X-ray fluorescence spectrometer. The composition reveals that silicon dioxide (SiO_2) is the major constituent while small amounts of iron oxide (Fe_2O_3) and aluminum oxide (Al_2O_3) are also present [32,33]. After modification, fossil shell flour can remove substances such as Lead (Pb^{2+}), Copper (Cu^{2+}), and other heavy metals optimally. One of the elements used in diatomite modification is magnesium oxide, which is achieved by treatment with sodium hydroxide and manganese chloride [34].

Table 3. Chemical composition of natural & modified diatomite (%) [32,33].

Compound	Natural Diatomite	Modified Diatomite
SiO	63.31	56.79
Al_2O_3	13.42	12.15
Fe_2O_3	12.58	10.11
Na_2O	0.74	2.37
CaO	0.49	0.08
Cl	<0.019	9.12
Loss on Ignition	6.54	6.73

3. Availability and Accessibility

Fossil shell flour (FSF), or diatomite, has been known for decades and several countries are actively involved in mining, milling, and transformation of this compound. The world production of diatomite in 1981 was 1.5 million tons, close to half of which came from North America [34]. Table4shows the major world producers of diatomite and the estimated annual quantity produced. The resources of crude diatomite all over the world are sufficient for the anticipatable future and its production worldwide is used in absorbents (9%), fillers (14%), cement (21%), filter aids (55%), other uses (1%), as well as specific pharmaceutical, biomedical, and agriculture uses.

Table 4. World diatomaceous earth production (in thousand metric tons) [34,35].

Country	2014	2015	2016	2017
USA	901	925	850	700
Argentina	100	55	200	200
China	420	420	420	420
Czech Republic	49	50	450	450
Denmark	95	95	440	440
France	75	75	75	75
Japan	90	100	100	100
Mexico	88	80	80	90
Peru	125	125	150	120
Russia	70	70	70	70
Spain	36	36	50	50
Turkey	85	90	60	60
Other countries	122	170	120	120

4. Types of Farmers Making Use of FSF

There are serious losses to stored grain suffered by communal farmers in sub-Saharan Africa because of insect damage. However, as many of these farmers cannot afford the cost of chemical protectants, they are resorting to using diatomaceous earth to preserve their produce for their household and livestock use [36]. Diatomaceous earth is cheap, readily available, effective against insects, and safe to use.

All farmers, whether commercial, small-scale, or communal, can make use of FSF. The numerous uses of FSF give room for all types of farmers to explore the various benefits and applications of fossil shell flour. Stathers et al. [36] and Mvumi et al. [37], observed in two different studies that small-scale farmers in Tanzania and Zimbabwe were able to preserve their grains for both their animals and their household by applying diatomaceous earth to the grain at the rate of 0.1% (*w*/*w*). Likewise, Badii et al. [38] reported that diatomaceous earth applied at 1.50 or 2.00 g kg^{-1} at 50% RH is a feasible substitute for preventing *C. maculates* infestation in stored Kersting's groundnut for commercial and small-scale farmers.

Another opportunity that is open to commercial, small-scale, and even communal farmers in the use of diatomaceous earth is that food grade diatomaceous earth can be put in a bowl and placed in the pen or barnyard as a mineral lick for the animal [39]. Farmers of different scales of production can therefore make use of diatomaceous earth for their animals to preserve feed ingredients as well as provide a source of minerals as well as other numerous uses.

5. Potentials of Fossil Shell Flour

5.1. Potential of FSF in Parasite Control in Livestock

Parasitism in small ruminants is a major problem for farmers. By their nature small ruminants, especially sheep, graze close to their own dung, which exposes them to parasitic ova and subsequently parasite load [40]. Consequently, parasitic gastroenteritis continues to be a health risk and constraint to the production of small stocks due to associated disease, mortality, control measures, and cost of treatment at clinical as well as subclinical levels [41]. Economically, a reduction in profitability levels of ~15%, including weight loss of ~50% on account of intestinal parasites, have been reported [42,43]. This loss is a result of low production on account of poor growth, low weight gains, and poor utilization of feed [44]. Parasites can cause hematological and biochemical turbulences in sheep [44], as well as anorexia, altered water and electrolyte balance, anemia, poor reproductive performance, and weight loss, which can consequently lead to an increase in the mortality rate of lambs [14]. Studies have revealed that GIT parasites are significant causes of production losses in sheep, particularly in sub-Saharan Africa, [14,45]. At any stage in the growth of sheep, they can be vulnerable to gastrointestinal nematodes, although lambs and peri-parturient ewes are most epidemiologically affected [46]. One of the main organisms that economically restricts sheep production worldwide are gastrointestinal nematodes [15]. The most important factor which limits the control of this parasitic organism is the stable increase of anthelmintic resistance globally, particularly if animals are underdosed or treated under preventative and suppressive treatment regimes. Therefore, alternative and/or complementary sustainable control programs would be beneficial [47,48].

For the past two decades' fossil shell flour (FSF) has been used to naturally deworm animals. The authors of a previous paper [5] clearly demonstrated that a 2% inclusion rate of FSF can be used with positive results in the destruction of internal parasites and worms. In the study, the inclusion of FSF increased the productivity and profit on dairy and beef cattle farms as a viable alternative to synthesized chemical products. The National Experimental Council and the National Council of Organic Standards in the USA discuss the disadvantages of chemical inoculations in their database of published products and suggest that Diatomite be used as an alternative [5]. A previous study [49], on the efficacy of fossil shell flour with ewes and lambs, reports that when feeding sheep with a combination of FSF and a mineral supplement, with no other anthelmintic used, at a ratio of 1:1 for three months, the incidence of Haemonchus in ewes was minimized compared with the control.

Lambs fed with 2% inclusion rate of FSF appeared to have an earlier gain in weight, tails that were cleaner, and shining wool [49]. Also, great improvement in the general body condition of the lambs was reported. Deutschlander [49] also performed a similar study using heifers. He reported that heifers that were five hundred-pound in weight that were fed FSF pasture on free-choice conditions showed no worms either mid- or late-season. The heifers consumed FSF of about one pound per week per heifer and when the dairy cows were not fed FSF for a few days they craved the substance and consumed several pounds as soon as it was made available to them. Despite the heavy feeding on FSF no side effects were recorded in the cows and they remained in good condition. He concluded that the lambs performed much better than the ewes and cattle while on pasture. In addition, the use of FSF recorded savings of $1.50 per head for both ewes and cattle as a dewormer over the use of conventional medicines. Deutschlander [49] observed that the body conditions of the heifer and ewes were very satisfactory when they were withdrawn from pasture. Another study Bernard et al. [50] observed that Spanish/Boer cross goats fed varying inclusion levels of FSF at 1.77 g, 3.54 g, and 5.31 g per kg had significant improvement in mean weight gain and fecal egg count as the inclusion levels increased. Similar observations were reported by Bennett et al., Mclean et al. [4,51]; both observed that FSF significantly increased body weight, feed conversion efficiency, and growth rate, and decreased parasite load of the experimental animals. However, they both postulated that for an effective and optimal outcome, FSF should be fed for a longer time period. They hypothesized that the abrasive edges of the diatom particles injured the cuticles of nematodes when in contact, resulting in dryness and eventually the death of the parasite. In line with these findings, Ahmed et al. [52], considered the use of biological control of gastrointestinal parasites in Merino sheep, feeding fossil shell flour as 2% of their diet. They also reported that fossil shell flour had an efficacy of 61%, although efficacy varied with time.

As an alternative to anthelmintic, fossil shell flour (FSF) was evaluated by [53] for its ability to inhibit the migration of *Oesaphagostomum dentatum* larvae using migration and inhibition assays in vitro, in unsheathed and sheathed third stage larvae. They observed that FSF was more effective in unsheathed larvae at 0.3 mg/mL after 20 h with 61.6% inhibition. With sheathed larvae, FSF had a significant effect of 1 mg/mL exposed within 24 h with 67.6% inhibition. This shows that the presence of cuticle could reduce the effectiveness of FSF as anthelmintic. There is therefore a need by researchers to investigate parasite cuticle abrasion by using equipment such as scanning electron microscopy to examine the cuticle integrity from treated versus untreated animals. In a separate experiment, Osweiler and Carson, Fernendez et al. [40,54] contrarily observed that FSF did not lower the parasite load in lambs.

Fossil shell flour has also been reported to reduce ectoparasites. Dawson [55] reported a significant reduction in bird flea population sizes (*Ceratophyllus* ideas) and several blow fly species (*Protocalliphora* spp.) in the same year. Fossil shell flour seemed to be more efficient at reducing the flea populations, perhaps because fleas have body sizes that are smaller and so are more susceptible to dehydration on account of FSF's abrasive action. It was also confirmed, in more recent researches by Martins and Mullens [56] and Amy [57], that FSF is able to suppress the activities of Northern fowl mites (*Acaris macronyssidae*). Kilpinen and Steenberg [23] reported that FSF, is one the commonly used substitute control methods for poultry red mites (*Dermanyssus gallinae*) in Europe because it kills the target host mainly by desiccation. The authors also observed large differences in the types of FSF versus red mites' mortality. Therefore, there is needs for other researchers to investigate difference FSF formulations head to head for parasite control

5.2. Potential of Fossil Shell Flour as Detoxifier in Livestock Feed

Animal feeds are often contaminated with many pathogenic microorganisms at different stages of the manufacturing process, despite concerted effort at averting these [10]. Chief among the microorganisms are Aspergillus and Fusarium genera that produce mycotoxins including Ochratoxin A, Aflatoxin B1, Deoxynivalenol, Fumonisin B1, and Zearalenone. Of the 300 to 400 known mycotoxins, deoxynivalenol (DON) and aflatoxin (AF) are known as the most common and most detrimental to animal industries [58]. According to Agag, Jones et al. [59,60] one-quarter of the world's crops are infected with mycotoxins. The North Carolina Cooperative Extension Service also established that close to one-fifth of tested corn contained levels greater than 20 μg/kg AF, and close to two-thirds of corn contains DON Weaver et al, Agag [10,59]. These mycotoxins are usually seen in the feed chain due to infection of crops by fungi, or use of moulds forage and grains as animal feed condiments [60].

Richard [61] reported that mycotoxins are produced on growing plants due to fungal invasion during preharvest, postharvest and during crop transportation and storage, and these have been shown to adversely affect man and animals at low levels, and have a substantial effect on international economies and trade. When consumed, these mycotoxins cause a reduction in the growth of animals, as well as immune and reproductive dysfunction and can damage organs [61,62]. These toxins also cause a pathogenic shift in vital organs such as liver, kidneys, and lymphoid tissues [63]. They have been reported in hepatic lesions in chickens causing such effects as enlargement, paleness, hydropic degeneration and necrosis, periportal fibrosis, and bile duct hyperplasia [64]. In addition, the spread of aflatoxin and its metabolites from feed to products such as liver and eggs as well as animal edible tissues [65] have become a predominant prospective hazard for human health.

Feed decontamination from aflatoxin is a major requirement in livestock industry. Physical, chemical, and biological methods for decontamination are used. Other approaches such as protective actions, good storage practices (GSP) as well as good agricultural practices (GAP) at both preharvest and postharvest periods, have been adopted to control the harmful mycotoxins in animal feed. As good as these options are in mitigating mycotoxin contamination; the use of best practices might not completely avoid or get rid of mycotoxins in the feed chain [66]. An acceptable detoxification procedure must be cost-effective and capable of eradicating all traces of toxin with no detrimental residues, and should not weaken the nutritional quality of the product [67]. One such procedure is the use of fossil shell flour, which has been reported to be effective against mycotoxins. The authors in their experiment using quail chicks observed that addition of FSF to an Aflatoxin (AF)-containing diet significantly reduced the deleterious effect of AF on food consumption, body weight gain, and feed conversion ratio. Food consumption was reduced by 14% in quail chicks consuming the AF diet without FSF, but by only 6% for quail chicks consuming the AF plus FSF diet. Similarly, overall body weight gain was reduced by 27% in birds consuming the AF diet without FSF, but by only 8% for birds consuming the AF plus FSF diet. Parlat [68], reported that broiler chickens fed a diet containing diatomaceous earth (DE) at 400 and 800 mg/kg, respectively, had significantly greater body weight and less feed conversion ratios and an increase in serum total protein and albumin values than those fed a diet containing T-2 toxin at 0.5 ppm and 1 ppm after 35 days of age. However, DE supplemented chickens were not significantly different in relative organ weights of kidney, liver, bursa of Fabricius, spleen and serum biochemical values of AST, ALT, cholesterol, triglycerides, and creatinine when compared to birds fed with only T-2 toxin. The study showed that the inclusion of DE as an additive in the diet was partly helpful in lessening the harmful T-2 toxin effects in broiler chickens. Furthermore, Shivashaankar et al. [69], reported that supplementation of fossil shell flour at 400 and 800 mg kg^{-1} in an aflatoxin-mixed diet (with 0.5 and 1 ppm of AF kg^{-1}, respectively) significantly reduced the deleterious effects of AF on the growth parameters of broiler chickens and the serum biochemical values by bringing about a boost in serum total proteins, albumin, triglycerides and cholesterol levels. The same author also recorded a significant increase in ALT, AST, BUN ALP, and creatinine levels in fossil shell flour supplemented broilers. [70], in another study with in vitro models to evaluate the properties of fossil shell flour and other natural absorbent agents on six mycotoxins, namely, ochratoxin A (OTA), aflatoxin B1 (AFL), zearalenone (ZON), diacetoxyscirpenol (DAS), deoxynivalenol (DON), and T-2 toxin using thin-layer chromatography (TLC), observed that fossil shell flour bound more than 95% of applicator AFL and 66.67% of OTA (only diatomite adsorbed this toxin). Binding of DON has also been observed [62], but only at pH 3.0 of an electrolyte. Its adsorption index varies from 25.00 to 50.00%, while the ZON adsorption index ranges from 12.20 to 37%, and for T-2 toxin the adsorption index ranged from 16.67 to 33.33%. These results appear to suggest that fossil shell flour is an effective absorption agent that can be used in the animal feed industry to decontaminate many mycotoxins in animal feed.

Fossil shell flour can also be used in the production of some mycotoxin adsorbents, and for diarrhea remediation in ruminants and birds [71]. The use of adsorbents such as fossil shell flour as a feed additive is the most effective, economical and healthy solution for the decontamination of mycotoxins livestock feed. It should be modified in accordance with current demand of farmers and should be widely monitored to ensure its safety and effectiveness [72].

5.3. Potential of Fossil Shell Flour as Animal Performance Enhancer

Growth promoters and enhancers are materials that facilitate the growth of farm animals, particularly swine, poultry, and livestock, where the value of such animals to the farmer depends partly on body weight. The market for animal growth promoters and enhancers is large and growing. The most widely used group of growth promoters in animal feed is antibiotics [73]. Other growth promoters include probiotics, prebiotics, essential oils, botanicals, enzymes, organic acids, phytochemicals, vaccines, RNAS, antibodies, bacteriophages, antimicrobials, innate defense molecules, immune enhancers, and combinations thereof [74]. There remains room for the development of new growth enhancers, particularly those that can be made in a cost-effective way. In line with this, Abd El-Tawab et al. [75] conducted a study using spent filter media containing ~35% moisture, 32% diatomaceous earth, 22% organic carbon, and 11% activated carbon, and reported that although their results were not definitive, spent filter media improved daily feed intake, weight gain, and efficiency. The animal feed compositions as described herein may be fed to different animals. It is believed that the use of spent filter media may advantageously increase the growth of the animals on a daily basis compared to identical amounts of conventional animal feed compositions, although this result is believed to be affected by other conditions, Abd El-Tawab et al.; Sarijit et al. [75,76], including the composition of the base feed. In addition, Abd El-Tawabet al. [75] also noted that these animal feed compositions did not adversely affect the lean mass percentage of the animals and did not appear to exhibit any harmful effects to the growth or the health of the animals. Likewise, Adebiyi et al. [25] reported that there was a substantial improvement in average weight gain, feed conversion efficiency and bone development of cockerel fed 6% fossil shell flour inclusion level as feed additives throughout the 16 weeks of the trials. Similarly, fossil shell flour significantly improved intake of feed (7.44%), body weight gain (9.51%), and improved ratio of feed conversion (2.08%), as well as the productive efficiency index (5.48%) in cockerel exposed to aflatoxin B1 [25,77]. Fossil shell flour also improved serum albumin (2.26%) and the action of serum LDH (44.4%) in a previous study [78].

Modirsane et al. [79] supplemented the feed of 252 piglets with silicon dioxide (a major component (79%) of FSF) and found improved feed intake by 4.13% and an improved mean daily gain by 3.26% during the general early period, compared to groups not given silicon dioxide. The outcome led to an increment of 2.2% in the piglets' weight at the expiration of the postweaning phase (24.52 kg vs. 23.99 kg). They concluded that under their study circumstances, an addition of 0.02% crystalline silicon dioxide (fossil shell flour) to piglet feed increases daily feed intake, growth rate, and piglet weight at the end of the weaning period. This mineral supplement could offer prospective financial gain to pig farmers.

5.4. Potential of Fossil Shell Flour as Water Purifier for Livestock Usage

The provision of poor quality drinking water for livestock in semi-arid and arid regions for several months of the year is very common [80,81]. These supplies originate from streams, canals, small wells, or water holes, which are also used for irrigation [82]. This water is often high in salt and toxic elements such as lead, copper, selenium, mercury, and arsenic [32]. They are hazardous to animal health and may cause physiological upset or render the animal products unsafe or unfit for human consumption, as well as possibly cause the death of the animal [83]. The bulk of heavy metals are identified to be poisonous and cancer-causing agents. Problems emanating from toxicity are aggravated by irrigation of forage with the same potentially toxic water. The plants make use of the salts, thus increasing the level of risk in toxicity to the animal when both feed and water sources surpass hazardous levels. This may also occur with selenium. Sources of these toxins and salts are animal feces, birds, animal carcasses, intensive livestock industry runoff from bare paddocks, sewerage waste, veterinary antibiotics, herbicides, and pesticide residues [32]. Diatomite that has been modified with manganese-oxide is an active and good adsorbent for removing heavy metal ions such as, Pb^{2+}, Cu^{2+}, Cd^{2+} Ni^{2+}, and Hg^{2+} [32]. The negative charges on the modified surface is an attribute that enhances adsorption performance. In addition, the enlargement of the surface area is believed to play a vital role in the overall removal process. Therefore, lower loading of diatomite has a superior performance over higher loading adsorbents [84]. Similarly, Al-Degs et al. [85] reported that heavy metal ions such as Pb, Ni, and Cu were removed from wastewater using manganese-oxide modified Iranian diatomite considered this to be an adsorbent and a filtration substance. Also, organic and inorganic substances can be removed from wastewater and surface water through the absorption of diatomite modified with Mn [86] and Bello et al. [32] reported that modified diatomite has a greater removal capacity for heavy metals from water than unmodified diatomite. Walker and; Weatherley [87] established that the use of diatomaceous earth is a promising technique in the removal of heavy metals from wastewater and surroundings. Hence, FSF can be used to treat water/wastewater for animal use, thereby reducing risk for human consumption. Hence, FSF has significant potentials in mitigating water scarcity and turning waste into wealth.

5.5. Potential of Fossil Shell Flour as Source of Minerals for Livestock

Minerals are naturally-occurring substances generally required in microscopic amounts from less than 1 to 2500 mg per day, depending on the mineral [88]. Just like vitamins and other essential food nutrients, mineral requirements vary with animal species. Large mammals require large amounts of calcium for the building and preservation of bones and standard function of nerves and muscles [89]. Phosphorus is an important constituent of adenosine triphosphate (ATP) and nucleic acid, and is also vital for acid–base balance, and the formation of teeth and bones. Red blood cells cannot function properly without iron in hemoglobin: the oxygen-carrying pigment of red blood cells. Iron is also an important component of the cytochromes that function in cellular respiration. Molybdenum, manganese, copper, zinc, magnesium, selenium, and iron are important cofactors found in the structure of certain enzymes and are crucial in many biochemical pathways. Mammals need iodine to make thyroid hormones. Sodium, potassium, and chlorine are imperative in the upholding of osmotic balance between cells and the interstitial fluid [90]. According to Soetan et al. [91] different microminerals, for example, Se, Mn, Zn, and Cu, are important for the optimal performance of the immune structure and for resilience against pathogens. Cobalt deficiency decreases the resistance of animals against helminth infections [92]. Molybdenum also plays an important role in immunity against endoparasite [93] and can decrease the worm burden in lambs [15,94]. Natural food grade FSF contains 15 macro and microminerals, which are important to animal diets. The inclusion of natural FSF to poultry diets has continuously revealed weight gains in production. This gain could be connected to a combination of factors such as the ability of natural food grade FSF to decrease parasite populations, which promotes a reduction in stress on the animal and improved food assimilation [25,95]. Natural food grade FSF contains a wide range of naturally present chelated minerals such as calcium, magnesium, iron, phosphate, sodium, titanium, and potassium. Based on the final optimal levels, the mineral constituents that it provides may replace a small proportion of the entire mineral premix or complex. However, its ability to increase the incorporation of other minerals and microminerals, (particularly the effects of fossil shell flour on improved general mineralization such as bone), may also be the reason for improved performance [96–98]. In a study conducted at the University of California, the importance of fossil shell flour for growth performance and maturity in chickens was established. It was reported that the silicon inclusion group had thicker legs and bigger combs relative to their body size, significantly superior to other groups without silicon supplementation [25,96] also reported that growing cockerels fed 6% inclusion levels of FSF had the highest values for Ca, P, and Ash than the control and other treatments. However, tibiotarsi weight, length, and robusticity index were not affected significantly. In livestock, production and health problems are often associated with calcium deficiency in many species of animal. Addition of natural food grade FSF to diets could, therefore, avert any calcium associated performance or health issues. Minerals present in natural food grade FSF can also help to meet the mineral constituents of lactating animals [88]. Table5shows the mineral constituent of food grade FSF, which includes major and trace elements needed by livestock for growth performance.

Fossil shell flour is the most abundant form of organic amorphous silica in the world (79% to 94% of silicon) [99]; it exists in silicon dioxide form, it is bioavailable, and is essential for good bone growth and nutritionally important for preventing certain forms of chronic diseases associated with aging [99,100]. Humans, animals, and plants have an essential need for silicon in order to sustain life, and regrettably, in today's world, our diets can easily become deficient in silicon.

Table 5. Mineral constituent of fossil shell flour (FSF).

Element	Quantity
Calcium (Ca)	0.40
Sodium (Na)	0.26
Manganese (Mn)	0.0052
Iron (Fe)	0.72
Copper (Cu)	0.0019
Vanadium (V)	ppm 43.8
Sulfate Sulfur (S)	0.062
Phosphorus (as P205)	0.037
Potassium (K)	0.16
Chloride	0.074% or 740 ppm
Zinc (Zn)	0.0022
Titanium (Ti)	ppm 420
MgO (calculated from % Mg)	0.34
Strontium (Sr)	ppm 59.9
Boron (B)	0.0023
Magnesium (Mg)	0.21
% CaO (calculated from % Ca)	0.55
Aluminum (Al) %	0.65

Sources: Adebiyi et al [25].

5.6. Potential of Fossil Shell Flour in Feed Storage

Over one-third of the total grains produced globally are lost annually due to pests during storage [101]. In sub-Saharan Africa farmers suffer significant losses to their products as a result of insect damage. These losses undermine their livelihood, food security, reduction in market returns, and also have an indirect effect on the feeding of animals [36]. This loss often increases the cost of production in livestock industries as a result of an increase in the cost of feeds. To prevent these losses, different traditional strategies have been adopted, including mixing grain with ashes or plant materials, and the use of synthetic chemicals (insecticides). Residual synthetic chemicals are the most frequently used protectants against stored-product pests in stored grain. They are often applied directly to the product and provide protection against stored-grain pests as long as the effect of these insecticidal persists [102]. Nevertheless, current frequently used protectants possess numerous disadvantages because of their toxicity to mammals, and the fact that they leave deposits in the product; in addition, many species of insect are resilient to some current protectants [102]. These shortcomings have made researchers assess the use of different control methods such as insect growth regulators, botanicals, biological control, microbial control, and inert dust. Fossil shell flour (FSF) is the most promising alternative that can replace insecticides successfully. This it does by absorbing the epicuticle lipids and fatty acids, thereby resulting in the dehydration of arthropods. FSF comes into direct contact with the insect bodies and absorbs the waxy cuticle of the outer layer, after which the insect loses water and dies [103–105]. FSF needs no specialized kit for application on grains, but one can use similar techniques as that used for insecticides. Sabbour and Abd-El-Aziz [106] reported that FSF is tenacious in its mode of operation, poses little or no pest resistance problems, and leaves no residue, but its efficacy depends on the influences of temperature, provenance, humidity, and the individuality of particular pests and substratum. Sabbour et al. [105] reported that calcium hydroxide modified diatomite (Ca-DE) and those modified with sodium hydroxide (Na-DE) were the most effective antidote against insects. Ca-DE yielded better results and accomplished the highest mortality percentages recorded at 88% and 96% for treatments against *R. dominica* and *B. incarnatus* with 1.0%, respectively. The lowest mortality rate was recorded for Al-DE at a concentration of 0.5% and achieved 21 and 15% mortality for the corresponding species, respectively. In a similar study conducted by the same researchers on the use of FSF in pest management in stored-grain, they reported that the rate of reproduction of tested insects was greatly reduced by both FSF and modified FSF, and egg production was greatly reduced by modified-FSF under stored conditions. The average quantity of eggs laid per female and percentage of adult emergence (F1) of each tested insect were appreciably affected by both natural FSF and modified-FSF in comparison to the control. The same authors found that Nano-FSF strongly reduced the number of eggs laid for *T. confusum* but reduced to a lesser extent for *T. castaneum* (3.8 ± 1.5, 17.8 ± 7.5, 26.6 ± 3.5 eggs/female and (13.8 ± 1.5, 37.8 ± 7.5, 46.6 ± 3.5 eggs/female) after 20, 90, and 120 days of storage interval respectively. The constant effect of nanoparticles showed numerous unusual modes of action such as falling oviposition, mature emergence (F1), and influx percentages of hardened insects. Sabbour et al. [105] concluded that FSF-nanoparticles and natural FSF can be a good instrument in pest management programs for *T. confusum* and *T. castaneum*. Badii et al. [38] in their studies on the prevention of *C. maculates* infestation in stored Kersting's groundnut, reported that mortality of the adults increased gradually with the increased quantity of FSF and the contact period. Grains that were treated at 2.00 or 1.50 g kg^{-1} documented considerably fewer eggs and a lower rate of F1 emergence compared with dosages with lower quantity. Increased FSF concentration constantly decreased produce weight as a result of low beetle counts however, a significant difference on seed viability was not recorded. FSF was more efficient at 50% relative humidity than at 80% relative humidity.

It appears that FSF can be used by communal farmers, animal feed mill operators and commercial farmers to preserve grains for their animals during the time of surplus to the time of scarcity. It is cheap, nontoxic, readily available and requires no equipment for administration. FSF likewise has broad uses as an anticaking agent in the storage of grain and in feed mixing. This enables for better flow ability, mixing and handling and prevention of particles from clumping together. According to Bannett et al. [4], food-grade fossil shell flour placed in livestock feed may help discourage the growth of fleas and other dangerous organisms.

5.7. Potential of Fossil Shell Flour on Quality of Wool and Mohair

In the face of demand for lighter weight fabrics by consumers and the rigorous challenge of competition from synthetic fabrics, producing quality wool that will translate to the yearning of the final consumer has been of utmost important to the sheep industry in the past 25 years. When looking for fine, uniform, and high-quality wool appropriate for the textile industry, Dohne-merino and other merino sheep come to mind [107]. Wool fiber diameter and fiber length are the key characteristics used to evaluate the processing route and final quality of the finished textile products [8]. Factors such as genotype, nutrition, age, and sex determine the quantity and quality of fleece from sheep. Cilek [108] reported that the fiber diameter and the proportion of kemp are greatly influenced by the genetic composition of the animal. Fiber diameter variability and proportion of medullated fiber are attributed to the sex of the animal. Shah and Khan [107] observed that females have a greater measure of fiber diameter variability and proportion of medullated fiber than males; and that age on all fleece traits was significant. The effect of sex was statistically noticeable for all fleece traits except for fiber tenacity and comfort factor. It can be generally concluded that younger sheep and rams have higher fleece yield and fleece quality than older sheep and ewes. Similarly, the age of the animal and the fiber diameter are directly proportional to each other, while age and fiber staple length are not affected by age in merino sheep. Fiber diameter is known to be affected by the quality and quantity of nutrients available to the animal, and is related to live weight [109–111].

Variation in staple length relates positively with live weight. McGregor et al. [112] observed that the highest correlation occurred between staple length and live weight that were measured simultaneously, with a lower correlation when comparing the two parameters at different time. Also fiber diameter variation mostly influences staple strength [82,110,111]. Staple strength and location of a break are greatly influenced by both the minimum fiber diameter and rate of variation along the fiber. However, nutrition, disease, stocking rate, genotype, and the physiological state of the animal all influence the staple strength. Likewise, climate, altitude, genotype, nutrition and soil, relative humidity, pH within the fleece, the level of perspiration, production of sebum by sebaceous glands, and time of shearing are factors that determine the color of wool [113–115].

The effect of gastrointestinal parasites and lice on fleece yield and quality have also been studied. Frequent treatment of antihelmintics increases the fleece yield, fiber diameter, staple length, and strength as well as crimp fleece weight [116,117]. *Bovicola ovis* (lice) infestation has an adverse effect on wool characteristics, and this is amplified as the number of lice present and duration of infestation increases. With lice infestation greasy wool changes color and becomes less bright, staple length is reduced and staple strength is negatively affected. Van Burgel et al. [117] also reported that sheep with modest to severe infestation produce less sound wool (1.7 vs. 3.0 kg/head) and more cast wool (0.4 vs. 0.1 kg/head) than sheep with very few lice.

Since fossil shell flour improves body weight gain, reduces intestinal parasites, and in general might be expected to suppress ectoparasites, it follows that inclusion of fossil shell flour to the diets of sheep will increase fleece yield and improve the quality of wool greatly for good market prices as well as the international standard.

5.8. Fossil Shell Flour Can Be a Replacement of Antibiotic in Animal Feed

Fossil shell flour might be able to replace antibiotics as a growth promoter [4,75,79], as a gastrointestinal nematodes eradicator [52,53] and as an eliminator of dangerous bacteria in the body system of an animal [118,119].

Most of the chemical constituents of FSF have a strong negative charge. Just like a magnet, the negatively charged elements of FSF attract all positive bodies that are sufficiently tiny to pass through the porous openings of FSF. These strong charges are capable of attracting a great quantity of oppositely charged substances, whether element, bacteria, or viruses. They pass through the digestive tract to remove these dangerous substances from the body system.

When diatomite is added to the feed of animals, Gram-positive bacteria, which are normally targeted in ruminant animals through the use of antimicrobial feed additives, may also bind to the negatively charged shells. This could promote FSF as an efficient replacement for antimicrobial and antibiotic products commonly used to carry out these roles. James et al. [118] in their study to evaluate the impact that diatomite (2% concentration) has on the adsorption of *Escherichia coli* (ATCC 25922), reported that the highest adsorption capability of 10.99 mg g^{-1} was realized within 26 h with a solution of 12 mg L^{-1} As (III) at pH 4. Another study carried out previously [119] on the adsorption ability of diatomite also demonstrated the superior capacity of diatomite to neutralize bacteria, comprising Gram-positive *Staphylococcus aureus* and Gram-negative *E. coli*.

5.9. Other Potentials of Fossil Shell Flour in Feed Industry and Livestock Production

Fossil Shell flour as adjuvants vaccine: Deactivated vaccines are normally used in poultry as part of a complete vaccination procedure. These vaccines are able to stir up antibodies of high titers, capable of protecting against general infections, and which are transmitted from parent stock to their offspring as maternal antibodies. In order to boost their immunogenicity, these vaccines have adjuvants. Common adjuvants often used in poultry vaccines are aluminum hydroxide ointment (Alum) and ASO_4 (oil-based type) [120,121]. Substances that are added to a vaccine to accelerate or enhance the body's immune response to the vaccine and to reduce the quantity of antigen needed for vaccines are referred to as adjuvants [122]. According to Waksman and Hunter [123,124] there are three different mechanisms to accomplished this. First, a prolonged immune response is induced by a slow release as a result of the deposit of antigens formed at the injection site. Second, particulate antigens are formed, which antigen-presenting cells can detect. Finally, local inflammation may be caused by adjuvants that initiate system-recognition receptors, activating antigen production. However, undesirable effects of adjuvants are an inflammation at the injection site. This effect is noticeable in farm animals such as poultry, resulting in reduction of meat quality and escalating condemnation of carcasses [125,126]. The price of vaccines is also considerably increased by adjuvants [127]. Discovering new, safe, steady, and cheap adjuvants is therefore vital to improving existing vaccines. Fossil shell flour has successfully met this need. Singh and O'Hagan [125] in their experiment using fossil shell flour as an adjuvants vaccine for Newcastle Disease Virus (NDV) for poultry, suggested that fossil shell flour could function as a prospective immunological agent for vaccines against poultry diseases. They established that using fossil shell flour as a vaccine adjuvant, causes no harmful effects to hatchability, quality of chicks, body weight, and meat quality. Although an apparent immune response was not observed when the vaccines were applied in ovo, subcutaneous boosters with NDV adjuvanted with diatoms generated NDV specific antibodies, starting at 7 d post the second booster in chickens. The effectiveness of diatoms as an adjuvant for INDV vaccines was similar to the action shown by aluminum hydroxide gel. The researchers proposed that fossil shell flour can be used as poultry adjuvant deactivated vaccines. A similar study Nazmi et al. [128] also looked at the efficacy of diatoms as adjuvants for the Ark-DPI live infectious bronchitis virus (IBV) vaccine after ocular or spray application. They observed that the addition of diatoms had no detrimental effect on the vaccine virus, hatchability, chick quality, live weight, and meat quality. However, the addition of diatoms to the vaccine did not stimulate higher IgG titers in the serum or IgA titers in tears. It also did not influence the occurrence of monocytes/macrophages in the blood and the spleen determined by flow cytometry. In addition, protection generated against IBV homologous challenges, measured by viral load in tears, respiratory signs and histopathology in tracheas, did not vary when diatoms were present in the vaccine formulation. Fossil shell flour can be postulated to be of potential as an adjuvants vaccine in Newcastle Disease Virus (NDV), although its efficacy on the Ark-DPI live infectious bronchitis virus (IBV) needs further research.

Prevention of Scours: Grazing animals frequently eat dark dirt, which contains a crystal-like type of silicon dioxide. The farmyard variety of dirt, contaminated with diseased organisms, bacteria and parasites, can lead to the death of calves and young animals. Providing stock with fresh water type of fossil shell flour that contains at least 80% pure silicon dioxide can prevent calf scours [129]. The purpose of providing fossil shell flour of free choice to the young animal is to prevent them from eating black dirt in the farmyard or elsewhere. Therefore, fossil shell flour serves as a better substitute to black dirt.

Feed additive: The nutrients and calories that are available in the diet are just as important as what the animal is fed. The digestion, health, cost-effectiveness of the diet and its related processes are also important. This is realized by maintaining a balanced intestinal process in the digestive system, and ensuring that bulk density of the feed and uniformity are within expected norms. Moreover, it is imperative to keep moisture out to avert the feed from rotting, clumping or caking.

To that effect, diatomaceous earth is a perfect animal feed additive for all livestock, with many benefits from preserving feed quality to improving livestock health and performance through better digestibility, acceptability and overall bioavailability. Diatomaceous earth will also provide cost-benefit advantages by improving mixing properties, as well as increasing the bulk density of some ingredients. The basic function of diatomaceous earth is to act as a natural preservative for the feed, absorbing moisture that may cause fungus, mold, or rot. In addition, due to its moisture reduction capacity, it reduces clumping and prevents caking of the feed. This helps to preserve feed without the need of chemicals, making it more acceptable to the animals during feeding and increasing processing and delivery efficiency.

Diatomaceous earth also helps to increase stock health, feed conversion, and ultimately, its performance. It is known to reduce internal and external parasites, bacterial infections, to control worms; its residual mineral content may also give animals a shinier coat.

However, for diatomaceous earth to work properly and efficiently as an animal feed additive, it has to be noncalcined and completely natural diatomaceous earth from fresh water. It should be organic (OMRI listed) and respect CFIA standards. Using fossil shell flour as an animal feed additive will increase return on investment by keeping the animal healthier, improving their feed conversion rates and ensuring the feed works harder and longer by preserving it naturally.

Source of natural Silica: Approximately 85% of food-grade fossil shell flour consists of silica. This significant mineral is needed by vital organs of the body for maintenance and development [97,100]. Before modern farming depleted the soil, food ingredients were the main source of natural silica. Nazmi et al. [130] and Martin [100] reported that plant-based food (natural) contains only one-third of the silica needed by mammals. With an increase in host resistance to many antihelmintics and antimicrobial drugs, coupled with health safety issues raised by the consumers of the products of animals that use the drugs, the use of fossil shell flour in livestock production is a promising substitute to these drugs [131].

In the testimony and report of [39] who experimented with FSF on his Merino sheep farm, allowing merino ewes to receive 300 g of FSF 2 weeks before lambing resulted in a good performance, and healthy, award-winning young.

Nonbeneficial effects of DE: Available information on the non-beneficial effects of DE showed that people who work with crystalline form of diatomaceous earth in large amounts such as miners, quarrymen, smelters, sandblasters, masons, and ceramic and glass manufacturers are likely to have lung problems [132,133]. Due to their very small density, Silica nanoparticles can be readily evaporated into air, and can be inhaled. Following inhalation, nanoparticles have been reported to rapidly cross the alveolar capillary barrier and penetrate into to the systemic circulation, reaching various organs [134]. Also, Cyrs et al [132] reported that after injection or skin application, nanoparticles can be distributed into the blood, causing a significant and dose-dependent platelet aggregation. Similarly, Nemmar et al. [134] observed that when rubbed on the skin, diatomaceous earth might cause wounds or loss of parts of the skin.

6. Conclusions

As a result of a global increase in organic farming, arising from the demand for organic livestock edible products by consumers and food safety campaign programs, diatomaceous earth has gained attention from scientists and commercial farmers. Its use has not yet been adopted in most countries, particularly in sub-Saharan Africa. There are relatively few studies investigating the potential of diatomaceous earth in livestock, especially in small stock. Besides an absence of authenticated statistical information from researchers, there is likewise a gap in knowledge concerning the application of these substances in an inorganic control program. There is a need for adequate scientific information and infrastructure to produce and continuously supply a naturally-occurring substance such as diatomaceous earth, for improving livestock production locally and internationally. There is also a need for awareness programs regarding diatomaceous earth use in the livestock industry for both farmers and consumers. Additional investigation is needed to motivate for the broader potential of diatomaceous earth as an agent of improvement in livestock production. In this context, it is assumed the diatomaceous earth will achieve a greater role in livestock production and food safety in the near future.

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References

1. FAO. *The Future of Food and Agriculture: Trends and Challenges*; FAO: Rome, Italy, 2017; ISBN 9789251095515.
2. Sejian, V.; Maurya, V.P.; Naqui, S.M.K. Adaptability and growth of Malpura ewes subjected to therma and nutritional stress. *Trop. Anim. Health Prod.* **2012**, *42*, 1763–1770. [CrossRef] [PubMed]
3. Ruiz-Garcia, L.; Lunadei, L. The role of RFID in agriculture: Applications, limitations and challenges. *Comput. Electron. Agric.* **2011**, *79*, 42–50. [CrossRef]

4. Bennett, D.C.; Yee, A.; Rhee, Y.J.; Cheng, K.M. Effect of diatomaceous earth on parasite load, egg production, and egg quality of free-range organic laying hens. *Poult. Sci.* **2011**, *90*, 1416–1426. [CrossRef] [PubMed]
5. Koster, H. Diatomite in Animal Feeds. 2013. Available online:agrisilica.co.za/pdf/eng/Diatoms%20in%20Animal%20Feeds%20HH%20Koster.pdf(accessed on 20 June 2018).
6. Dolatabadi, J.E.N.; de la Guardia, M. Applications of diatoms and silica nanotechnology in biosensing, drug and gene delivery, and formation of complex metal nanostructures. *Trends Anal. Chem.* **2011**, *30*, 1538–1548. [CrossRef]
7. Bakr, M.; Hossam, E.; Galal, M. Diatomite: Its Characterization, Modifications and Applications. *Asian J. Mater. Sci.* **2010**, *2*, 121–136.
8. Wang, Z.Y.; Jang, Y.X.; Zhang, L.P. Structural Investigation of some important Chinese diatomites. *Glass Phys. Chem.* **2009**, *35*, 673–679. [CrossRef]
9. Aw, M.S.; Bariana, M.; Yu, Y.; Addai-Mensah, J.; Losic, D. Surface-functionalized diatom microcapsules for drug delivery of water-insoluble drugs. *J. Biomater. Appl.* **2013**, *28*, 163–174. [CrossRef] [PubMed]
10. Weaver, A.C.; Todd See, M.; Hansen, J.A.; Kim, Y.B.; De Souza, A.L.P.; Middleton, T.F.; Kim, S.W. 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* **2013**, *5*, 1261–1281. [CrossRef] [PubMed]
11. Wiewiora, M.; Monika, Ł.; Justyna, B.; Mateusz, M.; Tomasz, N. Diatomaceous earth in the prevention of worm infestation in purebred pigeons. *Anim. Sci.* **2015**, *54*, 161–166.
12. Korunic, Z. Diatomaceous earths, a group of natural insecticides. *J. Stored Prod. Res.* **1998**, *34*, 87–97. [CrossRef]
13. Eldernawi, A.M.; Jamel Rious, M.; Al-Samarrai, K.I. Chemical, Physical and Mineralogical, Characterization of nAl-Hishal Diatomite at Subkhah Ghuzayil Area, Libya. *Int. J. Res. Appl. Nat. Soc. Sci.* **2014**, *2*, 165–174.
14. Hussain, H.U.; Usmani, R.H. *Livestock of Pakistan*, 1st ed.; Livestock Foundation: Islamabad, Pakistan, 2006.
15. Miller, P.J.; Afonso, C.L.; Attrache, J.E.; Dorsey, K.M.; Courtney, S.C.; Guo, Z.J.; Kapczynski, D. Effects of Newcastle disease virus vaccine antibodies on the shedding and transmission of challenge viruses. *Dev. Comp. Immunol.* **2013**, *41*, 505–507. [CrossRef] [PubMed]
16. Bernard, G.; Worku, M.; Ahmedna, M. The Effects of Diatomaceous Earth on Parasite Infected Goats. *Bull. Georgian Natl. Acad. Sci.* **2009**, *3*, 129–135.
17. Al-Ghouti, M.; Khraisheh, M.A.M.; Ahmad, M.N.M.; Allen, S. Thermodynamic behaviour and the effect of temperature on the removal of dyes from aqueous solution using modified diatomite: A kinetic study. *J. Colloid Interface Sci.* **2005**, *287*, 6–13. [CrossRef] [PubMed]
18. Akin, S.; Schembre, J.M.; Bhat, S.K.; Kovscek, A.R. Spontaneous imbibition characteristics of diatomite. *J. Pet. Sci. Eng.* **2000**, *25*, 149–165. [CrossRef]
19. Yuan, P.; Liu, D.; Fan, M.; Yang, D.; Zhu, R.; Ge, F.; Zhu, J.X.; He, H. Removal of hexavalent chromium [Cr(VI)] from aqueous solutions by the diatomite-supported/unsupported magnetite nanoparticles. *J. Hazard. Mater.* **2010**, *173*, 614–621. [CrossRef] [PubMed]
20. Gao, B.; Jiang, P.; An, F.; Zhao, S.; Ge, Z. Studies on the surface modification of diatomite with polyethyleneimine and trapping effect of the modified diatomite for phenol. *Appl. Surf. Sci.* **2005**, *250*, 273–279. [CrossRef]
21. Pookmanee, P.; Jansanthea, P.; Phanichphant, S. Adsorption of heavy metals onto natural and modified diatomite KMITL. *Sci. J.* **2008**, *8*, 1–8.
22. Angela, F.D.; El-Gamouz, A.; Sofia, F.; Vanina, M.; Liliana, V.; Oliver, A.W. Turning the volume down on heavy metals using tuned diatomite. A review of diatomite and modified diatomite for the extraction of heavy metals from water. *J. Hazard. Mater.* **2012**, *14*, 241–242.
23. Kilpinen, O.; Steenberg, T. Inert dusts and their effects on the poultry red mite. *Exp. Appl. Acarol.* **2009**, *48*, 51–62. [CrossRef]
24. Pirsaraei, A.; Reza, S.; Hasan, A.M.; Ahmad, J.J.; Zohreh, F.; Jafar, T. The Effect of Acid and Thermal Treatment on a Natural Diatomite. *Chem. J.* **2015**, *1*, 144–150.
25. Adebiyi, O.A.; Sokunbi, O.A.; Ewuola, E.O. Performance Evaluation and Bone Characteristics of Growing Cockerel Fed Diets Containing Different Levels of Diatomaceous Earth. *Middle-East J. Sci. Res.* **2009**, *4*, 36–39.
26. Safa, M.; Larouci, M.; Meddah, B.; Valemens, P. The sorption of lead, cadmium, copper and zinc ions from aqueous solutions on raw diatomite from Algeria. *Water Sci. Technol.* **2012**, *65*, 1729. [CrossRef] [PubMed]

27. Sheng, G.; Yang, S.; Sheng, J.; Hu, J.; Tan, X.; Wang, X. Macroscopic and microscopic investigation of Ni (II) sequestration on diatomite by batch, XPS and EXAFS techniques. *Environ. Sci. Technol.* **2011**, *45*, 7718. [CrossRef] [PubMed]
28. Koyuncu, M. Color removal from aqueous solution of tar-chromium green 3G dye using natural diatomite. *Physicochem. Probl. Miner. Process.* **2012**, *48*, 485.
29. Caliskan, N.; Kul, A.R.; Alkan, S.; Sogut, E.G.; Alacabey, I. Adsorption of Zinc (II) on diatomite and manganese-oxide-modified diatomite: A kinetic and equilibrium study. *J. Hazard. Mater.* **2011**, *193*, 27–36. [CrossRef] [PubMed]
30. Barakat, M.A. New trends in removing heavy metals from industrial wastewater. *Arab. J. Chem.* **2011**, *4*, 361–377. [CrossRef]
31. Goren, R.; Baykara, T.; Marsoglu, M. A study on the purification of diatomite in HCl. *Scand. J. Metall.* **2002**, *31*, 115–119. [CrossRef]
32. Bello, O.S.; Adegoke, K.A.; Oyewole, R.O. Insights into the Adsorption of Heavy Metals from Wastewater using Diatomaceous Earth. *Sep. Sci. Technol.* **2014**, *49*, 1787–1806. [CrossRef]
33. Pookmanee, P.; Thippraphan, P.; Phanichphant, S. Adsorption of copper ion (II) by manganese chloride modified diatomite via hydrother-mal method. In Proceedings of the 36th Congress on Science and Technology, Bangkok, Thailand, October 2010; pp. 26–28.
34. Crangle, R.J. Diatomite: United State Geological Survey 2007 Mineral Yearbook. Available online:https://minerals.usgs.gov/minerals/pubs/commodity/diatomite/index.html(accessed on 15 December 2018).
35. USA, Department of Environment. *Mineral Commodity Summaries 2016*; U.S. Geological Survey: Reston, VA, USA, 2016.
36. Stathers, T.E.; Riwa, W.; Mvumi, B.M.; Mosha, R.; Kitandu, L.; Mngara, K.; Kaoneka, B.; Morris, M. Do diatomaceous earths have potential as grain protectants for small-holder farmers in sub-Saharan Africa? The case of Tanzania. *Crop Prot.* **2008**, *27*, 44–70. [CrossRef]
37. Mvumi, B.; Stathers, T.; Riwa, W.; Morris, M.; Kitandu, L. *Diatomaceous Earth Dusts for Grain Protection by Small-Scale Farmers in Tanzania and Zimbabwe*; Department of Entomolgy, Kansas State University: Manhattan, KS, USA, 2004.
38. Badii, B.K.; Adarkwah, C.; Obeng-ofori, D.; Ulrichs, C. Efficacy of diatomaceous earth formulations against Callosobruchus maculatus (F.) (Coleoptera: Bruchidae) in Kersting's groundnut (Macrotyloma geocarpumHarms): Influence of dosage rate and relative humidity. *J. Pest Sci.* **2014**, *87*, 285–294. [CrossRef]
39. Van, P. An Award-wining Merino sheep enterprise with the use of diatomaceous earth. *Farmer's Weekly*, 30 July 2015.
40. Osweiler, G.D.; Carson, T.L. *Evaluation of Diatomaceous Earth as an Adjunct to Sheep Parasite Control in Organic Farming*; Leopold Center Completed Grant Reports; Iowa State University: Ames, IA, USA, 1997.
41. Nwosu, C.O.; Madu, P.P.; Richards, W.S. Prevalence and seasonal changes in the population of gastrointestinal nematodes of small ruminants in the semi-arid zone of north-eastern Nigeria. *Vet. Parasitol.* **2007**, *144*, 118–124. [CrossRef] [PubMed]
42. Shahnawaz, M.; Shahardar, R.A.; Wani, Z.A. Seasonal prevalence of platyhelminthosis of sheep in gandarbal area of Kashmir Vally. *Vet. Parasitol.* **2011**, *25*, 59–62.
43. Bhat, S.A.; Manzoor, R.M.; Qadri, S.; Allaie, I.; Khan, H.M. comparative resistance of Sheep Breeds to Strongyle spp. In pasture infection in Jammu and Kashmir. *Int. J. Vet. Sci. Technol.* **2011**, *10*, 2157–7579.
44. Ijaz, M.; Khan, M.S.; Avais, K.; Ashraf, M.; Ali, M. Infection rate and chemotherapy of various helminths in diarrhoeic sheep in and around Lahore. *J. Anim. Sci.* **2009**, *19*, 13–16.
45. Odoi, A.; Gathuma, J.M.; Gachuiri, C.K.; Omore, A. Risk factors of gastrointestinal nematode parasite infections in small ruminants kept in smallholder mixed farms in Kenya. *BMC Vet. Res.* **2007**, *3*, 1–11. [CrossRef]
46. Besier, R.B.; Kahn, L.P.; Sargison, N.D.; Van Wyk, J. The pathophysiology, ecology and epidemiology of Haemonchus contortus infection in small ruminants. *Adv. Parasitol.* **2016**, *93*, 95–143.
47. Jackson, F.; Bartley, D.; Bartley, Y.; Kenyon, F. Worm control in sheep in the future. *Small Rumin. Res.* **2009**, *86*, 40–45. [CrossRef]
48. Woodgate, R.G.; Besier, R.B. Sustainable use of anthelmintics in an Integrated Parasite Management Program for sheep nematodes. *Anim. Prod. Sci.* **2010**, *50*, 440–443. [CrossRef]

49. Deutschlander, D. *Evaluating Diatomaceous Earth as a Wormer for Sheep and Cattle*; Leopold Center Completed Grant Reports; Energy and Sustainable Agriculture Program—Minnesota Department of Agriculture: Saint Paul, MN, USA, 1993.
50. McLean, B.; Frost, D.; Evans, E.; Clarke, A.; Griffiths, B. The inclusion of diatomaceous earth in the diets of grazing ruminants and its effect on gastrointestinal parasite burdens. In Proceedings of the International Scientific Conference on Organic Agriculture, Adelaide, Australia; International Scientific Conference of Organic Agriculture Research, Bonn, Germany, 2005.
51. Ahmed, M.A.; Laing, M.D.; Nsahlai, I.V. Studies on the ability of two isolates of Bacillus thuringiensis, an isolate of Clonostachys rosea f. rosea and a diatomaceous earth product to control gastrointestinal nematodes of sheep. *Biocontrol. Sci. Technol.* **2013**, *23*, 1067–1082. [CrossRef]
52. Baltran, M.A.; Martins, R. Original article diatomaceous earth inhibited the. *Philipp. J. Vet. Anim. Sci.* **2015**, *41*, 135–140.
53. Fernandez, M.I.; Woodward, B.W.; Stromberg, B.E. Effect of diatomaceous earth as an anthelmintic treatment on internal parasites and feedlot performance of beef steers. *Anim. Sci.* **1998**, *66*, 635–641. [CrossRef]
54. Dawson, R.D. Efficacy of Diatomaceous Earth at Reducing Populations of Nest-Dwelling Ectoparasites in Tree Swallows/Eficacia de diatomáceas terrestres en reducir las poblaciones de ectoparásitos en nidos en Tachycineta bicolor. *J. Field Ornithol.* **2010**, *75*, 232–238. [CrossRef]
55. Martin, C.D.; Mullens, B.A. Housing and dustbathing effects on northern fowl mites (Ornithonyssus sylviarum) and chicken body lice (Menacanthus stramineus) on hens. *Med. Vet. Entomol.* **2012**, *26*, 323–333. [CrossRef] [PubMed]
56. Amy, C.M. Studies of Northern Fowl Mite Host-Parasite Interactions and Evaluation of Novel Control Strategies in Poultry. Thesis Submitted to Department of Entomology, University of California, CA, USA. 2016. Available online:https:escholarship.org/uc/item/1nh087m0(accessed on 15 December 2018).
57. Streit, E.; Schatzmayr, G.; Tassis, P.; Tzika, E.; Marin, M.; Taranu, I.; Tabuc, C.; Nicolau, A.; Aprodu, I.; Puel, O. Current situation of mycotoxin contamination and co-occurrence in animal feed—Focus on Europe. *Toxins* **2012**, *4*, 788–809. [CrossRef] [PubMed]
58. Agag, B.I. Mycotoxins in foods and feeds: 1-Aflatoxin. *Ass. Uni. Bull. Environ. Res.* **2004**, *7*, 173–206.
59. Jones, F.T.; Genter, M.B.; Hagler, W.M.; Hansen, J.A.; Mowrey, B.A.; Poore, M.H.; Whitlow, L.W. Understanding and Coping with Effects of Mycotoxins in Livestock Feed and Forage; NC, USA. In *North Carolina Cooperative Extension Service: Raleigh*; International Information System for the Agricultural Science and Technology: NC, USA, 2007.
60. Richard, J.L. Some major mycotoxins and their mycotoxicoses—An overview. *Int. J. Food Microbiol.* **2007**, *119*, 3–10. [CrossRef] [PubMed]
61. Meissonnier, G.M.; Pinton, P.; Laffitte, J.; Cossalter, A.; Gong, Y.Y.; Wild, C.P.; Bertin, G.; Altier, P.; Oswald, I. Immunotoxicity of aflatoxin B1: Impa1irment of the cell-mediated response to vaccine antigen and modulation of cytokine expression. *Toxicol. Appl. Pharmacol.* **2008**, *231*, 142–149. [CrossRef] [PubMed]
62. Chaytor, A.C.; See, M.T.; Hansen, J.A.; de Souza, A.L.P.; Middleton, T.F.; Kim, S.W. Effects of chronic exposure of diets with reduced concentrations of aflatoxin and deoxynivalenol on growth and immune status of pigs. *J. Anim. Sci.* **2011**, *89*, 124–135. [CrossRef]
63. Dafalla, R.; Yagi, A.; Adam, S.E. Experimental aflatoxicosis in hybro-type chicks; sequential changes in growth and serum constituents and histopathological changes. *Vet. Hum. Toxicol.* **1987**, *29*, 222–226. [PubMed]
64. Ledoux, D.R.; Rottinghaus, G.E.; Bermudez, A.J.; Alonso-Debolt, M. Efficacy of hydrated sodium calcium aluminosilicate to ameliorate the toxic effects of aflatoxin in broiler chicks. *Poult. Sci.* **1999**, *78*, 204–210. [CrossRef] [PubMed]
65. Bintvihok, A.; Banlunara, W.; Kaewamatawong, T. Aflatoxin detoxification by esterified glucomannan in ducklings. *Thai J. Heal. Res.* **2002**, *16*, 135–148.
66. Carraro, M.; Gregorio, D.; De Neeff, D.V.; Jager, A.V.; Corassin, C.H.; Cara, D.P.; Augusto, C.; Oliveira, F. Mineral adsorbents for prevention of mycotoxins in animal feeds Mineral adsorbents for prevention of mycotoxins in animal feeds. *Toxin Rev.* **2014**, *33*, 125–135. [CrossRef]
67. Parlat, S.S.; Yildiz, A.O.; Oguz, H. Effect of clinoptilolite on performance of Japanese quail (Coturnix coturnix japonica) during experimental aflatoxicosis. *Br. Poult. Sci.* **1999**, *40*, 495–500. [CrossRef] [PubMed]

68. Shivashaankar, B.P.; Narayanashwamy, H.D.; Satanarayana, M.L.; Rao, S.; Rathnamma, D.; Maniyellappa, H.K.; Sridhar, N. Effect of Diatomaceous Earth on Performance, Internal Organs and Biochemical Alterations in T-2 Toxicosis of Broiler Chickens. *J. Cell Tissue Res.* **2015**, *15*, 4983–4988.
69. Lakkawar, A.W.; Sathyanarayana, M.L.; Narayanaswamy, H.D.; Sugunarao, O.; Yathiraj, S. Efficacy of diatomacious earth in amelioration of aflatoxin induced toxicity in broiler chicken. *Indian J. Anim. Res.* **2016**, *50*, 529–536. [CrossRef]
70. Bocarov-Stancic, A.; Adamovic, M.; Salma, N.; Bodroza-Solarov, M.; Vuckovic, J.; Pantic, V. In vitro efficacy of mycotoxins adsorption by natural mineral adsorbents. *Biotechnol. Anim. Husb.* **2011**, *27*, 1241–1251. [CrossRef]
71. Manafi, M.; Narayanaswamy, H.D.; Pirany, N. In vitro binding ability of mycotoxic binder in commercial broiler feed. *Afr. J. Agric. Res.* **2009**, *4*, 141–143.
72. Vila-Donat, P.; Marín, S.; Sanchis, V.; Ramos, A.J. A review of the mycotoxin adsorbing agents, with an emphasis on their multi-binding capacity, for animal feed decontamination. *Food Chem. Toxicol.* **2018**, *114*, 246–259. [CrossRef]
73. Li, Y.X.; Zhang, X.L.; Li, W.; Lu, X.F.; Liu, B.; Wang, J. The residues and environmental risks of multiple veterinary antibiotics in animal faeces. *Environ. Monit. Assess.* **2013**, *185*, 2211–2220. [CrossRef] [PubMed]
74. Abd El-Tawab, M.M.; Youssef, I.M.I.; Bakr, H.A.; Fthenakis, G.C.; Giadinis, N.D. Role of probiotics in nutrition and health of small ruminants. *Pol. J. Vet. Sci.* **2016**, *19*, 893–906. [CrossRef] [PubMed]
75. Sarijit, J.; Steve, S.; Micheal, E.; Rodney, D. Animal Feed Compositions Including Spent Filter Media Containing Diatomaceous Earth. US Appl. US20160295884A1, 13 October 2016.
76. Schingoethe, D.J.; Kalscheur, K.F.; Hippen, A.R.; Garcia, A.D. Invited review: The use of distillers products in dairy cattle diets. *J. Dairy Sci.* **2009**, *92*, 5802–5813. [CrossRef] [PubMed]
77. Ewuola, E.O.; Sokunbi, O.A.; Adebiyi, A.O.; Lawal, T.T.; Akangbe, O. Physiological response of growing cockerels to dietary fossil shell flour. In Proceedings of the 14th Annual Conference of Animal Science Association of Nigeria, University of Ibadan, Ibadan, Nigeria, 14–17 September 2009; pp. 246–249.
78. Modirsanei, M.; Mansoori, B.; Khosravi, A.R.; Kiaei, M.M.; Khazraeinia, P.; Farkhoy, M.; Masoumi, Z. Effect of diatomaceous earth on the performance and blood variables of broiler chicks during experimental aflatoxicosis. *Sci. Food Agric.* **2008**, *632*, 626–632. [CrossRef]
79. Martel-Kennes, Y.; Lévesque, J.; Decaux, C. Effect of crystalline silicon dioxide in piglet feed on growth performance with different levels of growth promoters. *J. Anim. Sci.* **2016**, *94*, 488. [CrossRef]
80. National Research Council (NRC). *Nutrient Requeriments of Small Ruminants: Sheep, Goats, Cervids, and New World Camelids*; NRC: Washington, DC, USA, 2007.
81. Garcia, G.; De Araújo, L.; Voltolini, T.V.; Chizzotti, M.L.; Turco, H.N.; Fernando, F.; Carvalho, R. De Revista Brasileira de Zootecnia Water and small ruminant production. *R. Bras. Zootec.* **2010**, *39*, 326–336.
82. Schlink, A.C.; Nguyen, M.; Viljoen, G.J. Water requirements for livestock production: Water requirements for livestock. *Rev. Sci. Tech. Off. Int. Epiz.* **2010**, *29*, 603–619. [CrossRef]
83. Pick, T. *Assessing Water Quality for Human Consumption, Agriculture, and Aquatic Life Uses*; Environment Technical Note. No MT-1(Rev.2); United states Department of Agriculture Natural Resources Conservation Service: Washington, DC, USA, 2011; Volume 1, p. 31.
84. Al-Degs, Y.S.; Tutunju, M.F.; Shawabkeh, R.A. The feasibility of using diatomite and Mn-diatomite for remediation of Pb^{2+}, Cu^{2+}, and Cd^{2+} from water. *Sep. Sci. Technol.* **2000**, *35*, 2299–2310. [CrossRef]
85. Moleshi, P.; Nahid, P. Heavy metal removal from water and wastewater using raw and modified diatomite. *Int. J. Eng. Trans. B Appl.* **2015**, *20*, 141–146.
86. Walker, G.M.; Weatherley, L.R. Kinetic of acid dye adsorption on GAC. *Water Res.* **1999**, *33*, 1895. [CrossRef]
87. Oana, C.; Tănaselia, C.; Miclean, M.; Levei, E.; Senilă, M.; Senilă, L. Analysis of minor and trace element in Cow, Goat and Sheep milk in NW part of Romania. *Pro-Environment* **2016**, *9*, 87–90.
88. Alisson-Silva, F.; Kawanishi, K.; Varki, A. Molecular Aspects of Medicine Human risk of diseases associated with red meat intake: Analysis of current theories and proposed role for metabolic incorporation of a non-human sialic acid. *Mol. Asp. Med.* **2016**, *51*, 16–30. [CrossRef] [PubMed]
89. Soetan, K.O.; Olaiya, C.O.; Oyewole, O.E. The importance of mineral elements for humans, domestic animals and plants: A review. *Afr. J. Food Sci.* **2010**, *4*, 200–222. [CrossRef]
90. Galyean, M.L.; Perino, L.J.; Duff, G.C. Interaction of cattle health/immunity and nutrition. *J. Anim. Sci.* **1999**, *77*, 1120–1134. [CrossRef] [PubMed]

91. Ferguson, E.G.W.; Mitchell, G.B.B.; MacPherson, A. Cobalt deficiency and Osterfagia circumcincra infection in lambs. *Vet. Rec.* **1989**, *124*, 20. [CrossRef] [PubMed]
92. McClure, S.J. Mineral nutrition and its effects on gastrointestinal immune function of sheep. *Aust. J. Exp. Agric.* **2003**, *43*, 1455–1461. [CrossRef]
93. Qudoos, A.; Khan, M.N.; Sajid, M.S.; Muhammad, G. Correlation of trace mineral profiles with gastrointestinal worm burden in rangeland sheep of Chakwal District, Punjab, Pakistan. *Int. J. Agric. Biol.* **2017**, *19*, 140–144. [CrossRef]
94. Adeyemo, G.O. Growth performance of broiler chicken fed Fossil shell flour growth promoter. *Food Nutr. Sci.* **2013**, *4*, 26622.
95. Carlisle, E.M. In vivo requirement for silicon in articular cartilage and connective tissue formation in the chick. *J. Nutr.* **1976**, *106*, 478–484. [CrossRef]
96. Seaborn, C.D.; Nielsen, F.H. Boron and silicon: Effects on growth, plasma lipids, urinary cyclic amp and bone and brain mineral composition of male rats. *Environ. Toxicol. Chem.* **1994**, *13*, 941–947. [CrossRef]
97. Mertz, W. Trace Elements in Human and Animal Nutrition. In *Trace Elements in Human and Animal Nutrition*; Academic Press Inc.; Hacourt Brace Jovanovich Publishers: Orlando, FL, USA, 2012; Volume 2, pp. 133–154.
98. Martin, K.R. Silicon: The health benefits of a metalloid. *Met. Ions Life Sci.* **2013**, *13*, 451–471. [PubMed]
99. Martin, K.R. Chemistry of silica and its potential health benefits. *J. Nutr. Health Aging* **2007**, *11*, 94–97.
100. Singh, C.B.; Jayas, D.S.; Paliwal, J.; White, N.D.G. Detection of insect-damaged wheat kernels using near-infrared hyperspectral imaging. *J. Stored Prod. Res.* **2009**, *45*, 151–158. [CrossRef]
101. Arthur, F.H. Grain protectants: Current status and prospects for the future. *J. Stored. Prod. Res.* **1999**, *32*, 293–302. [CrossRef]
102. Ebeling, W. Sorptive dust for pest control. *Ann. Rev. Entomol.* **1971**, *16*, 123–158. [CrossRef] [PubMed]
103. Sabbour, M.M.; Abd-el-aziz, S.E.; Sherief, M.A. Efficacy of Three Entomopathogenic Fungi Alone or in Combination With Diatomaceous Earth Modifications For the Control of Three Pyralid Moths in Stored Grains. *J. Plant Prot. Res.* **2012**, *52*, 360–362. [CrossRef]
104. Sabbour, M.M.; Abd-El-Aziz, S.E. Control of Bruchidius incarnatus and Rhyzopertha Dominica using two entomopathogenic fungi alone or in combination with modified diatomaceous earth. *Elixir Entomol.* **2014**, *68*, 22239–22242.
105. Shah, M.A.; Khan, A.A. Use of diatomaceous earth for the management of stored-product pests. *Int. J. Pest Manag.* **2014**, *60*, 100–113. [CrossRef]
106. Cilek, S.; Breeding, A.; Medicine, V. Determination of Fleece Qualities of Malya Sheep (11/16 Akkaraman X 5/16 Deutsches Merinofleischschaf) and Effect of Age and Sex on These Qualities. *Pak. J. Agric. Sci.* **2015**, *52*, 545–552.
107. Esfandyari, H.; Aslaminejad, A.A.; Rafat, S.A. Wool characteristics in the third generation of Arkharmerino × Ghezel and Arkharmerino × Moghani crossbreed sheep. *Trop. Anim. Health Prod.* **2011**, *43*, 1337–1343. [CrossRef] [PubMed]
108. Allden, W.G.; Black, J.L.; Reis, P. *Feed Intake, Diet Compositions and Wool Growth: Physiological and Environmental Limitations to Wool Growth*; The University of New England Publishing Unit: Armidale, Australia, 1979.
109. Hatcher, S.; Atkins, K.D.; Thornberry, K.J. Age changes in wool traits of Merino sheep in western NSW. *Proc. Assoc. Adv. Anim. Breed. Genet* **2005**, *16*, 316–317.
110. McGregor, B.A.; de Graaf, S.P.; Hatcher, S. On-farm factors affecting physical quality of Merino wool. 1. Nutrition, reproduction, health and management. *Small Rumin. Res.* **2016**, *137*, 138–150. [CrossRef]
111. Huisman, A.E.; Brown, D.J. Genetic parameters for bodyweight wool, and disease resistance and reproduction traits in Merino sheep 2. Genetic relationships between bodyweight traits and other traits. *Aust. J. Exp. Agric.* **2008**, *48*, 1186–1193. [CrossRef]
112. Sumner, R.M.W.; Young, S.R.; Upsdell, M.P. Wool yellowing and pH within Merino and Romney fleeces. *Proc. N. Z. Soc. Anim.* **2003**, *63*, 155–159.
113. Sumner, R.M.W.; Ashby, M.G.; Craven, A.J. Relation between sweating and wool yellowing in Merino and Romney sheep. *Proc. N. Z. Soc. Anim. Prod.* **2004**, *64*, 286–292.
114. Sumner, R.M.W. Relationship of mean fibre diameter and time of shearing with wool yellowing in Merino sheep farmed under North Island conditions. *Proc. N. Z. Soc. Anim.* **2005**, *65*, 191–196.

115. Van Burgel, A.J.; Oldham, C.M.; Behrendt, R.; Curnow, M.; Gordon, D.J.; Thompson, A.N. The merit of condition score and fat score as alternatives to liveweight for managing the nutrition of ewes. *Anim. Prod. Sci.* **2011**, *51*, 834–841. [CrossRef]
116. James, P.J.; Horton, B.J.; Campbell, N.J.; Evans, D.L.; Winkleman, J.; McPhie, R. Population dynamics and production effects of sheep lice (Bovicola ovis Schrank) in extensively grazed flocks. *Anim. Prod. Sci.* **2011**, *51*, 753–762. [CrossRef]
117. Zhang, Q.; Shu, X.; Guo, X.; Mo, D.; Wei, S.; Yang, C. Effect of ions on sorption of tylosin on clay minerals. *RSC Adv.* **2016**, *6*, 53175–53181. [CrossRef]
118. Chen, Y.; Liu, K. Preparation of granulated N-doped TiO2/diatomites and its application of visible light degradation and disinfection. *Powder Technol.* **2016**, *303*, 176–191. [CrossRef]
119. Schijns, V.E.J.C.; Lavelle, E.C. Trends in vaccine adjuvants. *Expert Rev. Vaccines* **2011**, *10*, 539–550. [CrossRef] [PubMed]
120. Fox, C.B.; Haensler, J. An update on safety and immunogenicity of vaccines containing emulsion-based adjuvants. *Expert Rev. Vaccines* **2013**, *12*, 747–758. [CrossRef] [PubMed]
121. Mount, A.; Koernig, S.; Silva, A.; Drane, D.; Maraskovsky, E.; Morelli, A.B. Combination of adjuvants: The future of vaccine design. *Expert Rev. Vaccines* **2013**, *12*, 733–746. [CrossRef] [PubMed]
122. Waksman, B.H. Adjuvants and immune regulation by lymphoid cells. In *Springer Seminar Immunopathology*; Springer: Berlin, Germany, 1979; Volume 2, pp. 5–33.
123. Hunter, R.L. Overview of vaccine adjuvants: Present and future. *Vaccine* **2002**, *20*, 7–12. [CrossRef]
124. Singh, M.; O'Hagan, D.T. Recent advances in veterinary vaccine adjuvants. *Int. J. Parasitol.* **2003**, *33*, 469–478. [CrossRef]
125. Schat, K.A. Vaccines and vaccination practices: Key to sustainable animal production. In *Encyclopedia of Agriculture and Food Systems*; Elsevier Inc.: San Diego, CA, USA, 2014; Volume 5.
126. Meeusen, E.N.T.; Walker, J.; Peters, A.; Pastoret, P.P.; Jungersen, G. Current status of veterinary vaccines. *Clin. Microbiol. Rev.* **2007**, *20*, 489–510. [CrossRef]
127. Nazmi, A.; Hauck, R.; Davis, A.; Hildebrand, M.; Corbeil, L.B.; Gallardo, R.A. Immunology, health, and disease: Diatoms and diatomaceous earth as novel poultry vaccine adjuvants. *Poult. Sci.* **2017**, *96*, 288–294. [CrossRef]
128. Wildwood Enterprises. *Customer service Dealer Information Bullettin Board -Link*; Crystal City Manitoba Adrax Advertising: Crystal City, MB, Canada, 2003; p. 2.
129. Nazmi, A.; Hauck, R.; Corbeil, L.B.; Gallardo, R.A. The effect of diatomaceous earth in live, attenuated infectious bronchitis vaccine, immune responses, and protection against challenge. *Poult. Sci.* **2017**, *96*, 1–7. [CrossRef]
130. Sommer, R.S.; Nadachowski, A. Glacial refugia of mammals in Europe: Evidence from fossil records. *Mamm. Rev.* **2006**, *36*, 251–265. [CrossRef]
131. Cyrs, W.D.; Le, M.H.; Hollins, D.M.; Henshaw, D.M. Settling the Dust. *Heal. Hazards Peer-Rev.* **2014**, *38*, 38–44.
132. Möhner, M.; Pohrt, A.; Gellissen, J. Occupational exposure to respirable crystalline silica and chronic non—Malignant renal disease: Systematic review and meta—Analysis. *Int. Arch. Occup. Environ. Health* **2017**, *90*, 555–574. [CrossRef] [PubMed]
133. Pery, A.; Hoet, P.; Nemmar, A. Development of a physiologically based kinetic model for 99m-Technetium-labelled carbon nanoparticles inhaled by humans. *Inhal. Toxicol.* **2009**, *21*, 1099–1107. [CrossRef] [PubMed]
134. Nemmar, A.; Yuvaraju, P.; Beegam, S.; Yasin, J.; Dhaheri, R.A.; Fahim, M.A.; Ali, B.H. In vitro platelet aggregation and oxidative stress caused by amorphous silica nanoparticles. *Int. J. Physiol. Pathophysiol. Pharmacol.* **2015**, *7*, 27–33. [PubMed]

Animals

Communication

Effect of Varying Inclusion Levels of Fossil Shell Flour on Growth Performance, Water Intake, Digestibility and N Retention in Dohne-Merino Wethers

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Simple Summary: With the recent negative public opinion on chemical-based feed additive in many nations of the world (for the health implication and environmentally hazard it posed), naturally occurring feed additive are urgently needed to replace and support the sustainable development in livestock production. The potential of Fossil shell flour as performance enhancer was investigated in this study. The major finding was that fossil shell flour increased growth performance, apparent nutrient digestibility, N retention and make minimum use of water in Dohne-Merino wethers. Hence it could be an alternative to chemical-based feed additive in livestock production.

Abstract: This study was carried out to determine the effect of varying levels of Fossil shell flour (FSF) supplementation on growth performance, water intake, digestibility and N retention in Dohne Merino sheep pursuant to establishing the optimum inclusion rate of this supplement in Dohne Merino diets. Sixteen Dohne-Merino wethers (18 ± 1.5 kg body weight) were used in a complete randomized design with four animals per treatment. Sheep were fed a basal diet without FSF addition (control, T1), or with the addition of FSF (2%, T2), (4%, T3) or (6%, T4) of the diet for 105 days. Treatment 3 (4% FSF) has the highest values of dry matter intake, total weight gain, N retention and for most of the apparent digestibility nutrients (CP, EE and Ash) compared to treatment T1, T2 and T4 ($p < 0.05$). The urinary and fecal N excretion also significantly decreased in the FSF treated diets compared to the control ($p < 0.05$). Water intake values were highest in control and were significantly ($p < 0.05$) different from those in treatments 2 and 4, but not to treatment 3. It is concluded that 4% inclusion rate of FSF will give the best improvement on growth performance, diet digestibility and N retention of Dohne-Merino sheep. Also, the addition of FSF in the diets of sheep is a safe natural additive that can help to reduce environmental pollution by reducing fecal and urinary N excretion.

Keywords: Fossil shell flour; growth performance; digestibility; nitrogen utilization; Dohne-Merino

1. Introduction

The use of growth promoters in farm animal diets is growing at an increasing rate [1]. Growth promoters are used to improve feed efficiency and the growth performance of farm animals. Rumen regulation through the use of growth promoters is one of the most important methods for improving feed efficiency and, thus, growth performance. Likewise, feed additives in ruminant's nutrition has the potential to increase dry matter intake (DMI), feed conversion efficiency (FCE) and animal productivity [1,2]. There is a wide range of feed additives that include antibiotics, probiotics, antioxidants, enzymes, prebiotics, organic acids, mycotoxin binders, hormones, beta agonist, defaunation agents, essential oil and herbal feed additives, most of which are chemical based [2].

Although the use of growth promoters as feed additives has been a hallmark of modern animal husbandry, in recent years there have been increased concerns on chemical residues in meat and other animal products as a result of these chemical based feed additives [3,4]. There is also increase in ecological risk because of the accumulation of veterinary antibiotics residue in animal manure [5], bodies of water, sediments and soils [6,7]. Arikan [8] reported that antibiotics administered to farm animals either as growth promoter or medication were usually excreted without metabolism. Similarly, [9] observed that between 70 to 90% of tetracycline may be excreted as parent compounds through urine or feaces.

Because of the possible risks of chemical-based growth promoters, there have been increased interest in natural growth promoters (NGPs). Several plants and plant extracts, enzymes, organic acids and oils have received considerable attention recently as possible NGPs that are eco-friendly [10]. One of the major drawbacks to the use of these NGPs is the time and cost involved in harvesting them. One NGP that could be useful as a cost effective, readily available, health and eco-friendly feed additive is Fossil shell flour. Fossil shell flour is a naturally occurring silicate rich substance with important physical and chemical characteristics that enable its uses recently as feed additive in livestock production. The substance is nontoxic, cheap, and readily available in large quantity in many countries [11]. The mineral constituent of dietary fossil shell flour as reported by [12–14] are as follows: Sodium, 923 mg/kg; Copper, 30 mg/kg; Zinc, 118 mg/kg; Iron, 7944 mg/kg; Magnesium, 69 mg/kg; Calcium, 0.22%; Magnesium, 0.11%; Potassium, 0.08%; Aluminum, 0.065%; Sulfate sulphate, 0.062%, Boron, 23 mg/kg, and Vanadium, 438 mg/kg.

In the study conducted by Emeruwa et al. [15] using West Africa dwarf sheep, it was observed that inclusion of Fossil shell flour in the diet statistically affected the average daily weight gain with the highest value (0.20 kg/day) observed for the sheep fed the 4% inclusion level of Fossil shell flour and the lowest value of (0.11 kg/day) for those on 6% inclusion of fossil shell flour. Likewise, Sarijit et al., [16] reported that addition of diatomaceous earth (another name for FSF) to animal feeds (3.2% inclusion) improve daily feed intake, weight gain and feed efficiency.

On the effects of FSF on digestibility, [15] Emeruwa, (2016) observed that crude protein (%) digestibility was significantly higher (82.79) for sheep on 4% inclusion level of fossil shell flour than other levels. This author also reported that although N intake and the fecal and urinary N excretion were not significantly different among the treatment groups, N retention (% N intake) was significantly higher (72.4%) for sheep on 2% inclusion level of Fossil shell flour than for the other groups.

There is paucity of information on feedlot performance of Dohne-Merino supplemented with varying levels of FSF, and no reports are available on the effect of FSF on the water intake of Dohne-Merino. Considering that the use of this feed additive is cost effective, health and eco-friendly, the objective of this study was to assess the effects of 4 levels of FSF inclusion on growth performance, water intake, digestibility and N retention of Dohne-Merino sheep. We hypothesized that the inclusion of FSF at varying levels could increase the growth performance, digestibility and N retention of Dohne-Merino sheep.

2. Materials and Methods

2.1. Ethical Approval

The handling and the use of the animals was approved by University of Fort Hare, Animal ethics and Use Committee [Approval number (MPE041IKU01)].

2.2. Study Site Description

The experiment was conducted at the small ruminant unit of the University of Fort Hare teaching and research farm (animal section), Alice, Eastern Cape, South Africa. The research farm is located at about Km 5 along Alice-Kings Williams town, which lies at longitude 26°50′ E and latitude of 32°46′ S. The annual rainfall is between 480–490 mm and temperature range between 24.6 °C and 11.1 °C (average is 17.8 °C) at the altitude of 535 m above sea level.

2.3. Animal, Experimental Design and Management

Sixteen Dohne-Merino wethers (6 months old) weighing 20 ± 1.5 kg on average were selected from a commercial farm in Mitford village Tarkastad, Eastern Cape of South Africa, and were used for this study in a completely randomized design. All the 16 wethers were raised at the same facility in the same area under the same environmental conditions (University of Fort Hare, Teaching and Research Farm, Animal Section, Alice 5700, RSA). The wethers were randomly allotted into four treatment (n = 4). They were individually housed (1.5 m × 1.5 m) in a well-ventilated roofed animal building with concrete floor. The pens have similar temperature, relative humidity and sunlight conditions. The experiment lasted for 105 days, excluding 14 days of adaptation period. The animals have access to sufficient clean and fresh water over the trial.

2.4. Experimental Diets

The diets for the animals consisted of concentrate and hay at 40:60 ratio. The concentrate was made up of maize (8%), sunflower oil cake (10%), molasses (5%), wheat offal (15%), limestone (1.5%), salt 0.3% and sheep mineral-vitamin premix (0.2%), whereas the hay consisted of 30% teff and 30% Lucerne. All ingredients were thoroughly milled and mixed evenly together to form the basal diet. The feed was formulated to meet the nutritional (energy and protein) requirements of the used sheep [17]. The four dietary groups were: T1: Basal diet (Control); T2: Basal diet +2% FSF; T3: Basal diet +4% FSF, and T4: Basal diet +6% FSF. The animals were fed at 8:00 h and 15:00 h at 4% of the body weight (on dry matter (DM) basis). The Fossil shell flour (Food - Grade) was purchased from Eco-Earth (Pty) Ltd., Port Elizabeth, SA which produces this product under a license by Department of Agriculture, Forestry and Fisheries of South Africa.

2.5. Analytical Procedures

Dry matter content of the diets, orts and fecal samples was measured by drying samples in an air-forced oven at 135 °C for 24 h (method 930.15; [18]) Ash content was measured by placing samples into a muffle furnace at 550 °C for 5 h (method 938.08; [18]. Organic matter (OM) was measured as the difference between DM and the ash content. Nitrogen (N) was measured by the Kjeldahl method using Se as a catalyst and crude protein (CP) was calculated as 6.25 × N. Gross energy (GE) was measured using a bomb calorimeter (C200, IKA Works Inc., Staufen, Germany). Ether extracts (EE) were measured by weight loss of the DM on extraction with diethyl ether in Soxhlet extraction apparatus for 8 h (method 920.85) [18]. Crude fibre was determined by allowing the sample to boil with 1.25% dilute H_2SO_4, washed with water, further boiled with 1.25% dilute sodium hydroxide and the remaining residue after digestion was taken as crude fibre (method 978.10) as described by Thiex [19].

2.6. Feed Intakes and Growth Performance

During the 105 days of feeding trial, data on feed offered to each animal and the corresponding orts were recorded daily to estimate voluntary intake of DM and nutrients. Samples of feeds offered and orts were oven dried at 65 °C until a constant weight to determine DM concentration, and then ground to pass through 1-mm sieve (Wiley mill; Thomas Scientific, Philadelphia, PA, USA) and analyzed for organic matter (OM), CP, EE and CF by the procedures described above. The body weight (BW) of animals was individually recorded at the beginning of the trial, on weekly basis throughout the trial, and at the end of the experiment before the morning feeding. Feed intake, average daily gain (ADG) and feed efficiency were calculated from the data obtained.

2.7. Apparent Nutrients Digestibility and N Retention

Apparent digestibility coefficients of DM, OM, CP, EE and CF were determined by the total fecal collection method [20]. On day 91, 3 animals per treatment were placed into individual metabolism crates (0.5 m × 1.2 m), allowing feces and urine to be collected. The digestibility trial lasted for 14 days with 7 days for adaptation to metabolism crates and 7 days for the sample collection.

The amount of feed offered, refused, and feces were weighed daily and homogenized. A 10% sample of total feces was collected during a 7-day collection period as described by Ma et al. [21]. Urine was collected daily in buckets containing 100 mL of 10% (v/v) H_2SO_4. The volume was measured and a sample (10% of total volume) was collected and stored at −20 °C until analysis. Samples of feed, orts, feces, and urine were pooled to form a composite sample for each wether. Urinary N was analyzed by the Kjeldahl method [18], and N retention was calculated as daily N excretion (urinary N plus fecal N) subtracted from daily N intake.

2.8. Statistical Analysis

The data on apparent digestibility and N retention were analyzed using the PROC MIXED of SAS (version 9.1; SAS Inst. Inc., Cary, NC, USA). Because the experimental design was completely randomized, the model included only the fixed effect of the diet (treatment). Repeated measures were used to analyze data on feed and water intake and growth parameters. The effects of diet (treatment), weeks, and their interactions were considered fixed, whereas the wether was considered random. Data are presented as mean ± standard error of the mean, and significant differences were accepted if $p < 0.05$, Orthogonal polynomial contrasts were used to test the linear and quadratic effects of the diet on the parameters measured.

3. Results and Discussion

3.1. Chemical Composition of the Experimental Diets

The chemical composition of the basal experimental diets is shown in Table 1.

Table 1. Ingredients and chemical composition of the basal diet fed to Dohne-Merino sheep.

Items	Percentage (%)
Maize	8
Sunflower oil cake	10
Molasses	5
Wheat bran	15
Limestone	1.6
Sheep mineral-vitamin premix	0.20
Salt	0.30
Grinded Lucerne hay	30
Grinded teff hay	30
Chemical composition	
Dry Matter (% as fed)	95.5
Organic Matter (%DM)	85.2
Energy (MJ/kg DM)	24.7
Crude Protein (% DM)	14.6
Ash (%DM)	10.3
Ether Extract	1.70
Crude Fibre	22.60

3.2. Feed and Water Intake

As shown in Table2, the inclusion of FSF in the diet of Dohne-Merino wethers had a significant effect ($p < 0.05$) on feed intake. Animals in Treatments 3 and 4 consumed more feed than animals in the control group (linear $p = 0.04$; quadratic $p = 0.02$) while animals in treatment 2, consumed the least feed. This shows that the addition of FSF to the diets of Dohne-Merino wethers at 4% inclusion rate will increase the daily feed intake. This is similar to the results obtained by Emeruwa et al. [15], who recorded the lowest feed intake in the group fed 2% of FSF (900 ± 9.0 g/d) and the highest value for the 6% inclusion group (1009 ± 9.0 g/d). Also, Mclean et al. [22] reported significant variations in the feed intake of calves fed varying levels of FSF. Sheep requires about 25 to 40 ounces per head per day of NaCl, and will eat less feed when deprived of NaCl [23,24]. Similarly, it has been observed that minerals such as Na, Ca, K and Mg increase the palatability of the diet and the feed intake of the animal [25,26]. This could be the reason why animals fed FSF-supplemented diets had greater feed intakes compared with the controls, because FSF had been reported to be rich in minerals, including Na (923 ppm), Ca (0.22%), Mg (0.11%) and K (0.11%) [12–14]. Feed efficiency was highest in T3 while the control treatment has the lowest value ($p < 0.05$). However, the control was not statistically different from T2 and T4. The greater feed intake in T3 group was especially notable from week 7 until the end of the trial (Figure1). The absence of quadratic e ffect observed for various inclusion levels of FSF on nutrients intake shows that adding increasing levels of FSF up to 6% inclusion levels of the diets DM had no adverse effects on their consumption by the animals.

Table 2. Effects of varying levels of FSF on growth performance and water intake on Dohne-Merino wethers.

Parameter	Level of FSF in Diet (% of DM)				SEM	*P*-Values	
	0	2	4	6		Linear	Quadratic
Feed Intake(g)	593	572	694	648	39.9	0.045	0.024
Feed Efficiency (g/g)	0.14	0.18	0.19	0.17	0.01	0.011	0.023
ADG (g/d)	84.7	92.9	121.4	105.4	9.53	0.000	0.011
Water Intake (L)	1.95	1.67	1.8	1.45	0.10	0.000	0.113
Initial Weight (Kg)	19.2	19.4	19.5	19.4	0.43	0.667	0.221
Final Weight (Kg)	28.0	28.6	31.3	29.7	0.48	0.043	0.322
Total Weight Gain (Kg)	8.32	9.20	12.0	10.3	0.38	0.042	0.232

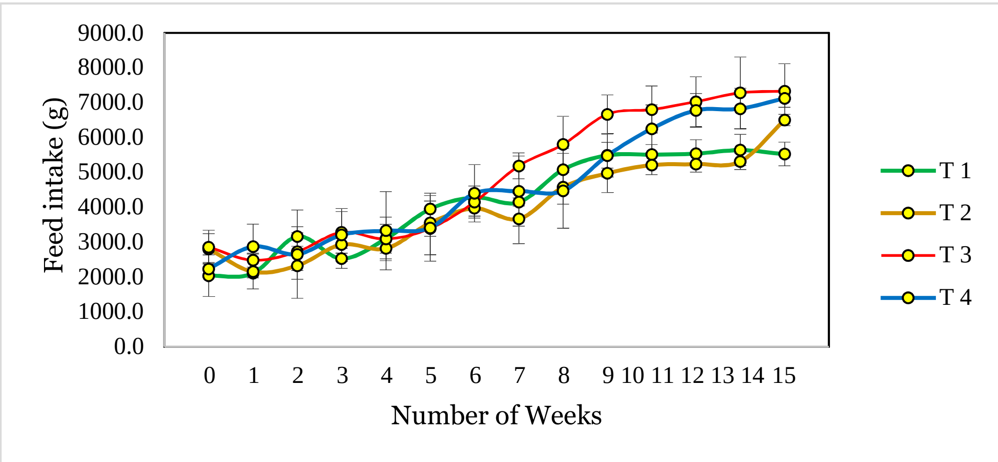

Figure 1. Graphical representation of feed intake of Dohne-Merino wethers fed varying levels of Fossil shell flour (FSF) shown as means ± standard errors. T1: 0% FSF diet; T 2: 2% FSF diet; T3: 4% FSF diet; T4: 6% FSF diet.

The water intake decreased linearly ($p < 0.001$) as the inclusion levels of FSF increased in the diets, except for 4% inclusion level (Table2). The control group has the greatest water intake, while T4 has the lowest value. Although the control group consumed slightly more water than T3 group, differences were not significant ($p > 0.05$). Increased water intakes usuallly encourage sheep to eat more feed [27,28].

3.3. Growth Performance

The effects of varying inclusion levels of FSF on growth performance are shown in Table2. Average daily weight gain was increased (linear $p = 0.00$; quadratic $p = 0.01$) and final weight was greater (linear $p = 0.04$) with increasing inclusion levels of FSF. The ADG was lowest for control group and greatest for T3. The lowest ADG and final BW in the control group (T1) is in agreement with the lower DMI recorded in the trial for this group.

3.4. Nitrogen Utilization and Nutrients Apparent Digestibility.

Table3 shows the nitrogen utilization and diet apparent digestibility in Dohne-Merino rams fed varying levels of FSF. Nitrogen intake, urinary N and N balance were not affected by the levels of FSF inclusion. However, fecal N was decreased (linear $p = 0.03$; quadratic, $p = 0.03$) as inclusion levels of FSF increased. This indicates that FSF could be used to reduce environmental pollution through reduction in fecal N excretion. This result was similar to the report obtained by Rajabi et al. [29] using pomegranate peel extract in fattening lambs as feed supplement. Vallejo et al. [30] reported that addition of natural feed additive such as xylanase (at 3 μL/g) to the diets of sheep could reduce excretion of N through fecal and urine.

Table 3. Nitrogen utilization (g/d) and apparent digestibility (%) of Dohne Merino fed varying levels of Fossil shell flour.

Parameter	Level of FSF in Diet, % of DM				SEM	*P*-Values	
	0	2	4	6		Linear	Quadratic
N Retention(g/d)							
N Intake	9.83	11.07	13.96	10.24	0.44	0.433	0.954
Fecal N	1.94	1.31	1.23	1.48	0.47	0.033	0.033
Urinary N	0.79	0.32	0.41	0.58	0.07	0.856	0.842
N Balance	7.15	9.41	12.35	8.18	0.46	0.454	0.965
Apparent Digestibility (%)							
Dry Matter	64.50	63.83	72.92	62.83	2.74	0.375	0.311
Organic Matter	65.58	66.24	73.15	63.13	2.81	0.353	0.367
Crude Protein	70.62	81.83	85.24	77.16	1.65	0.192	0.011
Ether Extract	85.54	89.69	93.01	90.19	1.41	0.247	0.222
Crude Fibre	51.10	51.17	64.79	52.73	3.88	0.712	0.312

The linear positive effect of the treatment on daily retained N in the present study was similar to that reported by Soroor et al. [31] when fattening Mehraban lambs were fed *Echium amoenum* extract up to 1.5 mL/kg of diet DM. Conversely, this result was not in agreement with the result observed by Emeruwa et al. [15] using FSF as supplement in West African dwarf sheep, as they observed that N retention was greater in control compared to the treatments. However, both in this study and in the study reported by Adebiyi et al. [12], FSF has no significant effect on N balance and N retention. The differences among studies could be due to different experimental conditions. This study was conducted in a semi-arid region while Emeruwa et al. conducted their trial in a tropical region. Other factors, as dietary and breed differences could have also influenced the results.

Increasing the inclusion levels of FSF had no effects on DM, OM, EE, and CF digestibility (Table3), but it has a quadratic effect on apparent CP digestibility ($p = 0.01$). The diet supplemented with 4% FSF (T3) showed the greatest value for all nutrient's digestibility and was significantly different ($p <$

0.05) from the control (T1) for all nutrients. However, there were no differences ($p > 0.05$) among T2, T3 and T4 treatments for CP and EE digestibility (Table 3). These results indicate that FSF enhanced the absorption of amino acids by the animal and thereby making them available at tissue levels [32]. These results are contrary to those obtained by Emeruwa et al. [15], who found that West African Dwarf sheep fed diets containing FSF at the same levels of inclusion than in the present study had numerically greater CP and EE digestibility values for control compared to other treatments, although differences did not reach the statistical significance. This difference in the results could be due to differences in breeds and diets composition. The lack of influence of FSF on DM, OM, EE and CF, may be attributed to similar digestibility of the experimental diets, particularly crude fibre digestibility [33]. Additionally, the relative similarity in the physical characteristics of the experimental diets may be the reason(s) for lack of no intake differences among the experimental sheep, because physical and nutrients composition of diets affect the feed intake [34].

4. Conclusions

The results from this study showed that adding 4% FSF to the diets of Dohne-Merino wethers gives a better improvement than other levels of FSF inclusion on DMI, feed efficiency, ADG, total weight gain, N retention and apparent nutrient digestibility of most of the nutrients. In addition, the wethers on this treatment consumed the lowest volume of water. These results suggested that inclusion of FSF at 4% in the diet could be potentially used as a performance enhancer.

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References

1. Frater, P. Feed Additives in Ruminant Nutrition 2014; Published by Agriculture and Horticulture Development Board, England (EBLEX). 2014.
2. Chaturvedi, I.; Dutta, T.K.; Singh, P.K.; Sharma, A.; Kumar, M.; Rao, B. Effect of Herbal Feed Additives on IVDMD, Methane and Total Gas Production Via in-vitro Study. *J. Agroecol. Nat. Resour. Manag.* **2014**, *1*, 108–112.
3. Tedeschi, L.O.; Callaway, T.R.; Muir, J.P.; Anderson, R.C. Potential environmental benefits of feed additives and other strategies for ruminant production. *Rev. Bras. Zootec.* **2011**, *40*, 291–309.
4. Jalaal, M.; Balmforth, N.J.; Stoeber, B. Slip of Spreading Viscoplastic Droplets. *Langmuir* **2015**, *31*, 12071–12075. [CrossRef] [PubMed]
5. Li, Y.X.; Zhang, X.L.; Li, W.; Lu, X.F.; Liu, B.; Wang, J. The residues and environmental risks of multiple veterinary antibiotics in animal faeces. *Environ. Monit. Assess.* **2013**, *185*, 2211–2220. [CrossRef] [PubMed]
6. Hu, X.; Zhou, Q.; Luo, Y. Occurrence and source analysis of typical veterinary antibiotics in manure, soil, vegetables and groundwater from organic vegetable bases, northern China. *Environ. Pollut.* **2010**, *158*, 2992–2998. [CrossRef] [PubMed]
7. Watanabe, N.; Bergamaschi, B.A.; Loftin, K.A.; Meyer, M.T.; Harter, T. Watanabe Use and environmental occurrence of antibiotics in freestall dairy farms. *Environ. Sci. Technol.* **2010**, *44*, 6591–6600. [CrossRef] [PubMed]
8. Arikan, O.A.; Mulbry, W.; Rice, C. Management of antibiotic residues from agricultural sources: Use of composting to reduce chlortetracycline residues in beef manure from treated animals. *J. Hazard. Mater.* **2009**, *164*, 483–489. [CrossRef]

9. Pils, J.R.V. The Role of Soil Clays and Clay-Humic Complexes in Processes Controlling Colloidal Stability and the Sorption and Degradation of Tetracyclines Recommended Citation. Ph.D. Thesis, Iowa State University, Ames, IA, USA, 2005.
10. Valero, M.V.; Do Prado, R.M.; Zawadzki, F.; Eiras, C.E.; Madrona, G.S.; Do Prado, I.N. Propolis and essential oils additives in the diets improved animal performance and feed efficiency of bulls finished in feedlot. *Acta Sci. Anim. Sci.* **2014**, *36*, 419–426. [CrossRef]
11. Ikusika, O.O.; Mpendulo, C.T.; Zindove, T.J.; Okoh, A.I. Fossil Shell Flour in Livestock Production: A Review. *Animals* **2019**, *9*, 70. [CrossRef] [PubMed]
12. Adebiyi, O.A.; Sokunbi, O.A.; Ewuola, E.O. Performance Evaluation and Bone Characteristics of Growing Cockerel Fed Diets Containing Different Levels of Diatomaceous Earth. *Middle East J. Sci. Res.* **2009**, *4*, 36–39.
13. Adeyemo, G.O. Growth performance of broiler chicken fed Fossil shell flour growth promoter. *Food Nutr. Sci.* **2013**, *4*, 26622.
14. Lakkawar, A.W.; Sathyanarayana, M.L.; Narayanaswamy, H.D.; Sugunarao, O.; Yathiraj, S. Efficacy of diatomacious earth in amelioration of aflatoxin induced toxicity in broiler chicken. *Indian J. Anim. Res.* **2016**, *50*, 529–536. [CrossRef]
15. Emeruwa, C.H. Growth Performance of West African Dwarf Sheep Fed Diets Supplemented with Fossil Shell Flour. Ph.D. Thesis, University of Ibadan, Ibadan, Nigeria, 2016.
16. Sarijit, J.; Steve, S.; Micheal, E.; Rodney, D. Animal Feed Compositions Including Spent Filter Media Containing Diatomaceous Earth. US Patent US20160295884A1, 13 October 2016.
17. National Research Council (NRC). *Nutrient Requeriments of Small Ruminants: Sheep, Goats, Cervids, and New World Camelids*; National Academy of Sciences: Washington, DC, USA, 2007.
18. Association of Officiating Analytical Chemists (AOAC). *Official Method of Analysis Association of Officiating Analytical Chemist*, 18th ed.; Association of Officiating Analytical Chemists: Washington, DC, USA, 2005.
19. Nancy, J. Thiex Evaluation of Analytical Methods for the Determination of Moisture, Crude Protein, Crude Fat, and Crude Fiber in Distillers Dried Grains with Solubles. *J. AOAC Int.* **2009**, *92*, 61–73.
20. Gebrehiwot, G.; Negesse, T.; Abebe, A. Effect of Feeding *Leucaena leucocephala* Leaves and Pods on Feed Intake, Digestibility, body Weight Change and Carcass Characteristic of Central-Highland Sheep Fed basal Diet Wheat bran and Natural Pasture Hay in tigray, Ethiopia. *Int. J. Agric. Environ. Biotechnol.* **2017**, *10*, 367. [CrossRef]
21. Ma, G.W.; Chu, Y.K.; Zhang, W.J.; Qin, F.Y.; Xu, S.S.; Yang, H.; Rong, E.G.; Du, Z.Q.; Wang, S.Z.; Li, H.; et al. Polymorphisms of FST gene and their association with wool quality traits in Chinese Merino sheep. *PLoS ONE* **2017**, *12*, e0174868. [CrossRef] [PubMed]
22. McLean, B.; Frost, D.; Evans, E.; Clarke, A.; Griffiths, B. The inclusion of diatomaceous earth in the diets of grazing ruminants and its effect on gastrointestinal parasite burdens. In Proceedings of the International Scientific Conference on Organic Agriculture, Adelaide, Australia, International Scientific Conference of Organic Agriculture Research, Bonn, Germany, 21–23 September 2005.
23. Digby, S.N.; Chadwick, M.A.; Blache, D. Salt intake and reproductive function in sheep. *Animal* **2011**, *5*, 1207–1216. [CrossRef] [PubMed]
24. Van, P. An Award-wining Merino sheep enterprise with the use of diatomaceous earth. *Farmer's Wkly.* **2015**, *30*, 1–15.
25. Garmyn, A.J.; Hilton, G.G.; Mateescu, R.G.; Morgan, J.B.; Reecy, J.M.; Tait, R.G., Jr.; Beitz, D.C.; Duan, Q.; Schoonmaker, J.P.; Mayes, M.S.; et al. Estimation of relationships between mineral concentration and fatty. *J. Anim. Sci.* **2011**, *89*, 2849–2858. [CrossRef]
26. Desimone, J.A.; Beauchamp, G.K.; Drewnowski, A.; Johnson, G.H. Sodium in the food supply: Challenges and opportunities. *Nutr. Rev.* **2013**, *71*, 52–59. [CrossRef]
27. Prasetiyono, B.W.H.E.; Sunagawa, K.; Shinjo, A.; Shiroma, S. Physiological relationship between thirst level and feed intake in goats fed on alfalfa hay cubes. *J. Anim. Sci.* **2000**, *13*, 1536–1541. [CrossRef]
28. Abioja, M.O.; Osinowo, O.A.; Adebambo, O.A.; Bello, N.J.; Abiona, J.A. Water restriction in goats during hot-dry season in the humid tropics: Feed intake and weight gain Nigerian livestock industries experience. *Arch. Zootec.* **2010**, *59*, 195–203. [CrossRef]
29. Rajabi, M.; Rouzbehan, Y.; Rezaei, J. A strategy to improve nitrogen utilization, reduce environmental impact, and increase performance and antioxidant capacity of fattening lambs using pomegranate peel extract. *J. Anim. Sci.* **2017**, *95*, 499–510. [CrossRef] [PubMed]

30. Vallejo, L.H.; Salem, A.Z.M.; Camacho, L.M.; Kholif, A.M.; Mariezcurrena, M.D.; Cipriano, M.; Alonso, M.U.; Olivares, J.; Lopez, S. Effects of xylanase supplementation on feed intake, digestibility and ruminal fermentation in Rambouillet sheep. *J. Agric. Sci.* **2016**, *154*, 1110–1117. [CrossRef]
31. Soroor, E.N.; Rouzbehan, Y.; Alipour, D. Effect of Echium amoenum extract on the growth rate and fermentation parameters of mehraban lambs. *Anim. Feed Sci. Technol.* **2013**, *184*, 49–57. [CrossRef]
32. Bahrami-yekdangi, M.; Ghorbani, G.R.; Khorvash, M.; Khan, M.A.; Ghaffari, M.H. Reducing crude protein and rumen degradable protein with a constant concentration of rumen undegradable protein in the diet of dairy cows: Production performance, nutrient digestibility, nitrogen efficiency, and blood metabolites. *J. Anim. Sci.* **2015**, *94*, 1–8. [CrossRef] [PubMed]
33. Van Soest, P.J. *Nutritional Ecology of the Ruminant*, 2nd ed.; Cornell University Press: Ithaca, NY, USA, 1994.
34. Riaz, M.Q.; Südekum, K.H.; Clauss, M.; Jayanegara, A. Voluntary feed intake and digestibility of four domestic ruminant species as influenced by dietary constituents: A meta-analysis. *Livest. Sci.* **2014**, *162*, 76–85. [CrossRef]